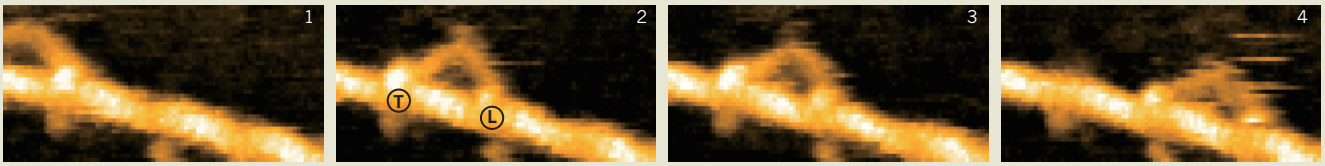
CELL BIOLOGY

Myosin in motion



Structural biologists are good at producing static snapshots of proteins, but seeing them in action is the ultimate goal. This is exactly what Kodera *et al.* have achieved in a remarkable study that appears elsewhere in this issue (N. Kodera, D. Yamamoto, R. Ishikawa and T. Ando *Nature* **468,** 72–76; 2010). They have been able to directly visualize myosin V, a cytoskeletal motor protein, as it 'walks' along actin filaments.

The authors have developed a high-speed atomic force microscopy (HS-AFM) method that enables them to generate rapid images of proteins at much higher resolution than light microscopy. Here they apply this technological advance to visualize the stepwise movement of myosin V. The protein consists of two heads connected by long necks (lever arms) to a globular tail domain through a coiled-coil helix. The tail binds to various cargo molecules to transport them along the actin filaments, the energy required being generated by hydrolysis of ATP to ADP.

The HS-AFM images showing the leading (L) and trailing (T) heads of the tail-truncated motor can be seen in Figure 1a of the paper on page 73, part of which is reproduced here. In frame 1, myosin is advancing from the left; in frames 2 and 3, it has marched into full view, the two heads and necks now being visible; in frame 4, it has moved on a step. See go.nature.com/DHBOY9 for the full movie.

The authors' analysis confirms the known behaviour of myosin motors, including the use of successive 36-nanometre steps along the actin filament and the 'hand-over-hand' movement of the motor heads. However, the study also provides convincing evidence for the hypothesized 'lever-arm swing'. In this feature, tiny changes in myosin's head domain are amplified by the lever arm to produce large displacements at the far end of the neck that translate into movement of the whole protein along the actin filaments.

The new analysis also uncovers novel characteristics of the myosin V mechanism, such as a 'stomping' behaviour, in which either the L or T head becomes detached then rebinds to actin. The stomp is observed more frequently for the L than for the T head, but the T-head stomp often leads to a forward movement on the actin filament.

The insight into the behaviour of myosin V that Kodera *et al.* reveal will have a major impact on understanding the mechanisms involved in biological molecular motors. More generally, the technological advance provided by HS-AFM looks set to take a prominent place in the field of biomolecular imaging. **Deepa Nath**

impaired insulin action. Drugs that decrease blood glucose, lower blood pressure or inhibit the actions of the hormone angiotensin can delay, but not eliminate, the onset of diabetic nephropathy.

To relay its signal from the bloodstream to a cell's interior, insulin binds to receptors on the cell surface. Welsh *et al.*[1] used mice in which the gene encoding the insulin receptor was deleted specifically from podocytes. At birth, kidney appearance in these animals was normal. At five weeks of age, however, they began to show excretion of albumin in the urine, shortening of the podocyte foot processes, increased deposition of components of the basal membrane, and a higher frequency of programmed podocyte death through apoptosis. Some animals even developed shrunken kidneys with prevalent scar tissue similar in appearance to the kidneys of humans with late-stage diabetic nephropathy.

These findings, however, are not just notable for their striking similarity to the pathology of diabetic nephropathy in humans. Welsh and colleagues' mice also showed mild worsening of kidney function. This observation is intriguing because, in the most commonly studied rodent model of diabetes, destruction of insulin-producing cells with the drug streptozotocin causes no significant change in kidney function, despite resulting in microscopic kidney abnormalities and albumin excretion in the urine[2].

Welsh *et al.* also report that insulin can reorganize the actin cytoskeleton in podocytes maintained in culture. This phenomenon resembles the actin remodelling seen when insulin causes translocation of glucose-transport proteins to the cell surface in fat or skeletal muscle cells[3]. Exactly how regulation of the cytoskeleton affects both podocyte foot processes and the filtration barrier requires more detailed investigation.

Remodelling of the actin cytoskeleton also does not explain the increased apoptosis of podocytes lacking insulin receptors. In this regard, the observation[4] that insulin enhances the expression of the protein vascular endothelial growth factor (VEGF) — a crucial survival factor as well as a regulator of blood-vessel formation — might be of relevance. Podocytes are the main source of VEGF in the kidney: mice lacking VEGF specifically in podocytes show partial loss of all major cell types in the glomerulus, including podocytes[5,6]. Moreover, VEGF expression is reduced in tissues such as heart muscle in animals with diabetes[7]. Insulin can also prevent apoptosis by other mechanisms[8,9], including inactivation of the transcription factor FoxO and inhibition of caspase-9, a signalling molecule that promotes apoptosis.

Whether insulin signalling to other cell types in the glomerulus is essential for maintenance of the filtration barrier is not known. Dysfunction of endothelial cells in the systemic circulation is associated with the initiation and progress of diabetic nephropathy, and endothelial cells respond to insulin by changing the production of factors that regulate blood-vessel tone and by decreasing oxidant production and increasing levels of antioxidant enzymes[10].

It also remains to be seen whether podocytes, or other renal cells, are insulin resistant in diabetes and metabolic syndrome in other animal models and in humans. It could be that some cell types or insulin-signalling pathways are more susceptible to insulin resistance than others. For example, insulin increases sodium transport in the kidney's tubular cells, but this aspect of its function is not affected in diabetes[11]. To understand whether insulin resistance in other renal cells contributes to diabetic nephropathy, researchers must study normal insulin action in kidney cells; whether these cells develop insulin resistance in metabolic syndrome and/or in diabetes; and what causes impaired insulin signalling.

With its focus on insulin resistance in glomerular cells, Welsh and co-workers' paper[1] helps to establish that diabetic nephropathy — the leading cause of chronic kidney