Pflugers Arch - Eur J Physiol (2017) 469:937–949
DOI 10.1007/s00424-017-2025-8

INVITED REVIEW

Stressed podocytes—mechanical forces, sensors, signaling and response

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Received: 1 June 2017 / Accepted: 22 June 2017 / Published online: 7 July 2017

Abstract Increased glomerular capillary pressure (glomerular hypertension) and increased glomerular filtration rate (glomerular hyperfiltration) have been proven to cause glomerulosclerosis in animal models and are likely to be operative in patients. Since podocytes cover the glomerular basement membrane, they are exposed to tensile stress due to circumferential wall tension and to fluid shear stress arising from filtrate flow through the narrow filtration slits and through Bowman's space. In vitro evidence documents that podocytes respond to tensile stress as well as to fluid shear stress. Several proteins are discussed in this review that are expressed in podocytes and could act as mechanosensors converting mechanical force via a conformational change into a biochemical signal. The cation channels P2X4 and TRPC6 were shown to be involved in mechanosignaling in podocytes. P2X4 is activated by stretch-induced ATP release, while TRPC6 might be inherently mechanosensitive. Membrane, slit diaphragm and cell-matrix contact proteins are connected to the sublemmal actin network in podocytes via various linker proteins. Therefore, actin-associated proteins, like the proven mechanosensor filamin, are ideal candidates to sense forces in the podocyte cytoskeleton. Furthermore, podocytes express talin, p130Cas, and fibronectin that are known to undergo a conformational change in response to mechanical force exposing cryptic binding sites. Downstream of mechanosensors, experimental evidence suggests the involvement of MAP kinases, Ca^{2+} and COX2 in mechanosignaling and an emerging role of YAP/TAZ. In summary, our understanding of mechanotransduction in podocytes is still sketchy, but future progress holds promise to identify targets to alleviate conditions of increased mechanical load.

Keywords Podocyte · Stretch · Mechanical stress · Mechanotransduction · Chronic kidney disease · Glomerulopathy

This article is part of the special issue on Functional Anatomy of the Kidney in Health and Disease in Pflügers Archiv—European Journal of Physiology.

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Forces acting on podocytes

Glomerular capillary pressure and glomerular blood flow are the driving forces of glomerular filtration. Renal hemodynamics that critically determine glomerular capillary pressure and glomerular blood flow are regulated by the renal vasculature [27, 28, 100]. Since podocytes cover the outer aspect of the glomerular capillaries, mechanical forces that act on and within the glomerulus are relevant for podocytes. We have provided a detailed description of the glomerular mechanical forces in a recent review [29]. There are three major glomerular forces that have to be considered for the podocyte (Fig. 1): (1) circumferential wall stress, (2) fluid shear stress in the filtration slit, and (3) fluid shear stress in Bowman's space.

Circumferential wall stress is generated by the pressure difference between the glomerular capillary lumen and Bowman's space, amounting to about 40 mmHg. The pressure difference tends to expand glomerular capillaries (Fig. 1a). The tensile stress exerted on the elements of the glomerular capillary wall (endothelium, glomerular basement membrane—GBM, podocyte foot processes) is estimated to amount to about

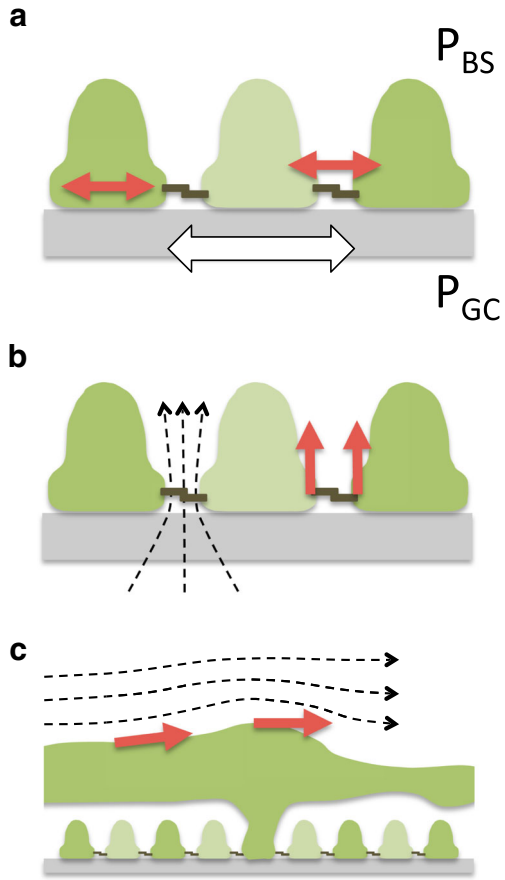

Fig. 1 Mechanical forces acting on podocytes. **a** The hydrostatic pressure difference between the glomerular capillary pressure (p_{GC}) and the pressure in Bowman's space (p_{BS}) creates circumferential wall stress (*white double arrow*). Tensile forces (red double arrows) act on the slit diaphragm and on podocyte foot processes in the transversal and the longitudinal direction (not shown) of foot processes. **b** Filtrate flow (*broken black arrows*) through the filtration slit exerts shear stress on foot processes. Forces (*red arrows*) are tangential to the surface of foot processes. **c** Filtrate flow (*broken black arrows*) in Bowman's space results in shear stress on the surface of podocytes. Forces (*red arrows*) are tangential to the surface of the processes and the cell body (not shown) of podocytes. The glomerular basement membrane (GBM) is depicted as a gray bar; the glomerular endothelium is omitted

50 kPa [29]. This is a rather high value due to the large hydrostatic pressure difference and the thin wall of glomerular capillaries. As podocyte foot processes show a random (isotropic) orientation, foot processes will experience tensile stress along the longitudinal and the transverse axes. Likewise, tensile stress will also act on the slit diaphragm. Neal and colleagues reported that an increased perfusion pressure selectively expanded the size of a special type of foot processes, so-called anchoring processes, which directly arise from the podocyte cell body [73]. The relevance of this finding is unclear.

After passing the open fenestrations of the glomerular endothelium, and after crossing the glomerular basement membrane, the filtrate squeezes through the filtration slit between the interdigitating foot processes (Fig. 1b). Making some rather crude assumptions, we estimated the shear stress, which is exerted on the foot processes by the flow of the filtrate through the filtration slit, to amount to about 8 Pa [29]. This magnitude of shear stress on foot processes is comparable to the magnitude of wall shear stress experienced by endothelial cells in blood vessels. The flow velocity of the filtrate in the filtration slit, which is of considerable length, is relatively low. However, it is the tiny width of the filtration slit of about 30 nm that causes the high shear stress.

Downstream of the filtration slit, the ultrafiltrate flows through Bowman's space (Fig. 1c). As described by Neal et al., Bowman's space can be subdivided into three spaces: the subpodocyte space below the major processes and the cell body of podocytes, the interpodocyte space, and the peripheral urinary space between the outermost podocytes and the glomerular parietal epithelial cells [73]. We developed a formula to estimate fluid shear stress arising from the flow of the ultrafiltrate in Bowman's space, or more exactly in the peripheral urinary space [35]. According to this formula, fluid shear stress is proportional to the fraction of the single glomerulus filtration rate that flows through the peripheral urinary space, whereas it is inversely related to the glomerular diameter and the square of the width of the peripheral urinary space. Thus, the magnitude of fluid shear stress in the peripheral urinary space is very sensitive to the latter parameter. Assuming a width of the peripheral urinary space of about 2 to 8 μm, we calculated values around 0.5 Pa [35]. According to intravital multiphoton studies of the glomerulus, our assumption of the width of the peripheral urinary space appears to be valid for the in vivo situation [8, 20, 42].

Our formula was developed for the peripheral urinary space. Reconstructions demonstrated that subpodocyte space drains through exit pores with a diameter of 0.2–0.3 μm and a length of 1.1 μm [73]. These pores are extremely narrow. The podocyte plasma membrane and its glycocalyx that delimits the pores will be exposed to considerable fluid shear stress. But not only the exit pores of the subpodocyte space are narrow, there are also exit pores of the interpodocyte space into the peripheral urinary space with a diameter of 0.7–1.4 μm and a length of 0.6–0.8 μm [73]. Modeling the flow in the subpodocyte space and experimental data by intravital two-photon microscopy suggest that the exit pores of the subpodocyte space impart a resistance to glomerular filtration of the same magnitude as that of the glomerular capillary wall [74, 86]. In conclusion, relevant shear stress of ultrafiltrate flow is likely to occur in all three compartments of Bowman's space (subpodocyte, interpodocyte, and peripheral urinary space).

In a review on the structural determinants of glomerular permeability, Deen et al. modeled the hydraulic resistances of the glomerular filtration barrier. They concluded that the hydraulic resistance of the glomerular endothelium is probably negligible and that the GBM and the slit diaphragm

contribute about 60 and 40%, respectively, to the resistance of the glomerular filtration barrier [16]. Taking this distribution of hydraulic resistance and a pressure difference of 40 mmHg over the glomerular filtration barrier, hydraulic pressure will drop by about 25 mmHg over the GBM and by about 15 mmHg over the slit diaphragm. As a result, hydraulic pressure at the basal side of foot processes will be higher than the hydraulic pressure at the apical side of foot processes. The pressure gradient over the foot processes tends to detach the foot processes from the GBM.

Currently, we do not have a clear view regarding the filtrate flow through the glomerular filtration barrier. Since the filtrate cannot flow perpendicularly to the GBM at all sites due to the obstructing effect of foot processes, filtrate flow assumes a more parallel orientation relative to the GBM below foot processes to reach the next filtration slit. In the filtration slit and close to the filtration slit below the foot processes, the streamlines of the filtrate flow are compressed and flow velocity increases. According to Bernoulli's principle, increased flow will produce forces that will pull foot processes toward the GBM and into the filtration slit. Whether the magnitude of these forces is of any physiologic or pathologic relevance cannot be answered at present. Sophisticated hydrodynamic simulations are needed to address this question and to clarify whether additional forces have to be considered at the level of podocyte foot processes.

Clinical relevance of tensile and fluid shear stress on podocytes

Increased glomerular capillary pressure, i.e., glomerular hypertension, leads to podocyte damage, podocyte loss, and glomerulosclerosis as confirmed in several animal models [3, 17, 24, 59, 61, 91]. Whether glomerular hypertension affects podocytes via an increased tensile stress and/or via increased fluid shear stress caused by hyperfiltration is unclear. Recently, Kriz and Lemley have proposed that increased shear stress through the filtration slit is the major determinant for podocyte damage and detachment [62, 63]. According to their attractive hypothesis, podocytes try to escape from excessive shear stress in the filtration slit by foot process effacement.

Srivastava and colleagues determined single glomerulus filtration rate and glomerular diameter in rats 2 and 8 weeks after unilateral nephrectomy [97]. Under the assumption that the width of the peripheral urinary space did not change, they calculated an almost twofold increased shear stress in Bowman's space using our formula [35]. The increased shear stress was mainly due to an increased single glomerulus filtration rate. The clinical relevance of hyperfiltration has long been acknowledged, especially in diabetic nephropathy [44, 107].

Mechanotransduction

A variety of cells in our body is able to sense mechanical forces. Podocytes are also capable of sensing and reacting to mechanical force. We demonstrated that cultured podocytes are sensitive to tensile stress as well as to fluid shear stress [30, 35]. This was later confirmed by other groups for tensile stress [33] as well as for fluid shear stress [2, 47, 98, 99]. Until very recently, all studies were performed in vitro using cultured podocytes or isolated glomeruli. Peti-Peterdi's group has reported the first in vivo evidence that podocytes are mechano-sensitive [40]. By means of two-photon in vivo microscopy, they observed an increase in intracellular Ca^{2+} concentration in podocytes in response to laser-induced thrombus formation in the efferent arteriole of mice. Whether the increase in intracellular Ca^{2+} concentration was caused by an elevation of the glomerular capillary pressure or by raising glomerular filtration rate cannot be discerned.

In order to create a complex response to any mechanical force, cells need to transduce mechanical forces into biochemical signals. This process is called "mechanotransduction." The first element in a mechanotransduction pathway converts mechanical energy into a conformational change and is called the "mechanosensor." The conformational change of the mechanosensor in response to forces in the piconewton range initiates a signaling cascade ("mechanosignaling"), which is purely biochemical. Therefore, the mechanosensor is the decisive element in the mechanotransduction process. Mechanosensors need to be localized at a strategic position, where they are directly exposed to mechanical force allowing them to absorb mechanical energy. Potential and known mechanosensors can be grouped into the following classes:

- Ion channels (and further membrane proteins)
- Proteins associated with the cytoskeleton
- Cell-cell and cell-matrix contact proteins
- Proteins of the extracellular matrix

It is much easier to experimentally verify the critical role of a protein in a mechanotransduction pathway than to experimentally verify that a protein is inherently sensitive to mechanical force and/or that mechanical force induces a conformational change representing the starting point of the mechanosignaling cascade. Therefore, our knowledge of bona fide mechanosensors is relatively limited. In the following, we will present known and potential mechanosensors and discuss them with regard to their mode of action and possible relevance in podocytes.

Ion channels as mechanosensors in podocytes

Electrophysiological recordings from whole cells or membrane patches revealed currents that are sensitive to

mechanical forces. Based on the electrophysiological characteristics of the currents, mechanosensitivity was attributed to various ion channels. However, it is unclear for many ion channels whether they are inherently sensitive to mechanical force or whether mechanosensitivity may be due to channel activation downstream of the "real" mechanosensor.

There are three conceivable mechanisms that change the conformation of membrane proteins: (1) tension of the lipid bilayer transmitted to the transmembrane protein domain(s); (2) tethering of the membrane protein, i.e., interaction with force transmitting intra- or extracellular proteins; and (3) changes in the mixing of the lipid bilayer by kinetic energy. The latter mechanism has been proposed only recently by demonstrating that shear stress disrupts lipid rafts, thereby releasing activatable phospholipase D2 from lipid rafts [77]. Since slit diaphragm proteins were reported to be spatially organized in lipid rafts [92], one could imagine that shear stress-mediated increases in kinetic energy at the slit diaphragm may lead to activation of membrane proteins via such a mechanism.

Inherent mechanosensitivity has been demonstrated for a few ion channels only: for the bacterial small and large conductance mechanosensitive channel (MscS and MscL) [101], for the eukaryotic two-pore domain K^+ channels TRAAK1 (or KCNA4) and TREK1 (or KCNK2) [7, 9], and for the eukaryotic non-selective cation channel PIEZO1 [103]. The MscS and MscL and the TRAAK1 and TREK1 channels as well as the PIEZO1 channel were expressed, purified, and reconstituted in liposomes. The reconstituted ion channels possessed the same characteristics as in cells and, most importantly, they still were mechanosensitive in the cell-free environment. These findings strongly support the hypothesis that tension in the lipid bilayer induces a conformational change of inherently mechanosensitive ion channels. This mechanism has been denoted the force-from-lipid principle [104].

In 2010, the piezo ion channels (PIEZO1 and PIEZO2) were discovered [14]. These channels are mechanosensitive, when expressed in cells. While initial experiments failed to prove mechanosensitivity in reconstitution experiments [15], Syeda and colleagues have succeeded to demonstrate the inherent mechanosensitivity of reconstituted PIEZO1 very recently [103]. So far, nothing is known about the expression and the function of piezo channels in podocytes. The same holds true for the inherently mechanosensitive K^+ channels TRAAK1 and TREK1, which are expressed in neuronal tissue. Since podocytes are non-excitable cells, one would expect that K^+ channels are of minor functional relevance as compared to non-selective cation channels.

Concerning the large family of Trp channels, Maroto et al. showed inherent mechanosensitivity of an enriched fraction of purified TRPC1 channels after reconstitution in 2005 [69]. However, this finding has neither been replicated nor extended to other Trp channels since then [104]. Moreover, there are conflicting results on the mechanosensitivity of TRPC6. Spassova et al. were able to activate TRPC6 in membrane patches of HEK and CHO cells by pressure pulses [95], whereas Gottlieb et al. failed to detect mechanosensitivity of TRPC6 in COS cells [39].

Mutations in TRPC6 were discovered to cause focal segmental glomerulosclerosis [80, 115]. TRPC6 is expressed in podocytes and located at the slit diaphragm and interacts with its C-terminus with the C-terminus of podocin [2, 80]. Podocin was shown to enhance the agonist-stimulated TRPC6 activity depending on its cholesterol-binding PHB domain [48]. In agreement with this finding, knockdown of podocin in cultured podocytes nearly abolished TRPC6 activation evoked by a diacylglycerol analog [2].

Applying mechanical force by osmotic swelling or by membrane indentation, the Dryer lab reported gating of TRPC6 in cultured podocytes [2]. Osmotic swelling also induced robust currents in CHO-K1 cells expressing wild-type TRPC6 but were absent if the TRPC6-N143S mutant, which causes familial focal segmental glomerulosclerosis, was expressed [114]. Forst et al. measured increased intracellular calcium concentrations and increased inward cation currents in response to mechanical stimulation by osmotic swelling and application of a positive pipette pressure in primary podocyte cultures [33]. In contrast to the findings of the Dryer lab, mechanically stimulated Ca^{2+} influx was identical in podocytes obtained from wild-type or TRPC6-deficient mice [33]. The P2X4 (or P2RX4) and not the TRPC6 channel was responsible for the mechanically stimulated Ca^{2+} influx in the experiments of Forst et al. [33]. Pharmacological blockade of P2X4 channels not only abrogated mechanically stimulated Ca^{2+} influx but also prevented the actin reorganization in stretched podocytes [33]. Further confusing is the finding that podocin was required to activate P2X4 channels via ATP release in response to mechanical stimulation [33], whereas the stretch-evoked activation of TRPC6 was markedly enhanced by knockdown of podocin in the experiments of Anderson et al. [2]. It is presently unclear how to reconcile these conflicting findings.

KCNMA1, a large conductance Ca^{2+}-activated K^+ channel (also called BK(Ca) channel or Slo1), was reported as the first ion channel in podocytes that could be activated mechanically [72]. Kim et al. demonstrated by pull-down experiments that TRPC6 interacts with KCNMA1 in podocytes and that expression of TRPC6 increases K^+ currents [56]. Of note, Ca^{2+}-activated BK channels in the distal nephron were proposed to be in a complex with mechanosensitive Ca^{2+} channels in the apical membrane [10]. In conclusion, mechanically induced Ca^{2+} influx most likely activates KCNMA1, suggesting that KCNMA1 does not act as a mechanosensor in podocytes.

Figure 2 summarizes our current knowledge of ion channels involved in the response of podocytes to mechanical

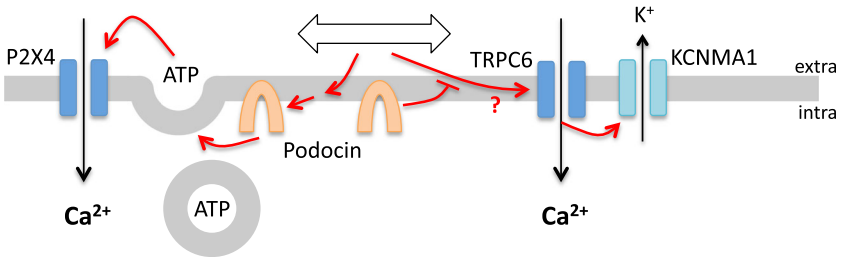

Fig. 2 Mechanotransduction by ion channels in podocytes. Membrane tension (white double arrow) induces release of ATP that binds to the P2X4 receptor, a cation channel, and triggers Ca^{2+} influx. The mechanotransduction pathway leading to ATP release depends on the presence of podocin but has not been further characterized. Membrane tension induces opening of the TRPC6 cation channel. TRPC6 is either directly gated by membrane tension or by a so far uncharacterized mechanotransduction pathway. Podocin, which has been shown to interact with TRPC6, attenuates tension-induced TRPC6 activation. TRPC6 also interacts with KCNMA1, a Ca^{2+}-activated K^+ channel. Ca^{2+} influx through TRPC6 activates KCNMA1, maintaining the driving force for Ca^{2+} influx

force. Whether TRPC6 acts as a mechanosensor or whether TRPC6 is—like P2X4—part of the downstream cascade of mechanotransduction awaits further studies. Osmotic swelling, membrane indentation, or inflation by positive pipette pressure have been employed to mechanically stimulate membrane currents in podocytes. How do these experimental maneuvers translate to the in vivo situation? Tensile stress generated by circumferential wall stress exerts a pulling force on the nephrin molecules that will lead to membrane deformation. Likewise, shear stress causes membrane deformation and, in addition, an increase in the kinetic energy of the membrane.

Actin filaments and actin-associated proteins as mechanosensors in podocytes

The cytoskeleton holds a key position in absorbing, distributing, and generating mechanical forces. Since microtubules and intermediate filaments are restricted to the cell body and major processes of podocytes, foot processes contain an actin cytoskeleton only [19]. The actin cytoskeleton of foot processes can be subdivided in the core, consisting of an actin filament bundle, and the sublemmal network of actin filaments [49]. The sublemmal network is connected to apical membrane proteins (e.g., podocalyxin), to the slit diaphragm proteins (e.g. nephrin) and to the cell-matrix contacts (e.g., integrin $\alpha_3\beta_1$) via linker proteins [32]. Thus, circumferential wall stress is transmitted via cell-matrix contacts from the GBM and via the slit diaphragm as tensile stress to the sublemmal network and the actin filament bundle in foot processes. Fluid shear stress, acting on the plasma membrane, on membrane proteins and the glycocalyx, is conveyed to the sublemmal actin network, which is also present in major processes and the cell body of podocytes.

Mechanical forces acting on podocytes could be sensed by the actin cytoskeleton as changes in tensile stress or as a deformation of the filamentous network. One could envisage actin-associated proteins to serve as mechanosensors and the actin filaments themselves, since their conformation may be altered by tension or bending [83]. An actin-associated protein, or a dimer thereof, could bind to two different actin filaments. If the actin network is deformed, the distance and/or the angle between the two actin filaments will change, resulting in a conformational change of the actin-associated protein. Force-induced unfolding of a domain, which contains otherwise inaccessible binding sites, represents such a conformational change.

Concerning the conformation of filamentous actin, it was shown that the binding of cofilin to actin filaments depends on filament tension [43]. The binding rate of cofilin to relaxed actin filaments was higher as compared to tensed filaments [43]. As a result, relaxed actin filaments will be more prone to depolymerization by cofilin. Cofilin is essential for maintaining the foot process architecture, as demonstrated by podocyte-specific cofilin-1 knockout in mice and by cofilin-1 knockdown in zebrafish [4, 36]. Moreover, an increased amount of inactive (phosphorylated) cofilin-1 in podocytes was observed in human glomerular diseases [4].

There are further actin-associated proteins, like zyxin and paxillin, which bind to filamentous actin depending on filament tension [93]. Colombelli et al. altered tension of actin filament bundles (stress fibers) by laser nanosurgery or by moving the tip of an atomic force microscope. Zyxin binding to stress fibers changed rapidly and was proportional to tension [12]. The LIM domain targets zyxin to actin stress fibers [108]. It is presently unclear whether the LIM domain directly binds to actin filaments and whether it senses force-induced conformational changes in the actin filament [93]. Zyxin-deficient fibroblasts show deficits in actin stress fiber remodeling, pointing to the functional importance of zyxin [46]. However, the role of zyxin in podocytes has not been defined as yet.

Concerning actin-associated proteins that are able to detect deformation of the actin network, it was demonstrated that filamin is such a mechanosensor. Filamin A consists of an N-terminal actin-binding domain and 24 Ig domains. Filamin A can dimerize at domain 24, which is located at the C-terminus, thereby connecting two actin filaments as a

V-shaped homodimer. In elegant single molecule experiments, Rognoni et al. demonstrated that the Ig domain pair 20–21 acts as an autoinhibited force-activatable mechanosensor [82]. Autoinhibition was relieved by piconewton forces, allowing interaction partners to bind [82]. It was shown that domain 21 of filamin A binds β-integrin at its cytoplasmic tail in direct competition with talin [55]. Furthermore, ARHGAP24 (or FilGAP), a Rho-regulated GTPase-activating protein (GAP) for Rac, binds to domain 23 of filamin A [76]. ARHGAP24 is expressed in podocytes and mutations of ARHGAP24 that impair its activity as a GAP for Rac cause focal segmental glomerulosclerosis [1]. Filamin A does not regulate ARHGAP24 activity but controls its localization [76]. Ehrlicher et al. reconstituted a minimal system, consisting of actin filaments, filamin A, the cytoplasmic tail of β-integrin, and ARHGAP24 [25]. Application of mechanical force resulted in increased binding of the cytoplasmic tail of β-integrin to filamin A and dissociation of ARHGAP24 from filamin A [25]. Interestingly, filamin is expressed in glomeruli [116], and filamin A was shown to be involved in the regulation of actin polymerization after nephrin clustering in podocytes [109]. Figure 3 summarizes the function of filamin A as a mechanosensor, which may play a hitherto undiscovered role in podocytes.

Cell contacts and extracellular matrix as mechanosensors in podocytes

Podocytes possess highly specific cell contacts. Cell-cell contacts are mainly mediated by the slit diaphragm protein nephrin; cell-matrix contacts are mainly mediated by integrins binding to the GBM. It was shown that α-catenin, which is part of the molecular complex of adherens junctions, acts as a mechanosensor, as piconewton forces unfold the vinculin binding domain of α-catenin [117]. It is tempting to speculate whether nephrin and slit diaphragm proteins might also possess inherent mechanosensitivity. The first evidence that nephrin phosphorylation feeds into the YAP/TAZ mechanotransduction pathway has been presented recently [54].

Adhesion to the GBM, which has a unique and complex composition of extracellular matrix (ECM) proteins [71, 78], relies primarily on integrin $\alpha_3\beta_1$ [29, 84]. However, podocytes express further integrins, like integrin $\alpha_V\beta_3$ [89, 111], which may become more relevant under pathological conditions. There are excellent reviews on the role of integrins in mechanotransduction (cf. the recent review by Fässler and colleagues [102]). As integrins interconnect the ECM and the actin cytoskeleton, they are in a strategic position for the transmission of intracellular as well as extracellular forces. Activation of integrins, which results in a conformational change, can be achieved by binding of proteins to the cytoplasmic domain of β-integrin ("inside-out signaling"), or by binding of ECM molecules to the extracellular domains of the integrin dimer ("outside-in signaling"). It was shown that mechanical force can activate integrins [34]. However, there is currently no evidence that force induces a conformational change in integrins directly [102]. Therefore, integrins act as hubs in mechanotransduction pathways, but probably they do not act as mechanosensors.

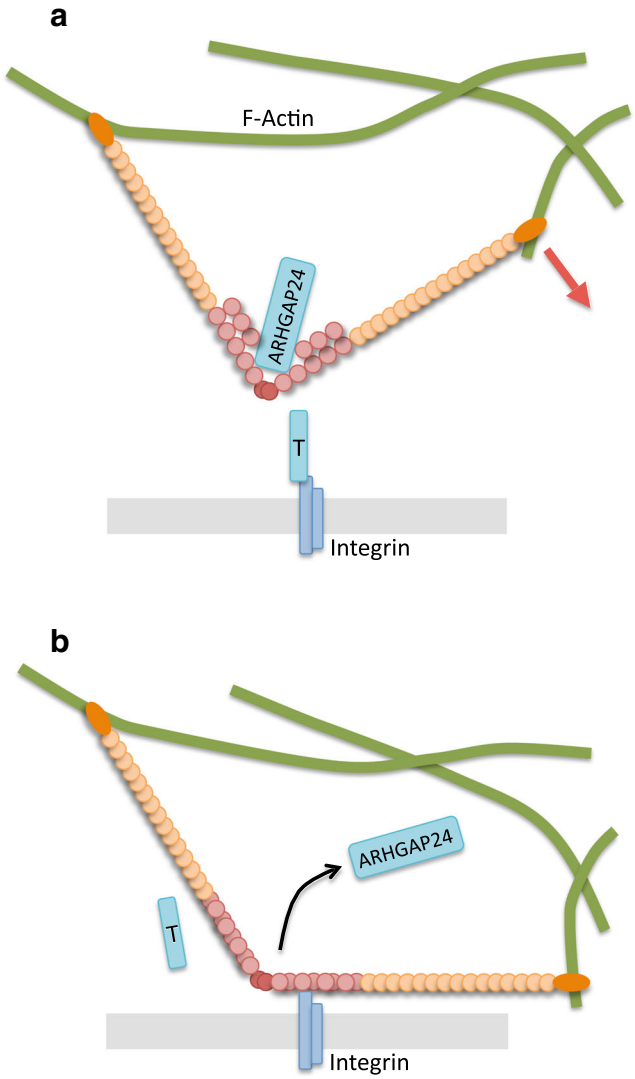

Fig. 3 The actin-associated protein filamin A as a mechanosensor. **a** Before deformation of the actin filament network and **b** after deformation due to mechanical force (*red arrow*). Filamin A contains an actin binding domain (*dark orange*) and 24 Ig domains (*light orange*, *light red*, and *dark red*). Filamin A dimerizes at Ig domain 24 (*dark red*). The V-shaped filamin A dimer attaches to two actin filaments. Deformation of the actin filament network results in a conformational change of filamin A (domains in *light red*), altering interactions with β-integrin and ARHGAP24. Talin (T) is displaced from β-integrin

Several proteins that bind to integrins or integrin-associated proteins were demonstrated to be bona fide mechanosensors, including talin, p130Cas (or BRCA1), and fibronectin. Talin features two integrin and three actin binding sites. As soon as talin connects to the cytoplasmic domain of β-integrin and to an actin filament, it becomes exposed to force. In single-

molecule experiments, del Rio et al. demonstrated unfolding of the talin rod domain by piconewton forces [18]. Talin unfolding exposed cryptic binding sites for vinculin [18]. Since vinculin binds also to F-actin, vinculin reinforces the actin-integrin connection by providing additional links between talin and F-actin. Mice lacking talin1 in podocytes develop foot process effacement in the second week after birth, massive proteinuria, and kidney failure [106]. This study demonstrates the essential role of talin1 in podocytes. Besides the function as a linker protein, the mechanosensor function of talin1 is most likely also crucial for the podocyte. Talin unfolding does not only expose cryptic binding sites for vinculin but for various other proteins with different functions [102]. Among the cryptic binding sites of talin, there is a binding site for kank proteins that are expressed in podocytes and cause nephrotic syndrome if mutated [38].

Sawada and colleagues demonstrated as early as 2006 that the scaffold protein p130Cas is a mechanosensor [87]. Stretching of p130Cas leads to unfolding of the substrate domain containing 12 cryptic YxxP motifs [87]. Upon exposure, these motifs are phosphorylated by Src-family kinases and serve as docking sites for SH2 domain-containing proteins like Crk and Nck. An N-terminal SH3 domain of p130Cas enables binding to several proteins, e.g., to vinculin and focal adhesion kinase [51]. At the C-terminus, p130Cas contains a conserved CCH (C-terminal Cas homology) domain. This domain may bind to hitherto unidentified proteins and/or mediate dimerization of p130Cas [51]. As soon as the p130Cas homodimer gets anchored with its N-terminal SH3 domains to focal adhesion proteins at two different sites, the substrate domain will unfold in the presence of sufficient force. Podocytes express p130Cas in vivo and it localizes to focal adhesions in cultured podocytes [113]. Levels of p130Cas were reported to be increased in glomeruli of nephrotic patients and to be diminished in diabetic patients [5, 41]. Whether p130Cas plays a role as a mechanosensor in podocytes remains to be established.

ECM proteins are often overlooked as mechanotransduction proteins or as mechanosensors. The ECM protein fibronectin (FN1) contains many fibronectin Type III (FNIII) domains that unfold in the presence of forces in the higher piconewton range as demonstrated by single-molecule force spectroscopy [75]. Unfolding of FNIII domains was shown in the ECM of living cells [6, 94]. Fibronectin is a 270-kDa glycoprotein, with the ability to bind simultaneously to cell surface receptors like integrins and ECM components such as collagen, fibrin, heparin, heparan sulfate proteoglycans (e.g., syndecan), and to itself [13, 50, 65, 90]. The ninth and the tenth FNIII domains, which can be unfolded by mechanical force, contain the synergy site and an RGD sequence for integrin binding as well as a cryptic fibronectin binding site that is essential for multimerization [37, 57, 58]. In addition, the interaction of fibronectin and collagen was shown to be regulated by mechanical force [64].

Mutations in fibronectin that result in defective heparin and cell binding cause glomerulopathy with fibronectin deposits in the glomerulus [11]. By proteomics analysis of the glomerular ECM, fibronectin was detected at an about fivefold lower amount as compared to the GBM-specific collagen IV chains [66]. Though podocytes express low levels of fibronectin under physiological conditions, fibronectin expression may become relevant in diabetes [67, 105]. Moreover, it was suggested that fibronectin binding to the α-dystroglycan induces an increase in intracellular Ca^{2+} concentration in podocytes [110]. The potential significance of fibronectin as an ECM mechanosensor for podocytes is summarized in Fig. 4.

Mechanosignaling

Mechanosensors are the starting point of the mechanotransduction signaling cascade (Fig. 5). With regard to tensile stress, we compiled all published data from stretch experiments with cultured podocytes in a recent review [29]. ERK, JNK, und p38 MAP kinases were activated in stretched podocytes or involved

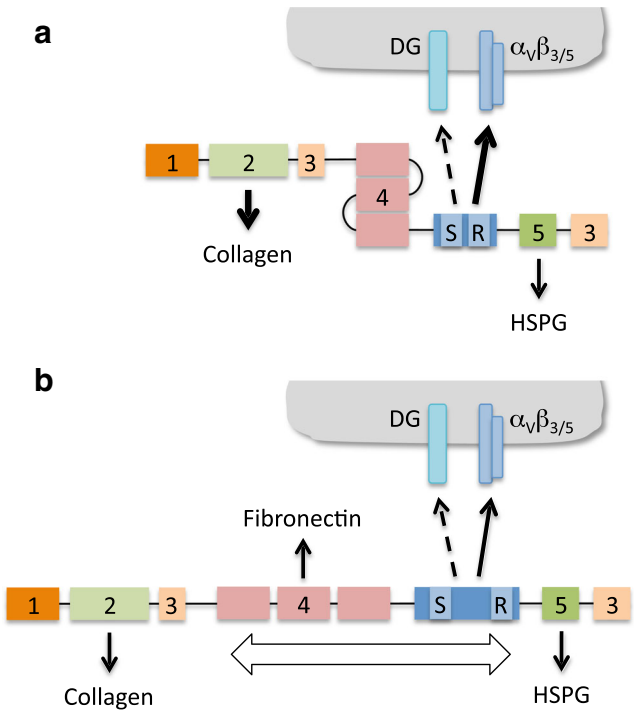

Fig. 4 The ECM protein fibronectin as a mechanosensor. **a** Fibronectin in the relaxed state and **b** in a tensed state. Tension (*white double arrow*) induces a conformational change. A cryptic binding site for fibronectin (*4*) is exposed, and the conformation of the cell surface receptor-binding domain (*blue*) is changed. The surface receptor-binding domain harbors a synergy site (S) and an RGD sequence (R) for integrin binding. Possibly, α-dystroglycan (DG) can also bind to fibronectin. Tension might reduce binding to cell surface receptors and to collagen. *1*—heparin-binding domain; *2*—collagen-binding domain; *3*—fibrin-binding domains; *5*—binding domain for heparan sulfate proteoglycans (HSPG)

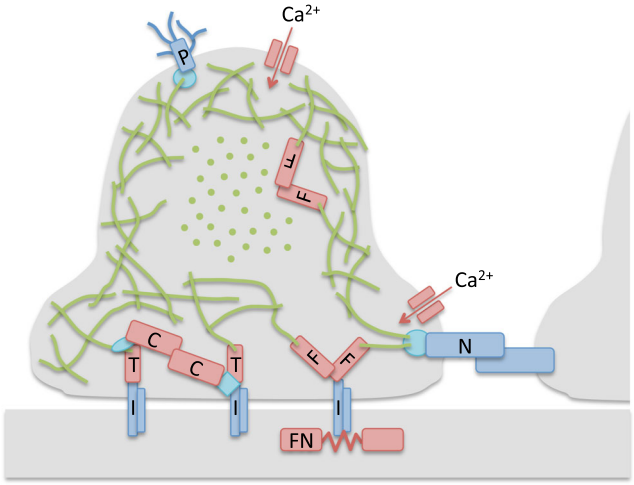

Fig. 5 Overview of possible mechanosensors in podocytes. The actin cytoskeleton (green) in foot processes consists of a filament bundle in the core and a sublemmal network. Integrins (I), slit diaphragm proteins (nephrin—N), and podocalyxin (P) are connected to actin filaments via linker proteins (*light blue*). Possible mechanosensors in podocytes are TRPC6 cation channels, filamin A (F), talin (T), p130Cas (C), and fibronectin (FN), which also binds to GBM components. The p130Cas dimer is connected to focal adhesion proteins (*light blue*)

in the stretch response in a larger number of experiments. It is interesting to note that unfolding und subsequent phosphorylation of the mechanosensor p130Cas leads to downstream activation of p38 MAPK via Rap1 [87]. Possibly, cell-matrix contacts of podocytes are of major importance for mechanotransduction signaling. Forst et al. were able to attenuate the typical stretch-induced reorganization of the podocyte actin cytoskeleton by a P2X4 antagonist [33]. This finding appears to argue against an involvement of mechanotransduction signaling from cell-matrix contacts. However, P2X4 activation was due to stretch-induced ATP release. Therefore, mechanotransduction signaling from cell-matrix contacts could well be responsible for the stretch-induced ATP release. Osteopontin-deficient podocytes showed an impaired stretch-induced actin reorganization [89]. This finding also supports the role of cell-matrix contacts for mechanotransduction signaling in podocytes, since osteopontin acts through α_V-integrins [89].

There are only a few reports that shed some light on the mechanotransduction pathway that is engaged in response to fluid shear stress in podocytes. In these studies, cultured podocytes were exposed to fluid shear stress, as a surrogate of the ultrafiltrate flow in Bowman's space. Huang et al. reported a phosphorylation of Src kinase that was involved in shear stress-induced apoptosis and in phospholipase D1 activation [47]. Though the actin reorganization in response to fluid shear stress was dependent on tyrosine phosphorylation, Src kinase inhibition was not involved [35]. In a series of studies, Srivastava and colleagues demonstrated that, in response to fluid shear stress, COX2 and the EP2 receptor are upregulated and PGE2 production is increased in podocytes [96, 98, 99]. Moreover, the authors confirmed the glomerular upregulation of COX2 and EP2 receptors in uninephrectomized and ROP Os+/− mice, which are born with a 50% lower number of nephrons [96, 99]. Of note, the authors further demonstrated that fluid shear stress increased the albumin permeability in isolated glomeruli and that COX2 inhibition could normalize the albumin permeability [96].

Finally, there is first indirect evidence that YAP/TAZ signaling may be involved in mechanotransduction in podocytes. YAP (or YAP1) and TAZ (or WWTR1) are transcriptional coactivators in the Hippo pathway [21, 68]. In 2011, evidence was presented that YAP/TAZ plays a key role in mechanotransduction [22]. YAP/TAZ is involved in a variety of processes related to cell mechanics such as regulation of cell size, exposure of cells to mechanical stress, and sensing of cellular contractility and ECM rigidity [21, 68]. The molecular mechanisms that link YAP/TAZ to the actin cytoskeleton and cell-matrix contacts are not well understood. Conditional knockout of YAP in the kidney cap mesenchyme revealed an important role of YAP for the morphogenesis of S-shaped bodies and for foot process formation in podocytes [79]. It was shown by Keyvani Chahi et al. that nephrin phosphorylation also regulates YAP/TAZ signaling [54]. Rinschen and colleagues identified increased activity and upregulation of YAP in podocytes in the puromycin aminonucleoside injury model in rats [81]. YAP/TAZ activity was also modulated by substrate stiffness and by actin stress fiber inhibition in cultured podocytes [81]. These findings are pointing to an important role of YAP/TAZ mechanosignaling in podocytes.

Response to tensile stress and fluid shear stress

Recent considerations suggest that podocytes cannot counteract circumferential wall stress [29, 63]. In brief, there are several arguments: the circumferential wall stress is remarkably high; the contractile capacity of foot processes is limited; glomerular capillaries in non-muscle myosin IIA (or MYH9) knockout mice are normal [52, 53]; no compensatory expression of non-muscle myosin IIB (or MYH10) in non-muscle myosin IIA knockout mice [53]; isotropic (random) orientation of foot processes on the GBM; and podocytes do not fully embrace the capillaries as opposed to pericytes. Therefore, it has been concluded that circumferential wall tension is passively counteracted by the GBM, which is held together by the contractile mesangium [29, 63]. Basement membranes are characterized by a high Young's modulus (2000–5000 kPa), when the basement membranes are stretched in a longitudinal direction [112]. Thus, basement membranes are stiff enough to withstand circumferential wall tension. So far, the elasticity of the GBM has been measured for perpendicular indentations or deformations only, giving a value for Young's modulus of about 2 kPa [26]. However, this value can be used neither to

estimate the contribution of the GBM to counteract circumferential wall tension nor to extrapolate the ECM rigidity that is experienced by podocytes.

If glomerular capillary surface area gets enlarged due to an increase of circumferential wall tension, what happens to the interdigitating foot processes? We as well as Kriz and Lemley [29, 63] analyzed morphological data from publications on isolated perfused rat kidneys [60, 85]. Increasing the perfusion pressure from 65 to 105 mmHg resulted in an expansion of pericapillary (podocyte-covered) GBM area by 55%, the length of the slit diaphragm increased by 59%, whereas there was no change in slit diaphragm and foot process width. As a logical consequence, foot processes have to lengthen and, due to the isotropic orientation, additional foot processes have to be formed. These findings and considerations suggest that the actin polymerization machinery in foot processes would be extremely relevant for the adaptation of podocytes to an expanded GBM surface area.

In stretch experiments with cultured podocytes, several groups observed a reorganization of the actin cytoskeleton with a significant loss of stress fibers [30, 33, 70]. In addition, stretched podocytes increased the production of matricellular proteins like SPARC and osteopontin, which reduce stress fibers and enhance dynamic actin structures [23, 31]. The stretch adaptation of osteopontin-deficient podocytes was impaired [89], and the development of glomerulosclerosis was aggravated in uninephrectomized osteopontin-deficient mice as compared to wild-type mice [88]. Thus, enhanced actin dynamics and plasticity appears to be an appropriate adaptive response of podocytes.

Reorganization of the actin cytoskeleton with a loss of stress fibers was also observed in podocytes exposed to fluid shear stress mimicking ultrafiltrate flow in Bowman's space [35, 98]. This is in contrast to endothelial cells that reorient and increase actin stress fibers in response to elevated fluid shear stress [45]. As a consequence, endothelial cells tolerate much higher levels of shear stress as compared to podocytes. Our knowledge about the cellular response of podocytes to fluid shear stress is very limited. The lack of podocytes with interdigitating foot processes, and a filtration slit in culture, is one reason that hampers studies on shear stress generated by filtrate flow.

Outlook

Defining the mechanosensor(s) and the signal transduction of mechanical forces in podocytes may unveil podocyte-specific mechanisms involving podocyte specific molecules. These molecules may represent ideal targets for controlling the response of podocytes to mechanical forces. In addition to preventing mechanical overload of podocytes via correction of renal hemodynamics, one could envisage to enhance adaptive responses and to inhibit maladaptive responses of podocytes by appropriate pharmaceutical compounds.

Acknowledgements The continuous support of the authors' work by grants of the German Federal Ministry of Education and Research (BMBF), the German Research Foundation (DFG), and the European Union (EU) is gratefully acknowledged.

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