SHORT COMMUNICATION

Light Microscopic Visualization of Podocyte Ultrastructure Demonstrates Oscillating Glomerular Contractions

Martin Höhne,^{*†} Christina Ising,^{*} Henning Hagmann,^{*} Linus A. Völker,^{*} Sebastian Brähler,^{*} Bernhard Schermer,^{*†‡} Paul T. Brinkkoetter,^{*} and Thomas Benzing^{*†‡}

From the Department II of Internal Medicine and the Center for Molecular Medicine Cologne,^{*} Systems Biology of Ageing Cologne (Sybacol),[†] and the Cologne Excellence Cluster on Cellular Stress Responses in Ageing-Associated Diseases,[‡] University of Cologne, Cologne, Germany

Accepted for publication
November 1, 2012.

Address correspondence to
Thomas Benzing, M.D.,
Department of Internal
Medicine II, University of
Cologne, 50937 Cologne,
Germany. E-mail: thomas.benzing@uk-koeln.de.

Podocytes, the visceral epithelial cells of the kidney glomerulus, elaborate primary and interdigitating secondary extensions to enwrap the glomerular capillaries. A hallmark of podocyte injury is the loss of unique ultrastructure and simplification of the cell shape, called foot process effacement, which is a classic feature of proteinuric kidney disease. Although several key pathways have been identified that control cytoskeletal regulation, actin dynamics, and polarity signaling, studies into the dynamic regulation of the podocyte structure have been hampered by the fact that ultrastructural analyses require electron microscopic imaging of fixed tissue. We developed a new technique that allows for visualization of podocyte foot processes using confocal laser scanning microscopy. The combination of inducible and mosaic expression of membrane-tagged fluorescent proteins in a small subset of podocytes enabled us to acquire light microscopic images of podocyte foot processes in unprecedented detail, even in living podocytes of freshly isolated glomeruli. Moreover, this technique visualized oscillatory glomerular contractions and confirmed the morphometric evaluations obtained in static electron microscopic images of podocyte processes. These data suggest that the new technique will provide an extremely powerful tool for studying the dynamics of podocyte ultrastructure. (*Am J Pathol* 2013, 182: 332–338; <http://dx.doi.org/10.1016/j.ajpath.2012.11.002>)

Visualizing the structure of glomerular cells has been the focus of kidney research for many years. Immunohistochemical analysis and indirect immunofluorescence are considered standard techniques in the field. However, thus far, the ultrastructure of podocytes could be visualized only by relying on transmission or scanning electron microscopy of fixed tissue. During the past decade, breathtaking advances, primarily the introduction of multiphoton microscopy, have opened the door for studying kidney morphology and function even in living rodents (see the article by Peti-Peterdi et al¹ for a review). Although these new techniques allow physiologic studies in living animals, the resolution is still insufficient to convincingly visualize primary and secondary processes of podocytes with their characteristic interdigitating pattern. Thus, podocyte research still depends on electron microscopy to which facility access is often limited. More feasible technical approaches are warranted that facilitate the study of podocyte foot process morphology at high resolution. Herein, we report a technique that allowed us to image podocyte foot processes in

unprecedented detail in fixed tissue and in living podocytes of freshly isolated glomeruli.

Materials and Methods

Mouse Breeding

The membrane-targeted tdTomato (mT)/membrane-targeted EGFP (mG) mouse (mixed genetic background including CD-1, 129 × 1/SvJ, and C57BL/6J; Jax mice stock number 007576; The Jackson Laboratory, Bar Harbor, ME)² was crossed with the podocin:Cre mouse³ or the tamoxifen-inducible podocin:Cre mouse (podocin-iCreER^{T2}; referred to as TPod:Cre)⁴ to obtain heterozygous podocin:Cre × R26mTmG or TPod:Cre × R26mTmG mice. The genetic background of the Cre lines was of C57BL/6. Heterozygous

Supported in part by the Deutsche Forschungsgemeinschaft SFB 635 and SFB 572. P.T.B. received funding from the Deutsche Forschungsgemeinschaft (BR2955/4-1).

podocin:Cre $\times$ R26mTmG mice were crossed with a homozygous R26mTmG mouse to obtain mice that are homozygous for R26mTmG and heterozygous for podocin:Cre. All genotyping was performed using PCR.

Mice bearing mTmG reporter construct in their genome express tdTomato driven through a chicken β -actin core promoter with a cytomegalovirus enhancer (pCA) in the Rosa26 locus. On Cre-mediated recombination, the tdTomato is cut out, and the cells with irreversible recombination events express membrane-tagged green fluorescent protein (GFP) under the pCA promoter.²

Tamoxifen Treatment

For i.p. injections, tamoxifen (T5548; Sigma-Aldrich, St. Louis, MO) was prepared in sesame oil (S3547; Sigma-Aldrich) at a concentration of 20 mg/mL. Eight-week-old mice were injected with 4 mg of tamoxifen daily for 8 consecutive days. For oral administration, 6 to 8-week-old mice were fed a tamoxifen-enriched diet (400 mg/kg; TD.55125; Harlan Laboratories, Indianapolis, IN) *ad libitum*. Based on a daily intake of 5 g of food per mouse, this corresponds to an oral dose of 2 mg/d per mouse.

Disease Model

Nephrotoxic nephritis was induced in 9-week-old male TPod:Cre $\times$ R26mTmG mice that were fed a tamoxifen diet for 18 days by a single i.p. injection of 200 μ L of sheep anti-rabbit glomerular antibody as described previously.⁵ Mice were sacrificed 3 days after the injection. Spot urine (before injection and 3 days after injection) was analyzed by SDS-PAGE and staining with Coomassie blue.

Tissue Preparation

Kidneys were isolated from anesthetized mice perfused with 4% paraformaldehyde (P6148; Sigma-Aldrich) in PBS and postfixed in 4% paraformaldehyde at 4°C for 24 hours. Cryoprotection was performed using 15% sucrose (S0389; Sigma-Aldrich) for 2 hours at 4°C and 30% sucrose overnight at 4°C before embedding in OCT (Tissue-Tek, 4583; Sakura Finetek USA, Torrance, CA). Sections (7 to 10 μ m) were obtained using a cryostat (Leica Mikrosysteme, Wetzlar, Germany). Slides were washed three times with PBS and were mounted in ProLong Gold antifade reagent (P36930; Invitrogen, Carlsbad, CA).

Isolation of Glomeruli

Glomeruli were isolated in 1 $\times$ HBSS (125 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L CaCl₂, 1.2 mmol/L MgSO₄ $\cdot$ 7H₂O, 25 mmol/L HEPES, and 6 mmol/L glucose) using Dynabead perfusion.⁶ DNase treatment was omitted, and collagenase treatment was restricted to 10 minutes. For image acquisition,

Figure 1 Selective visualization of podocytes using transgenic mice. **A:** R26mTmG mice were crossed with podocin:Cre mice, leading to GFP expression in podocytes (green) but not in other glomerular cells that express tdTomato (magenta). **B** and **C:** Tamoxifen-induced expression of GFP in podocytes in R26mTmG reporter mice crossed with the inducible TPod:Cre mice. The number of podocytes expressing GFP varies (green). In glomeruli with weaker Cre expression, single podocytes are clearly visible (**B**). Overview of a complete kidney section. Cortical and deeper glomeruli are GFP positive. Tamoxifen induction was achieved by injection of 4 mg of tamoxifen daily for 8 days (**C**). Scale bars: 20 μ m (**A** and **B**); 1000 μ m (**C**).

glomeruli were transferred to channel slides (μ -Slide VI; ibidi, Martinsried, Germany).

Image Acquisition

Images were acquired using an LSM 710/Axiobserver Z1 confocal microscope operated by ZEN 2009 software (all from Carl Zeiss MicroImaging GmbH, Jena, Germany). The following objectives were used: 10 $\times$ /0.3 (Figure 1C), 20 $\times$ /0.8 (Figure 1, A and B), 63 $\times$ /1.4 oil (Figures 2, 3, and 4), and 40 $\times$ /1.1 water immersion objective (Figure 4). For Figures 2 and 3, scan parameters were set to x:y:z = 1 with a voxel size of 95 nm³ (Figure 2, A and D), 75 nm³ (Figure 2B), and 89 nm³ (Figures 2C and 3, B and C). The time series of isolated glomeruli were acquired using an objective heater set to 38°C. Image stacks were deconvolved using AxioVision software version 4.8 (Carl Zeiss MicroImaging GmbH) and a constrained iterative algorithm. The theoretical point spread function that relates to the specific acquisition conditions (wavelength, numerical aperture, and refraction indexes) was used. Images were further processed using ImageJ/Fiji

software version 1.46 (NIH, Bethesda, MD).^{7,8} In particular, Fiji's bleach correction plugin (Exponential Fit method) was applied to time series. Figures were assembled using Adobe Illustrator CS5.1 (Adobe Systems Inc., San Francisco, CA).

Results

Recently, Grgic et al⁹ published a novel method for visualizing podocyte structure by using a tamoxifen-inducible GFP–Cre-ERT2 construct downstream of the collagen α 1(I) promoter combined with a tdTomato reporter mouse. Using submaximal doses of tamoxifen allowed genetic labeling of single podocytes, which could be studied by conventional fluorescence microscopy. However, the study was limited to the analysis of fixed tissue samples and did not allow the examination of living podocytes in nonfixed tissues. To address these issues, we generated mosaic animals for visualization of podocyte ultrastructure and mated podocin:Cre mice³ with R26mTmG reporter mice to irreversibly drive GFP expression in all podocytes from a constitutively active promoter,² whereas all other cells

Figure 2 Visualization of single podocytes using mosaic animals. **A:** In R26mTmG $\times$ podocin:Cre glomeruli, secondary foot processes are not clearly visible owing to too many GFP-expressing cells. Z-projection (average) of three consecutive confocal slices covering 0.3 μ m (z-step = 0.09 μ m). **B:** Generation of mosaic expression patterns of Cre recombinase by low-dose tamoxifen induction in R26mTmG $\times$ TPod:Cre glomeruli allows for the visualization of secondary processes. Z-projection (average) of 10 consecutive confocal slices covering 0.75 μ m (z-step = 0.075 μ m). Cre expression was induced by feeding with a tamoxifen diet for 6 weeks. **C:** Orthogonal view representation of an image stack showing a single capillary covered by a podocyte labeled with GFP. yz and xz views at the positions indicated by the green and orange lines, respectively, are shown. The xy view shows a maximum intensity projection of half of the stack [slice 1–50, covering 4.5 μ m (z-step = 0.09 μ m), indicated by gray bars next to the yz and xz views]. See also Supplemental Movies S3 and S4. **D:** Projection of three consecutive confocal slices in both channels covering 0.27 μ m (z-step = 0.09 μ m). **E:** Profile plot of magenta and green fluorescence of the region indicated with a white line in **D**. **F:** Transmission electron microscopic image of foot processes of a wild-type mouse. Foot processes are marked manually with alternating magenta and green lines. **G:** Profile plot of magenta and green fluorescence along the white line shown in **F**. Scale bars: 5 μ m (**A–C**); 2 μ m (**D**); 0.5 μ m (**F**).

Figure 3 Visualization of podocytes in a disease model. Cre recombinase was induced in R26mTmG × TPod:Cre mice by feeding with a tamoxifen diet for 14 to 18 days. Nephrotoxic nephritis was induced by a single i.p. injection of sheep anti-rabbit glomerular antibody. **A:** Coomassie blue-stained SDS-PAGE reveals massive proteinuria 3 days after injection of the serum. **B and C:** Z-projection of 10 consecutive confocal slices covering 0.9 µm (z-step = 0.09 µm). Foot process structure is completely lost in animals injected with nephrotoxic serum (**B**) but not in control animals (**C**). Scale bars: 5 µm.

continued to express tdTomato (Figure 1A). Next, we generated mosaic animals that were expected to show GFP expression in individual podocytes. In crosses with the TPod:Cre driver line, Cre expression was initially induced by i.p. tamoxifen injection for 8 days.⁴ Individual glomeruli exhibited a variable degree of Cre-mediated loxP recombination, ranging from only a few to almost all podocytes per glomerulus (Figure 1B). GFP expression was observed in cortical and deeper glomeruli in the kidney (Figure 1C). We next modified the induction scheme to feeding with a tamoxifen-containing diet. In these experiments, this approach proved to be more beneficial for the health of the mice, whereas the induction was comparable with that of the injection method. We recorded high-resolution confocal stacks of glomeruli from two groups of mice. In the first set, GFP expression in all podocytes was mediated by podocin:Cre-induced recombination (Figure 2A), whereas in the second set, recombination was mediated by the TPod:Cre transgene combined with a tamoxifen-containing diet, causing induction of GFP expression in only a small

subset of podocytes (Figure 2B). Whereas podocyte foot processes were not clearly detectable in glomeruli with full GFP expression (Figure 2A), induction of GFP expression in only a few podocytes allowed us to clearly visualize primary and secondary foot processes of individual podocytes in a three-dimensional stack (Figure 2, B and C, and Supplemental Movies S1, S2, S3, and S4).¹⁰ Single individual podocytes were detectable after feeding with a tamoxifen diet between 1 and 6 weeks (Supplemental Figure S1). In the experimental setting, an induction period of 2 to 3 weeks proved to be ideal.

The mutually exclusive expression of GFP or tdTomato allowed us to image areas where alternating foot processes are expressing tdTomato and GFP (Figure 2D). Morphometric analyses revealed that the distance between the individual foot processes is comparable with the distance measured in transmission electron microscopy, with dimensions ranging from 0.4 to 0.6 µm (Figure 2, D–G).

The loss of the elaborate structure with primary and secondary foot processes is a hallmark of podocyte damage. To test whether this technology would allow for the visualization of podocyte foot process effacement, we induced podocyte damage in animals that expressed GFP in a subset of podocytes by i.p. injection of a nephrotoxic sheep antibody (a gift from Friedrich Thaiss, Universitätsklinikum Hamburg-Eppendorf, Hamburg-Eppendorf, Germany).⁵ Three days after injection, the animals showed massive proteinuria (Figure 3A). We sacrificed the animals and recorded high-resolution confocal stacks of glomeruli. Podocyte foot process structure was completely lost in animals treated with the nephrotoxic serum compared with preserved podocyte ultrastructure in non-injected control animals (Figure 3, B and C).

As mentioned previously herein, the limitation to fixed tissue sections is a drawback of the described technique. We, therefore, tried to take this imaging technique one step further. We tested whether we could visualize the podocyte ultrastructure in living glomeruli. Therefore, we used freshly isolated glomeruli without fixation. The isolation protocol with magnetic beads allows for a comparably fast preparation while preserving the ultrastructure of the glomeruli⁶ (Supplemental Figure S2). Within a few hours after sacrifice, we could observe pulsatile contractions of the glomeruli using rapid preparation and imaging (Figure 4A, Supplemental Movies S5–S7, and Supplemental Figure S3). These oscillatory glomerular contractions did not seem to be restricted to the capillary loop but affected the entire glomerulus. Further analyses of the frequency of these contractions revealed that the contractions occur at an estimated frequency of 0.125 Hz (seven to eight contractions per minute) (Figure 4B and Supplemental Movies S5 and S6).

Discussion

The aim of the present study was to visualize podocyte ultrastructure at the resolution of podocyte foot processes in

A Pod:Cre x R26mTmG **B**

Figure 4 Visualization of podocytes in isolated glomeruli. **A:** Isolated glomeruli from an R26mTmG × podocin:Cre mouse were imaged with a time series of a single confocal slice. Cre-induced recombination did not occur in all podocytes of this animal. A region with only partial recombination was selected, where it is possible to see the secondary foot processes of a living podocyte that underwent recombination. The time series reveals a pulsatile movement of the glomerulus (see also [Supplemental Movies S5, S6, and S7](#)). **B:** Changes in intensity were used to generate a plot that depicts the frequency of the pulsatile movement. Scale bar = 5 μ m.

cryosections and in nonfixed, freshly isolated glomeruli. Using an inducible podocin:cre line and the well-established R26mTmG reporter mouse line, we could visualize podocyte foot processes using light microscopy in unprecedented detail. In contrast to the report by Grgic et al,⁹ where the R26mTmG mouse line combined with the Coll1 α 1GCE Cre line was found to be unsuitable for the visualization of distinct structures, the present data suggest that the R26mTmG mouse line can be used for selective visualization of different cells in the glomerulus when combined with a tamoxifen-inducible Cre line. The R26mTmG mouse expresses tdTomato in a pattern complementary to GFP as tdTomato and GFP expression are mutually exclusive in cells on a genetic level.² Although tdTomato-expressing podocytes were less clearly visible than GFP-expressing podocytes, most likely due to tdTomato expression in endothelial and mesangial cells, we could image parts where alternating foot processes expressed tdTomato and

GFP. These data clearly confirmed that adjacent foot processes derive from neighboring podocytes that project the processes in an alternating series. For this initial study, the use of a two-colored reporter model proved to be beneficial. However, the present approach is not limited to this particular reporter strain. Other reporter constructs that require only one fluorescent channel might be used in a similar way.

This pilot study also shows that this method can be used to track the change in podocyte ultrastructure in disease models. Although the ultimate proof for demonstrating (reversible) effacement would require time-lapse fluorescent imaging of a particular podocyte area before, during and after the injury, we envision that our current model will allow future development of sophisticated methods for monitoring podocyte ultrastructural changes in real time.

This method is not only suitable to study podocytes in renal sections: time series imaging of freshly isolated,

nonfixed glomeruli allows visualization of podocyte foot processes in living glomeruli as well as oscillatory glomerular contractions. There are two possible sources of origin of the glomerular contractions: mesangial cells and podocytes. Although it is possible that cyclic contractions of mesangial cells may be responsible for the glomerular oscillations, recent compelling data showed that there is a cell-to-cell calcium signaling that propagates as a calcium wave in the juxtaglomerular apparatus and beyond involving glomerular cells.¹¹ Peti-Peterdi¹² showed that these waves not only propagate to the afferent arteriole, causing vasoconstriction, but also simultaneously toward all cells of the glomerulus, including the most distant podocytes. It has been shown that oscillating contractions of the afferent arteriole occur at a similar frequency (0.1 to 0.2 Hz) to the one observed in the present study.^{13,14} Glomerular contractions in the present model did not seem to result from subtle laser-mediated injury as podocytes exposed to the laser beam did not show any laser-dependent artificial calcium influx (data not shown). Furthermore, work from several groups in the past has suggested that podocytes contain contractile proteins reminiscent of professional contracting smooth muscle cells and respond to mechanical strain, probably through a calcium-dependent actin response.^{15–21} Thus, the oscillatory glomerular contractions may reflect interplay among several cell types in the glomerulus, including podocytes, that control capillary diameter and/or fine-tune ultrafiltration. In rats, the glomerulus is subjected to a constant pulsatile capillary pressure of ~ 60 mm H₂O.²² It is possible that the lack of blood flow/pressure in the preparations has unmasked contractile activity in podocytes or mesangial cells.

Taken together, the results of this study not only confirm the method recently published by Grgic et al⁹ but also reflect a novel approach for visualizing podocyte secondary foot process structure by confocal microscopy in unprecedented detail without the need for previous fixation or electron microscopy. Nevertheless, the ability to monitor the structure and motility of living podocytes in freshly isolated, nonfixed glomeruli provides a powerful tool for a better understanding of the dynamics of the podocyte cytoskeleton. We envision that it will be possible to induce changes in the isolated glomeruli by the addition of inhibitors or stimulating substances and observe the immediate changes in podocyte morphology and/or motility in live imaging experiments. The method has some limitations. First, long exposure to the laser may result in substantial bleaching and loss of fluorescent intensity. However, in our experience, bleaching occurred only in recordings that required long exposure times. Second, an obvious limitation of the method is the current need for two transgenes, one of which is inducible. However, we are confident that future genetic animal work will result in the development of more ingenious mouse models built using this principle that are easier to handle and more practical to use in podocyte research.

Acknowledgments

We thank Sonja Kunath and Bettina Maar for excellent technical support. Mice used in this study were kindly provided by Farhad R. Danesh (Baylor College of Medicine, Houston, TX) and Lawrence B. Holzman (University of Pennsylvania, Philadelphia, PA).

Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2012.11.002>.

References

- Peti-Peterdi J, Burford JL, Hackl MJ: The first decade of using multiphoton microscopy for high-power kidney imaging. *Am J Physiol Renal Physiol* 2012, 302:F227–F233
- Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L: A global double-fluorescent Cre reporter mouse. *Genesis* 2007, 45:593–605
- Moeller MJ, Sanden SK, Soofi A, Wiggins RC, Holzman LB: Podocyte-specific expression of cre recombinase in transgenic mice. *Genesis* 2003, 35:39–42
- Wang J, Wang Y, Long J, Chang BHJ, Wilson MH, Overbeek P, Danesh FR: Tamoxifen-inducible podocyte-specific iCre recombinase transgenic mouse provides a simple approach for modulation of podocytes in vivo. *Genesis* 2010, 48:446–451
- Turner J-E, Paust H-J, Steinmetz OM, Peters A, Meyer-Schwesinger C, Heymann F, Helmchen U, Fehr S, Horuk R, Wenzel U, Kurts C, Mittrücker H-W, Stahl RAK, Panzer U: CCR5 deficiency aggravates crescentic glomerulonephritis in mice. *J Immunol* 2008, 181: 6546–6556
- Takemoto M, Asker N, Gerhardt H, Lundkvist A, Johansson BR, Saito Y, Betsholtz C: A new method for large scale isolation of kidney glomeruli from mice. *Am J Pathol* 2002, 161:799–805
- Schneider CA, Rasband WS, Eliceiri KW: NIH Image to ImageJ: 25 years of image analysis. *Nat Meth* 2012, 9:671–675
- Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A: Fiji: an open-source platform for biological-image analysis. *Nat Meth* 2012, 9: 676–682
- Grgic I, Brooks CR, Hofmeister AF, Bijol V, Bonventre JV, Humphreys BD: Imaging of podocyte foot processes by fluorescence microscopy. *J Am Soc Nephrol* 2012, 23:785–791
- Peng H, Ruan Z, Long F, Simpson JH, Myers EW: V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets. *Nat Biotech* 2010, 28:348–353
- Peti-Peterdi J, Sipos A: A high-powered view of the filtration barrier. *J Am Soc Nephrol* 2010, 21:1835–1841
- Peti-Peterdi J: Calcium wave of tubuloglomerular feedback. *Am J Physiol Renal Physiol* 2006, 291:F473–F480
- Holstein-Rathlou N-H, Leyssac PP: TGF-mediated oscillations in the proximal intratubular pressure: differences between spontaneously hypertensive rats and Wistar-Kyoto rats. *Acta Physiol Scand* 1986, 126:333–339
- Laugesen JL, Sosnovtseva OV, Mosekilde E, Holstein-Rathlou N-H, Marsh DJ: Coupling-induced complexity in nephron models of renal blood flow regulation. *Am J Physiol Regul Integr Comp Physiol* 2010, 298:R997–R1006
- Greka A, Mundel P: Calcium regulates podocyte actin dynamics. *Semin Nephrol* 2012, 32:319–326

16. Endlich N, Endlich K: The challenge and response of podocytes to glomerular hypertension. *Semin Nephrol* 2012, 32:327–341
17. Kriz W, Mundel P, Elger M: The contractile apparatus of podocytes is arranged to counteract GBM expansion. *Contrib Nephrol* 1994, 107: 1–9
18. Saleem MA, Zavadil J, Bailly M, McGee K, Witherden IR, Pavenstadt H, Hsu H, Sanday J, Satchell SC, Lennon R, Ni L, Bottinger EP, Mundel P, Mathieson PW: The molecular and functional phenotype of glomerular podocytes reveals key features of contractile smooth muscle cells. *Am J Physiol Renal Physiol* 2008, 295:F959–F970
19. Ichimura K, Kurihara H, Sakai T: Actin filament organization of foot processes in vertebrate glomerular podocytes. *Cell Tissue Res* 2007, 329:541–557
20. 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, 17:428–437
21. George B, Holzman LB: Signaling from the podocyte intercellular junction to the actin cytoskeleton. *Semin Nephrol* 2012, 32:307–318
22. Brenner BM, Troy JL, Daugharty TM: The dynamics of glomerular ultrafiltration in the rat. *J Clin Invest* 1971, 50:1776–1780