Beyond structure: do intermediate filaments modulate cell signalling?

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Summary

Intermediate filament (IF) proteins form the largest family of cytoskeletal proteins in mammalian cells. The function of these proteins has long been thought to be only structural. However, this single function does not explain their diverse tissue- and differentiation-specific expression patterns. Evidence is now emerging that IF also act as an important framework for the modulation and control of essential cell processes, in particular, signal transduction events. Here, we review the most recent developments in this growing and exciting new field. *BioEssays* 24:836–844, 2002. © 2002 Wiley Periodicals, Inc.

Introduction: the largest family in the cytoskeleton

Most molecular biologists consider the cytoplasm of the cells as a ''biological bag'' where important molecules are floating and interact with one another. However, careful inspection of this ''bag'' under the electron microscope (Fig. 1) reveals an astonishing net of fibrous proteins throughout the cytoplasm. This cytoskeleton is composed of three different classes of proteins: microfilaments of actin, microtubules formed by tubulins, and a third class denoted intermediate filaments (IF) due to their size (Fig. 1). This IF network comprises different proteins in the different cells of the body (for reviews see Refs 1–5). Collectively, the IF proteins are the largest family of cytoskeletal proteins and share a common structure (Fig. 2A). Each protein has a long central domain, which is principally α-helical and forms a coiled coil with a similar polypeptide. This domain, which is the building block of the IF, is subdivided into four blocks of helical structure (Fig. 2A boxes 1A, 1B, 2A and 2B) by short linker domains (Fig. 2A, L1, L12, L2), which are non-helical. Finally, non-helical terminal domains at the N terminus (head) and at the C terminus (tail) (Fig. 2A) flank the rod domain. These domains are highly variable in sequence and have been postulated to protrude from the core of the filaments and are assumed to specify the possible polypeptide-specific functions. Based on gene structure, sequence homology and immunological and/or assembly properties, they can be divided into six categories. With the clear exception of type V, the lamins, they all form cytoplasmic IF arrays. Types I and II include the epithelial IF proteins called cytokeratins or keratins and comprise more than 20 different polypeptides (35 if the keratins characteristic of hair and nail are included). Type III includes vimentin (expressed in cells of mesenchymal origin), desmin (characteristic of muscle cells), GFAP (in glial cells) and peripherin (in the peripheral nervous system). Type IV IF proteins are found in neurones and include the neurofilament proteins (NF-L, NF-M and NF-H) and α-internexin. The VI class may include, depending on different group criteria, nestin, synemin, paranemin and tanabin.

In the following sections of this review, we summarise new data that suggest possible roles for IF proteins in cell signalling. However due to length limitations, we will focus only on cytoplasmic, non-neuronal IF proteins, i.e., types I, II and III proteins. Those interested in the properties and functions of lamins and neurofilaments (type V and IV proteins, respectively) are referred to recent extensive reviews (see Refs. 6–15).

With respect to the functions of IF proteins in cells, data obtained in transgenic mice and genetic analyses of human hereditary syndromes in the early nineties, clearly demonstrated that IF proteins provide cells with mechanical resilience against physical stress (reviewed in Refs. 1, 16–23). However, can this function alone explain the wide diversity of this family of proteins and their elegant and complex tissue- and differentiation-specific expression patterns? The answer to this question could be positive: different IF proteins display subtle structural differences, in assembly for instance, so that they can fulfil tissue-specific protective functions (for a detailed

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Abbreviations: GFAP, glial fibrillary acidic protein; NF, neurofilaments; Vim, vimentin; TNF, Tumour necrosis factor; TGFβ, Tumor growth factor β; JNK, Jun kinase; PKC, protein kinase C; Rb, retinoblastoma; PI-3K, phophoinositide 3-kinase.

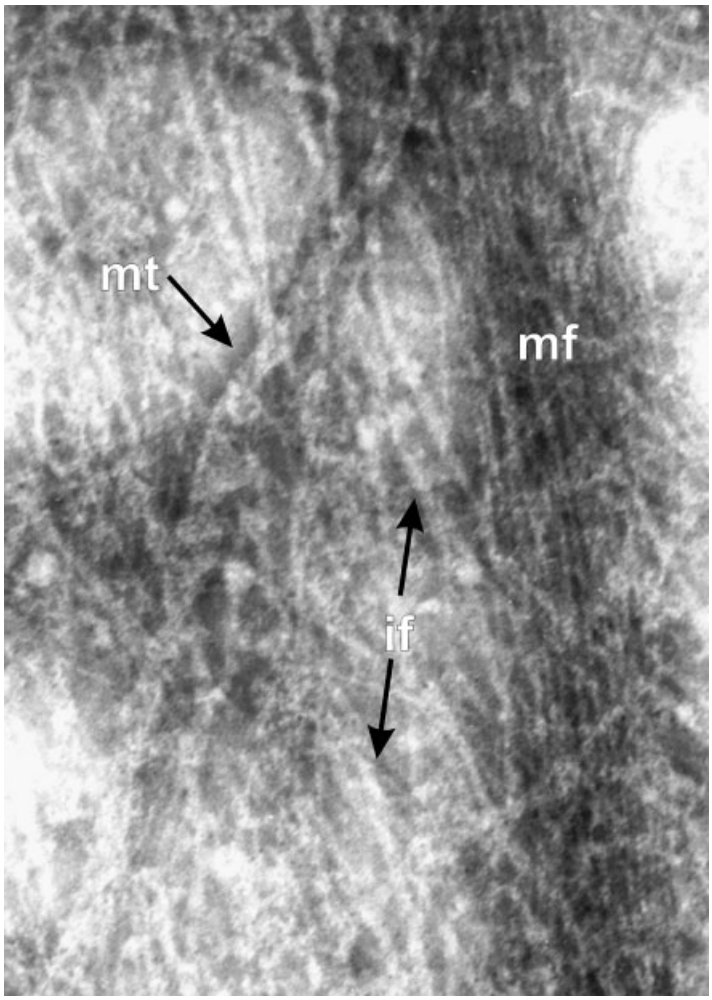

Figure 1. Whole-mount electron micrographs of the cytoplasm of a mammalian cell showing the highly ordered filamentous structures that form the cell cytoskeleton. Three main components can be discerned: microtubules (mt), microfilaments (mf) and intermediate filaments (if). Whereas the roles of mt and mf in cell signalling are well known, evidence that IF are also involved in these processes is just emerging. The amount and distribution of these proteins in cells makes them suitable candidates for compartmentalising and perhaps controlling molecular events.

discussion, see Ref. 2). But it is also possible that these cell-type-specific functions might also be related to other important roles in the cell. This long-standing hypothesis was based on the observations that cell shape, and so the cytoskeleton, is an important sensor and competent signal transducer,[24–26] and also was prompted by the appearance of the IF cytoskeleton (Fig. 2B). In fact, the IF cytoskeleton connects membrane and nucleus throughout the cell in a manner reminiscent of a network that regulates protein localisation and the transmission of signals (Fig. 2B), and displays, in many cases, tight interaction with the nuclear lamina and the nuclear cytoskeleton (Fig. 2C).

The high insolubility of IF proteins in physiological buffers, which has led them to be considered the most static structures of the cytoskeleton, however, there is recent evidence for a highly dynamic role in living cells. Moreover, the dynamic behaviour IF proteins is modulated through the interaction with a large number of proteins (reviewed in Refs 1–5,16,27). More importantly, many of these molecules belong to the so-called signalling intermediates. In this regard, the interaction of vimentin with Cdc42 and Rac1,[28] and phopholipase A2,[29] and the interaction of keratins with different protein kinases and phosphatases (reviewed in Ref. 30) and also with the 14-3-3 family of proteins in a phosphorylation-dependent manner have all been reported.[31–32]

Therefore it has been clearly demonstrated that the dynamic behaviour of IF proteins is controlled by a number of molecules that are themselves involved in the process of signal transduction. These interactions lead to reorganisation, solubilisation or the collapse of the IF cytoskeleton (Fig. 3). However, these interactions can also alter the properties of these signalling intermediates, and in particular can affect their cellular distribution, facilitating or precluding their translocation to other cell compartments where they can act on other molecules (Fig. 3). In this review, we focus on emerging data, derived form biochemical and transgenic approaches, that clearly point to this last possibility and indicate that some IF proteins participate in the control of cell signalling.

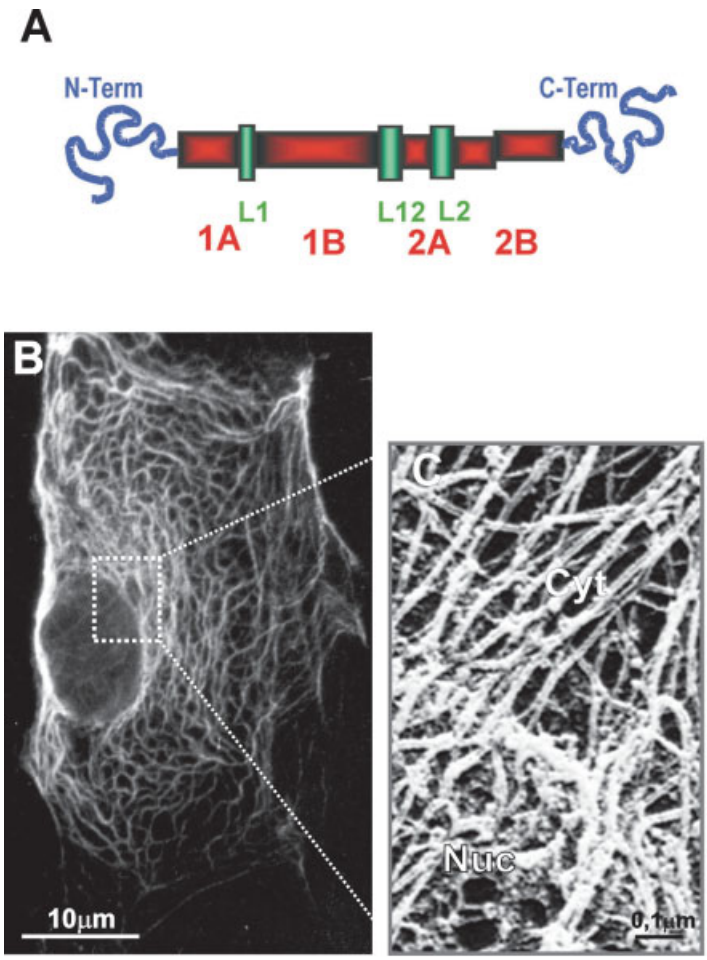

Figure 2. The IF proteins share a common structure (**A**) with a central rod domain with four α-helical domains (1A, 1B, 2A, 2B) disrupted by linker non-helical domains (L1, L12, L2), and two non-helical divergent domains at the amino and carboxy terminus. The IF cytoskeleton observed by immunofluorescence techniques (**B**) shows what appears to be a network of routes connecting the cell membrane and nucleus that might serve to canalise signal transmission. In addition, freeze-etching electron microscopy (**C**) demonstrates that IF in the cytoplasm (cyt) connect with the lamins in the nucleus (nuc). These observations have led to the hypothesis that cytoplasmic and nuclear IF may be mediators of a signalling pathway that, starting at the cell surface, leads to changes in gene expression.

Vimentin and GFAP: life without IF

Vimentin is the IF protein characteristic of mesenchymal cells, such as fibroblasts and endothelial cells (reviewed in Ref. 33). In addition, vimentin expression precedes the expression of other type III IF proteins during the differentiation and development of neural cells (later replaced by GFAP) and muscle (later replaced by desmin). This expression pattern during development suggested important functions for this protein in addition to its role as an intracellular scaffold. Initially, the structural similarities between vimentin and the DNA-binding region of certain transcription factors, such as c-fos, fra1, CREB and c-jun, led to the hypothesis of a regulatory

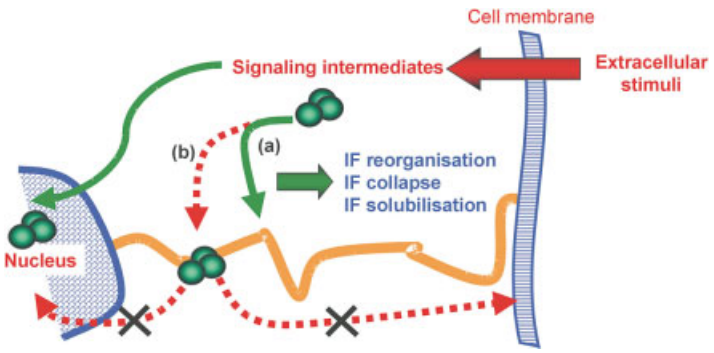

Figure 3. The IF cytoskeleton is affected by, and may participate in cell signalling. Upon extracellular stimuli determinate signalling intermediates, in particular protein kinases and phosphatases, are activated. These active molecules may interact with the IF cytoskeleton leading to different architectural alterations such as collapse, reorganisation or altered solubilisation of IF subunits. Alternatively, in some cases, the presence of specific subunits in the IF cytoskeleton, may serve as an anchoring site for these signalling intermediates and preclude their translocation to other cellular compartments.

role for vimentin.[34] Recently, it has been shown that vimentin acts as a functional perinuclear adapter for the cytosolic phospholipase A2, thus suggesting a role for the Vimentin IF in the modulation of prostaglandin biosynthesis.[29] Further, the increased nuclei density, cataract formation and impaired eye lens cell differentiation observed in transgenic mice overexpressing vimentin appeared to be in line with such a hypothesis.[35] However, a different, unexpected result was obtained when knockout mice were employed in studies.

Perhaps the most appropriate approach for elucidating protein functions involves targeted inactivation of the corresponding gene followed by analysis of the resulting phenotype in transgenic mice. Inactivation of the *vimentin* gene yielded surprising results; null animals displayed no overt anomalies in development and breeding, or in the structural and functional properties of several organs including the lens, a tissue that expresses vimentin as the sole IF protein.[36] The only difference between $vim^{+/+}$ and $vim^{-/-}$ mice was the inability of GFAP to form a well-developed IF network in astrocytes, indicating that the assembly of this protein requires a preexisting vimentin scaffold.[37,38] Thus, the overall conclusion derived from these experiments was that IF proteins are not indispensable for the normal life of mammals, since eye lens cells were completely devoid of IF. However, further studies suggested that this was not the complete picture and demonstrated additional defects in these knockout mice. For instance, $vim^{-/-}$ fibroblasts displayed reduced motility associated with disturbed organisation of actin and focal adhesion contact proteins.[39] This leads to impaired wound healing in $vim^{-/-}$ mice.[40] Hence, as the altered proteins are clearly associated with signalling processes, vimentin may mediate, albeit in an indirect manner, signalling processes through interaction with and the organisation of actin and focal adhesion proteins. Other relevant findings in these vimentin null animals include cerebellar defects, impaired motor coordination, and necrosis of Purkinje cells,[38] also indicating that important functions are impaired in these cells of the nervous system. Finally, it has been shown that $vim^{-/-}$ mice are more susceptible to nephrectomy than their wild-type counterparts due to impaired sustained vasodilatation of the renal vascular bed.[41] This defect is attributable to altered endothelin sensitivity, reduced responsiveness to NO-dependent vasodilators, and changes in gene expression.[41] These findings indicate that vimentin modulates endothelin–nitric oxide balance, which controls blood flow in renal endothelial cells, although no alterations in tubular regeneration and differentiation of the post-ischemic kidney were observed.[42] Collectively, the detailed analysis of $vim^{-/-}$ mice shows that many physiological aspects are disturbed and suggests possible alterations in signalling processes. However, there are no clues about how lack of vimentin causes these alterations on a molecular scale.

Another relevant feature is the long-time recognised association of vimentin expression with tumoral processes. Increased vimentin expression appears to enhance the malignancy of certain tumour types such as breast carcinoma.[43,44] In contrast, the behaviour of tumorigenic BHK cells is reverted when vimentin is overexpressed, suggesting an anti-tumoral role for this protein.[45] In an attempt to elucidate the possible role of vimentin in tumorigenesis, Langa et al.,[46] employed a teratocarcinoma model using exhaustive combinations of $vim^{-/-}$ and $vim^{+/+}$ embryonic stem cells and/or recipient mice. No differences were, however, observed,[46] indicating that vimentin is irrelevant for tumorigenesis in this model. Therefore, further work is required, in particular, specific tumorigenesis experiments (such as skin or breast) in transgenic mice, in order to determine whether vimentin has a role in the growth or invasiveness of cancer cells.

GFAP is the IF protein present in mature astrocytes and provides structural stability to astrocyte processes (reviewed in Ref. 47). A switch from vimentin to GFAP expression characterises astrocyte maturation. This change, which probably reflects the distinct ability of these proteins to build an IF network in these cells,[48] may explain the above-mentioned defect in forming GFAP IF shown by $vim^{-/-}$ mice[37] as well as other findings in GFAP-null mice. Early works failed to detect significant physiological abnormalities in these mice, which, like the $vim^{-/-}$ animals, develop and reproduce with apparent normality.[49,50] However, subsequent studies demonstrated that, in a high proportion of these mutant mice, GFAP expression is required for normal white matter architecture and blood–brain barrier integrity, and that its absence leads to late-onset CNS dysmyelination.[51] This suggests that the absence of GFAP actually impairs a major astrocyte function related to the formation and maintenance of myelin. Later on, it was found that the relatively mild phenotype of GFAP-null mice is further enhanced in the absence of vimentin. At this point, it should be explained that GFAP levels in astrocytes are regulated by developmental and pathological conditions. In higher vertebrates, these cells respond in a typical manner, termed astrogliosis, to injuries, either as a result of physical or chemical trauma or certain diseases. Astrogliosis is characterised by the rapid, increased synthesis of GFAP. However, in $GFAP^{-/-}$; $vim^{-/-}$ mice, the formation of glial scars was impaired, and accompanied by bleeding.[52] While these data could be interpreted in a restrictive manner as exclusively supporting a structural function for GFAP, other results more explicitly suggest the possibility that GFAP may perform further functions in astrocytes. For example, levels of glutamine, an important metabolite in the biology of these cells, are increased in cultured $GFAP^{-/-}$ glial cells. Interestingly, this effect is specifically dependent on GFAP expression rather than on the presence of IF, since a well-developed IF cytoskeleton is formed by vimentin and nestin in the absence of GFAP in cultured astrocytes.[53] Another line of indirect

evidence, which may suggest a possible role for GFAP in cell signalling, involves changes in its expression observed in glial tumours (reviewed in Ref. 54). It has been noted that increasing astrocytic malignancy is associated with a progressive loss of GFAP expression. Closely related to this, primary GFAP$^{-/-}$ astrocytes show increased cell saturation density in culture.[55] This would appear to indicate that GFAP modulates tumour cell growth and that its reduced expression may favour tumour progression to a more malignant phenotype. In agreement with this, it has been recently demonstrated that increased GFAP expression inhibits the growth of tumours induced by subcutaneous injection of C6 glioblastoma cells in nude mice.[56] The fact that GFAP overexpression does not alter the tumorigenic behaviour of L cells of fibroblast origin in the same type of experiments[56] lends strong support to the hypothesis that GFAP is a critical regulator of tumour growth of astrocytoma. Further studies using GFAP-deficient mice will help to confirm this interesting observation.

Desmin at the heart of IF

The IF cytoskeleton of mature muscle cells is composed of desmin (reviewed in Refs. 57,58). In mature striated muscle, desmin forms a scaffold across myofibers at the Z-discs. In addition, desmin IF also connect various Z-discs and associate with mitochondria, sarcolemma and nucleus. In cardiac muscle, they also link the Z-disc to the plasma membrane at the level of intercalated discs. Two main hypotheses regarding the role of desmin in muscle cells have persisted through the years. One is clearly supported by the morphological observation of the spatial distribution of desmin in muscle cells, and suggested that desmin's basic role is to allow the normal functioning of the contractile apparatus. The other was by far more provocative, and implicated desmin in signal transduction from the membrane to the nucleus. Studies using antisense RNA approaches demonstrated that the inhibition of desmin expression in C2C12 cells blocks myoblast fusion and myotube formation.[59] These defects, related to reduced expression of myoD and myogenin, two key elements governing muscle cell differentiation and development, were rescued by re-expression of desmin.[59] In agreement, the formation of striated and smooth muscle, but not cardiac muscle, is inhibited in embryoid bodies derived from desmin null embryonic stem cells, and linked to the absence of myoD, myogenin, myf5 and myosin heavy chain expression.[60] Such defective expression of myogenic helix–loop–helix transcription factors was hypothetically attributed to the connection of desmin with nuclear lamins, and the interaction of the latter with the nuclear matrix attachment regions (MARs) of DNA (see Refs. 57 and 58 for a detailed discussion).

However, when desmin null mice were generated, these observations were not confirmed. On the contrary, these mice are viable and fertile, though they display severe disruption of muscle architecture and myocardial degeneration associated with calcification and cardiac dilation, compromising systolic function.[61–63] Hence, as the expression of other IF proteins in adult muscle cells is lacking,[61,62] these results have been interpreted as demonstrating that desmin is only required for the integrity of the myofibrils, but not for myogenic commitment, differentiation and fusion of skeletal muscle.[61,64] However, as in the case of vimentin- and GFAP-null mice, this conclusion might be too simple. As a non-exclusive alternative, desmin may act indirectly in signalling, leading to the observed alterations in muscle cell physiology. For instance, desmin-null muscles show disturbed mitochondrial positioning and distribution, which gives rise to alterations in respiratory functions in situ.[65] Although these studies collectively revealed a dominant role for desmin as a structural integrator, whether desmin can or cannot modulate gene expression or signal transduction in these cells is yet to be established (using gene arrays for example) and cannot be totally ruled out.

Keratins: enlightening the dark side of IF

Keratins form the IF of all epithelial cells and are assembled from heterodimeric subunits of acidic type I [keratins 9 (K9) to K20] and basic type II (K1 to K8) IF proteins.[1,16–19] It is remarkable that the different types of epithelial cells can be basically characterised by the specific pair(s) of keratins that they express. Simple epithelial cells express keratins K8 + K18, and in some instances K19, K17 and K7. In stratified epithelia, keratins display a complex expression pattern that is widely assumed to reflect the structural requirements of distinct epithelial compartments.[16] The major keratins in the basal layer of these epithelia are K5 + K14, and when these cells begin terminal differentiation, they move upwards and start to express the keratin pairs K1/K10 in skin, K4/K13 in internal stratified epithelia and K3/K12 in corneal cells. These strictly regulated tissue- and differentiation-specific patterns of expression imply that keratins, as is the case of IF in general, might carry out specific functions in epithelial cells.

As commented above, the structural function of keratins was proposed in the face of the discovery of point mutations in human keratin genes, in patients suffering from different epithelial disorders.[1,17–20,22,23] The main characteristic feature of these inherited human diseases is the cytolysis of epithelial cells expressing the mutated keratins, resulting in blistering of the corresponding epithelial sheets. On the basis of these observations, it was suggested that the overall function of the keratins expressed in stratified epithelia is to reinforce the tissue and maintain cellular integrity under mechanical stress. These research efforts were preceded by studies on transgenic mice expressing mutant keratin subunits.[18,20,21] Several mice carrying null or dominant keratin mutations that affect epidermal integrity have added further support to this notion.

However, neither mid-gestational developmental defects nor the inflammatory bowel disease that develops in adult

K8-null mice appear to be consistent with a simple structural role for K8 and K18. The knockout of K8[66,67] has generated considerable confusion with regard to the function of keratins during embryonic development and in internal epithelia. In C57BL/6 mice, most K8-deficient embryos died at around E12.5[66] while, in FVB/N mice, embryonic lethality was overcome in almost half the offspring. In this genetic background, surviving mice developed colorectal hyperplasia and females showed reduced reproductive capacity.[67] These data may suggest that keratins of internal epithelia are not primarily required as cytoskeletal proteins, but are needed for other functions. It is of note that transgenic mice expressing a human K18 gene bearing a mutation equivalent to that found in K14 in patients suffering from epidermolysis bullosa simplex[1] displayed only discrete hepatocyte fragility but clear alterations in susceptibility to hepatotoxic injury.[68] The similarity between bowel diseases occurring in K8-null mice and T-cell receptor deficiency,[67] and liver susceptibility to griseofulvin and acetaminophen in transgenic mice expressing mutant K18,[68,69] clearly pointed to altered proinflammatory and apoptotic cytokines related to TNF. It has been recently demonstrated that K8 and K18 can affect TNF signalling. These keratins are both capable of binding the cytoplasmic domain of TNFR2 and moderate TNF-mediated NFκB and JNK activation. As a consequence, K8- and K18-deficient mice are more susceptible to TNF-dependent apoptotic liver damage.[70] Related observations that simple epithelium K8/K18 may modulate Fas targeting to the cell surface, providing resistance to Fas-mediated apoptosis,[71] and that K18 may sequester the TNF receptor type 1-associated death domain protein (TRADD),[72] also supports the involvement of K8 and K18 in the modulation of TNF signalling.

Further experimental findings have also suggested the participation of simple epithelial keratins in signalling, such as their interaction with different cytoplasmic proteins.[73] In particular, the interaction, in a phosphorylation-dependent manner, with 14-3-3 proteins[31,32] appears to partially modulate hepatocyte mitotic progression, in association with alterations in the nuclear redistribution of 14-3-3 proteins during mitosis.[74] In agreement with this observation, the distribution of the 14-3-3ξ protein was altered in K8 null mice, indicating that the absence of keratin filaments causes disturbances in cell-cycle regulation, driving cells into the $S-G_2$ phase and causing aberrant cytokinesis.[75] Another line of evidence is the increased migration and invasion observed in L fibroblasts upon transfection with K8/K18,[76] suggesting a role for these keratins in tumour cell motility, and hence, metastasis.[76] Finally, increased expression of human K8 in transgenic mice leads to severe exocrine pancreas alterations such as dysplasia, inflammation, altered differentiation, increased cell proliferation and apoptosis.[77] Interestingly, this phenotype is very similar to that displayed by transgenic mice expressing a dominant negative mutant TGFβ type II receptor.[78] In agreement, these dnTGFβRII transgenic mice exhibit increased levels of K8 and K18 in pancreatic acinar cells, suggesting the involvement of these keratins in TGFβ signalling.[77]

The behaviour of keratins in stratified epithelia is perhaps more complex. As mentioned above, mutations in the specific keratins expressed in the epithelial compartments that are affected in cell fragility syndromes demonstrated the structural role played by these proteins in providing epithelial resilience to physical trauma. However, once again, this does not seem to fully explain keratin diversity. As in the case of epithelial fragility syndromes, the epidermis has provided important clues on these alternative functions. In a manner similar to other stratified epithelia, mitotically active basal cells express K5 and K14. As keratinocytes enter the terminal differentiation program and become postmitotic, transcription of these keratins ceases, giving way to the expression of keratins K1 and K10. In wound healing and other hyperproliferative disorders, including cancer, the keratin pair K6 and K16 is expressed in the suprabasal layers substituting K1 and K10. These changes suggested that different keratin pairs exert specific functions in epidermal keratinocytes during the differentiation process. This aspect has also been highlighted by recent experiments in which K16 was ectopically expressed[79] to rescue the epidermal fragility promoted by inactivation of the keratin K14 gene.[80] The rescued animals show neither epidermal fragility nor neonatal mortality, but do exhibit strong phenotypic alterations such as alopecia, chronic epidermal ulcers, and alterations in other stratified epithelia,[79] indicating that the two proteins are not functionally equivalent. Further support for the idea of specific keratin functions is also provided by the finding that forced expression of human K16 in the skin of transgenic mice causes alterations in keratin organisation and cell–cell adhesion in keratinocytes, and also induces a transient hyperproliferation associated with increased epidermal growth factor receptor phosphorylation.[81–83] However, the most compelling evidence of a role for keratins in cell signalling comes from transfection experiments showing that the expression of particular keratin polypeptides may influence keratinocyte proliferation. We demonstrated that the ectopic expression of keratin K10 inhibits cell proliferation.[84] On the one hand, consistent with this observation, the expression of K10 under a keratin k6 promoter in transgenic mice delays tumour development in vivo.[85] On the other hand, our data also indicate that K16 expression facilitates cell proliferation and, more remarkably, that the cell growth arrest promoted by K10 is specifically rescued by co-expression of K16.[84] Keratins K10 and K16 achieve modulation of cell growth through mechanisms that lead to changes in the phosphorylation, and hence the activity of the retinoblastoma protein (pRb) and in the amount of cyclin D1, two critical molecules in the molecular machinery controlling cell cycle progression during G_1 phase.[84] Moreover, the relationship between Rb protein and K10 is bidirectional, as

transfection experiments have demonstrated that the overexpression of specific members of the Rb family can induce the expression of K10 in cultured human keratinocytes.[86]

We recently reported that the influence of K10 on the cell cycle machinery is due, at least in part, to the interaction of Akt/PKB and PKCξ with the K10 aminoterminus. This interaction sequesters the two kinases to the keratin cytoskelton, precluding their translocation to the membrane and subsequent activation.[87]

Akt and PKCξ play pivotal roles in the PI-3K signalling pathway. Interestingly, K16 could also act in this pathway, as transfection of this keratin rescues the growth arrest caused by specific PI-3K inhibitors, whereas it has no effect on MEK inhibitors.[87] In line with this rationale, primary keratinocytes derived from K16-transgenic mice display increased motility,[88] and PI-3K-dependent signalling is a well-known mediator of the migratory properties of the cells. However, we have also observed that Akt activity is highly relevant during the entire process of mouse skin carcinogenesis.[89] These results, together with the observed inhibition of Akt mediated by K10 in cultured cells, have prompted us to generate transgenic mice in which K10 is expressed in the basal layer of the epidermis.[90] In these mice, we observed, in a dose-dependent manner, that increased expression of K10 led to a hypoplasic and hyperkeratotic epidermis due to a dramatic decrease in skin keratinocyte proliferation in association with the inhibition of Akt and PKCξ activities.[90] More remarkably, we also observed a dramatic decrease in the tumorigenic susceptibility of these transgenic mice, even in non-phenotypic, low expressing animals.[90] The recently described K10-deficient mice[91] will provide an invaluable tool for precisely defining the in vivo role that this protein plays in skin. Finally, it remains to be discerned whether these functions are restricted to K10 and K16, or may also be ascribed to other keratin polypeptides expressed in stratified epithelia.

Concluding remarks

The possibility that IF proteins play specific roles in the transmission of signals from the membrane to the cell nucleus has long been considered. Although early transgenic approaches confirmed the role of IF as structural components of cells, subsequent analyses have demonstrated that other important cell functions are compromised as a consequence of the ectopic expression or absence of these proteins. Nonetheless, the molecular mechanisms underlying for these alterations have not yet been defined in many cases. An exception seems to be that of keratins, as proteins of this large family, expressed either in simple and stratified epithelia, have been shown to modulate specific signal transduction pathways involved in the control of functions as relevant as epithelial cell growth and apoptosis. It seems plausible to anticipate that the careful analysis of transgenic models, and the use of refined biochemical approaches will clarify this question in the near future and give rise to a new exciting field in cell biology. This line of research will no doubt serve to connect and integrate the IF cytoskeleton with the molecular machinery of signal transduction cascades and explain why different cell types or developmental states build up their, otherwise very similar, IF from different proteins.

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