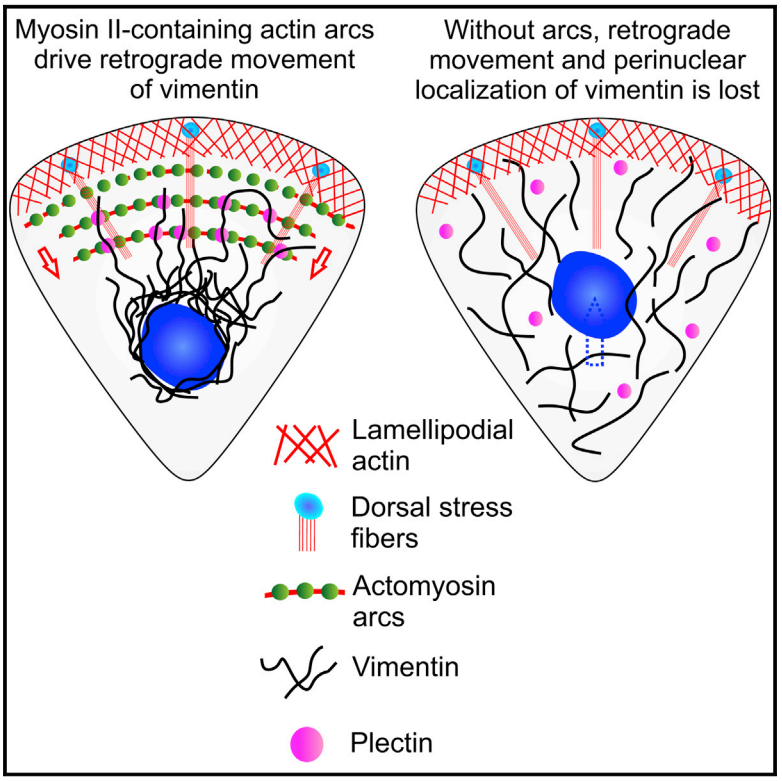
Cell Reports

Report

Bidirectional Interplay between Vimentin Intermediate Filaments and Contractile Actin Stress Fibers

Graphical Abstract



Highlights

- Vimentin filaments associate with actomyosin arcs to undergo retrograde flow
- Arc disruption leads to intermediate filament network spreading toward the cell edge
- Vimentin restricts retrograde flow of arcs and hence controls the lamellum width
- Plectins are essential for interplay between arcs and intermediate filaments

Authors

Yaming Jiu, Jaakko Lehtimäki, Sari Tojkander, ..., Markku Varjosalo, John E. Eriksson, Pekka Lappalainen

Correspondence

pekka.lappalainen@helsinki.fi

In Brief

Jiu et al. show that cytoplasmic vimentin intermediate filaments interact with contractile actomyosin arcs, which consequently drive the retrograde movement and perinuclear localization of the vimentin network. These dynamic interactions additionally control the localization of arcs and morphogenesis of flat lamellum in migrating cells.

Jiu et al., 2015, Cell Reports *11*, 1511–1518
June 16, 2015
http://dx.doi.org/10.1016/j.celrep.2015.05.008

CellPress

Cell Reports
Report

Bidirectional Interplay between Vimentin Intermediate Filaments and Contractile Actin Stress Fibers

Yaming Jiu,[1] Jaakko Lehtimäki,[1] Sari Tojkander,[1] Fang Cheng,[2,3] Harri Jäälinoja,[1] Xiaonan Liu,[1] Markku Varjosalo,[1] John E. Eriksson,[2,3] and Pekka Lappalainen[1,*]
[1]Institute of Biotechnology, P.O. Box 56, 00014 University of Helsinki, Helsinki, Finland
[2]Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, 20521 Turku, Finland
[3]Department of Biosciences, Åbo Akademi University, 20520 Turku, Finland
*Correspondence: pekka.lappalainen@helsinki.fi
http://dx.doi.org/10.1016/j.celrep.2015.05.008

SUMMARY

The actin cytoskeleton and cytoplasmic intermediate filaments contribute to cell migration and morphogenesis, but the interplay between these two central cytoskeletal elements has remained elusive. Here, we find that specific actin stress fiber structures, transverse arcs, interact with vimentin intermediate filaments and promote their retrograde flow. Consequently, myosin-II-containing arcs are important for perinuclear localization of the vimentin network in cells. The vimentin network reciprocally restricts retrograde movement of arcs and hence controls the width of flat lamellum at the leading edge of the cell. Depletion of plectin recapitulates the vimentin organization phenotype of arc-deficient cells without affecting the integrity of vimentin filaments or stress fibers, demonstrating that this cytoskeletal cross-linker is required for productive interactions between vimentin and arcs. Collectively, our results reveal that plectin-mediated interplay between contractile actomyosin arcs and vimentin intermediate filaments controls the localization and dynamics of these two cytoskeletal systems and is consequently important for cell morphogenesis.

INTRODUCTION

Cell migration and morphogenesis rely on a dynamic network of actin filaments, which provides force for the generation of membrane protrusions and contributes to cell adhesion to the extracellular matrix and neighboring cells. To fulfill these functions, actin filaments assemble into diverse protrusive and contractile structures in cells. These include dendritic actin filament networks, which generate lamellipodial membrane protrusions as well as thin filopodial actin filament bundles, which function as antennae for cells to probe their environment (Pollard and Cooper, 2009). Moreover, contractile actomyosin bundles called stress fibers contribute to cell morphogenesis, adhesion, and mechanosensing. They can be further divided into three categories. "Ventral stress fibers" are contractile myosin-II-containing actin bundles, which are anchored to focal adhesions at both ends. "Transverse arcs" are myosin-II-containing actin bundles that serve as precursors of ventral stress fibers. Arcs are generated at the lamellipodia-lamellum interface and undergo retrograde flow toward cell center. Arcs do not directly associate with focal adhesions but are linked to these cell-matrix interaction sites through "dorsal stress fibers" (radial fibers), which are non-contractile actin bundles connected to focal adhesions at their distal end (Tojkander et al., 2012; Burridge and Wittchen, 2013). Furthermore, eukaryotic cells contain an array of other actin-based structures that contribute to diverse cellular processes such as endocytosis, mitochondrial fission, and extracellular matrix degradation (Kaksonen et al., 2006; Schoumacher et al., 2010; Korobova et al., 2013). Importantly, actin filaments do not function in isolation but collaborate with two other cytoskeletal networks: intermediate filaments and microtubules (Huber et al., 2015).

Humans have >65 different intermediate filament proteins, which display cell-type-specific expression patterns and diverse biological functions. Vimentin and keratins are the major intermediate filament proteins in mesenchymal and epithelial cells, respectively (Eriksson et al., 2009; Snider and Omary, 2014; Loschke et al., 2015). They form motile networks, which undergo subunit exchange along the filaments (Vikstrom et al., 1992; Eriksson et al., 2009). Similarly to the actin cytoskeleton, keratins and vimentin contribute to cell migration and morphogenesis (Chu et al., 1993; Ivaska et al., 2007; Helfand et al., 2011; Busch et al., 2012). Intermediate filaments can also associate with actin filaments through direct binding, via cross-linking proteins and through steric effects (Correia et al., 1999; Schoumacher et al., 2010; Huber et al., 2015). For example, vimentin interacts with actin filaments through its C-terminal tail and associates with the actin cytoskeleton through plectins, which are large (~500 kDa) cytoskeletal cross-linkers (Svitkina et al., 1996; Esue et al., 2006; Wiche et al., 2015). Global pharmacological disruption of the actin cytoskeleton leads to decreased perinuclear density of vimentin and keratin intermediate filaments, demonstrating functional interplay between these cytoskeletal systems (Hollenbeck et al., 1989; Kölsch et al., 2009; Dupin

et al., 2011). However, the identity of actin filament structures that interact with and control the subcellular distribution of intermediate filaments has remained elusive. Additionally, whether intermediate filaments control the distribution of cellular actin filament structures and the possible roles of intermediate filament/actin cytoskeleton interplay in morphogenetic processes are not known.

RESULTS

Vimentin Filaments Undergo Retrograde Flow with Contractile Actin Arcs

To identify stress fiber components, we performed a proximity-dependent biotin identification (BioID) screen (Roux et al., 2012) on U2OS human osteosarcoma cells using a biotin ligase fused to tropomyosin-4 (Tm4). This actin-binding protein is a central component of stress fibers and co-localizes with myosin II in transverse arcs and ventral stress fibers (Tojkander et al., 2011). Among the Tm4 proximate and interacting proteins identified were the intermediate filament proteins vimentin and nestin as well as the cytoskeletal cross-linking protein plectin (Table S1).

Immunofluorescence microscopy demonstrated that endogenous vimentin filaments concentrated to the perinuclear region in U2OS cells, whereas actin filament structures were more pronounced at the cell periphery. However, vimentin filaments occasionally aligned with myosin-II-containing stress fibers (Figure 1A). Interestingly, live imaging revealed that while the vimentin filaments at the perinuclear region did not exhibit directional movements, many vimentin foci closer to cell periphery associated with transverse arcs for relatively long periods of time and displayed retrograde flow toward the cell center with these contractile actomyosin bundles (Figures 1B, S1A, and S1B; Movie S1). The arc-associated vimentin foci are typically sections of long vimentin filaments that are connected to the complex vimentin intermediate filament network (Movie S2). However, toward the cell periphery, many arc-associated vimentin foci also appear to represent shorter vimentin filaments called squiggles (Eriksson et al., 2009). Importantly, while majority of vimentin foci that overlapped with arcs displayed retrograde flow, the vimentin foci that did not co-localize with arcs exhibited mainly random movements (Figure 1C). Furthermore, specific disruption of arcs by Tm4 depletion resulted in loss of retrograde flow of vimentin, supporting the notion that arcs are essential for vimentin retrograde movements at the lamellum (Figure S2A).

Arcs Control the Subcellular Localization of Vimentin and Nestin Intermediate Filaments

To examine the role of arcs in perinuclear accumulation of vimentin filaments, we disrupted arcs and dorsal stress fibers from U2OS cells by small interfering RNA (siRNA) depletion of Tm4 and palladin, respectively. Tm4 depletion inhibits myosin II recruitment to arc precursors and thus specifically disrupts arcs without affecting dorsal stress fibers, whereas palladin is required for dorsal stress fiber elongation (Tojkander et al., 2011; Gateva et al., 2014). Endogenous vimentin filaments concentrated at the perinuclear region in control cells as well as in palladin knockdown cells, which did not display dorsal stress fibers but contained ventral stress fibers and arcs. However, perinuclear accumulation of vimentin was diminished in arc-deficient Tm4 knockdown cells (Figure 2A).

Because cells plated on coverslips display a large variation in their morphologies, it is not feasible to obtain quantitative information about subcellular localizations of actin and vimentin networks under these conditions. To overcome this obstacle, we plated control and knockdown cells on crossbow-shaped fibronectin micropatterns, where they display regular morphology and organization of the actin stress fiber networks (Vignaud et al., 2012). By dividing the cells into four segments (from segment 1, the leading edge, to segment 4, the trailing end; see Figure 2B), we could quantify the intensity of vimentin at different cell regions. These experiments provided quantitative validation that in wild-type and palladin knockdown cells, vimentin filaments indeed accumulated at the cell center around the nucleus, whereas in the Tm4 knockdown cells, the vimentin network spread toward the leading edge of the cell (Figures 2B and 2C). Similar expansion of the vimentin network was detected in U2OS cells and mouse embryonic fibroblasts expressing the dominant inactive GTPase Rif (Figures S2B and S2C), which likewise leads to specific depletion of transverse arcs (Tojkander et al., 2011). Also, perinuclear accumulation of another intermediate filament protein, nestin, which preferentially co-assembles with vimentin filaments (Sjöberg et al., 1994), was disrupted in dominant inactive Rif-expressing cells lacking arcs (Figures S3A and S3B). Thus, arcs are essential for proper perinuclear accumulation of vimentin and nestin filaments.

Vimentin Regulates Localization of Arcs and Width of the Lamellum

To study whether the vimentin network reciprocally influences actin stress fibers, we generated vimentin knockout U2OS cells by CRISPR/Cas9 technology. With two different target sites in the *vimentin* gene, we obtained several cell-lines lacking vimentin, one of which was chosen for further analysis (Figure 3A). The vimentin-deficient cells still contained all three categories of stress fibers (Figure 3B), but the distance of transverse arcs from the leading edge was moderately increased in vimentin-deficient versus control cells (8.3 μm versus 7.3 μm; Figure 3E).

A recent study provided evidence that transverse arc contraction mediates lamella flattening, and arc depletion thus decreases the width of the flat portion of the cell (lamellum) at the leading edge (Burnette et al., 2014). Analysis of Tm4 knockdown cells plated on micropatterns confirmed the reported decrease in the lamella width upon arc disruption. Importantly, vimentin deficient cells displayed ~15% wider lamella compared to control cells, and this phenotype could be rescued by expressing wild-type vimentin but not a vimentin mutant lacking 66 C-terminal residues (Figures 3C and 3D). The exogenously expressed wild-type vimentin displayed normal perinuclear accumulation in vimentin deficient cells, whereas the C-terminally truncated protein (vimentin C66) formed thick aberrant bundles that were deficient in perinuclear accumulation (Figures S3C and S3D).

Intermediate filaments and the actin cytoskeleton have been implicated in nuclear movement and positioning in many

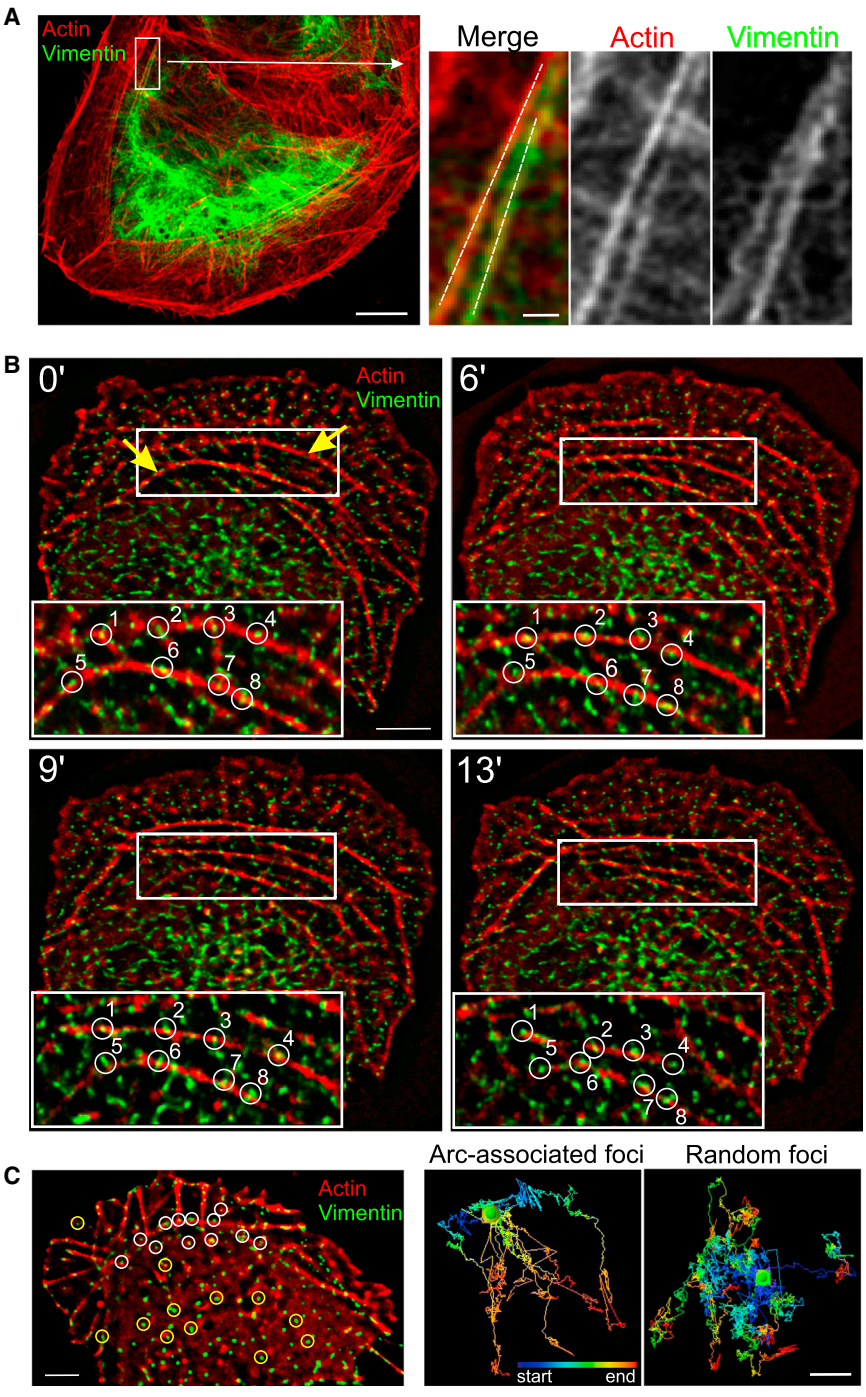


Figure 1. Interplay between Vimentin Intermediate Filaments and Contractile Actin Stress Fibers

(A) Localization of vimentin intermediate filaments (VIFs) and actin filaments in U2OS cells detected by polyclonal vimentin antibody and fluorescent phalloidin, respectively. Magnified region illustrates examples of co-localization (white dashed lines in the merged image) between VIFs and contractile actin stress fibers. Scale bars represent 10 μm and 1 μm in the left and right panels, respectively.

(B) Time-lapse imaging of U2OS cells expressing mCherry-actin and GFP-vimentin revealing co-localization and retrograde flow of vimentin foci along contractile arcs. White circles in the magnified regions indicate discrete vimentin spots, which flow toward the cell center with arcs (indicated by yellow arrows). Please note that arcs and some co-localizing vimentin foci moved a distance of ~4.4 μm toward the cell center during the 13 min imaging period. Scale bar, 10 μm.

(C) Time-lapse imaging demonstrating retrograde flow of stress fiber -associated vimentin foci (arc-associated spots) and non-directional movement of vimentin foci that are not clearly associated with stress fibers (random spots). White and yellow circles in the left panel indicate 12 selected vimentin foci overlapping with actin arcs and 12 vimentin foci that do not associate with actin arcs, respectively. Right panel shows the tracked trails of the vimentin foci when the starting points have been overlaid and color-coded from blue to red to indicate the starting and ending points, respectively. The recording time was 13.7 min, and the time interval was 30 frames/min. Scale bars represent 5 μm and 1 μm in left and right panels, respectively.

See also Figure S1.

cell-types (Ralston et al., 2006; Dupin et al., 2011; Gundersen and Worman, 2013). However, precisely how the cytoplasmic intermediate filaments contribute to nuclear positioning and which actin structures are involved in this process have remained elusive. Thus, we applied Tm4 knockdown and vimentin-deficient cells to examine the mechanisms of nuclear positioning. In control cells on micropatterns, the center of the nucleus located approximately 12 μm proximally from the intersection of the crossbow pattern (Figure S4A). In vimentin deficient cells, nuclei located slightly further toward the back of the cell, whereas disruption of arcs by Tm4 depletion resulted in significant forward positioning of the nucleus. Importantly, simultaneous depletion of arcs and vimentin resulted in similar positioning of the nuclei as observed in control cells (Figure S4B). Collectively, these experiments reveal that vimentin filaments restrict the retrograde flow of arcs and thus control the width of flat lamellum of the cells. Furthermore, both arcs and vimentin filaments appear to synergize in nuclear positioning.

Plectin Is Required for Functional Interplay between Vimentin Filaments and Actin Arcs

Because plectin was among the hits of the BioID screen for Tm4 interaction partners, we examined the role of this cytoskeletal cross-linker in the interplay between arcs and vimentin filaments. Immunofluorescence microscopy revealed that although plectin

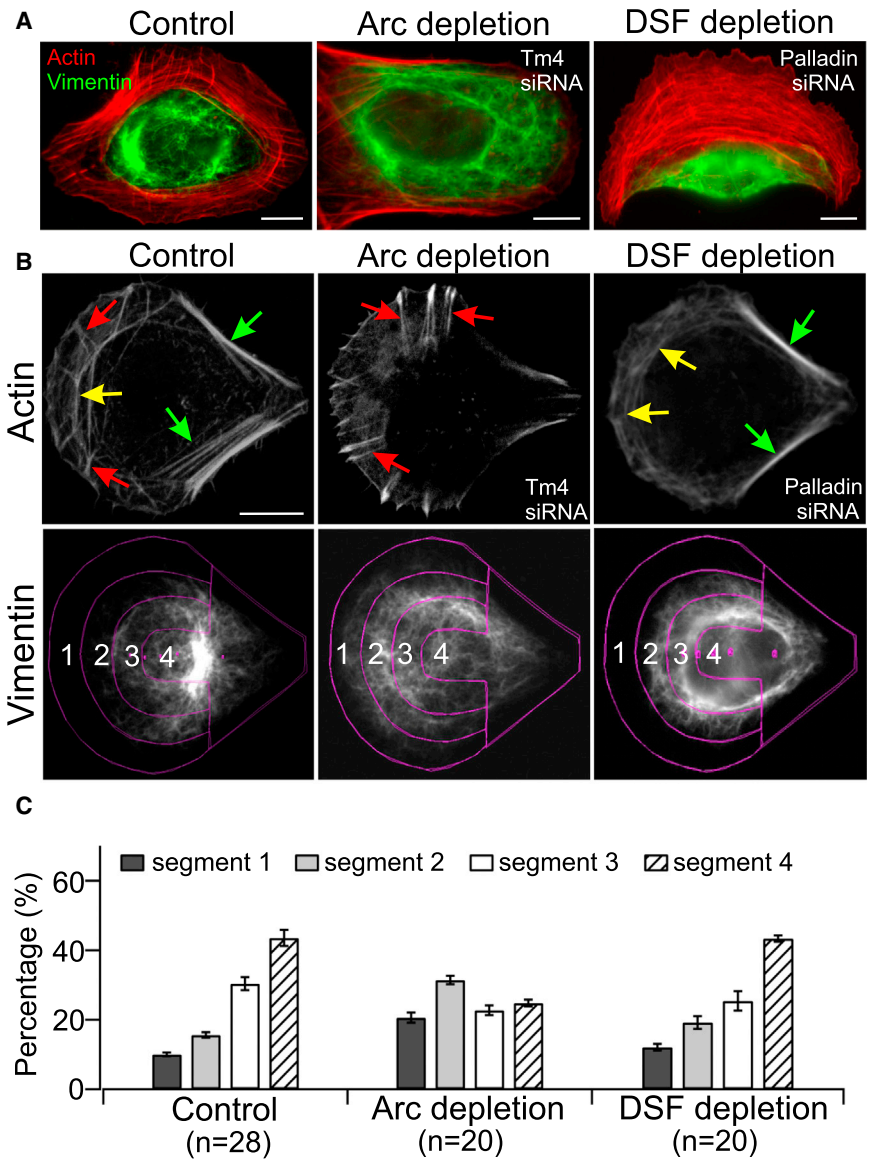


Figure 2. Transverse Arcs Regulate Subcellular Localization of the Vimentin Network

(A) Distinct localization patterns of vimentin in control, arc-depleted (Tm4 siRNA), and dorsal-stress-fiber-depleted (palladin siRNA) cells, stained with vimentin antibody (green) and fluorescent phalloidin (red) to visualize vimentin and F-actin, respectively. Scale bar, 10 μm.

(B) Localization of vimentin in control, arc-depleted, and dorsal-stress-fiber-depleted cells grown on "crossbow" shaped micropatterns. Cells were divided into four segments (purple lines in lower panels) from the leading edge (left) to cell rear (right). Red, yellow, and green arrows indicate examples of dorsal stress fibers, arcs, and ventral stress fibers, respectively. Scale bar, 10 μm.

(C) Quantification of vimentin intensity in four segments of control, arc-depleted, and dorsal-stress-fiber-depleted cells. In control and dorsal-stress-fiber-depleted cells, vimentin is largely excluded from the leading edge (segments 1 and 2), whereas in the absence of arcs, vimentin localizes more evenly across the cells. The data are presented as mean ± SEM.

See also Figures S2 and S3.

displayed relatively uniform punctual localization at the cytoplasm, it enriched to those vimentin filaments that aligned actomyosin stress fibers (Figure S4C). Depletion of plectin by siRNA did not result in gross effects on the actin cytoskeleton, and all three categories of stress fibers were readily visible (Figures 4A–4C). However, analysis of cells plated on crossbow-shaped micropatterns revealed that plectin depletion resulted in spreading of the vimentin network toward the cell edge similarly to the depletion of transverse arcs (Figures 4C and 4D). Thus, presence of plectin is required for functional interplay between vimentin intermediate filaments and actomyosin arcs.

DISCUSSION

Recent studies have revealed interplay between cytoplasmic intermediate filaments and the actin cytoskeleton in various cell-types (Huber et al., 2015). However, the identity of actin filament structures interacting with and controlling the intermediate filament network has remained obscure. Here, we reveal that (1) vimentin filaments associate and undergo retrograde flow with contractile transverse arcs; (2) integrity of arcs is required for correct perinuclear localization of the vimentin/nestin network; (3) vimentin filaments reciprocally control the retrograde flow of arcs, and consequently, vimentin depletion results in a small but significant decrease in lamella width in mesenchymal cells; and (4) the cytoskeletal cross-linker plectin is essential for functional interplay between vimentin and actomyosin arcs. Together, our results reveal a role for contractile stress fibers as regulators of the dynamics and localization of cytoplasmic intermediate filaments and uncover how the interplay between the actin cytoskeleton and intermediate filaments controls cell morphogenesis.

Three major components of the actin cytoskeleton undergo retrograde flow in migrating cells. From these, the retrograde flow of "lamellipodial actin networks" and dorsal stress fibers are powered by coordinated polymerization of Arp2/3- and formin-nucleated actin filament structures, whereas the retrograde flow of transverse arcs is driven by myosin-II-mediated contractility. Previous studies demonstrated that vimentin foci can associate with focal adhesions and suggested that the retrograde flow of keratin intermediate filaments is driven by focal-adhesion-attached dorsal stress fibers (Burgstaller et al., 2010; Kölsch et al., 2009). In contrast to these studies, our work

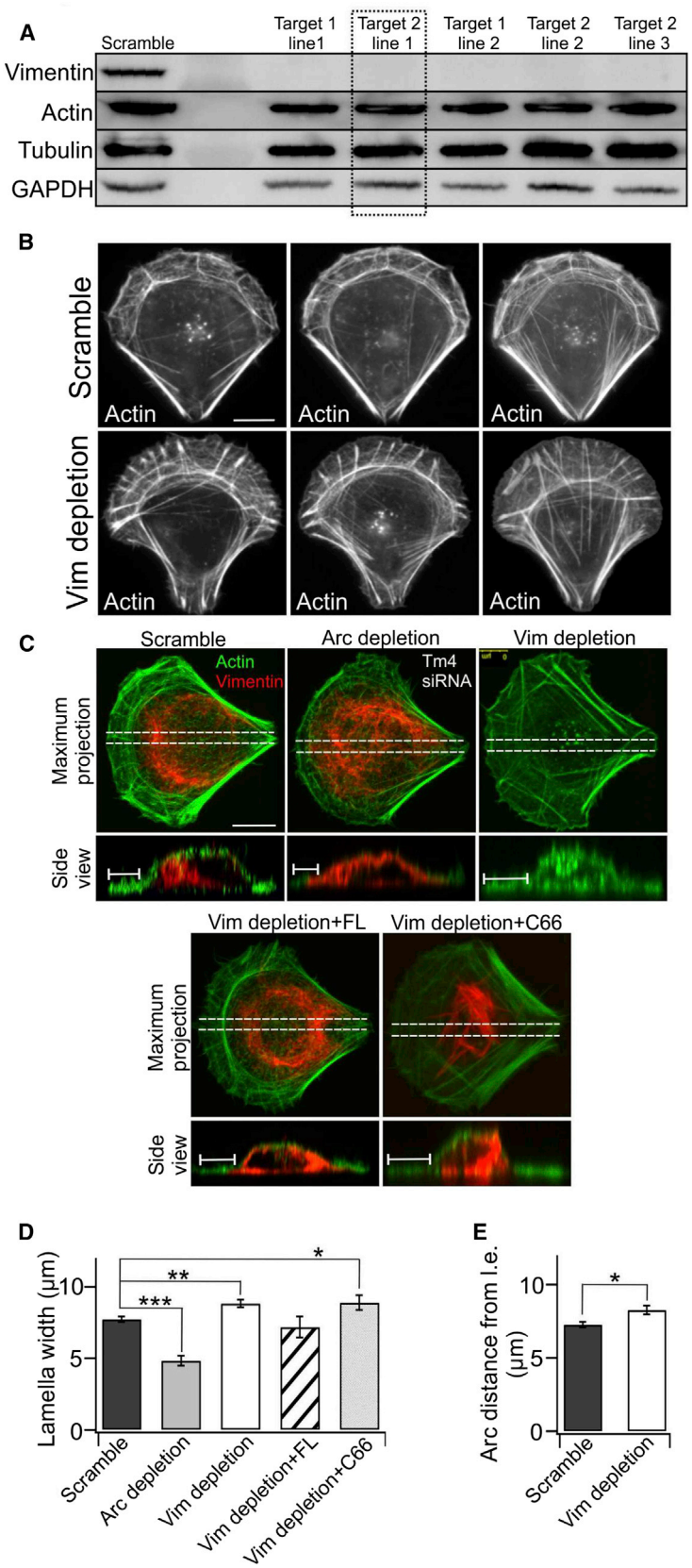


Figure 3. Effects of Vimentin Depletion on Stress Fiber Organization and Lamella Width

(A) Western blot analysis of vimentin expression levels in U2OS cell lines where the *vimentin* reading frame was disrupted by CRISPR/Cas using two target sequences. All vimentin-deficient lines displayed similar phenotypes, and target 2, line 1 was chosen for further analysis. The blot was also probed with actin, tubulin, and GADPH antibodies to verify equal sample loading.

(B) Three categories of stress fibers are preserved in vimentin-deficient cells plated on crossbow-shaped micropatterns and stained with fluorescent phalloidin. However, the arcs appear to span a wider region at the leading edge compared to control cells. Scale bar, 10 μm.

(C) Maximum z-projections (upper panels) and side views (lower panels) from 2 μm wide regions (indicated by white dashed lines in the upper panels) of confocal images from representative control (scramble CRISPR/Cas), arc-depleted (Tm4 RNAi), vimentin knockout, vimentin knockout/full-length (FL) vimentin rescue, and vimentin knockout/vimentin C66 mutant rescue cells. The lamellum was defined as a <2.5-μm-thick region at the leading edge of the cell (indicated by white lines in the side views). Bar, 10 μm.

(D) Quantification of lamella width. Arc deletion results in shortening of lamellum, whereas absence of vimentin results in widening of lamella. The latter phenotype can be rescued by expression of wild-type, but not C-terminally deleted (C66), vimentin. More than 25 cells were analyzed for each condition, and the data are presented as mean ± SEM.

(E) Distance of transverse arc network from the leading edge (l.e.) in control (scramble CRISPR/Cas) and vimentin knockdown (Vim depletion) cells. In the absence of vimentin, arcs can move further toward the cell center. Arc network positions were analyzed from 48 cells for both conditions, and the data are presented as mean ± SEM.

See also Figures S3 and S4.

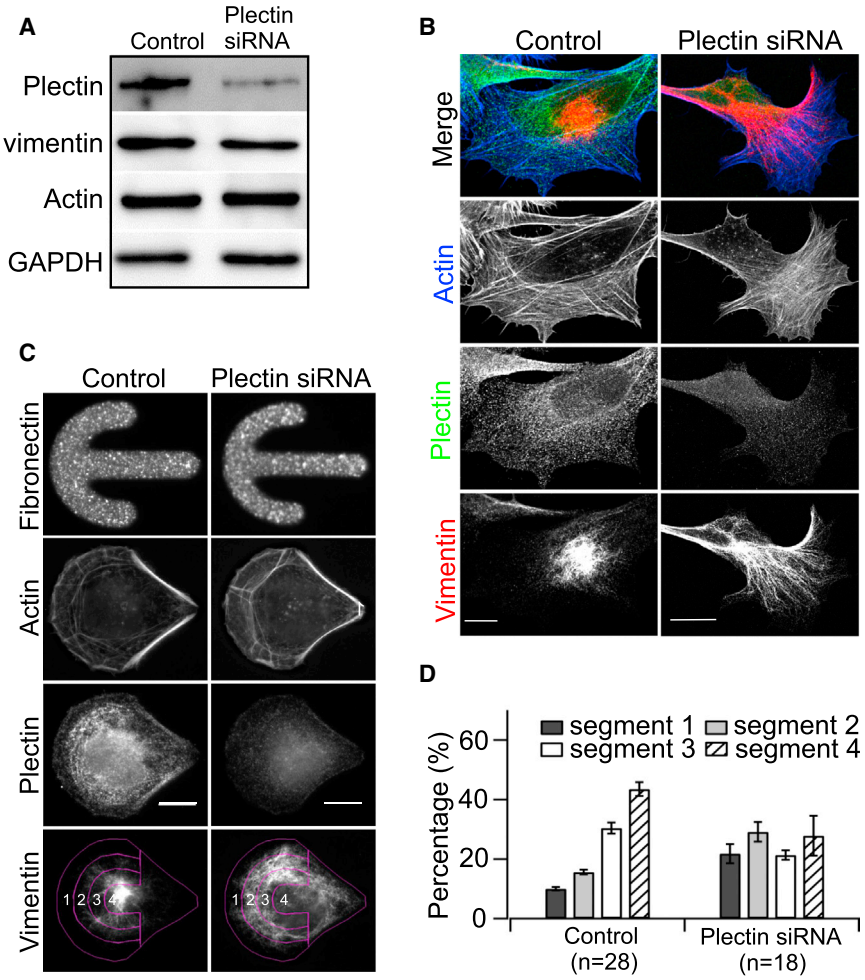


Figure 4. Plectin Is Required for Functional Interactions between Vimentin Filaments and Arcs

(A) Verification of the efficiency of plectin depletion by western blot with plectin antibody. The blot was also probed with vimentin, actin, and GADPH antibodies to verify equal sample loading.

(B) Control and plectin knockdown cells stained with phalloidin, plectin, and vimentin antibodies demonstrate expansion of the vimentin network in plectin RNAi cells. Scale bar, 10 μm.

(C) Comparison of vimentin localization divided into four segments in control and plectin knockdown cells grown on crossbow-micropatterned surfaces and stained additionally with fluorescent phalloidin and plectin antibodies. Note the co-localization of plectin with the contractile actomyosin arcs in control cells. Scale bar, 10 μm.

(D) Quantification of vimentin intensity in distinct segments as in Figure 2. Vimentin is largely excluded from segments 1 and 2 in control cells but displays nearly uniform distribution between all segments in plectin knockdown cells. The data are presented as mean ± SEM.

See also Figure S4.

provides evidence that cytoplasmic intermediate filaments associate and undergo retrograde flow with transverse arcs. These contractile actomyosin bundles assemble at the leading edge and undergo myosin-II-mediated retrograde flow toward the cell center (Tojkander et al., 2012), and they are thus ideally suited for transporting intermediate filaments from the leading lamellum toward the perinuclear region. Because not all cell types contain a dense network of transverse arcs, these findings may also explain the differences in the localizations of cytoplasmic intermediate filament networks between different cell types. Our study also demonstrates that, at least in U2OS cells, dorsal stress fibers, which elongate toward the cell center through actin polymerization at focal adhesions (Hotulainen and Lappalainen, 2006), are not required for the retrograde movement of intermediate filaments. Thus, myosin-II-based contractility of arcs, instead of actin polymerization at focal adhesions, appears to provide the force for the retrograde movement of intermediate filaments.

Previous work demonstrated a role of plectins in coupling intermediate filaments to focal adhesions (Burgstaller et al., 2010; Gregor et al., 2014). However, contribution of plectins in functional interplay between myosin-II-containing stress fibers and intermediate filaments has not been reported. We show that in U2OS cells plectins co-localize with vimentin at contractile stress fibers and that the presence of plectins is essential for linking retrograde flow of arcs to perinuclear localization of cytoplasmic intermediate filaments. Our results differ from the ones by Dupin et al., (2011), which proposed that depletion of plectins does not contribute to actin-dependent reorganization of vimentin filaments in astrocytes after Ca^{2+} induction. These differences may arise from different experimental conditions (constitutive retrograde flow of arcs and intermediate filaments versus calcium-induced reorganization of the actin cytoskeleton and intermediate filament network) or from variation in the efficiency of plectin depletion by siRNA.

We revealed that intermediate filaments control the subcellular localization of arcs by restricting their flow to the perinuclear, vimentin-dense region. Consequently, depletion of vimentin leads to relatively modest but significant increase in the width of flat lamellum at the leading edge. Thus, vimentin, and perhaps also other intermediate filaments, control cell morphology indirectly by affecting subcellular localization of transverse arcs. Interestingly, previous study demonstrated that vimentin also functions as a negative regulator of lamellipodium formation through a yet-to-be-identified molecular mechanism (Helfand et al., 2011). Thus, vimentin filaments can control both protrusive lamellipodial actin filament arrays and contractile actomyosin bundles of lamellum. Our data also reveal that both vimentin and transverse arcs control the nuclear positioning in U2OS cells. While the absence of arcs and vimentin leads to forward and rearward localizations of the nucleus, respectively, simultaneous depletion of both cytoskeletal structures recapitulated the wild-type situation of nuclear

positioning. Thus, vimentin filaments and transverse arcs appear to control nuclear positioning at least partially independently from each other, with arcs pushing nuclei toward the cell rear, while vimentin filaments pulling the nuclei toward the leading edge. In the future, it will be interesting to examine whether, in addition to Cdc42-induced actin cables (Gundersen and Worman, 2013), nuclear envelope components nesprin-2G and SUN2 can also associate with transverse arcs or if these contractile actomyosin bundles control nuclear movement and positioning through other protein-protein interactions or steric effects.

Collectively, our results reveal functional interplay between contractile actomyosin arcs, and vimentin filaments. While the two cytoskeletal structures affect each other's subcellular localization and motility, they do not appear to affect each other's assembly, because all three types of stress fibers are intact in vimentin-deficient cells and vimentin filaments appear to assemble normally in cells devoid of arcs. In the future, it will be important to examine the role of intermediate filament networks, and their interplay with contractile actomyosin bundles, in cell morphogenesis in a three-dimensional tissue environment, as well as to uncover the molecular mechanisms by which plectin mediates interaction between vimentin and tropomyosin-rich actomyosin arcs. Furthermore, because stress fibers are mechanosensitive structures (Discher et al., 2005; Bershadsky et al., 2006) and intermediate filaments, among other functions, provide mechanical integrity to cells, it will be interesting to study the role of stress fiber/intermediate filament interplay in mechanosensing and consequent morphological processes.

EXPERIMENTAL PROCEDURES

Immunofluorescence microscopy and live-cell imaging were performed as described previously (Hotulainen and Lappalainen, 2006; Tojkander et al., 2011). Identification of Tm4 interaction partners was carried out using the BioID method (Roux et al., 2012). Detailed protocols for these experiments and description of cell culture conditions, image analysis, plasmids, and antibodies are described in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, one table, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.05.008.

AUTHOR CONTRIBUTIONS

Y.J., M.V., J.E., and P.L. designed the study. Y.J., J.L., S.T., H.J., X.L., and M.V. performed the experiments and analyzed results. F.C. and J.E.E. provided reagents. Y.J., J.L., and P.L. wrote the paper.

ACKNOWLEDGMENTS

We thank Ville Paavilainen and Johan Peränen for discussions and critical reading of the manuscript and Kimmo Tanhuanpää (Viikki Light Microscopy Unit) for advice on data analysis. This study was funded by the Sigrid Juselius Foundation, Biocentrum Helsinki, and the Academy of Finland (to P.L.). J.L. was supported by a fellowship from the Integrative Life Science Doctoral Program.

Received: February 2, 2015
Revised: April 21, 2015
Accepted: May 4, 2015
Published: May 28, 2015

REFERENCES

Bershadsky, A., Kozlov, M., and Geiger, B. (2006). Adhesion-mediated mechanosensitivity: a time to experiment, and a time to theorize. Curr. Opin. Cell Biol. *18*, 472–481.

Burgstaller, G., Gregor, M., Winter, L., and Wiche, G. (2010). Keeping the vimentin network under control: cell-matrix adhesion-associated plectin 1f affects cell shape and polarity of fibroblasts. Mol. Biol. Cell *21*, 3362–3375.

Burnette, D.T., Shao, L., Ott, C., Pasapera, A.M., Fischer, R.S., Baird, M.A., Der Loughian, C., Delanoe-Ayari, H., Paszek, M.J., Davidson, M.W., et al. (2014). A contractile and counterbalancing adhesion system controls the 3D shape of crawling cells. J. Cell Biol. *205*, 83–96.

Burridge, K., and Wittchen, E.S. (2013). The tension mounts: stress fibers as force-generating mechanotransducers. J. Cell Biol. *200*, 9–19.

Busch, T., Armacki, M., Eiseler, T., Joodi, G., Temme, C., Jansen, J., von Wichert, G., Omary, M.B., Spatz, J., and Seufferlein, T. (2012). Keratin 8 phosphorylation regulates keratin reorganization and migration of epithelial tumor cells. J. Cell Sci. *125*, 2148–2159.

Chu, Y.W., Runyan, R.B., Oshima, R.G., and Hendrix, M.J. (1993). Expression of complete keratin filaments in mouse L cells augments cell migration and invasion. Proc. Natl. Acad. Sci. USA *90*, 4261–4265.

Correia, I., Chu, D., Chou, Y.H., Goldman, R.D., and Matsudaira, P. (1999). Integrating the actin and vimentin cytoskeletons: adhesion-dependent formation of fimbrin-vimentin complexes in macrophages. J. Cell Biol. *146*, 831–842.

Discher, D.E., Janmey, P., and Wang, Y.L. (2005). Tissue cells feel and respond to the stiffness of their substrate. Science *310*, 1139–1143.

Dupin, I., Sakamoto, Y., and Etienne-Manneville, S. (2011). Cytoplasmic intermediate filaments mediate actin-driven positioning of the nucleus. J. Cell Sci. *124*, 865–872.

Eriksson, J.E., Dechat, T., Grin, B., Helfand, B., Mendez, M., Pallari, H.M., and Goldman, R.D. (2009). Introducing intermediate filaments: from discovery to disease. J. Clin. Invest. *119*, 1763–1771.

Esue, O., Carson, A.A., Tseng, Y., and Wirtz, D. (2006). A direct interaction between actin and vimentin filaments mediated by the tail domain of vimentin. J. Biol. Chem. *281*, 30393–30399.

Gateva, G., Tojkander, S., Koho, S., Carpen, O., and Lappalainen, P. (2014). Palladin promotes assembly of non-contractile dorsal stress fibers through VASP recruitment. J. Cell Sci. *127*, 1887–1898.

Gregor, M., Osmanagic-Myers, S., Burgstaller, G., Wolfram, M., Fischer, I., Walko, G., Resch, G.P., Jörgl, A., Herrmann, H., and Wiche, G. (2014). Mechanosensing through focal adhesion-anchored intermediate filaments. FASEB J. *28*, 715–729.

Gundersen, G.G., and Worman, H.J. (2013). Nuclear positioning. Cell *152*, 1376–1389.

Helfand, B.T., Mendez, M.G., Murthy, S.N., Shumaker, D.K., Grin, B., Mahammad, S., Aebi, U., Wedig, T., Wu, Y.I., Hahn, K.M., et al. (2011). Vimentin organization modulates the formation of lamellipodia. Mol. Biol. Cell *22*, 1274–1289.

Hollenbeck, P.J., Bershadsky, A.D., Pletjushkina, O.Y., Tint, I.S., and Vasiliev, J.M. (1989). Intermediate filament collapse is an ATP-dependent and actin-dependent process. J. Cell Sci. *92*, 621–631.

Hotulainen, P., and Lappalainen, P. (2006). Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. J. Cell Biol. *173*, 383–394.

Huber, F., Boire, A., López, M.P., and Koenderink, G.H. (2015). Cytoskeletal crosstalk: when three different personalities team up. Curr. Opin. Cell Biol. *32*, 39–47.

Ivaska, J., Pallari, H.M., Nevo, J., and Eriksson, J.E. (2007). Novel functions of vimentin in cell adhesion, migration, and signaling. Exp. Cell Res. *313*, 2050–2062.

Kaksonen, M., Toret, C.P., and Drubin, D.G. (2006). Harnessing actin dynamics for clathrin-mediated endocytosis. Nat. Rev. Mol. Cell Biol. *7*, 404–414.

Kölsch, A., Windoffer, R., and Leube, R.E. (2009). Actin-dependent dynamics of keratin filament precursors. Cell Motil. Cytoskeleton *66*, 976–985.

Korobova, F., Ramabhadran, V., and Higgs, H.N. (2013). An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. Science *339*, 464–467.

Loschke, F., Seltmann, K., Bouameur, J.E., and Magin, T.M. (2015). Regulation of keratin network organization. Curr. Opin. Cell Biol. *32*, 56–64.

Pollard, T.D., and Cooper, J.A. (2009). Actin, a central player in cell shape and movement. Science *326*, 1208–1212.

Ralston, E., Lu, Z., Biscocho, N., Soumaka, E., Mavroidis, M., Prats, C., Lømo, T., Capetanaki, Y., and Ploug, T. (2006). Blood vessels and desmin control the positioning of nuclei in skeletal muscle fibers. J. Cell. Physiol. *209*, 874–882.

Roux, K.J., Kim, D.I., Raida, M., and Burke, B. (2012). A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J. Cell Biol. *196*, 801–810.

Schoumacher, M., Goldman, R.D., Louvard, D., and Vignjevic, D.M. (2010). Actin, microtubules, and vimentin intermediate filaments cooperate for elongation of invadopodia. J. Cell Biol. *189*, 541–556.

Sjöberg, G., Jiang, W.Q., Ringertz, N.R., Lendahl, U., and Sejersen, T. (1994). Colocalization of nestin and vimentin/desmin in skeletal muscle cells demonstrated by three-dimensional fluorescence digital imaging microscopy. Exp. Cell Res. *214*, 447–458.

Snider, N.T., and Omary, M.B. (2014). Post-translational modifications of intermediate filament proteins: mechanisms and functions. Nat. Rev. Mol. Cell Biol. *15*, 163–177.

Svitkina, T.M., Verkhovsky, A.B., and Borisy, G.G. (1996). Plectin sidearms mediate interaction of intermediate filaments with microtubules and other components of the cytoskeleton. J. Cell Biol. *135*, 991–1007.

Tojkander, S., Gateva, G., Schevzov, G., Hotulainen, P., Naumanen, P., Martin, C., Gunning, P.W., and Lappalainen, P. (2011). A molecular pathway for myosin II recruitment to stress fibers. Curr. Biol. *21*, 539–550.

Tojkander, S., Gateva, G., and Lappalainen, P. (2012). Actin stress fibers—assembly, dynamics and biological roles. J. Cell Sci. *125*, 1855–1864.

Vignaud, T., Galland, R., Tseng, Q., Blanchoin, L., Colombelli, J., and Théry, M. (2012). Reprogramming cell shape with laser nano-patterning. J. Cell Sci. *125*, 2134–2140.

Vikstrom, K.L., Lim, S.S., Goldman, R.D., and Borisy, G.G. (1992). Steady state dynamics of intermediate filament networks. J. Cell Biol. *118*, 121–129.

Wiche, G., Osmanagic-Myers, S., and Castañón, M.J. (2015). Networking and anchoring through plectin: a key to IF functionality and mechanotransduction. Curr. Opin. Cell Biol. *32*, 21–29.