View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

FEBS Letters 582 (2008) 2140–2148

Minireview

Providing cellular signposts – Post-translational modifications of intermediate filaments

Claire L. Hyder[a,b], Hanna-Mari Pallari[a,c], Vitaly Kochin[a,b], John E. Eriksson[a,b,*]

[a] *Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, P.O. Box 123, FIN-20521, Turku, Finland*
[b] *Department of Biology, Åbo Akademi University, Tykistökatu 6B, FI-20520, Turku, Finland*
[c] *Department of Biology, University of Turku, FI-20014, Turku, Finland*

Received 20 March 2008; revised 29 April 2008; accepted 30 April 2008

Available online 23 May 2008

Edited by Christos Stournaras

Abstract Intermediate filaments are dynamically regulated by their post-translational modifications. Initially these modifications were found to regulate filament dynamics and organization. In the last few years, their roles have extended significantly to facilitating, for example, the recruitment and sequestration of signaling molecules that regulate a wide range of cellular functions. While phosphorylation has been established as the principal post-translational modification regulating intermediate filament function, other modifications with co-operative roles are emerging, adding a further dimensions to intermediate filament-mediated signaling.
© 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: Intermediate filaments; Phosphorylation; O-Gl

1. Introduction

Intermediate filaments (IFs) are still the most enigmatic of the three cytoskeletal networks. The IFs, originally named based on their "intermediate" size (10–12 nm in diameter as compared to the 5–8 nm microfilaments and the 25 nm microtubules), were long considered as being rather static and rigid structures, with functions primarily related to structural integrity of cells and tissues. This view has drastically changed in the past few years, as IFs have been implicated in a highly diverse range of cellular functions unrelated to their presumed roles as structural guardians. Recent evidence has proven IFs to function in cell adhesion and migration [1], to be involved in cell organelle shaping and positioning [2], and to modify various cellular processes, such as stress response and tissue growth through their ability to regulate signaling molecules [3]. IFs are subjected to multiple post-translational modifications (PTMs) that have important consequences for their structure and properties. These PTMs have turned out to control various aspects of the observed IF functions and are also responsible for maintaining the dynamic properties of IFs.

In the years preceding sequencing of the human genome it was believed that genetic mutations would account for within species variation and disease. The human genome project revealed that genetic variation is considerably smaller than expected. Hence, PTMs are the key to functional diversity, adding an ever-increasing complexity to the functions of the cell. While there are tens of different established PTMs, phosphorylation is the most widely studied and is still regarded as the most consequential. While it is exceptionally important roles in all aspects of classical signaling pathways, it has also been established as a key regulator of IF dynamics and function. Recent studies have revealed novel and intriguing roles for phosphorylation-mediated PTMs in regulation of IFs, but O-linked glycosylation now emerges as an additional regulator IF phosphorylation.

2. Characteristic features of IF proteins

IFs are extensive polymerized networks comprised of differentially expressed IF proteins. The large family of IF proteins is divided into six subfamilies based on sequence comparison and expression patterns [4]. Accounting for the majority of the IF proteins are the type I and II keratins (K9–20 and K1–8, respectively). Keratins form heteropolymers consisting of type I and type II keratins and are found in epidermal cells and the cells of simple epithelia. Various diseases, including skin fragility disorders and hepatic ailments, arising from keratin mutations indicate keratins as essential for the integrity of epidermis and the functionality of simple epithelia [5,6]. Type III IFs consist of vimentin, desmin, glial fibrillary acidic protein (GFAP) and peripherin. Vimentin is derived from the cells of mesenchymal origin, its appearance in cells is often referred to as a hallmark of epithelial to mesenchymal transition [7]. Desmin expression is associated solely to muscle tissue, whereas GFAP and peripherin are crucial components in cells of the nervous system. Additionally, type IV IFs are expressed in the nervous system and consist of the neurofilaments heavy (NF-H), medium (NF-M), and light (NF-L), nestin, α-internexin, and synemin. Abnormal accumulation of type IV IF proteins is a hallmark of many neurodegenerative disorders [8]. Type V IFs, lamins, do not form cytoplasmic polymers but instead constitute an indispensable network surrounding the nucleus. Mutations in lamin A/C gene cause numerous diseases, laminopathies, showing a very broad spectrum of etiologies

*Corresponding author. Address: Department of Biology, Åbo Akademi University, Tykistökatu 6B, FI-20520, Turku, Finland. Fax: +358 2 215 4748.
E-mail address: john.eriksson@abo.fi (J.E. Eriksson).

0014-5793/$34.00 © 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2008.04.064

and symptoms, ranging from metabolic disturbances to myopathies and premature ageing disorders [9]. Phakinin and filensin belonging to the final type of IFs, type VI are exclusively expressed in the eye lens. First mentioned in the literature 14 years ago these proteins have been linked with myopia and cataracts and could prove to be an essential element in the optical quality of the lens [10].

Structurally IF proteins are characterized by their highly conserved α-helical rod domain, flanked by the amino (N)-terminal head and the carboxyl (C)-terminal tail domain. The nature of the IFs to dimerize and form coiled-coil structures is defined by the structural properties of the rod domain [11]. In contrast to the conserved rod domains, the N- and C-terminal domains are the sources of enormous sequence variability observed among IF protein family and, therefore, are thought be responsible for the cell-specific functions of different IF proteins [12]. Intriguingly, these domains are also targets of different PTMs, with a vast array of PTM motifs. In fact, many of the novel functions of IFs have emerged in conjunction with observed changes in their PTM status, which then in turn may, for example, alter the interaction with and affinity for associated signaling-related binding partners [11].

3. Post-translational modification of IF proteins

Phosphorylation has long been the most studied PTM of the IFs having a major influence on the dynamics of the IF structure. Reorganization of IFs during mitosis was initially demonstrated to be phosphorylation-dependent more than two decades ago and has been extensively characterized since [13]. Mitosis-associated phosphorylation among cytoplasmic IFs has been often linked to the N-terminus of IF proteins indicating the described indispensability of N-terminal domain in polymerization [14,15]. However, the large number of potential phosphorylation motifs in, for example, the C-terminal tail of vimentin (Fig. 1), suggests involvement in other regulatory roles than mitotic disassembly. Phosphorylation on the vimentin C-terminal ser458 by p37 kinase did not affect filament structure in vivo [16], while most N-terminal phosphorylation sites show effects on either vimentin dynamics or organization. While N-terminal phosphorylation sites are best characterized among all IFs, neurofilaments represent the rare branch of IF protein family, having a depicted function for C-terminal phosphorylation during neuronal development [17]. In addition to the differences between N- and C-terminal phosphorylation, phosphorylation effects maybe influenced by the surroundings and the ongoing cellular phase. While K18 can be phosphorylated on ser33 during mitosis [17], it exhibits a basal level of phosphorylation also during interphase where perhaps it has an alternative function [18]. This principle could extend to the other IFs, many of whose phosphorylation is modulated on a number of sites during both mitosis and interphase. Vimentin has numerous phosphorylation sites, the phosphorylation of which can be displayed both from interphase and mitotic cells (Fig. 1). Similarly to add several checkpoints of control, a common feature of many signal transduction systems, phosphorylation on several sites may

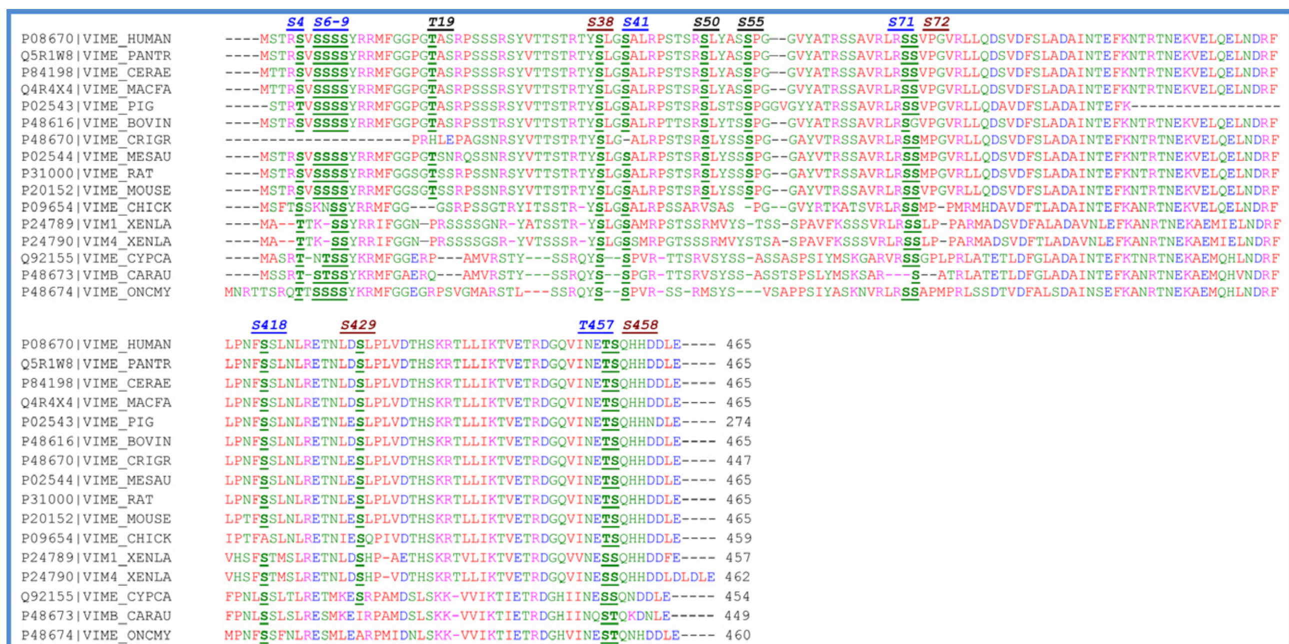

Fig. 1. Sequence comparison of vimentin from different species. The vimentin phosphorylation sites that have been established to be genuine in vivo [93,102,103] show striking sequence homology among vimentin from different species. One key feature of the vimentin phosphorylation sites in the head domain is to regulate vimentin assembly/disassembly in different circumstances. The head domain is essential for filament formation, ablation of which renders vimentin unable to dimerize and form homopolymers [104]. Ser55 is phosphorylated by CDK1 in early G_2/M phase inducing filament disassembly [95], followed by Plk-1 induced vimentin phosphorylation at ser82 remaining elevated from metaphase to the end of mitosis [99]. The latter phosphorylation step is important for segregation of vimentin filaments between the two daughter cells in co-ordination with phosphorylation at ser72 at the cleavage furrow by Aurora B kinase [97,98] and at ser71 by Rho-kinase [94]. The most detailed mechanisms of vimentin phosphorylation originate from mitosis studies. These have been extended to the role of vimentin phosphorylation in other events such as smooth muscle contraction [80], cell motility [88] and viral infection [100] (see Table 1 for list of putative functions of the different PTMs in the N-terminus). Yet very little is known about the roles of the C-terminal phosphorylation sites.

Table 1
In vivo vimentin phosphorylation sites and their associated in vivo kinases and cellular function

Site	Function	Kinase
N-terminus		
Ser4,6,7,8,9	Regulates cycling of β1-integrin and cell motility	PKC epsilon mediated [88]
Ser38	Decelerated assembly kinetics	PKA [93]
	Phosphorylation at the cleavage furrow during cytokinesis	Rho-kinase [94]
Ser50		
Ser55	Filament disassembly in early G_2/M phase	CDK1 [65,95]
	Reorganization of filaments during smooth muscle contraction	PAK-1[80]
	Regulation of the dissociation of Crk-associated substrate from vimentin	PAK-1[82]
Ser71	Phosphorylation at the cleavage furrow during cytokinesis	Rho kinase [94]
	Remodeling of filaments in neurites	Rho-kinase [96]
Ser72	Decelerated assembly kinetics	PKA [93]
	Phosphorylation at the cleavage furrow during cytokinesis	Aurora B [97,98]
Ser82	Sequential phosphorylation following CDK1 phosphorylation on S55 during mitosis. Essential for filament segregation between daughter cells in co-ordination with S72 phosphorylation by Aurora-B kinase	Plk-1 [99]
	Formation of a vimentin cage during African Swine Fever virus infection.	CamKII [100]
	Memory phosphorylation site in astrocytes. Suggested to decrease threshold for other phosphorylation signals to induce vimentin disassembly	CamKII [101]
C-terminus		
Ser418	Unknown function	
Ser429	Unknown function	
Ser457	Unknown function	p37 [16]
Ser458	Unknown function	p37 [16]

The brief descriptions of the functions of the vimentin phosphorylation sites clearly demonstrate a co-ordination between kinases and their site-specific phosphorylation during various cellular processes.

have to occur either sequentially or concurrently to elicit a drastic effect such as disassembly (see Table 1).

In recent years other PTMs have emerged as potent regulators of IF function. O-linked glycosylation involves the addition and removal of saccharides such as O-linked β-*N*-acetylglucosamine (O-GlcNAc) on the hydroxyl atoms of serine and threonine residues. An important post-translational modification of many proteins, it has a key role in mitosis. An extensive study which modulated O-GlcNAcase and O-GlcNAc transferase found that overexpression delayed G_2/M cell cycle progression and perturbed mitotic phosphorylation most likely due to disturbances of cyclin expression and proper function. Moreover, O-GlcNAcylation has been postulated to be a nutrient sensor [19], and consequently has an extensive role in signal transduction. This is logical from the viewpoint that how a cell reacts to external stimuli is highly dependent on its nutrient status. Thus, it has been shown to have a critical role in insulin signaling and diabetes [20]. Studies of O-GlcNAcylation and IFs have so far focused on keratins and neurofilaments. O-linked glycosylation is believed to act in competition with phosphorylation in a dynamic cycling known as the ''yin-yang'' hypothesis [21]. The dysregulation of this relationship in NF-M lead to their hyperphosphorylation and accumulation in the brains of Alzheimer's disease (AD) rats [22]. Accumulation of NF-M resulting from inhibited O-GlcNAcylation was speculated to be a result of impaired glucose uptake or metabolism in the brain which consequently could affect retrograde degeneration of neurons in AD [22]. The NF-M glycosylation site in question was localized to the KSP repeat motif in the carboxyl-terminal in a study on the role of NF-M post-translational modifications on ALS [23].

Reciprocal regulation of K18 ser48 O-GlcNAcylation and ser52 phosphorylation were postulated to be important counter regulators of each other [24]. Previous studies identified that mutations on the ser48 O-GlcNAcylation site did not have an effect on K18 filament formation [25], emphasizing the possibility for alternative functions for this site. One K18 head domain glycosylation site is associated with mitotic arrest in HT29 cells [26]. Furthermore, an O-GlcNAc site was recently mapped on vimentin at ser54 [27] which is adjacent to the mitotic phosphorylation site, ser55 (Fig. 1). However, no role for this site has been elucidated yet. These very preliminary but highly interesting findings on the reciprocal relationship between O-GlcNAcylation and phosphorylation are pointing towards an even more dynamic level of regulation of IF functions.

4. Stress-associated PTMs on IFs

Structural modifications of IFs, ranging from disassembly or reorganization to detrimental aggregation, is generalized to be a consequence of many forms of stresses and various diseases [28–30]. This kind of changes in filament network structure changes are often an outcome of extensive hyperphosphorylation, well exemplified by keratin reorganization occurring upon shear stress in alveolar epithelial cells. In this case, the reorganization has been observed to be mediated by phosphorylation on ser73 in K8 by PKCδ, a downstream effector of activated phospholipase *c* which resides on the cell membrane and acts as a signal transducer of extracellular stimuli, such as shear stress [31]. Hyperphosphorylation and consequent mod-

ifications in filament organization indicate a loss of mechanical integrity and emphasize the function of IFs in protecting epithelia against mechanical stimuli. The functions of stress-induced PTMs on IFs are more complicated as the different disease and stress states affecting IFs extend beyond the mechanical stress.

Site-dependent keratin phosphorylation has long been associated with liver injury both in a protective and exacerbating role [32,33]. During hepatotoxicity, K8/K18 are hyperphosphorylated. Further analysis in transgenic mice expressing K18 with ser52 mutation found that this phosphosite is critical in protecting hepatocytes against liver injury [33]. Phosphorylation on the K18 binding partner K8 occurs on ser73 during heat and drug induced stress [34] via recruitment of the stress activated kinase p38 [35], regulating filament organization in a spatio-temporal manner. Up-regulation of p38-mediated K8 phosphorylation leads to filament collapse and the formation of keratin granules, a characteristic trait of keratin response to stress stimuli while down-regulation allows the filaments to remain assembled [36]. The highly conserved motif surrounding ser73 is phosphorylated during stress also on other type II keratins, namely, K4, K5 and K6 [37] revealing a common function that could be relevant for other IF types. Individual kinases have many substrates; consequently one phosphosite can be phosphorylated by a number of kinases. In addition to p38, K8 ser73 is phosphorylated by the c-jun N-terminal kinase (JNK) in response to stimulation of the Fas receptor [38]. Intriguingly, in addition to JNK modulating K8/K18 filament organization, the observed association between keratin filaments and JNK could have a role in regulating JNK signaling since it affected the ability of JNK to phosphorylate c-Jun. The stress activated protein kinases (SAPKs), such as JNK, have involvement in many signaling pathways and removing them from the pool of functional kinases could severely attenuate the effects of signaling cascades during cellular stress. This concept was further demonstrated in a recent study, linking disturbed K8 ser73 phosphorylation by stress activated kinases with a K8 mutation at Gly61Cys predisposing mice to liver disease. This mutation may cause a conformational change inhibiting SAPKs' access to ser73 leading to increased liver disease and apoptosis susceptibility, resulting from a SAPK imbalance. Therefore far from a structural role, K8 serves to sequester SAPK activity, acting as "phosphate sponge" and protects tissues from injury [39]. A similar function could be discovered for the newly identified phosphosite on K20 at ser13. A marker of murine goblet cells and intestinal tissue injury, ser13 is hyperphosphorylated during stress. This is mediated by a member of the PKC family, although the specific kinase remains undefined [40].

This "phosphate sponge" hypothesis elegantly depicted with keratins has been expanded to other IFs as well. One of the first mentions of IFs acting as phosphate sinks was after finding that vimentin is preferentially hyperphosphorylated after okadaic acid treatment [41]. A similar function was proposed for neurofilament phosphorylation during ALS. Originally believed to be protective the neurofilaments were suggested to act as phosphorylation sinks for dysregulated Cdk5, to inhibit the hyperphosphorylation of tau, a hallmark of degeneration in both ALS and AD [42]. Further evidence using deletions in NF C-terminal phosphorylation sites found that the opposite is true [43]. The data, while sparse for this model, suggests that in specific circumstances such as stress then the functions of certain IFs could be to ameliorate and temper a phosphate imbalance in the cell. Whether this is protective or detrimental seems to be highly circumstantial and is almost certainly cell and tissue dependent.

5. The effect of IF PTMs on signal transduction

Recent research has changed the view of interactions between IFs and other cellular molecules broadening it to consider, in addition to cytoskeletal linker proteins also various signaling determinants such as kinases [1,3]. IFs ability to sequester or act as a scaffold for signaling molecules renders them exceptional organizers of different physiological processes. In many occasions PTMs seem to work as important regulator of IF signaling functions.

Lamins A/C interact with pRb, SMAD2 and PP2A. This interaction is critical for proper regulation of mesenchymal cellular physiology. $LMNA^{-/-}$ mouse embryonic fibroblasts (MEFs) display defective transcription regulation, such as aberrant pRb phosphorylation, accelerated S-phase transition, lack of proper inhibitory signaling normally activated in response to TGF-β1 [44], and aberrant NFκB activity [45]. While lamins may have some direct signaling functions many of them are mediated via their binding proteins [46] which function as linkers between the nuclear envelope and the cytoplasm or the nucleoplasm. It is possible that among uncharacterized lamin A/C phosphorylation sites, some might regulate interactions between lamins and other nuclear proteins, fulfilling signaling, architectural, or gene-regulatory functions. For instance, concrete binding regions in lamin A, especially in its C-terminal tail, are known for its multiple binding partners (reviewed in [46]). Some of the binding regions described contain highly conserved phosphoacceptor sites, which might be involved in regulation of these protein–protein interactions. Whether lamins' phosphorylation plays a role in their interactions with a series of connector proteins (such as UNC-83, UNC-84, nesprins, and plectin), which links lamins to IFs, actin and microtubule cytoskeletons [47,48] is also still unknown.

Vimentin is a substrate of members of the RAF-1/RhoA signaling pathway [49,50]. Rho-binding kinases (ROK), downstream effectors of RhoA, are involved in mediating actin dynamics, and focal adhesion formation [51]. Interestingly, ROKα is reported to interact with vimentin and mediate the collapse of vimentin filaments as part of the RhoA signaling cascade in response to extracellular stimuli [52]. Activated ROKα phosphorylates vimentin leading to filament collapse and consequently release of ROKα from filaments and its translocation to the periphery of the cell [52]. This study, as well as the keratin/JNK interaction described above, clearly demonstrate how IFs act as regulatory platforms for signaling molecules, controlling their intracellular distribution and, thereby, their ability to phosphorylate specific targets. Phosphorylation-mediated regulation of IF-kinase interactions can be expanded also to other IF proteins. K10 has been shown to sequester Akt and PKCζ to its N-terminal domain, preventing their activation. Since the majority of phosphorylation sites of IFs are on the N-terminus this indicates the mechanism of Akt and PKCζ sequestration to be phosphorylation mediated [53].

The members of 14-3-3 protein family modulate the functions of multiple proteins through direct phosphorylation-

dependent interactions. Phosphorylated vimentin has been demonstrated to interact with 14-3-3, resulting in the dissociation of 14-3-3 and Raf-1 thus, pointing to a PTM-based role of IFs as modulators of 14-3-3 interactions [54]. An interesting association between 14-3-3γ with GFAP originates from the observation that both have a protective role in reactive astrocytes during stress. 14-3-3γ binds to phosphorylated ser8 on both filamentous and soluble GFAP during S and G_2/M phases leading to the disassembly of GFAP, thereby, emphasizing the additional role of 14-3-3 as a regulator of IF dynamics [55].

Selective phosphorylation of K18 on ser33 during mitosis promotes interaction between 14-3-3ζ and K18 affecting the K18 organization and distribution [18,56]. Absence of K8 and accordingly loss of K8/K18 filaments shifts 14-3-3ζ distribution towards accumulation in the nucleus, consequently arresting cells in S/G^2 phase. The K18/14-3-3 interaction could regulate 14-3-3 binding to phosphorylated cdc25, a crucial mitotic checkpoint regulator indicating that keratin network has the capacity to prevent the uncontrolled and harmful 14-3-3 interactions [57]. Furthermore, the association between keratin and 14-3-3 is a basis of more complicated regulation of signal transduction. Keratin binds to Raf-1 [58], a prolific kinase involved in cdc25 phosphorylation [59]. In contrast, 14-3-3 binds to phosphorylated Raf-1 preventing its translocation to the plasma membrane [60]. Raf-1 is initially inactive upon binding to K8 but once activated it phosphorylates K18 on ser52, consequently being released from the filaments [58]. It seems that 14-3-3 binds to phosphorylated Raf-1, thereby, recruiting it to K8 where it phosphorylates K18, mediating its release. As with other examples herein, keratins sequester signaling molecules in a temporal manner, allowing timely modulation of their activation and interaction with each other.

The above-provided evidence that keratins modulation of signaling functions is highly dependent on type and differentiation state of the cell. While in earlier stages of differentiation or during injury, keratins ability to sequester and activate signaling moieties can modulate signaling interactions in both a positive and negative manner. The IF's involvement in mitosis and growth are becoming well characterized, particularly with their involvement with 14-3-3, Akt pathways and Raf-1/RhoA. Their involvement in other signaling networks remains to be characterized.

6. Modification of cell and tissue development through PTM of IFs

IFs are contributors to cell and tissue development. Disorders of IFs in humans result in severe phenotypes, ranging from the skin blistering diseases of the keratinopathies [61] to the accelerated aging diseases and muscular dystrophies of the laminopathies [62] to Parkinson's disease, whose pathology has been associated with neurofilaments [63]. These examples clearly demonstrate how essential IF's are for proper maintenance and growth of our tissues. While, in humans the mechanisms for these disorders are not always so clear. Studies in IF deficient or mutated animals and in cells reveal their ability to preserve the equilibrium when necessary and the subtleties of their reorganization whose knock-on effects can be more pronounced.

The expression of IF protein nestin, is characteristic to neuronal and myogenic stem and progenitor cells with high capacity to divide, migrate, and tolerate stress. Although little is known about the PTMs and the specific functions of nestin it is postulated that nestin could be engaged in regulating these features. Cdk1 has been revealed to regulate the disassembly of nestin network during mitosis [64]. More recently it has been suggested that nestin plays an important role in progenitor cell division regulating the disassembly of vimentin, the binding partner of nestin, phosphorylated on ser55 (Fig. 1) [65]. This implies the importance of timely phosphorylation-mediated regulation of nestin filaments lies in the control of subcellular localization of nestin associated molecules. Another kinase specifically targeting nestin is Cdk5, a crucial determinant of neuronal [66] and myogenic development [67,68]. In addition to contriving the phosphorylation-dependent reorganization of nestin in differentiating myoblast Cdk5 and its activator protein p35 complex on nestin scaffold [69]. Cdk5-mediated phosphorylation of nestin is sufficient for the release of the active Cdk5/p35 complex which consequently phosphorylates other targets. The significance of the nestin scaffolding properties upon development is further corroborated by the discovery that nestin acts by sequestering the cdk5/p35 complex also in neuronal progenitor cells upon oxidative stress, thus, protecting the cells from harmful Cdk5 activity during stress conditions [70].

Cell growth is a highly regulated process, integral to the cell cycle, organism size, and restoration of tissue functionality post-injury. While 14-3-3σ has been implicated in hyperproliferation and increase in keratinocyte size of K10 null mice [71] the mechanism by which this occurred and the relationship with keratin and 14-3-3 was not clarified. In a landmark paper K17 null mice showed a similar accumulation of 14-3-3σ in the nucleus. Cytoplasmic binding of 14-3-3σ to K17 was consequently found to be mediated by phosphorylation sites, Thr9 and ser44 on K17. This binding facilitates activation of mTOR/Akt signaling pathway, a process crucial for cell growth. Apart from the lamins, which are known to influence DNA replication [72], cytoplasmic IFs have never been considered before to influence the most basic cellular functions such as protein synthesis.

Axonal caliber is regulated by phosphorylation of neurofilaments however it has become a somewhat contentious issue [8,17]. NF phosphorylation regulates axonal transport. Phosphorylated NF are transported slower allowing them to accumulate in areas of the axon and contribute to its growth [73]. Phosphorylation could modulate the interaction between NF and kinesin, thereby regulating NF transport [74]. Expression of deleted of phosphomotifs from NF-H C-terminus in mice have consequently found no evidence of changes in growth of axons, although phosphorylated NF-M levels increased, perhaps providing a compensatory phosphorylation mechanism [75,76].

Peripherin, a type III IF protein, is expressed only in neurons of the peripheral nervous system and injured neurons of the CNS. Although associated with the pathology of ALS [77], its functions are relatively uncharacterized. Akt a modulator of cell survival and neuronal regeneration directly phosphorylates peripherin on the N-terminal ser66 in the cell bodies of injured neurons [78]. While the physiological effect of Akt phosphorylation on peripherin remains unknown, the author's speculated that peripherin could contribute to the rearrangement of other IFs. Considering the work of Kim et al., [105] on K17, 14-3-3σ and cell growth, peripherin could similarly

be involved in cell growth or cell cycle regulation of damaged neurons. Akt could phosphorylate peripherin in a spatiotemporal manner in the damaged neuron and facilitate signal transduction in a similar manner to IFs in other cellular processes [78].

In addition to growth and differentiation, IF-targetted PTMs have proven to be indispensable for the functionality of several tissues. In particular, vimentin and desmin are involved in signaling in smooth muscle [79]. Down-regulation of PAK1 upon 5-hydroxytryptamine stimulation of smooth muscle cells stimulates spatial reorganization of vimentin filaments [80]. Moreover, ser56 phosphorylation of vimentin by PAK1 regulates the dissociation of Crk-associated substrate (CAS) from vimentin. CAS is one of a family of adapter proteins involved in the recruitment and docking of signaling molecules and whose phosphorylation is reported to be involved in integrin mediated adhesion [81]. This interaction is crucial for contractile force development in smooth muscle. If PAK1 is downregulated then the disassociation of CAS from vimentin is inhibited and the active force generated significantly reduced [82]. It has been speculated that CAS disassociation from vimentin may facilitate its translocation to the cell border where it may be involved with cortical actin polymerization, a crucial event for smooth muscle force development. In differentiated smooth muscle (DMS), calcium/calmodulin-dependent protein kinase II (CaMKII), is bound to vimentin. Upon muscle stimulation CaMKII is activated by autophosphorylation and consequently phosphorylates vimentin, causing its dissociation from the filaments. Once unbound CaMKII translocates to dense cytoplasmic plaques where it colocalises with vinculin [83]. The authors speculated that binding to vimentin could be necessary, either to ensure proper activation of the kinase or alternatively to prevent inappropriate activation in unstimulated DMS cells. Interestingly, CaMKII phosphorylation in DMS muscle cells did not cause vimentin disassembly reiterating the function of IFs as signaling scaffolds.

Keratin is associated with desmosomes, complexes that provide cell-cell adhesion by connecting the cytoskeletal components of one cell to another. While the interaction between desmoplakin and keratin relies on phosphorylation on K8 ser24, which modulates where desmoplakin is deposited in the desmosome. The ser24 phosphorylation status was postulated to act as a rheostat, adjusting desmoplakin binding to keratin, a process essential to maintaining membrane integrity and intact signaling scaffolds during stress [84].

7. PTM of IFs during migration

It has been recently shown that vimentin structure is reorganized during transmigration of lymphocytes [85]. Although the mechanism for this has not yet been clarified, the structural changes suggest a role for phosphorylation. The early findings

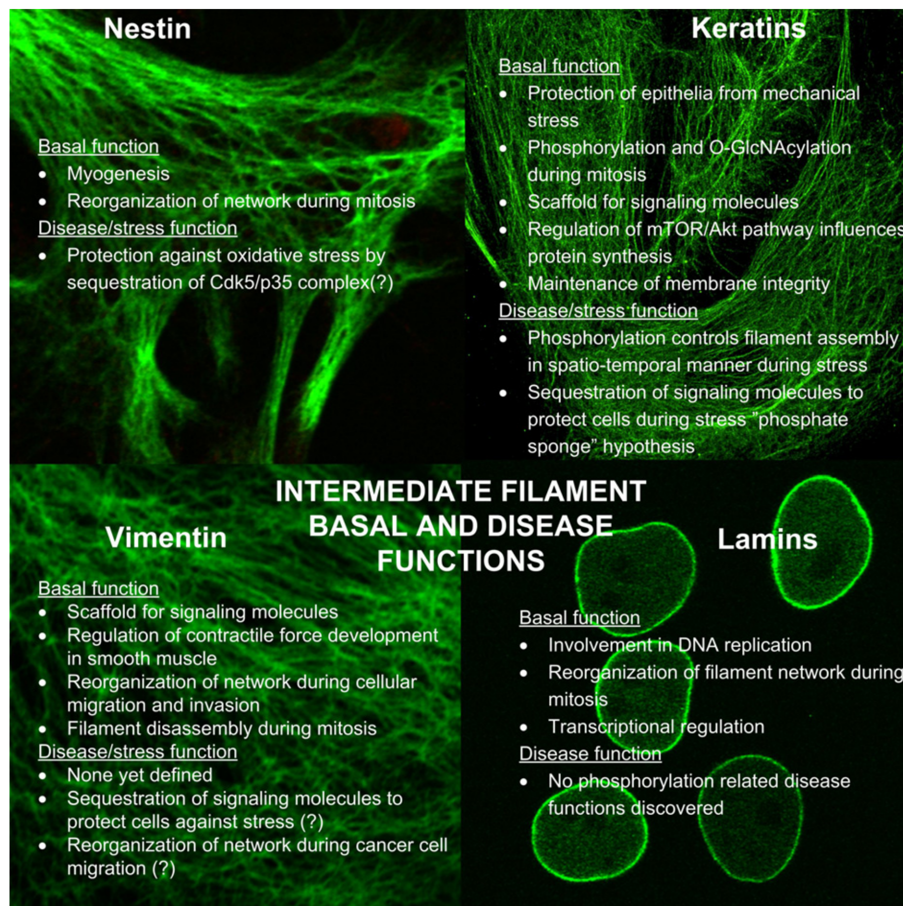

Fig. 2. Tentative roles and functions of IFs. Many of these functions will be actively regulated by dynamic PTMs of the involved IFs.

regarding the loss of motility from vimentin deficient animals [86] have been further corroborated by the findings that siRNA mediated knockdown of vimentin inhibited carcinoma cell migration [87]. Phosphorylation of vimentin dependent on PKCε is involved in β1-integrin recycling and cell motility [88]. If the corresponding phosphorylation sites on vimentin (a serine cluster of ser4,6,7,8,9) were abolished, then the filament network collapsed and cells were rendered immotile. Plk-1 which normally phosphorylates vimentin on ser82 during mitosis is active during invasion of breast carcinoma. The proposed model is while normally Plk-1 phosphorylates vimentin on ser82 during mitosis leading to filament segregation, during malignancy it could also activate an alternative pathway, which down-regulates surface expression of β1-integrin, promoting invasion [89]. Considering the importance of phosphorylation status to the structure of vimentin filaments, it is highly possible that new information on other less studied phosphosites and their contribution to filament reorganization during migration and other cellular processes could come to light.

8. PTM as regulator of IF degradation and stability

Protein turnover is also regulated via phosphorylation. Ubiquitination is involved in the degradation of K8 and K18. Phosphorylation on K8 ser23,73 and 431 and K18 ser33 has a protective effect, but does not completely preclude against it, while the relationship between phosphorylation and ubiquitination on other K18 sites remains unclear [90]. Apoptosis involves Lamin B phosphorylation by caspase-3 activated PKCδ. This instigates lamin disassembly, which exposes the lamins to caspase 6 for efficient lamin cleavage and degradation [91]. Keratins contain two amino acid sequences recognized for sequential cleavage by caspases. Hyperphosphorylation of K18 inhibits caspase-mediated cleavage of the second cleavage site, the function of which is believed to be protective, perhaps acting as a "phosphate sponge" allowing a controlled sequence of events [92].

9. Conclusions

The novel functions of IF proteins relate to the capacity of IFs to actively sequester, position, or act as scaffolds for signaling molecules, including stress-activated kinases. The relatively high abundance of IFs in most cells makes them highly efficient as signaling scaffolds, even for high-abundance signaling molecules. It seems that post-translational modifications play a significant role in regulating these interactions. The characterized PTM motifs are amazingly well conserved between species. Therefore, deciphering the molecular mechanisms and functions of these PTMs will pave the way towards a better understanding of the physiological roles of IFs as well as their roles in different pathological conditions and diseases (see Fig. 2 for overview).

References

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

[2] Toivola, D.M., Tao, G.-Z., Habtezion, A., Liao, J. and Omary, M.B. (2005) Cellular integrity plus: organelle-related and protein-targeting functions of intermediate filaments. Trends Cell Biol. 15, 608–617.

[3] Pallari, H.M. and Eriksson, J.E. (2006) Intermediate filaments as signaling platforms. Sci. STKE 2006, pe53.

[4] Stewart, M. (1990) Intermediate filaments: structure, assembly and molecular interactions. Curr. Opin. Cell Biol. 2, 91–100.

[5] Uitto, J., Richard, G. and McGrath, J.A. (2007) Diseases of epidermal keratins and their linker proteins. Exp. Cell Res. 313, 1995–2009.

[6] Ku, N.O., Strnad, P., Zhong, B.H., Tao, G.Z. and Omary, M.B. (2007) Keratins let liver live: mutations predispose to liver disease and crosslinking generates Mallory-Denk bodies. Hepatology 46, 1639–1649.

[7] Kokkinos, M.I., Wafai, R., Wong, M.K., Newgreen, D.F., Thompson, E.W. and Waltham, M. (2007) Vimentin and epithelial-mesenchymal transition in human breast cancer – observations in vitro and in vivo. Cells Tissues Organs 185, 191–203.

[8] Barry, D.M., Millecamps, S., Julien, J.P. and Garcia, M.L. (2007) New movements in neurofilament transport, turnover and disease. Exp. Cell Res. 313, 2110–2120.

[9] Broers, J.L.V., Ramaekers, F.C.S., Bonne, G., Yaou, R.B. and Hutchison, C.J. (2006) Nuclear lamins: laminopathies and their role in premature ageing. Physiol. Rev. 86, 967–1008.

[10] Sandilands, A., Prescott, A.R., Wegener, A., Zoltoski, R.K., Hutcheson, A.M., Masaki, S., Kuszak, J.R. and Quinlan, R.A. (2003) Knockout of the intermediate filament protein CP49 destabilises the lens fibre cell cytoskeleton and decreases lens optical quality, but does not induce cataract. Exp. Eye Res. 76, 385–391.

[11] Strelkov, S.V., Herrmann, H. and Aebi, U. (2003) Molecular architecture of intermediate filaments. Bioessays 25, 243–251.

[12] Goldman, R.D., Chou, Y.H., Prahlad, V. and Yoon, M. (1999) Intermediate filaments: dynamic processes regulating their assembly, motility, and interactions with other cytoskeletal systems. FASEB J. 13, S261–S265.

[13] Chou, Y.-H., Rosevear, E. and Goldman, R.D. (1989) Phosphorylation and disassembly of intermediate filaments in mitotic cells. Proc. Natl. Acad. Sci. 86, 1885–1889.

[14] Beuttenmuller, M., Chen, M., Janetzko, A., Kuhn, S. and Traub, P. (1994) Structural elements the amino-terminal head domain of vimentin essential for intermediate filament formation in vivo and in vitro. Exp. Cell Res. 213, 128–142.

[15] Ralton, J.E., Lu, X., Hutcheson, A.M. and Quinlan, R.A. (1994) Identification of two N-terminal non-alpha-helical domain motifs important in the assembly of glial fibrillary acidic protein. J. Cell Sci. 107, 1935–1948.

[16] Chou, Y.H., Opal, P., Quinlan, R.A. and Goldman, R.D. (1996) The relative roles of specific N- and C-terminal phosphorylation sites in the disassembly of intermediate filament in mitotic BHK-21 cells. J. Cell Sci. 109, 817–826.

[17] Sihag, R.K., Inagaki, M., Yamaguchi, T., Shea, T.B. and Pant, H.C. (2007) Role of phosphorylation on the structural dynamics and function of types III and IV intermediate filaments. Exp. Cell Res. 313, 2098–2109.

[18] Ku, N.O., Liao, J. and Omary, M.B. (1998) Phosphorylation of human keratin 18 serine 33 regulates binding to 14-3-3 proteins. Embo J. 17, 1892–1906.

[19] Slawson, C., Housley, M.P. and Hart, G.W. (2006) O-GlcNAc cycling: how a single sugar post-translational modification is changing the way we think about signaling networks. J. Cell. Biochem. 97, 71–83.

[20] Akimoto, Y., Hart, G.W., Hirano, H. and Kawakami, H. (2005) O-GlcNAc modification of nucleocytoplasmic proteins and diabetes. Med. Mol. Morphol. 38, 84–91.

[21] Wells, L., Kreppel, L.K., Comer, F.I., Wadzinski, B.E. and Hart, G.W. (2004) O-GlcNAc transferase is in a functional complex with protein phosphatase 1 catalytic subunits. J. Biol. Chem. 279, 38466–38470.

[22] Deng, Y., Li, B., Liu, F., Iqbal, K., Grundke-Iqbal, I., Brandt, R. and Gong, C.-X. (2008) Regulation between O-GlcNAcylation and phosphorylation of neurofilament-M and their dysregulation in Alzheimer disease. FASEB J. 22, 138–145.

[23] Ludemann, N., Clement, A., Hans, V.H., Leschik, J., Behl, C. and Brandt, R. (2005) O-Glycosylation of the tail domain of neurofilament protein M in human neurons and in spinal cord tissue of a rat model of amyotrophic lateral sclerosis (ALS). J. Biol. Chem. 280, 31648–31658.

[24] Tao, G.-Z. et al. (2006) Reciprocal keratin 18 Ser48 O-GlcNAcylation and Ser52 phosphorylation using peptide analysis. Biochem. Biophys. Res. Commun. 351, 708–712.

[25] Ku, N.-O. and Omary, M.B. (1995) Identification andmutational analysis of the glycosylation sites of human keratin 18. J. Biol. Chem. 270, 11820–11827.

[26] Chou, C.F. and Omary, M.B. (1993) Mitotic arrest-associated enhancement of O-linked glycosylation and phosphorylation of human keratins 8 and 18. J. Biol. Chem. 268, 4465–4472.

[27] Wang, Z., Pandey, A. and Hart, G.W. (2007) Dynamic interplay between O-linked *N*-acetylglucosaminylation and glycogen synthase kinase-3-dependent phosphorylation. Mol. Cell Proteomics 6, 1365–1379.

[28] Magin, T.M., Reichelt, J. and Hatzfeld, M. (2004) Emerging functions: diseases and animal models reshape our view of the cytoskeleton. Exp. Cell Res. 301, 91–102.

[29] Pekny, M. and Lane, E.B. (2007) Intermediate filaments and stress. Exp. Cell Res. 313, 2244–2254.

[30] Godsel, L.M., Hobbs, R.P. and Green, K.J. (2008) Intermediate filament assembly: dynamics to disease. Trends Cell Biol. 18, 28–37.

[31] Ridge, K.M., Linz, L., Flitney, F.W., Kuczmarski, E.R., Chou, Y.-H., Omary, M.B., Sznajder, J.I. and Goldman, R.D. (2005) Keratin 8 phosphorylation by protein kinase C {delta} regulates shear stress-mediated disassembly of keratin intermediate filaments in alveolar epithelial cells. J. Biol. Chem. 280, 30400–30405.

[32] Toivola, D.M., Ku, N.O., Resurreccion, E.Z., Nelson, D.R., Wright, T.L. and Omary, M.B. (2004) Keratin 8 and 18 hyperphosphorylation is a marker of progression of human liver disease. Hepatology 40, 459–466.

[33] Ku, N.O., Michie, S.A., Soetikno, R.M., Resurreccion, E.Z., Broome, R.L. and Omary, M.B. (1998) Mutation of a major keratin phosphorylation site predisposes to hepatotoxic injury in transgenic mice. J. Cell Biol. 143, 2023–2032.

[34] Liao, J., Ku, N.-O. and Omary, M.B. (1997) Stress, apoptosis, andmitosis induce phosphorylation of human keratin 8 at Ser-73 in tissues and cultured cells. J. Biol. Chem. 272, 17565–17573.

[35] Ku, N.-O., Azhar, S. and Omary, M.B. (2002) Keratin 8 phosphorylation by p38 kinase regulates cellular keratin filament reorganization. Modulation by a keratin 1-like disease-causing mutation. J. Biol. Chem. 277, 10775–10782.

[36] Woll, S., Windoffer, R. and Leube, R.E. (2007) p38 MAPK-dependent shaping of the keratin cytoskeleton in cultured cells. J. Cell Biol. 177, 795–807.

[37] Toivola, D.M., Zhou, Q., English, L.S. and Omary, M.B. (2002) Type II keratins are phosphorylated on a unique motif during stress and mitosis in tissues and cultured cells. Mol. Biol. Cell 13, 1857–1870.

[38] He, T., Stepulak, A., Holmstrom, T.H., Omary, M.B. and Eriksson, J.E. (2002) Theintermediate filament protein keratin 8 is a novel cytoplasmic substrate for c-Jun N-terminal kinase. J. Biol. Chem. 277, 10767–10774.

[39] Ku, N.-O. and Omary, M.B. (2006) A disease- and phosphorylation-related nonmechanical function for keratin 8. J. Cell Biol. 174, 115–125.

[40] Zhou, Q., Cadrin, M., Herrmann, H., Chen, C.-H., Chalkley, R.J., Burlingame, A.L. and Omary, M.B. (2006) Keratin 20 serine 13 phosphorylation is a stress and intestinal goblet cell marker. J. Biol. Chem. 281, 16453–16461.

[41] Lai, Y.K., Lee, W.C. and Chen, K.D. (1993) Vimentin serves as a phosphate sink during the apparent activation of protein kinases by okadaic acid in mammalian cells. J. Cell. Biochem. 53, 161–168.

[42] Nguyen, M.D., Lariviere, R.C. and Julien, J.-P. (2001) Deregulation of Cdk5 in a mouse model of ALS: toxicity alleviated by perikaryal neurofilament inclusions. Neuron 30, 135–148.

[43] Lobsiger, C.S., Garcia, M.L., Ward, C.M. and Cleveland, D.W. (2005) Altered axonal architecture by removal of the heavily phosphorylated neurofilament tail domains strongly slows superoxide dismutase 1 mutant-mediated ALS. Proc. Natl. Acad. Sci. USA 102, 10351–10356.

[44] Van Berlo, J.H. et al. (2005) A-type lamins are essential for TGF-{beta}1 induced PP2A to dephosphorylate transcription factors. Hum. Mol. Genet. 14, 2839–2849.

[45] Lammerding, J., Schulze, P.C., Takahashi, T., Kozlov, S., Sullivan, T., Kamm, R.D., Stewart, C.L. and Lee, R.T. (2004) Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. J. Clin. Invest. 113, 370–378.

[46] Zastrow, M.S., Vlcek, S. and Wilson, K.L. (2004) Proteins that bind A-type lamins: integrating isolated clues. J. Cell Sci. 117, 979–987.

[47] Gruenbaum, Y., Margalit, A., Goldman, R.D., Shumaker, D.K. and Wilson, K.L. (2005) The nuclear lamina comes of age. Nat. Rev. Mol. Cell Biol. 6, 21–31.

[48] Wilhelmsen, K., Litjens, S.H., Kuikman, I., Tshimbalanga, N., Janssen, H., van den Bout, I., Raymond, K. and Sonnenberg, A. (2005) Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin. J. Cell Biol. 171, 799–810.

[49] Ehrenreiter, K., Piazzolla, D., Velamoor, V., Sobczak, I., Small, J.V., Takeda, J., Leung, T. and Baccarini, M. (2005) Raf-1 regulates Rho signaling and cell migration. J. Cell Biol. 168, 955–964.

[50] Janosch, P. et al. (2000) The Raf-1 kinase associates with vimentin kinases and regulates the structure of vimentin filaments. FASEB J. 14, 2008–2021.

[51] Amano, M., Fukata, Y. and Kaibuchi, K. (2000) Regulation and functions of Rho-associated kinase. Exp. Cell Res. 261, 44–51.

[52] Sin, W.-C., Chen, X.-Q., Leung, T. and Lim, L. (1998) RhoA-binding kinase alpha translocation is facilitated by the collapse of the vimentin intermediate filament network. Mol. Cell. Biol. 18, 6325–6339.

[53] Paramio, J.M., Casanova, M.L., Segrelles, C., Mittnacht, S., Lane, E.B. and Jorcano, J.L. (1999) Modulation of cell proliferation by cytokeratins K10 and K16. Mol. Cell. Biol. 19, 3086–3094.

[54] Tzivion, G., Luo, Z.-J. and Avruch, J. (2000) Calyculin A-induced vimentin phosphorylation sequesters 14-3-3 and displaces other 14-3-3 partners in vivo. J. Biol. Chem. 275, 29772–29778.

[55] Li, H., Guo, Y., Teng, J., Ding, M., Yu, A.C.H. and Chen, J. (2006) 14-3-3{gamma} affects dynamics and integrity of glial filaments by binding to phosphorylated GFAP. J. Cell Sci. 119, 4452–4461.

[56] Liao, J. and Omary, M.B. (1996) 14-3-3 proteins associate with phosphorylated simple epithelial keratins during cell cycle progression and act as a solubility cofactor. J. Cell Biol. 133, 345–357.

[57] Toivola, D.M., Nieminen, M.I., Hesse, M., He, T., Baribault, H., Magin, T.M., Omary, M.B. and Eriksson, J.E. (2001) Disturbances in hepatic cell-cycle regulation in mice with assembly-deficient keratins 8/18. Hepatology 34, 1174–1183.

[58] Ku, N.-O., Fu, H. and Omary, M.B. (2004) Raf-1 activation disrupts its binding to keratins during cell stress. J. Cell Biol. 166, 479–485.

[59] Lee, M. and Yoo, H.S. (2007) Human Raf-1 proteins associate with Rad24 and Cdc25 in cell-cycle checkpoint pathway of fission yeast, *Schizosaccharomyces pombe*. J. Cell. Biochem. 101, 488–497.

[60] Dumaz, N. and Marais, R. (2003) Protein kinase a blocks Raf-1 activity by stimulating 14-3-3 binding and blocking Raf-1 interaction with Ras. J. Biol. Chem. 278, 29819–29823.

[61] Lane, E.B. and McLean, W.H. (2004) Keratins and skin disorders. J. Pathol. 204, 355–366.

[62] Worman, H.J. and Bonne, G. (2007) "Laminopathies": a wide spectrum of human diseases. Exp. Cell Res. 313, 2121–2133.

[63] Kruger, R. et al. (2003) Mutation analysis of the neurofilament M gene in Parkinson's disease. Neurosci. Lett. 351, 125–129.

[64] Sahlgren, C.M., Mikhailov, A., Hellman, J., Chou, Y.-H., Lendahl, U., Goldman, R.D. and Eriksson, J.E. (2001) Mitotic reorganization of the intermediate filament protein nestin involves phosphorylation by cdc2 kinase. J. Biol. Chem. 276, 16456–16463.

[65] Chou, Y.-H., Khuon, S., Herrmann, H. and Goldman, R.D. (2003) Nestin promotes the phosphorylation-dependent disassembly of vimentin intermediate filaments during mitosis. Mol. Biol. Cell 14, 1468–1478.
[66] Ohshima, T. et al. (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. Proc. Natl. Acad. Sci. 93, 11173–11178.
[67] Lazaro, J.B., Kitzmann, M., Poul, M.A., Vandromme, M., Lamb, N.J. and Fernandez, A. (1997) Cyclin dependent kinase 5, cdk5, is a positive regulator of myogenesis in mouse C2 cells. J. Cell Sci. 110, 1251–1260.
[68] Philpott, A., Porro, E.B., Kirschner, M.W. and Tsai, L.H. (1997) The role of cyclin-dependent kinase 5 and a novel regulatory subunit in regulating muscle differentiation and patterning. Genes Dev. 11, 1409–1421.
[69] Sahlgren, C.M., Mikhailov, A., Vaittinen, S., Pallari, H.-M., Kalimo, H., Pant, H.C. and Eriksson, J.E. (2003) Cdk5 regulates the organization of nestin and its association with p35. Mol. Cell. Biol. 23, 5090–5106.
[70] Sahlgren, C.M., Pallari, H.M., He, T., Chou, Y.H., Goldman, R.D. and Eriksson, J.E. (2006) A nestin scaffold links Cdk5/p35 signaling to oxidant-induced cell death. Embo J. 25, 4808–4819.
[71] Reichelt, J. and Magin, T.M. (2002) Hyperproliferation, induction of c-Myc and 14-3-3{sigma}, but no cell fragility in keratin-10-null mice. J. Cell Sci. 115, 2639–2650.
[72] Moir, R.D., Spann, T.P., Herrmann, H. and Goldman, R.D. (2000) Disruption of nuclear lamin organization blocks the elongation phase of DNA replication. J. Cell Biol. 149, 1179–1192.
[73] Shea, T.B., Jung, C. and Pant, H.C. (2003) Does neurofilament phosphorylation regulate axonal transport? Trends Neurosci. 26, 397–400.
[74] Jung, C., Lee, S., Ortiz, D., Zhu, Q., Julien, J.P. and Shea, T.B. (2005) The high and middle molecular weight neurofilament subunits regulate the association of neurofilaments with kinesin: inhibition by phosphorylation of the high molecular weight subunit. Brain Res. Mol. Brain Res. 141, 151–155.
[75] Rao, M.V. et al. (2002) Gene replacement in mice reveals that the heavily phosphorylated tail of neurofilament heavy subunit does not affect axonal caliber or the transit of cargoes in slow axonal transport. J. Cell Biol. 158, 681–693.
[76] Garcia, M.L. et al. (2003) NF-M is an essential target for the myelin-directed "outside-in" signaling cascade that mediates radial axonal growth. J. Cell Biol. 163, 1011–1020.
[77] Xiao, S., McLean, J. and Robertson, J. (2006) Neuronal intermediate filaments and ALS: A new look at an old question. Biochim. Biophys. Acta (BBA) – Molecular Basis of Disease 1762, 1001–1012.
[78] Konishi, H., Namikawa, K., Shikata, K., Kobatake, Y., Tachibana, T. and Kiyama, H. (2007) Identification of peripherin as a Akt substrate in neurons. J. Biol. Chem. 282, 23491–23499.
[79] Tang, D.D. (2008) Intermediate filaments in smooth muscle. Am. J. Physiol. Cell Physiol. 294, C869–C878.
[80] Tang, D., Bai, Y. and Gunst, S. (2005) Silencing of p21-activated kinase attenuates vimentin phosphorylation on Ser-56 and reorientation of the vimentin network during stimulation of smooth muscle cells by 5-hydroxytryptamine. Biochem. J. 388, 773–783.
[81] Defilippi, P., Di Stefano, P. and Cabodi, S. (2006) p130Cas: a versatile scaffold in signaling networks. Trends Cell Biol. 16, 257–263.
[82] Wang, R., Li, Q.-F., Anfinogenova, Y. and Tang, D.D. (2007) Dissociation of Crk-associated substrate from the vimentin network is regulated by p21-activated kinase on ACh activation of airway smooth muscle. Am. J. Physiol. Lung Cell Mol. Physiol. 292, L240–L248.
[83] Marganski, W.A., Gangopadhyay, S.S., Je, H.-D., Gallant, C. and Morgan, K.G. (2005) Targeting of a novel Ca+2/calmodulin-dependent protein kinase II is essential for extracellular signal-regulated kinase-mediated signaling in differentiated smooth muscle cells. Circ. Res. 97, 541–549.
[84] Loranger, A., Gilbert, S., Brouard, J.-S., Magin, T.M. and Marceau, N. (2006) Keratin 8 modulation of desmoplakin deposition at desmosomes in hepatocytes. Exp. Cell Res. 312, 4108–4119.
[85] Nieminen, M., Henttinen, T., Merinen, M., Marttila-Ichihara, F., Eriksson, J.E. and Jalkanen, S. (2006) Vimentin function in lymphocyte adhesion and transcellular migration. Nat. Cell Biol. 8, 156–162.
[86] Eckes, B. et al. (1998) Impaired mechanical stability, migration and contractile capacity in vimentin-deficient fibroblasts. J. Cell Sci. 111, 1897–1907.
[87] McInroy, L. and Maatta, A. (2007) Down-regulation of vimentin expression inhibits carcinoma cell migration and adhesion. Biochem. Biophys. Res. Commun. 360, 109–114.
[88] Ivaska, J., Vuoriluoto, K., Huovinen, T., Izawa, I., Inagaki, M. and Parker, P.J. (2005) PKCepsilon-mediated phosphorylation of vimentin controls integrin recycling and motility. EMBO 24, 3834–3845.
[89] Rizki, A., Mott, J.D. and Bissell, M.J. (2007) Polo-like kinase 1 is involved in invasion through extracellular matrix. Cancer Res. 67, 11106–11110.
[90] Ku, N.-O. and Omary, M.B. (2000) Keratins turn over by ubiquitination in a phosphorylation-modulated fashion. J. Cell Biol. 149, 547–552.
[91] Cross, T., Griffiths, G., Deacon, E., Sallis, R., Gough, M., Watters, D. and Lord, J.M. (2000) PKC-delta is an apoptotic lamin kinase. Oncogene 19, 2331–2337.
[92] Ku, N.O. and Omary, M.B. (2001) Effect of mutation and phosphorylation of type I keratins on their caspase-mediated degradation. J. Biol. Chem. 276, 26792–26798.
[93] Eriksson, J.E., He, T., Trejo-Skalli, A.V., Harmala-Brasken, A.-S., Hellman, J., Chou, Y.-H. and Goldman, R.D. (2004) Specific in vivo phosphorylation sites determine the assembly dynamics of vimentin intermediate filaments. J. Cell Sci. 117, 919–932.
[94] Goto, H., Kosako, H., Tanabe, K., Yanagida, M., Sakurai, M., Amano, M., Kaibuchi, K. and Inagaki, M. (1998) Phosphorylation of vimentin by Rho-associated kinase at a unique amino-terminal site that is specifically phosphorylated during cytokinesis. J. Biol. Chem. 273, 11728–11736.
[95] Tsujimura, K., Ogawara, M., Takeuchi, Y., Imajoh-Ohmi, S., Ha, M.H. and Inagaki, M. (1994) Visualization and function of vimentin phosphorylation by cdc2 kinase during mitosis. J. Biol. Chem. 269, 31097–31106.
[96] Nakamura, Y. et al. (2000) Localized phosphorylation of vimentin by Rho-kinase in neuroblastoma N2a cells. Genes Cells 5, 823–837.
[97] Yasui, Y., Goto, H., Matsui, S., Manser, E., Lim, L., Nagata, K.-i. and Inagaki, M. (2001) Protein kinases required for segregation of vimentin filaments in mitotic process. Oncogene 20, 2868.
[98] Goto, H., Yasui, Y., Kawajiri, A., Nigg, E.A., Terada, Y., Tatsuka, M., Nagata, K.-i. and Inagaki, M. (2003) Aurora-B regulates the cleavage furrow-specific vimentin phosphorylation in the cytokinetic process. J. Biol. Chem. 278, 8526–8530.
[99] Yamaguchi, T. et al. (2005) Phosphorylation by Cdk1 induces Plk1-mediated vimentin phosphorylation during mitosis. J. Cell Biol. 171, 431–436.
[100] Stefanovic, S., Windsor, M., Nagata, K.-i., Inagaki, M. and Wileman, T. (2005) Vimentin rearrangement during African swine fever virus infection involves retrograde transport along microtubules and phosphorylation of vimentin by calcium calmodulin kinase ii. J. Virol. 79, 11766–11775.
[101] Oguri, T. et al. (2006) Vimentin-Ser82 as a memory phosphorylation site in astrocytes. Genes Cells 11, 531–540.
[102] Kochin, V., Pallari, H.M., Pant, H. and Eriksson, J.E. (2004) Approaches to study posttranslational regulation of intermediate filament proteins. Meth. Cell Biol. 78, 373–409.
[103] Kochin, V., Imanishi, S.Y. and Eriksson, J.E. (2006) Fast track to a phosphoprotein sketch – MALDI-TOF characterization of TLC-based tryptic phosphopeptide maps at femtomolar detection sensitivity. Proteomics 6, 5676–5682.
[104] Herrmann, H. et al. (1996) Structure and assembly properties of the intermediate filament protein vimentin: the role of its head, rod and tail domains. J. Mol. Biol. 264, 933–953.
[105] Kim, S., Wong, P. and Coulombe, P.A. (2006) A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. Nature 441, 362–365.