Cellular functions of class IX myosins in epithelia and immune cells

Martin Bähler1, Kerstin Elfrink, Peter J. Hanley, Sabine Thelen and Yan Xu
Institute of Molecular Cell Biology, Westfalian Wilhelms University Münster, 48149 Münster, Germany

Abstract
Mammals contain two class IX myosins, Myo9a and Myo9b. They are actin-based motorized signalling molecules that negatively regulate RhoA signalling. Myo9a has been implicated in the regulation of epithelial cell morphology and differentiation, whereas Myo9b has been shown to play an important role in the regulation of macrophage shape and motility.

Class IX myosins
Helical actin filaments can serve as tracks for molecular motors owing to their polar structure. The actin-based molecular motors constitute the myosin superfamily. Members of this superfamily share a region with similar amino acid sequences that defines the motor region. Based on sequence similarities, the myosins have been grouped into different classes. Class IX myosins consist of a single member in invertebrates and of two members in mammals, called Myo9a (myr 7) and Myo9b (myr 5). As in the case of all myosin molecules, class IX myosins are composed of a motor region, a light-chain-binding region and a tail region [1–5]. The motor region of class IX myosins encompasses an N-terminal extension with structural similarities to Rassociation domains [6]. Another distinctive feature of the motor region of class IX myosins is an extended insertion at loop 2 (50–20 kDa junction). This insertion exhibits a CaM (calmodulin)-binding site [7]. The light-chain-binding region contains four evenly spaced IQ motifs in invertebrate Myo9 and mammalian Myo9b and six unevenly spaced IQ motifs in mammalian Myo9a. The extended tail region contains two frequently found domains, namely one (in the case of mammalian Myo9) or two C1 domains (invertebrate Myo9) and a Rho-GAP (Rho GTPase-activating protein) domain. The C1 domain is characterized by six cysteine and two histidine residues that co-ordinate two zinc ions. The typical C1 domains bind DAG (diacylglycerol), whereas the atypical ones do not [8]. The C1 domains of class IX myosins belong to the atypical subfamily and their functions are not yet known. The Rho-GAP domain activates the GTPase activity of the small G-protein RhoA and thereby switches it from the active, GTP-bound, form to its inactive, GDP-bound, form [1,3,9,10]. Hence class IX myosins negatively regulate Rho signalling. Since Rho signalling regulates the dynamics and organization of the actin cytoskeleton [11], class IX myosins might form feedback loops in that they move along actin filaments whose organization they regulate.

Unconventional motor properties of class IX myosins
To understand the cellular functions of class IX myosins, it is necessary to have mechanistic insight into the motor properties. So far, the in vitro motor properties of invertebrate Myo9 from Caenorhabditis elegans and mammalian Myo9b have been analysed to some degree. A property of special interest is that they both show characteristics of a processive motor, meaning that they take many consecutive steps along F-actin (filamentous actin) before they dissociate again [7,12–14]. This is truly remarkable, because they are single-headed. All other known processive myosins are double-headed and advance by a (co-ordinated) hand-over-hand mechanism. The mechanism of movement for class IX myosins is currently not well understood. The extended insertion at loop 2 in the head has been postulated to tether the myosin to the actin filament and thereby contribute to the processive movement. In agreement with this proposal, it could be shown that the insertion strengthens the head’s affinity and that the isolated insertion binds to F-actin with high affinity [15]. The affinity of the myosin head for F-actin is regulated by the nucleotide that is present in the binding pocket. Myosins have generally a high affinity for F-actin in the nucleotide-free and in the ADP-bound states. In the ATP-bound and the ADP-P,-bound states, they exhibit a weak affinity for F-actin [16]. Processive myosins spend a large fraction of their total ATPase cycling time in the strongly bound states and only a small fraction of time in the weakly bound states. This is commonly referred to as a ‘high duty ratio’. This minimizes the chances of diffusion away from the actin filament. Surprisingly, the rate-limiting step in the mammalian Myo9b ATPase cycle is ATP-hydrolysis [17,18], which implies that Myo9b spends most of its cycling time in the ATP-bound state. If this is a weak actin affinity state in class IX myosins as in other myosins, it would not be compatible with processive movement. However, a subpopulation of ATP-bound Myo9b was able to bind F-actin with high affinity [17,18]. Further work will be needed to obtain a detailed mechanistic understanding of class IX myosin movement along actin filaments.

Key words: cell migration, epithelium, macrophage, myosin, Rho-GAP, Rho signalling.
Abbreviations used: F-actin, filamentous actin; Rho-GAP, Rho GTPase-activating protein; ROCK, Rho kinase; wt, wild-type.
1 To whom correspondence should be addressed (email baehler@uni-muenster.de).
Cellular functions of Myo9a

Constitutive deletion of Myo9a in mice resulted in the development of a severe hydrocephalus [19] characterized by an accumulation of cerebrospinal fluid in the brain. The cerebrospinal fluid is produced by the choroid plexus within the ventricular system of the brain. It circulates through the ventricular system before it enters the spinal canal or gets resorbed. In the brains of Myo9a-deficient mice, the cerebrospinal fluid was retained in the lateral and third ventricles because there was a stenosis or closure in the ventral caudal third ventricle and in the adjacent aqueduct of Sylvius. Multiciliated ependymal epithelial cells line the ventricles and separate the cerebrospinal fluid from the brain tissue. These epithelial cells exhibited an irregular morphology and the intercellular tight junctions showed a loss of the protein occludin. The ependymal epithelial cells mature and become multiciliated at different time points during development depending on where they are located within the ventricular system [20]. The expression of Myo9a in ependymal epithelial cells is coupled with the maturation of these cells and becomes apparent as they mature. The alterations in the Myo9a-deficient mice were noticed precisely where the ependymal cells mature first. Analysis of ependymal epithelial cell maturation in the Myo9a-deficient mice revealed that it was impaired in these regions.

Why do mice that are lacking Myo9a develop hydrocephalus? The removal of a negative regulator of Rho might lead to increased Rho signalling. That in turn might lead to the overactivation of ROCK (Rho kinase), the best-characterized effector of Rho signalling. To test this idea, the drinking water of mice was supplemented with a ROCK inhibitor. Treatment with a ROCK inhibitor effectively attenuated the ventricle enlargement, and the impaired maturation of ependymal epithelial cells was rescued [19]. These results suggest that unregulated Rho and ROCK activities contribute to hydrocephalus formation. Myo9a was found to localize in cell–cell contacts and therefore might regulate specifically Rho signalling in cell junctions (Figure 1). As Myo9a is expressed throughout the brain, it remains to be determined whether hydrocephalus formation is an ependymal epithelial cell autonomous process or not. Furthermore, Myo9a might not only regulate Rho signalling, but could potentially modulate additional cellular pathways.

Cellular functions of Myo9b

Myo9b-deficient mice on average are ∼10% lighter than wt (wild-type) mice, but have no obvious phenotype and are fertile [21]. Since Myo9b is abundantly expressed in immune cells, including phagocytes, macrophages were analysed for potential differences in actin-mediated processes that are regulated by Rho signalling. Isolated Myo9b−/−peritoneal macrophages failed to spread in vitro and remained round [21]. In comparison with wt peritoneal macrophages, the migration of Myo9b-deficient macrophages was severely impaired in a chemoattractant gradient. Migration velocity and chemotactic orientation were both reduced. In agreement with the notion that removal of Myo9b, a negative regulator of Rho signalling, leads to increased Rho signalling, elevated levels of GTP-bound Rho were observed in Myo9b−/−macrophages. Unregulated Rho signalling in turn caused increased levels of phosphorylated myosin light chain and phosphorylated cofilin, indicative of increased acto-myosin contractility and reduced F-actin dynamics respectively.

Figure 1 | Schematic representation of the functions of the two mammalian class IX myosins

Class IX myosins produce directional force along actin filaments. The tail region consists of a GAP domain that catalyses the inactivation of the small monomeric G-protein RhoA. Myo9a localizes in epithelial cells to areas of cell-cell contact. It regulates the morphology and differentiation of ependymal epithelial cells. The differentiation is regulated through Rho and ROCK signalling. Myo9b localizes to dynamic F-actin at the extending cell front and regulates Rho signalling. Thereby it controls cell polarity and cell migration of macrophages.

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[11]. Treatment of wt and Myo9b−/− macrophages with a cell-permeant Rho inhibitor (TAT-C3 exotransferase) led to a similar migratory behaviour of the two cell types. The migration velocity was greater than in untreated wt macrophages, and macrophages of both genotypes were no longer able to orient themselves in a chemoaatractant gradient, because they were no longer able to retract their rear end properly and to maintain polarity and restrict actin probing activity to the front. An important effector of Rho is ROCK. When wt and Myo9b−/− macrophages were treated with a ROCK inhibitor, they still demonstrated differences in migration velocity and chemotaxis, suggesting that additional Rho effector(s) contribute to the phenotype. These results demonstrated that Myo9b is a major Rho-GAP in macrophages. In some cell types, there exists a cross-talk between Rho and Rac signalling [11]. Rac belongs to the rho subfamily of monomeric G-proteins and regulates lamellipodia formation [11]. However, no changes were observed in Rac signalling between wt and Myo9b−/− macrophages irrespective of whether they were exposed to chemotactant or not. Therefore lamellipodial extension in Myo9b−/− macrophages was impaired solely due to increased RhoA signalling that was competing with normal Rac signalling for regulation of the actin cytoskeleton. Not only in vitro migration of isolated Myo9b−/− peritoneal macrophages was impaired but also in vivo migration of monocytes/macrophages was significantly reduced in a peritonitis model. This finding indicates that innate immune responses are regulated by Myo9b in vivo.

How might regulation of Rho signalling by Myo9b contribute to directed cell migration? In migrating cells, Myo9b accumulates at the cell front in regions of dynamic actin filaments [22]. The enrichment of Myo9b depends on a functional motor region. Therefore processive movement of the motor region along actin filaments towards the cell front carrying as cargo the Rho-GAP domain could lead to local inactivation of Rho (Figure 1). Inactive Rho would not interfere with Rac-induced actin filament polymerization that drives cell extension. Rho activity at the sides and the back of the cells would allow retraction of the rear end. Myo9b might act in a positive feedback loop maintaining cell polarization and sustained extension at the front. The importance of local compared with a more global negative regulation of Rho by Myo9b in cells needs to be addressed in future experiments. These experiments will have to include a detailed analysis of Myo9b movement within lamellipodia and filopodia.

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