

# Cytoplasmic intermediate filaments revealed as dynamic and multipurpose scaffolds

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**Intermediate filaments are cytoskeletal polymers encoded by a large family of differentially expressed genes that provide crucial structural support in the cytoplasm and nucleus of higher eukaryotes. Perturbation of their function accounts for several genetically determined diseases in which fragile cells cannot sustain mechanical and non-mechanical stresses. Recent studies shed light on how this structural support is modulated to meet the changing needs of cells, and reveal a novel role whereby intermediate filaments influence cell growth and death through dynamic interactions with non-structural proteins.**

*Intermediate filaments may function to integrate mechanically the various structures of the cytoplasmic space in a way that is tailored to the differentiated state of the cell.*  
Elias Lazarides, 1980 (ref. 1)

Intermediate filaments represent one of the three major fibrous polymers of the cell and function as cytoskeletal scaffolds in the nucleus and cytoplasm<sup>2</sup>. Through molecular cloning and database mining, > 67 genes have been identified to date that encode proteins that can self-assemble into the characteristic 10–12-nm-wide intermediate filaments in humans and mice<sup>3,4</sup>. All intermediate-filament genes encode cytoplasmic proteins with the notable exception of lamins B<sub>1</sub>, B<sub>2</sub>, and A/C (Table 1), which are concentrated in the nuclear lamina meshwork<sup>5–7</sup>. Transcription of intermediate-filament genes is context-dependent and bears an obvious relationship to cell differentiation (Table 1)<sup>3,4,8,9</sup>. Similarly to microtubules and microfilaments, intermediate filaments are dynamically regulated, fully integrated within the cellular framework and interact with a range of cellular proteins (Table 1). However, there are several basic attributes that are unique to intermediate filaments. Other than their sequence diversity and substructure<sup>10–12</sup>, these properties include: a lack of the structural polarity that is inherent to actin and microtubule-based filaments; their mode and location of assembly and turnover in cells; the absence of nucleotide binding and hydrolysis as a means of regulating filament dynamics; the small size of the soluble pool *in vivo*; and their association with distinct adhesive structures<sup>2,3,8,13</sup>. Accordingly, intermediate filaments are expected to fulfill functions that differ from other cytoskeletal assemblies.

Deciphering the function of intermediate filaments has actually proved difficult, owing in part to a lack of useful chemical inhibitors, their unusual properties and complexity, the widely held notion that

they are missing from most genetically tractable organisms, and last but not least, functional redundancy within the family. A breakthrough took place in the early 1990s when the targeted expression of keratin mutants in the skin of mice was found to result in trauma-induced blistering<sup>14,15</sup>, highlighting a role for intermediate filaments in the maintenance of cell and tissue integrity in the face of mechanical stress (Fig. 1a). From these early studies, important lessons were learnt, including the relevance of manipulating intermediate-filament gene expression *in vivo* and the role of intermediate-filament-disrupting mutations in the context of dominantly inherited, fragility-based conditions. Since then, this function in protecting cells against mechanical stress has been extended to all major types of intermediate filament (Table 1) and, at last count, > 30 distinct human disorders had been found to be associated with mutations in intermediate-filament genes<sup>16</sup>. In particular, much progress has revealed that, in addition to their contribution to the integrity and shape of the nucleus<sup>6,7,17,18</sup>, lamin intermediate filaments bind to chromatin directly and associate with a large number of nuclear proteins, including general as well as differentiation-specific regulators of transcription<sup>19</sup>. Thus, lamins are poised to influence gene expression through several mechanisms<sup>6,18,19</sup>, including a direct influence on global chromatin organization, on transcription of specific genes, or on signalling pathways that regulate these processes. Similarly, much has been learnt about the structural and non-structural roles of cytoplasmic intermediate filaments, and how these rely on interactions with a versatile group of structural and non-structural proteins<sup>9,20–25</sup> (Table 1). Here, we focus on the newly found ability of cytoplasmic intermediate filaments to modulate cellular responses to metabolic stress, programmed cell death, cell migration and even tissue growth; the phenomenal progress that has taken place in the specific area of lamin biology and nuclear organization is covered elsewhere<sup>6,7,17,18,26</sup>.

## Evolutionary origins

Among the model organisms popular in cell biology research, *Danio rerio* (zebrafish) features a mammalian-like complexity in its intermediate-filament genes; *Caenorhabditis elegans* has at

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**Table 1** The intermediate filament family and associated proteins

Intermediate filament type <sup>1</sup>	Protein name <sup>2</sup>	Number of genes <sup>3</sup>	Main tissue distribution <sup>4</sup>	Interacting proteins <sup>5</sup>
I	Keratins (acidic)	>25	K9–K20 and K23, soft epithelia	<i>Crosslinkers:</i> filaggrin, trichohyalin
			Ha1–Ha8, hard epithelia	<i>Cytolinkers:</i> desmoplakin, plectin, BPAG1e, plakophilin, pinin, periplakin
			Irs1–4, inner root sheath (hair)	<i>Chaperones:</i> Hsp70, Hsp40, Hsp27, Grp78, Mrj <i>Enzymes:</i> PKCe, Akt/PKB, JNK, caspase9, 12-lipoxygenase, RNA Polymerase II
II	Keratins (basic)	>24	K1–K8, Soft epithelia	<i>Receptors:</i> TNFR2
			Hb1–Hb6, Hard epithelia	<i>Adaptors:</i> 14-3-3 $\zeta$ , TRADD, DEDD
			K6irs1–4, Inner root sheath (hair)	<i>Bacteria/virus:</i> E1–E4 (HPV); Us2 (HSV), Tir ( <i>E.coli</i> ); HTLV-Tax
				<i>Others:</i> bystin, eIF3-p150 (translation) Mrp8–Mrp14 (Calcium-binding proteins)
III	Vimentin	1	Fibroblast, endothelium, leukocytes	$\alpha$ B-crystallin and plectin bind to all type–III intermediate filament proteins; Hsp27 binds vimentin and GFAP;
	Desmin	1	Muscle	desmoplakin binds to both vimentin and desmin;
	GFAP	1	Astrocytes/glia	PKC, 14-3-3, PLA2, fimbrin, polycystin-1,
	Peripherin	1	PNS neurons	LMP (EBV) and Vmac all bind to vimentin; and
	Syncoilin	1	Muscle	nebulin binds desmin.
IV	NF-L	1	CNS neurons	BPAG1n, plectin, myosin Va, PKN, cdk5,
	NF-M	1	CNS neurons	hamartin and HTLV-Tax all interact with neurofilaments;
	NF-H	1	CNS neurons	$\alpha$ -actinin binds to synemin; $\alpha$ -dystobrevin binds
	$\alpha$ -internexin	1	CNS neurons	to desmuslin and syncoilin; and p35 kinase binds to
	Nestin	1	Heterogeneous	nestin.
	Synemin	1	Muscle	
	Desmuslin	1	Muscle	
V	Lamin A/C	1	Differentiated tissues (nucleus)	Lamins interact with a large group of nuclear
	Lamin B1	1	Ubiquitous (nucleus)	proteins; see reference 19.
	Lamin B2	1	Ubiquitous (nucleus)	
Orphan	Phakinin / CP49	1	Lens	$\alpha$ B-crystallin binds to CP49/CP115; tropomodulin
	Filensin / CP115	1	Lens	binds to CP115

<sup>1</sup>This classification was made on the basis of gene substructure and nucleotide sequence homology for the region coding for the central  $\alpha$ -helical rod domain shared by all intermediate filament proteins.

<sup>2</sup>Individual intermediate filament genes and proteins are highly homologous in related species. The relative molecular mass of individual intermediate filament proteins in human ranges between 40K (type I K19) and 240K (nestin).

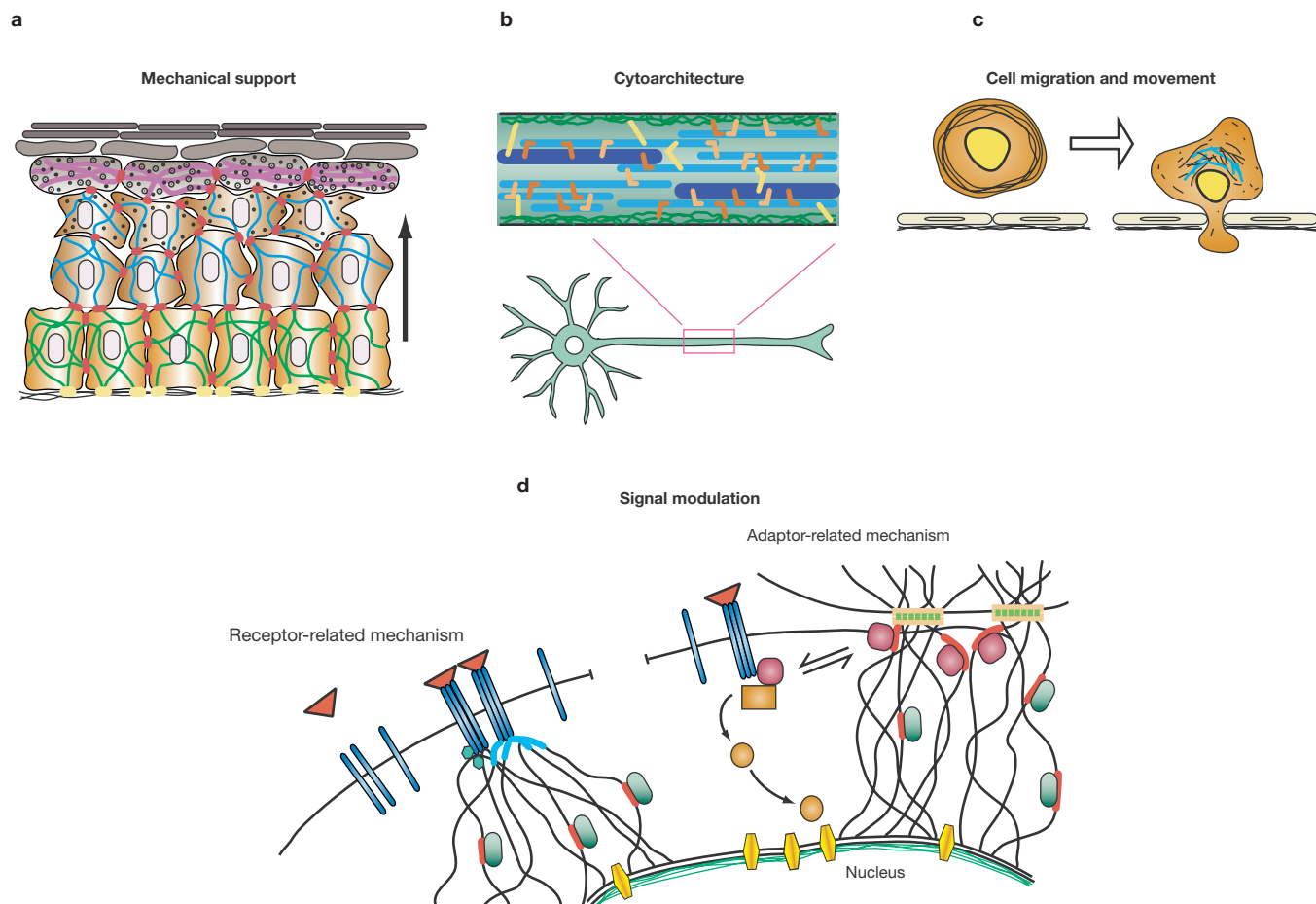
<sup>3</sup>All intermediate filament proteins are encoded by their own gene, with the notable exception of lamins A/C and peripherin, for which differential splicing of a common precursor mRNA occurs.

<sup>4</sup>This information pertains to mature tissues and cell types, and does not convey embryonic expression patterns. Type I/type II, type III/IV, type V and phakinin/filensin represent four distinct co-polymerization groups *in vitro* and *in vivo* (ref. 11). Type I and type II keratins, neurofilament-L, M and H subunits, and phakinin/filensin, each heteropolymerize in a strict obligatory way to form 10–12-nm filaments.

<sup>5</sup>Interactions were demonstrated by one or more of the following assays: co-immunoprecipitation, colocalization in cells or tissues, *in vitro* reconstitution coupled to co-sedimentation, or yeast two-hybrid analysis. More information about these interactions can be found in references 9, 11, 16, 20–23, 25 and 35. This listing is incomplete, but conveys the notion that intermediate filaments bind to a variety of other proteins.

least 12 genes (including a single nuclear lamin); *Drosophila melanogaster* has two genes encoding nuclear lamins, but no cytoplasmic intermediate filaments; whereas the yeast *Saccharomyces cerevisiae* is believed to have none<sup>5,27,28</sup>. Taking this into account, and the parallel discovery that protostomic invertebrates have

cytoplasmic intermediate-filament proteins with clear lamin-like features, it has been proposed that the ancestral intermediate-filament gene probably encoded a lamin-like nuclear protein<sup>27</sup>. One possibility is that subsequent loss of the small exons coding for nuclear targeting and membrane association in the 3' moiety of an



**Figure 1** Major cellular functions of cytoplasmic intermediate filaments. **(a)** Mechanical support. The epidermis is a good example to illustrate this function that is shared by all major types of intermediate filament (see Table 1). Keratin intermediate filaments are abundant in keratinocytes, ranging from > 10% of total proteins in progenitor basal cells to > 70% in late-differentiating cells. Changes in filament colour reflect differential expression and composition of keratins in basal, early- and late-differentiating cells (differentiation proceeds with the arrow). Keratin filament networks extend throughout the entire cytoplasm in individual keratinocytes and are integrated between cells by attachment at desmosome cell–cell junctions (red dots) and between basal cells and the basal lamina by attachment at hemidesmosomes (yellow dots). This organization maximizes the mechanical support provided by keratin filaments. **(b)** Cytoarchitecture. In motor neurons, the radial growth of axonal processes requires their interaction with neurofilaments (light blue) to exhibit correct stoichiometry between the light (NF-L), medium (NF-M) and heavy (NF-H) subunits (see Table 1). The large C-terminal tail domains of NF-H (red) and NF-M (orange) subunits are hyperphosphorylated and project away from the filament core, thereby determining interfilament spacing and axonal calibre. The many neurofilaments interact with the less frequent microtubules (dark blue) and with subcortical actin filaments (dark green) through cytoskeletal linker proteins, such as plectin and BPAG1 (yellow). Adapted from reference 32. A cytoarchitecture role has also been shown for nuclear lamins and other cytoplasmic intermediate filaments, such as desmin and keratin. **(c)** Cell migration. In circulation, lymphocytes resist haemodynamic and

mechanical stresses, owing in part to their vimentin intermediate filament network (see Table 1), which is organized in a cage configuration at the cytoplasmic periphery (left cell). After chemokine-induced chemotaxis, for example at sites of active inflammation, vimentin intermediate filaments are rapidly move to the perinuclear region at the cell uropod. This is made possible partly through the site-specific phosphorylation of vimentin subunits (depicted by a change in filament colour in the cell, right), and correlates with a softening of the viscoelastic properties of the cytoplasm, presumably to allow the pliability needed during extravasation. The same general principles underlie the ability of epithelial cells to migrate into a wound site after injury. **(d)** Signal modulation. Cytoplasmic intermediate filaments can bind and modulate the activity of signalling proteins, thereby influencing the flow of extracellular signals to relevant terminal effectors inside the cell. Two possible mechanisms are shown. Left, interactions of intermediate filaments with cell surface receptors, such as Fas, modulate their density and function; right, regulated interactions between intermediate filaments and an adaptor protein, such as TRADD (pink), near the cell surface limits the availability of this adaptor to a ligand-bound receptor that is poised to transmit a signal to the cell. Regulation of either type of interaction, conveyed here by a local change in filament colour (blue or red), can be mediated by dynamic post-translation modifications, association with other proteins or local differences in the composition of intermediate-filament subunits. Both of these mechanisms may participate in regulating the response of epithelial cells to pro-apoptotic signals and other signalling events.

ancestral lamin gene would have resulted in the appearance of intermediate-filament proteins in the cytoplasm. Presumably, such events were positively selected for in species whose constituent cells did not benefit from the protection afforded by an exoskeleton, as in arthropods. It has been noted in *D. melanogaster* that cells exposed to significant mechanical strain tend to show a higher-than-expected density of microtubules<sup>28</sup>. Why cytoplasmic intermediate-filament genes duplicated so much during the evolution of chordates, and whether and how this multiplicity of sequences reflects functional specialization, are important issues to resolve.

Enter crescentin, a protein recently shown to impart the vibroid/helical shape adopted by the bacterium *Caulobacter crescentus*<sup>29</sup>. Crescentin exhibits a heptad repeat-rich,  $\alpha$ -helical rod domain similar to the one shared by all eukaryotic intermediate-filament proteins, and can self-assemble into intermediate-filament-like fibres *in vitro*. However, crescentin does not feature many of the signature motifs of vertebrate intermediate-filament sequences, notably those found at the extremities of the central  $\alpha$ -helical domain. This situation may be analogous to the prokaryotic MreB<sup>30</sup> and FtsZ<sup>31</sup> proteins which, albeit distantly related to eukaryotic actins and tubulins in primary structure, are nonetheless highly similar in fold and function<sup>2</sup>. Here it is worth mentioning that neurofilaments foster the radial growth of axons and proper dendritic arborization of large motor neurons (Fig. 1b)<sup>9,32</sup>, whereas lamins influence the shape of the nucleus and the positioning of nuclear pores<sup>6,7,26</sup>. So, the crescentin story highlights particularly well the challenges that we face in assessing the occurrence of intermediate-filament-like systems in simpler organisms (for example, see ref. 33), considering the absence of a structural reference for the intermediate-filament polymer<sup>10,12</sup>. The structural relatedness of crescentin to eukaryotic intermediate filaments, and the evolutionary relationship of the genes encoding them, need to be ascertained through additional studies.

### Responding to metabolic stress and pro-apoptotic signals

Clues about novel functions of intermediate-filament polymers have arisen recently from the identification of proteins that are able to bind to them dynamically, and the assessment of these interactions in the context of transgenic mouse models. Many such proteins, including various kinases, receptors, adaptors and other types of effector (Table 1), function in signalling networks that regulate the cell cycle, programmed cell death and the cellular response to stress<sup>16,20,32,34–37</sup>. Central to this progress were studies of the keratin K8/K18 pair, which are expressed in the four-cell-stage embryo and during the early development of all types of epithelia, only to become restricted to simple epithelial linings in the adult (for example, gut, liver, pancreas, kidney and airways)<sup>34,35,37</sup>.

Demonstrating a mechanical role for intermediate filaments in liver hepatocytes — a well studied cell type whose cytoplasmic intermediate filaments are comprised only of K8/K18 — has proved difficult<sup>35,37–41</sup>. Conversely, evidence that K8/K18 intermediate filaments exert a cytoprotective role in hepatocytes during metabolic stress<sup>40,42–44</sup> is plentiful. Both K8 and K18 are rapidly and site-specifically phosphorylated under these conditions<sup>34</sup>. Remarkably, mutating the major phosphorylation site on K18 (Ser 52 to Ala) is sufficient to compromise the response of mouse hepatocytes to chemically induced liver damage<sup>45</sup>. How K8/K18 intermediate filaments afford cytoprotection in the liver is not yet understood at a mechanistic level<sup>34,35,37</sup>, but may involve their interactions with stress proteins such as hsp70 (ref. 46), Mrj<sup>47</sup>, Hsp27 and  $\alpha$ B-crystallins<sup>48</sup>, or kinases such as PKC- $\epsilon$  (ref. 49) and c-Jun<sup>50</sup> (Table 1). To date, the naturally occurring mutations discovered in the human K8/K18 genes differ from those found in keratin-encoding genes expressed in skin tissue, and are not causative, but are believed to

be a risk factor for, chronic diseases such as cryptogenic cirrhosis, hepatitis and inflammatory bowel disease<sup>51–53</sup>. This distinction may reflect the prominent cytoprotective role fulfilled by keratin intermediate filaments in adult liver hepatocytes.

Studies initially focused on K8/K18 (but since extended to other cytoplasmic intermediate filaments) have also revealed a role in modulating the response to specific pro-apoptotic signals. Simple epithelial cells lacking K8 or K18, or in which intermediate filaments are disrupted secondary to mutant K18 expression, are significantly more prone to apoptosis in specific circumstances<sup>54–57</sup>. The *in vivo* relevance of this role again had its origins in studies of the liver<sup>54,57</sup>, but was shown recently to apply also to K8/K18 filaments in extra-embryonic epithelia<sup>58</sup>. Similarly, mice carrying a null mutation in K17 — a type-I keratin expressed in specific cell types within all epithelial appendages including hair, nail and glands — exhibit severe and yet reversible alopecia, owing partially to the untimely apoptosis of matrix epithelial cells that are responsible for hair genesis<sup>59</sup>. Temporally and spatially orchestrated apoptosis allows hair follicles to transit from the growth stage to the resting stage of their cycle<sup>60</sup>, a phenomenon that may be accelerated in K17-null skin. Finally, the apoptotic death of transgenic mouse neurons containing peripherin aggregates also depends specifically on the presence of TNF- $\alpha$  (ref. 61).

Numerous mechanisms could potentially account for the effects of intermediate filaments on apoptosis, and some have already been substantiated experimentally. First, intermediate filaments could influence the display, density or function of death receptors at the cell surface (Fig. 1d). It has been shown that K8/K18 colocalize with tumour necrosis factor receptor 2 (TNFR2)<sup>54</sup>, reflecting their ability to interact specifically with its cytoplasmic tail domain, and that at least in cell lines, this interaction affects the TNF-dependent activation of downstream effectors such as JNK (Table 1) and NF- $\kappa$ B. Survival of K8-null embryos is improved significantly when the mother lacks either TNFR2 or TNF- $\alpha$  (ref. 58), supporting the significance of this interaction *in vivo*. However, these exciting findings do not preclude an additional contribution of trophoblast cell fragility to this phenotype<sup>11,62</sup>. In mouse hepatocytes in primary culture, Gilbert *et al.* showed that K8/K18 influence the density of Fas receptors at the cell surface, which correlated with the greater sensitivity of these mutant cells to a Fas-mediated apoptotic challenge<sup>55</sup>. Related to this, mutant K18-expressing mouse liver hepatocytes are more sensitive to Fas-, but not TNF- $\alpha$ -mediated, apoptosis *in vivo*<sup>57</sup>. Interactions with both TNFR2 and Fas receptors could be relevant *in vivo*, depending on cell type and context. Additional evidence strongly supporting the idea that K8/K18 intermediate filaments might target and/or distribute membrane proteins at the cell surface originated in studies focusing on the gastrointestinal tract epithelium<sup>63,64</sup>. Second, cytoplasmic intermediate filaments could regulate apoptosis through an influence on the formation of the death-inducing signalling complex (DISC) immediately after death-receptor engagement (Fig. 1d), or through more downstream events. Inada *et al.* showed that K18 interacts with TRADD through subdomain 1A in its rod domain<sup>56</sup>, which is highly conserved among type I keratins. TRADD (tumor-necrosis-factor-receptor-1-associated death domain protein) is a cytoplasmic adaptor molecule recruited to ligand-activated TNFR1, and is essential for downstream events that culminate in apoptosis<sup>65</sup>. Loss of this interaction greatly sensitizes K8-null cells to apoptosis through TNFR1, but not through other receptors, presumably because TRADD is no longer sequestered by K8/K18 and can readily be recruited for DISC formation<sup>56</sup>. As expected, TRADD also binds to the 1A subdomain of K14 (ref. 56) — an interaction postulated to be important for the pathogenesis of epidermolysis bullosa simplex<sup>66</sup> — and K17, possibly explaining the

heightened sensitivity of cultured K17-null keratinocytes to TNF- $\alpha$ -induced apoptosis (X. Tong and P.A.C., unpublished observations). Direct evidence for the physiological significance of the TRADD–keratin interaction *in vivo* is eagerly awaited. Again, there is emerging evidence in non-epithelial settings that cytoplasmic intermediate filaments bind to and regulate the activity of other types of effector molecules, for example, kinases<sup>67</sup>.

Considering now a different facet of apoptosis, several intermediate-filament proteins bind to, and are direct substrates for, caspases<sup>35,68,69</sup>. Caspase-mediated cleavage of lamin A/C<sup>70–72</sup>, vimentin<sup>73</sup>, and desmin intermediate filaments<sup>74</sup> promotes the timely completion of apoptosis in cultured cells. A specific caspase cleavage product of K18 (refs 75, 76) occurs in the serum of breast cancer patients<sup>77</sup>. An Asp residue located in the middle of the central rod domain of K14, essential for caspase cleavage<sup>76</sup>, is mutated in the context of epidermolysis bullosa simplex, although the implications for pathogenesis are unclear. Interestingly, Peter and colleagues<sup>68,69</sup> found that in cultured lung and breast epithelial cells undergoing apoptosis, several key effectors, including DEDD (death-effector-domain-containing DNA-binding protein), active caspases 3 and 9, and ubiquitin, are bound to K8/K18 intermediate filaments (Table 1); and they postulated that such interactions could assist in spatially and temporally organizing the degradation of cellular substrates. Because of the abundance of intermediate filaments and the structural support that they provide for cells, it is logical that their subunit constituents might be cleaved during the introverted process of programmed cell death. What could not be anticipated, however, is that intermediate filaments might participate in the response of cells to specific pro-apoptotic signals (Fig. 1), and potentially influence the execution of this programme.

### The control of tissue growth

Cytoplasmic intermediate filaments undergo significant and reversible remodelling during mitosis<sup>34,78–80</sup>, but are not otherwise believed to contribute to its regulation directly. However, there is emerging evidence to suggest that K10 — a type-I keratin expressed at high levels (along with its partner K1) in the post-mitotic, differentiating layers of the epidermis — influences cell proliferation *in vivo*. K10 interacts directly with Akt (ref. 81; Table 1), a versatile kinase important for the intracellular relay of signals that lead to cell growth or death. Forced expression of human K10 in cell lines and in the basal layer of mouse epidermis significantly and dose-dependently reduces cell proliferation<sup>82,83</sup>. This effect is believed to result from the sequestration of Akt by K10-containing intermediate filaments (Table 1), which thereby prevent it from properly regulating key downstream effectors such as Rb, PKC $\zeta$  and NF- $\kappa$ B (refs 83, 84). This scenario is conceptually identical to the proposed significance of the keratin–TRADD interaction during apoptosis. Paramio *et al.* postulated that the hypoproliferative skin phenotype observed in such mice reflects the normal function of K10 in the epidermis, that is, to prevent mitosis in keratinocytes as they trigger differentiation<sup>36</sup>. This hypothesis predicts that loss of K10 should result in enhanced proliferation in the basal layer of epidermis. This much is known to occur in epidermolytic hyperkeratosis (EHK), a disorder caused by missense mutations in either K10 or K1 (refs 9, 16). This also occurs in K10-null mice, albeit with a late onset (~6-weeks postnatally)<sup>85</sup>. Hyperproliferation occurs concomitantly with an increase in the levels of c-Myc and cyclin D1 in basal cells, a decrease in Akt activation, but no change in Rb (ref. 85), a result that is at odds with the study discussed above<sup>84</sup>. Notable alterations in the suprabasal layers of K10-null epidermis include marked cell hypertrophy, faster movement of differentiating cells towards the tissue surface, and increased levels of 14-3-3 $\sigma$ . Conspicuously absent, however, is the epidermal fragility

characteristic of EHK, a surprising outcome that correlates with increased levels of K5/K14 and K6/K16 keratin pairs, in K10-null epidermis<sup>86</sup>. Reichelt and Magin<sup>85</sup> favour a paracrine mechanism whereby suprabasal cells lacking K10 sense the altered cytoarchitecture and elaborate a signal that results in the enhanced proliferation of basal cells. Although these two sets of studies are conflicting in their mechanistic implications, they converge in showing that K10 influences proliferation of basal keratinocytes in epidermis. Defining when K10 protein appears relative to exit from the cell cycle in normal epidermis<sup>87</sup>, may help resolve the autocrine versus paracrine nature of its action.

Alterations in the cell cycle and mitotic aberrations have also been seen in liver tissue in which K8/K18 has been manipulated. A significant proportion of liver tissue taken from adult mice lacking K8 or K18 contains giant multinucleated cells that, along with an abnormally high proportion of S/G2-phase cells among hepatocytes, probably reflects cell-cycle alterations<sup>88</sup>. The distribution of 14-3-3 $\zeta$ , which K18 interacts with when phosphorylated at Ser 33 (ref. 89), is shifted abnormally to nuclei in these liver syncytia. Loss of this interaction, which peaks during mitosis<sup>89</sup>, may disrupt one of the many cell-cycle regulators to which 14-3-3 $\zeta$  binds<sup>90</sup>, such as the phosphatase Cdc25. Transgenic mice in which this phosphorylation site of K18 is mutated, also show aberrant mitotic spindles following hepatectomy, correlating with the retention of a speckled 14-3-3 $\zeta$  nuclear staining, instead of the normal diffuse pattern seen during mitosis<sup>91</sup>. Vimentin intermediate filaments also bind and sequester 14-3-3 isoforms in a phosphorylation-dependent manner, an interaction that influences the Raf-dependent response of Cos-7 cells to EGF (ref. 92). Although the mechanisms involved are still poorly defined, cytoplasmic intermediate filaments, or their dissociated subunits, are known to influence cell proliferation at least indirectly through their ability to bind and regulate the activity of key effectors, such as Akt, Raf, c-Jun, 14-3-3 proteins (Table 1), and probably others.

### Optimizing structural support during cell migration

The viscoelastic properties of the cytoplasm need to be modulated dynamically to meet the changing needs of a living cell. Consistently, the organization of cytoplasmic intermediate filaments changes rapidly and substantially in preparation for the cell migration that occurs after acute injury in several tissue settings, ranging from skin to the central nervous system<sup>32,34,93–95</sup>. Molecular analyses have revealed concomitant changes in the regulation of intermediate-filament proteins (for example, through phosphorylation) and/or in the transcription of intermediate-filament genes in cells located proximal to the wound margin. Whether these alterations indeed change the properties of the cell — viscoelastic or otherwise — has remained unclear. Recent studies suggest that this is indeed the case.

After skin injury, wound-proximal keratinocytes become substantially larger and polarized and, among a plethora of subcellular changes<sup>93</sup>, reorganize much of their keratin network to the perinuclear area<sup>96,97</sup>. This reorganization occurs concomitant with the induction of four keratins — K6 paralogues a and b, K16 and K17 — at the expense of K1/K10, which are downregulated<sup>97,98</sup>. The availability of K6-null mice, which die soon after birth owing to several oral lesions<sup>99,100</sup>, provided an opportunity to assess the importance of the K6/K16 pair in this setting (K16 protein is selectively downregulated in K6-null keratinocytes<sup>94</sup>). Surprisingly, these mutant keratinocytes exhibited enhanced migration in an *ex vivo* skin explant culture assay that mimics wound epithelialization<sup>94</sup>. This correlated with increased levels of total and tyrosine-phosphorylated p120 catenin, a situation previously linked to faster keratinocyte migration<sup>101</sup>. The enhanced migration potential of K6-null keratinocytes is not fulfilled *in vivo*, however. Analysis of grafted newborn skin tissue soon after acute injury showed that K6-null cells located proximal to, and within, the

wound site exhibit the characteristic signs of trauma-induced cell lysis, indicative of their fragility. These findings suggested that owing to the intermediate-filament reorganization they promote, the viscoelastic properties they specifically provide, and/or some property yet to be specified, accumulation of K6/K16 intermediate filaments provides the mechanical resilience needed by wound-edge keratinocytes at the cost of partially reducing their migratory potential<sup>94</sup>. Although this idea of a compromise<sup>94</sup> needs validation with direct experimental evidence, it is attractive in that it can account potentially for the general involvement of intermediate filaments after injury to various tissues in the adult<sup>32,34,93,95</sup> and in embryos<sup>102</sup>. It could also explain the more rapid movement of K10-null keratinocytes through the suprabasal layers of the epidermis<sup>85</sup>.

This concept makes the general prediction that cellular viscoelastic properties should be influenced significantly by the protein makeup and organization of cytoplasmic intermediate filaments. A study by Beil *et al.* provided direct support for this hypothesis<sup>103</sup>. Pancreatic tumour (panc-1) epithelial cells treated with sphingosylphosphorylcholine (SPC), a lipid found at high levels in solid tumours, reorganize their keratin intermediate filaments from a cytoplasmic- to a perinuclear-enriched localization, correlating with increased phosphorylation of Ser 52 in K18 and Ser 431 in K8. Such changes also take place in simple epithelial linings subjected to injury *in vivo*<sup>34</sup>. When tested in a Boyden-chamber-based migration assay, SPC treatment stimulated the migration of panc-1 cells by more than twofold<sup>103</sup>. Possibly related to this, Morley *et al.* found that skin keratinocyte cell lines established from patients suffering from epidermolysis bullosa simplex exhibit enhanced migration *ex vivo*<sup>104</sup>. What makes the contribution of Beil *et al.* original is their assessment of the viscoelastic properties of panc-1 cells using a novel assay in which live cells are stretched in a microscope chamber<sup>103</sup>. Not only did they find that SPC treatment significantly 'softened' the cytoplasm of panc-1 cells, but it did so to a greater extent than when the cells were treated with an F-actin-disrupting drug. Such *in vivo* measurements are needed to complement what is already known about the micromechanical properties of a pure suspension of intermediate filaments *in vitro*<sup>105–107</sup>.

Beil *et al.* concluded that exposure to SPC and other relevant signals may, through rapid and profound effects on the organization of keratin intermediate filaments, facilitate the squeezing of epithelial cells through size-restricted openings, and thus promote metastasis<sup>103</sup>. Remarkably, Brown *et al.* reached the same conclusion when assessing the role of vimentin intermediate filaments in lymphocytes<sup>108</sup>. Lymphocytes must be rigid while in circulation, and this property depends more on vimentin intermediate filaments, which form a spherical cage-like network in the peripheral cytoplasm (Fig. 1c), than on either microtubules or microfilaments<sup>108</sup>. When summoned by signals such as chemokines to extravasate into tissues, lymphocytes must rapidly and reversibly deform. Remarkably, under such conditions vimentin intermediate filaments retract and condense into a dense perinuclear aggregate at the uropod (Fig. 1c) — a process that requires plectin<sup>109</sup>, a versatile cytoskeletal linker protein<sup>23,110</sup>. Brown *et al.* showed that this reorganization leads to softer mechanical properties<sup>108</sup> which, analogous to the argument made for neoplastic epithelial cells<sup>103</sup>, enable lymphocytes to squeeze their way more easily through the vascular wall.

The need to modulate the mechanical scaffolding role of cytoplasmic intermediate filaments to meet the changing needs of cells may have contributed to the diversification of intermediate-filament primary structure during evolution, and to their transcriptional and post-translational regulation. Further characterization of how the viscoelastic properties of cells vary depending on the type of cytoplasmic intermediate filaments they contain, their modifications, associated proteins, and resulting organization, is greatly needed.

## Concluding remarks

In his classic 1980 paper<sup>1</sup>, Elias Lazarides, inspired from his studies on muscle, foresaw the major role of cytoplasmic intermediate filaments as a differentiation-dependent, adaptable and resilient meshwork that enables cells to cope with mechanical stress and, in specific settings, promote cytoarchitecture. Advances in molecular genetics, and in particular, gene manipulation in mice and other model organisms, have since confirmed and extended his vision, as well as revealing additional roles. The evidence in hand establishes that both cytoplasmic and nuclear intermediate filaments evolved the ability to organize and influence, through regulated interactions, diverse factors that participate in basic metabolic processes. Determining the extent of these newly defined functions, and how they are implemented at a mechanistic level, are among the exciting challenges that lay ahead of us.

There are excellent, although still limited, studies that point to additional functions that are no less exciting. Keratin<sup>63,64</sup> and vimentin<sup>111</sup> intermediate filaments have been implicated in the localization of membrane proteins to specific domains of the outer cell membrane. Vimentin is secreted by differentiated human macrophages in response to TNF- $\alpha$ , facilitating bacterial killing<sup>112</sup>. Loss of desmin intermediate filaments results in a decreased number and the gross mislocalization of mitochondria in cardiac and skeletal myocytes<sup>113,114</sup>, a phenotype rescued by overexpression of Bcl-2 (ref. 115). Neurofilaments bind to and establish the normal distribution of myosin Va, an F-actin-dependent motor, in central and peripheral neurons<sup>116</sup>. Because of this variety of functions, the diversity of intermediate-filament proteins and their tight regulation in a differentiation- and context-dependent manner suddenly appear as an obvious necessity.  $\square$

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