Published in final edited form as:

Semin Nephrol. 2012 July ; 32(4): 319–326. doi:10.1016/j.semnephrol.2012.06.003.

Regulation of podocyte actin dynamics by calcium

Anna Greka, MD, PhD and

Assistant Professor of Medicine, Harvard Medical School, Division of Nephrology, Massachusetts General Hospital, Boston MA, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, 02129

Peter Mundel, MD

Associate Professor of Medicine, Harvard Medical School, Division of Nephrology, Massachusetts General Hospital, Boston, MA, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, 02129

Abstract

Ca²⁺-mediated remodeling of the actin cytoskeleton is a dynamic process that regulates cell motility through the modulation of Rho GTPase signaling. Kidney podocytes are unique, pericyte-like, cells with a complex cellular organization consisting of a cell body, major processes, and foot processes (FP). The FPs form a characteristic interdigitating pattern with FPs of neighboring podocytes leaving in between the filtration slits that are covered by the slit diaphragm (SD). The actin-based FP and the SD form the final barrier to proteinuria. Mutations affecting several podocyte proteins cause disruption of the filtration barrier and rearrangement of the highly dynamic podocyte actin cytoskeleton. Proteins regulating the plasticity of the podocyte actin cytoskeleton are therefore of critical importance for sustained kidney barrier function [1]. Dynamic regulation of the actin-based contractile apparatus in podocyte FPs is essential for sustained kidney filter function [2]. Thus, the podocyte represents an excellent model system to study calcium signaling and actin dynamics in a physiologic context. Here we discuss the regulation of podocyte actin dynamics by angiotensin or bradykinin mediated calcium influx and downstream Rho GTPase signaling pathways and how these pathways are operative in other cells including fibroblasts and cancer cells.

Keywords

Cdc42; fibroblasts; synaptopodin; TRPC channels; Rac1; RhoA; tropomyosin

Calcium and the cytoskeleton

In the late 1800s, Sydney Ringer published seminal papers establishing the relative importance, both qualitative and quantitative, of calcium ions in cardiac conduction [3]. He postulated a “saline” instrumental for life, which is now widely known as “Ringer’s solution.” This was the first glimpse of the importance of Ca²⁺ signaling in cellular motility.

© 2012 Elsevier Inc. All rights reserved.

Corresponding authors: Anna Greka, M.D., Ph.D., or Peter Mundel, M.D., Division of Nephrology, Massachusetts General Hospital and Harvard Medical School, 149 13th Street, Charlestown, MA 02129, USA, Tel 001-617-726-9363 (A.G.) or 001-617-643-8710 (P.M.); greka.anna@mgh.harvard.edu or mundel.peter@mgh.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

In muscle cells, Ca^{2+} entering the myoplasm is tightly and selectively sequestered by troponin's Ca^{2+} binding sites [4]. Together with calmodulin, these proteins have evolved into powerful detectors of sub-micromolar changes in cytosolic $[\text{Ca}^{2+}]$ [4]. Ca^{2+} binding leads to the activation of enzymes, and thus the tiny Ca^{2+} signal is amplified to match the potency of entire proteins. For example, activation of the actomyosin ATPase results in shortening of the contractile filament, an essential step in muscle contraction, and activation of the Ca^{2+} -calmodulin dependent kinase (CaMK) leads to phosphorylation of a plethora of downstream signaling molecules. Indeed, the shape of each cell is dependent on the activities of Ca^{2+} /calmodulin through control of myosin's interaction with actin filaments [4].

Vascular smooth muscle cells contract when voltage gated Ca^{2+} channels open, Ca^{2+} bound calmodulin activates myosin light chain kinase (MLCK) to phosphorylate the myosin head light chain and trigger myosin ATPase activity leading to contraction [4–6]. In a matter of milliseconds, homeostatic mechanisms prevail, the cytosolic $[\text{Ca}^{2+}]$ drops, and MLCK phosphatases now mediate smooth muscle relaxation [4–6]. The theme that emerges from these conserved pathways is that Ca^{2+} is a selective activator of kinases that mediate contraction, and that a decline or change in localized $[\text{Ca}^{2+}]$ may fuel phosphatases to mediate relaxation and/or increased motility.

The Ca^{2+} -mediated activation of downstream enzymatic activity to promote changes in cell morphology is further illustrated in cardiac hypertrophy models [7]. Increases in cytosolic $[\text{Ca}^{2+}]$ in response to vasoactive hormones such as Endothelin and Angiotensin II (Ang II) contribute to cardiomyocyte hypertrophy through the activation of the Ca^{2+} activated serine/threonine phosphatase calcineurin [7–10]. Here calcineurin signals the activation of the nuclear transcription factor of activated T cells (NFAT), which in turn induces the expression of hypertrophic genes such as b-myosin heavy chain (b-MHC), altering myocyte contractility [8–10]. Of note, the sources of Ca^{2+} influx in these models of cardiac hypertrophy are members of the Transient Receptor Potential Canonical (TRPC) family of non-selective, Ca^{2+} -conducting channels (which are reviewed in detail below) [8–10]. Triggered by upstream G-protein coupled receptors (GPCRs) such as the Angiotensin Type 1 receptor (AT1R), TRPC channels allow the influx of Ca^{2+} in a spatially and temporally defined manner, to activate calcineurin's downstream transcriptional program. Impressively, the cardiac myocyte, replete with thousands of voltage gated Ca^{2+} channels to maintain the contraction-relaxation cycle of systole-diastole, relies on the many 1000-fold smaller Ca^{2+} influx through TRPC channels to activate transcriptional pathways. This example thus illustrates once again the remarkable specificity of Ca^{2+} signals in space and time.

Podocytes and Calcium

The podocyte FPs with the interposed SD) that cover the outer aspect of the glomerular basement membrane [11–13]. Over the past decade, a prevalent hypothesis has therefore emerged that proteins regulating the plasticity of the podocyte actin cytoskeleton are critical for sustained glomerular filter function [2, 13]. This has been supported by a growing list of human mutations leading to proteinuric kidney disease. Congenital proteinuria [14, 15] can arise from mutations in phospholipase C epsilon [16], podocin [14], or nephrin [15]. Additionally, adult-onset focal segmental glomerulosclerosis (FSGS) is associated with podocytes mutations in genes encoding α -actinin-4 [17], CD2AP [18], INF2 [19], TRPC6 [20, 21] and synaptopodin [22]. Common variations in glypican-5 associate with acquired nephrotic syndrome [23] and mutations in the non-muscle class I myosin *MYOIE* gene were lined to childhood proteinuric disease and FSGS [24, 25].

Podocytes allow the study of Ca^{2+} -regulated actin dynamics in a physiologic context

The podocyte's need to adapt its cytoskeleton to environmental cues [13, 26–30] makes it a physiologically relevant model system to study Ca^{2+} -dependent actin dynamics. Indeed, podocyte FP effacement required the rearrangements of the actin cytoskeleton [2, 26, 27]. Three decades ago, Kerjaschki proposed that a rise in $[\text{Ca}^{2+}]_i$ may be an early event in podocyte injury [31]. An increase in $[\text{Ca}^{2+}]_i$ and activation of phospholipase C are associated with complement C5b-9 complex-mediated podocyte damage [32]. Protamine sulfate, which can cause FP effacement *in vivo* [33] and disruption of stress fibers *in vitro* [34], increases podocyte $[\text{Ca}^{2+}]_i$ *in vitro* [35]. Differentiated mouse podocytes in culture increase $[\text{Ca}^{2+}]_i$ in response to bradykinin [36], an effect also observed in cultured rat podocytes in response to Ang II [37]. In 1997, a non-selective cationic current in podocytes was identified by detailed analysis of rat whole glomerulus recordings [38]. This finding, together with the observation that TRPC6 channel mutations contribute to familial FSGS [20, 21], led to the discovery of TRPC5 and TRPC6 as channels downstream of the Ang II-evoked non-selective cationic conductance [39], initially identified more than a decade ago.

Increased Ang II signaling in podocytes causes proteinuria and FSGS-like kidney disease

The detrimental effect of increased Ang II signaling on podocytes was established by showing that podocyte-specific AT1R overexpression in transgenic rats is sufficient to cause proteinuria and FSGS-type lesions [40]. This *is vivo* finding can now be coupled, at a cellular level, to the discovery that AT1R signaling is upstream of pathways mediated by TRPC channel signaling [39]. Calcineurin activation appears to be downstream of AT1R signaling [8]. Since calcineurin is a negative regulator of synaptopodin signaling in podocytes [41, 42], a unifying hypothesis for the above observations calls for AT1R, TRPC, calcineurin and the actin cytoskeleton to form a lineal signaling cascade of central importance for the emergence of proteinuria and progressive glomerular disease. In support of this idea, Ang II activates calcineurin in a TRPC5-dependent manner [39] and induces membrane ruffling and loss of stress fibers [43], similar to the depletion of synaptopodin [41, 44] or TRPC6 [39].

Ca^{2+} signaling through TRPC channels

The TRP superfamily consists of 28 members segregated into seven families with diverse mechanisms of activation, tissue-specific distribution, and functional properties [45, 46]. The canonical TRPC channels include seven members, TRPC1 to TRPC7 [45, 46] divided in two major subfamilies, TRPC1/4/5 and TRPC3/6/7 (TRPC2 is a pseudogene in humans) [46]. A cation conducting pore that is either a homomeric or heteromeric tetramer is formed by four TRP subunits [46]. While specific modulators of channel activity are still the subject of investigation, a common theme for TRPC channel activation is the initial activation of phospholipase C (PLC) leading to the cleavage of phosphatidylinositol biphosphate (PIP_2) which gates the channels either directly, as in TRPC4 and TRPC5, or indirectly through PIP_2 hydrolysis into diacylglycerol (DAG), as in TRPC3, TRPC6 and TRPC7 [45, 47].

In podocytes, TRPC channels garnered attention when gain-of-function TRPC6 mutations were linked to familial FSGS [20, 21]. In other systems, TRPC6 signaling inhibits endothelial cell migration [48], increases smooth muscle cell contraction [49–52], contributes to pulmonary arterial hypertension [53] as well as cardiac hypertrophy and fibrosis [8, 54–56]. Notably, the elevated blood pressure and increased vascular smooth muscle contractility in aortic rings from TRPC6 knockout mice resulted from the

upregulation of constitutively active TRPC3 channels [57]. TRPC5, in addition to TRPC6, was recently identified as a mediator of cytoskeletal changes in podocytes [39]. Loss of TRPC5 in the amygdala leads to impaired fear responses in young mice [58], suggesting a role for TRPC5 in innate fear. In podocytes, TRPC5 channels have been studied *in vitro* [59], but *in vivo* studies are needed to demonstrate the physiologic relevance of TRPC5 in kidney filter function. Of note, TRPC5 channels have a well-established role in generating Ca^{2+} transients during neuronal growth cone motility [59, 60] and smooth muscle cell migration [61]. Vesicular insertion of TRPC5 from a reserve pool of vesicles occurs downstream of EGFR signaling in a Rac1 and phosphatidylinositol 4-phosphate 5-kinase-dependent manner [59]. The current-voltage relation for homomeric TRPC5 channels is unique and characteristic, showing substantial inward current, little outward current up to +40 mV and steeply outwardly rectifying current above +40 mV [62].

Regulation of Rho GTPase signaling by synaptopodin

The Rho family of small GTPases (RhoA, Rac1 and Cdc42) controls signal transduction pathways that influence many aspects of cell behavior, including cytoskeletal dynamics [63–65]. Rac1 and Cdc42 promote cell motility through the formation of lamellipodia and filopodia, respectively. RhoA promotes the formation of contractile actin:myosin containing stress fibers in the cell body and at the rear [63, 64, 66]. Synaptopodin is the founding member of a unique class of actin binding proteins. It is strongly expressed in highly dynamic cell compartments such as podocytes FPs and telencephalic dendrites [67]. Gene silencing of synaptopodin in podocytes causes the loss of stress fibers and the concomitant development of aberrant, non-polarized, filopodia [44, 68]. These observations prompted a series of studies into the role of synaptopodin as regulator of Rho GTPase signaling that led to the discoveries that synaptopodin is positive regulator of RhoA and a negative regulator of Cdc42 signaling [44, 69]. Synaptopodin can directly bind to RhoA and induces stress fibers in podocytes by competitively blocking the Smurf1-mediated ubiquitination of RhoA, thereby preventing the targeting of RhoA for proteasomal degradation [44]. Of note, synaptopodin not only increases total but also activated RhoA levels, raising the possibility that synaptopodin may also contribute the activation/deactivation of RhoA by guanine nucleotide exchange factors (GEFs) or GTPase-activating proteins (GAPs) [44]. The general relevance of this mechanism for the regulation of RhoA signaling beyond highly specialized podocytes was explored by turning to tropomyosin (Tm), a family of widespread actin binding protein with more than 40 isoforms that influence numerous cellular functions including actin dynamics, cell migration, tumor suppression and *Drosophila* oocyte development [70–72]. Similar to synaptopodin, (Tm) can promote stress fiber formation, but the precise mechanisms remained elusive [71–73]. Tm also has a well-characterized role in *Drosophila* development. During oogenesis, the assembly of the posterior pole plasm requires the polarized microtubule-mediated transport of *oskar* (*osk*) mRNA to the posterior pole [74–78]. *Osk* mediates the nucleation of the pole plasm, which is required for germ cell and abdomen development [79, 80]. Failure to localize and enrich the pole plasm results in offspring lacking germ cells, referred to as grandchildlessness. Besides mutations in other factors that are needed for the assembly the pole plasm (e.g. *Osk*), *TmII* loss of function also causes grandchildlessness [81]. In developing oocytes of *TmII* mutant females, the germline determinants do not get localized to the posterior pole plasm because *osk* mRNA is not properly transported to and localized at the posterior pole, subsequently giving rise to very few germ cells [81]. Tm and synaptopodin both induce stress fibers, suggesting that these two structurally distinct actin-binding proteins may be interchangeable. This was shown *in vivo* by the rescue of the grandchildless phenotype of *Drosophila TmII* mutants [82]. Synaptopodin can rescue TmII deficiency in *Drosophila* by regulating *osk* mRNA localization and function, thereby showing that these two structurally distinct proteins share functional properties *in vivo* [82]. The functional similarity was underscored by the

observation that synaptopodin was also sufficient to restore stress fibers in *TPMa* depleted NIH3T3 fibroblasts and human MDA-MB 231 breast cancer cells, through the inhibition of Smurf1 mediated ubiquitination of RhoA [82]. These findings suggest that the regulation of actin dynamics by Smurf1-mediated degradation of RhoA is not only essential in highly specialized cell such as podocytes [44], but represents a conserved mechanism for the regulation of stress fiber dynamics and cell motility [82] (Fig. 1). These findings further support the notion that, the proteasomal degradation of RhoA is a second conserved mechanism for the control of RhoA signaling and cell motility, upstream of the regulation by GEFs, GAPs and guanine nucleotide-dissociation inhibitors (GDIs) [65].

These data also raise the question why podocytes express synaptopodin and Tm, if both proteins can serve overlapping functions? In podocytes, Tm is localized in the cell body [83], whereas synaptopodin is targeted to FPs [2, 67]. FPs are highly dynamics, structures, so rapid changes in FP morphology require dynamic control of their actin cytoskeleton, a function thought to be subserved by synaptopodin [2]. Synaptopodin undergoes rapid degraded by TRPC5/ Ca^{2+} induced [39] and calcineurin/cathepsin L mediated proteolysis [41]. On the other hand, similarly to cardiomyocytes [84], Tm in podocytes is highly stable [82], and therefore not well suited to mediate a fast remodeling of the actin cytoskeleton. In keeping with this notion, the half-life of synaptopodin in podocytes is markedly shorter than that of Tm [82].

In addition to promoting RhoA signaling [44], synaptopodin can suppress Cdc42 signaling in podocytes [69]. Synaptopodin binds to IRSp53 and suppress bradykinin induced, Cdc42:IRSp53:Mena mediated filopodia formation by blocking the binding of Cdc42 and Mena to IRSp53 [69]. Moreover the Mena inhibitor FP4-Mito suppresses aberrant filopodia formation in synaptopodin knockdown podocytes and when delivered into mice protects against LPS-induced proteinuria [69].

Ca^{2+} dependent signaling in podocytes: TRPCs, calcineurin and NFAT

The Ca^{2+} -dependent activation of calcineurin triggers the cathepsin L-mediated cleavage of synaptopodin, thereby causing proteinuria [41]. The calcineurin inhibitor cyclosporine A (CsA) prevents synaptopodin degradation *in vitro*, and mice resistant to cathepsin-mediated synaptopodin degradation are protected from LPS induced proteinuria *in vivo* [41]. Conversely, the activation of calcineurin in podocytes is sufficient to cause degradation of synaptopodin and proteinuria [41]. Taken together, the latter study revealed a T-cell and NFAT-independent mechanism for the long known anti-proteinuric effect of CsA: preservation of synaptopodin and the podocyte actin cytoskeleton [41]. Synaptopodin mutant mice lacking Synpo-short and Synpo-long upregulate Synpo-T protein expression in podocytes, thereby rescuing kidney filter function during development [68]. Moreover, bigenic heterozygosity for synaptopodin and CD2AP results in proteinuria and FSGS-like glomerular damage, highlighting the importance of synaptopodin and CD2AP for sustained kidney filter function [85]. In humans, heterozygous mutations in the promoter of the *Synpo* gene in patients with idiopathic FSGS reduce gene transcription *in vitro* and protein abundance *in vivo* [22]. FSGS-causing gain of function TRPC6 mutations but not wild type TRPC6 induce constitutive activation of calcineurin-NFAT-dependent gene transcription [86]. This study supports an important role for TRPC channel mediated Ca^{2+} influx in activating calcineurin and its downstream effectors in podocytes, converging on the actin cytoskeleton. Similar to cardiac myocytes [8], podocytes possess a positive feed loop linking TRPC6 channels to NFAT activation and subsequent *TRPC6* channel transcriptional upregulation [87]. Moreover, expression of constitutively active NFAT in podocytes is sufficient to cause glomerulosclerosis in mice [88]. It should be noted that while calcineurin-NFAT-mediated increases in *TRPC6* transcription promote pathologic cardiac remodeling

[8], other TRPC channels, including TRPC3, TRPC4 (which is highly homologous and functionally similar to TRPC5 [62, 89]) and TRPC7, also mediate calcineurin-NFAT activation in cardiac myocytes [8, 55, 56]. This finding provides strong evidence that this pathway is not specific to TRPC6, but that cell type specific TRPC:calcineurin:NFAT pairs are likely possible.

Regulation of Rho GTPases in podocytes by Ca^{2+} signaling

RhoA promotes the formation of stress fibers and focal contacts, generating a contractile phenotype [63]. The predominance of RhoA activity produces a stationary podocyte phenotype, interpreted as stable FPs, while predominance of Cdc42/Rac1 activity mediates a disease-associated motile phenotype, suggesting unstable or retracted FPs [2]. Rac1 is also important in the emergence of proteinuria. Rho GDP dissociation inhibitor α (Rho GDI α) knockout mice [90] develop albuminuria, which was attributed to increased, Rac1 signaling in podocytes [91]. Rac1-dependent accumulation of mineralocorticoid receptor (MR) into the podocyte nucleus through p21 activated kinase phosphorylation is the mechanism invoked to explain this finding [91]. The Rac1 inhibitor NSC23766 diminished MR hyperactivity and ameliorated proteinuria and renal damage in the Rho GDI α knockout mice [91].

To date, Ca^{2+} and synaptopodin-mediated have been identified as essential modulators of Rho GTPase signaling in podocytes. However, many steps in these pathways are still unknown. In mouse embryonic fibroblasts (MEFs), spatially and temporally restricted changes in the concentration of free calcium (Ca^{2+} flickers) mediated by TRP channels, are enriched near the leading edge of migrating cells [92, 93]. This supports the notion that TRP channels are the Ca^{2+} influx pathways responsible for the initiation of this signaling cascade. But which channels serve this role in podocytes? TRPC5 and TRPC6 are antagonistic regulators of actin dynamics and cell motility in podocytes through the regulation of RhoA and Rac1, respectively [39]. AT1R-mediated TRPC5 and TRPC6 channel activity was shown to control the balance between motile and contractile phenotypes [39]. Gene silencing of *TRPC6* results in loss of stress fibers, activation of Rac1, and increased motility, which is rescued by constitutively active RhoA [39]. The loss of stress fibers and synaptopodin in TRPC6-depleted cells was rescued by CsA [39], similar to previous studies [41]. Conversely, gene silencing of *TRPC5* results in enhanced stress fiber formation, activation of RhoA, and decreased motility, which is reversed by constitutively active Rac1 [39]. In contrast to TRPC6-depleted cells, synaptopodin expression is preserved in TRPC5-depleted podocytes [39]. The spatial and temporal coincidence of the paired signals is easier to understand given data showing that TRPC5 specifically interacts with and activates Rac1 in a signaling microdomain, whereas TRPC6 specifically interacts with and activates RhoA in another, distinct molecular complex [39]. These data suggest that while genetics has steered us in the direction of TRPC6 in podocytes, a clearer understanding of Ca^{2+} signaling and cytoskeletal changes in podocytes may be gained by focusing on the *balance* between TRPC5 and TRPC6 [1, 94].

We now know that TRPC5 can activate Rac1 and TRPC6 can activate RhoA [39] (Fig. 2) but the intermediate, Ca^{2+} dependent steps that regulate Rho GTPase activity remain elusive. One likely hypothesis is that the Ca^{2+} dependent activation/inactivation of the Rho GTPases is mediated by GEFs, catalyzing the exchange of bound GDP with free GTP, and GAPs catalyzing the hydrolysis of GTP to GDP, [63]. The Rho GEF, Arhgef1, can regulate vascular tone and blood pressure in a Ca^{2+} -dependent manner in vascular smooth muscle cells *in vivo* [95]. In podocytes the RhoA and ROCK-activated Rac1 GTPase-activating Arhgap24/FilGAP [96] inactivates Rac1 in podocytes, and a mutant form of Arhgap24/FilGAP is associated with familial FSGS [97]. An emerging concept thus links Ca^{2+} influx,

FilGAP and Rho GTPase-mediated cytoskeletal reorganization to modulate podocyte cell shape and motility.

Calcium signaling in podocyte health and disease: a balancing act?

Despite this recent progress, many open questions remain. For example, how is it possible that constitutive Rac1 leads to proteinuria [91], while constitutive RhoA is similarly promoting proteinuria and FSGS-type lesions [98]? The idea of a tipped-over balance between TRPC5/Rac1 versus TRPC6/RhoA (Fig. 2) may be the only way to reconcile these discrepant findings [94]. TRPC6 is the predominant TRPC channel in the plasma membrane of healthy podocytes, which supports the notion that at baseline podocytes maintain an adaptive TRPC6 and RhoA-predominant, contractile actin cytoskeleton [39]. The balance is tipped over and becomes severely biased toward TRPC6 in the presence of gain of function *TRPC6* mutations leading to FSGS, likely due to Ca^{2+} overload-mediated cellular injury, podocyte detachment or cell death [94]. In keeping with this idea, mice overexpressing wild type *TRPC6* or *TRPC6* gain-of-function mutants develop albuminuria and FSGS-type lesions around 6–8 months of age [99]. Further support for the notion that the TRPC6-RhoA axis can cause FSGS comes from the observation that overexpression of constitutive RhoA activity in podocytes is sufficient to induce FSGS-type lesions [98]. Additional support comes from the finding that *TRPC6* null mice are protected from Ang II induced proteinuria [100]. Constitutively active Rac1 signaling also results in proteinuria [91], suggesting that the tipping of the balance toward TRPC5/Rac1 signaling may mediate disease-associated podocyte actin remodeling and increased motility. Intriguingly, TRPC5 channels depend on Rac1 for their insertion into the plasma membrane [59], which may point to a positive feedback loop whereby Rac1 mediates enhanced insertion and overactivity of TRPC5 channels in the podocyte plasma membrane. Ongoing *in vivo* studies on TRPC5 should unveil its precise role in podocyte health and disease.

Conclusions

This review illustrates the importance of understanding not only the cell biology of the podocyte actin cytoskeleton but also its physiologic regulation. The tiny but potent Ca^{2+} ion appears to be critical for the regulation of podocyte homeostasis and plays a pivotal role in the emergence of kidney disease. Clearly, we need more work to understand Ca^{2+} signaling in podocytes, but an emerging paradigm calls for careful consideration of the crosstalk between bradykinin/Cdc42, TRPC5/Rac1 and TRPC6/RhoA (Fig. 2) as mediators of cytoskeletal changes that lead to proteinuria, podocyte loss or detachment, and ultimately, FSGS.

The ability of synaptopodin to functionally replace tropomyosin in non-podocytes [82] is intriguing and is tempting to draw parallels between Ca^{2+} -dependent calmodulin/calcineurin/synaptopodin signaling in podocytes and troponin/myosin phosphatase/tropomyosin in muscle cells. TRPC5 and TRPC6 can regulate stress fibers in podocytes that express synaptopodin, and also in fibroblasts [39] that express tropomyosin but not synaptopodin [82]. Thus, it is tempting to speculate whether tropomyosin is a downstream target of TRPC5 and TRPC6 in fibroblasts, similar to synaptopodin in podocytes [39]. This concept revisits a theme presented in the beginning of this review, namely that Ca^{2+} is a selective activator of kinases that mediate contraction, and that a decline or change in localized $[\text{Ca}^{2+}]$ may fuel phosphatases to mediate relaxation and/or increased motility. This process is likely to apply to any contractile cell, including the podocyte, and may therefore be a fruitful prism through which we view Ca^{2+} signaling and podocyte biology. Finally this work also illustrates how the study of calcium and Rho GTPase signaling in the highly

specialized podocytes can offer novel insight into the regulation of actin dynamics in diverse cell systems from *Drosophila* oocytes to fibroblast to human cancer cells.

Acknowledgments

Supported in part by an American Society of Nephrology Gottschalk Grant and NIH grant DK083511 to AG, and NIH grants DK57683 and DK062472 to PM

We thank the current and former members of the Greka and Mundel laboratories and our collaborators who were involved in the studies from our laboratories discussed above.

References

1. Greka A, Mundel P. Cell Biology and Pathology of Podocytes. *Annu Rev Physiol*. 2011
2. 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; 17:428–37. [PubMed: 17804239]
3. Ringer S, Phear AG. The Influence of Saline Media on the Tadpole. *J Physiol*. 1895; 17:423–32.
4. Clapham DE. Calcium signaling. *Cell*. 2007; 131:1047–58. [PubMed: 18083096]
5. Hille, B. Ionic channels of excitable membranes. 1992.
6. Mandegar M, Fung YC, Huang W, Remillard CV, Rubin LJ, Yuan JX. Cellular and molecular mechanisms of pulmonary vascular remodeling: role in the development of pulmonary hypertension. *Microvasc Res*. 2004; 68:75–103. [PubMed: 15313118]
7. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nature reviews Molecular cell biology*. 2006; 7:589–600.
8. Kuwahara K, Wang Y, McAnally J, Richardson JA, Bassel-Duby R, Hill JA, et al. TRPC6 fulfills a calcineurin signaling circuit during pathologic cardiac remodeling. *J Clin Invest*. 2006; 116:3114–26. [PubMed: 17099778]
9. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, et al. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell*. 1998; 93:215–28. [PubMed: 9568714]
10. Molkentin JD, Olson EN. GATA4: a novel transcriptional regulator of cardiac hypertrophy? *Circulation*. 1997; 96:3833–5. [PubMed: 9403603]
11. Kriz W, LeHir M. Pathways to nephron loss starting from glomerular diseases-insights from animal models. *Kidney Int*. 2005; 67:404–19. [PubMed: 15673288]
12. Mundel P, Kriz W. Structure and function of podocytes: an update. *Anat Embryol (Berl)*. 1995; 192:385–97. [PubMed: 8546330]
13. Somlo S, Mundel P. Getting a foothold in nephrotic syndrome. *Nat Genet*. 2000; 24:333–5. [PubMed: 10742089]
14. 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; 24:349–54. [PubMed: 10742096]
15. 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; 1:575–82. [PubMed: 9660941]
16. Hinkes B, Wiggins RC, Gbadegesin R, Vlangos CN, Seelow D, Nurnberg G, et al. Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. *Nat Genet*. 2006; 38:1397–405. [PubMed: 17086182]
17. 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; 24:251–6. [PubMed: 10700177]
18. 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; 300:1298–300. [PubMed: 12764198]

19. Brown EJ, Schlondorff JS, Becker DJ, Tsukaguchi H, Uscinski AL, Higgs HN, et al. Mutations in the formin gene INF2 cause focal segmental glomerulosclerosis. *Nat Genet.* 42:72–6. [PubMed: 20023659]
20. 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; 308:1801–4. [PubMed: 15879175]
21. 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; 37:739–44. [PubMed: 15924139]
22. Dai S, Wang Z, Pan X, Wang W, Chen X, Ren H, et al. Functional analysis of promoter mutations in the ACTN4 and SYNPO genes in focal segmental glomerulosclerosis. *Nephrol Dial Transplant.* 2010; 25:824–35. [PubMed: 19666657]
23. Okamoto K, Tokunaga K, Doi K, Fujita T, Suzuki H, Katoh T, et al. Common variation in GPC5 is associated with acquired nephrotic syndrome. *Nat Genet.* 2011; 43:459–63. [PubMed: 21441931]
24. Mele C, Iatropoulos P, Donadelli R, Calabria A, Maranta R, Cassis P, et al. MYO1E mutations and childhood familial focal segmental glomerulosclerosis. *N Engl J Med.* 2011; 365:295–306. [PubMed: 21756023]
25. Sanna-Cherchi S, Burgess KE, Nees SN, Caridi G, Weng PL, Dagnino M, et al. Exome sequencing identified MYO1E and NEIL1 as candidate genes for human autosomal recessive steroid-resistant nephrotic syndrome. *Kidney Int.* 2011; 80:389–96. [PubMed: 21697813]
26. Garg P, Verma R, Nihalani D, Johnstone DB, Holzman LB. Nephl cooperates with nephrin to transduce a signal that induces actin polymerization. *Mol Cell Biol.* 2007; 27:8698–712. [PubMed: 17923684]
27. 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. *J Clin Invest.* 2006; 116:1346–59. [PubMed: 16543952]
28. Barletta GM, Kovari IA, Verma RK, Kerjaschki D, Holzman LB. Nephrin and Nephl co-localize at the podocyte foot process intercellular junction and form cis hetero-oligomers. *J Biol Chem.* 2003
29. Tryggvason K, Pikkarainen T, Patrakka J. Nck links nephrin to actin in kidney podocytes. *Cell.* 2006; 125:221–4. [PubMed: 16630808]
30. Kurihara H, Anderson JM, Kerjaschki D, Farquhar MG. The altered glomerular filtration slits seen in puromycin aminonucleoside nephrosis and protamine sulfate-treated rats contain the tight junction protein ZO-1. *Am J Pathol.* 1992; 141:805–16. [PubMed: 1415478]
31. Kerjaschki D. Polycation-induced dislocation of slit diaphragms and formation of cell junctions in rat kidney glomeruli. The effects of low temperature, divalent cations, colchicine, and cytochalasin B. *Lab Invest.* 1978; 39:430–40. [PubMed: 104090]
32. Cybulsky AV, Bonventre JV, Quigg RJ, Lieberthal W, Salant DJ. Cytosolic calcium and protein kinase C reduce complement-mediated glomerular epithelial injury. *Kidney Int.* 1990; 38:803–11. [PubMed: 2266662]
33. Seiler MW, Venkatachalam MA, Cotran RS. Glomerular epithelium: structural alterations induced by polycations. *Science.* 1975; 189:390–3. [PubMed: 1145209]
34. Reiser J, Pixley FJ, Hug A, Kriz W, Smoyer WE, Stanley ER, et al. Regulation of mouse podocyte process dynamics by protein tyrosine phosphatases. *Kidney Int.* 2000; 57:2035–42. [PubMed: 10792622]
35. Rudiger F, Greger R, Nitschke R, Henger A, Mundel P, Pavenstadt H. Polycations induce calcium signaling in glomerular podocytes. *Kidney Int.* 1999; 56:1700–9. [PubMed: 10571778]
36. Mundel P, Reiser J, Borja AZ, Pavenstadt H, Davidson GR, Kriz W, et al. Rearrangements of the cytoskeleton and cell contacts induce process formation during differentiation of conditionally immortalized mouse podocyte cell lines. *Exp Cell Res.* 1997; 236:248–58. [PubMed: 9344605]
37. Henger A, Huber T, Fischer KG, Nitschke R, Mundel P, Schollmeyer P, et al. Angiotensin II increases the cytosolic calcium activity in rat podocytes in culture. *Kidney Int.* 1997; 52:687–93. [PubMed: 9291188]

38. Gloy J, Henger A, Fischer KG, Nitschke R, Mundel P, Bleich M, et al. Angiotensin II depolarizes podocytes in the intact glomerulus of the Rat. *J Clin Invest.* 1997; 99:2772–81. [PubMed: 9169508]
39. 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. *Sci Signal.* 2010; 3:ra77. [PubMed: 20978238]
40. Hoffmann S, Podlich D, Hahnel B, Kriz W, Gretz N. Angiotensin II Type 1 Receptor Overexpression in Podocytes Induces Glomerulosclerosis in Transgenic Rats. *J Am Soc Nephrol.* 2004; 15:1475–87. [PubMed: 15153558]
41. Faul C, Donnelly M, Merscher-Gomez S, Chang YH, Franz S, Delfgaauw J, et al. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med.* 2008; 14:931–8. [PubMed: 18724379]
42. Vassiliadis J, Bracken C, Matthews D, O'Brien S, Schiavi S, Wawersik S. Calcium Mediates Glomerular Filtration through Calcineurin and mTORC2/Akt Signaling. *J Am Soc Nephrol.* 2011; 22:1453–61. [PubMed: 21784900]
43. Hsu HH, Hoffmann S, Endlich N, Velic A, Schwab A, Weide T, et al. Mechanisms of angiotensin II signaling on cytoskeleton of podocytes. *J Mol Med.* 2008; 86:1379–94. [PubMed: 18773185]
44. 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. *Nat Cell Biol.* 2006; 8:485–91. [PubMed: 16622418]
45. Clapham DE. TRP channels as cellular sensors. *Nature.* 2003; 426:517–24. [PubMed: 14654832]
46. Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. *Annu Rev Physiol.* 2006; 68:619–47. [PubMed: 16460286]
47. Gees M, Colasoul B, Nilius B. The Role of Transient Receptor Potential Cation Channels in Ca²⁺ signaling. *Cold Spring Harbor perspectives in biology.* 2010
48. Chaudhuri P, Colles SM, Bhat M, Van Wagoner DR, Birnbaumer L, Graham LM. Elucidation of a TRPC6-TRPC5 channel cascade that restricts endothelial cell movement. *Mol Biol Cell.* 2008; 19:3203–11. [PubMed: 18495872]
49. Inoue R, Jensen LJ, Jian Z, Shi J, Hai L, Lurie AI, et al. Synergistic activation of vascular TRPC6 channel by receptor and mechanical stimulation via phospholipase C/diacylglycerol and phospholipase A2/omega-hydroxylase/20-HETE pathways. *Circ Res.* 2009; 104:1399–409. [PubMed: 19443836]
50. Inoue R, Okada T, Onoue H, Hara Y, Shimizu S, Naitoh S, et al. The transient receptor potential protein homologue TRP6 is the essential component of vascular alpha(1)-adrenoceptor-activated Ca(2+)-permeable cation channel. *Circ Res.* 2001; 88:325–32. [PubMed: 11179201]
51. Jung S, Strotmann R, Schultz G, Plant TD. TRPC6 is a candidate channel involved in receptor-stimulated cation currents in A7r5 smooth muscle cells. *Am J Physiol Cell Physiol.* 2002; 282:C347–59. [PubMed: 11788346]
52. Welsh DG, Morielli AD, Nelson MT, Brayden JE. Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ Res.* 2002; 90:248–50. [PubMed: 11861411]
53. Yu Y, Fantozzi I, Remillard CV, Landsberg JW, Kunichika N, Platoshyn O, et al. Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *Proceedings of the National Academy of Sciences of the United States of America.* 2004; 101:13861–6. [PubMed: 15358862]
54. Nishida M, Onohara N, Sato Y, Suda R, Ogushi M, Tanabe S, et al. Galpha12/13-mediated up-regulation of TRPC6 negatively regulates endothelin-1-induced cardiac myofibroblast formation and collagen synthesis through nuclear factor of activated T cells activation. *J Biol Chem.* 2007; 282:23117–28. [PubMed: 17533154]
55. Onohara N, Nishida M, Inoue R, Kobayashi H, Sumimoto H, Sato Y, et al. TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy. *The EMBO journal.* 2006; 25:5305–16. [PubMed: 17082763]
56. Wu X, Eder P, Chang B, Molkentin JD. TRPC channels are necessary mediators of pathologic cardiac hypertrophy. *Proceedings of the National Academy of Sciences of the United States of America.* 2010; 107:7000–5. [PubMed: 20351294]

57. Dietrich A, Mederos YSM, Gollasch M, Gross V, Storch U, Dubrovskaya G, et al. Increased vascular smooth muscle contractility in TRPC6^{-/-} mice. *Mol Cell Biol*. 2005; 25:6980–9. [PubMed: 16055711]
58. Riccio A, Li Y, Moon J, Kim KS, Smith KS, Rudolph U, et al. Essential role for TRPC5 in amygdala function and fear-related behavior. *Cell*. 2009; 137:761–72. [PubMed: 19450521]
59. Bezzerides VJ, Ramsey IS, Kotecha S, Greka A, Clapham DE. Rapid vesicular translocation and insertion of TRP channels. *Nat Cell Biol*. 2004; 6:709–20. [PubMed: 15258588]
60. Greka A, Navarro B, Oancea E, Duggan A, Clapham DE. TRPC5 is a regulator of hippocampal neurite length and growth cone morphology. *Nat Neurosci*. 2003; 6:837–45. [PubMed: 12858178]
61. Xu SZ, Muraki K, Zeng F, Li J, Sukumar P, Shah S, et al. A sphingosine-1-phosphate-activated calcium channel controlling vascular smooth muscle cell motility. *Circ Res*. 2006; 98:1381–9. [PubMed: 16675717]
62. Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE. TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron*. 2001; 29:645–55. [PubMed: 11301024]
63. Etienne-Manneville S, Hall A. Rho GTPases in cell biology. *Nature*. 2002; 420:629–35. [PubMed: 12478284]
64. Raftopoulou M, Hall A. Cell migration: Rho GTPases lead the way. *Developmental biology*. 2004; 265:23–32. [PubMed: 14697350]
65. Jaffe AB, Hall A. RHO GTPases: Biochemistry and Biology. *Annu Rev Cell Dev Biol*. 2005
66. Jaffe AB, Hall A. Cell biology. Smurfing at the leading edge. *Science*. 2003; 302:1690–1. [PubMed: 14657480]
67. Mundel P, Heid HW, Mundel TM, Kruger M, Reiser J, Kriz W. Synaptopodin: an actin-associated protein in telencephalic dendrites and renal podocytes. *The Journal of cell biology*. 1997; 139:193–204. [PubMed: 9314539]
68. Asanuma K, Kim K, Oh J, Giardino L, Chabanis S, Faul C, et al. Synaptopodin regulates the actin-bundling activity of alpha-actinin in an isoform-specific manner. *J Clin Invest*. 2005; 115:1188–98. [PubMed: 15841212]
69. Yanagida-Asanuma E, Asanuma K, Kim K, Donnelly M, Young Choi H, Hyung Chang J, et al. Synaptopodin protects against proteinuria by disrupting Cdc42:IRSp53:Mena signaling complexes in kidney podocytes. *Am J Pathol*. 2007; 171:415–27. [PubMed: 17569780]
70. Cooper JA. Actin dynamics: tropomyosin provides stability. *Curr Biol*. 2002; 12:R523–5. [PubMed: 12176375]
71. O'Neill GM, Stehn J, Gunning PW. Tropomyosins as interpreters of the signalling environment to regulate the local cytoskeleton. *Semin Cancer Biol*. 2008; 18:35–44. [PubMed: 17942320]
72. Hitchcock-DeGregori SE, Greenfield NJ, Singh A. Tropomyosin: regulator of actin filaments. *Adv Exp Med Biol*. 2007; 592:87–97. [PubMed: 17278358]
73. Gunning PW, Schevzov G, Kee AJ, Hardeman EC. Tropomyosin isoforms: divining rods for actin cytoskeleton function. *Trends Cell Biol*. 2005; 15:333–41. [PubMed: 15953552]
74. Clark I, Giniger E, Ruohola-Baker H, Jan LY, Jan YN. Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr Biol*. 1994; 4:289–300. [PubMed: 7922338]
75. Shulman JM, Benton R, St Johnston D. The *Drosophila* homolog of *C. elegans* PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. *Cell*. 2000; 101:377–88. [PubMed: 10830165]
76. Tomancak P, Piano F, Riechmann V, Gunsalus KC, Kempfues KJ, Ephrussi A. A *Drosophila melanogaster* homologue of *Caenorhabditis elegans* par-1 acts at an early step in embryonic-axis formation. *Nat Cell Biol*. 2000; 2:458–60. [PubMed: 10878812]
77. Doerflinger H, Benton R, Torres IL, Zwart MF, St Johnston D. *Drosophila* anterior-posterior polarity requires actin-dependent PAR-1 recruitment to the oocyte posterior. *Curr Biol*. 2006; 16:1090–5. [PubMed: 16753562]
78. Zimyanin VL, Belaya K, Pecreaux J, Gilchrist MJ, Clark A, Davis I, et al. In vivo imaging of oskar mRNA transport reveals the mechanism of posterior localization. *Cell*. 2008; 134:843–53. [PubMed: 18775316]

79. Ephrussi A, Dickinson LK, Lehmann R. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell*. 1991; 66:37–50. [PubMed: 2070417]
80. Kim-Ha J, Smith JL, Macdonald PM. oskar mRNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell*. 1991; 66:23–35. [PubMed: 2070416]
81. Erdelyi M, Michon AM, Guichet A, Glotzer JB, Ephrussi A. Requirement for *Drosophila* cytoplasmic tropomyosin in oskar mRNA localization. *Nature*. 1995; 377:524–7. [PubMed: 7566149]
82. Wong JS, Iorns E, Rheault MN, Ward TM, Rashmi P, Weber U, et al. Rescue of tropomyosin deficiency in *Drosophila* and human cancer cells by synaptopodin reveals a role of tropomyosin alpha in RhoA stabilization. *The EMBO journal*. 2011
83. Drenckhahn D, Schnittler H, Nobiling R, Kriz W. Ultrastructural organization of contractile proteins in rat glomerular mesangial cells. *Am J Pathol*. 1990; 137:1343–51. [PubMed: 2260624]
84. Martin AF. Turnover of cardiac troponin subunits. Kinetic evidence for a precursor pool of troponin-I. *J Biol Chem*. 1981; 256:964–8. [PubMed: 7451483]
85. Huber TB, Kwok C, Wu H, Asanuma K, Godel M, Hartleben B, et al. Bigenic mouse models of focal segmental glomerulosclerosis involving pairwise interaction of CD2AP, Fyn, and synaptopodin. *J Clin Invest*. 2006; 116:1337–45. [PubMed: 16628251]
86. Schlondorff J, Del Camino D, Carrasquillo R, Lacey V, Pollak MR. TRPC6 mutations associated with focal segmental glomerulosclerosis cause constitutive activation of NFAT-dependent transcription. *Am J Physiol Cell Physiol*. 2009; 296:C558–69. [PubMed: 19129465]
87. Nijenhuis T, Sloan AJ, Hoenderop JG, Flesche J, van Goor H, Kistler AD, et al. Angiotensin II Contributes to Podocyte Injury by Increasing TRPC6 Expression via an NFAT-Mediated Positive Feedback Signaling Pathway. *Am J Pathol*. 2011
88. Wang Y, Jarad G, Tripathi P, Pan M, Cunningham J, Martin DR, et al. Activation of NFAT signaling in podocytes causes glomerulosclerosis. *J Am Soc Nephrol*. 2010; 21:1657–66. [PubMed: 20651158]
89. Strubing C, Krapivinsky G, Krapivinsky L, Clapham DE. Formation of novel TRPC channels by complex subunit interactions in embryonic brain. *J Biol Chem*. 2003; 278:39014–9. [PubMed: 12857742]
90. Togawa A, Miyoshi J, Ishizaki H, Tanaka M, Takakura A, Nishioka H, et al. Progressive impairment of kidneys and reproductive organs in mice lacking Rho GDIalpha. *Oncogene*. 1999; 18:5373–80. [PubMed: 10498891]
91. Shibata S, Nagase M, Yoshida S, Kawarazaki W, Kurihara H, Tanaka H, et al. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. *Nat Med*. 2008; 14:1370–6. [PubMed: 19029984]
92. Collins SR, Meyer T. Calcium flickers lighting the way in chemotaxis? *Dev Cell*. 2009; 16:160–1. [PubMed: 19217416]
93. Wei C, Wang X, Chen M, Ouyang K, Song LS, Cheng H. Calcium flickers steer cell migration. *Nature*. 2009; 457:901–5. [PubMed: 19118385]
94. Greka A, Mundel P. Balancing calcium signals through TRPC5 and TRPC6 in podocytes. *J Am Soc Nephrol*. 2011; 22:1969–80. [PubMed: 21980113]
95. Guilluy C, Bregeon J, Toumaniantz G, Rolli-Derkinderen M, Retailliau K, Loufrani L, et al. The Rho exchange factor Arhgef1 mediates the effects of angiotensin II on vascular tone and blood pressure. *Nat Med*. 2010; 16:183–90. [PubMed: 20098430]
96. Ohta Y, Hartwig JH, Stossel TP. FilGAP, a Rho- and ROCK-regulated GAP for Rac binds filamin A to control actin remodelling. *Nat Cell Biol*. 2006; 8:803–14. [PubMed: 16862148]
97. Akilesh S, Suleiman H, Yu H, Stander MC, Lavin P, Gbadegesin R, et al. Arhgap24 inactivates Rac1 in mouse podocytes, and a mutant form is associated with familial focal segmental glomerulosclerosis. *J Clin Invest*. 2011; 121:4127–37. [PubMed: 21911940]
98. Zhu L, Jiang R, Aoudjit L, Jones N, Takano T. Activation of RhoA in Podocytes Induces Focal Segmental Glomerulosclerosis. *J Am Soc Nephrol*. 2011
99. Krall P, Canales CP, Kairath P, Carmona-Mora P, Molina J, Carpio JD, et al. Podocyte-specific overexpression of wild type or mutant trpc6 in mice is sufficient to cause glomerular disease. *PLoS One*. 2010; 5:e12859. [PubMed: 20877463]

100. Eckel J, Lavin PJ, Finch EA, Mukerji N, Burch J, Gbadegesin R, et al. TRPC6 Enhances Angiotensin II-induced Albuminuria. *J Am Soc Nephrol*. 2011

Figure 1. Proteasomal degradation of RhoA is an upstream regulator of the RhoA cycle

According to the classical Rho GTPase cycle, the activity of Rho GTPases is regulated by a switch between an inactive GDP-bound and an active GTP-bound form. This cycle is tightly controlled by guanine exchange factors (GEF), GTPase activating proteins (GAP) and guanine dissociation inhibitors (GDI). Synaptopodin (Synpo) and tropomyosin (Tm) promote stress fiber formation by blocking the Smurf1 mediated ubiquitination of RhoA. Thus, the regulation of RhoA degradation is a widespread conserved mechanism for the regulation of RhoA signaling, upstream of the GDP/GTP switch.

Figure 2. Regulation of podocyte Rho GTPase signaling by Ca^{2+}

Stimulation of AT1R induces both TRPC5 and TRPC6 mediated Ca^{2+} influx, thereby activation of RhoA and Rac1, respectively. Activation of the TRPC5-Rac1 complex inhibits RhoA and mediates stress fiber disassembly, thus promoting cell motility. In contrast, activation of the TRPC6-RhoA complex inhibits Rac1 activity and promotes stress fiber formation. TRPC6 increases synaptopodin protein levels, which in turn increases RhoA abundance and activation. TRPC5 decreases synaptopodin abundance by stimulation the calcineurin-mediated dephosphorylation of synaptopodin. Stimulation of the bradykinin receptor (BkR) increases $[\text{Ca}^{2+}]_i$ from yet unidentified sources, thereby inducing Cdc42 dependent filopodia formation, which can be blocked by synaptopodin.