ANALYSIS

Integrating molecular and network biology to decode endocytosis

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The strength of network biology lies in its ability to derive cell biological information without a priori mechanistic or molecular knowledge. It is shown here how a careful understanding of a given biological pathway can refine an interactome approach. This permits the elucidation of additional design principles and of spatio-temporal dynamics behind pathways, and aids in experimental design and interpretation.

Clathrin-mediated endocytosis (CME) is an important vesicle biogenesis pathway[1,2]. Cargo is packaged with high fidelity into vesicles of defined size that are surrounded by a coat predominantly made of the protein clathrin and adaptor protein complexes. This coat is visible by electron microscopy and intermediate stages of vesicle formation have been documented (Fig. 1a)[3]. An extensive protein machinery works alongside the scaffolding clathrin and adaptor protein complexes to build a vesicle (Fig. 1b and Supplementary Tables).

The CME pathway was quickly recognized to be important for nutrient and growth factor entry into cells, and, despite its cargo specificity and regulation, some toxins and viruses can still hijack it to gain entry into cells[1,4]. At the synapse, clathrin-coated vesicles (CCVs) participate in retrieval of synaptic vesicles following exocytosis, in which potentially multiple copies of more than 10 different transmembrane proteins must be packaged, in appropriate stoichiometries, into vesicles of uniform size to maintain synaptic transmission[5].

We define CME as a 'pathway' because between the input (cargo) and the output (cargo-containing vesicle) there is an organized set of intermediate sub-reactions. In this analysis when we refer to a pathway we mean a macroscopic series of discrete events with a functional outcome and we do not mean higher order functions like cell division or vesicle trafficking, which are composed of orchestrated interactions of one or more pathways.

The CME interactome

At the morphological level, CME is seen as the pathway of cargo recruitment into patches of membrane, which become progressively invaginated to form CCVs. These vesicles are subsequently detached from the parent membrane and then uncoated (Fig. 1a). On closer inspection, this linear depiction is an oversimplification, because at the molecular level there are many micro-reactions occurring simultaneously in this pathway. For example, cargo recruitment, membrane bending and clathrin-polymerization all happen in parallel in the early stages of vesicle formation[6].

Appreciating the considerable variety of proteins known to be involved in CME, we set out to learn about the pathway from a network approach. Using biochemical, structural and proteomic data[6–12], the major protein interactions for CME can be organized as a pathway protein interactome (Fig. 1b)[6,10] giving us the 'CME-interactome'. This may not be the complete description of all possible interactors but, given the intensity of work on CME, it is likely that a very large percentage of protein–protein interactions are already described and therefore presented in this interactome.

The basic elements of the interactome (individual proteins and lipids, and so on) are called nodes and their interactions are depicted as links connecting the nodes[13–15]. Stable protein complexes, like the adaptor protein complex AP2 or a clathrin triskelion, are considered as single nodes (a position that has support in the literature[16]). To emphasize the core machinery of endocytosis we peeled away interactions that point outwards from the pathway to distinct processes like exocytosis and actin polymerization (Fig. 1b, grey-shaded areas). Thereby we distinguish our pathway from other network modules[17] that can be connected pathways (for example, exocytosis) or modular attachments ('plug-ins'). Such attachments can be used in many diverse pathways (for example, actin polymerization associated with vesicle scission and motility[18–20]). Also, for simplicity, in our CME interactome we concentrated on protein nodes. Changes in second messengers like calcium, in phosphorylation levels, and in membrane lipid composition could also be described as nodes in the pathway and changes to these components would have wide implications for this and other trafficking pathways. Disregarding the overall regulation is crucial for identifying the core machinery. These plots deliberately underestimate the ways in which pathways are directly or indirectly sensitive to other pathways in the cell when embedded in a larger network.

The first thing that becomes apparent within this newly generated pathway-network is that clathrin and AP2 have disproportionately more interactions than other proteins in this pathway and so fall into the definition of being 'hubs'[13,21] (see Fig. 1b).

Hubs have been subdivided into 'party' and 'date' subtypes[22]. Date hubs are biomolecules that have many partners but interact with them at different times or locations (dynamic hubs), whereas party hubs can interact with many biomolecules at once (static hubs). Date hubs are proposed to organize the proteome, connecting biological processes to each other, whereas party hubs are proposed to function within modules (which may be pathways). Although recognizing the descriptive benefits of 'party hub' and 'date hub' nomenclature we call clathrin and AP2 'pathway hubs' because this does not immediately ascribe any behaviour to these hubs, but simply states that they appear within the studied pathway.

CME pathway hubs

The AP2 hub belongs to the family encoding heterotetrameric adaptor protein complexes. Such adaptor protein complexes have a trunk domain that binds to cargo and lipids, and two appendage domains, positioned on flexible linkers that bind to accessory proteins via two distinct binding sites on each appendage (Fig. 2a)[7,8,23,24].

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It is these appendage domains that form the protein interaction surface for the many accessory proteins (for binding motifs see Table 1 and Supplementary Tables), when concentrated in forming coated pits[10]. Adaptor protein complexes do not self-polymerize and so concentration and stabilization of adaptor protein complexes is via binding partners (as shown schematically for the binding partner Eps15; Fig. 2a).

Clathrin is the second hub in the network (Fig. 1b). Clathrin binds to most of its interaction partners via a β-propeller domain found at the end of each leg of the clathrin triskelion (Fig. 2b). During coated vesicle formation, clathrin becomes concentrated by interactions both with adaptor protein complexes and with accessory proteins[6,12].

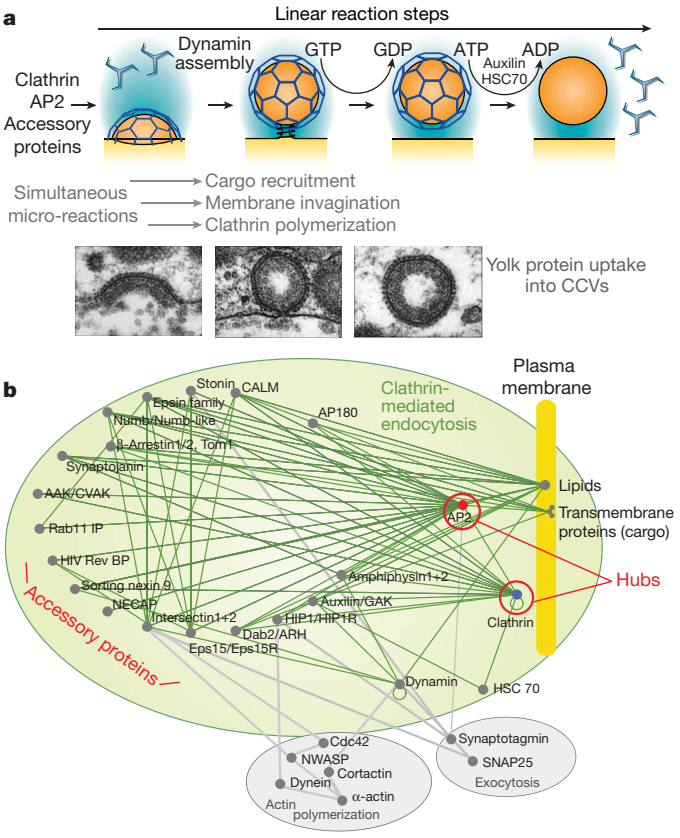

Figure 1 | Clathrin-coated vesicle formation: molecular detail versus a network view. a, Budding of a CCV. The initial membrane invagination occurs in concert with cargo recruitment and clathrin polymerization. Accessory proteins, such as AP180/CALM and epsin, are responsible for clathrin recruitment and size-constrained polymerization as well as membrane bending. The AP2 adaptor protein complex links the plasma membrane cargo into the forming clathrin coat. Dynamin is recruited by the accessory proteins amphiphysin, sorting nexin 9 and/or intersectin to the neck of the vesicle to enact membrane scission on GTP hydrolysis, whereas auxilin and the ATPase HSC70 are involved in the uncoating. The stages depicted are also evident in electron microscopy pictures taken from the work on yolk protein endocytosis in chicken[3] and reproduced with permission of the Company of Biologists. **b**, The CME interactome: the protein interactions occurring during coated vesicle formation. We have grouped many proteins with similar functions together, for example some of the kinases involved in CME (AAK and CVAK) and alternative cargo adaptors (Dab2 and ARH) are illustrated as single nodes. Different kinds of cargo molecules are also illustrated as one node, which should not lead to the assumption that cargo is a hub. This type of plot shows that AP2 and clathrin are the most common protein interaction points in the network, and therefore are of importance in the organization of the network, and these are surrounded by accessory proteins. The CME interactome is likely to be slightly different in each cell type and will be adapted to the ligands to be endocytosed and the speed with which this is required. Also, differences between species are easy to accommodate within the network, given its flexibility.

Table 1 | Domain structures and functions of CME proteins

Proteins enriched in CME	Proposed function and domain structures	*R. norvegicus*	*D. rerio*	*D. melanogaster*	*C. elegans*	*S. purpuratus*	*P. falciparum*
		Conservation					
Clathrin heavy chain	Self-polymerizing support around vesicle						
	β-Propeller — Clathrin heavy chain repeat	+	+	+	+	+	+
AP2 (α, β2, μ2, σ2)	Links plasma membrane, cargo, clathrin and accessory proteins	Blast searches for α-subunit only					
	Trunk — Appendage (α)	+	+	+	+	+	+
	Scaffolding molecule						
Eps15	EH EH EH — UIMs	+	+	+	+	+	–
Eps15 R	EH EH EH — UIMs	+	–	–	–	–	–
	Membrane bending molecule						
Epsin 1	ENTH UIMs X X	+	+	+	+	+	–
Epsin 2	ENTH UIMs X	+	+	–	–	–	–
	Membrane binding, clathrin recruitment and vesicle size determination						
AP180	ANTH — X X X	+	+	–	–	–	–
CALM	ANTH — X	+	+	+	+	+	–
	Dynamin recruitment						
Amphiphysin 1	N-BAR — X X — SH3	+	+	–	–	–	–
Amphiphysin 2	N-BAR — X X — SH3	+	+	*	–	–	–
	Scission molecules						
Dynamin 1		+	+	+	+	+	–
Dynamin 2	GTPase — PH — GED — PRD	+	+	–	–	–	–
Dynamin 3		+	+ ?	–	–	–	–
	Alternative adaptors for GPCR receptors						
β-Arrestin 1	Arrestin Arrestin X	+	+	+	+	+	–
β-Arrestin 2	Arrestin Arrestin X	+	+	+	–	–	–
	Alternative adaptor for the LDL receptor						
ARH	PTB	+	+	+	+	+	–
	Alternative adaptor for the LDL receptor						
Dab2	PTB X X	+	+	+	+	+	–
	Alternative adaptors for the Notch receptor						
Numb	PTB	+	+	+	+	+	–
Numb-like	PTB	+	+	–	–	–	–

and AP2 appendage binding motifs — 100 amino acids — X Clathrin binding sites

Hub proteins are conserved; but where there are multiple isoforms of accessory proteins in mammals and fish, one seems to be sufficient in invertebrates, even in those with nervous systems. A more comprehensive version of this Table is given in Supplementary Tables 1 and 2. Clathrin interaction motifs (X) are incomplete as they are difficult to detect given their sequence variations. Question marks indicate uncertainty. When we do not find clathrin or adaptin interaction motifs in homologues then we generally assume that the protein is not involved in CME. For amphiphysin, we know that a *Drosophila* form of amphiphysin does not have any clathrin or adaptor interaction and does not function in CME and so is not annotated in this Table (see asterisk). ANTH, API80 N-terminal homology; AP180, adapter protein 180 kDa; ARH, autosomal recessive hypercholesterolaemia; BAR, Bin/amphiphysin/Rvs; CALM, clathrin assembly lymphoid myeloid leukaemia protein; *C. elegans*, *Caenorhabditis elegans*; Dab2, disabled 2; *D. melanogaster*, *Drosophila melanogaster*; *D. rerio*, *Danio rerio*; EH, Eps15 homology; ENTH, epsin N-terminal homology; Eps15/R, epidermal growth factor receptor pathway substrate 15/related; *P. falciparum*, *Plasmodium falciparum*; PRD, proline rich domain; PTB, phosphotyrosine binding; *R. norvegicus*, *Rattus norvegicus*; SH3, Src homology 3; *S. purpuratus*, *Strongylocentrotus purpuratus*; UIM, ubiquitin interacting motif.

Concentrated clathrin self-polymerizes into a lattice, forming an interaction surface (beneath the outer shell of clathrin legs) made up of many β-propeller domains (Fig. 2b), allowing the lattice to bind to many adaptor protein complexes and accessory proteins simultaneously. This, and clathrin's interaction with itself, provides the required stability to form the budding vesicle and is also likely to be important in the concentration of cargo into the coated pit and in the binding of proteins involved in uncoating.

Functional versus connectivity view of CME

Interactomes give a static picture of pathways that we know to be dynamic[25]. Thus we attempt to show the changes in connectivity

associated with the different stages of vesicle formation (Fig. 3). In the static interactome (Fig. 1b), we present a network for plasma membrane CCV formation where AP2 is the major heterotetrameric adaptor complex involved in cargo binding[23]. We now further focus on one form of plasma membrane CCV formation—that of synaptic vesicle retrieval in nerve terminals[26]—to emphasize the dynamics of what can happen in one vesicle, but the dynamics of the network will be similar for all types of CCV formation (Fig. 3).

To form a cargo-containing vesicle, cargo needs to be concentrated at patches on the membrane of the appropriate lipid environment. This is likely to happen by the clustering of adaptor protein complexes bound to phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) on the plasma membrane[27] (Fig. 3a, functional view), which become stabilized by accessory proteins that bind multiple AP2s simultaneously[10] and also aid in recruitment of clathrin. Clathrin polymerization leads to coat formation. AP2 loses its importance at this stage because clathrin now drives vesicle formation (Fig. 3c, functional view). As soon as the formation of the coated vesicle is completed, it is detached from the parent membrane, and subsequently uncoated (Fig. 3d, functional view).

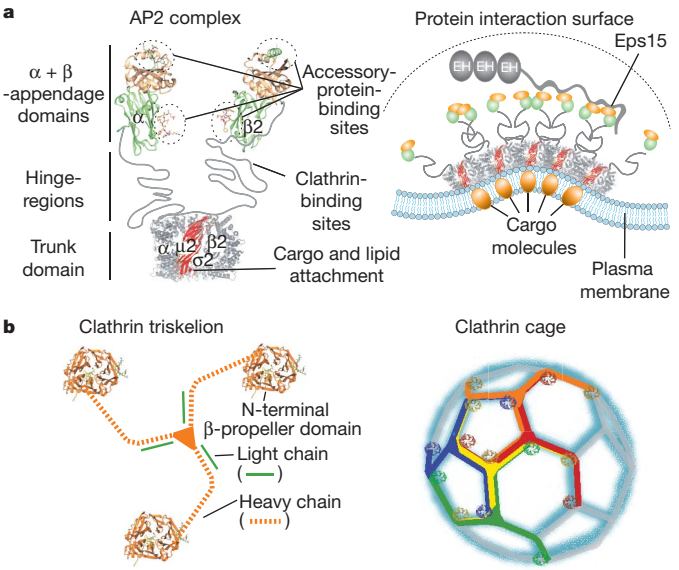

Figure 2 | Structural organization of the hubs in CME. a, The major adaptor protein complex in brain-derived CCVs is the AP2 complex. This is composed of four subunits (α, β2, μ2, σ2). Membrane cargo interacts predominantly with the μ-subunit (in red), whereas the appendage domains (orange and green), are responsible for binding to accessory proteins (four different peptide interactions are highlighted by dotted lines). These domains are positioned on long flexible linkers and thus they are perfectly designed to be protein recruitment domains bringing the needed partners from the cytosol to the forming coated pit. The right panel shows a representation of a cargo-containing plasma membrane patch accommodating closely packed AP2 complexes, generating a dense appendage protein interaction surface. AP2s are stabilized by clustering molecules such as Eps15, which have been shown to bind up to four appendages simultaneously. **b**, A clathrin triskelion consisting of three clathrin heavy and light chains. Clathrin interacts with ligands via its N-terminal β-propeller domain. When clathrin self-polymerizes into a cage (right panel), these domains form a dense interaction surface composed of at least 180 β-propeller domains per vesicle[2]. From a structural point of view, these propellers are tucked underneath the forming cage (unlike the adaptor protein complex appendage domains that point away from the vesicle) and are thus more likely to have an organizational role in coated pit formation, rather than a protein recruitment role. The clathrin cage on the right is not modelled on a 50 nm vesicle but comes from cryo-electron microscopy reconstruction of isolated cages[42]. The protein data bank accession numbers for the structures are as follows: α-appendage, 1W80; β-appendage, 2IV8, 2Iv9; AP2 core, 1GW5; clathrin β-propeller, 1C9L and 1UTC.

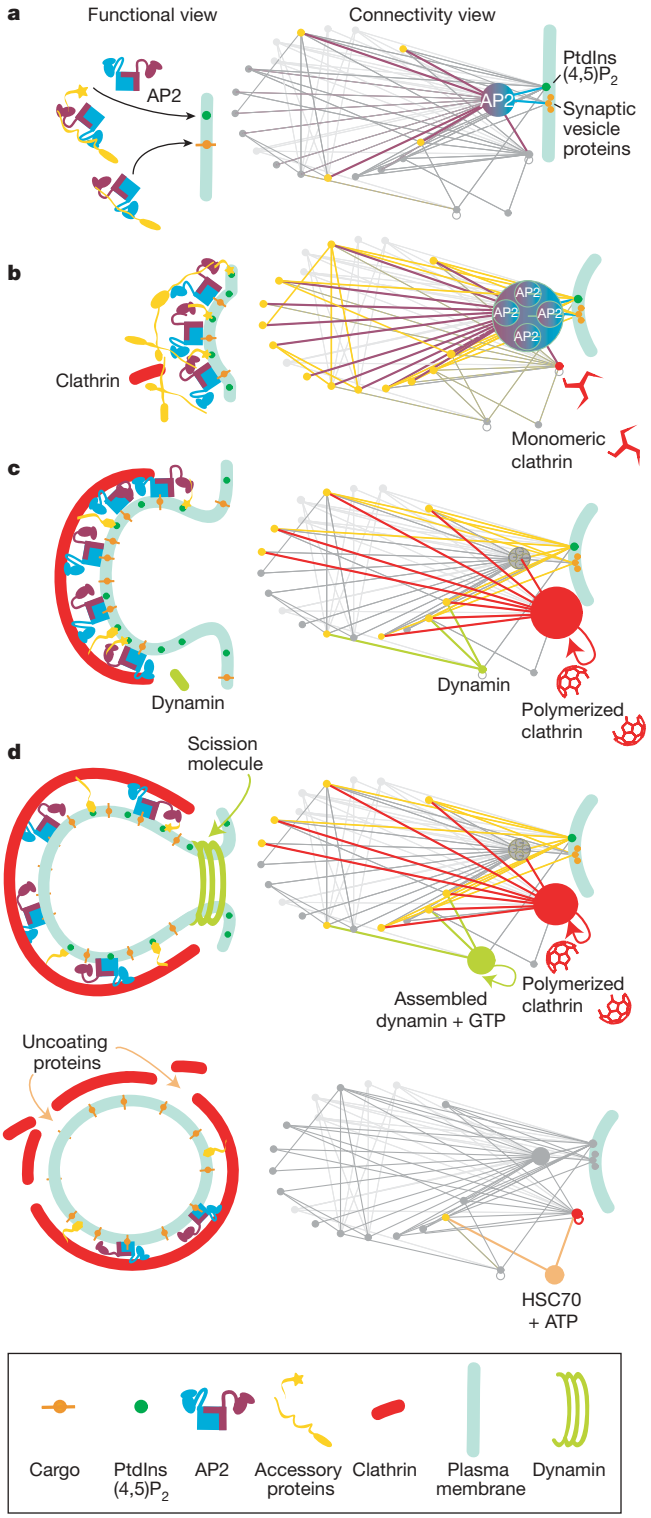

Figure 3 | The steps of synaptic vesicle CCV formation. Functional and connectivity views of CCV formation in nerve terminals. In the network view of Fig. 1b, we included all plasma membrane accessory proteins, but here we are concerned with the changing interactions during synaptic CCV formation (non-synaptic CCV nodes are in light grey). **a**, AP2 recruitment to membranes, by cargo and lipid interactions. **b**, Building the AP2 hub assembly zone: the AP2 pathway hub is stabilized by cross-linking through accessory proteins. **c**, Changing hubs: clathrin is recruited and polymerization stabilizes the forming vesicle, displacing the AP2 recruitment hub and becoming the new organizing hub. **d**, Assembled dynamin and other late-stage interaction partners (like uncoating factors) begin to function.

Connectivity interactomes are a different presentation of pathway progression, emphasizing the dynamic nature of the CME-network (Fig. 3, connectivity view). With each step of CME there is a corresponding change in protein connectivity. The increasing number of purple lines from Fig. 3a to 3b (connectivity view) illustrates the increasing number of interactions AP2 can achieve by being clustered on the membrane. One AP2 molecule can at most interact with four accessory proteins at once, one clathrin molecule, and a limited number of cargoes and lipid head groups. By assembling many AP2s at the membrane these interactions expand and lead to the fully engaged interactome. We would suggest that AP2 only functions as a hub when it is concentrated, forming an AP2 hub assembly zone[6].

Many of the accessory proteins successfully recruited by AP2 to the site of endocytosis also have interactions with each other and with lipids (yellow lines), further stabilizing this intermediate and allowing the recruitment of proteins that are present in lower concentrations and that bind with lower affinities. In addition, many accessory proteins have clathrin interaction sites and therefore they can participate (with AP2) in clathrin recruitment and concentration, leading to polymerization. Because there is steric overlap between most clathrin and AP2 sites in accessory proteins, AP2 loses its significance and there is a rewiring of the network because the newly formed clathrin hub takes over from the AP2 hub as the organizing centre (red lines to polymerized clathrin; Fig. 3c, connectivity view). Finally the conditions are ripe for the assembled scission molecule dynamin and subsequently the uncoating molecule auxilin to function (Fig. 3d). More information on predicting the timescale of events from topological properties of interactomes can be found in Supplementary Information Part 1 and Supplementary Fig. 2.

What we learn from integration

Plotting the CME pathway protein interactome allows one to immediately identify the key players (hubs) and approximately where they work in the pathway.

Knowing what status a protein has in the network (hub versus a regular node) allows us to predict experimental outcomes. It has previously been suggested that depletion of proteins with many interaction partners (by RNA interference) is more likely to give phenotypes[28]. Complete depletion of a pathway hub will probably eliminate the pathway. Partial depletion of the AP2 hub affects AP2-specific cargo internalization[29]. Additional modules (alternative cargo adaptors) lead to robustness in the network allowing alternative cargoes to be taken up with less or no dependence on AP2. Depletion of regular nodes is likely to be less deleterious than depletion of hubs. Depletion of a protein positioned in-between pathways (like dynamin, see below) may well inhibit many different pathways[30,31].

We would suggest that a good test for proteins that function as hubs only when clustered in space and time is that overexpression of such a protein should not have any phenotype on the pathway. Conversely, overexpression of nodes that bind directly to hubs would be predicted to have disastrous affects owing to titration of hub interaction points (see Supplementary Information Part 2, for further discussion).

Fine-tuning hub nomenclature

We describe the pathway hubs, AP2 and clathrin, as the control centres of the CME pathway. We do not ascribe hub status to dynamin in the CME interactome because it has only four links into this pathway. However on the Human Protein Reference Database (http://www.hprd.org) dynamin is reported to have approximately 50 interaction partners. Thus, in a proteome network, dynamin would also be a hub and would seem to connect many different pathways together. This is misleading, because from a functional point of view dynamin is simply a module ('plug-in') that is attached to many different pathways in a cell because of its function as a scission molecule, and it is not the connection point between these pathways. According to the network nomenclature[22], dynamin would be called a date hub, but in our view these hubs do not necessarily organize the proteome. On this point we would urge caution because the network nomenclature has preceded a functional understanding of hubs. In a network, the node for dynamin should be present in as many copies as there are pathways in which it is involved, and each of these nodes should not be linked to each other.

We would suggest that hubs could alternatively be named according to where they are positioned in a pathway at a given time, rather than the number and type of interactions. The notion that deletion of party/pathway hubs has much more limited effects than deletion of date hubs[22] can now be explained, because deletion of date-hubs will lead to more severe effects on cell viability owing to the additive result of disrupting a frequently used module (for example, dynamin[30–32]) and thus affecting disparate pathways, whereas deletion of a pathway hub (for example, AP2; refs 29, 33) will have more limited consequences (see also ref. 34 and Supplementary Information Part 2).

Hubs in proteomes are generally not directly connected to other hubs[34] and this may mean that the organization of each pathway is independent, with the loss of one hub not affecting other pathways in the cell[22]. A more recent observation was that party hubs, being the core of highly clustered functional modules, are frequently directly connected[21]. This fits with the direct connection between AP2 and clathrin in the CME interactome. A useful functional feature of this direct connection is seen in the biochemical observation that the clathrin hub displaces the AP2 hub interaction partners and thus ensures directionality to the dynamic protein interactions occurring during CCV formation[6].

One important property of the pathway hubs AP2 and clathrin is that neither are hubs at the beginning of CME, but mature into hubs by clustering either on the membrane or through polymerization. It is likely that many pathway/party hub proteins will oligomerize or cluster to function as pathway hubs. 'Clustered hubs' are a new subtype of hubs not previously described (Supplementary Information Part 3 and Supplementary Fig. 3).

Consequences of hub-centric pathways

The hub-centric organization of a pathway has at least four major consequences, described below.

(1) Ease of module attachment. An appealing feature of a network is the ease with which modules of functionality can be attached/evolve. Hubs act as the control points where these modules can be plugged into the system to give extra capabilities. In the CME interactome, we find additional alternative cargo adaptors/clathrin-associated sorting proteins (CLASPs) (see Supplementary Tables) added to the network by binding to the AP2 and clathrin hubs, when specific cargo needs to be endocytosed[6,12,35]. Thus by adding a small module, the repertoire and potentially independent regulation of this specialist cargo[36] is accommodated while still using the same overall network. Not all pathways will have such complicated networks. For example, in COPI and COPII vesicle budding the network of interactions is much simpler[23]. Simpler and less flexible networks should have hubs with fewer connecting partners and not all hubs can be expected to be regulated by oligomerization. Indeed post-translational modifications such as phosphorylation could well be regulators. Phosphorylation controls the overall flux through CME at the synapse by influencing the ability to form protein networks and so phosphorylation in this context is a master regulator of CME, but it is also clear that phosphorylation can fine-tune individual steps[27,37,38].

Modularity can also be seen from looking at the conservation of the CME network across species. We observe that most participating proteins are conserved in the animal kingdom (Table 1 and Supplementary Information Part 4). Species evolutionary distant from mammals, like the malaria parasite *Plasmodium falciparum*, tend to only conserve the hub proteins and not other nodes, an observation previously made more generally from proteome analysis[39].

(2) Dynamic instability. In a network of interacting proteins most interactions are of necessity low affinity, and from biochemical measurements of affinities we know that many individual steps are dependent on coincidence of multiple interactions[10,27]. Even the initial event of adaptor protein complexes binding to cargo is dependent on the presentation of binding sequences in membrane cargo and binding of the adaptor protein complexes to a specific lipid. Coincidence is seen at all stages in the process because affinities are frequently low enough that multiple interactions are required to give an avidity that can allow an interaction to occur. This gives rise to a dynamic instability in the network, because the network assembly will depend on whether sufficient adaptor protein complexes are recruited and sufficient accessory proteins are present to crosslink and organize these molecules and, subsequently, whether sufficient clathrin is recruited to stabilize the forming coated pit.

(3) Pathway progression. Dynamic instability leads to the question of how directionality can be imposed on the overall pathway of CCV formation. At some point there must be commitment to coated pit assembly and the pathway needs directionality to reach the end product of a free vesicle.

In CME, we are concerned with a network of interactions that can only form when the first hub protein AP2 is recruited and concentrated on the membrane at sites for endocytosis. The strengths of network interactions around clustered AP2s are described as avidities, as they are the combination of many different interactions. To progress in vesicle formation, these avidities have to be overcome. Normally, this would be overcome with a still stronger interaction, or by weakening the previous interaction (perhaps via post-translational modification). In CME, clustered adaptor protein complexes, cross-linked by high avidity interactions with accessory proteins, need to progress to a mature CCV. In immunofluorescence experiments, it is observed that clathrin is recruited and polymerized around nascent vesicles while accessory proteins (for example, eps15; ref. 6) are lost. This is reinforced by the absence of most accessory proteins from purified CCVs[40]. Biochemical experiments on the other hand tell us that clathrin competes poorly with accessory proteins for AP2 (refs 6, 8, 12) and so it would seem that clathrin is unlikely to be responsible for this loss of accessory proteins. The answer may lie in the different polymerization states of clathrin: in the biochemical competition experiments, clathrin is not polymerized but on the vesicle, clathrin is polymerized (Fig. 3b, c). Polymerized clathrin will have different properties and needs to be viewed as a semi-solid matrix. Neither affinity nor avidity interactions describe the interactions of this matrix with its binding partners sufficiently, and therefore we have previously described this mass-action as 'matricity'. Matricity describes a situation in which the matrix does not have traditional diffusion kinetics, and thus a very low-affinity interaction appropriately positioned may well be highly significant. Thus, the network moves from affinity-based interaction, to avidity-based interaction, to matricity-based interaction. This gives directionality to the pathway (Supplementary Fig. 4).

One problem posed by this increasing stability of the CME interactome is that eventually components have to be recycled and reused for the next CCV formation. It is at this point that ATP hydrolysis by the uncoating proteins gives the required injection of energy to re-prime the molecules so that they can start the cycle again, when required. Of course, there are many other required inputs of energy into the system, such as in making the appropriate lipids, but it is interesting that this major input of energy occurs after vesicle-formation is committed (dynamin GTP hydrolysis) or completed (ATP hydrolysis by HSC70 during uncoating) (Fig. 3d). In exocytosis, we also see a network of protein interactions in which ATP hydrolysis is mostly seen after vesicle fusion has occurred, and this is used to reprime the molecules for the next round of fusion. This use of ATP late in the cycle also adds to the dynamic instability of the pathway.

(4) Fidelity and flexibility. In CME, there is an interesting observation that hubs, AP2 and clathrin, bind to overlapping partners, and both hubs compete with each other for these partners. Evolution has neither resulted in these hubs being dispensed with, nor combined them into one, because the AP2 hub also has a specialized function of specific cargo interaction and clathrin has the specific cage-forming function. If these hubs were to become one, then the pathway would lose the dynamic instability, and hence the directionality and flexibility. We argue that AP2 binding modules must surely result in the expansion of cargo recruitment possibilities and if cells had no adaptors, but clathrin were to include a cargo binding domain, then this would reduce the flexibility of the cargo repertoire. Also, if clathrin were to polymerize on cargo binding then the process is more likely to cascade in a forward direction because clathrin self-polymerizes, forming empty cages. Natural selection has, instead, resulted in non-self-polymerizing modules to capture cargo, and a non-membrane-binding module to form the polymerized coat around this module. Thus the pathway has in-built fidelity.

Conclusion

Networks and interactome pathway maps are useful tools to help explain a biological pathway, but they need to be anchored in biological contexts and experimental fact. This can initially be done by embedding interactomes within well-characterized pathways and using this information to inform us in other pathway investigations. We have made many observations about the nature of the CME interactome—about what constitutes a hub, the modularity of the system, the possible evolution of the network, and about dynamic instability, directionality and robustness within networks. We need to understand more about how proteins function together and are organized into pathways, and how these pathways are integrated into yet larger contexts. For this, a fresh perspective and a vision for conceptual thinking, coupled with technical advances to map out dynamic interactomes[41] and in visual immunoprecipitation[25], is needed, which ultimately will lead to a better understanding of complex biological processes.

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