

# The 'ins' and 'outs' of intermediate filament organization

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*A major function shared by several types of cytoplasmic intermediate filaments (IFs) is to stabilize cellular architecture against the mechanical forces it is subjected to. As for other fibrous cytoskeletal arrays, a crucial determinant of this function is the spatial organization of IFs in the cytoplasm. However, very few crossbridging proteins are specific for IFs – most IF-associated proteins known to exert a structural role act to tether IFs to other major cytoskeletal elements, such as F-actin, microtubules or adhesion complexes. In addition, IFs are endowed with the ability to participate in their own organization. This intriguing property is probably connected to the unusual degree of sequence diversity and sequence-specific regulation that characterize IF genes and their proteins. This dependence upon a combination of extrinsic and intrinsic determinants contributes to distinguish IFs from other fibrous cytoskeletal polymers and is key to their function.*

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Eukaryotic cells feature two ubiquitous fibrous cytoskeletal polymers in their cytoplasm: F-actin and microtubules<sup>1</sup>. A third fibrous polymer, intermediate filaments (IFs), is more recent in its appearance during evolution and its characterization. Until the recent identification of unconventional actins and tubulins, IF proteins stood out by virtue of their remarkable heterogeneity at the level of sequence and distribution.

The >50 known human genes encoding IF proteins can be partitioned into six major classes based on gene structure, sequence homology, and/or assembly properties<sup>2</sup>. The human IF genes have been found to be highly conserved in sequence and, usually, in regulation in mammals and other higher vertebrates<sup>3,4</sup>. Lamins, a.k.a. type V IF sequences, are the primary constituents of the fibrous lamina that

is apposed against the inner membrane of the nucleus. Several lamin sequences have been discovered in species spanning the entire metazoan phylum, including *Drosophila*<sup>5</sup>. Cytoplasmic IF arrays, with which this review is concerned, are made of type I–IV and/or type VI IF proteins. Their existence has been documented in many but not all orders within the metazoan phylum<sup>4</sup>. All genes encoding cytoplasmic IFs are regulated in a cell-type- or differentiation-specific fashion *in vivo*. Type I and type II IF genes each encode a group of >20 keratin proteins usually found in epithelial cells. Type III IF genes include vimentin (found in cells of mesenchymal origin), desmin (expressed in all muscles), GFAP (glial cells) and peripherin (peripheral nervous system). Type IV IF genes are expressed in neurons and encode proteins including  $\alpha$ -internexin and the neurofilament triplet proteins NF-L, NF-M and NF-H. A group of 'newer IF genes', including nestin, synemin, paranemin and tanabin, have been classified to a sixth type or to an existing type depending on the criteria applied<sup>2,6,7</sup>. Two additional IF proteins, filensin and phakinin, stand out by virtue of their sequence as well as their polymerization properties, given that they are the core constituents of the unusual beaded filaments found in the lens (see Ref. 8 for review).

In contrast to F-actin and microtubules, cytoplasmic IFs do not exert true housekeeping functions in the cell. Consistent with this, unicellular eukaryotes such as *Saccharomyces cerevisiae* do not have genes encoding cytoplasmic IFs, and vertebrate cells can grow in the absence of a cytoplasmic IF network *ex vivo*<sup>9</sup> and even *in vivo*<sup>10</sup>. What, then, do cytoplasmic IFs do? A major role they fulfil, at least in higher vertebrates, is to provide 'protection' against mechanical stress. Epithelial cells, muscle cells or astrocytes deprived of an intact IF network are prone to rupture when subjected to shearing forces. Experimental evidence in support of this comes from two complementary sources. One is the phenotype of transgenic mouse strains carrying null mutations in specific IF genes or engineered to express dominant-negative mutant versions of IF proteins<sup>11,12</sup>. The other consists of the clinical presentation of patients whose genome harbours mutations affecting the coding sequence of specific IF gene types<sup>13,14</sup>. Other than structural scaffolding, cytoplasmic IFs also fulfil cell-type-specific functions, such as the contribution of neurofilaments to the radial growth of axons<sup>15</sup>, of vimentin IFs to sphingolipid synthesis<sup>16</sup> and of K8–K18 filaments in protecting the liver hepatocyte against drug-induced and apoptotic stress<sup>17,18</sup>. Together, these findings suggest that cytoplasmic IFs contribute to several fundamental processes in the cell, albeit in a context-dependent fashion.

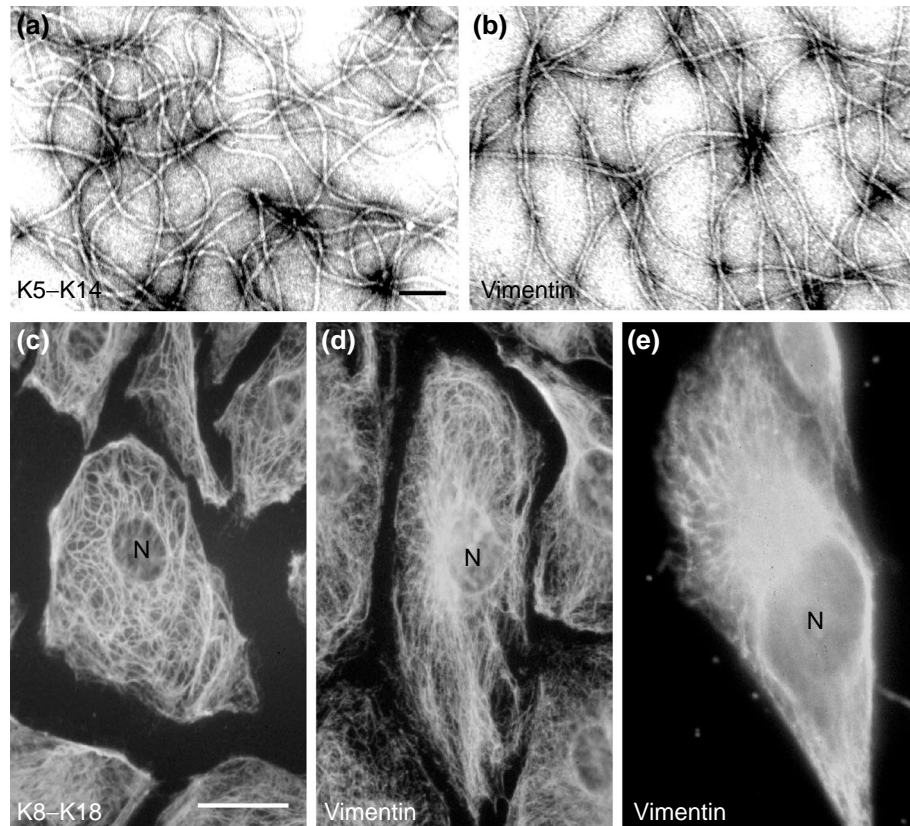
The spatial organization of cytoskeletal arrays is a major determinant of their function(s) in the cell<sup>1</sup>. Here, we review the recent literature pertaining to the organization of cytoplasmic IFs. The emerging lesson is that IF organization is largely determined by two types of influences. On the one hand, associated, non-IF proteins (defined as

extrinsic determinants, or 'outs') act to tether IFs to other cytoskeletal elements and thus influence the spatial arrangement of IFs within the cytoplasm. On the other, sequence determinants located within IF protein themselves (here defined as intrinsic determinants, or 'ins') influence both IF distribution as well as IF-IF interactions in the cytoplasm.

**Surprisingly few cytoplasmic proteins specifically crossbridge IFs**

Like polymerized (F-) actin, cytoplasmic IFs (see Fig. 1 and Box 1) probably function as groups of filaments. In support of this, specialized cell types as diverse as keratinocytes, neurons or myocytes feature prominent bundles of IFs in their cytoplasm. Dozens of proteins impact in a specific fashion upon the organization of F-actin, and this proficiency has contributed to shape our view of how cytoskeletal fibres are put to work in the cell<sup>1</sup>. Accordingly, IF researchers have long postulated the existence of a corresponding class of IF-associated proteins that would function to produce the highly organized networks seen in cells (Fig. 1). In fact, a rather modest number of proteins satisfying these simple criteria have been identified so far. These proteins are listed in Table 1.

Only two of these proteins, filaggrin and trichohyalin, are believed to be specific for IFs in their crossbridging activity. Filaggrin is a small, highly charged protein that features a short, repeated sequence motif that bundles keratin and vimentin IFs *in vitro*. This interaction is probably electrostatic and has been postulated to involve charged amino acids exposed at the surface of keratin IFs<sup>19</sup>. Filaggrin, whose expression is restricted to keratinizing epithelia, is made initially as an inactive polyprotein precursor that is stored in cytoplasmic granules in keratinizing epithelia. Processing and activation of profilaggrin occurs at a late stage of keratinocyte differentiation and results in macrofibril formation<sup>20</sup>. Trichohyalin is also a highly charged protein that initially is stored in granules. This 200-kDa protein, believed to adopt an elongated  $\alpha$ -helical structure, can bundle keratin IFs into tight parallel arrays in a subset of hair follicles and tongue epithelial cells<sup>21</sup>. In both instances, the binding site(s) on the relevant IF proteins are not known. Given their restricted distribution and their storage as inactive precursors, these two proteins cannot be viewed as functional equivalents of  $\alpha$ -actinin or fimbrin, two examples of ubiquitous F-actin bundling proteins<sup>22</sup>. As it turns out, most of the IF-associated proteins listed in Table 1 link cytoplasmic IFs to other elements of the cytoskeleton.



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**FIGURE 1**

Examples of intermediate filament (IF) polymer structure and organization. (a, b) Filaments reconstituted from purified human recombinant protein and visualized through negative staining and electron microscopy. (a) K5-K14 filaments; (b) vimentin filaments. Bar, 200 nm. (c, d) Indirect immunofluorescence staining of the IF network in cultured cells. (c) K8-K18 network and (d) vimentin network in PtK2 kidney epithelial cells. Note the differences in the architecture of the two IF networks in PtK2 cells – the vimentin network resembles a microtubule array. (e) Vimentin network in a baby hamster kidney (BHK-21) fibroblast. Abbreviation: N, nucleus. Bar, 20  $\mu$ m.

**The plakin/cytolinker proteins crossbridge IFs to other cytoskeletal elements**

Members of an emerging family of intriguing proteins, designated as plakins or cytolinkers<sup>23,24</sup>, possess binding determinants for at least two major cytoskeletal elements. The three founding members of this family, desmoplakin, plectin and the bullous pemphigoid antigen 1 (BPAG1), attach cytoplasmic IFs to F-actin, microtubules and/or adhesion complexes. Each is encoded by a single gene whose primary transcript is subjected to differential splicing in a cell-type-specific fashion, thereby producing isoforms having the ability to crossbridge different elements<sup>25-27</sup>. Desmoplakin is a major structural constituent of the cytoplasmic 'plaque' domain of desmosomes, a cell-cell adhesion complex, where it plays an important role in anchoring keratin and other types of cytoplasmic IFs<sup>28,29</sup>. The BPAG1e isoform is a component of the cytoplasmic plaque of hemidesmosomes, which mediate the adhesion of epithelial cells to the extracellular matrix in an  $\alpha_6\beta_4$ -integrin-dependent fashion. In a manner similar to the role of desmoplakin, BPAG1e plays a crucial role in anchoring keratin IFs to the hemidesmosomal plaque<sup>30</sup>. Other BPAG1 isoforms, designated n1, n2 and n3 occur in subsets of neurons, where they

**BOX 1 – A PRIMER ON THE BASIC STRUCTURAL FEATURES OF CYTOPLASMIC INTERMEDIATE FILAMENT POLYMERS**

The signature element of cytoplasmic intermediate filament (IF) proteins is a centrally located domain of ~310 amino acids that features long-range heptad repeats of hydrophobic residues (abcdefg, where positions *a* and *d* are often occupied by apolar amino acids). Not only does this central domain foster the formation of parallel coiled-coil dimers, the first step towards assembly, but it also represents the major driving force that sustains the entire polymerization reaction<sup>2,6</sup>. The rod domain is flanked by nonhelical sequences at both ends – the N-terminal head and C-terminal tail domains. The size and amino acid sequence of these domains, which are poised to play major roles in the functional specialization and regulation of IF polymers, vary extensively among IF proteins.

Early on, it was discovered that keratin IFs and neurofilaments are copolymers of type I and type II keratins and NF-L, -M and -H, respectively, whereas type III IF proteins such as vimentin and desmin can homopolymerize into IFs both *in vitro* and *in vivo*. Recent contributions from many sources, however, suggest that most IF proteins are part of heteromeric filaments *in vivo* (see Ref. 6 for review). The biochemical basis and functional relevance of heteropolymerization in either an obligatory or facultative fashion are not known but probably are key to the role(s) and regulation of IF polymers in their natural setting.

The structural features of purified IFs can be likened to a microscopic version of cooked spaghetti. Individual filaments are 10–12 nm in diameter and several microns in length (Fig. 1). Unlike F-actin and microtubules, IFs do not feature an obvious polarity, and their shorter persistence length implies greater flexibility. The molecular architecture of the IF backbone is still debated, and might in fact vary depending upon IF sequence type and polymerization conditions. The prevailing model for the 'ideal' IF polymer includes 16 coiled-coil dimers equally partitioned into four intertwined protofibrils per filament cross-section<sup>6</sup>.

Purified type III IF proteins can be made to self-assemble into a complete polymer *in vitro* in the presence of physiological amounts of salt and pH. Polymerization of type I-type II keratins is peculiar, at least *in vitro*, in that it 'occurs best' in low ionic strength buffer at physiological pH, in the absence of salt<sup>66</sup>. In all instances, polymerization is highly efficient – that is, the critical concentration for polymerization is low (<1  $\mu\text{M}$ )<sup>66</sup>.

A new model accounting for cytoplasmic IF assembly has recently been proposed based upon *in vitro* studies involving purified proteins<sup>6</sup>. Unit-length filaments of 10–12 nm width and 100 nm length would assemble initially and then proceed to form IFs several microns long through end-to-end annealing (see Ref. 6 and references therein).

Relatively little is known about IF polymerization in the *in vivo* setting. As is the case *in vitro*, the fraction of non-filamentous IF proteins in the cytoplasm is very small, being <5% of the IF protein pool (as defined by centrifugation-type assays; see Ref. 42 and references therein). The steady-state dynamics displayed by cytoplasmic IFs<sup>2,42</sup> is not linked to nucleotide binding and hydrolysis and has not yet been explained by regulated interaction(s) with associated proteins, as is the case for F-actin and microtubules<sup>1</sup>. Whatever the precise mechanisms are, it most certainly involves dynamic phosphorylation, with or without modulation by associated proteins<sup>42</sup>.

mediate direct linkages between neurofilaments and either cortical F-actin or microtubules (see Ref. 31 and references therein). Plectin is also found near desmosomal and hemidesmosomal plaques, but, in part due to a wider range of interacting partners (see Table 1), it can also be found in the cytoplasm, where it is often associated with IFs<sup>23</sup>. That desmoplakin, BPAG1e and plectin play a strategic role in the function of the adhesion complexes, and in particular in the attachment of IFs at these sites, has been confirmed in studies involving the creation of null mutations in the corresponding genes in mouse<sup>29,30,32</sup>.

The functional relatedness of the cytolinker/plakin family members has its basis in a shared domain organization at the protein level. The C-terminus of family members is encoded by a single large exon and features varying numbers of a novel sequence motif. This domain contains the binding site(s) for IFs – in the case of plectin, this determinant

has been narrowed down to ~50 amino acid residues<sup>33</sup>. The N-terminal domain, on the other hand, largely determines the subcellular localization and specific function(s) of these proteins. As an example, the neuronal isoforms of BPAG1 can contain binding domains for F-actin or microtubules, depending on differential splicing of the 5'-most exons of the nascent transcript (see Ref. 31 and references therein). Again, here, relatively little is known about the sequences to which these proteins bind onto IF proteins (see Refs 34 and 35). At another level, how large the family of plakins/cytolinkers really is, and how versatile these crosslinkers might be, remains to be determined as well. Two additional members, envoplakin and periplakin, have been discovered as components of desmosomes and cornified envelopes<sup>24</sup>. A recently discovered member, ACF7, binds to both microtubules and F-actin and is expressed in the epidermis<sup>36,37</sup>. The structural and functional relatedness of ACF7 to Kakapo, a

**TABLE 1 – A LIST OF PROTEINS WITH DEMONSTRATED ROLES IN ORGANIZING IF ARRAYS IN THE CYTOPLASM<sup>a,b</sup>**

Class	Protein <sup>c</sup>	No. genes/ No. mRNAs Protein size	Major sites of expression	<i>In vivo</i> binding partners <sup>d</sup>	Subcellular localization	Refs
IF bundling proteins	Filaggrin	1/1 37 kDa	Keratinizing epithelia	Keratin IFs	Cytoplasm	19, 20
	Trichohyalin	1/1 220 kDa	Differentiating keratinocytes in hair follicle, tongue	Keratin IFs	Cytoplasm	21
Cytolinker/ plakins	Plectin	1/18 >500 kDa	Nearly all cell types	Various IFs, fodrin/spectrin, desmoplakin, integrin $\beta_4$ , actin, microtubules	Cytoplasmic (with IFs) Sub-plasma membrane Desmosomes Hemidesmosomes Focal contacts	23, 25
	Desmoplakin	1/2 250/215	Epithelia; muscle	Keratin, desmin, vimentin IFs, plakoglobin, plakophilin1, desmosomal cadherins?	Desmosomes Z-lines (muscle)	26, 29, 34, 35
	BPAG1	1/4 230–280	Epithelia; neurons	Keratin IFs, neurofilaments F-actin, microtubules	Hemidesmosomes Axonal cortex	27, 31
Actin-binding proteins	Fimbrin/Plastin	3/3 68 kDa	All replicating cells	Vimentin subunits F-actin	Cell surface specializations	40
	Calponin	3/3 28–36 kDa	Muscle and many other cell types	Desmin IFs F-actin, myosin tropomyosin, caldesmon	Colocalizes with F-actin or actomyosin	41
Others	$\alpha$ B-crystallins	1/1 each <30 kDa	Lens, muscle, astrocytes	Vimentin IFs Peripherin IFs	Cytoplasm	62, 63
	14-3-3 proteins	7/7 28–33 kDa	Isoform-dependent; several cell types	Keratin IFs	Cytoplasm; nucleus	64, 65
	Plakophilin 1	1/2 81–83 kDa	Complex epithelia	Keratin IFs	Desmosomes; nucleus	38, 39

<sup>a</sup>Abbreviations: BPAG1, bullous pemphigoid antigen 1; F-actin, filamentous actin; HSP27, heat shock protein 27 kilodaltons; IF, intermediate filament; kDa, kilodaltons.

<sup>b</sup>This list ignores the important fact that keratin IFs are covalently bound to several structural components of the cornified envelope, a specialized polymer that forms against the inner side of the plasma membrane at a late stage of epidermal keratinocyte differentiation.

<sup>c</sup>An excellent source of general information about many of these proteins is provided in a book edited by T. Kreis and R. Vale (Ref. 22).

<sup>d</sup>Some of the interacting partners listed interact with specific isoforms.

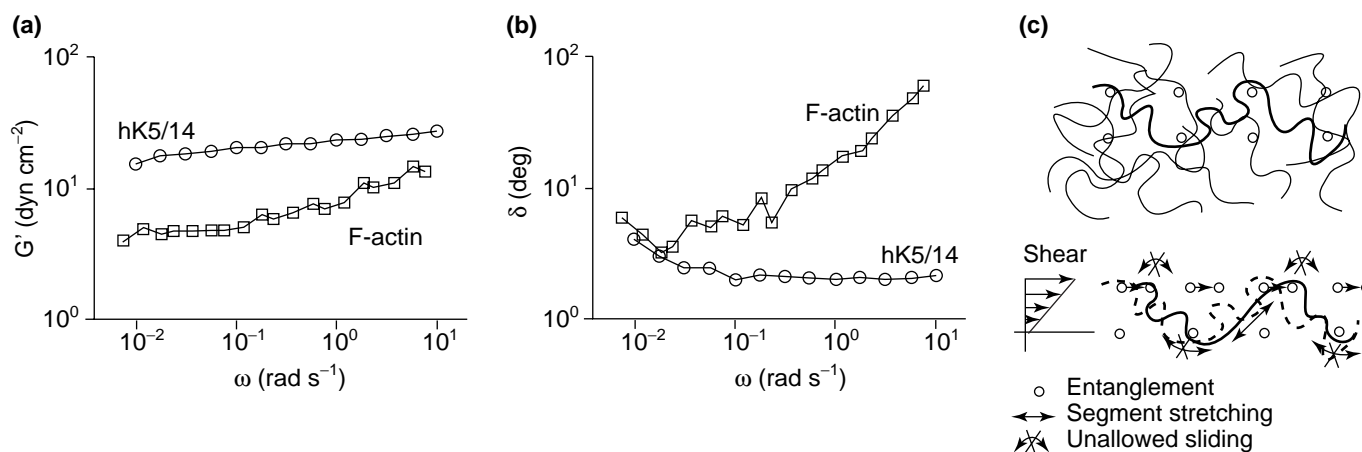
*Drosophila* protein, places the appearance of this protein family at an interestingly early stage of metazoan evolution (see Ref. 27 for review).

### Other proteins link IFs to adhesion complexes and other cytoskeletal elements

Like plakins/cytolinker proteins, most other known IF-associated proteins listed in Table 1 are at least bivalent with regards to the types of cytoskeletal elements they interact with. Like desmoplakin, plakophilin 1<sup>38,39</sup> is a structural constituent of desmosomes and binds to keratin IFs directly (and to vimentin, albeit to a lesser extent). Two classical F-actin binding proteins, fimbrin/plastin<sup>40</sup> and calponin<sup>41</sup>, have recently been shown to bind to vimentin and/or desmin *in vivo* and *in vitro*. Additional IF-associated proteins, while not involved in an obvious fashion in the organization of cytoplasmic IFs, are just as interesting for a different set of reasons. This group includes several isoforms of protein kinase C (see Ref. 42 for review), the

70-kDa heat shock proteins<sup>43</sup>, bystin<sup>44</sup> and tumour necrosis factor receptors<sup>18</sup>. The functional significance of the association of these proteins with cytoplasmic IFs has not yet been established, although many of them point to an involvement in modulating signalling events.

An emerging theme and perhaps a typifying feature, therefore, is that cytoplasmic IFs frequently rely on interactions with other elements of the cytoskeleton for their organization. This principle extends exciting recent studies showing that the peripheral extension of certain types of cytoplasmic IFs depends upon an interaction with a kinesin-type, microtubule-dependent motor (e.g. Refs 45 and 46). The latter finding substantiates an observation, made over thirty years ago by Holtzer and colleagues<sup>47</sup>, that colcemid treatment of myoblasts collapses IFs in addition to microtubules. Given that the appearance of IFs during evolution postdates that of F-actin, microtubules and at least some types of cell–cell adhesion complexes, it seems logical that



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**FIGURE 2**

Comparing the rheological properties of suspensions of F-actin and keratin IFs *in vitro*. (a, b) Frequency dependence of the elastic modulus  $G'$  (a) and phase angle  $\delta$  (b) for suspensions of rabbit skeletal muscle F-actin and human K5–K14 filaments. Each sample was tested at  $1 \text{ mg ml}^{-1}$ . In this experiment, the amplitude of the oscillatory strain was fixed at  $\gamma = 1\%$ , and sweeping was done from low to high frequency  $\omega$ . The keratin sample is significantly stiffer than F-actin, as evidenced by a higher  $G'$  value, a lower phase angle, and a lesser dependence upon the frequency of the input strain. (c) Model of keratin rheology. At equilibrium, flexible keratin filaments exhibit fluctuations between entanglements formed by neighbouring filaments (top). After a shear deformation, entanglements are moved and some of the segments between entanglements stretch (bottom). However, owing to crosslinking interactions at entanglement points that prevent sliding of filaments past one another, the filaments resist further deformation, which causes strain-hardening.

IF proteins took advantage of already existing constituents of the cell to evolve an organization that best suits their function(s).

**IF organization in the cytoplasm: emerging evidence for intrinsic determinants**

There is accumulating evidence pointing to an important role for IF proteins in fostering their own organization in the cytoplasm. Recent studies focusing on unusual IF proteins and on the mechanical properties of IF suspensions *in vitro* provide additional support for this interesting notion.

A subset of ‘odd’ cytoplasmic IF proteins, including nestin, synemin, paranemin, NF-H and tanabin, feature an unusually long nonhelical tail domain at their C-termini. As it turns out, many of these proteins depend upon copolymerization with other IF proteins for integration into filaments. For NF-H (the heavy subunit of neurofilaments) and synemin, strong evidence supports a role for their long nonhelical tail domain in organizing the IFs they are part of. The tail domain of NF-H, which at 661 amino acid residues is 4.5-fold longer than that of NF-L (the ‘lighter’ neurofilament subunit and major constituent of the NF polymer), projects away from the filament surface in a phosphorylation-dependent fashion and contributes to the interfilament spacing in the axoplasm through a steric exclusion mechanism<sup>15</sup>. As a result, the NF-H content of neurofilaments influences the radial growth of axons, and axonal calibre in turn directly impacts conduction velocity<sup>15</sup>. In the case of synemin, a protein discovered in muscle more than 20 years ago, a recent study showed that its 1290-residue tail domain features binding sites for desmin, with which it can readily copolymerize to form IFs, and for the F-actin bundling protein  $\alpha$ -actinin<sup>48</sup>. These findings implicate synemin as a potential integrator of the vimentin/desmin

and F-actin polymers at the Z-lines of differentiated muscle cells. Synemin and NF-H thus represent examples of IF proteins that are an intrinsic part of the IF core polymer and which, through the presence of determinants located in their tail domain, profoundly influence IF organization in the cytoplasm. Nestin and paranemin, whose nonhelical tail domains are 1306 and 1282 residues, respectively, possibly belong to this group as well (see Refs 7 and 49 for interesting findings along these lines).

**Rheological studies provide insights into interactions between IF polymers in solution**

Another line of experimental evidence supporting the concept of direct IF–IF interactions comes from an entirely different source, that is, rheological studies. The mechanical properties of several types of cytoplasmic IFs in solution including vimentin<sup>50,51</sup>, neurofilaments<sup>52</sup> and keratin<sup>51</sup>, are typical of viscoelastic solids, a hardly surprising notion. In contrast to F-actin (see Fig. 2 and Boxes 2 and 3) and microtubules, however, the elastic properties of keratin and vimentin IF suspensions cannot be entirely explained by steric forces, that is, forces that arise from mechanical interactions between overlapping/entangled polymers in solution<sup>51</sup>. Quite remarkably, in fact, the properties of keratin IF suspensions are best related to those displayed by F-actin in the presence of exogenous crosslinker proteins (see Box 2 and Fig. 2). This notion is particularly satisfying given the important role of mechanical scaffolding that has so far been demonstrated for keratins and a few other cytoplasmic IF polymers *in vivo*. These rheological findings raise the possibility that the ability of IFs to self-interact represents a general property of this type of fibrous polymer and not an exception restricted to a few members of the family featuring exceptionally long nonhelical end domains.

**BOX 2 – COMPARING THE RHEOLOGICAL PROPERTIES OF F-ACTIN AND KERATIN INTERMEDIATE FILAMENT SUSPENSIONS**

When tested by rheological methods *in vitro*, suspensions of pure keratin intermediate filaments (IFs) display a higher visco-elastic modulus and a lower phase angle than suspensions of F-actin at the same protein concentration (Fig. 2 and Table I; see also Box 3 for a glossary of rheological terms). This implies that, relative to F-actin, keratin IFs form a stiffer, more solid-like gel. Likewise, the visco-elastic modulus of keratin IF suspensions is less dependent upon the (i) frequency of the deformation applied (Fig. 2); (ii) polymer concentration; and (iii) length of individual polymers. Both keratin IF and F-actin suspensions yield (i.e. 'break') relatively rapidly when subjected to progressively larger deformations. While F-actin recovers its original properties several hours after cessation of the input strain, keratin IF suspensions recover their virtually immediately (see Refs 51 and 67 and references therein).

**TABLE I – COMPARISON OF MECHANICAL PROPERTIES OF F-ACTIN AND KERATIN INTERMEDIATE FILAMENTS**

Property	F-actin	Keratin
Elastic modulus $G'$ (dynes $\text{cm}^{-2}$ ) <sup>a,b</sup>	≈ 10	≈ 40–60
Phase angle $\delta$ (degrees) <sup>c</sup>	≈ 20–40	≈ 8–10
Dependence of $G'$ upon		
(a) Frequency of deformation	Significant	Weak
(b) Polymer concentration	$G \sim C^{1.4}$	$G \sim C^{0.5}$
(c) Individual polymer length	Significant	Weak
Strain hardening	No	Yes
Recovery after yield	Hours	Immediate

<sup>a</sup>See Box 3 for a definition of many of these rheological terms.

<sup>b</sup>The magnitude of  $G'$  is directly proportional to the stiffness of a material.

<sup>c</sup>A phase angle of 90° is characteristic of viscous liquids (e.g. water), whereas solids display a phase angle of 0° (see Box 3).

What do such differences mean? The dynamical behaviour of a network is related directly to the propensity of its constitutive filaments to move<sup>68</sup>. If motion of the filaments is allowed, an applied deformation will generate a stress that can completely relax: this is the case of pure F-actin networks. If the motion of the filaments is disallowed owing to strong intermolecular interactions (as illustrated in the model presented in Fig. 2 c), the stress cannot relax even at long time scales: this is the case of keratin IFs<sup>51</sup> and crosslinked F-actin networks (see Ref. 69 and references therein). Recent rheological, light-scattering and microscopy studies suggest that entanglements formed by actin filaments with their closest neighbours dominate the mechanical behaviour of F-actin<sup>67</sup>. By contrast, keratin IF suspensions display little stress relaxation, which results in a higher plateau modulus. This suggests that the motion of keratin filaments is highly restricted owing to non-steric interactions (Fig. 2c and Box 3). A similar behaviour is observed for F-actin- $\alpha$ -actinin networks, especially at low temperatures, because of long-lived interfilament interactions mediated by the long lifetime of binding of  $\alpha$ -actinin to F-actin<sup>69</sup>. Crosslinked F-actin networks and keratin IF suspensions exhibit a plateau modulus that depends weakly on concentration, which suggests that steric interactions (i.e. topological constraints) play a minor role. Non-steric interactions are probably responsible for the strain-hardening behaviour (i.e. an increase of the modulus with strain amplitude) exhibited by both keratin IFs<sup>51</sup> and crosslinked F-actin but not F-actin alone (Y. Tseng and D. Wirtz, unpublished).

The notion that IF polymers can self-interact, possibly through determinants localized in their non-helical end domains, is hardly new. For instance, a plethora of studies in which the nonhelical end domains of these proteins was altered through mutagenesis has collectively revealed their potential involvement in filament-filament interactions. *In vitro* and/or *in vivo* evidence to that effect exists for keratin-, vimentin-, desmin-, neurofilament- and  $\alpha$ -internexin-containing polymers<sup>2</sup>. Additional lines of evidence suggest that the end-domains are accessible at the surface of polymerized IFs and thus are available to participate in these interactions. For instance, the end domains are preferentially 'clipped off' when pre-assembled IFs are subjected to limited

proteolysis. Moreover, the non- $\alpha$ -helical end domains of virtually all IF proteins (and not the central rod domain) are subjected to a variety of post-translational modifications<sup>42</sup>, implying that they are exposed to the relevant enzymatic effectors. In particular, phosphorylation of IF proteins occurs in a site-specific and highly regulated manner and affects several aspects of IF polymer structure and organization<sup>42,53</sup>. Finally, the C-terminal tail domain of the type I keratin K14, which is not required for its copolymerization with a type II keratin partner<sup>54,55</sup>, makes a significant contribution to the elastic properties of keratin IF suspensions *in vitro* (O. Bousquet, S. Yamada and P.A. Coulombe, unpublished). Likewise, the C-terminal tail domain of

**BOX 3 – A GLOSSARY OF RHEOLOGICAL TERMS**

**Rheology** – Derived from the Greek word *rheos*, which means ‘to flow’. Rheology is concerned with the flow properties of materials, and attempts to establish the relationship between forces and deformation in materials.

**Input strain** – Strain (deformation) is often applied as a sinusoidal oscillation of frequency  $\omega$  and strain amplitude  $\gamma$ , and the resulting stress incurred within the material is measured as the output.

**Elastic modulus,  $G'$**  – Elastic response of a material to a deformation.  $G'$  is directly related to the fraction of the input energy (deformation) that is stored within the material. The units of  $G'$  are expressed in Pascal or dynes  $\text{cm}^{-2}$ .

**Loss modulus,  $G''$**  – Viscous response of a material to a deformation, which originates from the dissipation of the input energy within the material. The units of  $G''$  are also expressed in Pascal or dynes  $\text{cm}^{-2}$ .

**Phase angle,  $\delta$**  – Delay in the material response owing to energy dissipation.  $\delta$  is formally related to  $G'$  and  $G''$  [ $\delta = \arctan(G''/G')$ ] and is expressed in degrees. The stress response of elastic solids is perfectly in-phase with the imposed strain (deformation), and thus  $\delta = 0^\circ$ . The stress response of viscous liquids is out of phase with the strain, and  $\delta = 90^\circ$ .

**Plateau modulus** – A regime of moduli ( $G'$ ,  $G''$ ) that remains (relatively) constant over a range of frequency at a given strain.

**Strain hardening** – Increase in elasticity in response to strain. Strain hardening is a characteristic of crosslinked networks (e.g. rubber).

**Steric vs nonsteric forces** – Steric forces generate elasticity owing to entanglements between individual polymers within a network or suspension. Nonsteric forces stem from chemical interactions between individual polymers and promote the formation of an interconnected network.

vimentin has been shown to modulate the elastic properties of vimentin IF suspensions *in vitro*<sup>56</sup>. Studies are needed to explore further the biochemical basis and functional significance of this potential for self-interaction.

**The diversity of IF sequences might relate to the modulation of IF organization**

Whether obligatory or facultative (see Box 1), the coassembly of different IF proteins within the IF core polymer represents a simple way to tailor IF-IF interactions and thus IF organization in the cytoplasm. Several examples support this idea. In the epidermis of skin, a dramatic enhancement of bundling of keratin IFs occurs as early-differentiating keratinocytes begin to synthesize the high-molecular-weight keratin pair K1 and K10<sup>57</sup>, a phenomenon that can be reproduced using purified proteins *in vitro*<sup>58</sup>. The pancytoplasmic organization of K1-K10-rich filaments is disrupted in wound-edge keratinocytes whenever the epidermis withstands acute injury, an event that is concomitant with the rapid induction and accumulation of keratins K6, K16 and K17 at the expense of K1 and

K10<sup>59</sup>. The progressive replacement of vimentin by desmin, two related type III IF sequences, accompanying cell differentiation within striated muscle is paralleled by a dramatic reorganization of the IF network from a pancytoplasmic distribution to a regional concentration towards Z-lines and sites of myofibril-membrane attachment (see Ref. 60 and references therein). Yet another example is found in astrocytes, in which the organization of the IF array and IF-IF relationships are influenced by the content in three distinct IF proteins – nestin, GFAP and vimentin<sup>61</sup>. It is possible to conjecture, therefore, that the need for various configurations of cytoplasmic IF arrays depending on the cell's agenda might have contributed to the evolutionary forces that led to the duplication and diversification of IF sequences.

**Regulating self-interactions among IF polymers**

Given the abundancy and cytoplasm-wide distribution of IFs in many cell types (see Fig. 1), problems would obviously arise if such filament-filament interactions were allowed to take place in an unrestricted and unregulated fashion. Several mechanisms possibly contribute to ensure that this is not the case. First, the filament-filament interactions involving IF protein subdomains, whatever they are, are likely to be of low affinity and stereospecific, in that they might be favoured at a post-rather than a pre-assembly stage. Second, through its ability to locally alter the properties of target sequences, phosphorylation and other modifications are poised to regulate both IF-IF and IF-IF-associated protein interactions. Third, interactions between IFs and non-IF proteins probably impact on these interactions as well. In support of this, Perng *et al.*<sup>62</sup> showed that the binding of  $\alpha$ B-crystallins/HSP27 to vimentin filaments *in vitro* effects a gel-sol transition, suggesting that these small heat-shock proteins might antagonize filament-filament interactions in solution (see also Ref. 63). Likewise, the phosphorylation-dependent binding of 14-3-3 proteins to K8/K18 filaments<sup>64,65</sup> could alter IF-IF interactions as well. Altogether, therefore, there is a genuine potential for a variety of mechanisms that could effectively regulate these ‘interactions from within’.

**Conclusion and future prospects**

The mechanisms governing the organization of cytoplasmic IF polymers represent yet another trait that distinguish them from F-actin and microtubules. Of significant interest, no crossbridging protein that is widely distributed and specific for IF polymers has been described to date. Cytoplasmic IF polymers appear to rely on a fascinating combination of multivalent crosslinkers and intrinsic determinants to adopt the configuration that best serves their function(s).

The challenge of understanding these interactions at a biochemical and structural level lies ahead of us. Deciphering the IF protein domain(s) and type of interaction(s) involved stands out as a pressing issue. With this information in hand, experiments

can be designed to, first, test for the functional importance of interactions involving IF polymers and other proteins, or self-interactions, and, second, characterize their regulation *in vivo*. Such knowledge might also help further our understanding of the functional significance of the remarkable diversity and tissue-specific regulation that typifies IF sequences. This, along with the atomic structure of the IF polymer, arguably represents the Holy Grail in this field of research.

With regards to the remarkable micromechanical properties of keratin IFs, two open issues need our particular attention. The first one concerns the behaviour of IFs as they are subjected to strain. What happens as IF suspensions 'yield' to the applied strain? And what accounts for the ability of IFs to recover their original properties upon cessation of the strain? The second one consists of the impact of naturally occurring mutations on the micromechanical properties of IF assemblies. This latter issue speaks directly to the mechanistic basis for the cell fragility observed in the context of keratinopathies and other IF disorders, an important topic that has yet to receive adequate attention.

## References

- 1 Alberts, B. *et al.* (1994) *Molecular Biology of the Cell* (3rd edn), Garland Publishing
- 2 Fuchs, E. and Weber, K. (1994) Intermediate Filaments: Structure, Dynamics, Function, and Disease. *Annu. Rev. Biochem.* 63, 345–382
- 3 Markl, J. and Schechter, N. (1998) in *Subcellular Biochemistry: Intermediate Filaments* (Harris, J.R. and Herrmann, H., eds), pp. 1–34, Plenum Publishing
- 4 Erber, A. *et al.* (1998) Molecular phylogeny of metazoan intermediate filament proteins. *J. Mol. Evol.* 47, 751–762
- 5 Stuurman, N. *et al.* (1998) Nuclear lamins: Their structure, assembly, and interactions. *J. Struct. Biol.* 122, 42–66
- 6 Herrmann, H. and Aebi, U. (2000) Intermediate filaments and their associates: Multi-talented structural elements specifying cytoarchitecture and cytodynamics. *Curr. Opin. Cell Biol.* 12, 79–90
- 7 Steinert, P.M. *et al.* (1999) A high molecular weight intermediate associated protein in BHK-21 cells is nestin, a type VI intermediate filament protein. *J. Biol. Chem.* 274, 9881–9890
- 8 Georgatos, S.D. *et al.* (1997) To bead or not to bead? Lens-specific intermediate filaments revisited. *J. Cell Sci.* 110, 2629–2634
- 9 Venetianer, A. *et al.* (1983) Cessation of cytokeratin expression in a rat hepatoma cell line lacking differentiated functions. *Nature* 305, 730–733
- 10 Colucci-Guyon, E. *et al.* (1994) Mice lacking vimentin develop and reproduce without an obvious phenotype. *Cell* 79, 679–694
- 11 Magin, T.M. (1998) in *Subcellular Biochemistry: Intermediate Filaments* (Harris, J.R. and Herrmann, H., eds), pp. 141–165, Plenum Publishing
- 12 Takahashi, K. *et al.* (1999) Using transgenic models to study the pathogenesis of keratin-based inherited diseases. *J. Dermatol. Sci.* 21, 73–95
- 13 Fuchs, E. and Cleveland, D.W. (1998) A structural scaffolding of intermediate filaments in health and disease. *Science* 279, 514–519
- 14 Irvine, A.D. and McLean, W.H.I. (1999) Human keratin diseases: The increasing spectrum of disease and subtlety of the phenotype–genotype correlation. *Br. J. Dermatol.* 140, 815–828
- 15 Lee, M.K. and Cleveland, D.W. (1996) Neuronal intermediate filaments. *Annu. Rev. Neurosci.* 19, 187–217
- 16 Gillard, B. K., *et al.* (1998) Decreased synthesis of glycosphingolipids in cells lacking vimentin intermediate filaments. *Exp. Cell Res.* 242, 561–572
- 17 Ku, N.O. *et al.* (1998) Mutation of a major phosphorylation site predisposes to hepatotoxic injury in transgenic mice. *J. Cell Biol.* 143, 2023–2032
- 18 Caulin, C. *et al.* (2000) Keratin-dependent, epithelial resistance to tumor necrosis factor-induced apoptosis. *J. Cell Biol.* 149, 17–22
- 19 Mack, J.W. *et al.* (1993) The mechanism of interaction of filaggrin with intermediate filaments. The ionic zipper hypothesis. *J. Mol. Biol.* 232, 50–66
- 20 Dale, B.A. *et al.* (1978) Assembly of stratum corneum basic protein and keratin filaments in macrofibrils. *Nature* 276, 729–731
- 21 Lee, S.C. *et al.* (1993) The structure of human trichohyalin. Potential multiple roles as a functional EF-hand-like calcium-binding protein, a cornified cell envelope precursor, and an intermediate filament-associated (cross-linking) protein. *J. Biol. Chem.* 268, 12164–12176
- 22 Kreis, T. and Vale, R. (1999) *Guidebook to Cytoskeletal and Motor Proteins*, Oxford University Press
- 23 Wiche, G. (1998) Role of Plectin in cytoskeleton organization and dynamics. *J. Cell Sci.* 111, 2477–2486
- 24 Ruhrberg, C. *et al.* (1997) Periplakin, a novel component of cornified envelopes and desmosomes that belongs to the plakin family forms complexes with envoplakin. *J. Cell Biol.* 139, 1835–1849
- 25 Fuchs, P. *et al.* (1999) Unusual 5' transcript complexity of plectin isoforms: Novel tissue-specific exons modulate actin binding activity. *Hum. Mol. Genet.* 13, 2461–2472
- 26 Green, K.J. *et al.* (1999) Analysis of the desmoplakin gene reveals striking conservation with other members of the plakin family of cytolinkers. *Exp. Dermatol.* 8, 462–470
- 27 Fuchs, E. and Yang, Y. (1999) Crossroads on cytoskeletal highways. *Cell* 98, 547–550
- 28 Stappenbeck, T.S. *et al.* (1993) Functional analysis of desmoplakin domains: specification of the interaction with keratin versus vimentin intermediate filament networks. *J. Cell Biol.* 123, 691–705
- 29 Gallicano, G.I. *et al.* (1998) Desmoplakin is required early in development for assembly of desmosomes and cytoskeletal linkage. *J. Cell Biol.* 143, 2009–2022
- 30 Guo, L. *et al.* (1995) Gene targeting of BPAG1: abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. *Cell* 81, 233–243
- 31 Yang, Y. *et al.* (1999) Integrators of the cytoskeleton that stabilize microtubules. *Cell* 98, 229–238
- 32 Andra, K. *et al.* (1997) Targeted inactivation of plectin reveals essential function in maintaining the integrity of skin, muscle, and heart cytoarchitecture. *Genes Dev.* 11, 3143–3156
- 33 Nikolic, B. *et al.* (1996) Basic amino acid residue cluster within nuclear targeting sequence motif is essential for cytoplasmic plectin–vimentin network junctions. *J. Cell Biol.* 134, 1455–1467
- 34 Kouklis, P.D. *et al.* (1994) Making a connection: Direct binding between keratin intermediate filaments and desmosomal proteins. *J. Cell Biol.* 127, 1049–1060
- 35 Meng, J.-J. *et al.* (1997) Two-hybrid analysis reveals fundamental differences in direct interactions between desmoplakin and cell-type-specific intermediate filaments. *J. Biol. Chem.* 272, 21495–21503
- 36 Leung, C.L. *et al.* (1999) Microtubule actin cross-linking factor (MACF): A hybrid of dystonin and dystrophin that can interact with the actin and microtubule cytoskeleton. *J. Cell Biol.* 147, 1275–1285
- 37 Karakesiosoglou, I. *et al.* (2000) An epidermal plakin that integrates actin and microtubule networks at cellular junctions. *J. Cell Biol.* 149, 195–208
- 38 Hoffmann, I. *et al.* (2000) Interaction of plakophilins with desmoplakin and intermediate filament proteins: an *in vitro* analysis. *J. Cell Sci.* 113, 2471–2483
- 39 Hatzfeld, M. *et al.* (2000) The function of plakophilin 1 in desmosome assembly and actin filament organization. *J. Cell Biol.* 149, 209–222
- 40 Correia, I. *et al.* (1999) Integrating the actin and vimentin cytoskeletons: Adhesion-dependent formation of fimbrin–vimentin complexes in macrophages. *J. Cell Biol.* 146, 831–842
- 41 Mabuchi, K. *et al.* (1997) Association of calponin with desmin intermediate filaments. *J. Biol. Chem.* 272, 22662–22666
- 42 Omary, M.B. *et al.* (1998) in *Subcellular Biochemistry: Intermediate Filaments* (Vol. 31) (Herrman, H. and Harris, J.R., eds), pp. 105–140, Plenum Press
- 43 Liao, J. *et al.* (1995) The 70-kDa heat shock proteins associate with glandular intermediate filaments in an ATP-dependent manner. *J. Biol. Chem.* 270, 915–922
- 44 Suzuki, N. *et al.* (1998) A cytoplasmic protein bystin, interacts with troponin, tasin, and cytokeratin and may be involved in troponin-mediated cell adhesion between trophoblast and endometrial epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5027–5032
- 45 Prahlad, V. *et al.* (1998) Rapid movements of vimentin on microtubule tracks: Kinesin-dependent assembly of intermediate filament networks. *J. Cell Biol.* 143, 159–170
- 46 Kreitzer, G. *et al.* (1999) Detyrosination of tubulin regulates the interaction of intermediate filaments with microtubules *in vivo* via a kinesin-dependent mechanism. *Mol. Biol. Cell* 10, 1105–1118
- 47 Ishikawa, H. *et al.* (1968) Mitosis and intermediate-sized filaments in developing skeletal muscle. *J. Cell Biol.* 38, 538–555

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- 48 Bellin, R.M. *et al.* (1999) Molecular characteristics and interactions of the intermediate filament protein synemin. *J. Biol. Chem.* 274, 29493–29499
- 49 Hemken, P.M. *et al.* (1997) Molecular characteristics of the novel intermediate filament protein paranemin. *J. Biol. Chem.* 272, 32489–32499
- 50 Janmey, P.A. *et al.* (1991) Viscoelastic properties of vimentin compared with other filamentous biopolymer networks. *J. Cell Biol.* 113, 155–160
- 51 Ma, L. *et al.* (1999) Keratin filament suspensions show unique micromechanical properties. *J. Biol. Chem.* 274, 19145–19151
- 52 Leterrier, J.F. *et al.* (1996) Mechanical effects of neurofilament cross-bridges. Modulation by phosphorylation, lipids, and interactions with F-actin. *J. Biol. Chem.* 271, 15687–15694
- 53 Inagaki, M. *et al.* (1996) Dynamic properties of intermediate filaments: regulation by phosphorylation. *BioEssays* 18, 481–487
- 54 Albers, K. and Fuchs, E. (1987) The expression of mutant epidermal keratin cDNAs transfected in simple epithelial and squamous cell carcinoma lines. *J. Cell Biol.* 105, 791–806
- 55 Coulombe, P.A. *et al.* (1990) Deletions in epidermal keratins leading to alterations in filament organization *in vivo* and in intermediate filament assembly *in vitro*. *J. Cell Biol.* 111, 3049–3064
- 56 Rogers, K.R. *et al.* (1995) Truncation mutagenesis of the non-alpha-helical carboxyterminal tail domain of vimentin reveals contributions to cellular localization but not to filament assembly. *Eur. J. Cell Biol.* 66, 136–150
- 57 Coulombe, P.A. *et al.* (1989) Expression of keratin K14 in the epidermis and hair follicle: insights into complex programs of differentiation. *J. Cell Biol.* 109, 2295–2312
- 58 Eichner, R. *et al.* (1986) The role of keratin subfamilies and keratin pairs in the formation of human epidermal intermediate filaments. *J. Cell Biol.* 102, 1767–1777
- 59 Paladini, R.D. *et al.* (1996) Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin 16. *J. Cell Biol.* 132, 381–397
- 60 Cary, R.B. and Klymkowsky, M.W. (1995) Disruption of intermediate filament organization leads to structural defects at the intersomite junction in *Xenopus* myotomal muscle. *Development* 121, 1041–1052
- 61 Eliasson, C. *et al.* (1999) Intermediate filament protein partnership in astrocytes. *J. Biol. Chem.* 274, 23996–24006
- 62 Perng, M.D. *et al.* (1999) Intermediate filament interactions can be altered by HSP27 and  $\alpha$ B-crystallins. *J. Cell Sci.* 112, 2099–2112
- 63 Djabali, K. *et al.* (1997)  $\alpha$ B-crystallin interacts with intermediate filaments in response to stress. *J. Cell Sci.* 110, 2759–2769
- 64 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
- 65 Ku, N.O. *et al.* (1998) Phosphorylation of human keratin 18 serine 33 regulates binding to 14-3-3 proteins. *EMBO J.* 17, 1892–1906
- 66 Steinert, P.M. *et al.* (1976) Self-assembly of bovine epidermal keratin filaments *in vitro*. *J. Mol. Biol.* 108, 547–567
- 67 Palmer, A. *et al.* (1999) Diffusing wave spectroscopy microrheology of actin filament networks. *Biophys. J.* 76, 1063–1071
- 68 Doi, M. and Edwards, S.F. (1989) *The Theory of Polymer Dynamics*, Clarendon Press
- 69 Xu, J. *et al.* (1998) Dynamic cross-linking by alpha-actinin determines the mechanical properties of actin filament networks. *J. Biol. Chem.* 273, 9570–9576

**Author correction**

The authors of the August 2000 review 'Calmodulin: a prototypical calcium receptor' (*Trends Cell Biol.* 10, 322–328) wish to correct some errors in their legend to Figure 3 on p. 324. The figure is actually three views of a metaphase spindle from the sea urchin *Lytechinus variegatus*.

Panel (a) is a polarization micrograph showing contrast by the spindle and astral fibre birefringence; panel (b) is a phase-contrast micrograph of the same spindle; and panel (c) is an immunofluorescence micrograph showing the distribution of calmodulin. The images were kindly provided by Edward D. Salmon, UNC, Chapel Hill, NC, USA. The authors apologize to readers for any confusion caused by the wording in this figure legend.

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**Author correction**

The authors of the September 2000 review 'Signalling by semaphorin receptors: cell guidance and beyond' (*Trends Cell Biol.* 10, 377–383) regretfully acknowledge that the text of the article contains a typing error on page 382. The first column, 4th line, should read: 'Npn-2 as a *Sema3F* receptor', instead of '*Sema4F* receptor' as erroneously stated. The authors apologize to readers for any confusion that this mistake might have created.

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