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Intermediate filaments and the regulation of focal adhesion

Rudolf E Leube, Marcin Moch and Reinhard Windoffer

Focal adhesions are localized actin filament-anchoring signalling centres at the cell–extracellular matrix interface. The currently emerging view is that they fulfil an all-embracing coordinating function for the entire cytoskeleton. This review highlights the tight relationship between focal adhesions and the intermediate filament cytoskeleton. We summarize the accumulating evidence for direct binding of intermediate filaments to focal adhesion components and their mutual cross-talk through signalling molecules. Examples are presented to emphasize the high degree of complexity of these interactions equipping cells with a precisely controlled machinery for context-dependent adjustment of their biomechanical properties.

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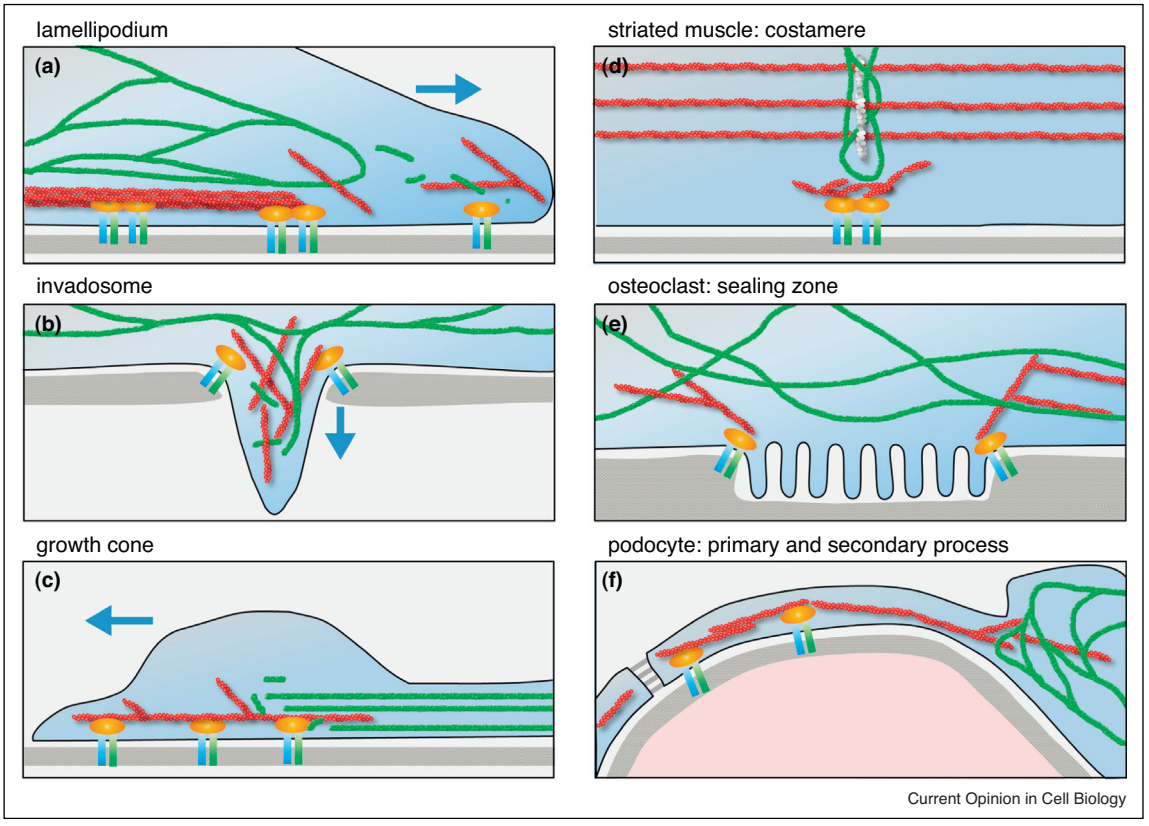
Introduction

The metazoan cytoskeleton fulfils two seemingly incompatible tasks. It provides both cellular rigidity and flexibility. This challenge is met in part by biomechanically unique filamentous components that are expressed in cell type-specific admixtures thereby supporting a broad spectrum of cellular properties ranging from static/resilient to dynamic/fragile for engagement in various tissue functions. Situations requiring rapid changes in cellular mechanics, however, require additional mechanisms. One such situation is the conversion of a sessile, tightly integrated epithelial cell into a highly motile migratory cell upon wounding or malignant transformation. The change is brought about by activation and formation of cell surface receptors, which cluster together with cytoplasmic proteins to build focal adhesions (FAs) at the cell–extracellular matrix (ECM) interface. FAs induce major alterations in cytoskeletal organization: they promote localized actin stress fibres to provide pulling and pushing forces in conjunction with myosin motors and they redirect microtubules to facilitate polarized trafficking [1,2]. Comparatively little is known, however, in which way and how FA formation affects the intermediate filament (IF) cytoskeleton and vice versa. We will present examples for the growing evidence of direct molecular links and for signalling molecule-mediated cross talk between FA components and IFs in different functional settings supporting context-dependent dynamic and static interactions.

Functional and structural diversity of focal adhesion–cytoskeletal interactions

FAs consist of localized membrane-associated multicomponent complexes, which couple the actin cytoskeleton to the ECM and serve as major mechanosensory signalling nodes to control multiple cell functions [1–3]. The dynamics of FAs have been well characterized in cultured cells. They appear as nascent adhesions of less than 0.25 μm diameter in active lamellipodia, enlarge into focal complexes at the lamellipodial-lamellar transition zone and give rise to bona fide focal adhesions, which further mature into fibrillar adhesions [1,2,4•]. At the core of these different FA types are clustered integrin receptors in the plasma membrane linking the ECM to the cell interior. A variable ensemble of proteins regulates cytoskeletal filament attachment and dynamics and also generates signals to modulate other cell functions [1,2,4•]. The wealth of knowledge on these prominent structures in cultured cells is contrasted by rather limited insights into the functional diversity of related structures *in vivo* (Figure 1). For example, they have been described in podosomes and invadopodia, together referred to as invadosomes, supporting migration of fibroblasts, immune cells or epithelial cells through ECM and have been noted in neuronal growth cones guiding axonal outgrowth. Compositionally similar structures function in other cells as stabilizing architectural devices, for example, costameres in striated muscle, pedicle basement membrane attachments in renal podocytes and the sealing zones of osteoclasts. Only few reports are available on the IF organization in these different scenarios (e.g., [5–8,9•,10]). But an emerging theme is that IFs are backbones of the respective structures serving as counter bearings for local actomyosin-related forces. It is obvious that the structural diversity and functionality of the different FA types must be reflected by specialized molecular and organizational principles involving the cytoskeletal filament networks in a context-dependent fashion. The isotype-specific and cell

Figure 1



Coordination between actin-rich FA-like structures and the IF cytoskeleton may either drive cell motility (a–c) or stabilize cell-ECM association (d–f). **(a)** Migrating cells develop lamellipodia, which foster the formation of nascent focal complexes at the lamellipodial-lamellar transition zone. These adhesions assemble actin filaments and support the assembly of IFs. **(b)** Invadosomes facilitate invasion of cells into the ECM, which is degraded with the help of secreted proteases. FAs are localized in a ring-like fashion at their base. **(c)** Axonal pathfinding is coupled to the formation of growth cones, which are characterized by a well-developed actin cytoskeleton in conjunction with defined ECM contact sites. Axonal IFs extend a central core presumably recruiting filament particles at the tips. **(d)** Striated muscle cells anchor their contractile apparatus at Z-disks that are laterally attached to the ECM through prominent adhesion sites referred to as costameres. They are associated with actin filaments and connect to desmin IFs via multiple linkers. **(e)** Osteoclasts are tightly bound to bone ECM in the sealing zone through FA-like attachments. These may be stabilized by interaction with the vimentin IF cytoskeleton. The sealing zone surrounds microvilli-rich osteclast membrane domains abutting the resorptive pit that is devoted to matrix degradation. **(f)** Renal podocytes extend multiple secondary processes, which are tightly attached to the basement membrane through FA-like structures and encase the intercellular filtration slit. The IF cytoskeleton serves as a stabilizer in the adjacent primary processes. Green filaments, IFs; red filaments, actin microfilaments; blue-green rods, integrins; orange-yellow ellipsoids, integrin-associated FA proteins.

type-specifically regulated IF cytoskeletons are manifestations of this complexity.

Direct molecular links between intermediate filaments and focal adhesions

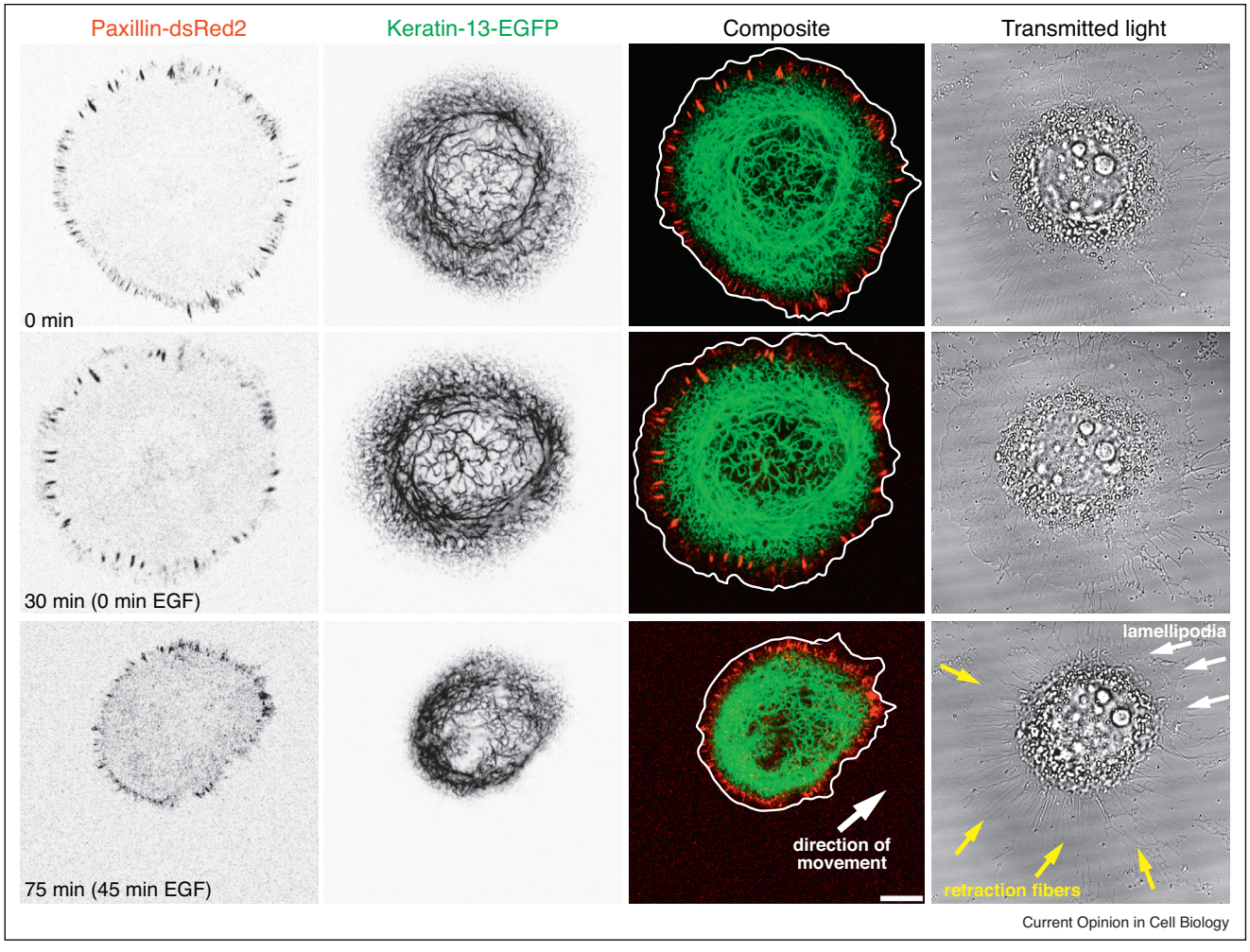
In mesenchymal cells type III vimentin IFs interact directly with the FA-specific cytoskeletal cross linker plectin splice variant 1f [11,12]. Time-lapse fluorescence recordings revealed that motile vimentin precursors are captured by plectin 1f-positive FAs and subsequently serve as seeds for IF network biogenesis and growth [11]. IF polymerization reduces FA turnover. FAs mature into fibrillar adhesions in the cell centre where they anchor the rather stable perinuclear vimentin cage [11]. IF-FA cross talk is further modulated by binding of vimentin to integrins [13–15] and to the actin-binding cytoskeletal cross linker filamin A [16–18]. These interactions also involve protein kinase C which has additional consequences for cell adhesion, spreading and migration [14–18].

Particularly intriguing are the links between the broadly expressed IF proteins α and β synemin and FA components. Synemins, also referred to as desmuslins, are synthesized in many different tissues and cannot assemble on their own into filaments but co-assemble efficiently with type III IFs [19]. On the other hand, it has been demonstrated that synemins bind to multiple FA components including talin [20,21], vinculin/metavinculin [20,22], plectin [23], α-actinin [24], and zyxin [25] as well as to costameric α-dystrobrevin [26] and dystrophin/utrophin [27]. In striated muscle synemins may thereby

serve as a bridge between desmin type III IFs and costameres [23]. Interestingly, overexpression of the β-synemin carboxyterminus comprising the zyxin binding site led to zyxin-depletion of FA sites resulting in reduced adhesion and cell motility [25]. In GFAP type III IF-expressing astrocytoma cells synemins are localized to the leading edge and their down regulation slows down migration [28]. A fascinating possibility is that synemins not only function as static IF anchors but also serve as active IF nucleation sites.

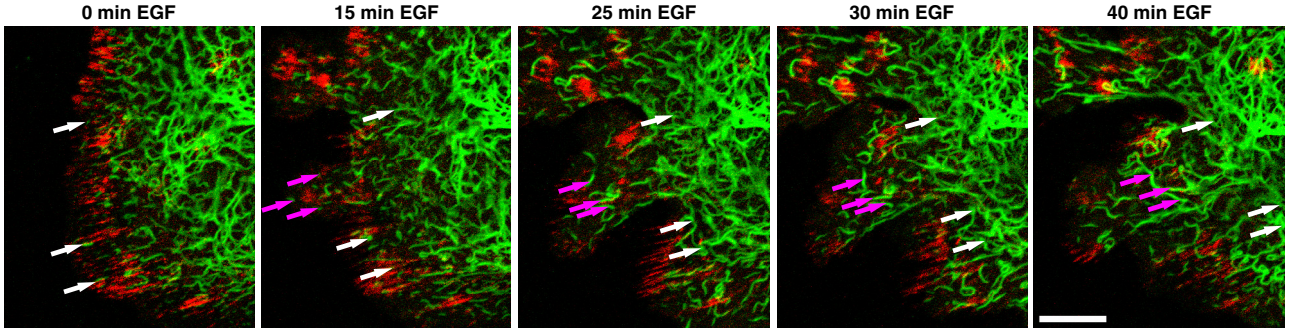
The available evidence from co-localization and double fluorescence *in vivo* monitoring indicates that interactions between keratins and FAs are rare and only transient in epithelial cells, although keratins are known to bind directly to plectin [29,30]. A spatial correlation between focal adhesions and the appearance of keratin filament particles has been noted [31]. The images presented in Figures 2 and 3 and the corresponding Videos 1 and 2 show the emergence of keratin particles in close vicinity of FAs. They subsequently enlarge, fuse with each other and move toward the cell interior where they integrate into the peripheral network. Addition of epidermal growth factor leads to increased cell motility with novel FAs in extending lamellipodia and enhanced keratin particle formation [4•,32•].

Figure 2



Keratin IF network organization and dynamics are coupled to FAs. The images are taken from a live cell recording (Video 1) of an epithelial vulva carcinoma-derived A431 cell clone producing the fluorescently tagged FA protein paxillin (Paxillin-dsRed2) and keratin 13 (Keratin 13-EGFP). The images show the recorded fluorescence signals in inverse black and white presentation and as merged color micrographs (Composite) together with the corresponding contrast micrographs at right (Transmitted light). Treatment with 50 ng ml^{-1} EGF resulted in cell polarization (white arrow) with overall reorganization of FAs and the keratin cytoskeleton. Note the increased FAs and keratin dynamics at the leading edge (white arrows) that is also characterized by high ruffling activity and lamellipodia formation. By contrast, long retraction fibres develop at the trailing edge, where FAs disassemble and the keratin network compacts (yellow arrows). Bar, 10 μm.

Figure 3



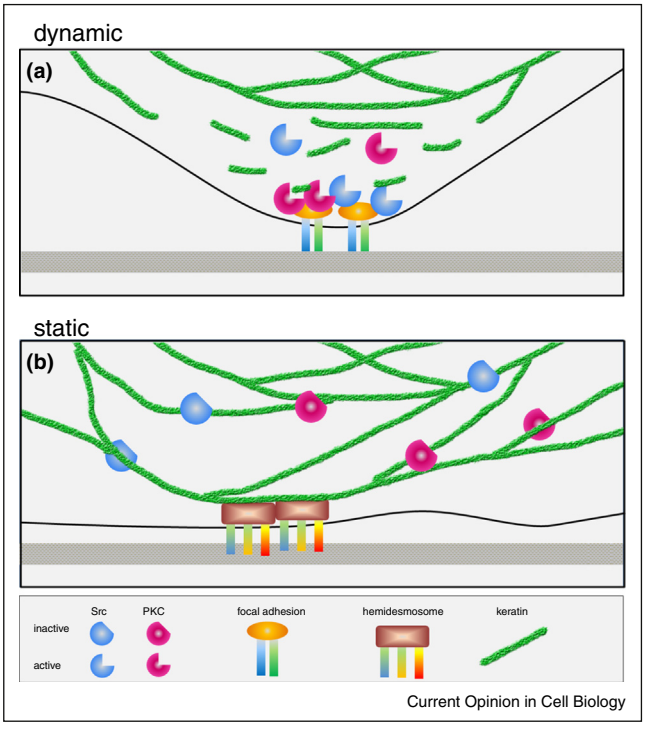
EGF increases lamellipodia formation with abundant and highly dynamic FAs and nascent keratin IF particles, which enlarge, fuse and attach to the peripheral IF cytoskeleton. The composite fluorescence micrographs are taken from Video 2. They depict fluorescent keratin IFs (Keratin 13-EGFP; green) and FA protein paxillin (Paxillin-dsRed2; red) in the periphery of a vulva carcinoma-derived A431 cell. Arrows track newly-formed keratin particles, which grow, integrate into the keratin network and are transported toward the cell interior. Note the appearance of large lamellipodia with new FAs and multiple KF particles upon EGF addition Bar, 10 μm.

Cross talk between intermediate filaments and focal adhesions by signalling molecules

Although direct linkage between IFs and FAs may prevail in some situations, signal mediated cross talk appears to determine mutual regulation in other situations. Triggers are posttranslational modifications that are particularly abundant in the IF system and are known to be generated through FA signalling [33]. Posttranslational modifications affect IF assembly states, turnover dynamics and interactions with associated proteins and thereby modulate the IF scaffolding function. Thus, IF modification-induced and assembly-dependent sequestration or release of signalling molecules controls a broad spectrum of cellular reactions. The two following examples illustrate aspects of this mechanism (Figure 4):

- Keratin 6 has been shown to bind inactive Src kinase [34•]. Deletion of keratins 6a and 6b therefore increased active Src kinase with coincident tyrosine phosphorylation of Src substrates including the FA components focal adhesion kinase (FAK), p130Cas and paxillin resulting in elevated motility and migration [34•]. These observations were taken as an explanation for the negative effect of keratin 6 on keratinocyte migration during wound repair.
- In keratin-free keratinocytes PKCα activity is elevated because of increased cytoplasmic receptor for activated C kinase 1 (RACK1), which is usually bound to keratins [35]. PKCα is known to regulate FA dynamics, especially during cell spreading [36]. Similarly, Bordeleau *et al.* [37] observed altered kinetics of complex formation between RACK1, PKC, β1 integrin, plectin and Src complex in keratin-depleted hepatocytes with effects on FAK residency in FAs, adhesion and migration. Interestingly, Dave *et al.* [38••] recently

Figure 4



The FA-mediated plasticity increase of the keratin IF cytoskeleton **(a)** and hemidesmosome-mediated stabilization of the keratin IF cytoskeleton **(b)** are mutually interdependent and linked to signalling. Keratin IFs (green) serve as scaffolds for sequestration of signalling molecules such as Src and PKC (through RACK1). Modulation of the keratin cytoskeleton results in release and activation of these signalling molecules which act on FA and keratin organization and dynamics.

reported also on a multimeric complex between vimentin and RACK1, which, in addition, included FAK and was enhanced upon invasion of endothelial cells. The association of vimentin and FAK was shown to be dependent on RACK1 and needed for vessel sprouting. Focal contact formation was downregulated upon silencing of vimentin and RACK1. Similar mechanisms have been described for plectin-deficient fibroblasts and keratinocytes [39,40]. The observations indicate that the absence of plectin abrogates RACK1 sequestration on the respective vimentin and keratin IF scaffolds thereby contributing to deregulated PKC signalling.

It should be stressed, that the signalling between IFs and FAs is bidirectional. For example, analysis of the transport of assembly-incompetent mutant vimentin recently revealed that FA signalling affects vimentin dynamics. The Rho-kinase ROCK reduced microtubule-dependent transport of vimentin particles whereas the antagonistic GTPase-regulated p21-activated kinase PAK enhanced it [41•]. Both types of enzyme, which have been localized to FAs [3], have been shown to phosphorylate vimentin [42].

Coordination between extracellular matrix adhesions and intermediate filaments in motile and migrating cells

In epithelial cells, the epidermal growth factor (EGF)-stimulated transition from the sessile to a motile polarized phenotype has been subject of numerous studies and has been shown to induce substantial, though poorly characterized re-structuring of the keratin cytoskeleton (cf. [4•,43]). To better understand the consequences of growth factor stimulation on keratin network organization and dynamics, tools were developed to quantitatively measure motility and turnover of keratin IFs in living cells [32•]. With the help of these tools it was demonstrated that inward-directed transport of keratins originating in the vicinity of FAs is upregulated upon EGF application. This coincided with an increased turnover rate because of elevated assembly in the cell periphery and increased disassembly in the perinuclear domain. At the same time, phosphorylation of keratins was significantly enhanced. The underlying signalling pathways remain to be worked out. One possibility is that increased FAK signalling is involved, since EGF induces Src-FAK signalling [44–46]. At the same time, ERK1/2 activation by EGF leads to the mobilisation of α6β4 integrins from hemidesmosomes by integrin phosphorylation [47,48]. Similar effects can also be induced by PKC activation [47]. Interestingly, PKC-dependent phosphorylation has been shown to regulate the formation of an actin/keratin/14-3-3 complex which supports breast tumour invasion by providing a pool of available complexes for polarized network assembly [49••]. Conversely, activation of the integrin-associated nucleotide receptor P2Y2R has been shown to counteract EGF signal transduction by stabilising α6β4 integrin, plectin, BPAG1, BPAG2 and CD151 in hemidesmosomal plaques [48].

Directed cell migration relies on the transmission of ECM signals to all of the cytoplasmic filament systems via FAs thereby ensuring a coordinated response. The hitherto neglected contribution of IFs to this process has entered the limelight through a number of recent studies (review in [43]). The selected examples in Figure 1 highlight that this cross talk can either result in a stabilization of FA–IF interaction employing direct proteinaceous links or may result in increased plasticity of the network through elevated component recycling. Thus, a finely graded response in cell shape and mechanics can be attained for distinct cytoskeletal-ECM coupling. Cell type and IF isotype further increase the cellular tool box. The diversity of interactions may explain why vimentin-deficient cells migrate slower [50,51] whereas keratin-deficient cells or cells producing keratin mutants present a pro-migratory phenotype [52,53•].

The physical linkage between IFs and FAs presumably serves to precisely sense alterations in force load and to elicit appropriate responses through associated signalling molecules. Thus, fluid shear is known to increase vimentin-rich focal contacts in endothelial cells [54]. This mechanosensory function was explored in a recent publication by Gregor *et al.* [55••]. In the absence of either vimentin or plectin, they noted an attenuated activation of FAK and its downstream targets Src, ERK1/2 and p38. This resulted in impaired directional migration presumably by diminished cytoskeletal tension. A model was put forward by which vimentin imposes physical constraints on actomyosin gels through its capacity to couple FAs with the peripheral vimentin network. The physiological relevance of vimentin–FA interaction was recently further unveiled in a wound repair model using explanted lens epithelium [56••]. The observation that local phosphorylation-dependent vimentin modifications affect lamellipodia formation [57] are also evidence of a pro-migratory function of vimentin.

A further layer of complexity in the regulation of cell migration is introduced in epithelial cells by hemidesmosomal adhesion which likely exerts an inhibitory effect (Figure 4; [59]). Thus, disruption of integrin α6β4-mediated hemidesmosomal IF anchorage by PKC-activation or wounding leads to release of α6β4 integrin into developing actin-rich membrane ruffles and lamellipodia and Rac1 activation to subsequently facilitate cell migration [60,61]. This may explain the observed increased motility of epithelial cells lacking an intact keratin cytoskeleton [52,53•]. In line, SPS-induced retraction of keratins resulted in altered mechanical properties and increased migration [58]. Furthermore, loss of plectin-mediated keratin anchorage at hemidesmosomes

leads to upregulation of MAPK signalling and increased migration [39]. For additional details on plectin's role in the regulation of hemidesmosomal organization in migrating cells see the article by Wiche and Osmanagic-Myers, in this issue. The complex interplay between hemidesmosomes and FAs in cell motility is also highlighted by observations in keratin 8-deficient hepatocytes [62]. They exhibit slowed down spreading and present defective distribution of vinculin in addition to altered plectin and RACK1 localization.

Synemin/zyxin interaction has been shown to support cell migration [25]. A close relationship between nestin type VI IF expression levels and migration was also reported [63]. Experimental down regulation of nestin was shown to induce a redistribution of phosphorylated FA kinase (pFAK/PTK2) to FAs and alterations in FA turnover [64•]. Levels of FA integrin α5β1 were increased at the plasma membrane, integrin β1 was activated and an overall increased integrin clustering was noted [64•]. All of this related to increased invasiveness.

An elegant example of IF–extracellular matrix interaction has been described for the outgrowth of the excretory canal in *Caenorhabditis elegans* [65••]. Here, the dense subapical IF-based cytoskeletal network was shown to contribute to straight lumen outgrowth. This and the other examples (e.g., Figure 1) underscore a role of the IF cytoskeleton as a stabilizing element and as a counter bearing for cell translocation.

A still poorly explored regulatory mechanism of IF–FA interaction is vesicle trafficking. Evidence has been presented that IFs affect vesicle trafficking and that this, in turn, has consequences for integrin deposition at the cell surface [14,15,18,62,66,67]. In support, recent evidence suggests that endocytotic recycling of junctional components is severely impaired in keratin-deficient keratinocytes [35,53•]. Inhibition of endocytotic membrane fission reversed the phenotype [35].

Conclusions

Coordination between IF networks and actin-rich FAs presents an amazing degree of complexity. The mutual interdependencies in structural organization and turnover kinetics are regulated by direct molecular links and bidirectional signalling. The resulting tool box enables cells to precisely adjust their shapes and biomechanical properties to changing microenvironments. A main challenge at present is the dissection of the cell type-specific and context-specific interaction patterns of the different IF proteins and FA components. This is particularly true for the different *in vivo* scenarios with their highly variable functionalities that are not only relevant for maintenance of normal cell and tissue physiology but also for disease, for example, wounding, metastasis and inflammation.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ceb.2014.09.011.

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- •• of outstanding interest

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