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Intermediate filaments in mammalian cell motility
Úloha středních filament v pohybu savčích buněk

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

Declaration

I honestly declare that I wrote this thesis on my own and that I stated all used literature and other information sources. This work or its significant part was not used to previously acquire any other or same academic title.

V Praze / In Prague, 03.08.2023

Abstract

Cell migration is crucial for the formation and maintenance of a multicellular mammalian organism, contributing to important processes such as embryonic development, tissue renewal, and immune surveillance. It is a complex phenomenon involving a plethora of processes, including relevant signalization. An impairment of those processes could be projected into innumerable pathological states, from wound healing disruption to tumour metastasis and invasiveness, thus the interest of many researchers has turned toward migration.

The migration of mammalian cells is dependent on a cytoskeleton, which is being considerably rearranged in motile cells. This thesis aims to summarise the role of intermediated filaments in cell motility – the less understood cytoskeletal network in this context.

Cytoplasmic, as well as nuclear intermediate filaments, due to their unique mechanical properties, affect cell mechanics. They protect against physical stresses (as cells squeeze through confined pores in a complex intercellular microenvironment) and modulate and direct actomyosin-generated forces, which are the main driving force of migration. In addition, they contribute to important migration-involved steps, such as cell polarisation, cell adhesion to surrounding surfaces, cohesion in collective migration, and navigation through an intricate extracellular environment.

Keywords

Cell migration, intermediate filaments, cytoskeleton, cell adhesion

Abstrakt

Buněčná migrace hraje klíčovou roli ve formování a udržování mnohobuněčného savčího těla. Podílí se na tak zásadních jevech, jakými jsou embryonální vývoj, obnova tkání nebo imunitní odpověď. Jedná se o komplexní děj, zahrnující celou škálu procesů, včetně příslušné signalisace. Narušení těchto procesů se může manifestovat širokou škálou patologických stavů, od narušeného hojení ran až po nádorovou invasivitu, proto jsou děje provázející migraci cílem výzkumu mnoha týmů.

Migrace buněk je závislá na cytoskeletu, který je v migrujících buňkách zásadně přestavován a neustále přizpůsobován pohybu. Tato práce si klade za cíl shrnout poznatky o úloze středních filament v migraci savčích buněk. Jedná se o nejméně probádaný cytoskeletární systém v tomto kontextu.

Cytoplasmatická i jaderná střední filamenta, díky svým unikátním mechanoelastickým vlastnostem, významně ovlivňují mechaniku buňky. Poskytují jí fyzickou ochranu před silami, působícími při migraci skrze stísněné prostory komplexního mezibuněčného prostředí. Zároveň ovlivňují a usměrňují kontraktilitu aktomyosinové sítě, jež je hnacím motorem buněčného pohybu, a dále se podílí na zásadních krocích migrace, jakými jsou polarisace, přichycení buněk k okolním povrchům nebo navigace skrze spletité extracelulární prostředí.

Klíčová slova

Migrace buněk, střední filamenta, cytoskelet, adheze buněk

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition
ERK1/2	Extracellular signal-regulated kinase 1/2
FA	Focal adhesion
FAK	Focal adhesion kinase
GEF	Guanin nucleotide exchange factor
GFAP	Glial fibrillary acidic protein
IF	Intermediate filament
INM	Inner nuclear membrane
KASH	Klarsicht/ANC1/SYNE homology
LINC	Linker of nucleoskeleton and cytoskeleton
L_P	Persistence length
Nesprin	Nuclear envelope spectrin repeat protein
NTP	Nucleotide triphosphate
ONM	Outer nuclear membrane
PKC	Protein kinase C
RACK1	Receptor for activated C kinase 1
SEM	Scanning electron microscope
SUN	Sad1/UNC-84
ULF	Unit length filament

Table of Contents

1. Introduction

Cell migration is essential for maintaining integrity and homeostasis in the multicellular mammalian organisms. It has been involved in ontogenesis from the early stages of embryonic development in processes as pivotal as gastrulation and neural crest cells distribution, as well as in many others, as reviewed by Scarpa and Mayor (2016). In addition, it is involved in a broad spectrum of physiological processes for maintaining a complex multicellular body, such as tissue renewal and wound healing, as reviewed by Shaw and Martin (2009). In order to exert proper immunological surveillance, it is necessary for particular immune cell subpopulations to migrate to and from the actual site of the immune response (reviewed by Luster *et al.*, 2005).

Finally, impaired migration may result in some, potentially serious, conditions such as immune or wound healing malfunction (Wojcik, Bundman and Roop, 2000; Snapper *et al.*, 2005). Notably, excessive motility of cancer cells is directly connected with increased tumour invasivity and metastasizing to adjacent tissues (for reviews see Polacheck, Zervantonakis, and Kamm, 2013; Raudenská *et al.*, 2023). Changes in the expression of proteins linked to migration, and its correlation with tumour invasivity and metastatic potential, have been observed in some types of tumours, such as those found in breast, colorectal, or prostate cancer – therefore, in some cases, it could be considered a hallmark of malignancy (Willis *et al.*, 2008; Zhao *et al.*, 2008; Yamashita *et al.*, 2013). Although an indisputably important field of research, the particular mechanisms involved in tumour metastasis are beyond the scope of this thesis.

The motility of mammalian cells is dependent on the cytoskeleton. At least three major types of cytoskeletal fibres can be distinguished: microfilaments, microtubules, and intermediate filaments (Ishikawa, Bischoff and Holtzer, 1968). Changes in the expression and remodelling of all three of the networks mentioned above have been demonstrated in migrating mammalian cells (e.g. Hendrix *et al.*, 1996; Ganguly *et al.*, 2012; Letort *et al.*, 2015; Costigliola *et al.*, 2017). A multitude of recent studies have started to elucidate the effect of IFs on cell motility – the less explored cytoskeletal component in this context.

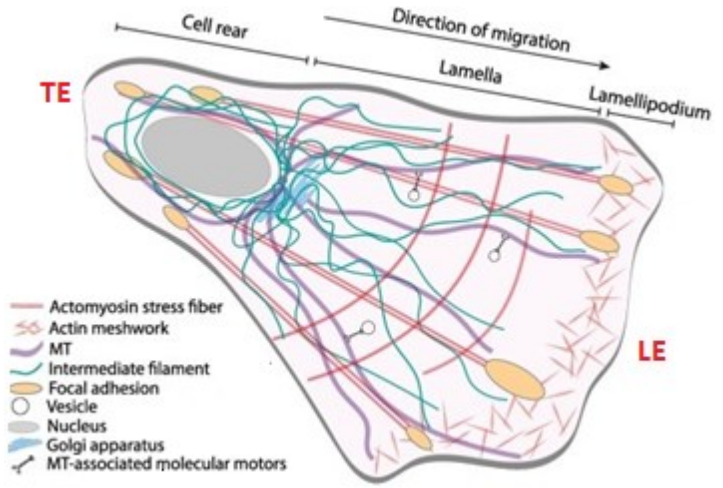
This bachelor thesis summarises current knowledge of the IF network and the importance of its dynamics in the context of orchestrating such a complex and indispensable mammalian cell migration phenomenon. For this purpose, the topic is divided into an introductory part to obtain an understanding of the general features of migration and IF structure, followed by several functionally defined thematic units outlining IF contribution to the mechanics of the cell, adhesivity, and finally the nuclear cytoskeletons' involvement.

2. The Many Forms of Migration

Because cellular migration is a highly polarised event, so must be the inner organisation of motile cells. At the anterior part (in the direction of locomotion), also known as the leading edge, cells protrude forward and, generally, attach to the substrate. The posterior part is referred to as the trailing edge and it is where adhesive complexes disassemble. The preceding polarisation involves also a massive cytoskeletal rearrangement. For a review see Lauffenburger and Horwitz, (1996).

Although quite unusual in a physiological context and offering only limited modes of motility, simplified 2D migration of cells cultured on glass or plastics remain the most studied and best understood (reviewed by Yamada and Sixt, 2019). The 2D migration (see Fig. 1) is composed of a few consecutive, cyclically repeated steps described below in simplified form (For reviews see Lauffenburger and Horwitz, 1996; Yamada and Sixt, 2019; Seetharaman and Etienne-Manneville, 2020):

Fig. 1: Distribution of cytoskeletal networks in migrating mammalian cells. LE – leading edge, TE – trailing edge. (Adopted from Seetharaman and Etienne-Manneville, 2020; modified)



(1) Protruding forward at the leading edge: A dynamic cytoskeletal meshwork gives rise to new protrusions (Lauffenburger and Horwitz, 1996). Membrane protrusions are driven by the polymerisation of actin microfilaments towards the membrane (reviewed by Condeelis, 1993). Broad, flat, sheet-like protrusions, characterised by a dense mesh of differently oriented microfilaments, are called lamellipodia, whereas filopodia are the narrow, needle-like, protrusions exceeding the lamellipodia and characterised by actin filaments bundled in a parallel manner (reviewed by Lauffenburger and Horwitz, 1996).

(2) Attachment of newly formed protrusions to the surface: Cells attach the progressing leading edge to the extracellular matrix (ECM) via an assembly of transmembrane multiprotein complexes that are referred to as focal adhesions (FAs) (Izzard and Lochner, 1980). Adhesive complexes will be discussed later.

(3) Rear detachment and contraction: The trailing edge must be released from the substrate (through adhesive complexes disassembly) and subsequently retracted to the rest of the cell (Chen,

1981; Lauffenburger and Horwitz, 1996). FAs-anchored contractile actomyosin stress fibres carry out the contraction (Chen, 1981).

2.1 Motile cells fine tune the migration mode in 3D environment

In general, solitary cells migrating through a complex, more native, 3D environment are forced to opt either for generating their own path by ECM degradation or squeezing through pre-existing ECM pores. Based on these described strategies, two major modes of 3D migration can be distinguished: the mesenchymal and the amoeboid (see Fig. 2). The mesenchymal mode, a 'make the own path' strategy, is characterised by a strong and persistent polarisation, an elongated spindle-like cell shape, and well-developed actin-rich lamellipodium at the one stable leading edge. Mesenchymal cells display stronger adhesion, due to abundant mature FAs, and a higher degree of ECM degradation at the leading edge, exerted by enzymes such as matrix metalloproteases (Packard *et al.*, 2009). The other strategy, amoeboid mode (either protrusion- or actomyosin contractility-based) is typically faster 'sliding', preferably through pre-existing ECM pores, and has both a lower strength and total number of adhesive complexes. For reviews see Yamada and Sixt, (2019); Pawluchin and Galic, (2022); Raudenská *et al.*, (2023).

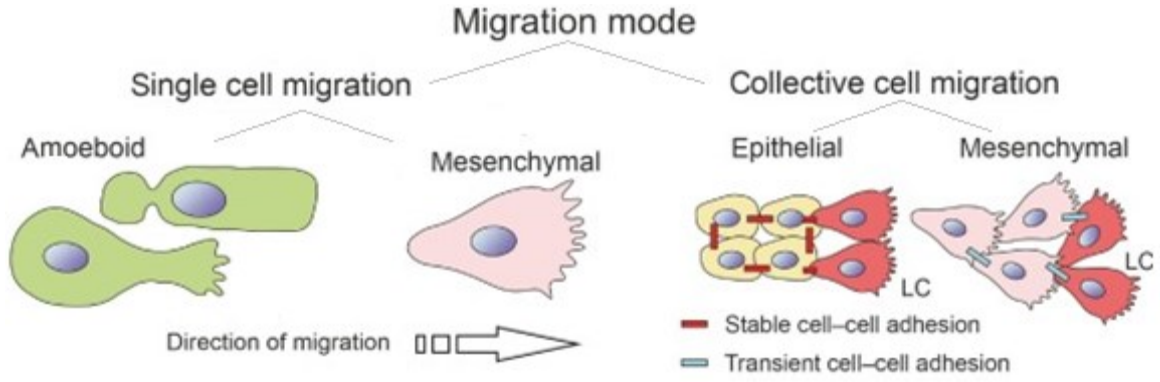
Amoeboid and mesenchymal modes constitute two extremes of a continuum rather than mutually excluding categories (reviewed by Pawluchin and Galic, 2022). Fine tuning of a particular mode is dependent on innumerable, intrinsic and external factors such as energetic balance, intercellular contacts, chemical cues, substrate adhesion, and ECM properties (Petrie *et al.*, 2012; Liu *et al.*, 2015; Lehmann *et al.*, 2017; Talkenberger *et al.*, 2017; Pietrovito *et al.*, 2018). Although best-understood, simplified 2D migration displays only a limited diversity, usually resembling the mesenchymal mode. Current research has focused on studying migration inside 3D matrices, which better represents the real environment (reviewed by Yamada and Sixt, 2019).

2.2 Cells could cooperate and migrate collectively

Cells migrate either individually or collectively as a cooperating mass (see Fig. 2). In epithelial collective migration, cells preserve their epithelial character and stable cell-cell adhesion, migrating as an interconnected sheet. Another option is to undergo epithelial-mesenchymal transition (EMT) - a process resulting in mesenchymal characteristics, such as a loss of apicobasal polarisation, decreased adhesivity and promoted migration and invasiveness (reviewed in Haensel and Dai, 2018). Desmosomes and another cell-cell junction disassembly during the initiation of migration has been observed and it is considered to be a part of EMT (Paladini *et al.*, 1996; Garrod *et al.*, 2005; for review see Moreno-Bueno, Portillo and Cano, 2008). In addition, EMT is accompanied by IFs rearrangement (reviewed by Yang *et al.*, 2020). Mesenchymal cells could migrate completely

detached (although still cooperatively) or display only transient cell-cell adhesion (reviewed by Yamada and Sixt, 2019; Raudenská *et al.*, 2023). Similar to the amoeboid-mesenchymal continuum, the epithelial/mesenchymal character of collective migration also takes on many transitional states (Polioudaki *et al.*, 2015).

Fig. 2: Simplified migration mode classification. Cells migrate either individually or collectively. In a real-life complex environment, categories are rather a continuum and cells exert apparent plasticity in the mode they particularly choose. LC - leader cell. (Raudenská *et al.*, 2023; modified)



3. Intermediate Filaments

Intermediate filaments (IFs) are the third characterised constituent part of the cytoskeleton after microfilaments and microtubules. Because the reported diameters peaked at 100 Å (10 nm), which was intermediate in comparison with other thin sarcoplasmic fibres, Ishikawa, Bischoff and Holtzer (1968) started to use the term 'intermediate filaments'. The ubiquitous nature and versatility of IFs have subsequently been shown, as they have been observed in the cytoplasm of various non-muscular mammalian cells such as neurons or fibroblasts, being related to cell-cell connection in an epithelium, and also in records of similarly-structured fibres as a part of the nuclear envelope (Wuerker, 1970; Brecher, 1975; Starger and Goldman, 1977; Aebi *et al.*, 1986). Later, based on sequence similarity, all of these IF proteins have been affiliated to the same large protein superfamily (McKeon, Kirschner and Caput, 1986; Hesse, Magin and Weber, 2001).

IFs are built of a broad spectrum of different proteins, which belong to a large protein superfamily, encoded by at least 70 functional genes in the human genome, and their combination is cell-type and context specific (McKeon, Kirschner and Caput, 1986; Hesse, Magin and Weber, 2001; Szeverenyi *et al.*, 2008). Based on sequence similarity and an expression pattern, five or six (depending on the author) distinct IF classes have been differentiated. However, not all of the authors are in agreement and references such as Fuchs and Weber (1994), Herrmann and Aebi (2000), and Sanghvi-Shah and Weber (2017) have grouped them slightly differently. Tables 1 provides an overview of IF classes.

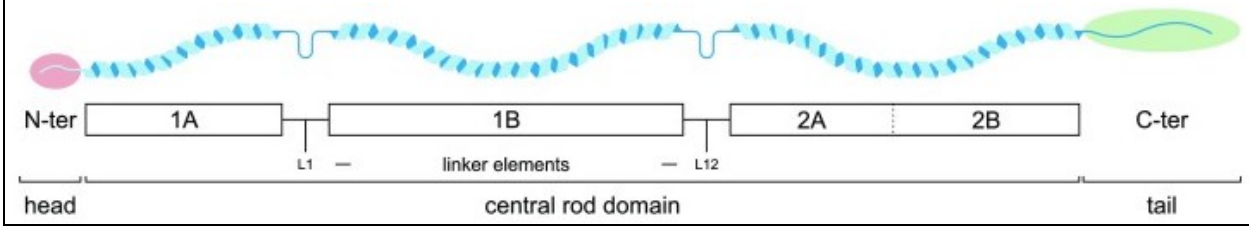
Table 1: Brief overview of IF protein classes. The table primarily shows IF proteins mentioned in this thesis. (Classification adopted from (Strelkov, Herrmann and Aebi, 2003; Sanghvi-Shah and Weber, 2017; Russell, 2020; Infante and Etienne-Manneville, 2022).

CLASS	CATEGORIZATION	REPRESENTATIVES	ROLE IN MIGRATION	REFERENCES
I, II	Acidic keratins (type I), basic keratins (type II)	Type I and II keratins (I: 28 genes in humans, II: 26 genes in humans)	Regulation of adhesive complexes (hemidesmosomes, desmosomes, FAs):	(Kröger *et al.*, 2013; Seltmann *et al.*, 2015; Pora *et al.*, 2019)
III	Homodimerizing cytoplasmatic IFs (sometimes form heterodimers with class IV/VI IFs)	Vimentin	Regulation of cell mechanics, cytoplasmic stiffening	(Guo *et al.*, 2013)
			Provides the mechanical stress resistance	(Eckes *et al.*, 1998)
			Protection of the nucleus, preventing its rupture	(Patteson *et al.*, 2019)
			Keratin rearrangement and expression regulation	(Dmello *et al.*, 2017)
			EMT regulation	(Sivagurunathan *et al.*, 2022)
			FAs regulation	(Tsuruta and Jones, 2003)
			FA-related mechanosensing	(Gregor *et al.*, 2014)
			Polarity establishment and maintenance	(Helfand *et al.*, 2011; Gan *et al.*, 2016)
			Regulation of stress fibres	(Jiu *et al.*, 2017)
			Absorption/redistribution of actomyosin-generated forces	(Costigliola *et al.*, 2017)
		GFAP	FAs regulation	(Moeton *et al.*, 2016)
IV	IFs xpressed mainly in neural or muscular tissues	synemin (α and β)*	FAs regulation	(reviewed by Russell, 2020)
V	Nuclear IFs (lamins)	A-type lamins (A,C)	Increasing nuclear stiffness	(Lammerding *et al.*, 2006)
			Nuclear durability in confined spaces	(Harada *et al.*, 2014)
			Nuclear durability in confined spaces	(Pieuchot *et al.*, 2018)
		B-type lamins (B1, B2)	Promoting migration via perinuclear actin reorganization	(Fracchia *et al.*, 2020)

* Some authors classify synemins as a separate group (VI), yet they share particular similarity with neurofilament proteins, some authors categorise synemins to the same group (IV) as neurofilaments (Peter and Stick, 2015; Russell, 2020).

IF proteins share a conserved central rod-domain, approximately 310-350 amino acids long, which is composed of an alpha-helical structure (Geisler and Weber, 1982; McKeon, Kirschner and Caput, 1986). Flanking non-helical N-terminal heads and C-terminal tails are more variable, differentiating IF functions, and reaching highly variable sizes, ranging between 8 and 1,200 amino acids in length (Dahlstrand *et al.*, 1992; Titeux *et al.*, 2001; Esue *et al.*, 2006). A monomer general structure is depicted in Fig. 3. Unlike actin and tubulin (both globular proteins with NTPase activity), IFs monomers are characterised by an elongated structure, do not involve an NTP binding site and possess no enzymatic activity (for review see Herrmann and Aebi, 2016; Ndiaye, Koenderink and Shemesh, 2022).

Fig. 3: Nestin monomer structure. The same architecture – based on an alpha-helical central rod domain, and more variable N-terminal head and C-terminal tail – is shared within the whole IF protein family. (Adopted from Bernal and Arranz, 2018)



3.1 Assembly and Dynamics of IFs

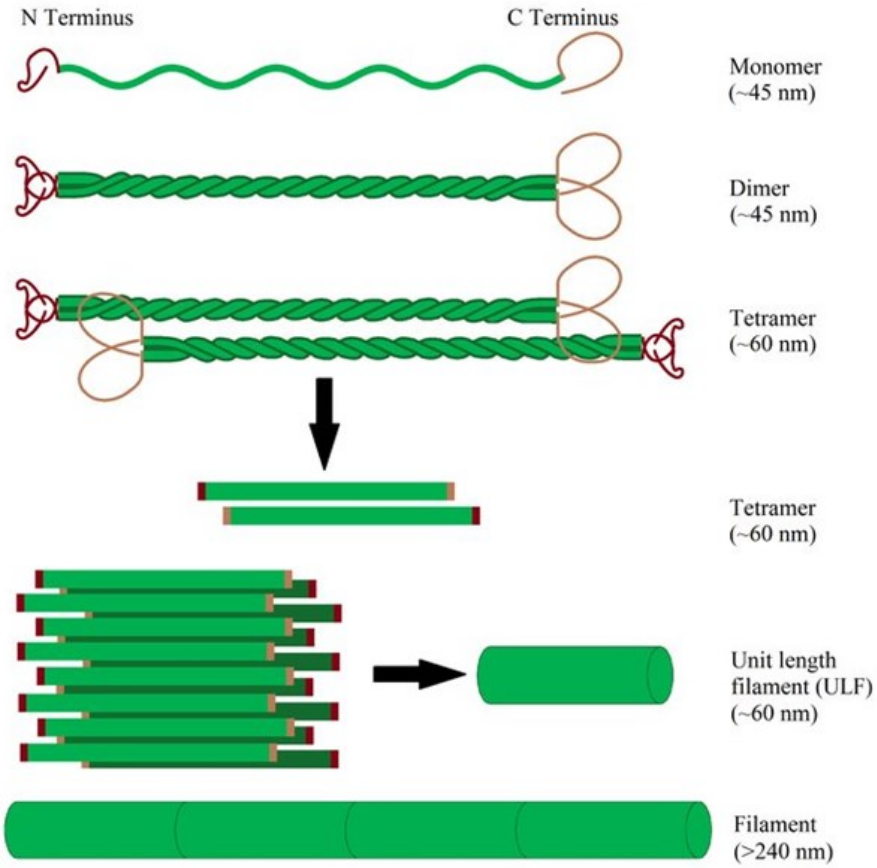
Elongated IF protein monomers associate together to form higher hierarchical structures, able to possess either a heteropolymeric or homopolymeric character (Steinert *et al.*, 1981; Steinert, 1990). In early IF assembly stages, lateral attachment and reciprocally winding helical central 'rod' domains of two monomers spontaneously form dimers, displaying an extremely stable coiled-coil structure (McLachlan, 1978; Coulombe and Fuchs, 1990). Subsequently, two dimers attach in an antiparallel manner, resulting in a non-polarised tetramer unit with two equivalent endings (Geisler, Kaufmann and Weber, 1985). Afterwards, the tetramers rapidly (~ seconds) congregate together to form a so-called "unit-length filament" (ULF), the final building blocks able to anneal end-to-end and elongate into a final long fibre (Çolakoğlu and Brown, 2009; Czeizler *et al.*, 2012). The process, depicted in Fig. 4, produces non-polarised resilient fibres whose diameter ranges roughly between 8 and 12 nanometres (Engel, Eichner and Aebi, 1985).

The whole assembly is cofactor-independent under *in vitro* conditions (Herrmann, Kreplak and Aebi, 2004). *In vivo* assembly is more complex, guided by chaperones and enzymes exerting posttranslational modification of IFs proteins (Nicholl and Quinlan, 1994; Beil *et al.*, 2003). Notably, unlike microtubules and microfilaments (fast, ~ seconds-minutes, treadmill driven by NTP

hydrolysis), the dynamics of an IF cytoskeleton is not directly NTP-hydrolysis-dependent as IFs lack any intrinsic hydrolase activity (reviewed in Ndiaye, Koenderink and Shemesh, 2022). On the other hand, a slower (~ hours) IF re-modulation happens via tetramer subunit exchanges along the whole length of the fibres (Çolakoğlu and Brown, 2009). The IF's building blocks occur in the cytoplasm as tetramers, larger particles, or short fibres (Soellner, Quinlan and Franke, 1985; Prahlad *et al.*, 1998; Yoon *et al.*, 1998).

Phosphorylation has been the most studied IF posttranslational modification, especially in the context of mitosis. For decades, lamin phosphorylation and subsequent nuclear disassembly during mitosis have been known to occur in mammalian cells (Gerace and Blobel, 1980). In addition, cytoplasmic IFs undergo regulating (de)phosphorylation (Strnad, Windoffer and Leube, 2002; Beil *et al.*, 2003). Other posttranslational IFs modifications, such as acethylation, sumoylation, and ubiquitinylation, have been described that likewise affect IF dynamics (Ku and Omary, 2000; Snider *et al.*, 2011, 2013).

Fig. 4: IFs general assembly model. In the beginning, dimers and then tetramers are rapidly and spontaneously formed. Tetramers laterally associate to create an ULF. Finally, ULFs anneal together (end-end joining) to elongate the final fiber. The hierarchical assembly model produces a non-polarised fibre (with two equivalent endings) that is moreover extremely durable. (Adopted from Hohmann and Dehghani, 2019)



3.2 IFs are unique in mechanical properties and force-response

Persistence length (L_P) expresses the flexibility of biopolymers. Informally, the persistence length corresponds to a measure of the distance over which a filament appears approximately straight (as reviewed by Riveline, 2013; Charrier and Janmey, 2016). IFs display exceptional flexibility with L_P (~ 0.3-2 μm) several orders of magnitude lower compared with semi-flexible microfilaments and rigid microtubules (Gittes *et al.*, 1993). Besides extraordinary flexibility, IFs could be extended up to 300 % along their length without rupturing, whilst microtubules and microfilaments are able to bear only an extension not exceeding 50 % of their length (Janmey *et al.*, 1991; Qin, Kreplak and Buehler, 2009). In addition, IFs tend to stiffen while extended, something not observed in microtubules and microfilaments, which predestine IFs as the cell mechanics contributor (Janmey *et al.*, 1991). Vimentin fibres are also efficient in dissipating of input energy - up to 70 % (Block *et al.*, 2018).

Considering their unique rheological properties, IFs seem to be an ideal candidate for being a carrier of a cell's mechanical properties. Ultra-resolution microscopy has substantially revealed these extraordinary features to be not due to sliding IF subunits, but rather secondary structure changes when α-helical structure is unfolded and replaced by β-sheets (Fleissner *et al.*, 2020; Nunes Vicente *et al.*, 2022). Moreover, Block *et al.* (2018) have demonstrated vimentin IFs to have a certain 'tensile memory', displaying long-lasting changes in a viscoelastic response of individual fibres, while being exposed to cyclical stretching. This memory is contained within long-term conformational changes based on incomplete restoration of switches from α-helices to β-sheets while exposed to repetitive mechanical stress (Block *et al.*, 2018). Furthermore, *in vivo* strain-exposed cells displayed vimentin IFs with a long-term altered secondary structure and changed pattern in particular phosphorylations correlated with tension (Fleissner *et al.*, 2020). This opens up an interesting viewpoint on vimentin as a mechanosensor itself, although more research in this field is needed.

4. IFs as Part of an Interconnected Cytoskeletal Network

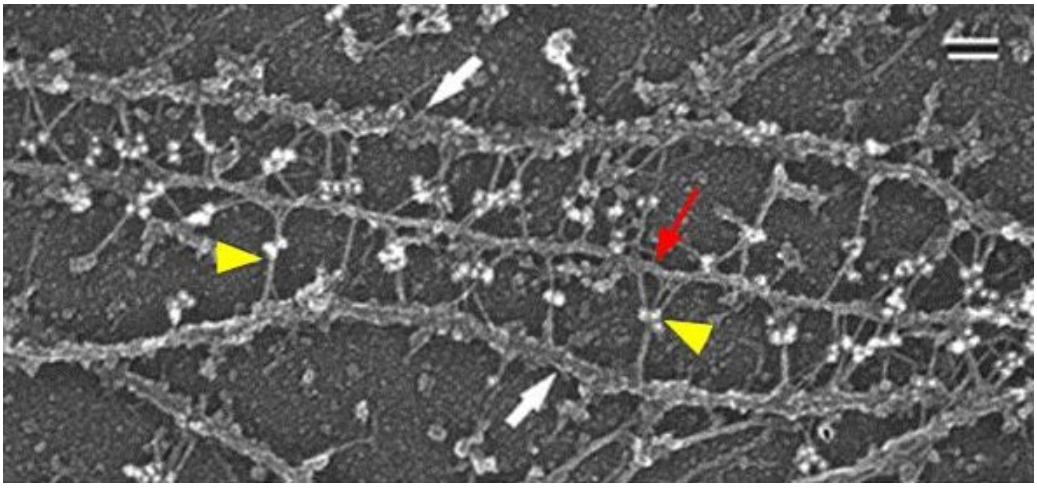
In vivo, the three main cytoplasmic skeletal networks are not independent, but abundantly interconnected - either physically bonded by direct interaction or numerous cross-linking proteins, or indirectly interdependent via a multitude of signalisation pathways (Svitkina, Verkhovsky and Borisy, 1996; Esue *et al.*, 2006; Bocquet *et al.*, 2009; Schaedel *et al.*, 2021).

The first evidence of physical association of IFs with microtubular cytoskeleton was observed in fibroblasts, where IFs and microtubules occur in parallel arrays throughout the cytoplasm (Goldman, 1971; Ball and Singer, 1981). Vimentin IFs protect microtubules from depolymerisation, support

microtubular rescue and affect microtubular mechanics - microtubules are able to undergo higher compressive forces due to lateral IF reinforcement under both *in vitro* and *in vivo* conditions (Brangwynne *et al.*, 2006; Schaedel *et al.*, 2021). Similarly, microfilaments have been known to interact with IFs as well (Svitkina, Verkhovsky and Borisy, 1996; Jiu *et al.*, 2015).

Plectin, with binding sites for microfilaments, microtubules, and various IFs, has been identified as a versatile cytoskeletal cross-linker (see Fig. 5), interacting with rod-domain of type III IFs and multiple domains of keratins (Seifert, Lawson and Wiche, 1992; Svitkina, Verkhovsky and Borisy, 1996; Favre *et al.*, 2011; Bouameur *et al.*, 2014). Besides plectin, a multitude of other different molecules interconnect IFs with at least one other cytoskeletal system such as fimbrin, filamin A, dynactin or APC (reviewed by Sanghvi-Shah and Weber, 2017). IFs and their precursor are also bidirectionally transported along microtubules and microfilaments, via interacting with molecular motors kinesin, dynein and myosin (IIA) (Prahlad *et al.*, 1998; Helfand *et al.*, 2002; Wang *et al.*, 2018; Ostrowska-Podhorodecka *et al.*, 2022).

Fig. 5: SEM microphotography capturing numerous plectin bridges (yellow arrowhead) between microtubules (white arrow) and vimentin IFs (red arrow). For scale, the bars represent 0.1 μm. (Svitkina, Verkhovsky and Borisy, 1996, modified)



4.1 IFs regulate cytoplasmic stiffness, thus affecting motility and mechanical resilience

Vimentin IFs contribute considerably to cytoplasmic stiffness, although they are little involved in the cortical mechanics of migrating fibroblasts, where microfilaments carry the majority of mechanical properties (Eckes *et al.*, 1998; Guo *et al.*, 2013; Vahabikashi *et al.*, 2019). In vimentin-depleted mesenchymal stem cells, cytoplasmic mechanics is only partially compensated by the remaining, less-flexible cytoskeletal networks and the affected cells displayed decreased deformability (Sharma *et al.*, 2017). These observations correspond with the mechanical properties

of particular cytoskeletal fibres that were earlier mentioned in chapter 3.2 and show vimentin IFs to be at least in some cell types crucial for proper flexibility. Vimentin-dependent changes in the mechanical properties of cytoplasm are not only crucial to the mechanics of the whole cell, but also could affect particular signalling processes because they retard the movement of subcellular compartments (Guo *et al.*, 2013).

There is accumulating evidence that promigratory changes, predetermining the cells for the squeezing through confined pores, are accompanied by IF alterations that contribute to cytoplasmic stiffness. For example, diapedesis of T-lymphocytes to an inflammatory site, requiring them to squeeze through the endothelium, is accompanied by a collapse of vimentin IFs - normally spread throughout cytoplasm and carrying most of the lymphocyte cytoplasmic stiffness - into a form of juxtanuclear aggregate (Brown *et al.*, 2001)

EMT is accompanied by both rearrangement of the IF network and changes in particular IF proteins expression. Vimentin (a reliable EMT marker) is expressed, whilst expression of (epithelium-related) keratin IFs is supressed (Dmello *et al.*, 2017; Yang *et al.*, 2020; Sivagurunathan *et al.*, 2022). Intriguingly, vimentin itself plays a pivotal role in EMT regulation; intact vimentin IFs are capable of inducing EMT in epithelial cells and regulates expression of migration-contributed genes (Sivagurunathan *et al.*, 2022). Keratin reorganisation and changes in cytoplasmic stiffness have been observed by Sivagurunathan *et al.* (2022) after inducing a vimentin expression in epithelial cells. During promigratory changes, IFs rearrange, partially retract from the cell periphery, and localise more perinuclearly (Paladini *et al.*, 1996). Beil *et al.* (2003) concluded that cytoplasmic ‘softening’ and elasticity elevation, caused by keratin reorganisation, facilitates migration through size-restricted spaces, thereby increasing migratory potential. Indeed, keratin 10-depleted keratinocytes *in vivo* showed accelerated migration through a confined environment of epidermal structures, whereas intact keratin 10 IFs retarded this motility (Reichelt and Magin, 2002).

Finally, IFs are required for successful resistance to shape distortion, which accompanies the squeezing through tight EMC pores. Vimentin IFs contribute to cell mechanical stability and prevent cells from rupturing while they are deformed (Maniotis, Chen and Ingber, 1997; Eckes *et al.*, 1998). Moreover, perinuclear cytoplasmic stiffness is important in protecting the nucleus. In migrating fibroblasts, vimentin IFs create a perinuclear ‘cage’ which protects the nucleus from deformation, thus hindering migration through restricted pores while considerably reducing nuclear-rupture frequency, DNA damage, and the necrosis rate (Patteson *et al.*, 2019).

4.2 IFs assist a polarity establishment and maintenance

At the leading edge, dynamics of actin and vimentin IFs are profoundly intertwined. Actin microfilaments are responsible for vimentin retrograde flow, observed in a migration-related relocalisation to the perinuclear area (Jiu *et al.*, 2015). Vimentin IF phosphorylation and subsequent disassembly drives oriented lamellipodium formation while, in the immediate proximity of the leading edge, only vimentin particles and no intact long IFs are present (Helfand *et al.*, 2011). In addition, vimentin arranges the localisation of contractile actomyosin arcs, which contribute to the determination of lamella orientation and morphology (Jiu *et al.*, 2015).

Interestingly, the preservation of established polarisation and directional persistence are microtubule-dependent, yet individual lamellar microtubules in migrating fibroblasts possess too fast a turnover on a migration event scale (Shelden and Wadsworth, 1993; Gan *et al.*, 2016). However, longer-lived vimentin IFs align with protrusions and overall cell direction, as well as microtubules, which serve as a clue to directional IFs assembly (Gurland and Gundersen, 1995; Gan *et al.*, 2016; Costigliola *et al.*, 2017). Gan *et al.* (2016) proposed that longer-lasting vimentin IFs be a template for microtubular organisation memory, therefore assisting polarisation persistence. Indeed, the inability of persistence in directional movement was repeatedly described in both vimentin and microtubules-distorted cells (Eckes *et al.*, 1998; Takesono *et al.*, 2010).

4.3 IFs regulate actomyosin, thus govern contractile forces

A precise regulation of contractility is important for migration. Multiple IFs have been observed to affect contractile forces stemming from stress fibre activity (e.g. Jiu *et al.*, 2017; Fujiwara *et al.*, 2019). Vimentin-KO fibroblasts displayed changes in cortical contractile forces and contractile capacity to deform adjacent ECM (Eckes *et al.*, 1998; Vahabikashi *et al.*, 2019; Sivagurunathan *et al.*, 2022). A deterioration of the cell-ECM connection explains some of these observations (see chapter 5 for IF contribution to adhesion), but vimentin IFs have also been observed to affect the stress fibres themselves by modulating RhoA activity (Bordeleau *et al.*, 2012; Jiu *et al.*, 2017). RhoA is a small GTPase that promotes stress-fibre assembly and contractility (Ridley and Hall, 1992; Katoh *et al.*, 2001; Sarasa-Renedo, Tunç-Civelek and Chiquet, 2006). Vimentin suppresses RhoA via binding GEF-H1 (RhoA-targeting GEF) to prevent its activation, thus diminishing stress fibres assembly and contractility (Jiu *et al.*, 2017).

Vimentin IFs absorb and redistribute actomyosin-generated forces, making them more homogenously distributed throughout the cell with decreased local forces maxima (Costigliola *et*

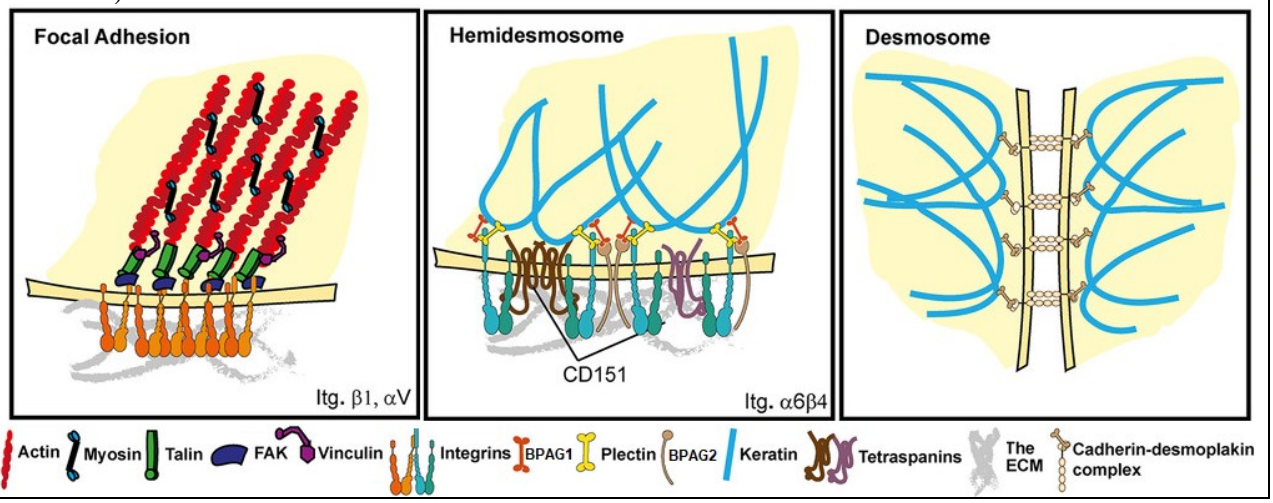
al., 2017). In addition, Costigliola *et al.*, (2017) showed that directionality of forces applied by a cell to the surrounding matrix is tightly coupled with a local IF orientation.

Keratins contribute to actomyosin and contractility regulation in epithelial cells. Keratin 6 binds and stabilises myosin IIA, which modulates actomyosin contractility (Wang *et al.*, 2018). Another possible way it acts is by affecting RhoA, but in a different manner than vimentin. Solo (also referred as ARHGEF40) is another RhoA-targeting GEF promoting stress fibre formation and reinforcement (Fujiwara *et al.*, 2019). For Solo, proper localisation and function is necessary to bind to intact keratin 8/18 fibres (Fujiwara *et al.*, 2019).

5. IF Contribution to Cell Adhesion

For migrating cells, the ability to adhere to surrounding surfaces is indispensable. This chapter focuses on three important groups of adhesive complexes where IFs are known to have contributed: actin-linked focal adhesions (connecting cell-ECM) whose regulating agents are the IFs themselves, and IFs-linked desmosomes (cell-cell junction) and hemidesmosomes (cell-ECM junction). Fig. 6 provides an overview of these adhesive complexes.

Fig. 6: Schematic representation of focal adhesion, hemidesmosome, and desmosome. Not all the discussed molecules are involved in this depiction. (Zuidema, Wang and Sonnenberg, 2020; modified)



FAs are multiprotein complexes, occurring in various protein combinations, which often, but not always, involve regulatory intermediate filaments (DiPersio, Shah and Hynes, 1995; Uyama *et al.*, 2006; Leube, Moch and Windoffer, 2015). FAs are known for being a dynamic signalisation hub, comprehending regulation proteins such as kinases (FAK, Src) and small GTPases (Ridley and Hall, 1992; Schaller *et al.*, 1992). The entire structure is connected to contractile (myosin-containing) actin stress fibres, driving the contraction of motile cells (Chen, 1981).

IFs-linked hemidesmosomes connect cells with ECM. A hemidesmosomal plaque is formed from a few proteins including adaptor proteins – plectin and BPAG1 – linking hemidesmosomes with keratin (Rezniczek *et al.*, 1998; Fontao *et al.*, 2003). Desmosomes, in contrast to the previously described complexes, exert cell-to-cell connection via transmembrane proteins from a cadherin group (desmocollin, desmoglein). Intracellularly, a desmosomal plaque is formed, composed of various proteins including keratin-binding desmoplakin. For a review, see Green and Jones, (1996).

Cell-to-ECM adhesion is dependent on a large family of transmembrane receptor proteins – integrins, which form heterodimers involving different combinations of α and β subunits and ensure cell adhesion to matrices (reviewed by Hynes, 1987). The hemidesmosomal-specific integrin is integrin α6β4 (Stepp *et al.*, 1990). In the case of FAs, more possible combinations of α/β subunits are possible (DiPersio, Shah and Hynes, 1995; Cai *et al.*, 2009).

5.1 IFs are engaged in focal adhesions and their regulation

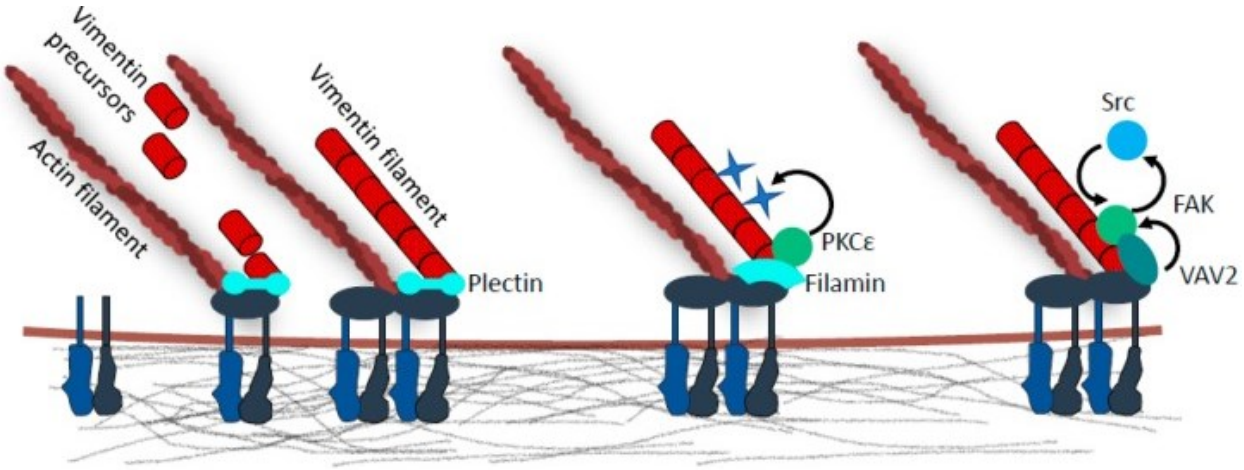
IF-microfilament coupling could be dependent on adhesive sites; a vimentin-fimbrin association has been observed to be focal adhesion-dependent (Correia *et al.*, 1999). Gonzales *et al.*, (2001) demonstrated that endothelial cells have abundant αvβ4-integrin-rich FAs, often accompanied by vimentin IF protein. In astrocytes, different GFAP isoforms accompany FAs (Moeton *et al.*, 2016). Keratin-6 depleted keratinocytes displayed a faster migration accompanied by a higher FA disassembly rate (Wang *et al.*, 2018).

Some proteins involved in FA complexes are known to be binding IF proteins, such as plectin and filamin A (Svitkina, Verkhovsky and Borisy, 1996; Kim *et al.*, 2010). Plectin, a versatile cytoskeletal cross-linker described in the previous chapter, has many splice variants, from which a splice variant plectin f1 is FA-specific (Svitkina, Verkhovsky and Borisy, 1996; Burgstaller *et al.*, 2010). Fimbrin, a phosphoprotein localised at adhesion sites, possibly generates fimbrin-vimentin complexes (Messier *et al.*, 1993; Correia *et al.*, 1999). Synemins (α and β) are interesting IF proteins, often called ‘exotic’, incapable of assembling homopolymeric fibres themselves, but only to copolymerise with group III IFs (e.g. vimentin, GFAP) (reviewed by Russell, 2020). They are known interaction partners for several FAs proteins like plectin, talin or vinculin (Uyama *et al.*, 2006; Hijikata *et al.*, 2008; Sun *et al.*, 2008; for review see Russell, 2020).

As described, physical coupling of IF proteins and FAs is evident, and its relevance for FAs regulation and cell migration has been demonstrated as well. Vimentin-deficient fibroblasts possessed impaired actin cytoskeleton organisation and FAs distribution, resulting in perturbed migration (Eckes *et al.*, 1998). Tsuruta and Jones (2003) showed that vimentin depletion in

immortalised bone marrow endothelial cells results in changes in FAs morphology and a reduction of adhesion strength. Moreover, the flow-exposed cells flexibly reacted by assembling larger FAs, interconnected with thick vimentin bundles (Tsuruta and Jones, 2003). To reveal the exact IF function within FAs seems to be more challenging. The enumeration of the possibly involved mechanisms given below demonstrates the complexity of this regulation (also see Fig. 7 for possible IF-FAs interactions schemes).

Fig. 7: Vimentin-containing focal adhesion. Vimentin IF emerges right at FAs through end-to-end joining of precursors; then serves as a signaling platform for FAs-related kinases. (Adopted from: Strouhalova *et al.*, 2020)



- **Impact on PKC signalisation**

  Vimentin is targeted by several kinases, including PKC (Eriksson *et al.*, 2004). Filamin A is crucial for the association of PKC and vimentin on FAs (Kim *et al.*, 2010). PKCε has a crucial role in the FA's integrin-β1 return to the membrane after endocytosis and inhibition of PKCε signalling arrests migration (Ivaska *et al.*, 2002). Results from Ivaska *et al.* (2005) indicated that PKCε's phosphorylation of vimentin holds results in integrin, therefore FAs, turnover within the cell, thus affects adhesivity and migratory potential.

- **Involvement in the Src/FAK signalling pathway**

  Another important kinase observed to interact with vimentin is FAK, interacting with vimentin via the scaffolding molecule RACK1 (Dave *et al.*, 2013). RACK1 and vimentin are upregulated during angiogenesis-related migration, and create a complex with FAK, which affects FAK activity and regulates proper cell invasion in angiogenesis. Simultaneously, vimentin recruits VAV2, which promotes FAK activity (Havel *et al.*, 2015). Moreover, woundhealing-associated keratin 6 directly binds and inactivates Src kinase, which phosphorylates the mentioned FAK, suggesting another mechanism affecting Src/FAK (Rotty and Coulombe, 2012). In the collective migration of keratinocytes, Src activity is inactivated by binding directly to wound-induced

keratin 6, while keratin-loss-contributed Src enhancement increased FAs turnover (Rotty and Coulombe, 2012; Wang *et al.*, 2018).

- **Extensible IFs help with integration of the mechanical signal at FAs**

  The process of apprehending mechanical forces and transforming them into a downstream signalisation and a proper response is referred as mechanotransduction. Multiple mechanotransduction mechanisms have been described; involving membrane receptors, focal adhesions, deformation-sensitive ion channels and some cytoplasmic structures such as sarcomeric and cytoskeleton-related proteins (reviewed by Hahn and Schwartz, 2009; Chen *et al.*, 2017). Vimentin IFs contribute to FA mechanotransduction: an intact vimentin cytoskeleton and its anchorage to FAs are necessary prerequisites for tensional FAK activation (Gregor *et al.*, 2014). Although the exact mechanism of vimentin-dependent FAK activation in mechanosensing remains elusive, the response of extensible IFs fibres to tensile forces has been described (presented in chapter 3.2) and vimentin itself could possibly act as the mechanosensor. More about cell response to mechanical stimuli is presented in chapter 6.3.

5.2 IFs contribute to EMT and regulate desmosomes in collective migration

Initially, IFs-linked desmosomes were considered to be only a highly stable structure, ensuring hyper-adhesive intercellular connection, as well as the durable mechanics of the entire tissue, and impeding migration. This is generally true in intact homeostatic tissues (Windoffer, Borchert-Stuhlträger and Leube, 2002; Garrod *et al.*, 2005; Thomason *et al.*, 2012). But, as mentioned in chapter 2.1, cells could migrate also as an interconnected cell mass, sealed together, for instance, by desmosomes attached to keratin IFs. An example of this is what happens during the deeply studied process of wound healing re-epithelisation. Garrod *et al.* (2005)

IFs play an important role in desmosomal stabilisation and the regulation of function, rather than in assembly (Vasioukhin *et al.*, 2001; Kröger *et al.*, 2013). It turned out that keratin IFs stabilise desmosomes by sequestrating PKCα in cytoplasm through the scaffolding molecule RACK1. Subsequently, PKCα is not available for a phosphorylation of the protein desmoplakin (Kröger *et al.*, 2013; Seltmann *et al.*, 2015; Loschke, Homberg and Magin, 2016). The desmoplakin C-terminus associates with keratin IFs (Stappenbeck and Green, 1992; Kouklis, Hutton and Fuchs, 1994) and its phosphorylation via PKCα is linked to desmosomal complex destabilisation and internalisation (Kröger *et al.*, 2013). During premigratory changes, keratin IFs rearrange, partially retract from the cell periphery, and diminish their contact with desmosomal components, which leads to an observable decrease in the desmosomal count (Paladini *et al.*, 1996).

(Garrod *et al.*, 2005) noted that keratinocytes subjected to promigratory conditions alter the remaining desmosomes to be Ca2+ dependent and changed their microstructure to be less organised, leading to lower adhesivity and resembling the more dynamic FAs. Indeed, desmosomes vary between two states – a Ca2+ independent, well-organised, hyper-adhesive state and a less adhesive, Ca2+ dependent state, where the switch between these two modes is connected with PKCα activity which, as mentioned previously, is regulated by keratin IFs (Wallis *et al.*, 2000; Kimura, Merritt and Garrod, 2007; Thomason *et al.*, 2012).

Finally, cells change the keratin isotypes' expression pattern with keratin 6, 16 and 17 induced in promigratory, wound-healing settings (Paladini *et al.*, 1996). Migration-contributed keratins (namely keratin 6 and 17) do not sequestrate PKCα (Loschke, Homberg and Magin, 2016). It is still not clear whether a reduction of PKCα sequestration itself or even a decreased IF's physical link to desmosomes plays a role in desmosomal destabilisation in migrating keratinocytes.

5.3 IFs are involved in FAs-hemidesmosomal cross-talk

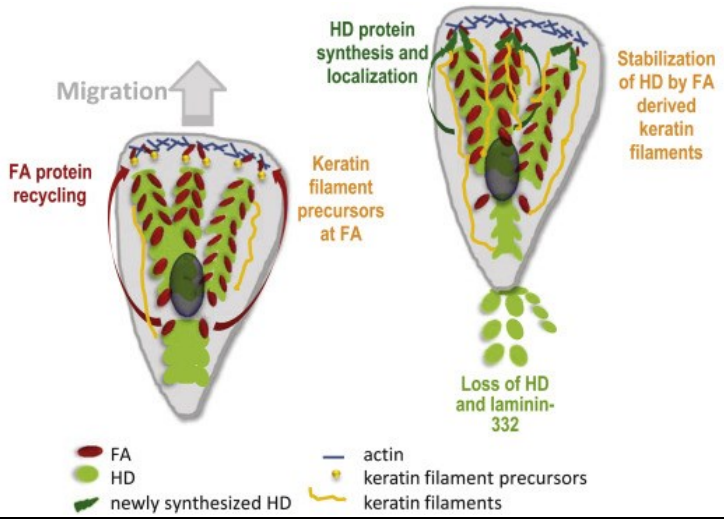
Keratin IF anchorage to a hemidesmosomal plaque is required for proper migration of some motile cells, e.g. leading cells in collective migration, where a loss of keratin anchorage significantly retards epithelial migrating cells in wound re-epithelisation, (Guo *et al.*, 1995; Rigort *et al.*, 2004; Ozawa *et al.*, 2010; Pora *et al.*, 2019).

IF presence changes hemidesmosomal localisation and stabilises the hemodosmosomes by facilitating the binding of them to plectin (Seltmann *et al.*, 2013, 2015). Furthermore, IFs stabilise hemidesmosomes by regulating integrin-β4 turnover from the impact by ERK1/2 (influenced by PKCα signalisation) on integrin phosphorylation, which leads to the destabilisation and internalisation of desmosomal components whilst keratin IFs prevent this phosphorylation, thus stabilising the desmosomes (Frijns *et al.*, 2010; Seltmann *et al.*, 2015).

Multiple authors have reported mutual FA interdependence along with hemidesmosomal assembly and dynamics. In addition, hemidesmosomes in motile keratinocytes (both solitary migrating and leading, collectively migrating cells) are distributed toward the leading edge in a regularly alternating chevron-like pattern with intercalated FAs (as could be seen in Fig. 8). (Ozawa *et al.*, 2010; Colburn and Jones, 2017; Pora *et al.*, 2019). This distribution likewise applied in the study of Pora *et al.* (2019).

Although the exact nature of FAs-hemidesmosomal interdependence remains unknown and involves likely multiple mechanisms (as discussed in Hatzfeld and Magin, 2019; Pora *et al.*, 2019), some studies highlight the prominent function of keratin IFs themselves and their dynamics in the establishment of hemidesmosome-FA interdependence. While the hemidesmosomal assembly disrupted by downregulation of keratin-14, FAs exerted an impaired size, velocity, and direction. In addition, affected cells lacked any polarisation of their movement And *vice versa* an abrogation of FA formation changed hemidesmosal shape and turnover (Pora *et al.*, 2019). Despite being primarily connected to keratin IFs, it has been shown that the dynamics of assembling hemidesmosomes, prior to keratin binding, is dependent on actin microfilaments and keratin IFs assembly happens in actin-rich areas such as the lamellipodium (Windoffer and Leube, 2001; Windoffer *et al.*, 2004; Roberts *et al.*, 2011). Indeed, there is a notable spatiotemporal coupling of FAs and formation of keratin precursor particles, which are subsequently transported along actin stress fibres before merging together to form long keratin fiber. FAs act as a hotspot for keratin cytoskeleton precursors formation (Windoffer *et al.*, 2006). This could explain the observations made by Ozawa *et al.* (2010), who demonstrated that hemidesmosome-FA cross-talk requires ATP and is myosin dependent. It is also consistent with Pora *et al.* (2019), which reported that FAs are adhesive structures first established at the leading edge and later followed by hemidesmosomes. Only then do the keratin filaments attach to a hemidesmosome. Subsequently, intact keratin IFs seem to be crucial for intgrin-β4 (hemidesmosomal) dynamics and hemidesmosomal stability (Seltmann *et al.*, 2015).

Fig. 8: Migrating keratinocyte visible chevron-patterned hemidesosomes and FAs. (Adopted from Hatzfeld and Magin, 2019; based on Pora *et al.*, 2019)



6. Nuclear Intermediate Filaments Impact in Migration

A nucleus occupies a remarkable volume of a cell, usually being the largest organelle, and simultaneously possesses a quite rigid shape, being approximately 5-10 times stiffer than the surrounding cellular structures (Lammerding, 2011). Thus, an unmalleable nucleus becomes a

considerable obstacle during migration, when the cell needs to squeeze itself through confined environment of dense ECM.

The nucleus is encircled by two membranes – the outer nuclear membrane (ONM) and the inner nuclear membrane (INM) (Callan, Tomlin and Waddington, 1950). Besides these two membranes, there is a nuclear envelope separating the nuclear space and the cytoplasm, consisting of a protein filamentous framework – nuclear lamina (NL) – adjacent to the INM and consisting of IF class V proteins (see table 1), generally referred as lamins (Fawcett, 1966; Gerace, Blum and Blobel, 1978).

Based on sequence homologies, two subclasses of lamins could be differentiated: A-type (Lamin A, C) and B-type (Lamin B1, B2) (McKeon, Kirschner and Caput, 1986; Höger *et al.*, 1990). Beside the major lamin isoforms mentioned above, a few minor isoforms have been described, including the minor isoform AΔ10 and the germ-line specific lamins B3 and C2 (Furukawa and Hotta, 1993; Furukawa, Inagaki and Hotta, 1994; Machiels *et al.*, 1996). Whilst type-B lamins are considered to be constitutively expressed in all mammalian cells (although Yang *et al.*, 2011 have shown dispensability of B-type lamins in some cell types), lamins A/C are acquired late in development, and lamin A/C is absent in early embryonic development and in some specialised or cancer cells (Röber, Weber and Osborn, 1989; Harborth *et al.*, 2001).

Nuclear lamina exert innumerable key functions: affecting nuclear mechanics, genome stability, DNA spatial organisation, telomere maintenance, and regulation of transcription and replication (Moir *et al.*, 2000; Lammerding *et al.*, 2006; Guelen *et al.*, 2008; Shimi *et al.*, 2008; Gonzalez-Suarez *et al.*, 2009; Kychygina *et al.*, 2021). Mutations in all lamin genes (most commonly in LMNA) could lead to a broad spectrum of rare genetic disorders, generally referred to as laminopathies and including such severe diseases as Hutchinson-Gilford progeria syndrome and muscular dystrophies (reviewed by Worman and Bonne, 2007). Moreover, nuclear IFs are known to regulate migratory abilities of cells and affect cell orientation, to be discussed in the following chapter.

6.1 Nuclear lamina is linked with other cytoskeletal components

Although enclosed in two membranes, the nuclear envelope scaffold is not completely isolated from other cytoplasmic cytoskeletal structures. In fact, a connection between the nucleoskeleton and microtubules, actin microfilaments, and cytoplasmic IFs has been described (reviewed in Liu *et al.*, 2016).

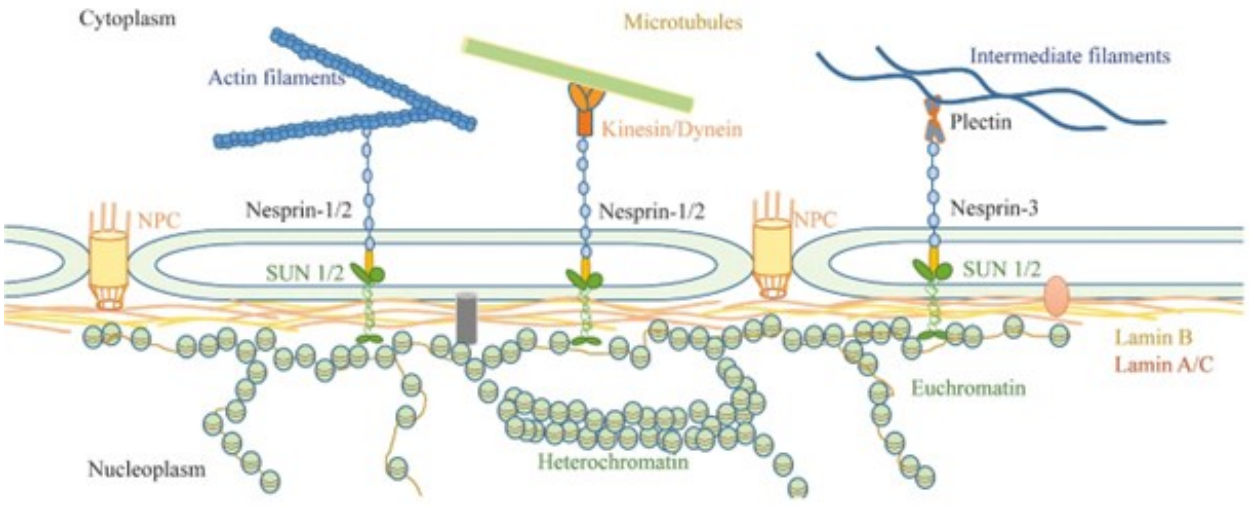
To connect the nuclear lamina with plasmatic cytoskeletal structures requires bridging two membranes, which is accomplished by so-called linker of nucleoskeleton and cytoskeleton (LINC)

protein complexes. The LINC complex is composed of two protein families with conserved transmembrane domains: SUN (Sad1/UNC-84) and KASH (Karsicht/ANC-1/SYNE homology) (reviewed by Starr and Fridolfsson, 2010). In a perinuclear space, the SUN domain interacts directly with the KASH domain, creating a physical bridge over the two nuclear membranes (Haque *et al.*, 2006). Figure 9 depicts and reviews the complexes that extend from the nucleoskeleton to the cytoskeleton.

SUN-domain proteins interact with the nuclear lamina and chromatin (Haque *et al.*, 2006). The mammalian KASH-domain protein family includes nuclear envelope spectrin repeat proteins (nesprins). Nesprin-1 and -2 directly bind actin microfilaments and indirectly interact with microtubules through molecular motors dynein and kinesins (Zhang *et al.*, 2002, 2009). Roux *et al.*, (2009) have recorded the most recently described nesprin, nesprin-4, to bind kinesin, while nesprin-3 mediates interaction of cytoplasmic and nuclear IFs via cross-linker plectin (Wilhelmsen *et al.*, 2005).

LINC complexes are involved in a range of processes such as nuclear anchorage, nucleus positioning, and the transfer of information about mechanical stimuli into the nucleus (Zhang *et al.*, 2010; reviewed by Rothballer, Schwartz and Kutay, 2013; Pieuchot *et al.*, 2018). How this impacts migration will be discussed in the next subchapter.

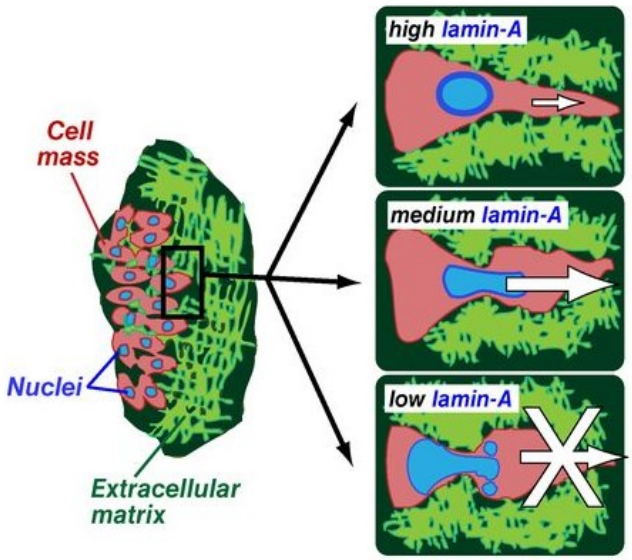
Fig. 9: LINC complexes connecting the nuclear skeleton with the cytoplasmic cytoskeletal component. SUNs and nesprins, the elements of LINC, physically bridge the nuclear mebranes. NPC - nuclear pore complex. (Liu *et al.*, 2016; modified)



6.2 Stiff nucleus, reinforced with the nuclear lamina, acts as a mechanical barrier

Different lamins affect nuclear mechanics in various manners. Nuclear envelope stiffness relies primarily on A-lamin type (Lamin A/C), whereas B-type lamins do not affect stiffness itself yet remains important in signalisation and retaining a nuclear integrity (Lammerding *et al.*, 2006). Either lamin A/C deficiency or mutation changes the nuclear mechanics. It loses its stiffness, its morphology is disrupted, and it becomes more susceptible to deformation under pressure (Lammerding *et al.*, 2006; Davidson *et al.*, 2014; Yang *et al.*, 2023). Lamin A tyrosine-phosphorylation stimulates disassembly of the nuclear lamina even in interphase cells, resulting in higher nuclear compliance and elevated migratory tendency (Chu *et al.*, 2021).

Fig. 10: Effect of different lamin A levels on migration. Higher levels of lamin A hinder migration, whilst low lamin A levels cause mechanical instability of the nucleus. (Harada et al., 2014)



Under physiological conditions, lamin A/C is absent in some highly motile cell types such as granulocytes, in which a softer, pliable nucleus probably facilitates diapedesis and chemotaxis to the inflammatory site (Olins *et al.*, 2008). In contrast with Lamin A/C, lamin-B upregulation could paradoxically promote migration by inhibiting interaction between the nuclear envelope and perinuclear microfilaments, which may limit nuclear deformability (Fracchia *et al.*, 2020).

Although higher nuclear stiffness impedes locomotion through confined pores in complex 3D environment and lamin A downregulation promotes migratory potential, cells with decreased lamin A expression seem to be less resistant to migration-related stress, resulting in a higher mortality rate for the afflicted cells, an effect similar to what has been observed in cells with a disrupted ‘outer’ protective perinuclear vimentin cage. (Harada *et al.*, 2014; Patteson *et al.*, 2019). Thus, cells have to compromise between higher motility and stress resistance – as reviewed in Fig. 10.

6.3 Nuclear lamins lie on the crossroad of mechanosensing

Although the orientation of motile cells based on various chemical gradients has been known for many decades (Carter, 1965), more recently a cell's ability to perceive mechanical stimuli, and to use them, for example as directing cues for precise navigation, has become more evident. When they migrate, cells need to sensitively react to subtle changes in the mechanical forces acting upon them and to evaluate possible corridors for navigating through tight pores in a complex 3D environment. Lo *et al.* (2000) revealed the movement of some cells according to a mechanical strain in an environment and the use an ECM rigidity for their orientation. Recent studies have recorded cells evaluating nanometre-scale nuances in the surrounding surface topography and they furthermore reflect its gradients (Park *et al.*, 2016). The perception of cell-scale topography deviation results in precise nuclear and FA positioning and a leading-edge navigation preferably through concave landscape "valleys" rather than "terrain hills". The authors referred this ability as a curvotaxis (Pieuchot *et al.*, 2018).

Though the nucleus has long been known to affect cell motility, it was first considered rather a plain mechanical barrier than an actively responding regulator. Intriguingly, recent findings suggest the nucleus itself to be capable of mechanical sensing. Transferring mechanical forces from the cellular surface (created by pulling on integrin receptors) directly into a deformation of the nucleus has been described by Maniotis, Chen and Ingber (1997), showing a direct physical connection between the nucleus and FAs located in the cytoplasmic membrane provided by the cytoskeleton. Furthermore, an experiment conducted by Pieuchot *et al.* (2018) have showed that impaired actin, the LINC complex and nuclear IFs led to an abrogation of curvotactical navigation, suggesting that the nucleoskeleton and LINC complexes, as well as actin microfilaments, are all required for proper response to a mechanical force.

Some authors proposed the possible exertion of a nuclear response through the mechanical alteration of a lamin meshwork and subsequent chromatin changes. In fact, reorganisation of inner nuclear sub-compartments caused by external forces has already been recognised (Maniotis, Chen and Ingber, 1997; Poh *et al.*, 2012). A nuclear lamina meshwork, interconnected with chromatin and a variation in a lamina structure, has been observed to change heterochromatin/euchromatin organisation (Shimi *et al.*, 2008; chromatin and lamina association reviewed by Dechat, Adam and Goldman, 2009) A rigid A-type lamin network and changes in chromatin organisation and condensation itself are known to modulate a stiffening of the nucleus (Stephens *et al.*, 2017).

In addition, changes in lamins and the following chromatin organisation are known to alter RNA polymerase II activity (Shimi *et al.*, 2008). Tajik *et al.*, (2016) demonstrated that mechanical forces

applied to a cell surface are propagated into the nucleus, leading to an upregulation of gene transcription, while disrupting actin microfilaments inhibits this effect. These data suggest that a direct mechanical deformation of chromatin could be projected to changes in protein expression, thus potentially enabling cell adaptation to the mechanical properties of its surrounding environment.

Altogether, these findings are consistent with other studies, indicating that the integrin-actin-LINC-lamina-chromatin axis is pivotal to nuclear mechanosensing. It is possible that either chromatin mechanics and altering gene transcription participate in nuclear mechanosensing. Although recent years have brought deeper insight into this process, our understanding is still not complete and the exact mechanism of nuclear mechanosensing is yet to be revealed.

7. For conclusion: summary and discussion

This thesis demonstrates that intermediate filaments are a versatile, representative-rich, component of cytoskeleton, and, with the same versatility, they participate in a plethora of processes accompanying migration. Regardless of inner diversity, almost all IFs groups contribute to migration. Moreover, either nuclear or cytoplasmic IF affect cellular motility. Highlights summarised from this thesis are given below:

- IFs possess unique physical properties and contribute to cytoplasmic mechanics, ensuring the entire cell's mechanical stability and deformability (Eckes *et al.*, 1998; Sharma *et al.*, 2017). Motile cells favor such cytoplasmic IF rearrangement, which facilitates squeezing through intricate, narrow, extracellular spaces (Brown *et al.*, 2001). IFs abundantly crosstalk with remaining cytoskeletal systems in establishing polarity and maintaining a persistent cell directionality (Helfand *et al.*, 2011; Gan *et al.*, 2016). Although not contractile by themselves, cytoplasmic IFs modulate stress fibre-based contractility, and redistribute actomyosin-generated forces more equally throughout the cytoplasm (Costigliola *et al.*, 2017).
- For migrating cells, the ability to adhere to surrounding surfaces is pivotal. IFs exert an active role in different types of adhesive complexes like some types of (primarily actin-linked) focal adhesion and (primarily IF-linked) desmosomes and hemidesmosomes (for review see Zuidema, Wang and Sonnenberg, 2020). In stress fibre-connected, focal adhesions, IFs participate in complex signalisation and mechanotransduction, which, among other things, regulate FAs turnover. And *vice versa*, FAs drive IFs dynamics, acting as an IF assembly hotspot (Ivaska *et al.*, 2002; Windoffer *et al.*, 2006). Although desmosomes and

hemidesmosomes remain present in some migrating cells, they still are required to undergo some rearrangement and become more dynamic in order to enable proper migration and IFs contribute to the regulation (Kimura, Merritt and Garrod, 2007; Pora *et al.*, 2019).

- In a confined 3D intercellular environment, a quite rigid nucleus acts as a mechanical barrier that hinders the pushing through narrow spaces. Lamin IFs are the main contributor to nuclear stiffness, and cells remodulate the lamin network either to impede or facilitate migratory potential (Harada, Swift, Irianto, Shin, Spinler, Athirasala, Diegmiller, P. C. Dave P. Dingal, *et al.*, 2014). Nuclear IFs hold a special position on the nuclear function crossroad while nuclear lamina is not only a mechanical obstacle. Nuclear lamins provide protection against mechanical stress, prevent nuclear envelope rupturing and DNA damage, and actively contribute to the mechanosensitive response (Harada, *et al.*, 2014; Pieuchot *et al.*, 2018).

This thesis provides an overview of possible mechanisms and processes in which IFs contribute to mammalian cell migration, and concurrently indicates the main directions in which research of IFs in cell motility is heading. Although many IF-dependent processes in migration have been revealed, the exact mechanisms of those interactions are not fully understood. IFs are a still less researched cytoskeletal system in this context. Whereas a contractile and highly dynamic actomyosin network has been over time identified and researched as the main force-generator in cell movements (Chen, 1981), indirect involvement of relatively stable, molecular motor-lacking, IFs have been less obvious. Finally, IFs with ~ 70 functional genes, expressed in tissue and context-dependent manner, display much higher diversity than microfilaments or microtubules, making it harder to comprehend such complexity (McKeon, Kirschner and Caput, 1986; Hesse, Magin and Weber, 2001; Szeverenyi *et al.*, 2008).

IF disruption is linked to many serious pathological conditions, some of them probably linked to impaired migration (Seifert, Lawson and Wiche, 1992; Fuchs and Weber, 1994; Fuchs and Cleveland, 1998; Wojcik, Bundman and Roop, 2000). Excessive migration is perceived to be in a causal link with between tumour invasivity, and in some contexts IFs are considered to be a reliable prognosis marker (Zhao *et al.*, 2008; Yamashita *et al.*, 2013; Polioudaki *et al.*, 2015). Deeper insight into IFs in cancer cell migration could enhance an early diagnosis, assess the prognosis and unlock possible pharmacological intervention. Some IFs are perceived to be a possible candidate for a being targeted by new migrastatic drugs (for reviews see Strouhalova *et al.*, 2020; Raudenská *et al.*, 2023). On the other hand, pharmacological support of motility where it is demanded could be also beneficial (de Pablo *et al.*, 2018).

I am aware of the complexity of intermediate filaments in a cell migration, so an approach has been chosen that summarise major migratory processes and demonstrates where IFs - with their unique physical properties - play an essential role. Some different approaches are presented below that could possibly follow up on or provide additional information about this thesis and each of its chapters:

- IFs act as a versatile signalling platform; operating as a scaffolding molecule (briefly reviewed by Pallari and Eriksson, 2006). Though less dynamic than microfilaments and microtubules, IFs still need to be continually rearranged in motile cells. More emphasis ought to be put on how IFs are involved in particular migration-related signalisation cascades and/or to focus on how motile cells control IFs dynamics.
- A simplified 2D migration, though uncommon in a physiological context, is still the best understood, as experimental settings involving cultivation on plastic/glass 2D surfaces are actually the most available. On the contrary, 2D migration displays only a limited stereotypical, mode and does not take into consideration some crucial phenomena required in a more native condition of complex 3D matrices such as navigation through ECM pores and the contribution of ECM mechanics (reviewed by Yamada and Sixt, 2019). To demonstrate a broader range of described processes, findings from 2D and 3D environments have been included. However, a more systematic comparison of IFs in 2D and 3D migration is not available, despite the existence of numerous reviews comparing general 2D/3D migration. More evaluation of findings from 2D in the context of 3D migration is necessary.
- Similarly, IF dynamics in particular modes of migration (described in chapter 2.1) could differ, but considerably less is known about the amoeboid mode.
- Finally, paying attention to the differences between IFs in a physiological state and particular pathologies would attract interest.

In summary, intermediate filaments in mammalian cell migration and all their contributed aspects are a highly promising and applicable area of research with many mechanisms yet to be revealed.

8. References

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