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The function of endocytosis in podocytes

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Abstract

Purpose of review—In this review we discuss the role of endocytosis, a fundamental process internalizing molecules from the plasma membrane, and its critical importance in podocyte biology.

Recent Findings—Endocytic clathrin and nonclathrin coated pits have been visualized in podocytes using electron microscopy, but the functional biological relevance has not been well defined. Recent evidence suggests that loss of key clathrin endocytic regulatory apparatus, such as dynamin, synaptojanin 1, or endophilin, in genetic mouse models of disease, results in severe proteinuria and foot process effacement. Additionally, several genes implicated in human nephrotic syndrome, directly or indirectly associate with these endocytic proteins, thus creating a protein network, that is linked in actin dynamics, signaling, and endocytosis.

Summary—This review summarizes our current understanding of membrane trafficking specifically in podocytes, thus giving further novel insights into the molecular mechanisms and pathogenesis of nephrotic syndrome.

Keywords

endocytosis; podocytes; nephrotic syndrome

Introduction

A major function of the glomerulus is to filter plasma to generate urine while retaining essential solutes in circulation. Podocytes, the cells of the glomerulus that line the capillary loops, play a critical role in carrying out this function. Their interdigitated foot processes, connected by a slit diaphragm, together with the fenestrated endothelial cells, and the intervening basement membrane form the filtration barrier. The impairment in the glomerular filtration barrier often manifests as massive protein loss in the urine which is often a sequelae of chronic kidney disease (1). The importance of podocyte integrity in the pathogenesis of nephrotic syndrome is best illustrated by the identification of human disease-causing mutations in podocyte-specific genes, nephrin and podocin, two membrane proteins that span and stitch together foot processes of neighboring podocytes (2-4). Other

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Conflict of Interest

The authors disclose no conflict of interest

key genetic mutations in alpha 3 integrin, CD2-associated protein (CD2AP), alpha actinin-4, Myo1e, *APOL1* and *TRPC 6* have further illustrated the complex regulation of podocytes during health and disease (5-12). Over the last several years an interesting concept has emerged regarding the relationship between glomerular podocytes and neuronal synapses (13, 14). For example, a subset of Charcot-Marie-Tooth disease, a hereditary disorder affecting the peripheral nervous system, has been linked to nephrotic syndrome (15). To some extent, such similarities may reflect organizational similarities: foot processes, like axon terminals and dendritic spines, are peripheral extensions of branched cellular processes. They also participate in very precise cell-cell contacts whose function is critically dependent upon protein networks that regulate the cortical cellular cytoskeleton, with roles, often interconnected, in signaling, actin dynamics, and endocytosis. This fundamentally important pathway that has been extensively studied in neurons, appears to be recapitulated and equally critical in podocytes. In this review, we will focus on the recent new findings on clathrin and nonclathrin mediated endocytosis within podocytes, concentrating on the link between endocytosis and the actin cytoskeleton, and its role in slit diaphragm recycling.

Overview of endocytic pathways

Endocytic pathways serve as a gateway for all cells by orchestrating extracellular proteins that are internalized. Utilization of various discrete forms of endocytosis can regulate the numerous cellular processes and this fundamental process appears to be conserved in podocytes, as abundant endocytic vesicles have been observed and described in glomerular cross sections by electron microscopy (**Fig 1**) (16, 17). Endocytosis is often categorized into clathrin dependent and clathrin independent pathways. For example, clathrin mediated endocytosis (CME) is observed where the plasma membrane invagination is decorated by clathrin illustrated by the lattice like coat. This process has been characterized to occur in a multi-step fashion. Initially, a phosphatidylinositol-4,5-bisphosphate(PI(4,5)P2) enriched region of the plasma membrane allows for recruitment of clathrin adaptor protein, AP2 along with BAR domain proteins, such as endophilin that sense and generate membrane curvature, to initiate clathrin nucleation and polymerization. Following clathrin coat assembly, the BAR domain protein and the actin machinery, sculpts the neck of the budding vesicle, resulting in GTPase dynamin self assembly, constricting the neck. Through GTP hydrolysis, fission occurs, resulting in the internalization of the clathrin-coated vesicle. Lastly, synaptojanin, an inositol 5' phosphatase, dephosphorylates its main substrate PI(4,5)P2 at the plasma membrane, and along with auxillin/GAK and hsc70, participate in shedding of the clathrin coat (18).

The other major mechanism of internalization that has been described in podocytes is lipid raft dependent, clathrin independent endocytosis, where plasma membrane buds are composed of flask or omega shaped smooth invaginations that are enriched in cholesterol, and sphingolipids. Tyrosine phosphorylation of caveolin 1 initiates the initial budding and internalization (19). Raft mediated endocytosis appears also to be dependent on the actin machinery and dynamin, which triggers the fission reaction. Yet, recent evidence suggests in fibroblasts lacking dynamin, there is reduced caveolae found in the cell, rather than an increase, as would be expected (20, 21).

Other clathrin independent endocytic mechanisms that likely contribute to podocyte homeostasis, but have not been clearly elucidated are macropinocytosis, Arf6, flotilin, and Clathrin independent carrier (CLIC)/GPI-AP-enriched early endosomal compartment (GEEC) mediated endocytosis (19).

Recent evidence of the importance of CME in podocytes

Using an in-vitro gene delivery approach, it has been previously reported that cathepsin induced proteolysis of dynamin modulates the podocyte actin cytoskeleton (22). However, the physiological relevance of endocytosis in-vivo has not been studied until recently. The loss of dynamin 1 and 2 (dynamin DKO) specifically in podocytes revealed massive proteinuria and kidney failure with histological features suggestive of focal segmental glomerulosclerosis (23). By ultrastructural examination, the glomeruli of kidneys showed marked collapse of the podocyte foot processes, with highly irregular and thickened basement membranes. Interestingly the constitutive loss of two proteins that form a close functional and interactive partnership with dynamin at neuronal synapses, namely, synaptojanin 1 (24, 25), or endophilin, (26, 27), also resulted in severe proteinuria and foot process effacement at birth (23). The functional and ultrastructure changes produced by the lack of dynamin and synaptojanin 1 or endophilin were strikingly similar (28). Another protein implicated to be critical in CME is the *class II PI 3-kinase*, which phosphorylates PI(4,5)P₂ into PI(3,4,5)P₃. Mice lacking PI3KC2 γ , the gene encoding this kinase, develop glomerulonephropathy with proteinuria and renal failure (29). Recent evidence also suggests in kidney biopsy samples from Fabry disease patients, α -galactosidase A, binds with the mannose 6 phosphate receptor, sortilin, and megalin in podocytes, which undergoes subsequent endocytosis (30). Furthermore, increased podocyte expression of endosomal H⁺/Cl⁻ exchanger CIC-5 has been observed in human kidney biopsies from patients with nephrotic syndrome (31). This suggests that podocytes utilize CME pathways, during development, maintenance, and in disease states.

Actin cytoskeletal regulation in podocytes is linked with CME

The maintenance of a normal glomerular filtration barrier requires an intact and properly functioning podocyte actin cytoskeletal meshwork (32). Hence defects in the actin framework are often connected to the pathogenesis of nephrotic syndrome (8, 33-36). Podocyte damage often results in foot process collapse where actin cytoskeletal reorganization takes place transforming parallel contractile bundles into a dense disorganized network. There are several proteins implicated to participate in CME, which also directly or indirectly regulate the actin cytoskeleton (37). This connection has been demonstrated in fission yeast where actin assembly is a critical participant during endocytosis (38, 39). However, the obligatory recruitment of actin and its cooperative role with endocytic proteins have been highly contested in mammalian cells (40-43) but a link between actin and clathrin-mediated endocytosis appears to exist in podocytes (23). This is best represented in live podocytes, where the dynamics of actin at endocytic buds, can be studied with high temporal and spatial resolution (20, 41, 42). A link between dynamin and actin at endocytic sites has been demonstrated through its direct interaction with actin (44), as well as through colocalization with actin nucleating proteins such as actin-related protein

2/3 (Arp2/3) and Neuronal Wiskott-Aldrich syndrome protein (N-WASP) (45, 46). In podocytes, dynamin also colocalizes with clathrin selectively at late stage clathrin-coated pits, just prior to the disappearance of the clathrin. Interestingly, podocytes that lack dynamin result in accumulation of Arp2/3 at or around the necks of the arrested clathrin coated pits, similar to what has been observed in fibroblasts (20). Moreover, synaptojanin 1-170 and endophilin also colocalizes with actin (23, 47). As PI(4,5)P₂ stimulates actin polymerization while inhibiting the actin-severing abilities of both gelsolin and actin depolymerizing factor/cofilin, one can posit that an increase in PI(4,5) P₂ leads to an overall increase in actin filaments. Dynamin and synaptojanin 1 interacting partner, Myo1e (48), whose loss of function also results in nephrotic syndrome, and proteinuria in mice and humans (9, 49), also arrives at late-stage clathrin coated pits where it colocalizes with a pool of F-actin present at these sites (23). CD2AP, a protein whose functional impairment results in nephrotic syndrome in both mice and humans, has been shown to co-localize with cortactin and late endosomal marker Rab4 (50). Moreover, CD2AP has also been shown to interact and colocalize with endophilin in podocytes (23). Lastly, loss of Nck, an endocytic adaptor that binds to both dynamin and synaptojanin (51), results in congenital nephrotic syndrome in mice, and through its interactions with nephrin, serves to regulate the actin cytoskeleton in podocytes (33, 34). What mechanisms underlie the link between clathrin and actin in the formation and maintenance of the podocyte foot processes? A plausible explanation is this network of proteins that regulate protein trafficking help control the steady-state cell surface area through its interaction with the actin, and perturbations to this system, underwrite the severe podocyte dysfunction observed in nephrotic syndrome.

The regulation of slit diaphragm proteins by endocytosis

The slit diaphragm, a modified tight junction connects neighboring foot processes, and has been demonstrated to serve as a signaling platform (52). As podocytes develop from discs of columnar epithelium, the slit diaphragm is formed as the apical junctional complex, migrates toward the base of the cell as the lateral cell attachments become absent (53). Human mutations in slit diaphragm protein, nephrin, has brought to the forefront, further studies on the regulation of this complex which also includes proteins such as, podocin, Neph1, P cadherin, and FAT1 and 2 (54-56). Nephrin, is a transmembrane protein with an extracellular N terminal domain consisting of immunoglobulin domain repeats, and a C terminal cytoplasmic domain which associates with various interacting partners such as the p85 regulatory subunit of the phosphoinositide 3-OH kinase, Nck adaptor protein, CD2AP, and podocin (33, 34, 52, 57, 58). Podocin, appears to tether nephrin at the lipid raft domain (59), and human mutations of this gene results in childhood nephrotic syndrome, possibly due to mislocalization of nephrin (60). Moreover, during podocyte injury, recent evidence suggests that nephrin is also mislocalized, thus providing further evidence that endocytosis may play a vital role in the internalization and recycling of nephrin (61). Through podocyte cell culture work, it has been postulated that nephrin can undergo both clathrin and raft mediated endocytosis (62). The phosphorylation status of nephrin is one mechanism that regulates its internalization (62, 63). The Src family of tyrosine kinases, Fyn and Yes has been shown to phosphorylate nephrin. The Yes dependent nephrin phosphorylation at Y1193, results in enhanced association with podocin and enhances nephrin signaling (63).

Conversely, dephosphorylation of this tyrosine residue initiates β arrestin induced endocytosis. Further reports have demonstrated that podocytes stimulated with high glucose concentration result in increased protein kinase C α (PKC α) expression, thus promoting nephrin internalization, which was inhibited by pretreatment with PKC α inhibitor (61). This was attributed to PKC α ability to phosphorylate nephrin at threonine residues 1120 and 1125, which propagates β arrestin interaction with nephrin and its subsequent internalization (64). However, it remains unclear whether β arrestin induced nephrin internalization involves clathrin dependent and or clathrin independent mechanisms. Yet, its plausible that β arrestin is involved in both pathways as current evidence suggests that nephrin internalization involves two distinct endocytic routes where fast endocytosis is regulated by CME, and slower uptake is dependent on raft mediated endocytosis (62). Nephrin endocytosis also relies on CIN85/RukL, the homologue of CD2AP. CIN85/RukL has been thought to have overlapping cellular functions but partially different tissue distribution. However, in podocytes, CD2AP and CIN85 have functionally compensatory roles. In wild type podocytes, CD2AP appears to downregulate CIN85 expression through posttranslational modification by SUMOylation at lysine 598 (65). Conversely in podocytes lacking CD2AP expression, there is increased CIN85/RukL expression, which results in ubiquitination of nephrin. Other investigators have suggested that CME of nephrin is regulated by increased Notch activation in podocytes, as cholesterol depletion had minimal effect on nephrin surface retention, in comparison to podocytes pretreated with dynamin inhibitor, dynasore (66). Furthermore, nephrin endocytosis has been shown to be important in glucose regulation through a complex generated by septin 7, nephrin, and CD2AP (67), and in pancreatic beta cells where dynamin regulation of nephrin endocytosis results in insulin release (68). Moreover, in drosophila, *sns* and *kirre*, which are orthologs of mammalian nephrin and *Neph1*, regulate the formation of nephrocyte diaphragm, as knockdown of these two genes results in reduced protein uptake into the labyrinthine channels(69). Hence, nephrin endocytosis appears to be a tightly regulated process, as overabundance of nephrin endocytosis following podocyte injury results in its removal from the slit diaphragm causing proteinuria. Yet, reduced nephrin endocytosis due to the loss of clathrin mediated adaptors may also be playing a role in the pathogenesis of nephrotic syndrome (23). Therefore, we hypothesize that as the human kidney filters nearly 180 liters of plasma daily, causing the slit diaphragm to work unceasingly, thus relying on the endocytic machinery (**Fig2**) for maintenance and renewal of a normal filtration barrier.

Conclusion

Although the study of endocytosis specifically in podocytes remains in its infancy, there is accumulating evidence that this pathway plays a critical role during podocyte health and disease. However, many questions remain to be answered. It would be of interest to delineate the critical factors being internalized, and regulated by endocytic pathways within podocytes. One could speculate that defects in endocytosis could alter a plethora of cellular processes. For example, the Par3/Par6/atypical PKC complex along with RhoGTPase CDC42 appear to be critical in efficient clathrin and nonclathrin mediated endocytosis (70). Hence a better understanding of how membrane trafficking regulates podocyte polarity proteins, Par3/Par6/atypical PKC, and CDC42, would be of relevance as loss of atypical

PKC and CDC42 in mice results in nephrotic syndrome (71-73). Moreover, the role of endocytic regulation of cell adhesion proteins, alpha and beta integrins (6, 74, 75), channel proteins, TRPC5 and 6 (11, 12, 76), junctional proteins, Nephrin (54), or tyrosine kinase or G protein coupled receptors, may further impact our understanding of podocyte biology. Another area to delineate is the link between endocytosis and autophagy as recent mice models have elegantly highlighted the importance of autophagy for podocyte homeostasis (28, 77-81). Future challenges also include exploration of proteins that are localized at sites of endocytosis such as sFLT1, and how they modulate gangliosidases (82). Lastly, further studies addressing podocyte endocytosis as a means of clearing large serum proteins, hence preventing the clogging of the filtration barrier is also enticing (83). Collectively, these studies suggest the importance of endocytosis in podocyte biology thus motivating further investigation to elucidate its precise role during the development, maintenance, and following injury of the glomerular filtration barrier.

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Bibliography

* Manuscript of Special Interest

** Manuscript of Outstanding Interest

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1. Jarad G, Miner JH. Update on the glomerular filtration barrier. Current opinion in nephrology and hypertension. 2009; 18(3):226–32. Epub 2009/04/18. [PubMed: 19374010]
2. Kestila M, Lenkkeri U, Mannikko M, Lamerdin J, McCready P, Putaala H, et al. Positionally cloned gene for a novel glomerular protein--nephrin--is mutated in congenital nephrotic syndrome. Mol Cell. 1998; 1(4):575–82. Epub 1998/07/14. [PubMed: 9660941]
3. Ruotsalainen V, Ljungberg P, Wartiovaara J, Lenkkeri U, Kestila M, Jalanko H, et al. Nephrin is specifically located at the slit diaphragm of glomerular podocytes. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96(14):7962–7. Epub 1999/07/08. [PubMed: 10393930]
4. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, et al. NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nat Genet. 2000; 24(4):349–54. Epub 2000/03/31. [PubMed: 10742096]
5. Has C, Sparta G, Kiritsi D, Weibel L, Moeller A, Vega-Warner V, et al. Integrin alpha3 mutations with kidney, lung, and skin disease. The New England journal of medicine. 2012; 366(16):1508–14. Epub 2012/04/20. [PubMed: 22512483]
6. Nicolaou N, Margadant C, Kevelam SH, Lilien MR, Oosterveld MJ, Kreft M, et al. Gain of glycosylation in integrin alpha3 causes lung disease and nephrotic syndrome. The Journal of clinical investigation. 2012; 122(12):4375–87. Epub 2012/11/02. [PubMed: 23114595]
7. Kim JM, Wu H, Green G, Winkler CA, Kopp JB, Miner JH, et al. CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. Science. 2003; 300(5623):1298–300. Epub 2003/05/24. [PubMed: 12764198]
8. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, et al. Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet. 2000; 24(3):251–6. Epub 2000/03/04. [PubMed: 10700177]

9. Mele C, Iatropoulos P, Donadelli R, Calabria A, Maranta R, Cassis P, et al. MYO1E mutations and childhood familial focal segmental glomerulosclerosis. *The New England journal of medicine*. 2011; 365(4):295–306. Epub 2011/07/16. [PubMed: 21756023]
10. Genovese G, Tonna SJ, Knob AU, Appel GB, Katz A, Bernhardt AJ, et al. A risk allele for focal segmental glomerulosclerosis in African Americans is located within a region containing APOL1 and MYH9. *Kidney Int*. 2010; 78(7):698–704. Epub 2010/07/30. [PubMed: 20668430]
11. Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, et al. A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science*. 2005; 308(5729):1801–4. Epub 2005/05/10. [PubMed: 15879175]
12. Reiser J, Polu KR, Moller CC, Kenlan P, Altintas MM, Wei C, et al. TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. *Nat Genet*. 2005; 37(7):739–44. Epub 2005/06/01. [PubMed: 15924139]
13. Rastaldi MP, Armelloni S, Berra S, Calvaresi N, Corbelli A, Giardino LA, et al. Glomerular podocytes contain neuron-like functional synaptic vesicles. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2006; 20(7):976–8. Epub 2006/04/06.
14. Shen K, Fetter RD, Bargmann CI. Synaptic specificity is generated by the synaptic guidepost protein SYG-2 and its receptor, SYG-1. *Cell*. 2004; 116(6):869–81. Epub 2004/03/24. [PubMed: 15035988]
15. Boyer O, Nevo F, Plaisier E, Funalot B, Gribouval O, Benoit G, et al. INF2 mutations in Charcot-Marie-Tooth disease with glomerulopathy. *N Engl J Med*. 2011; 365(25):2377–88. Epub 2011/12/23. [PubMed: 22187985]
16. Farquhar MG, Vernier RL, Good RA. An electron microscope study of the glomerulus in nephrosis, glomerulonephritis, and lupus erythematosus. *The Journal of experimental medicine*. 1957; 106(5):649–60. Epub 1957/11/01. [PubMed: 13475621]
17. Kerjaschki D, Farquhar MG. Immunocytochemical localization of the Heymann nephritis antigen (GP330) in glomerular epithelial cells of normal Lewis rats. *The Journal of experimental medicine*. 1983; 157(2):667–86. Epub 1983/02/01. [PubMed: 6337231]
18. McMahon HT, Boucrot E. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol*. 2011; 12(8):517–33. Epub 2011/07/23. [PubMed: 21779028]
19. Doherty GJ, McMahon HT. Mechanisms of endocytosis. *Annu Rev Biochem*. 2009; 78:857–902. Epub 2009/03/26. [PubMed: 19317650]
20. Ferguson SM, Raimondi A, Paradise S, Shen H, Mesaki K, Ferguson A, et al. Coordinated actions of actin and BAR proteins upstream of dynamin at endocytic clathrin-coated pits. *Dev Cell*. 2009; 17(6):811–22. Epub 2010/01/12. [PubMed: 20059951]
21. Ferguson, SaDC. Dynamin, a membrane remodeling GTPase. *Nature Reviews Molecular Cell Biology*. 2011 P. [Review]. In press.
22. Sever S, Altintas MM, Nankoe SR, Moller CC, Ko D, Wei C, et al. Proteolytic processing of dynamin by cytoplasmic cathepsin L is a mechanism for proteinuric kidney disease. *J Clin Invest*. 2007; 117(8):2095–104. Epub 2007/08/03. [PubMed: 17671649]
- 23**. Soda K, Balkin DM, Ferguson SM, Paradise S, Milosevic I, Giovedi S, et al. Role of dynamin, synaptojanin, and endophilin in podocyte foot processes. *The Journal of clinical investigation*. 2012; 122(12):4401–11. Epub 2012/11/29. [PubMed: 23187129]
24. **This study demonstrates the importance of key regulators of clathrin mediated endocytosis for a properly functioning glomeruli and further implicates the intimate link between actin regulation and endocytosis.
24. McPherson PS, Garcia EP, Slepnev VI, David C, Zhang X, Grabs D, et al. A presynaptic inositol-5-phosphatase. *Nature*. 1996; 379(6563):353–7. [PubMed: 8552192]
25. Cremona O, Di Paolo G, Wenk MR, Luthi A, Kim WT, Takei K, et al. Essential role of phosphoinositide metabolism in synaptic vesicle recycling. *Cell*. 1999; 99(2):179–88. Epub 1999/10/27. [PubMed: 10535736]
26. Milosevic I, Giovedi S, Lou X, Raimondi A, Collesi C, Shen H, et al. Recruitment of endophilin to clathrin-coated pit necks is required for efficient vesicle uncoating after fission. *Neuron*. 2011; 72(4):587–601. Epub 2011/11/22. [PubMed: 22099461]

27. Gallop JL, Jao CC, Kent HM, Butler PJ, Evans PR, Langen R, et al. Mechanism of endophilin N-BAR domain-mediated membrane curvature. *EMBO J*. 2006; 25(12):2898–910. Epub 2006/06/10. [PubMed: 16763559]
28. Inoki K, Mori H, Wang J, Suzuki T, Hong S, Yoshida S, et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *J Clin Invest*. 2011; 121(6):2181–96. Epub 2011/05/25. [PubMed: 21606597]
29. Harris DP, Vogel P, Wims M, Moberg K, Humphries J, Jhaveri KG, et al. Requirement for class II phosphoinositide 3-kinase C2alpha in maintenance of glomerular structure and function. *Mol Cell Biol*. 2011; 31(1):63–80. Epub 2010/10/27. [PubMed: 20974805]
30. Prabakaran T, Nielsen R, Larsen JV, Sorensen SS, Feldt-Rasmussen U, Saleem MA, et al. Receptor-mediated endocytosis of alpha-galactosidase A in human podocytes in Fabry disease. *PLoS One*. 2011; 69:e25065. Epub 2011/09/29. [PubMed: 21949853]
- 31*. Ceol M, Tiralongo E, Baelde HJ, Vianello D, Betto G, Marangelli A, et al. Involvement of the tubular CIC-type exchanger CIC-5 in glomeruli of human proteinuric nephropathies. *PLoS One*. 2012; 7(9):e45605. Epub 2012/10/03. [PubMed: 23029130]
33. *This paper shows CIC-5 is expressed in podocytes and may play a critical role in retrieval of albumin by endocytosis.
32. Faul C, Asanuma K, Yanagida-Asanuma E, Kim K, Mundel P. Actin up: regulation of podocyte structure and function by components of the actin cytoskeleton. *Trends Cell Biol*. 2007; 17(9):428–37. [PubMed: 17804239]
33. Verma R, Kovari I, Soofi A, Nihalani D, Patrie K, Holzman LB. Nephrin ectodomain engagement results in Src kinase activation, nephrin phosphorylation, Nck recruitment, and actin polymerization. *The Journal of clinical investigation*. 2006; 116(5):1346–59. Epub 2006/03/18. [PubMed: 16543952]
34. Jones N, Blasutig IM, Eremina V, Ruston JM, Bladt F, Li H, et al. Nck adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. *Nature*. 2006; 440(7085):818–23. Epub 2006/03/10. [PubMed: 16525419]
35. Asanuma K, Yanagida-Asanuma E, Faul C, Tomino Y, Kim K, Mundel P. Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling. *Nature cell biology*. 2006; 8(5):485–91. Epub 2006/04/20.
36. Yaddanapudi S, Altintas MM, Kistler AD, Fernandez I, Moller CC, Wei C, et al. CD2AP in mouse and human podocytes controls a proteolytic program that regulates cytoskeletal structure and cellular survival. *J Clin Invest*. 2011; 121(10):3965–80. Epub 2011/09/14. [PubMed: 21911934]
37. Galletta BJ, Mooren OL, Cooper JA. Actin dynamics and endocytosis in yeast and mammals. *Curr Opin Biotechnol*. 2010; 21(5):604–10. Epub 2010/07/20. [PubMed: 20637595]
38. Berro J, Sirotkin V, Pollard TD. Mathematical modeling of endocytic actin patch kinetics in fission yeast: disassembly requires release of actin filament fragments. *Mol Biol Cell*. 2010; 21(16):2905–15. Epub 2010/07/01. [PubMed: 20587776]
39. Sirotkin V, Berro J, Macmillan K, Zhao L, Pollard TD. Quantitative analysis of the mechanism of endocytic actin patch assembly and disassembly in fission yeast. *Mol Biol Cell*. 2010; 21(16):2894–904. Epub 2010/07/01. [PubMed: 20587778]
40. Boulant S, Kural C, Zehe JC, Ubelmann F, Kirchhausen T. Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. *Nat Cell Biol*. 2011; 13(9):1124–31. Epub 2011/08/16. [PubMed: 21841790]
41. Taylor MJ, Perrais D, Merrifield CJ. A high precision survey of the molecular dynamics of mammalian clathrin-mediated endocytosis. *PLoS Biol*. 2011; 9(3):e1000604. Epub 2011/03/30. [PubMed: 21445324]
42. Taylor MJ, Lampe M, Merrifield CJ. A Feedback Loop between Dynamin and Actin Recruitment during Clathrin-Mediated Endocytosis. *PLoS Biol*. 2012; 10(4):e1001302. Epub 2012/04/17. [PubMed: 22505844]
43. Yarar D, Waterman-Storer CM, Schmid SL. A dynamic actin cytoskeleton functions at multiple stages of clathrin-mediated endocytosis. *Mol Biol Cell*. 2005; 16(2):964–75. Epub 2004/12/17. [PubMed: 15601897]

44. Gu C, Yaddanapudi S, Weins A, Osborn T, Reiser J, Pollak M, et al. Direct dynamin-actin interactions regulate the actin cytoskeleton. *The EMBO journal*. 2010; 29(21):3593–606. Epub 2010/10/12. [PubMed: 20935625]
45. Krueger EW, Orth JD, Cao H, McNiven MA. A dynamin-cortactin-Arp2/3 complex mediates actin reorganization in growth factor-stimulated cells. *Mol Biol Cell*. 2003; 14(3):1085–96. Epub 2003/03/13. [PubMed: 12631725]
46. Merrifield CJ, Qualmann B, Kessels MM, Almers W. Neural Wiskott Aldrich Syndrome Protein (N-WASP) and the Arp2/3 complex are recruited to sites of clathrin-mediated endocytosis in cultured fibroblasts. *European journal of cell biology*. 2004; 83(1):13–8. Epub 2004/04/17. [PubMed: 15085951]
47. Perera RM, Zoncu R, Lucast L, De Camilli P, Toomre D. Two synaptojanin 1 isoforms are recruited to clathrin-coated pits at different stages. *Proc Natl Acad Sci U S A*. 2006; 103(51):19332–7. [PubMed: 17158794]
48. Krendel M, Osterweil EK, Mooseker MS. Myosin 1E interacts with synaptojanin-1 and dynamin and is involved in endocytosis. *FEBS Lett*. 2007; 581(4):644–50. [PubMed: 17257598]
49. Krendel M, Kim SV, Willinger T, Wang T, Kashgarian M, Flavell RA, et al. Disruption of Myosin 1e promotes podocyte injury. *J Am Soc Nephrol*. 2009; 20(1):86–94. [PubMed: 19005011]
50. Welsch T, Endlich N, Gokce G, Doroshenko E, Simpson JC, Kriz W, et al. Association of CD2AP with dynamic actin on vesicles in podocytes. *American journal of physiology Renal physiology*. 2005; 289(5):F1134–43. Epub 2005/06/16. [PubMed: 15956777]
51. Fawcett JP, Georgiou J, Ruston J, Bladt F, Sherman A, Warner N, et al. Nck adaptor proteins control the organization of neuronal circuits important for walking. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(52):20973–8. Epub 2007/12/21. [PubMed: 18093944]
52. Zhu J, Sun N, Aoudjit L, Li H, Kawachi H, Lemay S, et al. Nephrin mediates actin reorganization via phosphoinositide 3-kinase in podocytes. *Kidney Int*. 2008; 73(5):556–66. Epub 2007/11/23. [PubMed: 18033240]
53. Quaggin SE, Kreidberg JA. Development of the renal glomerulus: good neighbors and good fences. *Development*. 2008; 135(4):609–20. Epub 2008/01/11. [PubMed: 18184729]
54. Liu G, Kaw B, Kurfis J, Rahmanuddin S, Kanwar YS, Chugh SS. Nephrin and nephrin interaction in the slit diaphragm is an important determinant of glomerular permeability. *J Clin Invest*. 2003; 112(2):209–21. [PubMed: 12865409]
55. Barletta GM, Kovari IA, Verma RK, Kerjaschki D, Holzman LB. Nephrin and Nephrin co-localize at the podocyte foot process intercellular junction and form cis hetero-oligomers. *The Journal of biological chemistry*. 2003; 278(21):19266–71. Epub 2003/03/21. [PubMed: 12646566]
56. Fukasawa H, Bornheimer S, Kudlicka K, Farquhar MG. Slit diaphragms contain tight junction proteins. *J Am Soc Nephrol*. 2009; 20(7):1491–503. Epub 2009/05/30. [PubMed: 19478094]
57. Huber TB, Hartleben B, Kim J, Schmidts M, Schermer B, Keil A, et al. Nephrin and CD2AP associate with phosphoinositide 3-OH kinase and stimulate AKT-dependent signaling. *Mol Cell Biol*. 2003; 23(14):4917–28. Epub 2003/07/02. [PubMed: 12832477]
58. Huber TB, Kottgen M, Schilling B, Walz G, Benzing T. Interaction with podocin facilitates nephrin signaling. *J Biol Chem*. 2001; 276(45):41543–6. Epub 2001/09/20. [PubMed: 11562357]
59. Schwarz K, Simons M, Reiser J, Saleem MA, Faul C, Kriz W, et al. Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest*. 2001; 108(11):1621–9. Epub 2001/12/26. [PubMed: 11733557]
60. Nishibori Y, Liu L, Hosoyamada M, Endou H, Kudo A, Takenaka H, et al. Disease-causing missense mutations in NPHS2 gene alter normal nephrin trafficking to the plasma membrane. *Kidney international*. 2004; 66(5):1755–65. Epub 2004/10/22. [PubMed: 15496146]
61. Tossidou I, Teng B, Menne J, Shushakova N, Park JK, Becker JU, et al. Podocytic PKC- α is regulated in murine and human diabetes and mediates nephrin endocytosis. *PLoS One*. 2010; 5(4):e10185. Epub 2010/04/27. [PubMed: 20419132]
62. Qin XS, Tsukaguchi H, Shono A, Yamamoto A, Kurihara H, Doi T. Phosphorylation of nephrin triggers its internalization by raft-mediated endocytosis. *J Am Soc Nephrol*. 2009; 20(12):2534–45. [PubMed: 19850954]

63. Quack I, Rump LC, Gerke P, Walther I, Vinke T, Vonend O, et al. beta-Arrestin2 mediates nephrin endocytosis and impairs slit diaphragm integrity. *Proc Natl Acad Sci U S A*. 2006; 103(38): 14110–5. [PubMed: 16968782]
64. Quack I, Woznowski M, Potthoff SA, Palmer R, Konigshausen E, Sivritas S, et al. PKC alpha mediates beta-arrestin2-dependent nephrin endocytosis in hyperglycemia. *J Biol Chem*. 2011; 286(15):12959–70. Epub 2011/02/16. [PubMed: 21321125]
65. Tossidou I, Niedenthal R, Klaus M, Teng B, Worthmann K, King BL, et al. CD2AP regulates SUMOylation of CIN85 in podocytes. *Mol Cell Biol*. 2012; 32(6):1068–79. Epub 2011/12/29. [PubMed: 22203040]
66. Waters AM, Wu MY, Huang YW, Liu GY, Holmyard D, Onay T, et al. Notch Promotes Dynamin-Dependent Endocytosis of Nephrin. *J Am Soc Nephrol*. 2011 Epub 2011/11/05.
- **67. Wasik AA, Polianskyte-Prause Z, Dong MQ, Shaw AS, Yates JR 3rd, Farquhar MG, et al. Septin 7 forms a complex with CD2AP and nephrin and regulates glucose transporter trafficking. *Mol Biol Cell*. 2012; 23(17):3370–9. Epub 2012/07/20. [PubMed: 22809625]
70. **This study addresses the role of Septin 7 which is found in vesicles, through its interactions with nephrin and CD2AP, modulate glucose transport. This highlights the importance of vesicular transport mechanisms in podocytes regulating glucose uptake.
68. Jeon J, Leibiger I, Moede T, Walter B, Faul C, Maignel D, et al. Dynamin-mediated Nephrin phosphorylation regulates glucose-stimulated insulin release in pancreatic beta cells. *J Biol Chem*. 2012; 287(34):28932–42. Epub 2012/06/22. [PubMed: 22718751]
69. Zhuang S, Shao H, Guo F, Trimble R, Pearce E, Abmayr SM. Sns and Kirre, the Drosophila orthologs of Nephrin and Neph1, direct adhesion, fusion and formation of a slit diaphragm-like structure in insect nephrocytes. *Development*. 2009; 136(14):2335–44. Epub 2009/06/12. [PubMed: 19515699]
70. Balklava Z, Pant S, Fares H, Grant BD. Genome-wide analysis identifies a general requirement for polarity proteins in endocytic traffic. *Nat Cell Biol*. 2007; 9(9):1066–73. Epub 2007/08/21. [PubMed: 17704769]
71. Hartleben B, Schweizer H, Lubben P, Bartram MP, Moller CC, Herr R, et al. Nephrin proteins bind the Par3-Par6-atypical protein kinase C (aPKC) complex to regulate podocyte cell polarity. *J Biol Chem*. 2008; 283(34):23033–8. Epub 2008/06/20. [PubMed: 18562307]
72. Hirose T, Satoh D, Kurihara H, Kusaka C, Hirose H, Akimoto K, et al. An essential role of the universal polarity protein, aPKC λ , on the maintenance of podocyte slit diaphragms. *PLoS One*. 2009; 4(1):e4194. Epub 2009/01/15. [PubMed: 19142224]
73. Scott RP, Hawley SP, Ruston J, Du J, Brakebusch C, Jones N, et al. Podocyte-specific loss of Cdc42 leads to congenital nephropathy. *J Am Soc Nephrol*. 2012; 23(7):1149–54. Epub 2012/04/21. [PubMed: 22518006]
74. Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, et al. Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development*. 1996; 122(11):3537–47. Epub 1996/11/01. [PubMed: 8951069]
75. Pozzi A, Jarad G, Moeckel GW, Coffa S, Zhang X, Gewin L, et al. Beta1 integrin expression by podocytes is required to maintain glomerular structural integrity. *Dev Biol*. 2008; 316(2):288–301. Epub 2008/03/11. [PubMed: 18328474]
76. Tian D, Jacobo SM, Billing D, Rozkalne A, Gage SD, Anagnostou T, et al. Antagonistic regulation of actin dynamics and cell motility by TRPC5 and TRPC6 channels. *Science signaling*. 2010; 3(145):ra77. Epub 2010/10/28. [PubMed: 20978238]
77. Riediger F, Quack I, Qadri F, Hartleben B, Park JK, Potthoff SA, et al. Prorenin receptor is essential for podocyte autophagy and survival. *J Am Soc Nephrol*. 2011; 22(12):2193–202. Epub 2011/10/29. [PubMed: 22034640]
78. Oshima Y, Kinouchi K, Ichihara A, Sakoda M, Kurauchi-Mito A, Bokuda K, et al. Prorenin receptor is essential for normal podocyte structure and function. *J Am Soc Nephrol*. 2011; 22(12): 2203–12. Epub 2011/11/05. [PubMed: 22052048]
79. Hartleben B, Godel M, Meyer-Schwesinger C, Liu S, Ulrich T, Kobler S, et al. Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. *J Clin Invest*. 2010; 120(4):1084–96. Epub 2010/03/05. [PubMed: 20200449]

80. Cina DP, Onay T, Paltoo A, Li C, Maezawa Y, De Arteaga J, et al. Inhibition of MTOR disrupts autophagic flux in podocytes. *J Am Soc Nephrol*. 2012; 23(3):412–20. Epub 2011/12/24. [PubMed: 22193387]
- **81. Chen J, Chen MX, Fogo AB, Harris RC, Chen JK. mVps34 Deletion in Podocytes Causes Glomerulosclerosis by Disrupting Intracellular Vesicle Trafficking. *J Am Soc Nephrol*. 2013 Epub 2013/01/08.
85. **This study demonstrates the critical role of yeast vacuolar protein sorting defective 34 (mVps34), for the integrity of the glomerular filtration barrier, suggesting the connection between autophagy with endocytic trafficking.
- **82. Jin J, Sison K, Li C, Tian R, Wnuk M, Sung HK, et al. Soluble FLT1 binds lipid microdomains in podocytes to control cell morphology and glomerular barrier function. *Cell*. 2012; 151(2):384–99. Epub 2012/10/16. [PubMed: 23063127]
87. **This study carefully examines the role of soluble FLT1 that is localized at lipid rafts, and interacts with glycosphingolipid GM3 which may not only regulate glomeruli but also other vascular beds within the body.
83. Akilesh S, Huber TB, Wu H, Wang G, Hartleben B, Kopp JB, et al. Podocytes use FcRn to clear IgG from the glomerular basement membrane. *Proc Natl Acad Sci U S A*. 2008; 105(3):967–72. Epub 2008/01/17. [PubMed: 18198272]

Summary Points

1. Loss of key CME proteins, dynamin, synaptojanin 1 or endophilin result in proteinuria and foot process effacement.
2. A relationship between actin and CME exists within podocytes.
3. Endocytosis is likely critical in regulating turnover of nephrin.
4. Components of endocytic protein networks are implicated in nephrotic syndrome.

Figure 1.
Clathrin Coated Pits in Podocytes- An electron microscopic image of a clathrin coated pits and vesicles

Figure 2.
Cartoon depicting the actin and clathrin machinery along with group of interacting genes implicated to be causal for proteinuria in mice and/or human nephrotic syndrome.
Endo-Endophilin, Dnm-Dynamin, Myo1E-Myosin 1E, Synj1-Synaptojanin1
SD-slit diaphragm