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REVIEW

Focal adhesion-mediated cell anchoring and migration: from *in vitro* to *in vivo*

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ABSTRACT

Cell-extracellular matrix interactions have been studied extensively using cells cultured *in vitro*. These studies indicate that focal adhesion (FA)-based cell-extracellular matrix interactions are essential for cell anchoring and cell migration. Whether FAs play a similarly important role *in vivo* is less clear. Here, we summarize the formation and function of FAs in cultured cells and review how FAs transmit and sense force *in vitro*. Using examples from animal studies, we also describe the role of FAs in cell anchoring during morphogenetic movements and cell migration *in vivo*. Finally, we conclude by discussing similarities and differences in how FAs function *in vitro* and *in vivo*.

KEY WORDS: Cell adhesion, Cell migration, Focal adhesion, Integrin

Introduction

The ability of cells to adhere to the extracellular matrix (ECM) is a fundamental property seen in most multicellular organisms. It allows cells to anchor or migrate, and plays essential roles in development (Brown et al., 2002), immunity (Weber et al., 2013), homeostasis (Hara et al., 2014) and disease (Schaffer et al., 2018; Cheung et al., 2013). These processes have been studied intensively and, as such, a detailed picture of how cells adhere to the ECM to firmly attach or pull themselves forward has emerged.

At sites of ECM adhesion, cells localize hundreds of proteins that can interact with each other as part of an 'adhesome' (Horton et al., 2015; Horton et al., 2016; Zaidel-Bar et al., 2007a). These macromolecular assemblies form electron-dense structures called focal adhesions (FAs; Fig. 1). Two essential FA components are integrins and talins. Integrins are transmembrane proteins consisting of one α subunit and one β subunit (Barczyk et al., 2010). There are multiple α and β subunits, and the specific pairing of the subunits determines which component on the outside of the cell a certain integrin heterodimer will bind to (Barczyk et al., 2010; Humphries, 1990). Integrin binding partners in the ECM and on adjacent cells comprise fibronectin, collagen, laminin and cell adhesion molecules (CAMs). On the cytoplasmic side, integrins bind to – among other proteins – talins, which connect integrins to the filamentous actin (F-actin) network of the cell (Legate and Fassler, 2009). When under tension, talins unfold to expose binding sites for vinculin, another F-actin binder. The recruitment of vinculin strengthens the bridge between the ECM, integrins and the F-actin network (Dedden et al., 2019; Wegener et al., 2007; Yao et al., 2016; Goult et al., 2018). These bridges allow cells to withstand and transmit tension. They are thus at the core of the ability of the cell to anchor to the outside when remaining in place and to pull themselves forward when migrating.

Classically, the role of FAs and integrin-mediated ECM adhesion in cell anchoring and cell migration has been investigated using cells cultured on two-dimensional substrates (Jockusch et al., 1995; Burridge and Chrzanowska-Wodnicka, 1996). FA-like structures have also been observed in cells cultured in three-dimensional environments (Kubow and Horwitz, 2011; Doyle et al., 2015), although not in all cases (Fraley et al., 2010). More recently, the role of FAs and integrins has also been studied *in vivo* (Green and Brown, 2019; Bouvard et al., 2013) using a variety of different techniques (see Box 1). These studies have complemented the findings of studies in cultured cells, but also uncovered that migrating cells and tissues do not require integrins in some settings (Paluch et al., 2016).

In this Review, we do not focus on the molecular machinery that mediates the interaction between cells and the ECM; we refer the reader to excellent reviews on this topic (Huveneers and Danen, 2009; Kechagia et al., 2019; Yamada and Sixt, 2019; Sun et al., 2019; Moreno-Layseca et al., 2019). Rather, we review the formation and function of FAs in cultured cells and discuss how forces are transmitted across FAs. We then compare the role of FAs in cell anchoring and migration in cultured cells and in animals, and discuss possible reasons for similarities and differences in how FAs are used in these contexts.

Focal adhesion formation during cell anchoring and migration *in vitro*

When cells spread or migrate on two-dimensional substrates, they form FAs (Fig. 1). FA formation is initiated through the binding of integrins to their ligands on the substrate (Sun et al., 2019). Within 1 to 3 min of substrate exposure, adhering cells assemble clusters of around 50 ligated integrins. These clusters, which grow to a uniform size of about 100 nm in diameter (Fig. 2), are termed nascent adhesions (Zaidel-Bar et al., 2007b; Nayal et al., 2006; Changede et al., 2015; Choi et al., 2008; Yu et al., 2011). In migrating cells, actin filaments push unligated integrins into small clusters at the leading edge of the cell (Galbraith et al., 2007). In both cases, initiation of clustering requires actin polymerization (Changede et al., 2015; Zhang et al., 2014; Alexandrova et al., 2008), but precedes detectable tension buildup (Zhang et al., 2014). It also occurs independently of myosin II activity (Zhang et al., 2014; Choi et al., 2008; Changede et al., 2015; Schiller et al., 2013) and substrate rigidity (Changede et al., 2015). In addition, F-actin is recruited before talin to the membrane and accumulates at nascent adhesions after ligated integrins have formed clusters (Yu et al., 2011; Choi et al., 2008; Bachir et al., 2014; Rossier et al., 2012). Thus, nascent adhesions likely form in the absence of tension (Fig. 2). Many nascent adhesions are short-lived and disassemble within 2-3 min. Others recruit additional proteins, grow in size, and

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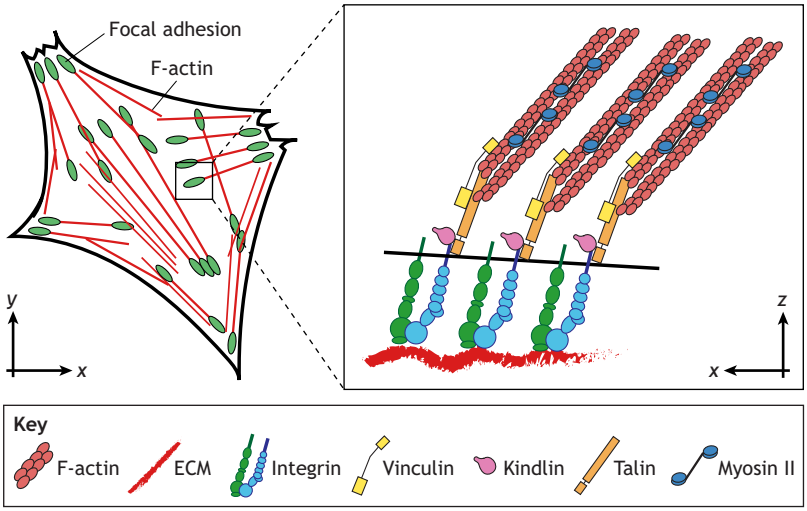

Fig. 1. Overview of focal adhesions. In culture, cells form focal adhesions in order to adhere to their substrate. This adhesion is mediated by integrins, which form a bridge between the extracellular matrix (ECM) outside the cell and the actomyosin network inside the cell, acting via various adaptor proteins.

mature into FAs (Fig. 2). The maturation of nascent adhesions into FAs requires myosin II, tension across the integrin-talin bridge between extracellular ligands and the intracellular actin network (Choi et al., 2008), and the integrin-binding adaptors kindlin (Orre et al., 2021; Bottcher et al., 2017; Theodosiou et al., 2016) and talin for substrate adhesion (Mathew et al., 2017; Zhang et al., 2008; Conti et al., 2009; Theodosiou et al., 2016; Atherton et al., 2015; Fischer et al., 2021a).

Ligand binding by integrins, and thus integrin activation, can be initiated from inside the cell (inside-out activation) or from outside the cell (outside-in activation) (Sun et al., 2019). Integrin heterodimers exist in three conformations: a bent-closed state, an extended-closed state or an extended-open state (Kim et al., 2011). Most integrins reside in the bent-closed state because this conformation is energetically favorable (Li and Springer, 2018; Li et al., 2017b). Importantly, the rare extended-open state displays a hundred- to thousand-fold higher ligand affinity than the other two states and, energetically, is slightly favored over the extended-closed state. During inside-out activation, G protein-coupled receptor signaling activates talin by relieving it from its closed auto-inhibitory conformation (Lagarrigue et al., 2016). Activated open talin then binds to integrin's cytoplasmic tail. This converts integrin from its inactive bent-closed to its active extended-open confirmation, which then engages with the ECM (Su et al., 2016; Springer and Dustin, 2012; Luo et al., 2007).

During outside-in activation, the rare extended-closed confirmation of integrin becomes stabilized by talin binding to its cytoplasmic tail and can convert to the energetically favored, high ligand-affinity extended-open confirmation. Upon ligand binding, transduction of small forces across the integrin-ligand complex then slightly separates the α and β integrin subunits and shifts the equilibrium strongly in favor of the extended-open confirmation. This renders integrin-mediated adhesion exquisitely sensitive to forces of 1-4 pN across the integrin-ligand complex (Li and Springer, 2018). Ligand engagement also causes more integrins to pause (up to 80 s) and not diffuse in the membrane (Rossier et al., 2012; Tsunoyama et al., 2018). This allows the activation and clustering of integrins to be regulated by the concentration of talin and other integrin effectors and by the forces applied across the integrin-ligand complex (Li et al., 2017a; Zhu et al., 2008). Such rapid activation and inactivation of integrins by force and adaptor proteins likely endows cells with the ability to make and break nascent adhesions rapidly during cell migration. When the forces across integrin-ligand complexes increase, integrin-ligand complexes show two responses: they strengthen their binding and they form larger clusters (Strohmeyer et al., 2017; Chang et al., 2016; Chen et al., 2010; Friedland et al., 2009; Kong et al., 2009; Rosetti et al., 2015; Benito-Jardon et al., 2017). Stronger binding through catch bonds – bonds that unbind slower with increasing force – counteracts bond slippage and bond rupture, whereas increased integrin clustering distributes the load across more integrin-ligand complexes and increases the likelihood of dissociated integrins rebinding, possibly aided by condensation of FA proteins (Wang et al., 2021).

Anchoring to the substrate can be resolved in several ways. The anchoring can break before integrins unbind their ECM ligand, resulting in membranous integrin footprints on the substrate (Laukaitis et al., 2001; Regen and Horwitz, 1992) or uptake of the ECM ligand still complexed with integrin by the cells (Sarkar et al., 2020). However, it is thought that integrin-ligand complexes mostly dissolve through integrin-ECM bond rupture, cleavage of complex components (Franco et al., 2004; Flevaris et al., 2007), and internalization (Mendoza et al., 2013; Ezratty et al., 2009; Ezratty et al., 2005). Additionally, bond dissolution is regulated by the recruitment of negative integrin effectors that stabilize integrin in its bent-closed confirmation (Webb et al., 2002; Parsons et al., 2010) or weaken the link to the actomyosin network (Sun et al., 2016b).

Force transmission and sensing across focal adhesions *in vitro*

The mechanical clutch model

FAs transmit force from inside the cell to the substrate outside the cell and thus mediate cell-substrate adhesion and cell motility (Case and Waterman, 2015). The force across FAs is largely generated by the cell's actin network, although low forces (of 3 pN or less) can be maintained across integrins independently of the actin cytoskeleton (Chang et al., 2016) and in the absence of detectable FAs (Reinhart-King et al., 2005). Actin filament assembly and elongation close to the cell membrane pushes out lamellipodial protrusions. Two mechanisms cause the elongating actin filaments to slide backward relative to the front of the protrusion: the physical resistance of the membrane and, in some cell types, myosin II-mediated contraction and disassembly of the actin network in the center and back of the cell (Renkawitz et al., 2009; Ponti et al., 2004; Lin and Forscher, 1995; Wilson et al., 2010; Yolland et al., 2019; Vallotton et al., 2004). The transmission of this 'actin retrograde flow' to the cell's

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Box 1. Techniques to study FA protein dynamics *in vivo*

Fuorescence recovery after photobleaching (FRAP)

This technique measures the rate of diffusion and trafficking of fluorescently tagged proteins. Upon photobleaching of a region, fluorescence recovery through transport of unbleached fluorescent proteins from regions neighboring the bleached region is monitored. By fitting the recovery of fluorescence to a model, one can calculate the mobile fraction and the diffusion rate of the fluorescently tagged protein. Using FRAP, the role of mechanical forces in FA component turnover at *Drosophila* myotendinous junctions was deciphered (Pines et al., 2012; Yuan et al., 2010; Hakonardottir et al., 2015) and the asymmetric sorting of integrins to basal cell sides of the optic cup was discovered (Bogdanovic et al., 2012).

Fluorescence correlation spectroscopy (FCS)

This approach can measure the concentration, diffusivity and oligomerization of fluorescently tagged proteins. Its extension to two proteins tagged with differently colored fluorescent proteins, termed fluorescence cross-correlation spectroscopy (FCCS), allows for the determination of protein-protein associations (Ries and Schwille, 2012). These techniques were recently used to determine the concentration of talin in *Drosophila* muscle (Lemke et al., 2019) and to show that inactive integrin α5 proteins from adjacent cells interact in the zebrafish mesoderm (Julich et al., 2015).

Förster resonance energy transfer by fluorescence lifetime imaging (FRET/FLIM)

An excited fluorescent protein (donor) can transfer energy to a non-excited fluorescent protein (acceptor); this is called Förster resonance energy transfer (FRET). The efficiency of energy transfer is, among other things, a function of the distance between the donor and the acceptor fluorescent protein, and thus a measure of this distance. This approach has been used to analyze the activated state of different integrin heterodimers in zebrafish embryos (Sun et al., 2021a).

FRET-based molecular tension sensors (TSs)

These were developed and validated in cultured cells (Fischer et al., 2021b), but, because they are genetically encodable, can be used in *in vivo* settings (Lemke et al., 2019; Lagendijk et al., 2017). As more proteins with TSs become available, our knowledge of tension across FA proteins will grow.

Endogenous protein tagging

Antibody staining against endogenous proteins provides static, but not dynamic, information. Moreover, fixation can distort tissues and protein distribution. Heterologous fluorescently tagged protein expression allows for the study of protein dynamics but may lead to protein mislocalization or mask protein localization because of high protein levels (Kubow and Horwitz, 2011). These caveats can be avoided by tagging proteins with fluorescent proteins at their endogenous locus. Owing to the rapid development of genome-editing techniques, more endogenously tagged FA proteins should become available in different model organisms (Lemke et al., 2019; Levic et al., 2021; Green et al., 2018).

Antibody labeling

Antibodies against the extracellular domain of integrin – or any other protein – can be used to follow protein internalization and trafficking *in vivo*. In the case of optic cup morphogenesis, embryos expressing the tail of integrin β1 fused to GFP on the extracellular domain were incubated with an anti-GFP antibody and the distribution of the GFP-integrin fusion protein was assessed, showing that the integrin β1 tail relocalizes to the basal sides of cells in this tissue (Bogdanovic et al., 2012).

substrate for cell anchoring or cell movement has been hypothesized to occur in a manner akin to a mechanical clutch (Fig. 3) (Mitchison and Kirschner, 1988). According to this hypothesis, FAs mediate transient interactions between the actin cytoskeleton and substrate-bound integrins. This interaction is proposed to be governed by two behaviors of the molecular players involved in force transmission. First, the actin network flows backward (at a constant speed of around 2-10 μm/min) when not interacting with FAs; interaction with FAs will slow and, if strong enough, stall actin retrograde flow (Fig. 3). Second, increasing the force across the ECM/integrin/adaptor/F-actin bonds changes their lifetimes and, if large enough, will break the weakest bonds. This is also true for catch bonds, which initially unbind more slowly with increasing force, but, once the force exceeds a specific threshold, also unbind faster. These properties cause the force transmission across FA proteins to behave in interesting ways. When FA proteins are not interacting with the F-actin network or ECM, no force is transmitted to the substrate and actin flow is unopposed. As ECM-bound FA proteins engage with the moving F-actin network, the FA proteins transmit force from the network to the ECM, slow actin retrograde flow, and – if the substrate is pliable – deform the substrate. With increasing force, greater tension builds across individual bonds and actin retrograde flow slows further. Eventually, tension becomes so high that a bond in the complex breaks. This increases the load across the remaining bonds, and thus increases the chance of the next bond to break. In this manner, higher loads shift to fewer remaining bonds. This ultimately causes the abrupt unbinding of all FA proteins. Without engaged FA proteins, force is not transmitted any longer, the actin retrograde flow resumes its original speed and, if elastic, the substrate relaxes back to its original shape, and force loading can start again (Case and Waterman, 2015; Chan and Odde, 2008; Elosegui-Artola et al., 2018).

The role of cellular properties and substrate stiffness in force transmission

The behavior of these 'load and fail' cycles depends on cellular properties and the rigidity of the substrate. Both factors influence the force loading rate – the speed at which force builds across engaged FA proteins – which in turn influences the efficiency of force transmission (see below). Reduced actin retrograde flow slows the loading rate (Elosegui-Artola et al., 2016; Bangasser et al., 2017). Conversely, reduced load sharing increases the loading rate. This happens as a result of reduced recruitment of integrins (Elosegui-Artola et al., 2014) or adaptor proteins (Elosegui-Artola et al., 2016; Andreu et al., 2021), reduced integrin clustering, or decreased ECM ligand density (Oria et al., 2017). Slowed loading rates favor engaged FAs whereas accelerated loading rates favor disengagement of FAs. In contrast to these cellular properties, the effect of the substrate's stiffness on the force loading rate is biphasic. There is an optimal substrate stiffness at which force transmission is maximal. Below this, the substrate is so soft that it provides little resistance to the pulling forces from engaged FA proteins. Therefore, the force across the FA proteins builds up so slowly that FA proteins unbind before they carry large loads. Under this regimen, force transmission from the actin network to the substrate is low.

At the optimal stiffness, the force across adhesion proteins builds up faster than the adhesion proteins unbind the substrate but slowly enough for them to recruit additional FA proteins. This distributes the load across more FA proteins such that each individual protein carries less of the total load of the entire FA, a phenomenon that likely underlies the increased counter-pulling by cells on beads with increasing load (Choquet et al., 1997). In this manner, the load across individual adhesion proteins builds up more slowly, and loads that cause abrupt FA protein unbinding are reached more slowly than in the absence of load sharing. Hence, force transmission from the actin network to the substrate is maximal.

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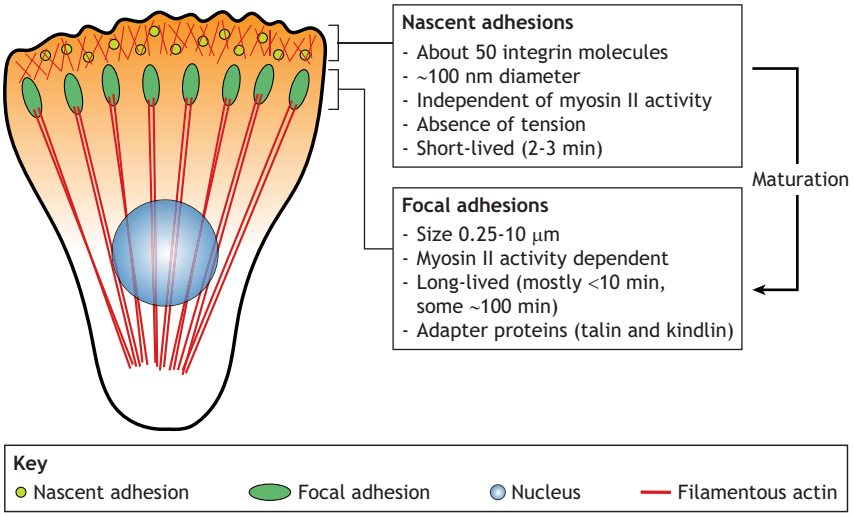

Fig. 2. Properties of nascent adhesions and focal adhesions. In a migrating cell in culture, nascent adhesions form at the leading edge of the cell. Nascent adhesions are small, short-lived adhesion complexes that do not require myosin II activity. Some nascent adhesions grow in size, recruit additional proteins and mature into focal adhesions in a myosin II-dependent manner.

Above the optimal stiffness, the force across FA proteins builds up so quickly that FA proteins unbind the substrate before additional FA proteins are recruited and the load is not shared with newly recruited FA proteins. This results in decreased force transmission and 'frictional slippage'. Importantly, not all substrates are elastic; they can be viscoelastic or viscous. Viscoelastic substrates do not maintain the same stiffness over time. They remodel and reduce their initial stiffness with time when pulled or pushed on. If the stiffness of viscoelastic substrates relaxes within the time frame that the force builds up across FA proteins, the force loading rate is reduced, which can influence the way the cell moves (Adebowale et al., 2021). Similarly, the viscosity of substrates influences the force loading rate; more viscous substrates present higher resistance to FA proteins and less viscous substrates provide reduced resistance to FA proteins such that the force loading rate increases with the viscosity of the substrate (Bennett et al., 2018). Given that physiological substrates are mostly viscoelastic, stress relaxation is probably an important factor during force transduction across FAs *in vivo*.

Biphasic behaviors on substrates with different stiffnesses – predicted by the mechanical clutch model – have been observed in some (Chan and Odde, 2008; Bangasser et al., 2017), but not all (Schiller et al., 2013; Elosegui-Artola et al., 2016; Choquet et al., 1997; Ghibaudo et al., 2008; Califano and Reinhart-King, 2010; Ghassemi et al., 2012), instances. At least in some cases, this discrepancy is due to decreased force loading rates caused by load sharing through the recruitment of adaptor proteins (Elosegui-Artola et al., 2016) or decreased actin flow rates (Bangasser et al., 2017).

Focal adhesion protein organization, orientation and force sensing

The clutch-like mechanism is also consistent with the layered organization, global flow, and kinetics of FA proteins. It is known that FA proteins are organized in layers (Figs 2 and 3). Closest to the membrane is the integrin signaling layer, followed by the force transduction layer, and then – 40 nm away from the membrane – the actin regulator layer (Kanchanawong et al., 2010). Flow speed

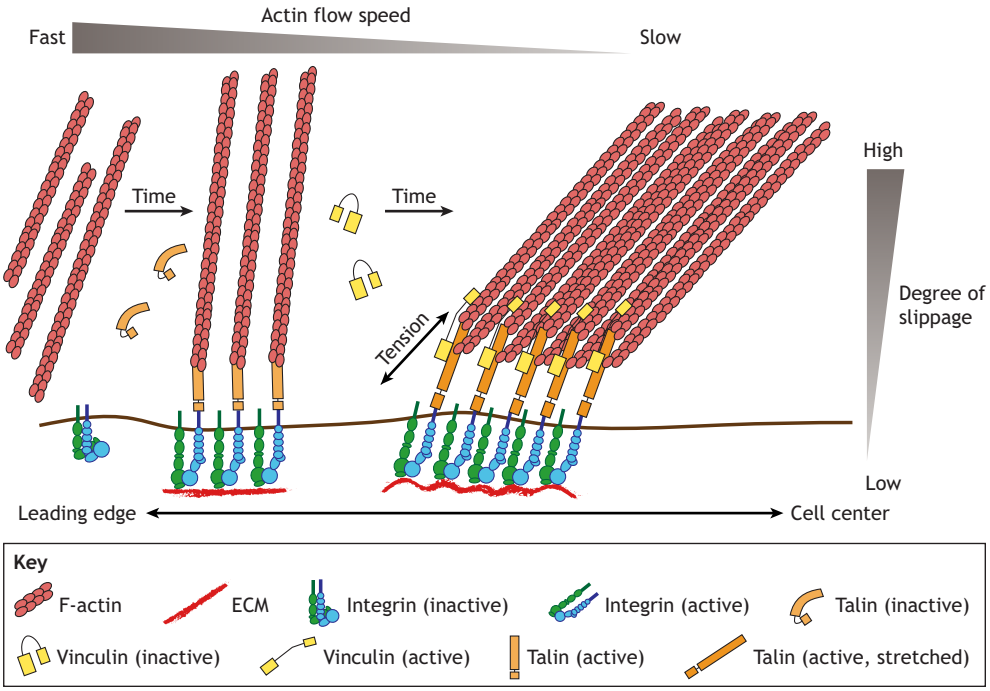

Fig. 3. Molecular clutch model. The binding of integrin to the ECM recruits talin and couples it to the retrograde flowing actin network. An increase in tension across talin exposes vinculin-binding sites in talin and recruits vinculin. Vinculin couples talin to the actin network, strengthens the bridge between the ECM and the actin network, and slows retrograde actin flow.

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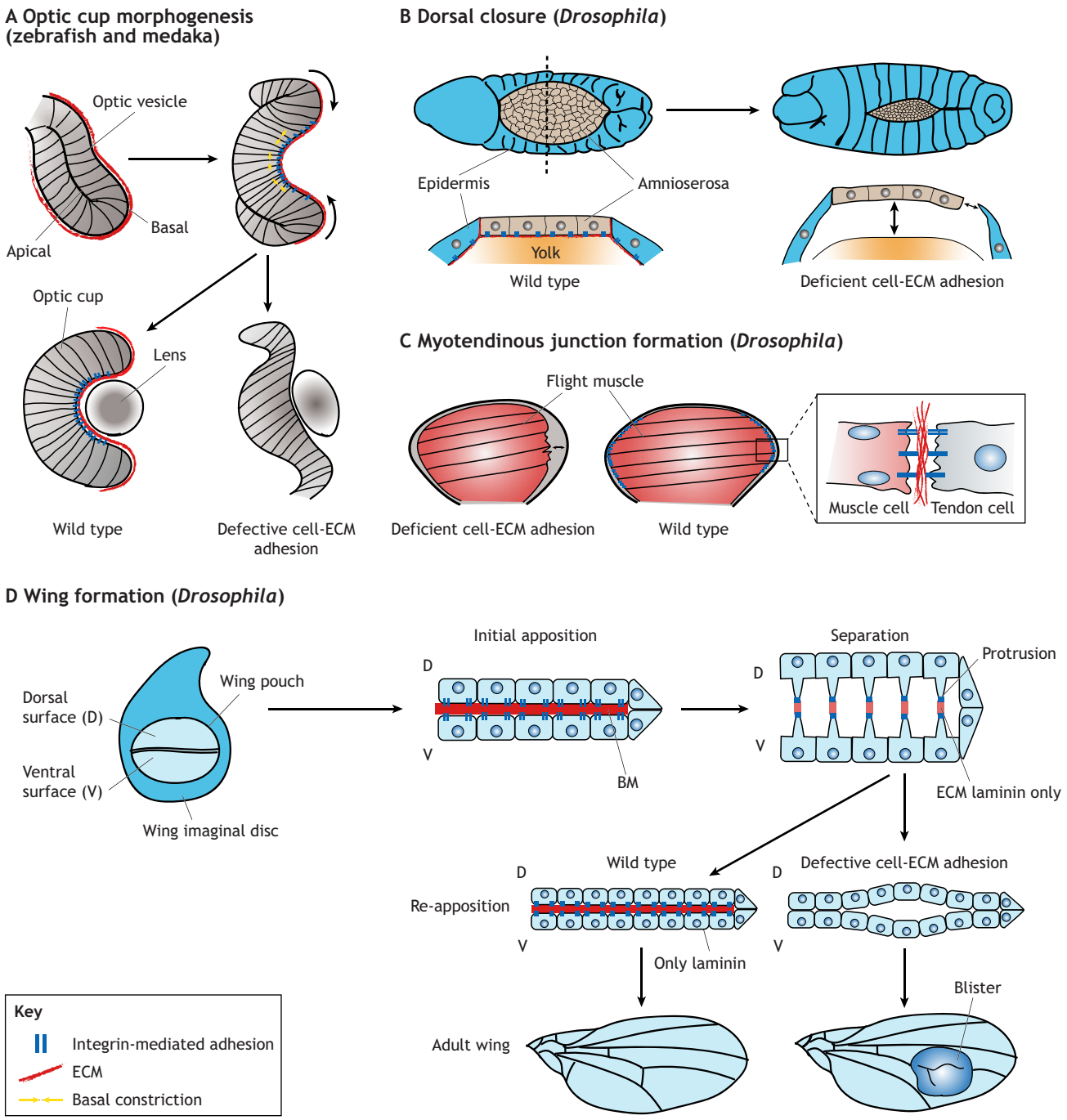

Fig. 4. Focal adhesion-mediated cell anchorage *in vivo*. (A) Optic cup morphogenesis in fish (zebrafish and medaka). The basal sides of retinal neuroepithelial cells in the optic vesicle form focal adhesion-like clusters and attach to the ECM. Myosin II-mediated constriction of the basal cell sides indents the epithelium and induces its cup shape-like form (bottom left). Defects in cell-ECM interactions block this process (bottom right). (B) *Drosophila* dorsal closure. Focal adhesion proteins localize to the basal sides of amnioserosa cells and epidermal cells. To close the gap in the epidermis, amnioserosa cells use myosin II to constrict their apical sides. This pulls the two sides of the epidermis towards each other. Focal adhesion-like structures on the basal cell sides tether the tissue to the yolk cell (bottom left). Without these tethers, the amnioserosa separates from the yolk cell and the epidermis (bottom right). Dashed line indicates the plane shown in the cross-sections below. (C) Myotendinous junction formation in *Drosophila* flight muscles. Muscle cells and tendon cells adhere to each other by binding to the ECM via integrins. Defective cell-ECM adhesion results in rupture of the muscle-tendon connection. (D) Wing formation in *Drosophila*. The fly wing forms via outgrowth and apposition of two epithelia from the initially planar epithelium of the wing disc (left). The apposed epithelia adhere initially and then separate again and only stay connected to each other via long protrusions. These long protrusions then pull the two epithelia together for final adhesion and re-apposition of the wing. Failure of the epithelia to adhere for initial or final apposition leads to wing blisters. BM, basement membrane.

decreases across these layers; actin flows the fastest, talin flows more slowly and integrin flow is the slowest. Thus, there is slippage between each layer, indicating a declining efficiency of motion transmission from the actin to the integrin layer (Gardel et al., 2008; Hu et al., 2007) (Fig. 3). Such slippage has been observed not only in cells cultured on two-dimensional substrates but also in cells cultured in three-dimensional contexts (Owen et al., 2017). The difference in coupling between the different layers is also reflected

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in the acute behavior of FA proteins in response to substrate stretching. Whereas integrins and the N-terminal head domains of talin follow substrate deformations closely and return to their initial position, talin's C-terminal rod domain and F-actin track the deformation with a lag and often do not return to their initial position because of talin unfolding and, possibly, slippage (Massou et al., 2020; Margadant et al., 2011). However, although FA components are clearly under tension (Chang et al., 2016; Austen et al., 2015; Grashoff et al., 2010; Kumar et al., 2016), dynamic force measurements on single integrin molecules rarely show a continuous load build-up as predicted by the mechanical clutch model. Modeling suggests that a possible reason for this could be load sharing not only through talin recruitment but also through reversible cross-linking of actin filaments (Tan et al., 2020).

The molecular model of force transmission across FAs suggests that FA proteins should reorient, change their configuration and exert tension on the substrate. Indeed, engaged integrins and talins orient with their axes – ligand-binding domain to cytoplasmic domain for the integrin β subunit, and N-terminal head domain to the C-terminal rod for talin – in the direction of the pulling force between the outside ligand and actin retrograde flow. That is, the major axis of the proteins tilts towards the membrane and towards the center of the cell (Nordenfelt et al., 2017; Brockman et al., 2018; Kanchanawong et al., 2010; Paszek et al., 2012; Liu et al., 2015) (Fig. 3). Intriguingly, tilting in the direction of retrograde actin flow might be supported by the higher affinity of vinculin for actin filaments moving in the direction of the pointed (−) end, the filament end that moves away from the edge of the cell protrusion during retrograde actin flow (Huang et al., 2017). Forces across FA proteins – and in the case of integrin activation across the membrane (Kim et al., 2020) – not only reorient proteins but also stretch and unfold proteins such that buried sites become accessible for protein binding and phosphorylation (Elosegui-Artola et al., 2016; Austen et al., 2015; Kumar et al., 2016; Sawada et al., 2006; Le et al., 2017; Cheng et al., 2020; Zhou et al., 2021; Bauer et al., 2019) (Fig. 3). This enables many FA proteins to sense and communicate tension. Indeed, cultured cells periodically pull on the distal portion of their FAs (Plotnikov et al., 2012) with high forces (LaCroix et al., 2018; Legant et al., 2013), possibly to sample the stiffness of the substrate.

From force transmission to cellular behaviors

Once engaged, FAs pull on their substrate. This pulling can buckle thin substrates and create wrinkles (Harris et al., 1981, 1980; Burton and Taylor, 1997), displace beads or marks seeded in the substrate (Balaban et al., 2001; Butler et al., 2002; Lendenmann et al., 2019; Gaudet et al., 2003; Marganski et al., 2003; Oliver et al., 1999; Bergert et al., 2016), or bend pillars underneath cultured cells (Tan et al., 2003; Trichet et al., 2012). Quantification of wrinkle length, bead displacement and pillar bending provides a measure of the forces or stresses (force per area) that cells exert on their substrate – techniques that are referred to as wrinkle or traction-force microscopy. These approaches have shown that most cells and tissues exert the highest traction of up to 1 kPa on their substrate with their front parts (Maskarinec et al., 2009; du Roure et al., 2005) or via leading cells (Serra-Picamal et al., 2012; Vedula et al., 2012; Brugues et al., 2014; Saez et al., 2010; Kim et al., 2013), respectively, but some singly migrating cells generate most of the thrust on their lateral sides (Oliver et al., 1999; Legant et al., 2013) and some tissues exert the highest traction in the rear part (Trepat et al., 2009; Yamaguchi et al., 2022).

The ability to sense the load across FA proteins enables cells to respond to the substrate stiffness around them in different ways. For example, some cell types migrate fastest at a certain substrate stiffness (Bangasser et al., 2017; Riaz et al., 2016). They also adjust their contractile and adhesive protein levels and remodel the substrate, and thus its stiffness, in response to changes in substrate stiffness (Moro et al., 2019; Calvo et al., 2013). It is also known that cells differentiate into different fates at different substrate stiffnesses (Engler et al., 2006), form larger colonies with increasing substrate stiffness (Paszek et al., 2005), and increase glycolysis with increasing substrate stiffness (Park et al., 2020). These responses highlight the close interplay between cells and their surroundings, a feature that is likely key for cell anchoring and cell migration in physiological settings.

Focal adhesions anchor cells to the ECM *in vivo* during morphogenesis

In vivo, cells and tissues need to withstand constant pulling and pushing as animals develop, maintain homeostasis and move about. This requires firm adhesions between cells and their substrate. Impairment of FA components disrupts ECM adhesion of tissues that are under high tension, such as muscles (Conti et al., 2009; Schwander et al., 2003; Hayashi et al., 1998; Mayer et al., 1997; Taverna et al., 1998), the skin (van der Neut et al., 1996) and the cardiovascular system (Manso et al., 2017; Carlson et al., 2008), whereas removal of most integrin activity is early embryonic lethal (Stephens et al., 1995; Monkley et al., 2000). To compare the role of FAs in cell adhesion *in vitro* with their role *in vivo*, we first introduce four examples in which cell-substrate adhesion is key in animals: optic cup formation in vertebrates, dorsal closure in *Drosophila*, myotendinous junction formation in *Drosophila*, and wing development in *Drosophila*. Based on these examples, we then compare FA-like structures found *in vivo* with those occurring *in vitro*.

Optic cup formation

The optic cup gives rise to the neural retina and retinal pigment epithelium of the vertebrate eye (Casey et al., 2021). After evagination from the diencephalic neuroectoderm, the optic vesicle – the optic cup precursor – consists of two epithelia that are sandwiched together with their apical sides facing each other and their basal sides pointing outwards. The basal sides of the cells touch an ECM that surrounds the optic vesicle. A combination of cell migration and cell constriction sculpts the optic vesicle into its cup-like form (Casey et al., 2021). Cells from the inner epithelium of the optic vesicle use the basement membrane as a substrate to migrate around the rim of the vesicle and join the outer epithelium that faces the future lens. At the same time, outer epithelium cells constrict their basal sides, become wedge-shaped and cause the outer epithelium to curve inwards (Fig. 4A). This process is driven by actomyosin contraction and cell-substrate adhesion. Myosin II and F-actin localize at the basal cell sides of the outer epithelium where FA proteins also form long-lived complexes and interact with the basement membrane (Sidhaye and Norden, 2017; Nicolas-Perez et al., 2016; Soans et al., 2022 preprint). Pulsed membrane contractions are thought to drive basal shrinkage via a ratchet-like mechanism (Nicolas-Perez et al., 2016), similar to the mechanism underlying apical cell constriction in other contexts (Clarke and Martin, 2021), although Myosin II accumulation – and not Myosin II pulsing – correlates with basal constriction in optic cup morphogenesis. Importantly, this shrinkage, and thus cup morphogenesis, requires integrins, ECM components and Myosin II activity both *in vivo* (Martinez-Morales et al., 2009; Bogdanovic et al., 2012; Sidhaye and Norden, 2017) and, at least partly, in organoid cultures (Nakano et al., 2012). In their absence, the optic

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cup does not adapt its cup-like shape and folds aberrantly (Fig. 4A), suggesting that basement membrane anchorage is essential for proper contraction of basal cell sides and, thus, tissue folding.

Dorsal closure

Dorsal closure is the process by which a gap in the dorsal epithelium of *Drosophila* embryos is sealed. Before closure, the dorsal gap is covered by an epithelium called the amnioserosa. The aminoserosa overlies the yolk cell and abuts two epithelial cell sheets on either side of the dorsal gap. When the aminoserosa contracts and ingresses, it pulls the two epithelial cell sheets towards the dorsal midline. At the midline, the leading edges of the two cell sheets then fuse with each other and seal the dorsal gap (Fig. 4B) (Kiehart et al., 2017). Shrinkage of the aminoserosa is driven by pulses of actomyosin activity (Ducuing and Vincent, 2016; Pasakarnis et al., 2016; Young et al., 1993; Fischer et al., 2014; Franke et al., 2010; Blanchard et al., 2010; David et al., 2010), which contract the apical surface of aminoserosa cells (Solon et al., 2009; Gorfinkiel et al., 2009; Kiehart et al., 2000). Owing to a ratchet-like mechanism, the apical surfaces do not expand to the same extent after the last pulse as before the last pulse (Pasakarnis et al., 2016; Blanchard et al., 2010). Together with cell volume reduction (Saias et al., 2015) and cell extrusion (Toyama et al., 2008), this causes the apical side of the aminoserosa tissue to shrink and pull the adjacent epithelial sheets across the dorsal gap.

Integrins form clusters on the basal sides of amnioserosa cells. These clusters colocalize with talin and F-actin and increase in size with increasing load. Although smaller in size, these clusters are likely thus the *in vivo* equivalent of FAs in cultured cells (Goodwin et al., 2016). They anchor the amnioserosa tissue basally to a basement membrane that overlies the yolk cell and laterally to the leading edge of the abutting epithelial sheets (Narasimha and Brown, 2004). Without integrin-mediated anchorage, aminoserosa cells fail to resist the apical pulling forces that the actomyosin pulses generate, and the aminoserosa tears off from the yolk cell (Newman and Wright, 1981; Wright, 1960; Narasimha and Brown, 2004). Similarly, rips form between the aminoserosa and the leading edge of the epithelial sheets in the absence of integrin because adhesion between these two tissues does not withstand the contractile pulling forces of the amnioserosa (Fig. 4B) (Stark et al., 1997; Wright, 1960; Narasimha and Brown, 2004; Schock and Perrimon, 2003; Ellis et al., 2013; Brown et al., 2002; Camp et al., 2018). Thus, integrins serve as tethers to ensure force transmission among these tissues during cell sheet fusion (Goodwin et al., 2016, 2017).

Myotendinous junction formation

The myotendinous junction connects muscle to tendon. During its formation, muscle and tendon cells secrete ECM proteins into the extracellular space between them (Fogerty et al., 1994; Martin et al., 1999; Subramanian and Schilling, 2014; Subramanian et al., 2007; Aparecida de Aro et al., 2012; Bajanca et al., 2006; Gilsohn and Volk, 2010). Integrins on muscle and tendon cells bind these ECM proteins on the outside and – through talin and other adapter proteins – actin filaments on the inside in a layered fashion akin to the layered architecture of focal adhesions (Lemke and Schnorrer, 2017; Schweitzer et al., 2010; Green et al., 2018). This creates a junction from muscle to ECM to tendon (Fig. 4C). Without integrins, integrin ligands or talins, myotendinous junctions do not form or rupture under tension (Leptin et al., 1989; Prokop et al., 1998; Conti et al., 2009; Schwander et al., 2003; Martin-Bermudo et al., 1998; Tanentzapf and Brown, 2006), whereas the absence of other integrin-associated proteins does not lead to rupture but causes defective actin organization (Green et al., 2018; Maartens et al., 2016). As force across the myotendinous junction increases during muscle formation or muscle use, more integrin and talin proteins are recruited to the junction. This is achieved by reducing the turnover of integrins and talins on the membrane (Pines et al., 2012; Yuan et al., 2010; Hakonardottir et al., 2015). Counterintuitively, only about one in ten talin proteins is mechanically engaged at myotendinous junctions during muscle development in flies. Moreover, during force build-up and talin recruitment, the fraction of load-bearing talin proteins does not increase at the junction (Lemke et al., 2019). This is consistent with the observation that residual amounts of talin activity still support cell adhesion in culture (Zhang et al., 2008; Elosegui-Artola et al., 2016) and in animals (Tanentzapf and Brown, 2006). One possible reason for only 10% of talin proteins being mechanical engaged is that the other 90% of talin proteins serves as a mechanical buffer. Together with the force-dependent unfolding and refolding of individual talin proteins (Yao et al., 2016), this likely provides an efficient shock absorber that ensures that myotendinous junctions do not rupture during peak mechanical load upon muscle contraction, as happens when talin levels are reduced (Lemke et al., 2019). Thus, integrin-mediated adhesion provides a robust connection between muscle and tendons.

Wing development

The fly wing forms from the monolayered epithelium of the wing disc. In larvae, the wing disc folds into a bilayered pouch such that the epithelia's basal sides come to lie next to each other and adhere through a shared basement membrane. Intriguingly, the two epithelia of the wing then separate and only stay connected through long, slender processes, to then re-appose again (Fristrom et al., 1993) (Fig. 4D). The initial and final adhesion of the two wing epithelia requires integrin and talin (Sun et al., 2021b; Brabant and Brower, 1993; Brower and Jaffe, 1989; Wilcox et al., 1989; Zusman et al., 1990; Brabant et al., 1996); lack of these and other focal adhesion components leads to wing blisters in adult flies (Walsh and Brown, 1998; Prout et al., 1997; Bilousov et al., 2014). Counterintuitively, initial adhesion requires both basement membrane formation (Sun et al., 2021b) and degradation (Diaz-de-la-Loza et al., 2018; De Las Heras et al., 2018; Sun et al., 2021b). Indeed, during wing formation, the four core basement membrane components laminin, nidogen, perlecan and collagen IV are degraded by matrix metalloproteases (Sun et al., 2021b; Diaz-de-la-Loza et al., 2018; De Las Heras et al., 2018), but only laminin levels recover, with laminin protein localizing to dots between the basal sides of the two epithelia (Sun et al., 2021b). Consistent with these expression patterns, all four basement membrane components are individually dispensable for initial and final adhesion of the wing epithelia (Sun et al., 2021b; Wolfstetter et al., 2019) except for laminin, the absence of which causes final adhesion to fail (Sun et al., 2021b; Henchcliffe et al., 1993; Martin et al., 1999). A possible explanation for these seemingly contradictory observations is that basement membrane components are required redundantly for initial adhesion but their degradation is also required to refine and focus initial adhesion to laminin dots. The laminin dots are at the tip of processes from the opposing wing epithelia (Sun et al., 2021b; Fristrom et al., 1994, 1993). These processes harbor F-actin and microtubule bundles (Fristrom et al., 1993; Sun et al., 2021b; Mogensen et al., 1989; Tucker et al., 1986) and localize integrin and talin to their tips (Sun et al., 2021b; Brabant et al., 1996; Fristrom et al., 1993). In this manner, processes from opposing wing epithelia – through talin-integrin-laminin-integrin-talin bridges – continue to connect the two wing epithelia during epithelia separation and likely

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pull the two wing epithelia together again for final adhesion. During initial adhesion, the two wing epithelia utilize a typical basement membrane to adhere to each other but then only rely on laminin for final adhesion, similar to the basement membrane between muscle and tendon (Maartens and Brown, 2015). The reason for this could be that adhesion needs to support high loads during final adhesion of the two epithelia.

A comparison between FA-like structures *in vivo* and *in vitro*

The examples above highlight how integrins anchor cells to their substrates in animals. Amnioserosa cells form punctate, optic cup cells more continuous, and wing epithelia and muscle larger FA-like structures that are similar in composition, organization and dynamics to FAs of cultured cells. In each case, these FA-like structures stably anchor cells to their surroundings to withstand tension, and failure to do so results in tissue rupture. The forces across mechanically engaged talin proteins in the myotendinous junction (Lemke et al., 2019) are similar to those seen in cultured cells (Kumar et al., 2016; Austen et al., 2015) and comparable to the forces observed across integrins in adherent cells in culture (Morimatsu et al., 2013; Zhang et al., 2014; Brockman et al., 2018; Tan et al., 2020; Sarkar et al., 2020; Liu et al., 2013; Chang et al., 2016; Blakely et al., 2014), ranging from 3 to 10 pN and peaking at about 30 pN. In contrast, the fraction of mechanically engaged talins at the fly myotendinous junction – the only place where forces across FA-like structures have been measured *in vivo* – is much smaller than that in cultured cells; at the myotendinous junction, about 10% of talin proteins are mechanically engaged (Lemke et al., 2019) whereas about 70% of talin and integrin proteins in FAs of adherent cells bear forces *in vitro* (Ringer et al., 2017; Morimatsu et al., 2013). These similarities at the molecular level and differences at the cellular level likely reflect the advantages and limits of studying *in vitro* systems. Although the proteins utilized to adhere cells to their substrates are mostly the same *in vitro* and *in vivo*, the substrate for cultured cells does not fully recapitulate the mechanical environment of cells in animals, and thus likely accounts for the discrepancies in the number of load-bearing FA proteins. To solidify our understanding of the role of FAs in cell anchorage, it is therefore important to assess the degree to which we can apply our knowledge about how cells adhere in culture to our understanding of cell adhesion in different tissues in animals. This should yield important confirmations and likely some surprises.

Do migrating cells *in vivo* form FAs to pull themselves forward?

Migrating cells can propel themselves forward in different ways. They can couple retrograde actin flow across the membrane to their substrate through FAs, nonspecific friction between membrane proteins and the substrate, or bleb-like membrane protrusions that push against the surroundings (Yamada and Sixt, 2019). Although integrins can couple actin flow inside the cells to the surroundings (Sun et al., 2016a), the degree to which migrating cells in animals rely on such coupling for propulsion is poorly understood. Below, using five different examples, we discuss the role of integrins and FA-like structures in cell migration in animals.

Distal tip cell migration

Caenorhabditis elegans distal tip cells sit on the tips of two U-shaped gonad arms (Fig. 5A), where they provide a niche for germline stem cells. The two distal tip cells are born on the ventral side in the middle of the worm. During development, they migrate in opposite directions towards the head and tail, make a U-turn from the ventral to the dorsal side, and migrate back to the middle of the worm on the dorsal side (Wong and Schwarzbauer, 2012). They are therefore associated with different basement membranes along their migratory route, and disrupting the basement membrane stalls tip cell migration (Kao et al., 2006). Distal tip cells require talin (Cram et al., 2003) and integrins (Lee et al., 2001) for their migration. Interestingly, worms possess only two integrin receptors – one is required for motility (Baum and Garriga, 1997; Meighan and Schwarzbauer, 2007) and the other for directionality (Fig. 5A) (Meighan and Schwarzbauer, 2007). Besides providing cell-substrate coupling for propulsion, this indicates that integrins also provide directional information for distal tip cells, presumably by reading out differences in the composition or physical properties of the basement membrane. Owing to technical challenges in imaging distal tip cell migration by time-lapse microscopy, it is unclear whether integrins form FA-like structures and couple them to actin flow in this context; this is a hurdle that can hopefully be overcome with the advent of microfluidics and more gentle imaging techniques (Keil et al., 2017).

Enteric neural crest migration

The enteric nervous system controls peristaltic movements of the gastrointestinal tract. It is formed by two different neural crest populations. The larger of the two populations migrates from the dorsal side of the neural tube behind the head to the foregut (Fig. 5B). Once they reach the foregut, these cells migrate caudally as interconnected strands through the laminin- and collagen-rich gut mesenchyme (Chevalier et al., 2016; Breau et al., 2009) to the junction between the small and large intestine. At this junction, enteric neural crest cells pause before they resume migration mostly as single cells to cover the large intestine; they then reassemble into interconnected strands (Young et al., 2004; Druckenbrod and Epstein, 2005, 2007). This process partly requires integrin activity. In mice, neural crest cells lacking integrin β1 colonize the small intestine and other tissues normally (Breau et al., 2006; Fassler and Meyer, 1995). However, they invade the large intestine slowly, do not cover the entire large intestine – a hallmark of Hirschsprung's disease (Kapur, 2009) – and aggregate in clusters instead of forming a regular network (Fig. 5B) (Breau et al., 2006). This is because fewer enteric neural crest cells detach from the strands, and those that do detach migrate more slowly and with less directionality (Breau et al., 2009). Increased integrin activity perturbs enteric neural crest cell migration in a similar manner (Zhang et al., 2012).

The requirement for integrin in enteric neural crest cells to invade the large intestine is also reflected in differential ECM composition; the mesenchyme of the large, but not the small, intestine harbors high levels of fibronectin and tenascin (Breau et al., 2009), two proteins that are known to affect neural crest migration *in vitro* (Breau et al., 2009; Leonard and Taneyhill, 2020). Thus, integrin activity is likely required for enteric neural crest cells to overcome the repulsive environment of the large intestine and to maintain directionality and speed, but not for motility per se. Although vinculin has also been associated with defects in enteric neural crest migration (Lai et al., 2017), the degree to which enteric neural crest cells use FA-like structures for their migration is unclear.

Macrophage migration in flies

Macrophages engulf and digest cellular debris of dying or non-healthy cells and contribute to the innate immune response (Wood and Martin, 2017). In flies, macrophages (hemocytes) are born in

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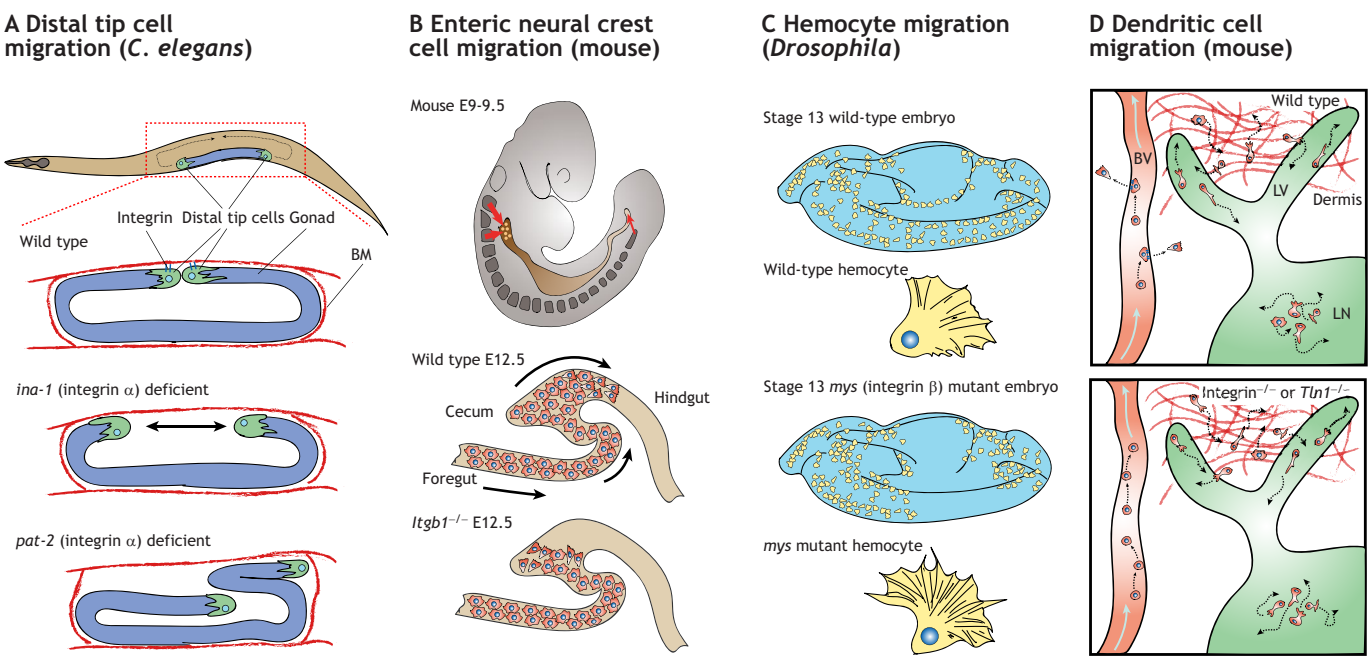

Fig. 5. The role of focal adhesions in cell migration *in vivo*. (A) Distal tip cell migration in *C. elegans*. The two distal tip cells are located at each end of the gonad and migrate along a U-shaped route through the embryo (top). During their migration, distal tip cells express *ina-1* and *pat-2*, the sole two α integrin subunits found in worms. These two α integrin subunits serve different functions: *ina-1* is required for the generation of traction and *pat-2* for pathfinding. Therefore, distal tip cells lacking *ina-1* are slowed down and do not reach their target (middle) whereas distal tip cells lacking *pat-2* are less directional and veer off their migratory course (bottom). BM, basement membrane. (B) Enteric neural crest cell (ENCC) migration in mice. A large population of ENCCs (top, yellow cells indicated by thick red arrows; the location of the smaller population is indicated by the thin red arrow) migrates from the dorsal side of the neural tube to the foregut, then to the hindgut. By embryonic day (E) 12.5 (middle) this population colonizes the entire length of the gut. Depletion of integrin β1 from ENCCs (e.g. in *Itgb1*$^{-/-}$ embryos; bottom) results in abnormal migration and a premature stop around the cecum, the junction between the fore- and hindgut. Black arrows indicate direction of migration. (C) Hemocyte migration in fly embryos. Hemocytes (yellow) rely on integrin-mediated adhesion for efficient migration but not for directionality. Because they are slowed in the absence of integrin activity, hemocytes fail to spread through the embryo in *myospheroid* (*mys*/integrin beta) mutants and extend more filopodia than in wild-type embryos. (D) Dendritic cell migration in mice. Focal adhesion-mediated cell-ECM adhesion is required for dendritic cells to extravasate from blood vessels (BV; top). By contrast, interstitial migration in the dermis, entry into lymphatic vessels (LV) and migration in the lymph node (LN) do not require integrins; as such, these processes are unaffected in integrin $a_v^{flox/flox}$, integrin $b_1^{flox/flox}$, integrin $b_2^{-/-}$, integrin $b_7^{-/-}$, Mx1:Cre$^{+/-}$ or *Tln1*$^{-/-}$ mutants whereas extravasion is blocked (bottom).

the head of the embryo. From there, they spread along different routes throughout the embryo (Tepass et al., 1994) (Fig. 5C) and, upon wounding, emigrate to the site of lesion (Stramer et al., 2005). Macrophages in the fly embryo need integrin activity for their dispersal. In its absence, macrophages still migrate in a directional manner and extend protrusions, but move at reduced speed and fail to spread in the embryo (Comber et al., 2013; Siekhaus et al., 2010) (Fig. 5C). This is likely due to inefficient coupling of actin flow inside the macrophages to the ECM. Fly macrophages extend large, F-actin-dependent protrusions that do not require integrin activity; the number of microspikes and filopodia in fact increases in the absence of β-integrin (Evans et al., 2013; Verboon et al., 2015; Wood et al., 2006; Comber et al., 2013). In these protrusions, actin flows from the leading edge towards the cell body (Stramer et al., 2010; Yolland et al., 2019). Actin flow speed is fast at the leading edge and slows towards the cell body. This suggests that actin flow becomes gradually more coupled to the substrate towards the cell body, probably through integrins. Consistent with this idea, the actin network is under more stress towards the macrophage cell body (Yolland et al., 2019; Davis et al., 2015) and macrophages deform the ECM during their migration (Matsubayashi et al., 2017). Although the distribution of integrin – or any other FA protein – is unclear in migrating macrophages, these cells likely use integrins to couple actin flow to the ECM for speed but not directionality. However, given that macrophage protrusions are short-lived – they turn over within 30-60 s (Yolland et al., 2019) – it is likely that fly macrophages use nascent adhesion-like structures rather than long-lived FAs for movement.

Leukocyte migration in mice

Leukocytes detect and fight pathogens. To do this, they scatter throughout the body and can infiltrate most tissues. Although they respond to guidance cues, leukocytes often move randomly through diverse environments (Kameritsch and Renkawitz, 2020). Although leukocytes require integrins to leave the vasculature, enter the interstitial tissues, and migrate on two-dimensional substrates *in vitro* (Nourshargh and Alon, 2014), T cells and dendritic cells that lack integrin or talin activity home to the lymph nodes in mice as efficiently as do wild-type cells (Fig. 5D) (Lammermann et al., 2008; Hons et al., 2018). *In vitro* studies indicate that the reason why these cells can still migrate efficiently without integrins is that they upregulate the rate of actin polymerization and thus actin flow; faster actin flow compensates for less-efficient coupling to the substrate in the absence of integrin adhesion such that integrin-deficient and wild-type cells move equally fast (Renkawitz et al., 2009; Hons et al., 2018). Integrin-independent migration has also been observed in other cell types, for example in fat body cells navigating through the hemolymph in *Drosophila* embryos (Franz et al., 2018) and in migrating cells in the early zebrafish embryo (Ruprecht et al., 2015). Fat body cells use actomyosin-driven peristaltic movements to swim through the hemolymph, and early embryonic zebrafish cells use frictional coupling of actin flow to move through the embryo. Thus,

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in contrast to cells migrating on two-dimensional substrates, leukocytes – and likely other cells – can migrate efficiently in the absence of integrin and, thus, FA-mediated adhesion in animals.

Follicle cell migration in flies

Drosophila follicle cells form an epithelium that surrounds a ball-shaped cluster of germ cells: the oocyte and its nurse cells. Together, the follicular epithelium and germ cells make up the egg chamber, which matures into an egg. During oogenesis, follicle cells start to crawl along the basal membrane that surrounds the egg chamber such that the chamber starts revolving around its own axis (Cetera et al., 2014; Haigo and Bilder, 2011). While migrating along the basement membrane, follicle cells deposit additional basement membrane material (Chen et al., 2017; Isabella and Horne-Badovinac, 2015, 2016; Lovegrove et al., 2019; Zajac and Horne-Badovinac, 2022) and align the basement membrane into fibrils (Chlasta et al., 2017; Gutzeit et al., 1991) in a migration- and dystroglycan-dependent manner (Campos et al., 2020; Cetera et al., 2014; Isabella and Horne-Badovinac, 2016). This results in stiffening of the basement membrane with alternating softer and oriented stiffer areas (Chlasta et al., 2017; Crest et al., 2017; Loza et al., 2017; Pearson et al., 2016). Inside follicle cells, actin stress fibers cover the basal side of the cells (Campos et al., 2020; Chen et al., 2016; Delon and Brown, 2009; Gutzeit et al., 1991; He et al., 2010; Lewellyn et al., 2013; Sherrard et al., 2021; Squarr et al., 2016) and partly align with basement membrane fibrils on the outside of cells (Cetera et al., 2014; Isabella and Horne-Badovinac, 2015; Loza et al., 2017). These stress fibers form at the leading edge and disassemble at the trailing edge of cells. At their front, the stress fibers assemble into adhesions – composed of many components also seen in FAs in cultured cells (Cetera et al., 2014; Delon and Brown, 2009; Sherrard et al., 2021) – that slide backwards as the cell crawls along the basement membrane. Once the adhesions reach the second to last position, they disassemble; the last adhesion is not stationary but slides along with the migrating cell (Sherrard et al., 2021). The peculiar behavior of these adhesions depends on cell migration; immotile follicle cells only form adhesions at the ends of stress fibers but not along them (Sherrard et al., 2021). To move, the follicle cells require integrin and talin activity. Both not enough and too much integrin activity block migration whereas a slight reduction in integrin activity or basement membrane levels increase migration speed above that of wild-type follicle cells (Haigo and Bilder, 2011; Lewellyn et al., 2013; Loza et al., 2017). Together, these observations suggest that at least some migrating epithelial cells in animals use focal adhesion-like structures – both sliding and stationary ones – to pull themselves along their substrate and while doing so remodel and align it, possibly to ensure efficient movement.

Taken together, these observations suggest that migrating cells in animals differ with regard to how much they rely on integrins and FA-like structures for motility. Whereas some cells seem to use integrins for directional information, others use integrins to couple to adhesive substrates but can migrate equally efficiently in non-adhesive environments by, based on *in vitro* studies, increasing the rate of actin flow and transmitting force to their environment through unspecific friction or by pushing against crevices in their environment (Renkawitz et al., 2009; Hons et al., 2018; Reversat et al., 2020). In support of this notion, the posterior lateral line primordium, a migrating tissue in zebrafish embryos, uses integrin-mediated adhesion to the basement membrane for efficient force transmission and propulsion. In the absence of integrins, primordium cells upregulate the rate of actin flow such that the tissue continues to migrate, albeit with less efficient force transmission and at a slower speed (Yamaguchi et al., 2022). These examples indicate that cells are able to adjust actin flow rates according to how well they can transmit the actin flow to their surroundings, such that they can propel themselves forward through heterogeneous environments, for example those encountered along migratory routes in animals.

Conclusions and perspectives

FA-mediated adhesion of cells to the ECM underlies cell anchorage and cell motility. It is essential for human development and homeostasis, and drugs targeting integrins are increasingly being used to interfere with a wide range of diseases (Slack et al., 2022). Consistent with their role in cell mechanics, integrins respond to force across them. *In vitro* studies have delineated how mechanical force regulates integrin activation and clustering, protein recruitment and signaling, and thus, the strength of FA-mediated adhesion. More recently, *in vivo* studies have begun to refine our understanding of the interplay between force and FA-mediated adhesion in physiological contexts. Although integrins are essential for many processes in animals, these studies also revealed that cells are surprisingly versatile *in vivo*. FA-like structures, often smaller and shorter lived than *in vitro*, clearly anchor cells to their surroundings and aid cells in pulling and pushing themselves forward. However, migrating cells can shift rapidly between integrin-mediated adhesion and integrin-independent adhesion, sometimes with surprisingly little reduction in migration speed. Similarly, FA-mediated cell anchoring is often buffered through redundancy in protein type and number. This endows cells in animals with robustness to internal and external fluctuations and perturbations.

The molecular players and interactions that underlie robustness in cell migration and cell anchoring are just beginning to be revealed. Further studies are therefore needed to address several different aspects of FA-mediated cell anchoring and migration. Guided by our detailed understanding of FA *in vitro*, we need to delineate which FA components are required for cell anchoring and cell migration and how these components interact to assemble into FAs, respond to changing loads and disassemble *in vivo*. Additionally, studies are required to decipher the mechanisms of cross-talk between cells and tissues and the ECM through FAs; cells and tissues likely influence the abundance, molecular composition and architecture and, thus, the chemical and physical properties of the ECM and vice versa. These clarifications will expand our understanding of FA-mediated cell adhesion and should unravel the logic underlying the perplexing flexibility and robustness of cell migration and anchoring in animals, including humans.

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Competing interests

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