The role of myosin Va in secretory granule trafficking and exocytosis


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Abstract

It emerges that myosin Va plays multiple roles in the trafficking of SGs (secretory granules). In addition to a function in the capture and transport of newly formed SGs in the F-actin-rich cortex, myosin Va is implicated in late transport events of these organelles, which precede their exocytosis. Consistent with these roles, interactions of myosin Va with an array of well-known proteins involved in regulated protein secretion have been documented.

Introduction

The neuropeptide- and hormone-containing SGs (secretory granules) of neuroendocrine cells are generated at the trans-Golgi network as immature SGs [1]. They are transported to the cell cortex, where they complete maturation including homotypic fusion as well as removal of excess membrane and proteins not destined for mature SGs in clathrin-coated vesicles [1]. Upon an appropriate stimulus leading to the influx of Ca\(^{2+}\), SGs fuse to the PM (plasma membrane) and release their contents to the extracellular space [2]. In contrast to biogenesis and exocytosis of SGs, which have been studied intensively over the last decades, detailed investigations of the transport of these organelles were carried out only during the last years. These studies confirmed previous indications [3] for an MT (microtubule)-dependent transport of SGs [4,5]. Furthermore, several lines of evidence suggested that SGs are transported actively along cortical actin filaments [6] driven by the motor protein myosin Va [7,8]. In this review, we summarize the current knowledge of the role of myosin Va in the different trafficking steps during the life span of SGs.

Key words: dilute mouse, exocytosis, F-actin cortex, Griscelli syndrome, myosin Va, secretory granule.

Abbreviations used: CaM, calmodulin; MT, microtubule; MyRIP, myosin VIIa- and Rab-interacting protein; NSF protein, N-ethylmaleimide-sensitive fusion protein; PM, plasma membrane; SG, secretory granule; Slp, synaptotagmin-like protein; Slac2-a, Slp lacking C2 domains-a; SNAP, soluble NSF-attachment protein.

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Myosin Va is a homodimer consisting of two identical heavy chains. The N-terminal head contains the actin-binding, ATP-dependent motor domain, whereas the C-terminal globular tail is the cargo-binding domain. The neck is a regulatory domain with six light-chain-binding domains.

In search of linker proteins recruiting myosin Va to SGs, research on melanosome trafficking provided again a suitable guiding model. In a pivotal paper, Hammer and co-workers [14] demonstrated that myosin Va is linked to melanosomes via a ‘receptor’ consisting of the small GTPase Rab27a and melanophilin/Slac2-a [Slp (synaptotagmin-like protein) lacking C2 domains-a] that also binds directly to actin [15]. The alternatively spliced form of myosin Va must contain exon F for this interaction [14]. In accordance with this model, it has been shown that Rab27a associates with SGs in both chromaffin and PC12 cells [16] and with insulin-containing SGs in pancreatic β-cells [17,18]. The interaction partners of Rab27a on SGs in these cases are also similar to melanophilin, namely Slac-2c/MyRIP (myosin VIIa- and Rab-interacting protein) in chromaffin and PC12 cells [16] and Slp4/granulophilin in pancreatic and MIN6 β-cells [17,18]. Slac-2c/MyRIP has been shown to interact with Rab27a/b, myosin Va and actin in vitro [19]. However, a functional link between this complex and myosin Va remains to be shown in vivo [16,20]. In this context, it is of note that both Rab proteins and melanophilin-related Rab effectors display broad diversity (60 human Rab proteins, [21]), five Slp proteins and three Slac2 proteins, rabphilin, Noc2 and Munc13-4 [22]). Therefore it is conceivable that different combinations of these linker proteins provide organelle-specific complexes for the recruitment of myosin Va. The composition of these receptor complexes may even change in a temporal fashion to regulate myosin Va function and future research on this issue should take into account this presumed complexity.

The Ca2+- and ATP-dependence of late steps in exocytosis of SGs [23] suggested the involvement of a molecular motor, which was subsequently identified as myosin Va [7,8]. Myosin Va has a significantly higher ATPase activity in the presence of Ca2+ than in the absence of Ca2+, which is accompanied by a large conformational change involving the entire motor protein. The conformation of inhibited myosin Va in the absence of Ca2+ is compact, in which the globular tail domain interacts with the head–neck region. In the presence of a high Ca2+ concentration, myosin Va displays an extended conformation [24–26]. It has been demonstrated that the influx of Ca2+ upon depolarization-induced stimulation also activates the F-actin severing protein scinderin [27]. This is thought to clear the way for myosin Va-dependent transport of marker proteins of SGs isolated from chromaffin cells [8]. In a similar study carried out with pancreatic MIN6 β-cells, comparable effects on docking, exocytosis and clustering of insulin-containing SGs were found upon expression of a dominant-negative truncated form of myosin Va as well as by silencing of myosin Va by RNA interference [11]. Also these granules were associated with myosin Va [11]. Thus the 'dual transport' model applies to SGs as well. In line with this model, it was shown in permeabilized chromaffin cells that the inhibition of myosin Va by specific antibodies against the head domain reduced the amount of regulated exocytosis after sustained stimulation but not within the first 2 min of the stimulus [8]. This supported the long-standing idea that SGs move myosin Va-dependently from a reserve pool towards the PM to replenish the readily releasable pool [12,13].
of SGs from the reserve pool along the remaining fine actin filaments to the PM (Figure 2B) [8]. Evidence from in vitro experiments indicates that myosin Va might interact with components of the docking complex after its arrival at the PM. Micromolar Ca\(^{2+}\) concentrations lead to a replacement of CaM light chains at the IQ motifs of myosin Va by \(\alpha\)-SNAP (Figure 2C) [28]. Furthermore, the presence of antibodies against the myosin Va-neck or myosin Va-binding fragment of syntaxin-1A reduced the frequency of exocytotic events predominantly in the sustained phase, but not during the first minute [28]. These results support the view that the interaction of myosin Va and syntaxin-1A confines SGs at the exocytotic site (Figure 2C). It is conceivable that the observed reduction of regulated exocytosis upon expression of truncated myosin Va [11] (R. Rudolf and H.-H. Gerdes, unpublished work) or silencing of myosin Va by RNA interference [11] may be caused by the impairment of the aforementioned late myosin Va-dependent steps in exocytosis. Myosin Va does not seem to play a role in the final exocytotic event, since its interaction with the fusion machinery constituents NSF (N-ethylmaleimide-sensitive fusion) and SNAP (α-soluble NSF-attachment protein) was shown to release myosin Va from the SNARE (SNAP receptor) complex in vitro [28] and cell depolarization induced the dissociation of myosin Va from chromaffin vesicles [29]. Two possible fates of released myosin Va have been proposed: calpain-mediated degradation [30,31] or recycling by passive treadmilling towards the minus-end of F-actin [32].

Myosin Va-related diseases

The important role of myosin Va in the trafficking of numerous organelles, e.g. small synaptic vesicles, SGs and melanosomes, suggests that mutations in the myosin Va gene should lead to severe pathological phenotypes. It is therefore not surprising that mice with mutated myosin Va gene, dilute, display, in addition to a lighter coatcolour due to a failure in the delivery of melanosomes to keratinocytes [33], neurological impairments, namely seizures and ataxia, and they die within a few weeks after birth [34]. More detailed studies revealed defects in retinal neurotransmitter release [35] and absence of long-term synaptic depression [36]. It should be noted, however, that the loss of myosin Va function in dilute mice seems not to affect all secretory processes, since hippocampal synaptic transmission and plasticity were shown to be unaffected [37]. This lack of effect may be due to low expression levels of myosin Va in the hippocampus compared with other brain regions [38]. In humans, mutations of myosin Va (MYOSA) lead to Griscelli syndrome, a rare autosomal recessive disorder, with two different types of clinical manifestation, hypopigmentation and neurological impairment. MYOSA mutations that result in a deletion of exon F lead to Griscelli syndrome type 3 and are characterized by hypopigmentation Only [39]. MYOSA mutations that affect other regions of exon F lead to Griscelli syndrome type 1, which is characterized by severe primary neurological impairment in addition to hypopigmentation [40]. The finding that SGs undergo a myosin Va-dependent transport provides a clue as to how the defect of this motor may contribute to the observed neurological impairments.

Outlook

The identification of myosin Va and its multiple roles in transport and exocytosis of SGs has set the stage to study the various functions of this motor protein in more detail. In particular, the recruitment of myosin Va to SGs, the regulation of its motor activity and its interaction with molecules leading to the presumed roles in regulated protein secretion need to be addressed. Understanding of these dynamic processes at the molecular level will help to elucidate acute and subtle defects in hormone and neuropeptide secretion.

References


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Received 24 July 2006