Regulation of ZBP1 transport dynamics in axons by MyosinVa

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Abstract

Directed transport of the mRNA binding protein, zipcode binding protein1 (ZBP1), into developing axons is believed to play an important role in mRNA localization and local protein synthesis. The role of molecular motors in this process is unclear. We elucidated a role for Myosin Va (MyoVa) to modulate the axonal localization and transport of ZBP1 in axons. Using cultured rat hippocampal neurons, ZBP1 colocalized with MyoVa in axons and growth cones. Interaction of MyoVa with ZBP1 was evident by co-immunoprecipitation of endogenous and overexpressed proteins. Inhibition of MyoVa function with the globular tail domain (GTD) of MyoVa protein or short hairpin RNA led to an accumulation of ZBP1 in axons. Live cell imaging of mCherryZBP1 in neurons expressing GTD showed an increase in the number of motile particles, run length and stimulated anterograde moving ZBP1 particles, suggesting that MyoVa controls availability of ZBP1 for microtubule-dependent transport. These findings suggest a novel regulatory role for MyoVa in the transport of ZBP1 within axons.

Introduction

Localized translation of mRNA transcripts serves to spatially and temporally control cell motility, developmental polarity during oogenesis and embryogenesis, axon guidance and synaptic plasticity (Martin and Ephrussi, 2009). Transport of mRNA to distal compartments in neurons, such as growth cones and dendritic spines, ensures local translation of proteins in response to receptor signaling. mRNA binding proteins may serve as adapters to facilitate the microtubule-dependent transport of mRNA in dendrites, and actin-dependent anchoring of mRNA within dendritic spines (Dictenberg et al., 2008; Yoshimura et al., 2006). RNA binding proteins not only transport, but also regulate translation of mRNAs (Kiebler and Bassell, 2006). Zipcode binding protein (ZBP1) binds to a 54-nucleotide zipcode sequence in the 3′ UTR of β-actin mRNA, which is essential for mRNA localization in fibroblasts and neurons (Ross et al., 1997; Zhang et al., 2001). Neurotrophin signaling stimulates an increase in the transport of ZBP1 particles containing β-actin mRNA into developing axons and growth cones (Zhang et al., 2001). The Src kinase-dependent phosphorylation of ZBP1 removes translational inhibition (Huttelmaier et al., 2005), which is stimulated by BDNF in neurons to promote local translation of β-actin mRNA required for growth cone guidance (Sasaki et al., 2010; Welshhans and Bassell, 2011; Yao et al., 2006). Hence, the precise spatio-temporal control of ZBP1 localization is crucial for neuronal functions.
The ‘tug of war’ model for bidirectional cargo transport proposes a coordination between opposing motors moving on microtubules (Hendricks et al., 2010; Holzbaur and Goldman, 2010; Ross et al., 2008) as well as actin (Ali et al., 2011) networks. In vitro and in vivo measurement of defined cargoes and motors indicated that opposing motors are simultaneously engaged on cargoes that undergo bidirectional transport and suggest a potential for regulation during activation by controlling motor type and number. It could be proposed therefore that directed ZBP1 transport is carried out by and requires a fine-tuned regulation of multiple microtubule and actin motors. Myosin Va (MyoVa) is a fast processive myosin that transports diverse cargoes such as vesicles (Kogel et al., 2010; Wu et al., 1997), neurofilaments (Pathak et al., 2010) and ER (Wagner et al., 2011), as well as RNA (Krauss et al., 2009; Salerno et al., 2008; Yoshimura et al., 2006). Mutations of MyoVa cause Griscelli syndrome in humans and dilute-lethal phenotype in mice, which display defects in skin/coat color and have neurological phenotypes (Pastural et al., 1997; Takagishi and Murata, 2006). While MyoVa is important for neuronal development (Lewis et al., 2009; Tamada et al., 2010), its contribution to the trafficking of mRNA binding proteins important for mRNA localization is not well understood. We hypothesized that the prominent brain myosin, MyoVa, can modulate the axonal localization and transport of ZBP1. Our results indicate that MyoVa plays an inhibitory role and thus regulates ZBP1 accumulation in axons of cultured hippocampal neurons. MyoVa inhibition resulted in increased transport dynamics and reversal of orientation of ZBP1 particles in neurons favoring anterograde movement.

Materials and Methods

Cell Culture

Hippocampal and cortical neurons were prepared from rat E18 embryos of either sex as before (Sasaki et al., 2010).

DNA constructs and transfection

MyoVa GTD was amplified from mouse cDNA using primers 5′
CCAAGCTTTGCATCTTGAGGTCGCAGCTGGTGAGCCAAAAAGAAGCCATCC 3′
and 5′
CGGAATTCTTATCAGTAGCTCACTGGAAACAACTGTTCATATTCTGAGGAGAAA
TG C 3′ and cloned into Hind III-EcoR I-sites of the plasmid pEGFP-C1. An Age I-EcoR I fragment from this was cloned into corresponding sites in pFUGW to generate a lentiviral transfer vector and produce lentivirus expressing MyoVa GTD fused to GFP. Lentiviruses were prepared at the ENNCF viral vector core facility. Photactivatable green fluorescent protein tagged GFP (PAGFP-ZBP1) was generated by replacing 3XFLAGmCherry with PAGFP in the 3XFLA-GmCherryZBP1 vector (Sasaki et al., 2010). MyoVa-GFP, MyosinVa short hairpin RNA (shRNA), and control shRNA containing vectors have been described before (Yoshimura et al., 2006). Neurons were transfected by either nucleofection (AMAXA) or magnetofection (OZ biosciences) according to manufacturer’s instructions. For nucleofection, 5×10^6 cells were used for electroporation using 3–5 μg of DNA containing mCherryZBP1(Sasaki et al., 2010) for particle tracking or PAGFP-ZBP1, either control shRNA or MyoVa shRNA and mCherry for identification of transfected cells for live cell imaging with PAGFP-ZBP1. For magnetofection, DIV 1 rat hippocampal neurons were incubated on a magnetic plate (OZ biosciences) for 20 minutes with DNA-magnetic bead (Neuromag) complexes formed with either control shRNA or MyoVa shRNA and pMaxGFP to identify transfected neurons. The complexes were washed and neurons were incubated for 48 hours.
Immunofluorescence (IF) and image analysis

Transfected or transduced hippocampal neurons (DIV3) were fixed in 4% formaldehyde and labeled by IF with guinea pig anti-ZBP1 antibody (Sasaki et al., 2010) and anti-MyoVa antibody. Multiple Z-slices of neurons were imaged on a Nikon Eclipse T500 inverted microscope equipped with a motorized stage with a 60X DIC oil objective. The longest (>50–100 μm) process having an axon-like morphology (DIV3) was analyzed for each neuron. IF signal intensity for ZBP1 was measured in a distal (>50 μm) axonal segment using ImageJ. To disrupt F-actin, cytochalasin-D (5 μg/ml) was added 30 minutes before fixation. For quantification of colocalization, analysis was carried out on growth cones of multiple 3D slices in the ImarisColoc module (Bitplane). Briefly, the algorithm calculated an automatic threshold for each of the channels by randomizing pixels from one channel and verifying that >95% of the randomized iterations do not have a Pearson’s correlation coefficient value greater than the original channel.

Live cell imaging and analysis

Rat cortical neurons were transfected with mCherryZBP1 at DIV0 by nucleofection and cultured on glass bottomed dishes (Bioptechs). After 2 days in culture, they were transduced with lentiviral vectors expressing GFP-GTD for 12–16 hrs. At DIV3, neurons expressing only mCherryZBP1 or mCherryZBP1 and GFP-GTD were imaged on a Nikon TE2000 inverted widefield microscope using a 60X DIC oil objective every 500 msec for 3 min. ZBP1 particles were tracked using ImarisTrack (Bitplane) where mCherryZBP1 particles were used to build spots, and after filtering background, the spots were automatically tracked over time applying an algorithm for autoregressive motion. Tracks with a defined straightness (0.8) and gaps (<3 frames) were selected and the statistics were extracted. The orientation of the spots was measured manually by counting directional particles moving towards the cell body as retrograde and away from the cell body as anterograde. In other experiments, PAGFP-ZBP1 with mCherry and either MyoVa shRNA or control shRNA were used to transfect cortical neurons by nucleofection. Transfected cells were identified by mCherry fluorescence and the cell body was selectively photoactivated by 405 Laser at 40% power for 7 pulses of 2 seconds each on a Nikon A1R laser confocal microscope. Cells were imaged for 5 minutes every 2 seconds and the GFP fluorescence accumulated in the non-activated neurite was measured using NIS Elements. After background subtraction, data from multiple neurites was normalized as fold increase over basal GFP intensity of that neurite.

Immunoprecipitation and Immunoblotting

Neuro2a cells transfected with either GFP or MyoVa-GFP and in other experiments with FLAG-tagged mCherry or FLAG-tagged mCherry-ZBP1 for 24 hours were used for immunoprecipitation using anti-GFP antibody (Abcam) or anti-MyoVa (Sigma) antibody respectively. DIV3 cultured high-density cortical neurons were used for immunoprecipitation with anti-MyoVa antibody (Sigma). The immunoprecipitates were screened by western blot with anti-ZBP1 antibody or anti-FLAG antibody. For RNase treatment, cell lysates were treated with 2μg/ml RNase A for 30 min at 37°C before incubation with anti-MyoVa antibody.

Results

ZBP1 associates with Myosin Va in axons and growth cones of cultured neurons

To examine if ZBP1 and MyoVa were colocalized in neurons, high-resolution immunofluorescence imaging experiments were performed to visualize endogenous MyoVa and ZBP1 in cultured hippocampal neurons (3DIV). Automated analysis of colocalization
using ImarisColoc (Bitplane) revealed 27.5 ± 9.2% of ZBP1 particles colocalized with MyoVa in growth cones. Colocalized particles could be detected both in neurites, filopodia and lamellopodia of growth cones (Figure 1A, B and D). As a negative control, colocalization between ZBP1 and Fragile X mental retardation protein, FMRP, another non-overlapping (Narayanan et al., 2007) RNA binding protein that does not bind beta-actin RNA (Muddashetty et al., 2007) was very low or not detected (4.2 ± 0.76) (Figure 1C,D). To further assess a possible interaction between MyoVa and ZBP1, we performed immunoprecipitation (IP) of MyoVa using multiple strategies. Endogenous ZBP1 was detected following immunoprecipitation of GFP-MyoVa using anti-GFP antibody in Neuro2a cells transfected with GFP-MyoVa and not in cells with GFP alone (Figure 1E). Similarly, endogenous ZBP1 was also detected following immunoprecipitation of endogenous MyoVa using anti-MyoVa antibody applied to high-density cortical neurons (Figure 1F). In order to investigate whether the interaction of ZBP1 and MyoVa might be dependent on an indirect interaction through RNA, we performed MyoVa IP experiments with lysates of Neuro2a cells expressing FLAG-mCherryZBP1 with or without pretreatment with RNase A. ZBP1 was detected in IPs of both RNAse treated and untreated cells, suggesting an interaction between MyoVa and ZBP1 that may be independent of RNA binding (Figure 1G).

MyosinVa restricts ZBP1 localization and regulates its dynamics in axons

The globular tail domain (GTD) of MyoVa has been used as an inhibitor of myosin function since it lacks the motor domain and acts as a dominant negative construct (Li et al., 2006). Mouse brain cDNA was used to amplify the GTD and generate lentiviral vector particles expressing GTD. High-resolution imaging and quantitation of endogenous ZBP1 immunofluorescence (IF) intensities in axons and growth cones of hippocampal neurons transduced with GFP-GTD showed 45.7±14.4 and 65.7±18.6 % increases in ZBP1 signal, respectively, compared to GFP only transduced neurons (Figure 2A,B). Transfection of neurons with the full-length GFP-MyoVa did not change the levels of ZBP1 in axons, whereas transfection with GFP-GTD construct resulted in a ~ 2-fold increase in the axonal ZBP1 IF signal over the untransfected control (Figure 2C,D). Further, depletion of MyoVa by shRNA resulted in a 61.5 ±20.4 % increase in axonal ZBP1 levels compared to control shRNA treated neurons (Figure 2E,F). Quantification of ZBP1 signal in the cell bodies of transfected neurons indicated a 26.6 ± 5.4% decrease in ZBP1 levels in the cell body indicating that the accumulation of ZBP1 in neurites may be due to transport (Figure 2E, F). Collectively, these data suggest that MyoVa may inhibit transport and accumulation of ZBP1 into axons. To examine if the effect of MyoVa perturbation or knockdown on ZBP1 localization might also be apparent by disruption of F-actin, neurons were treated with cytochalasin D. ZBP1 levels in axons of cytochalasin D treated cells were 54.1±11.91 % higher as compared to the untreated cells (Figure 2 G,H), suggesting that F-actin and associated myosinV motors may limit ZBP1 localization and levels within the axon.

To investigate the role of MyoVa in ZBP1 transport, live cell imaging was performed on neurons transfected with mCherryZBP1. ZBP1 particles were dynamic and exhibited bidirectional transport with a bias in the retrograde orientation (Figure 3A,B). As previously reported, they displayed a maximum average speed of 0.1 – 2.0μ/s consistent with the speed of fast microtubule (MT) and actin based motors (Zhang et al., 2001). Inhibition of MyoVa by transduction with a GFP-GTD construct resulted in a 1.6-fold increase in the number of motile ZBP1 particles in the neurites (Figure 3C). However, there was no alteration in the maximum average speed of the particles (data not shown). Analysis of orientation of motile particles in untransfected cells indicated a bias towards retrograde orientation (Figure 3D). While mCherryZBP1 particles displayed a bias towards retrograde orientation at basal state, this was removed in GTD expressing neurons where there was a concomitant increase in the
anterograde oriented tracks (Figure 3 D). Inhibition of MyoVa also led to a moderate but significant (14.9±4.4%) increase in the average run length of all motile ZBP1 particles, which included directed, bidirectional and oscillatory (Figure 3E). To further examine the effect on run-lengths, the run-lengths of all directed ZBP1 particles, both anterograde and retrograde were compared. There was a marked increase in run-lengths (3.19 ± 1.1 fold) in the anterograde orientation in the GFP-GTD transduced cells (Figure 3F). This suggests that MyoVa may be involved in limiting anterograde ZBP1 particle runs. In order to gather further evidence for the effect of Myosin Va inhibition on orientation, we performed additional live cell experiments on PAGFP-ZBP1 expressed in neurons. PAGFP-ZBP1 expressing cells were selectively photomobilized in the cell body and accumulation of GFP signal in the neurites as a measure of anterograde transport was quantified in cells expressing MyoVa shRNA or control shRNA (Figure 4 A and B). Cells transfected with MyoVa shRNA showed a 1.9 fold higher GFP signal accumulation in neurites by 5 minutes over control shRNA transfected cells (Figure 4C), further confirming that inhibition of MyoVa leads to increased anterograde movement of ZBP1 and that MyoVa plays a restraining role on anterograde movement of ZBP1 from the cell body.

Discussion

The ability of a neuron to regulate steady state expression and transport dynamics of mRNA granules in axons and dendrites is believed to be modulated by mRNA binding proteins acting as adapters for molecular motors. While previous work has shown that ZBP1 mediated localization of β-actin mRNA into axons depends on microtubules (Zhang et al., 1999; Zhang et al., 2001), it remains unclear what molecular motors are involved in the directed transport of ZBP1. Kinesin motors have been shown to play an important role in the transport of mRNAs and mRNA binding proteins in neurons (Dictenberg et al., 2008; Kanai et al., 2004). In these studies, perturbation of kinesin leads to a reduction in RNA granule localization in neuronal processes and impaired dynamics. Here, we report that perturbation of myosin Va leads to an opposite phenotype, characterized by increased levels and transport dynamics of ZBP1 in axons. These findings suggest that transport dynamics for ZBP1 and perhaps other mRNA granule components are regulated by both microtubule and actin based motors. Our data indicate a novel role for MyoVa to regulate ZBP1 transport in axons. Previous work has shown that β-actin mRNA localization in the absence of MyoVa is altered (Salerno et al., 2008). However, the requirement of MyoVa to regulate the transport dynamics and axonal localization of an mRNA binding protein necessary for mRNA localization has not been previously shown.

Here we show that inhibition of MyoVa resulted in an overall increase in anterograde transport and accumulation of ZBP1 protein in axons, suggesting that MyoVa plays a restraining role on ZBP1 protein motility in the axon. The increased accumulation of ZBP1 in axons following perturbation or knockdown of MyoVa could be attributed to increased availability of ZBP1 for microtubule-dependent anterograde transport, perhaps resulting from release of ZBP1 from subcortical actin docking sites in the cell body or neuronal processes, resulting in a change in the frequency and dynamics of ZBP1 particles moving along microtubules at steady state. These results bear some similarities to other examples of MyoVa inhibition leading to an accumulation of cargoes, like the neurofilaments (Alami et al., 2009) where MyoVa regulates transport by decreasing the frequency of pauses. MyoVa is believed to be a short-range motor in neurons owing to the fact that F-actin filaments are small and of mixed polarity in neuronal processes. In the case of ZBP1, short-range tracks by MyoVa might serve to regulate the availability of ZBP1 for transport to the axon and ultimately the growth cone.
Although we have previously observed the bidirectional transport of ZBP1 in axons and growth cones (Zhang et al., 2001; Welshhans et al., 2011), here we analyze particle direction and reveal a retrograde bias of ZBP1 transport in axons. In Drosophila embryos, apically localized RNAs, which should move retrogradely on MTs, do move in both directions; the resultant retrograde motility is only because of more frequent retrograde runs due to increased numbers of dynein motors (Bullock et al., 2006). These observations exemplify the ‘tug-of-war’ hypothesis for bidirectional transport where multiple motors similarly or oppositely oriented are present on a cargo and the resultant direction of motility is a sum of forces. Multiple motors may be engaged in a ‘tug-of-war’ on RNA or other cargoes and the resultant orientation may be a function of the number of the anterograde or retrograde motors (Holzbaur and Goldman, 2010). While MT-based motors are likely the primary transporters along neuronal processes, the role of MyoVa might be tether or limit interactions with microtubule based motors. We speculate that in neurons MyoVa may facilitate a retrograde orientation of ZBP1 particles by tipping the balance to a retrograde motor like dynein (Figure 4D). Inhibition of MyoVa by expressing a dominant negative thus leads to more frequent and longer anterograde runs. This may or may not involve direct interactions with dynein since it is also suggested that MyoVa and dynein share the same light chains (Espindola et al., 2000; Hodi et al., 2006). Functionally, this may be significant for axon guidance since RNA binding proteins like ZBP1 are negative regulators of their target RNA translation and an additional control to keep the translation of key molecules dormant would be to restrict their transport unless required. Retrograde transport may also facilitate retrograde signaling or recycling of ZBP1.

ZBP1 may associate with MyoVa directly and independent of RNA. Thus, RNA binding proteins like ZBP1 may be considered as adapters for RNA to associate with motors. The mechanism that governs MyoVa association remains unknown. Phosphorylation at Y396 stimulates release of ZBP1 from β-actin mRNA (Huttelmaier et al., 2005), but did not alter ZBP1 binding to MyoVa (data not shown). There may be other post-translational modifications that determine the binding of ZBP1 to MyoVa. It is also likely that post-translational modifications on MyoVa itself may regulate ZBP1 binding. It is known that phosphorylation, calcium and cargo binding can alter the motility of MyoVa (Krementsov et al., 2004; Sellers and Knight, 2007). However, it has been found recently, using in vitro and in vivo single-molecule RNA imaging, that localization elements in the RNA were sufficient to decide the fate of localized RNA by increasing the number of dynein-dynactin recruited on the RNA (Amrute-Nayak and Bullock, 2012).

In summary, our results suggest a model (Figure 4D) whereby MyoVa plays a restraining role for ZBP1 transport to and from axons and growth cones by regulating recruitment into the neurite and run-lengths along microtubules. Like other neuronal cargoes, motility along the neurite may involve predominantly MT-based motors. We still do not know what types of MT-based motors may be involved. Both KIF1 and KIF5 motors are involved in the transport Fragile X mental retardation protein (Davidovic et al., 2007;Dictenberg et al., 2008) and kinesins, dynein and myosin motors are all bound to localized RNAs like oskar in drosophila (Bullock, 2011). Further work is needed to identify other motors and elucidate their cooperative involvement in ZBP1 transport and its regulation by physiological signals, which will lead to a clearer understanding of motor dynamics during basal and activated states.

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References


Figure 1. ZBP1 associates with MyoVa in neurons
A. Double label immunofluorescence of ZBP1 (panel 1) and MyoVa (panel 2) and their colocalization in distal axon and growth cone of cultured hippocampal neuron (panels 3 and 4). Neuronal morphology is shown by F-actin staining. Panel 3 shows an overlap of MyoVa and ZBP1 channels with the colocalized channel and panel 4 depicts the overlap of the colocalized channel with F-actin staining. B. Double label immunofluorescence image of ZBP1 and MyoVa in a subregion of the growth cone shown in A, displayed at the automatic thresholds used for colocalization analysis by ImarisColoc. Neuronal morphology is shown by F-actin staining. C. Double label immunofluorescence staining of ZBP1 and FMRP and their overlap with F-actin in a growth cone. D. Histogram represents mean ± SEM of % colocalization of ZBP1 with MyoVa or FMRP in deconvolved and 3D reconstructed Z-stacks from multiple growth cones (n=3, p<0.0001, t-test). E. Immunoblot screened with
anti-ZBP1 antibody or anti-GFP antibody in duplicates of immunoprecipitates with an anti-GFP antibody of Neuro2a cell lysates expressing GFP alone or GFP-MyoVa. Position of molecular weight standards in kilodaltons is indicated on the left. F. Immunoblots screened with anti-ZBP1 or anti-MyoVa antibody of immunoprecipitate with anti-MyoVa antibody or IgG from DIV3 high-density cortical neurons. Position of molecular weight standards in kilodaltons is indicated on the left. G. Immunoblots screened with anti-FLAG antibody in duplicates of immunoprecipitate with anti-MyoVa antibody of Neuro2a cells transfected with FLAG-mCherry alone or FLAG-mCherry-ZBP1 with or without treatment with RNase. Protein input was screened by IB with anti-FLAG and anti-ZBP1 antibodies. Lower panel shows an RNA gel of RNA extracted from the lysate post-RNase treatment. Position of molecular weight standards in kilodaltons is indicated on the left.
Figure 2. Inhibition of MyoVa function results in increased accumulation of ZBP1 in neurites

A. Images showing GFP and ZBP1 immunofluorescence staining of DIV3 rat hippocampal neurons transduced with GFP only or GFP-GTD for 12–16 hrs. Arrows (axon) and arrowheads (g.cone) indicate increased ZBP1 staining in GTD expressing neurons. B. Histogram represents mean ± SEM of normalized ZBP1 intensity in axons and growth cones of neurons expressing GFP alone or GFP-GTD (n=3, p=0.0084, One-way ANOVA, Bonferroni). C. Images showing GFP and ZBP1 immunostaining of untransfected neurons or neurons transfected with GFP-GTD or GFP-MyoVa. Arrows indicate increased ZBP1 staining in GTD expressing neurons. D. Histogram shows mean ± SEM of ZBP1 intensities in multiple untransfected and transfected neurons (n=3, p<0.0001, One way ANOVA, Bonferroni). E. Immunofluorescence images of ZBP1 and MyoVa staining in DIV3 neurons co-transfected with a construct expressing only GFP and a construct encoding control shRNA or MyoVa shRNA for 48 hrs. GFP signal was used to identify transfected neurons. Arrows indicate increased ZBP1 staining in MyoVa depleted neurons. F. Histogram representing mean ± SEM of % ZBP1 intensities relative to control shRNA in neurites and cell bodies of control shRNA and MyoVa shRNA transfected neurons (n=3, p=0.0003, One way ANOVA, Bonferroni). G. Immunofluorescence images of ZBP1 staining in DIV3 neurons with or without treatment with cytochalasin-D. Arrows (axon) and arrowheads (g.cone) indicate increased ZBP1 staining in cytochalasin-D treated neurons. Yellow asterisk indicates the position of the growth cone. F-actin staining with Alexa488-phalloidin.
was used to identify neuronal morphology as well as F-actin integrity. H. Histogram represents mean ±SEM of % ZBP1 intensities in untreated or cytochalasin-D treated neurons (n=3, p<0.0001, t-test)
Figure 3. Inhibition of myosin function increases number of motile ZBP1 particles and changes orientation and run length

A. Sequential time lapse images of neurites of rat cortical neurons transfected with mCherryZBP1 for 3 days without or with transduction with lentiviral vector expressing GFP-GTD. Arrows indicate retrograde (orange and green) or anterograde (red and blue) orientation in an untransduced or GFP-GTD transduced neurons. B. Kymographs are shown from a 3-minute movie with 360 frames from untransduced or GFP-GTD transduced neurons. C. Histogram represents mean±SEM of number of motile ZBP1 particles/μm of neurite in multiple untransduced or GTD transduced neurons [n (#neurons=15, #particles 400–500), p=0.025, t-test]. D. Histogram represents mean± SEM percentage of ZBP1 particles in anterograde or retrograde orientation (p=0.0029; **, *P<0.05, One-way ANOVA, Bonferroni) from multiple neurons untransduced or transduced with GTD. E. Histogram represents mean ± SEM of average run-lengths of total tracked ZBP1 particles (directed, bidirectional and oscillatory) in untransduced neurons or neurons transduced with GFP-GTD (p=0.045, t-test). F. Histogram represents mean ± SEM of average anterograde or retrograde run-lengths of ZBP1 particles showing persistent, directional movement/micrometer of neurite in untransduced or neurons transduced with GFP-GTD (p=0.0268, One-way ANOVA, Bonferroni).
Figure 4. Inhibition of MyoVa leads to increased anterograde motility of ZBP1 from the cell body
A. Images showing cortical neurons transfected with mCherry and PAGFP-ZBP1 and with MyoVa shRNA or control shRNA at time 0 (first panel), before photoactivation (second panel), 2 seconds after photoactivation (third panel) and 5 minutes after photoactivation (fourth panel) of the cell body indicated by red and green boxes in MyoVa or control shRNA treated neurons respectively. B. Sequential time-lapse images of a heat map of PAGFP-ZBP1 intensities accumulated in neurites marked by black boxes in neurons transfected with MyoVashRNA and Control shRNA in panel A. Arrows indicate accumulated PAGFP-ZBP1 signal in distal neurites. C. Histogram shows average fold-basal PAGFP-ZBP1 intensities in multiple neurons transfected with MyoVa shRNA or control shRNA as a function of time. D. A model to depict the proposed role of MyosinVa in ZBP1 transport. Myosin Va may tether ZBP1 on subcortical F-actin and compete with kinesin mediated anterograde transport along microtubules. Myosin Va may regulate ZBP1 traffic by restraining anterograde transport in the cell body (1), along the axon (2) or within the growth cone (3).