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Review Article

Type III intermediate filaments in redox interplay: key role of the conserved cysteine residue

María A. Pajares and Dolores Pérez-Sala

Department of Structural and Chemical Biology, Centro de Investigaciones Biológicas Margarita Salas, C.S.I.C., Ramiro de Maeztu, 9, 28040 Madrid, Spain

Correspondence: María A. Pajares (mapajares@cib.csic.es) or Dolores Pérez-Sala (dperezsala@cib.csic.es)

Intermediate filaments (IFs) are cytoskeletal elements involved in mechanotransduction and in the integration of cellular responses. They are versatile structures and their assembly and organization are finely tuned by posttranslational modifications. Among them, type III IFs, mainly vimentin, have been identified as targets of multiple oxidative and electrophilic modifications. A characteristic of most type III IF proteins is the presence in their sequence of a single, conserved cysteine residue (C328 in vimentin), that is a hot spot for these modifications and appears to play a key role in the ability of the filament network to respond to oxidative stress. Current structural models and experimental evidence indicate that this cysteine residue may occupy a strategic position in the filaments in such a way that perturbations at this site, due to chemical modification or mutation, impact filament assembly or organization in a structure-dependent manner. Cysteine-dependent regulation of vimentin can be modulated by interaction with divalent cations, such as zinc, and by pH. Importantly, vimentin remodeling induced by C328 modification may affect its interaction with cellular organelles, as well as the cross-talk between cytoskeletal networks, as seems to be the case for the reorganization of actin filaments in response to oxidants and electrophiles. In summary, the evidence herein reviewed delineates a complex interplay in which type III IFs emerge both as targets and modulators of redox signaling.

Introduction

The cytoskeleton is a complex and dynamic network of filamentous structures that altogether support cell integrity and are tightly regulated. These filamentous structures are classified according to their diameter into microfilaments, intermediate filaments (IFs) and microtubules, but differences also concern their composition, assembly and elongation. Microfilaments and microtubules are composed by the globular proteins actin and tubulin, respectively, which form regular chains with a defined orientation and elongation directions. The interaction surfaces of the monomers are known, as well as their crystal structures. In contrast, the human IF family comprises proteins encoded by more than 70 genes, which based on their expression pattern, sequence homology and predicted structure, are subdivided into six classes as will be described below (reviewed in [1,2]). Distinctively, IF form non-polar filaments, the precise structure and assembly of which are not fully understood. Importantly, the introduction of posttranslational modifications (PTMs) on the basic building blocks controls the assembly and function of the cytoskeletal elements and their interplay [3]. These are also modulated by a huge variety of associated proteins. This makes cytoskeletal networks highly responsive to environmental factors and cell needs. In particular, cytoskeletal structures are finely modulated under oxidative stress.

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Intermediate filaments

The IF network expands throughout the cytoplasm from the nucleus to the cell membrane, but some proteins show specific subcellular locations [1,2], as is the case of the type V IFs class, comprising lamins, that are ubiquitous and locate to the cell nucleus. Other classes show cell-type specific

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expression. Type I and II IFs (keratins) show a preferential expression in epithelial cells, whereas class VI (filensin and phakinin) proteins are detected in the lens. Type III and IV IF proteins display a larger diversity of expression patterns. Type III IFs include vimentin, expressed in mesenchymal cells, glial fibrillary acidic protein (GFAP), specific of glial cells, desmin, found in muscle cells, and peripherin, expressed in neurons. Type IV IFs include three types of neurofilaments expressed in neurons, and two longer proteins, nestin (240 kDa) and synemin (150–180 kDa), that are identified in stem cells and muscle cells, respectively.

Mutations on IF proteins have been associated with a plethora of diseases, examples of which include epidermolysis bullosa (keratins) [4], dominant cataract (vimentin, filensin, phakinin) [5,6], myopathies and cardiomyopathies (desmin) [7], different types of neurodegenerative diseases (GFAP, peripherin, neurofilaments) [8–10] and progeria syndromes (lamins, vimentin) [11,12]. For reviews on this subject, please see [4,7,13–15]. Mutated proteoforms often exhibit altered assembly and/or associate into diverse aggregates that are deposited in the damaged cells in association with disease. Additionally, anomalously high expression levels of wild type proteins can also become pathogenic or serve as markers of pathology, as occurs with GFAP in the rare neurodegenerative Alexander disease or in reactive astrogliosis [16], or with vimentin in cancer and fibrosis [17]. Such a variety of clinical manifestations reflect the wide array of functions exerted by IFs and that comprise structural support, mechanotransduction, response to stress, regulation of gene expression, organelle positioning and function, cytoskeletal interplay, intercellular communication and tissue homeostasis [13,18]. A fine regulation of IF functions in these diverse roles is achieved through a wealth of PTMs that can be enzymatically catalyzed or incorporated non-enzymatically. Among the former we can highlight phosphorylations regulating filament assembly/disassembly and certain oxidative PTMs such as glutathionylation, whereas non-enzymatic incorporations can be induced by certain oxidants and electrophiles (reviewed in [14,19,20]).

In this review, we will focus on type III IF proteins, and more specifically in the prototypic type III IF protein vimentin, which is probably the most studied member of this class. In early studies, *Vim* knockout ($Vim^{-/-}$) mice showed no apparent phenotype affecting development or reproduction, thus initially suggesting that vimentin was a non-essential protein (reviewed in [21]). However, as research progressed it became evident that vimentin played a crucial role under stress conditions, to cope, among others, with wound healing or mechanical stress in the central nervous system. Analysis of these effects proved vimentin involvement in cell adhesion and migration, cell structural support and resistance to mechanical stress, as well as its interactions with many proteins through which vimentin regulates signaling and metabolism, the position and function of several organelles and pathogen infection [21–23]. In cells, vimentin can form both filamentous and non-filamentous structures, the specific structure and function of which are not completely understood [23,24]. Moreover, the interconversion between the diverse forms appears to be controlled by multiple PTMs that, as we should see below impact its assembly, filament morphology, distribution and function.

IF assembly

IFs are non-polar, and hence, able to elongate at either end and shed and incorporate subunits at any point along their length (reviewed in [1]). There is no complete crystal structure of any of the IF proteins and the information available has been obtained from the crystallization of fragments and structural models [1]. IF monomers share a basic organization with a central α-helical rod domain flanked by disordered head (N-terminal) and tail (C-terminal) domains of different sizes (Figure 1A). A highly simplified view of IF assembly, exemplified for vimentin, implies the association of IF monomers into parallel coiled-coil dimers in a process favored by the presence of hydrophobic residues on the rod surface [25–28]. These parallel dimers then align into staggered antiparallel tetramers linked by electrostatic interactions between residues of opposite charge that appear periodically at the surface [1,29] (Figure 1A). Subsequently, tetramers, apparently 8–10 [1,30], bind laterally to generate the unit length filaments (ULFs). Filament formation then proceeds with an elongation step that occurs by head-to-tail association of ULFs at either end (Figure 1A), followed by filament compaction to attain the final diameter [1]. Within vimentin filaments, tetramers can be found in different conformations, as revealed by cross-linking studies [27,31]. This type of studies has also provided information on the proximity of certain residues in the filaments and predicted some overlap of head and tail domains upon filament assembly.

Conformational changes of the disordered head and tail domains likely occur in response to PTM incorporation or protein-protein interactions. Indeed, insight into the disposition of these domains, obtained through deuterium exchange and electron paramagnetic resonance, indicates reorganization of the head and tail segments during filament assembly [25,32]. Interestingly, the combination of tail mutants and immunological

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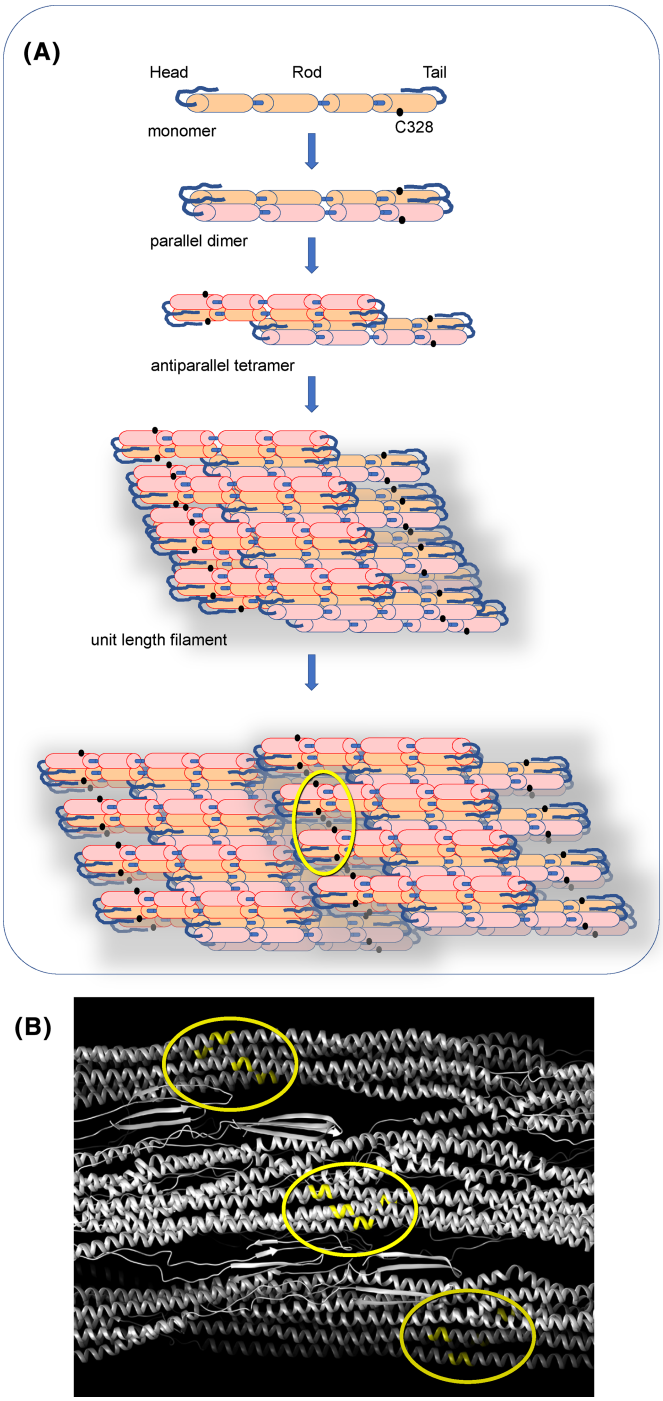

Figure 1. Assembly and features of vimentin Intermediate filaments.

(**A**) Schematic cartoon displaying the features of the vimentin monomer with the head, rod and tail domains. The approximate position of C328 is denoted by a black circle. The assembly process of the filament with the formation of parallel dimers, association into antiparallel tetramers and their organization into unit length filaments (ULFs) is schematically shown. Connection of several ULFs will lead to filament formation. In this process, cysteine residues from different subunits could putatively coincide near the region of overlap (encircled by the yellow oval). Please note that this representation does not intend to be structurally accurate. Elements of this panel were adapted from Monico et al. [75], published under CC BY license. (**B**) Model obtained from the cryoelectron tomography structure of vimentin filaments in mouse embryonic fibroblasts [30], highlighting the position of the cysteine residues (yellow). Courtesy of Prof. O. Medalia. See text for details.

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approaches allowed observation of a differential accessibility of tail segments in cells, depending on the subcellular location and the exposure to electrophiles. These observations suggest the existence of at least two conformations of the tail domain, putatively 'extended' or 'packed', according to the exposure of the vimentin segment comprising residues 419–438 [33]. Combination of different tetramer dispositions in the filaments, together with the disordered nature of head and tail domains and the putative diversity of their conformations seems responsible for the variety of structures and shapes that IFs can adopt and that make them highly moldable. Importantly, a recent work has shed light on filament structure by analyzing straight filament vimentin segments in murine embryonic fibroblasts using cryoelectron tomography [30] (Figure 1B). The model obtained confirms the intricate interactions between the α-helical segments and the head and tail domains in the organization of the mature filament.

IFs are finely tuned by posttranslational modifications

As already mentioned, IF proteins are the subject of a large variety of modifications (reviewed in [3,14]). For instance, vimentin is a target for glutathionylation [34], ubiquitination [35,36], sumoylation [37,38] and acetylation [39], as well as for citrullination which creates a neoepitope involved in autoimmune recognition (e.g. in rheumatoid arthritis) [40]. However, phosphorylation has been probably the most studied PTM on vimentin. Mapping of its phosphorylated residues has shown the existence of many such sites along the whole vimentin sequence [14,41–43], although those involved in the control of filament assembly appear mainly concentrated on the head domain (reviewed in [44]). A detailed summary of phosphorylation sites, together with some of the kinases responsible of the modification can be obtained at PhosphoSitePlus (https://www.phosphosite.org). Vimentin is phosphorylated in a cell-cycle dependent manner and this regulates its assembly state, which is critical for its dynamics in mitosis, particularly in cytokinesis [45]. Introduction or removal of phosphorylations can depend on conditions such as oxidative stress, known to regulate several kinases and phosphatases [46,47]. Thus, a cross-talk between different PTMs can take place on IFs to further regulate their assembly, subcellular localization and interactions.

A wide number of oxidative and electrophilic PTMs have been detected on vimentin both under control conditions and during oxidative stress [20,48], In fact, reactive oxygen species (ROS) are continuously generated during the production of ATP, mainly by complexes I and III of the mitochondrial electron transport chain, but also by other oxygen-consuming reactions. Importantly, low levels of ROS serve as signaling molecules [49], and certain oxidative modifications of vimentin have been clearly detected under standard cell culture conditions [48]. Nevertheless, in many pathologies (e.g. cancer and neurodegeneration), but also in the natural aging process, ROS are produced in excess that may surpass the enzymatic and small molecule antioxidant systems of cells, leading to oxidative stress. Cytoskeletal proteins such as actin, tubulin and vimentin have been identified as frequent targets for oxidative PTMs during oxidative stress and related pathologies, as well as in senescence and aging.

Oxidative PTMs can be highly varied structurally, and affect many residues throughout the sequence of the proteins (reviewed in [50,51]). As ROS are able to interconvert into a diversity of species and lead to additional free radicals, their effects can be multiplied and the damage extended not only to proteins, but also to the DNA and lipids. Secondary processes induced by ROS include protein and lipid peroxidation (reviewed in [50,52]). Protein peroxides can in turn oxidize other targets, including other proteins, and are slowly removed, which contributes to their accumulation in cells [50]. In lipid peroxidation, polyunsaturated fatty acids such as arachidonic acid or γ-linolenic acid are the targets for lipid radical formation. This is the initial step of a cascade of reactions in which they evolve into peroxyl radicals that, in turn, lead to lipid peroxides. Electrophilic lipids exhibit a large structural variety, including reactive aldehydes, nitrated lipids, or species bearing α,β-unsaturated carbonyl moieties, and can be detected at concentrations that range from picomolar (e.g. 15-oxo-eicosatetraenoic acid) to micromolar levels (e.g. acrolein or 4-hydroxynonenal). The modification of proteins by electrophilic lipids is known as lipoxidation, and usually occurs through the formation of Schiff bases or the more stable Michael adducts (reviewed in [53]). The structural variety of the resulting modifications also influences the diversity of their functional outcome. Although many lipoxidation targets have been identified, proteomic studies have shown that this PTM affects a preferred set of cellular proteins, to extents not correlating with their abundance, and among which cytoskeletal proteins such as vimentin, actin and tubulin can be highlighted [54,55]. Moreover, within a single protein, nucleophilic residues, such as cysteines, histidines or lysines, are the preferred targets.

In this context, actin, tubulin and vimentin are important targets for lipoxidation [48]. From a functional point of view, oxidants and electrophiles have been shown to elicit multiple morphologically distinct vimentin arrangements that apparently depend on the structure of the modifying moiety. In cells, vimentin rearrangements can range from fragmentation of filaments into dots or squiggles (e.g. diamide) [56], to filament bundling (e.g. elicited by the electrophilic prostaglandin 15-deoxy-$\Delta^{12,14}PGJ_2$) [57], accumulation at certain particular locations (e.g. aggresomes induced by 4-hydroxynonenal), solubilization yielding diffuse patterns (e.g. combination of oxidants and phosphatase inhibitors) and parallel linear arrays (e.g. certain electrophiles). In vitro assays also showed production of various forms of aggregates, bundles, shorter or thicker filaments in response to those compounds. Moreover, certain studies found correlations between the structure of the chemical modification and the nature of filament remodeling, as well as between the modification extent and the severity of the assembly defect observed [56,58,59]. Nevertheless, establishing the structure-function relationships of these complex modifications requires further work. On the one side, both endogenous and exogenous reactive species and agents can have multiple target residues (e.g. cysteine, methionine, tyrosine, etc.) in the same subunit or filament, with overlapping or opposite morphological effects on the filaments. On the other, indirect modifications can also occur as the result of the generation of additional reactive species or of their direct or indirect modulation of signaling pathways or the coexistence of different PTMs.

Type III IFs contain a redox-sensitive cysteine residue, which is critical for the remodeling elicited by oxidants and electrophiles

Redox modifications can occur by enzymatic and non-enzymatic mechanisms, may be reversible or irreversible and may coexist with additional PTMs, establishing a cross-talk to control protein function, as already mentioned. Type III IF proteins contain a conserved cysteine residue, which is also the only cysteine in vimentin (C328), desmin (C333) and GFAP (C294) (numbered according to the human sequences; reviewed in [60]). Available structural data and models indicate that this conserved cysteine is exposed at the outer surface of the dimer, and hence is susceptible to modification (see for instance [29,61]) (Figure 1). Among oxidative modifications, a certain proportion of disulfide bonded homo-dimers of vimentin, GFAP and desmin [56,59,61–64], as well as heterodimers of vimentin and the other two proteins [65–67], have been identified in several settings, indicating the exposed location of some cysteines in the filaments and their proximity in certain conformations. The proportion of these disulfide-bonded dimers may rise under oxidative conditions or in the presence of mutant proteoforms that carry additional cysteines, putatively decreasing the flexibility of the filament structures and/or favoring more compact conformations, and/or bundling.

Besides disulfide bond formation, more than a dozen oxidative or lipoxidative modifications of C328 have been identified in numerous biochemical and proteomic studies, employing different experimental models, both in cells and *in vitro* (Figure 2). In fact, in a recent study, up to seven different PTMs were identified by mass spectrometry during nitroxidative stress in primary rat cardiac cells at this precise position (e.g. sulfenation, *S*-glutathionylation or *S*-nitrosation), some of which accumulated during cell treatment, while others were transient [48]. Of note, several of these modifications can be interrelated (e.g. nitrosation or sulfenic acids are often intermediates in disulfide formation). Therefore, transient detection of certain PTMs may result from their interconversion into more stable modifications or the consequence of their removal. These results suggested cross-talk between PTMs on C328 allowing redox sensing, and indicate the importance of this conserved cysteine at the rod domain and its role as a hub for modification.

Agents or treatments eliciting these modifications produce diverse rearrangements of vimentin filaments, some of which have been described above. These reorganizations occur not only in cells but also *in vitro*, and depend, at least in part, on the structure of the modifying moieties. Thus, it is possible that, even in the case of potentially interrelated PTMs, each of the individual modifications has unique functional consequences, as it seems to be the case with nitrosation, glutathionylation and disulfide formation [56,58]. Although vimentin rearrangements can be the result of multiple factors, the observation that all morphological alterations of filaments are drastically attenuated in vimentin conservative cysteine mutants, e.g. C328S or C328A [56,57], both *in vitro* and in cells, pose C328 as a master regulator of vimentin remodeling. Likewise, this single cysteine is also a hot spot for modification by a variety of drugs, chemicals and natural products [34,57,59,68,69]. All these modifications have roles in physiological processes such as aging or during hypoxia, but also in the pathological context of neurodegeneration, cataracts, etc.

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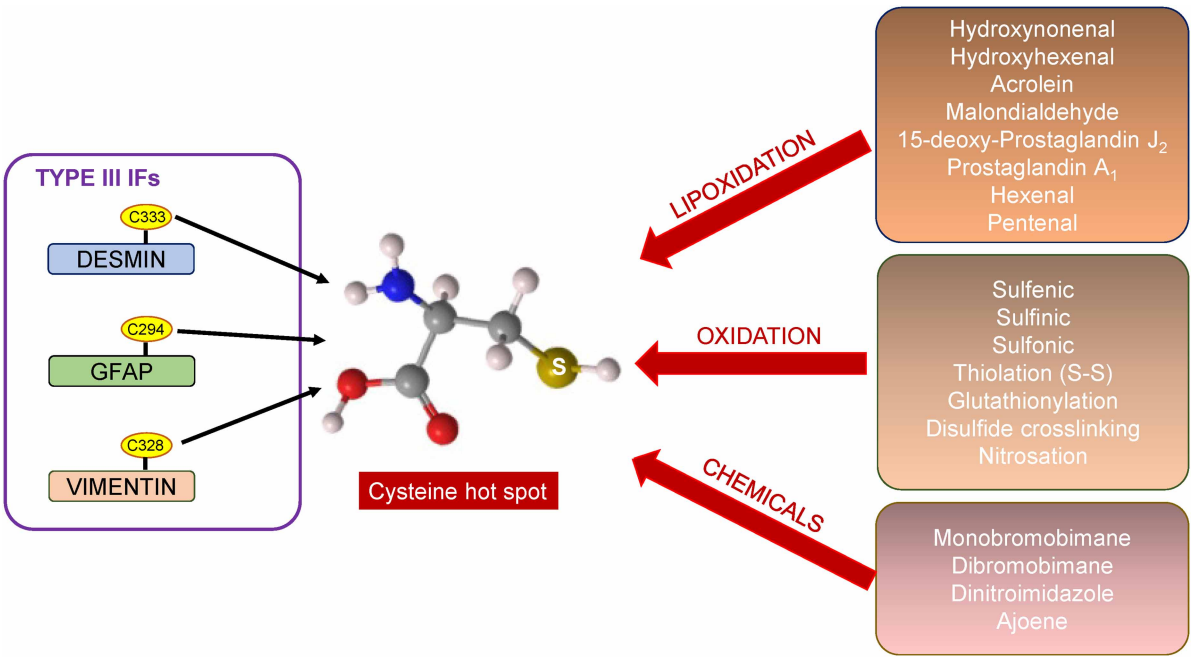

Figure 2. Modifications of the single cysteine residues of desmin, GFAP and vimentin.

The type III intermediate filament proteins that possess a single cysteine residue fully conserved between species are shown at the left. These cysteine residues are hot spots for PTMs. Various types of lipoxidative and oxidative modifications, as well as modifications by chemicals, natural products and drug metabolites, shown in boxes at right, have been identified, mainly in the case of vimentin C328. These various modifications may elicit structure-dependent functional consequences.

The importance of C328 in filament organization is also evidenced by the observation that local perturbations at this position introduced through mutation determine the morphological impact on vimentin filaments depending on the type of lateral chain of the residue used to substitute cysteine [59]. The rearrangements obtained include a variety of filamentous patterns (short filaments, curls or bundles) or aggregates. These experiments also showed that a C328H mutation can be accepted at this position, while being resistant to electrophilic modification and filament disruption [59]. Altogether, experimental and structural evidence suggests that C328 is strategically located in such a manner that its modifications influence filament assembly, especially, considering the apparent proximity of cysteine residues from several monomers (Figure 1B).

Importantly, the single cysteine residues on GFAP (C294) and desmin (C333) seem to have the same key role than vimentin C328 by regulating their rearrangement upon oxidative modifications [63,70–72]. Studies combining cell models and *in vitro* assays have shown that their modification leads to several types of filament rearrangements including bundles and network retraction towards the nuclear periphery, as well as the impairment of these effects in proteoforms lacking their conserved cysteine. Moreover, the use of mutants including additional cysteine residues, as those involved in Alexander disease, allowed demonstration of the increased susceptibility of those filaments to lipoxidation that, in turn, may contribute to the severity of the pathological outcome [73].

Modification of vimentin C328 is modulated by context factors

Modification of proteins by oxidants and electrophiles, as well as their functional consequences depend on context factors, such as the local concentration of antioxidants, divalent cations or pH. The polyanionic character of IFs allows them to bind a range of cations, such as calcium and magnesium. These divalent cations have been shown to modulate vimentin filament assembly [74]. In particular, the interaction of IF with zinc may entail special interest. A combination of oxidation and cross-linking assays together with molecular dynamic simulations support zinc binding to the thiolate form of vimentin C328, aided by the stabilization effects of the nearby residues E329 and D331 in the coordination of this ion [57,75]. In contrast, simulations using

magnesium placed a lower number of these cations in the region surrounding C328 [75]. Such differences in cation binding may lay at the basis of the protection provided by low zinc concentrations against vimentin C328 alkylation, lipoxidation and disulfide formation, as well as the selective prevention provided by zinc against *in vitro* cross-linking by cysteine-reactive compounds, but not by amino-reactive agents [75]. Nevertheless, zinc has multiple cellular actions, including the modulation of antioxidant systems, and hence indirect effects on the control of oxidative PTMs can be also exerted. Notably, the region surrounding vimentin C328 is conserved in other members of the type III IF family, such as GFAP and desmin. Therefore, zinc interaction through this same region can be expected for these other members of the IF type III class.

As noted above, molecular dynamic studies indicate that C328 would be involved in zinc binding when in its thiolate form. Interestingly, *in vitro* data suggest the existence of two cysteine subpopulations in vimentin, exhibiting different degrees of ionization at physiological pH [75]. Therefore, intracellular pH variations could affect the proportion of the thiolate, more reactive form of C328, and its susceptibility to modification. In fact, it has been recently reported that in several cell lines acidification of the intracellular pH provides selective protection against vimentin modification by oxidants, while its alkalinization favors oxidative disruption of this particular type of IFs in a manner dependent on the presence of C328 [76].

Finally, the susceptibility to modification could depend on additional factors, including the subcellular localization of the filament, the occurrence of PTMs at nearby residues, the degree of filament 'packing' or cytoplasmic crowding, the establishment of protein-protein interactions or the formation of biomolecular condensates. All these factors may affect the accessibility of the cysteine residue, and/or the rate or extent of modification [20,60,77–80], although their precise effects need further investigation.

Redox interplay of type III IF with cytoskeletal structures and organelles

The main cytoskeletal networks, actin, tubulin and IFs, are highly sensitive to redox regulation. This has been extensively studied in the case of actin, for which multiple mechanisms, affecting both actin filaments and regulatory proteins have been identified [81–85]. Vimentin interacts with actin in various ways, for instance at the actin arcs [86], and at the cell cortex, both in interphase and mitotic cells, where both protein filaments appear intimately interwoven and vimentin influences the properties of the actin cortex [87,88]. Lack of vimentin has been shown to increase actin stress fibers in various models [59,89]. Hence, the interest to analyze whether vimentin has a role in the response of those structures to electrophiles. In this line, treatment of cells with certain electrophiles led to the production of actin stress fibers aligned with vimentin [59]. Both patterns were prevented in cells expressing vimentin C328H or C328A mutants [59]. In several vimentin-depleted cellular models, an increase in actomyosin contractility and RhoA activity has been detected, and therefore the possibility that vimentin controls RhoA and its downstream targets was hypothesized [89]. Results of assays combining electrophile modification with inhibitors or activators of the Rho pathway further supported that vimentin may exert a break on this pathway regulating actin stress fiber formation [59]. As some functions of vimentin on cytoskeletal interplay appear to depend on its presence in filamentous or non-filamentous structures, modulation of its assembly state through modifications of C328 may impact this role [59,89]. Redox mechanisms could also contribute to the interplay between vimentin and microtubules, although this aspect has been less explored.

Type III IF play a key role in organelle homeostasis. Vimentin C328 could also be important for this function, as suggested by the observation that cells expressing a vimentin C328S mutant display altered organelle position [57]. Of particular interest is the case of mitochondria, which are both source and targets of ROS, and the position and function of which is affected by contacts established with IFs ([90] and reviewed in [60]). Mitochondrial damage arising from oxidative stress can lead to an increase in their ROS production, thus potentially generating a deleterious feedback loop. Certain pathogenic mutations of desmin and GFAP have been shown not only to cause alterations of the filament and network morphologies, but also on mitochondria [73,91]. Precisely, certain desmin mutants led to misfolding and/or filament disruption and, subsequently, impact calcium signaling [91,92]. In the case of GFAP, expression in astrocytoma cells of the Alexander disease GFAP R239C mutant (carrying two cysteines), responsible of a severe form of the disease, disrupted the IF network leading to altered mitochondrial morphology (i.e. elongated and interconnected mitochondria) and increasing mitochondrial ROS production [73]. Several observations indicate that functional disruptions due to this mutation may be related to additional redox PTMs involving this extra cysteine. In control cells, cysteine availability of the GFAP R239C mutant is proportionally lower than that of the wild type, suggesting a higher degree of modification.

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Moreover, this mutant shows a higher proportion of disulfide-bonded oligomers, and a two-fold higher incorporation of a biotinylated analog of 15-deoxy-$\Delta^{12,14}$-PGJ_2 in cells. In addition, GFAP R239C filaments suffer a stronger retraction and/or aggregation in response to 15-deoxy-$\Delta^{12,14}$-PGJ_2, 4-hydroxynonenal or H_2O_2 than the wild type. Moreover, the GFAP R239C mutant increased the cell susceptibility to H_2O_2 damage, leading to cell death, whereas cells expressing wild type GFAP were able to recover [73]. Therefore, there seems to be a correlation between the increased susceptibility of the GFAP R239C mutant to oxidative or lipoxidative modifications, and the functional alterations. Nevertheless, intrinsic alterations in GFAP R239C assembly likely also play a role. Thus, it would be interesting to assess whether other Alexander disease GFAP mutants, containing or not additional cysteines, also show an increased susceptibility to oxidative and electrophilic stress.

Concluding remarks and future perspectives

From recent research on type III IFs the concept emerges that the position occupied by the conserved cysteine residue is important for the regulation of their assembly and functions. Interestingly, different effects on the filaments are obtained by mutations changing the amino acid lateral chain or by modifications of the thiol group introducing moieties of diverse charge or size. The single cysteine residue of type III IF proteins has already been identified as the target of numerous PTMs. Nevertheless, the diversity of PTMs potentially targeting cysteine residues (e.g. glutathionylation, persulfidation, phosphorylation) is ever growing. Coexistence of different PTMs on the cysteines along the filaments may allow a variety of assemblies, and facilitate subunit exchange or accessibility for additional modifications at other positions in the filament. Understanding the structure-function relationships of oxidative PTMs of type III IFs will require complementary high resolution techniques potentially combining spatial proteomics, microscopy, chemical tools, and reconstitution approaches. Future research will shed light into new potential PTMs, as well as on the interplay between diverse PTMs occurring not only on cysteines, but also in other residues, their spatiotemporal regulation, and the impact of environmental factors under physiological and pathological situations.

Perspectives

- IFs are arising as key elements in the integration of cellular functions, above all in response to stress. The conserved cysteine residue of type III IFs is a critical target for modifications that impact the structure and function of the filaments and the organization of the network.
- There is a need to map redox-dependent PTMs of IFs and to study their cross-talk with additional modifications both in the physiological and pathological contexts.
- Understanding the structural implications of cysteine modification in type III IFs will contribute to unveil their full role in cytoskeletal cross-talk and essential cellular processes. Moreover, this knowledge will be critical to assess its potential as a drug target.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

GFAP, glial fibrillary acidic protein; IF, intermediate filament; PTM, posttranslational modifications; ROS, reactive oxygen species; ULF, unit length filament.

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