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Invited Review

Type III intermediate filaments as targets and effectors of electrophiles and oxidants

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ABSTRACT

Intermediate filaments (IFs) play key roles in cell mechanics, signaling and homeostasis. Their assembly and dynamics are finely regulated by posttranslational modifications. The type III IFs, vimentin, desmin, peripherin and glial fibrillary acidic protein (GFAP), are targets for diverse modifications by oxidants and electrophiles, for which their conserved cysteine residue emerges as a hot spot. Pathophysiological examples of these modifications include lipoxidation in cell senescence and rheumatoid arthritis, disulfide formation in cataracts and nitrosation in endothelial shear stress, although some oxidative modifications can also be detected under basal conditions. We previously proposed that cysteine residues of vimentin and GFAP act as sensors for oxidative and electrophilic stress, and as hinges influencing filament assembly. Accumulating evidence indicates that the structurally diverse cysteine modifications, either per se or in combination with other posttranslational modifications, elicit specific functional outcomes inducing distinct assemblies or network rearrangements, including filament stabilization, bundling or fragmentation. Cysteine-deficient mutants are protected from these alterations but show compromised cellular performance in network assembly and expansion, organelle positioning and aggresome formation, revealing the importance of this residue. Therefore, the high susceptibility to modification of the conserved cysteine of type III IFs and its cornerstone position in filament architecture sustains their role in redox sensing and integration of cellular responses. This has deep pathophysiological implications and supports the potential of this residue as a drug target.

1. Introduction

Intermediate filaments (IFs) are cytoskeletal structures critical for cell mechanics, which maintain a close interplay with the other cytoskeletal systems, namely, actin microfilaments and microtubules [1,2]. IFs act as integrators of cytoskeletal responses and cell behavior. They are constituted by homo- or heterooligomers of proteins, which are classified into six different families depending on their structure. Keratins (types I and II), vimentin (type III) and nuclear lamins (type V) are examples of the different protein families and illustrate the critical roles of IFs in epithelial biology, cytoplasmic and nuclear mechanics and stress sensing, and nuclear support and function, respectively [3]. Mutations in many IF proteins lead to devastating diseases, ranging from epidermolysis bullosa (keratin) [4] to premature aging syndromes (lamins) [5], which highlight their key function in cell and organism homeostasis.

The type III IF protein family comprises vimentin, glial fibrillary acidic protein (GFAP), desmin and peripherin, which are important components of the cytoplasmic cytoskeleton and are expressed in specific cell types. Vimentin is present mainly in cells of mesenchymal origin and is the most widely expressed type III IF protein [6,7], whereas the expression of desmin, GFAP and peripherin is mainly restricted to the muscle and glial cells and neurons of the peripheral system, respectively [6]. Vimentin is also the most thoroughly studied member and is frequently considered as a prototype for the assembly, regulation and implications of the constituents of this family [7]. Therefore, many of the features outlined below derive from research on vimentin.

Type III IF proteins form a dynamic network that typically extends from the periphery of the nucleus to the cell membrane, interacting with and seemingly modulating the behavior of the actin and tubulin cytoskeletons [7–10]. Vimentin influences many critical cellular processes including cell plasticity and mechanics, deformability, cell cortex properties in interphase and mitosis, migration and adhesion, cell division, organelle positioning, and nucleus and DNA integrity under stress [6–8,11–13]. In turn, GFAP has been involved in astrocyte

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Abbreviations

AD	Alzheimer's disease
AxD	Alexander's disease
15d-PGJ$_2$	15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$
cyPG	cyclopentenone prostaglandin(s)
eNOS	endotelial nitric oxide synthase
IF	intermediate filament
GFAP	glial fibrillary acidic protein
HNE	4-hydroxynonenal
MDA	malondialdehyde
PCF	protein carbonyl formation
PTM	posttranslational modification
RAGE	receptor for advanced glycation end products
ULF	unit length filament
WFA	withaferin A

migration, integrity and signaling [14], whereas desmin is important for striated muscle cell differentiation and survival, mitochondrial function and mechanochemical signaling [6,15]. Therefore, these proteins function in the maintenance of normal cell homeostasis and their alterations are involved in pathology, including cancer aggressiveness and invasion, heart disease and neurodegenerative disease in the case of vimentin, desmin and GFAP, respectively [15–17].

1.1. Structural aspects of type III IFs

IF proteins share a three domain structure comprising a disordered N-terminal head, a central α-helical rod domain and a disordered C-terminal tail (Fig. 1). The rod domain is in turn divided into several α-helical segments joined by linkers. Several excellent reviews have covered the vast work performed on the structure and assembly of these proteins [3,18]. Therefore, only a simplified view will be provided in this review. Exhaustive in vitro work has outlined the process of filament formation. The disposition of hydrophobic residues of the rod towards the same side of the α-helix favors monomer association into parallel coiled-coil dimers. Then, dimers associate into staggered antiparallel tetramers, a process favored by electrostatic interactions due to the periodical arrangement of positive and negative charge spots. Tetramers then associate laterally to form short “cylinders”, known as “unit-length filaments” (ULFs), typically constituted by eight tetramers. These units connect end to end to form filaments. However, given the staggered disposition of dimers in tetramers, considerable imbrication of dimer extremities needs to occur during filament elongation. In fact, superposition of N-and C-terminal ends of the rod domains of consecutive ULFs, involving an approximately 30-residue overlap, has been proposed for vimentin [19].

The complete crystal structures of vimentin, or other type III IF proteins, are not available yet. Nevertheless, integration of the information from a variety of structural techniques, together with molecular modeling, have rendered an advanced view of the configuration of tetrameric vimentin and have provided models for the disposition of vimentin in filaments [18,20]. Importantly, early crosslinking experiments already indicated that vimentin undergoes important reorganization during filament assembly and elongation [21,22]. It is accepted that, within filaments, different vimentin tetramer conformations coexist, depending on the relative positions of dimers, which can adopt N-terminal or C-terminal overlaps or N-terminus-C-terminus coupling, known as A11, A22 and ACN conformations, respectively (Fig. 1). Recently, strategies like deuterium exchange, electron paramagnetic resonance and molecular dynamics have provided further insight into IF protein organization [20,23,24].

Type III IF proteins share a high degree of sequence identity, not only among the members of the family but also between species. Therefore, the positions of hydrophobic residues and clusters of charged amino acids are highly conserved. Moreover, these proteins present numerous target residues for phosphorylation, mainly in the head domain, which are very important for the regulation of filament assembly. Remarkably, all members of this family possess a cysteine residue, located at positions 328, 294, 333 and 324 (numbered from the initial methionine) in the sequences of human vimentin, GFAP, desmin and peripherin, respectively, which in the first three members is also the only cysteine residue. Moreover, this residue is conserved in all species, from zebra fish to humans. The conserved cysteine residue is located in the last coil segment of the rod, facing outwards from the vimentin dimer [25]. Notably, whereas in the A11 tetramer cysteine residues from different dimers will be far apart, in the A22 tetramer conformation, the cysteine residues would be closer. Nevertheless, information on the three-dimensional organization of tetramers in the body of the filament would be necessary in order to ascertain the relative positions of cysteine residues from different tetramers. From the existing models of vimentin [19,24], it could be hypothesized that the cysteine residue would occupy a position close to the space needed for the interdigitation of adjacent ULFs during elongation (Fig. 1). Therefore, bulky modifications of this residue could lead to alterations in assembly. In the context of oxidative modifications, other nucleophilic residues prone to oxidation or adduction of electrophiles, including several histidine, lysine and arginine residues, as well as the single tryptophan (W290 in vimentin), are also conserved among members of the type III IF family, as well as between species [26,27].

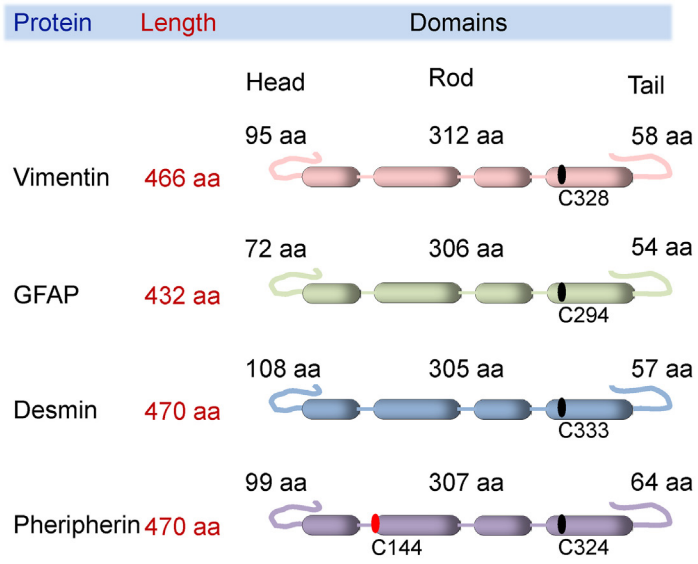
1.2. Posttranslational modifications of type III IFs

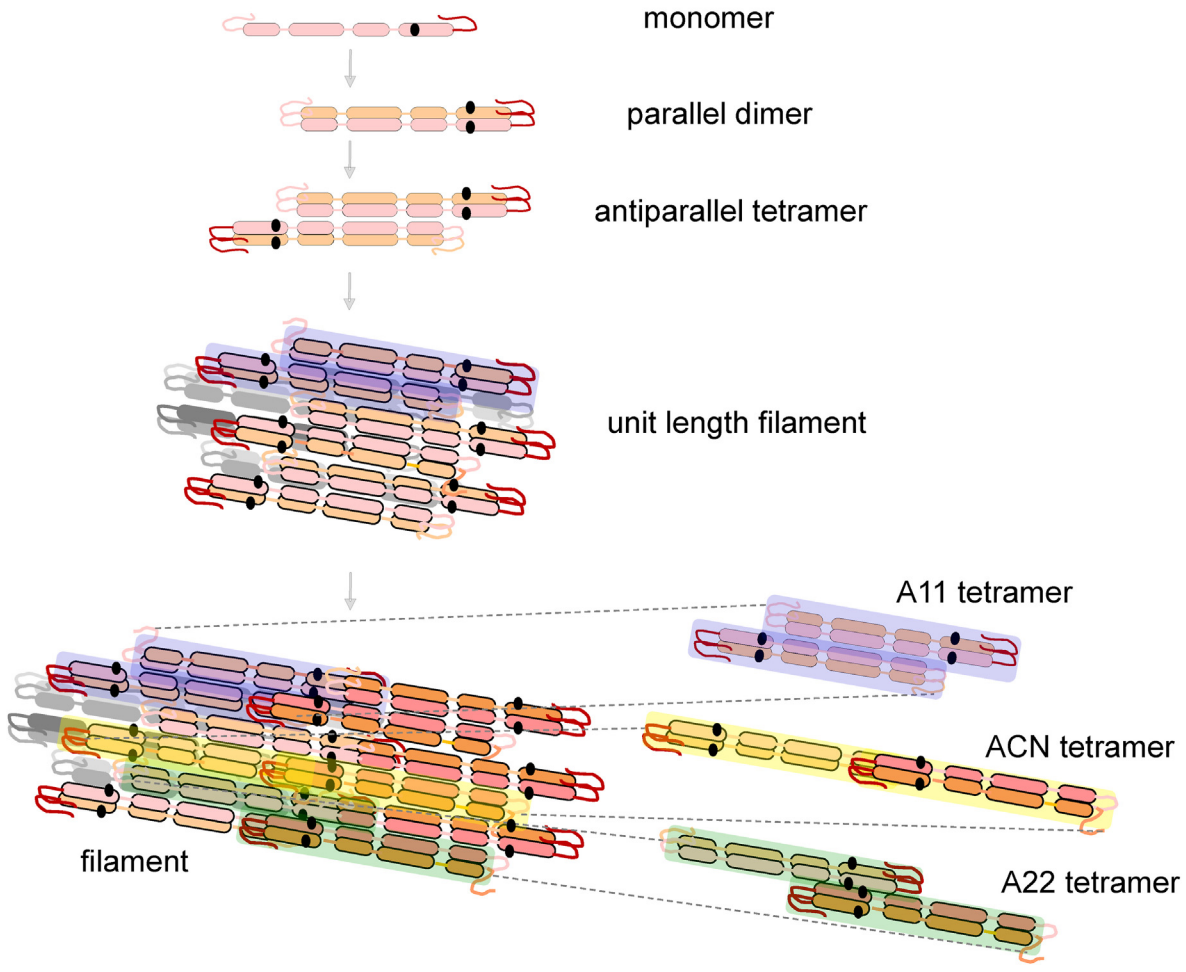
In cells, type III IFs are under constant remodeling through the exchange between the assembled polymers and the pool of soluble subunits, and their dynamics is tightly controlled by posttranslational modifications (PTMs), mainly phosphorylation [28]. Regulation of vimentin by phosphorylation is critical in mitosis, where the spatiotemporally concerted action of several kinases allows its reorganization, which is important for completion of cytokinesis [29]. In addition, the extent of phosphorylation together with protein-protein interactions influences whether vimentin is disassembled in mitosis or remains in filaments [30] that intertwine with and modulate the actin cortex, allowing proper mitosis progression [8].

Type III IFs can suffer diverse enzymatic PTMs, including ubiquitination and sumoylation [31], glycosylation [32], proteolysis [33], and acetylation [34]. One important modification with significant pathophysiological consequences is citrullination [35]. Citrullinated vimentin is an autoantigen involved in the pathogenesis of rheumatoid arthritis and in antitumor immunity [36,37]. In turn, citrullinated GFAP has been detected in several diseases, including Alzheimer's disease (AD) and multiple sclerosis, and it has been reported to constitute an early response to retinal injury [38,39]. Enzymatic PTMs of type III IF proteins have been considered in several important reviews [31,40]. Nevertheless, type III IFs are also subjected to numerous non-enzymatic modifications, including those induced by oxidants and electrophiles, which are arising as important determinants in filament structure and function and will be considered in more detail below.

1.3. General aspects of PTMs frequently associated with oxidative stress

All cells are exposed to reactive oxygen and/or nitrogen species (ROS/RNS) and possess defense mechanisms to avoid excessive oxidation. Oxidative stress arises when oxidant levels, either from endogenous or exogenous sources, surpass the cellular antioxidant defenses. Protein oxidation can occur upon exposure to diverse oxidant species and induce multiple structural alterations. Several excellent





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recent reviews have addressed the oxidative modifications of proteins [41,42], and of cysteine residues in particular [43,44], and provided detailed methodological information for their assessment. In addition, oxidative stress can increase the production of electrophilic species eliciting additional PTMs.

Depending on their type and/or extent, oxidative and electrophile-induced PTMs can contribute to cell homeostasis, signaling or stimulation of antioxidant defenses, or result in protein dysfunction,

Fig. 1. Schematic features of type III intermediate filament proteins and cartoon view of their assembly. The length of the full protein and of the head, rod and tail domains is shown for the four members of the type III class of IF (upper panel). Dots indicate the position of the conserved cysteine residue (black) and that of the additional cysteine of peripherin (red). Amino acid numbering for the various domains corresponds to the following entries from Uniprot: vimentin, P08670; GFAPα, P14136; desmin, P17661; peripherin, P41219. The lower panel depicts a cartoon view interpretation of the assembly process of type III IFs, as inferred from numerous in vitro works (see the text for details). Please note that in unit length filaments (ULFs), individual tetramers are deliberately not perfectly aligned. The 2-ULF "filament" shown at the bottom illustrates the potentially critical position of the cysteine residue, which could lie close to the region required for the "plugging" of two ULFs. Modifications of this residue with bulky moieties may have important consequences for filament assembly or elongation. Although the A11 tetramer assembly is considered the starting unit, once assembled in filaments, vimentin dimers can fall in different relative positions, giving rise to several tetrameric configurations, including those outlined in the figure by color shadowing: A11 tetramer (blue), ACN tetramer (yellow) and A22 tetramer (green). These tetrameric configurations have been schematically extracted at the right for easier visualization. Depictions of IF monomer and ULF are modified from Ref. [13]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

misfolding or aggregation, and to the impairment of protein interactions and functions, in the cell or in the extracellular medium [45–47]. Importantly, these PTMs can lead to structure-specific outcomes. Thus, depending on the protein, certain modifications can have virtually no consequences on protein function, whereas others can lead to activation or inhibition, or to the modulation of protein-protein interactions or subcellular localization [45,48,49].

Cysteines are the most nucleophilic residues and readily attack and form adducts with electrophiles of very diverse structure. Therefore, the moieties bound to a protein through cysteine residues can be very varied, and range from the addition of one oxygen atom or one oxygen plus one nitrogen atom, in the case of sulfenylation (sometimes called sulfenation) and nitrosation (frequently referred to as nitrosylation) [50], respectively, to bulky substitutions by endogenous reactive species or drugs (Fig. 2). Formation of disulfide bonds can result in covalent binding of the protein to small molecules, like cysteine or glutathione (GSH) (cysteinylation and glutathionylation, respectively), or to other proteins. Importantly, cysteine modifications can sometimes interconvert. Disulfide exchanges between proteins and small molecules can occur. Moreover, the nitroso moiety can be transferred between proteins or lead to NO release. Interestingly, nitrosoglutathione (GSNO) can induce transnitrosation or glutathionylation of target cysteine residues depending on their nucleophilicity. Thus, highly nucleophilic thiols break the S–NO bond leading to glutathionylation, whereas moderately nucleophilic thiols are more likely to become transnitrosated [51]. In turn, oxidized and electrophile adducted moieties can suffer further transformations leading to derived species and/or protein crosslinks [41].

An important aspect of cysteine oxidative modifications is stability. While some modifications are readily reversible (e.g. sulfenylation, disulfide formation and nitrosation), others are considered irreversible due to their high stability under biological conditions (e.g. sulfonylation). Some reversible modifications of cysteine residues, including glutathionylation or nitrosation have been considered as protective mechanisms preventing the occurrence of "irreversible" modifications, thus preserving protein function [52]. Cysteine residues can also undergo a plethora of enzymatic PTMs, including some catalyzed oxidative modifications, acylations, prenylation and methylation (for C-terminal isoprenylated cysteine residues), etc. [53–55]. Therefore, in certain cases there can be interplay between these PTMs leading to structurally and functionally diverse proteoforms [49].

The term **lipoxidation** refers to the modification of proteins by reactive products of lipid peroxidation [56]. These electrophilic lipids are generated under physiological conditions at moderate levels and they participate in signaling pathways and adaptive and cytoprotective responses that have been recently reviewed [57]. However, under pathological situations commonly associated with oxidative stress, the levels of these agents increase, favoring their reaction with other biomolecules including other lipids, DNA and proteins. The structures and concentrations of electrophilic lipids can be very varied (Fig. 3), and can range from small aldehydes, like acrolein or malondialdehyde (MDA) (56 and 72 Da, respectively), to cyclopentenone prostaglandins (cyPG) or isoprostanes (300–400 Da), or even oxidized or nitrated phospholipids (700–900 Da). Indeed, the number of reactive lipid species expands to several hundred [56]. Adducts with protein residues occur mainly through Schiff base formation with amino groups or

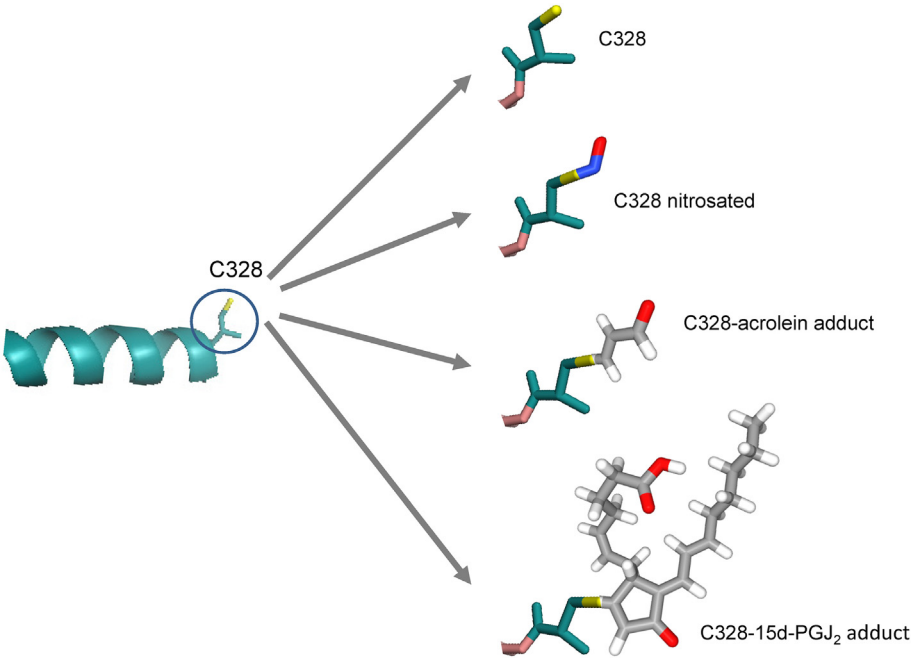

Fig. 2. Schematic representation of several modifications of vimentin C328. The figure shows a cartoon of a stretch of the alpha helical structure of a segment of vimentin and the C328 lateral chain prepared using PyMol and data from the PDB entry 3KLT [202]. Structures of nitrosocysteine, acrolein and 15d-PGJ_2 have been obtained from Pubmed Compound. This schematic representation illustrates the diverse size of the moieties that can bind to C328, potentially imposing steric hindrances or altering interactions required for filament assembly.

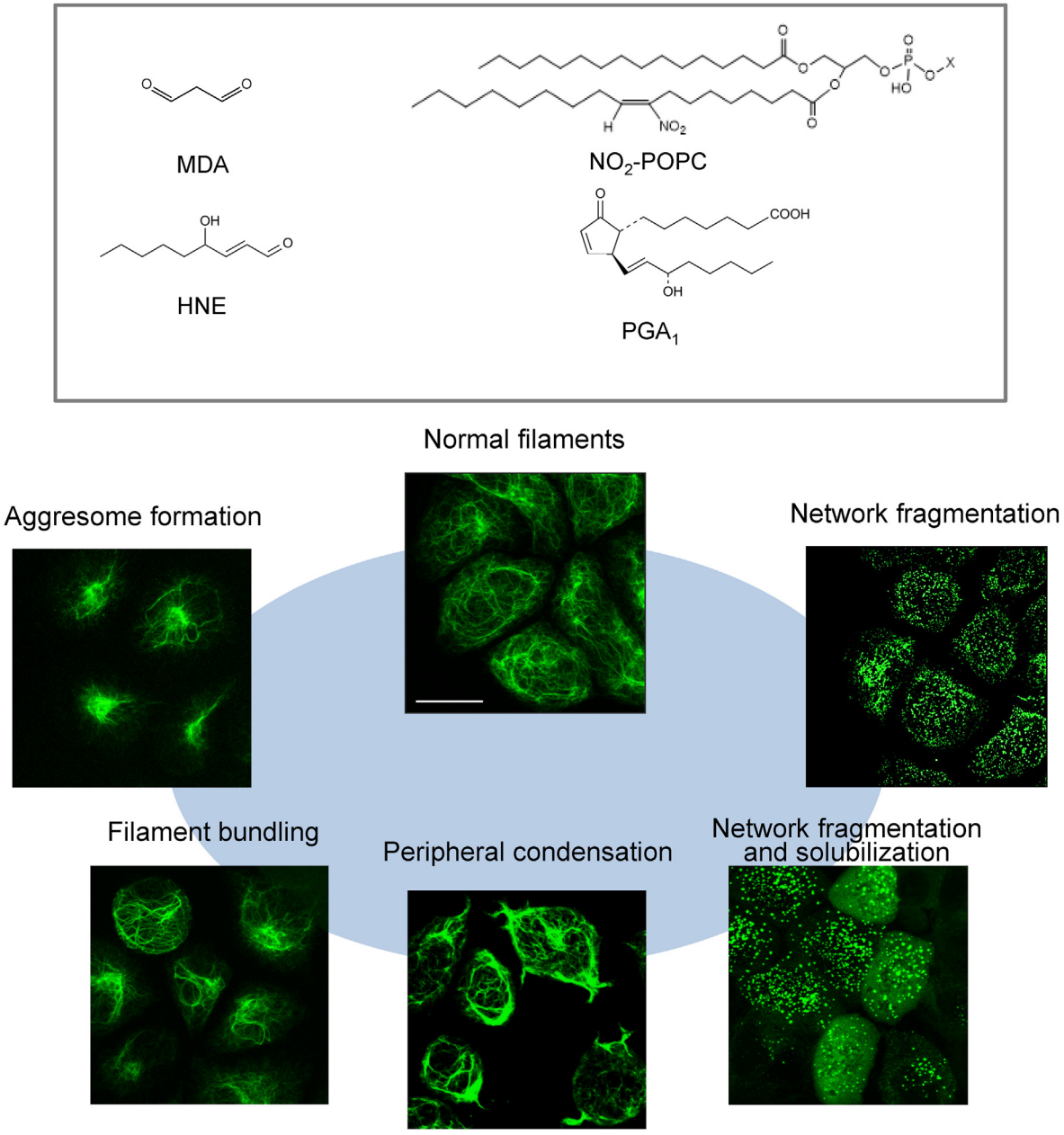

Fig. 3. Structures of diverse electrophilic species involved in lipoxidation and examples of cytoskeletal type III IF rearrangements. Upper panel, the structures of malondialdehyde (MDA), 4-hydroynonenal (HNE), prostaglandin A_1 (PGA_1), and nitrated 1-palmitoyl-2-oleyl-phosphatidyl choline (NO_2-POPC), are shown. Lower panel, confocal fluorescence microscopy images of IF reorganization in response to several strategies aimed at inducing cysteine modification in cells. A normal vimentin network together with examples of previously reported functional outcomes, observed upon treatment of cells with various electrophilic agents, are shown. Aggresome formation is illustrated by HNE treatment, as in Ref. [13]; network fragmentation alone or accompanied by solubilization, characterized by the diffuse background, correspond to treatment with diamide or diamide plus calyculin A, respectively, as in Ref. [91]; filament bundling is exemplified by treatment with 15d-PGJ_2 as in Ref. [91], and peripheral condensation, by treatment with NO_2-POPC as in Ref. [115]. Scale bar, 20 μm. Please note that the effects observed in cells can be the consequence of direct and/or indirect modifications of IF proteins, as well as of PTM interplay.

through Michael addition with various residues bearing nucleophilic moieties, like the thiol group of cysteine, imidazole of histidine and amino of lysine residues [48]. The stability of adducts formed will be influenced by several factors including the nature of the adducted residue and the occurrence of additional intramolecular reactions [58]. Moreover, both, non-enzymatic and enzymatic reversal of certain electrophilic lipid-protein adducts has been reported in vitro as well as in cellular models [59–61]. Therefore, lipoxidation can constitute a dynamic modification contributing to signaling mechanisms [60,61]. Given the structural variety of adducts their functional consequences (structure-activity relationships) can be diverse. There are several examples of differential effects of lipoxidation of cysteine residues, depending on the adducted moiety. Adduction of distinct electrophilic lipids to Ras proteins can differentially influence activation and subcellular localization [49,62]. Similarly, the aldo-keto reductase enzyme AKR1B1 is activated by addition of small moieties but inhibited by larger ones [63].

CyPG are electrophilic eicosanoids derived from prostaglandins by non-enzymatic dehydration [64]. They are characterized by the presence of an α,β-unsaturated carbonyl group in the cyclopentane ring that allows Michael adduct formation, preferentially with cysteine residues. One example of these eicosanoids is 15-deoxy-$\Delta^{12,14}$-

prostaglandin J_2 (15d-PGJ_2) (Fig. 2), which has been broadly studied both in cell culture systems and in vivo. The effects of cyPG, as those of many lipoxidation agents and oxidants, can have a dual or hormetic nature, depending on time or concentration. Low concentrations can activate the cellular antioxidant defenses and potentiate the initial inflammatory response, whereas high levels can elicit anti-inflammatory and antiproliferative effects and induce cytotoxicity [65,66]. Intracellular GSH is an important determinant of cyPG distribution and effects through various mechanisms, including the formation of cyPG-GSH adducts that can be involved in cyPG transport or sequestering, leading to a differential modification of proteins depending on the subcellular compartment [49].

Numerous studies on protein oxidative damage use the term carbonylation to refer to the formation of carbonyl groups on proteins. Chemically, carbonylation refers to the addition of carbon monoxide to a compound leading to the appearance of a carbonyl group. Appearance of carbonyl groups on proteins can occur by several mechanisms, including oxidation of amino acid lateral chains, oxidative deamination or formation of certain adducts with compounds that retain a free carbonyl group, as in some types of lipoxidation, like Michael addition of cyPG or of HNE, or after glycation or glycoxidation. As none of these processes involves carbon monoxide addition the term "formation of protein carbonyls", should be preferred instead of protein carbonylation, which, as stated above is not selective for a particular type of modification, but can arise by multiple mechanisms, as detailed in Ref. [67].

2. Vimentin

Early functional studies in cells lacking vimentin showed altered nuclear morphology and impaired distribution of certain organelles [68]. However, the finding that vimentin knockout mice were fertile and apparently developed normally [69] dimmed the interest in this protein. Nevertheless, subsequent works have established the role of vimentin as a key player in cellular organization and as a modulator and effector of numerous biological and pathophysiological processes. Indeed, vimentin knockout animals do not respond normally to many kinds of stress and show alterations in immune function [70], autophagy, cell migration and division, wound healing, function of the central nervous system, and arterial remodeling [71], among others. In addition, vimentin is an important marker and player of epithelial mesenchymal transition and its expression in certain types of cancer can be considered a sign of malignancy and invasiveness [16]. It is also necessary for cellular entry and/or toxic cellular actions of diverse bacterial and viral pathogens [72–74]. In contrast to other IF proteins, only a few mutations in the vimentin sequence have been identified. The E151K mutation [75], the heterozygous frameshift from V6C [76] and the missense (Q208R) [77] mutation have been associated with

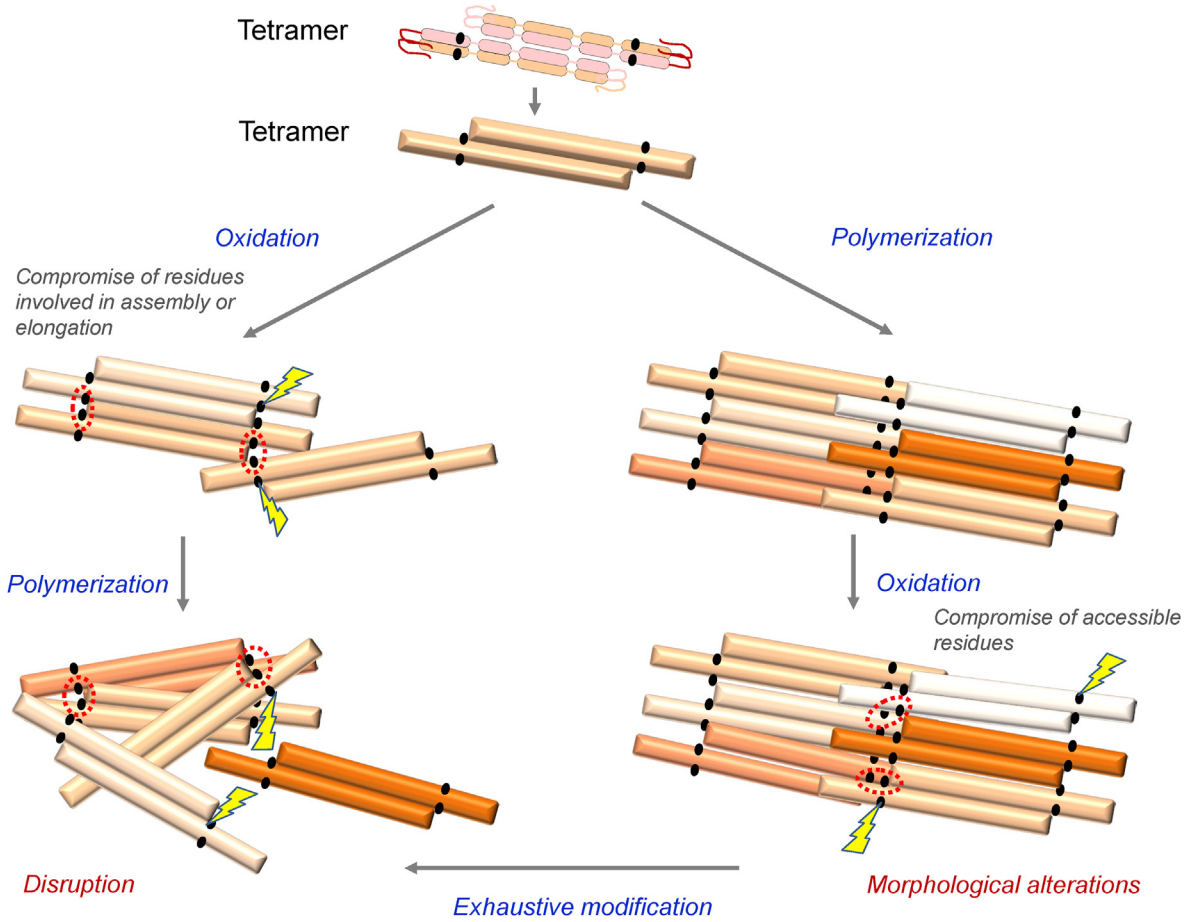

Fig. 4. Schematic illustration of the potential effects of oxidative modifications on type III intermediate filament assembly. A hypothetical model to conciliate the differential effects of certain oxidants on the assembly of soluble IF proteins and preformed filaments is shown. A very simplified representation of IF dimers (rectangles) and IF tetramers is depicted, showing the approximate positions of the cysteine residues (black dots). On the left, disulfide formation (red ellipses) or modification of cysteine residues by bulky moieties (yellow shapes) in soluble IF tetramers may interfere with their subsequent assembly in ULFs or with ULF elongation upon induction of polymerization, or may impede conformational rearrangements needed for proper assembly. Conversely, on the right, preformed filaments could undergo a certain degree of modification on accessible residues, or of disulfide formation between close cysteine residues, without suffering substantial disruption of their assembly. Nevertheless, modification above a certain threshold could lead to filament disruption. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cataract development. Recently, a dominant vimentin variant (L387P) causing a rare syndrome with premature aging has been reported [78].

Vimentin is the target of numerous enzymatic and non-enzymatic PTMs, some of which have been outlined above. Accumulating evidence indicates that vimentin behaves as a sensor for several kinds of stress, including oxidative stress and generation of lipid- or sugar-derived electrophiles that target several residues leading to versatile network remodeling [56,79] (Fig. 3). The single cysteine residue of vimentin (C328) appears to be highly susceptible to oxidation or addition of diverse species, both in vitro and in cells [25,80–84], suggesting that it is a preferred site of modification. Moreover, modifications of C328 play a key role in redox-related reorganizations of vimentin filaments, with diverse consequences on cell behavior.

Disulfide formation and other cysteine oxidations. Early studies using cytoskeleton preparations from several cell types subjected to oxidative in vitro crosslinking with Cu^{2+}-phenanthroline showed the formation of homo- and heterodimers of either vimentin and desmin or vimentin and GFAP, which led to propose that both proteins were present in hybrid filaments [85,86]. These studies indicated that cysteine residues in the "crosslinkable" units were exposed to the protein surface at a distance sufficiently close to allow interaction. Oxidized oligomeric species of vimentin were also detected in fibers isolated from eye lenses of several species, when extracted in the absence of beta-mercaptoethanol [87]. Later studies putatively identified vimentin as one of the proteins undergoing disulfide bonding in fibroblast-like synoviocytes subjected to oxidative stress by treatment with H_2O_2 [88], and soon after, Traub et al., showed that oxidized vimentin and desmin species, including homo- and heterooligomers of both proteins, formed abnormal filaments, which could be restored by reducing agents [89].

The involvement of the cysteine residue in disulfide formation and its impact on assembly was confirmed by using a C328A vimentin mutant that showed resistance to crosslinking by oxidation with H_2O_2 and $CuCl_2$ and formed apparently normal filaments in vitro [25]. It was then postulated that crosslinking of vimentin wild type could occur between neighboring dimers arranged in the A22 conformation, in which the cysteine residues fall in a closer position (Fig. 1). However, it was noted that this arrangement was still not ideal for disulfide bond formation unless additional flexibility or molecular motion within the tetramer occurred to allow a closer contact between the cysteine lateral chains [25].

Dimeric species of vimentin have also been observed in SDS-PAGE gels upon treatment with bifunctional cysteine crosslinkers, like dibromobimane (spacer arm 4.66 Å), both in vitro and in cells [13], indicating that in intact cells cysteine residues within filaments can also be present at a close distance. Moreover, the use of this reagent allowed obtaining vimentin-GFAP heterodimers from intact astrocytoma cells [90], thus suggesting the presence of both proteins in the same filaments as proposed in earlier in vitro works [85,86].

Recently, the electrophilic reagent diamide has been used to induce vimentin disulfide bonding [91]. This reagent reacts sequentially with two thiol groups facilitating disulfide bond formation. Diamide-treated purified vimentin appears as monomer and dimer species in non-reducing SDS-PAGE gels in approximately a 1:1 proportion. Notably, disulfide formation profoundly impaired subsequent polymerization into filaments of vimentin wild type, but not of a cysteine-deficient mutant. However, preformed filaments underwent disulfide formation to a similar extent than the soluble protein but were not disrupted by short diamide treatments. Therefore, these results suggest that promoting disulfide bond formation in soluble tetramers may lead to their association in conformations that are not productive for subsequent assembly, whereas once filaments are formed and the subunits are in place, a certain number of disulfide bonds can form between cysteines that fall within close distance without disrupting the whole filament (Fig. 4). Remarkably, although resistant to oxidation by diamide, filaments formed in vitro by a C328S mutant showed subtle, but significant, differences with vimentin wild type, calling attention on the importance of this residue for filament assembly [91,92]. Interestingly, diamide induces a major disruption of vimentin filaments in cells in such a way that filaments rapidly reorganize into discrete dots [13,91]. In cells, both soluble and polymerized vimentin coexist and exchange. Therefore, oxidation of soluble vimentin could hamper its incorporation into filaments leading to network disruption. Moreover occurrence of additional PTMs as a consequence of oxidative stress, could also contribute to diamide-induced filament fragmentation. Nevertheless, the fact that the C328S vimentin mutant is completely resistant to this effect, confirms the critical role of the modification of the cysteine residue in vimentin reorganization [91].

Formation of mixed disulfides can also occur between vimentin and small thiol-containing molecules, like GSH (glutathionylation) or cysteine (cysteinylation). Vimentin **glutathionylation** has been observed in several experimental models. Stimulation of isolated aortic rings with acetylcholine led to vimentin glutathionylation through an eNOS-dependent mechanism [93]. Notably, in this model system, glutathionylation can occur directly or after denitrosation. As eNOS possesses basal activity in endothelial cells, these observations suggest that glutathionylation could occur under basal conditions and constitute a physiological regulatory mechanism. Indeed, vimentin glutathionylation has been detected in control mouse embryonic fibroblasts [94]. Nevertheless, this modification increases under conditions of oxidative stress. In particular, loss of function of the *KRIT1* gene, which is associated with the pathogenesis of cerebral cavernous malformations, induces a decrease in the GSH/GSSG ratio and increased glutathionylation of several proteins, including vimentin, in cellular models of the disease [94]. Glutathionylation of vimentin has also been observed in oxidatively stressed T-lymphocytes or COS7 cells exposed to NO donors [93,95], and in human senescent fibroblasts, where it has been connected with cell growth arrest and cell death [96]. Moreover, data mining of the oxidized thiol proteome, has found a high proportion of vimentin C328 involved in disulfide bonding in aging and cataractous lenses [82]. From the functional perspective, the vimentin cysteine residue has been compared to cryptic cysteines in other proteins, in which glutathionylation could contribute to protein elasticity [97]. In addition, recent studies indicate that quantitative vimentin glutathionylation impairs vimentin elongation and provokes pre-formed filament severing [98], supporting the view that modification of C328 with bulky moieties could have important consequences for filament assembly. Nevertheless, the functional implications of vimentin glutathionylation in cells have not been fully elucidated and could depend on the proportion of the protein modified, and/or represent a protective mechanism against irreversible modifications.

The oxidation of vimentin in the extracellular space deserves special attention. Vimentin can be exposed at the cell surface or secreted by several cell types [99,100]. Cell surface vimentin can be labeled by cell-impermeable cysteine reagents. The observation that labeling can be increased by pretreatment with the reducing agent N-acetyl-cysteine suggests that part of the protein exposed is reversibly oxidized [101]. Interestingly, oxidative stress increases extracellular exposure of vimentin, frequently in an oxidized form [100], and it has been proposed that this phenomenon could constitute an early response of cells to oxidants from the extracellular space, and at the same time, generate oxidized and secreted species of vimentin that could be involved in pathogenesis of autoimmune diseases or act as a vehicle amplifying oxidative stress and lipid oxidation [74,102].

Nitrosation. The above observations show that vimentin cysteine can be modified under (patho)physiological conditions. Shear flow is a physiological stimulus that activates eNOS in endothelial cells leading to increased NO levels that can induce protein S-nitrosation. Indeed, vimentin was identified among the S-nitrosated proteins detected in endothelial cells after treatment with an exogenous NO donor or upon stimulation of endogenous NO production [103]. Moreover, C328 has been identified as the nitrosated residue in endothelial cells after shear stress, depending on eNOS activity [104], and after NO-donor exposure

[105]. Vimentin nitrosation can also occur under basal conditions and increase as a consequence of drug treatment. Statins, drugs widely used to control cholesterol levels, modulate eNOS expression in endothelial cells [106], and lead to increased eNOS activity and nitrosation of endothelial proteins, including vimentin [107]. Nitrosation has been proposed to occur in hydrophobic environments, and indeed, hydrophobicity plots predict C328 to be located in a hydrophobic motif, QSLTCEVDA [104]. Interestingly, the sequence -[I/L]-X-C-X_2-[D/E]-, has been reported to constitute a consensus motif for iNOS-S100 catalyzed transnitrosation [108], that can take place for instance in activated peripheral blood monocytes. Vimentin C328 is located in one such motif and has been validated as a target for nitrosation through this mechanism. The functional significance of vimentin nitrosation is not fully understood, although in endothelial cells it has been proposed to contribute to endothelial remodeling under flow. A recent study indicates that S-nitrosation of vimentin at C328 does not hinder, but slows down, vimentin elongation in vitro. These results shed light on the different functional outcomes of structurally different C328 modifications [98]. In addition, being a readily reversible modification, S-nitrosation could also act as a defense mechanism protecting proteins from deleterious oxidative modifications.

Lipoxidation. The stability of some adducts formed between vimentin and electrophilic lipids has allowed obtaining structural and functional information on the role of the lipoxidation of vimentin cysteine, both in vitro and in cells. Modification of vimentin by cyPG in vitro occurs selectively at C328 [80,91], and disrupts subsequent NaCl-elicited polymerization, inducing filament widening and bundling and formation of aggregates, in wild type but not C328S vimentin. Notably, these differential effects occurred in conditions under which the extent of vimentin lipoxidation was estimated to be approximately 10% [80], which is in the range of that estimated after treatment of cells with biotinylated cyPG analogs [109]. Nevertheless, at high 15d-PGJ_2 concentrations this selectivity is lost, since these compounds can form adducts with other nucleophilic residues, namely, histidine. Interestingly, as observed with oxidants, preformed filaments appear to be protected from disruption in vitro, even when modified to the same extent, potentially due to a different topology of the modification (Fig. 4).

The amenability of cyPG to labeling with biotin or other moieties has allowed their use as probes for lipoxidation. Using biotinylated 15d-PGJ_2 or PGA_1, vimentin was first identified as one of the major targets for these eicosanoids in mesangial cells, in which they induce a marked cytoskeletal rearrangement leading to perinuclear condensation of vimentin filaments into thick bundles [109]. Besides filament condensation, cyPG provoke the dislodgement of vimentin from the actin cell cortex in mitosis, which could have deleterious consequences for cell division [8]. The use of a vimentin C238S mutant has allowed identifying the cysteine residue as the key site for adduct formation and/or for the induction of vimentin filament disruption by cyPG in several cell types [13,80]. As other electrophilic lipids, cyPG covalently bind to multiple cellular targets and induce oxidative stress on their own, thus the possibility exists that some of their effects on the vimentin network may be indirect. Indeed, in addition to lipoxidation, 15d-PGJ_2 has been shown to induce reversible cysteine oxidation (cysteinylation) of certain proteins [110]. Nevertheless, the fact that the effects of cyPG are attenuated in the C328S mutant, both in vitro and in cells, indicate that this residue is a critical site for the action of these eicosanoids.

HNE is one of the most studied electrophilic lipids. The levels of HNE, and of HNE-protein adducts, increase under many pathophysiological conditions, including inflammatory, neurodegenerative diseases and aging [56,111], in some of which HNE can reach micromolar concentrations [112]. Lipoxidation of vimentin by HNE has been detected in human monocytic THP1 cells treated with this lipid by means of immunological and derivatization methods, and C328 was identified as the modification site using LC-MS/MS [81]. HNE-vimentin adducts have also been identified in senescent fibroblasts, mainly in soluble vimentin and vimentin fragments [113]. Notably, HNE can form several types of adducts with proteins, both through Michael addition and Schiff base formation [114]. In addition, HNE adducts can give rise to protein crosslinks. Consistent with this broad reactivity, HNE can modify both vimentin wild type and C328S in vitro [91]. However, the functional consequences of the modification are different; whereas HNE-pretreated vimentin wild type formed thicker and shorter filaments with a tendency to laterally associate, C328S vimentin was protected from these effects [91]. These results suggest that even though HNE can bind to other residues, the modification of C328 is functionally the most important. This is also supported by observations in cells showing that HNE treatment induces a juxtanuclear accumulation of vimentin wild type in thick bundles or aggregates, whereas in cells expressing vimentin C328S, the network is preserved and filament retraction is attenuated [13,56].

Among other lipoxidation agents, vimentin can be modified by MDA. Interestingly, immunization of mice with senescent cells led to the isolation of a polyreactive antibody that recognized vimentin at the surface of senescent primary human fibroblasts. In addition, an increase in vimentin modified by MDA at C328 was observed at the cell surface of senescent cells and in plasma of aged senescence-accelerated mice [83]. Based on these findings it was proposed that MDA-modified vimentin could serve as a senescence marker and potentially be recognized by the innate immune system to eliminate senescent cells. Other electrophilic lipids potentially interacting with vimentin include nitrated phospholipids (Fig. 3), which alter the cellular vimentin network in a manner dependent on the presence of C328, and shield this residue from alkylation in vitro [115].

Importantly, although the C328S mutant is protected from oxidative insults in cells, it shows a lower efficiency than the wild type at forming extended networks, supporting aggresome formation and promoting organelle position [13]. Therefore, even conservative substitutions of the cysteine residue have a measurable impact on vimentin function.

In spite of the importance of C328 in vimentin lipoxidation, modification of K235, but not of C328, by nonealdehyde has been detected in hepatic tissue from patients with end stage alcoholic liver disease or non-alcoholic steatohepatitis [116]. This modification eliminates the positive charge of K235, disrupting its electrostatic interaction with E230 [117]. This brings about the potential of lipoxidation to alter protein-protein interactions, with consequences for cell activation and/or cytotoxicity. Indeed, lipoxidized proteins are ligands for the receptor for advanced glycated end products (RAGE), which has been involved in the pathogenic consequences of hyperglycemia or inflammation. By abolishing the positive charges of lysine residues, lipoxidation disrupts the ion pairs with negative residues in their vicinity, enabling the latter to interact with positive residues in RAGE, favoring the activation of this receptor [118]. Through this mechanism, lipoxidized proteins could contribute to propagate the damage.

Protein carbonyl formation (PCF). Formation of protein carbonyls on vimentin has been reported in numerous studies using derivatization approaches, although, in many instances, the sites of modification have not been identified. Among evidence from cellular models, PCF on vimentin has been detected in rat aortic smooth muscle cells treated with homocysteine or bone morphogenic protein-4 (BMP-4) [119,120], HUVEC treated with sodium arsenite [121], and lung epithelial cancer cells exposed to acrolein or smoke extracts [122,123], these latter cells showing also vimentin crosslinked products [122]. Vimentin bearing protein carbonyls has been identified in non-tumor lung specimens of patients with lung cancer with or without chronic obstructive pulmonary disease [124]. Increased vimentin PCF has also been detected in cultured fibroblasts from AD patients with respect to those of control subjects, after exposure to Aβ(25–35). These observations suggest a dysfunction of antioxidant responses in AD [125], and raise the importance of vimentin as a target for oxidation in CNS diseases [126], together with GFAP, as it will be detailed below. PCF can occur also as a consequence of glycation. In primary cultured human dermal

fibroblasts vimentin was glycated predominantly at lysine residues located in linker regions likely exposed to the cytosol. This caused vimentin redistribution into perinuclear aggregates, which was accompanied by loss of fibroblast contractile capacity. Glycated aggregated vimentin was also detected in facial skin biopsies. Therefore, the accumulation of life-long vimentin glycation was proposed to contribute to loss of skin contractile properties during aging [79].

Other modifications. Vimentin tyrosine **nitration** has been detected by immunoprecipitation with an anti-3-nitrotyrosine antibody in mitochondrial diseases [127]. A similar approach was used to identify tyrosine nitrated vimentin in lung cancer [128]. However, in these studies, identification of the nitration sites was not achieved.

Tryptamine-4,5-dione is a product of the oxidation of serotonin that is highly reactive with thiols. Vimentin has been identified as a target for this putative neurotoxin in SH-SY5Y neuroblastoma cells, suggesting the modification of the thiol group, although this was not confirmed [129].

In summary, a plethora of vimentin modifications has been identified to date in many pathophysiological situations. Oxidative and non-oxidative modifications can coexist in many instances allowing their interplay, but an understanding of their structural and functional consequences requires further investigation.

3. GFAP

GFAP is the main IF expressed in mature astrocytes, which also express vimentin [130,131]. GFAP can be expressed in other cell types outside the nervous system, including fibroblasts and hepatic stellate cells [80,132]. A particular feature of GFAP among type III IF proteins is the existence of multiple isoforms that arise by alternative splicing and differ mainly in the tail domain, some of which are not able to form filaments on their own [14]. Here, we will focus on the most abundant isoform that is GFAPα (Fig. 1). This protein plays complex functions in astrocyte homeostasis which, to date, have not been totally unraveled. Studies in knockout animals have yielded diverse results pointing to the complexity of GFAP functions and their dependence on the experimental system. Roles in astrocyte migration, organelle distribution and mitosis have been defined. The presence of GFAP also influences neuronal functions, axonal and neuronal regeneration and several aspects of neurotransmission [14]. GFAP plays an important role in the response to damage, including inflammation, physical or ischemic trauma. In these processes, astrocytes become activated, overexpress GFAP and resume the expression of other IFs, like nestin and synemin [130]. GFAP appears to play a dual role in trauma, favoring the formation of a glial scar that limits the damage at the early stages, but slowing down regeneration at later stages [133]. The importance of GFAP in brain homeostasis is highlighted by the devastating consequences of its mutations, which lead to Alexander disease (AxD), a rare neurodegenerative disease that causes leukodystrophy, epilepsy, loss of brain white matter and ultimately death [17]. In AxD, GFAP forms aggregates known as Rosenthal fibers that accumulate other proteins as well, and show oxidative damage [134]. Interestingly, AxD associated mutations often involve substitutions of the wild type residues by cysteines [135], which raises the possibility that they undergo additional oxidative modifications.

GFAP is also a hub for PTMs and it has been reported to undergo phosphorylation at multiple residues. Phosphorylation plays an important role in the regulation of filament assembly and in the progression of mitosis and cytokinesis [136,137]. Moreover, increased phosphorylation of certain residues, in particular S13, has been associated with pathological conditions including frontotemporal lobar degeneration, AxD and hypoxia [138–140]. Other PTMs have also been associated with disease, including glycosylation and citrullination, the latter, rendering GFAP an autoantigen for autoimmune disease or appearing in conditions such as AD or ischemia reperfusion injury [38,39]. Moreover, GFAP degradation products have been observed in neurodegenerative diseases [141] as well as in models of viral infection [33].

GFAP can be targeted by numerous oxidative modifications and appears frequently in proteomic studies of pathophysiological conditions. Nevertheless, most studies focus on the variations of its levels as a marker of nervous system injury and less attention has been paid to its role as a target of damage. As specified above, **disulfide formation** between GFAP monomers, likely belonging to different tetramers, has been observed in vitro upon oxidative crosslinking [86]. Moreover, bifunctional cysteine reagents also lead to homo- and heterodimers of GFAP and vimentin in vitro and in cells [90]. These studies have substantiated the complex nature of these filaments in astrocytes and in astrocyte models. Moreover, results from cellular models have shown the requirement for the presence of the cysteine residue in GFAP for proper filament assembly, which highlights the functional importance of this residue. Interestingly, in primary astrocytes from mice deficient in both GFAP and vimentin, the requirement for the cysteine residue was more marked for GFAP than for vimentin, and this latter protein seemed to rescue the defect in C294S GFAP assembly to some extent [90]. GFAP has been found both in reduced and in reversibly oxidized forms in brain tissue from a subject with mild AD, by employing a differential thiol labeling protocol followed by 2D electrophoresis and MS identification [142]. Finally, the appearance of high molecular weight oligomers containing GFAP has been noted in several models of disease, including AxD [143], although the nature of these oligomers has not been characterized.

GFAP appears **S-nitrosated** in several conditions. A marked increase in GFAP nitrosation has been observed in synaptosomes of transgenic mice overexpressing mutated human amyloid precursor protein compared to wild type, suggesting its involvement in pathology [144]. However, S-nitrosation of GFAP has also been detected under control conditions and found not to increase in experimental models of murine autoimmune encephalomyelitis [145]. Therefore, the role of this modification in pathogenesis or as a defense mechanism requires further investigation.

Lipoxidation. Evidence on GFAP lipoxidation is gathering in recent years. Pick's disease is a type of fronto-temporal dementia characterized by severe atrophy of frontal and temporal lobes, neuronal loss and astrogliosis [146]. GFAP has been shown to be a major target of lipoxidation by HNE and glycoxidative damage in brain samples from Pick's disease patients compared to control subjects [146]. Down's syndrome patients develop symptoms of premature aging, including neuropathological features of AD. Therefore, observations in this syndrome have been considered clues for the understanding of AD. A 3.3-fold increase in HNE-modified GFAP has been observed in brain tissue from Down's syndrome patients compared to controls by means of immunological detection with an *anti*-HNE antibody [147]. HNE also targets elements of the protein degradation machinery and quality control, the impairment of which could contribute to accumulation of damaged or misfolded proteins. GFAP has also been recognized as a major target for HNE-protein adduct formation in frontotemporal lobar degeneration [148], although the site(s) of modification have not been identified. Notably, an 8-fold increase in MDA-modified GFAP has also been reported in brain samples from AD patients compared to aged-matched controls [149], whereas non-significant differences were found for vimentin modification in this study. Nevertheless, it should be taken into account that many of these reports use whole tissue samples for the proteomic studies. Although the evidence for lipoxidation is solid, novel approaches able to assess oxidative damage in specific regions and/or cell types or subpopulations in the brain may shed valuable information on pathogenic mechanisms [150]. Using cellular models such as fibroblasts and astrocytes, we have recently identified GFAP as a target for modification by cyPG [80,90]. In astrocytoma cells, cyPG produced an intense disruption of the GFAP network with retraction of filaments from the cell periphery and formation of curly bundles or aggregates in the proximity of the nucleus, whereas in

mouse primary astrocytes the most obvious effect was filament retraction and perinuclear condensation [90]. Biotinylated analogs of cyPG bind to GFAP in a manner dependent on the presence of its single cysteine, C294. Moreover, a cysteine deficient GFAP mutant, although showing altered filament assembly per se, is protected against network disruption induced by these electrophilic lipids. Similarly, treatment of cells in the presence of DTT attenuated network disruption, pointing to the importance of thiol modification in cyPG effect [90].

Protein carbonyl formation. Most of the evidence on protein carbonyl formation on GFAP has been obtained through the use of derivatization techniques combined with immunological detection after immunoprecipitation or 2D analysis, or by using adduct-specific antibodies (reviewed in Ref. [151]). These approaches have demonstrated PCF in AD, tautopathies including progressive supranuclear palsy and Pick's disease, and in Huntington disease [146,151–153]. An 8-fold increase in GFAP protein carbonyl levels has been found in samples from the frontal cortex of Down's syndrome patients compared to controls [154]. Moreover GFAP has been identified as the major carbonyl containing protein in the cerebral cortex of a patient with aceruloplasminemia, the hallmark of which is intracellular iron overload [155]. From all this evidence, it seems clear that GFAP constitutes a highly susceptible target for oxidative modifications in various neurodegenerative diseases. Nevertheless, whether the modifications lead to significant network rearrangements and whether they represent a toxic or a defense mechanism requires further study.

Nitration. Nitrated GFAP has been identified in normal rat cortex [156,157], and marked increases in nitration have been observed in a model of hypobaric hypoxia and reoxygenation [157]. In this model, intense nitration of other cytoskeletal components, including tubulin and γ-actin has been observed, for which a cooperation of these proteins in cytoskeletal disruption has been proposed.

Although in this review we have not considered the modifications of GFAP isoforms, the study of their specific modifications could yield valuable pathogenic information. Indeed, in proteomic studies, up to 46 forms of soluble GFAP could be separated by 2D immunoblotting from control and AD brain samples, which differed in size, phosphorylation, glycosylation and oxidation state [158].

4. Desmin and peripherin

Desmin is the main IF protein of mature striated muscle, where it is located in the sarcolemma, Z-lines, neuromuscular and myotendinous junctions. This protein provides strength to the muscle fiber during contraction and facilitates mechanochemical signaling [15,159]. Desmin and its protein binding targets form a scaffold that links the contractile system to the nucleus, mitochondria and other organelles through the Z-discs, allowing their crosstalk. This interconnecting role also enables transport between the extracellular and nuclear matrix [15,159]. More than 70 mutations in the desmin gene (*DES*) have been identified, which lead to different types of myopathy (e.g. myotilinopathies and desminopathies), including cardiomyopathy or isolated myopathies. Mutations have been identified in all the protein domains. Skeletal and cardiac muscle phenotypes involve mainly mutations affecting rod 2B, abnormal phosphorylation levels, and some oxidative modifications that correlate with desmin related myopathies (DRMs) and cardiac disease [15,159].

Desmin is a target for a variety of PTMs including phosphorylation, ADP-ribosylation, ubiquitination, glycation, oxidation and nitration. Most of them cause disassembly of the desmin network [160]. Also in this case, phosphorylation is by far the most studied PTM. Although alterations in desmin levels or distribution have been frequently encountered in pathophysiological situations associated with oxidative stress or in cellular models of electrophilic stress [161], the precise characterization of oxidative modifications and their impact on the function of the protein is not always available. Indeed, muscle activity induces thermal, mechanical and redox stress, which in cellular models of desminopathies elicit aggregation of desmin mutants [162] that can be attenuated by treatment with antioxidants, like, N-acetyl-cysteine or tocopherol [163].

As described above, oxidative crosslinking-induced dimers of desmin or of desmin and vimentin have been generated in vitro and showed impaired assembly [85]. Moreover, purified desmin subjected to in vitro oxidation with H_2O_2 and Fe^{2+} increases its content of random coil and β-sheet, in turn, decreasing its α-helix content according to far-UV CD spectra [164]. Also, oxidation can alter its susceptibility to proteolytic cleavage [164]. In myocytes, several redox modifications of desmin have been reported including disulfide linked and sulfenylated forms of the protein, as has been identified in studies using diamide, dimedone analogs or peroxide [165,166]. Desmin oxidation has also been confirmed in muscle biopsies from patients with desminopathies or myotilinopathies [159].

Desmin is also a target for lipoxidation. Functional derangement by HNE treatment has been reported [161], and acrolein-modified desmin has been identified in myocytes exposed to this toxic agent [167].

Among other modifications, desmin has also been identified as a target for nitration [127,159], and increased formation of protein carbonyls in skeletal muscle has been detected in sepsis, ischemia-reperfusion, diabetes and chronic obstructive pulmonary disease (reviewed in Ref. [168]). However, the functional consequences of these modifications are not yet clear.

Peripherin expression is mainly restricted to the peripheral nervous system, but also occurs in neurons of the CNS. There are several forms of peripherin generated by alternative splicing, some of which increase in response to traumatic neuronal injury [169]. The role of this protein is unclear, but it seems to contribute to IF cytoskeleton organization and axonal elongation. The protein is a major autoantigen in type 1 diabetes [170] and appears in compact inclusions in several diseases, including amyotrophic lateral sclerosis (ALS). Screening for sequence variants in the peripherin gene (*PRPH*) has been performed in patients with ALS and several SNPs have been identified [171]. These include an insertion in intron 8 (IVS8-36insA), a 1 bp deletion in exon 1 (228delC) leading to a truncated form of the protein, and a homozygous mutation in the first linker generating the D141Y mutation. Peripherin overexpression induces motor neuron degeneration in transgenic mice, whereas the knockout mice seem to compensate lack of *PRPH* expression by inducing that of vimentin and α-internexin, apparently leading to decreased myelination of only a subset of sensory fibers [172].

Peripherin is by far the less studied of the type III IF class, and is the only one that possesses two cysteine residues. Nevertheless, several oxidative modifications have been reported. A disulfide-linked peripherin dimer of 130 kDa has been detected in rat sciatic nerve and dorsal root ganglia [173]. Non-differentiated N2a neuroblastoma cells treated with hydrogen peroxide showed concentration-dependent changes in the levels of peripherin, together with alterations in the proportion of the peripherin forms, Per-58 and Per-45. Moreover, peroxide (100–500 μM) induced loss of filament structure and inclusions in non-differentiated cells, whereas retraction of neurites was detected at 10 μM. However, peripherin filaments and aggregates did not colocalize with carbonyl markers, despite increased total protein carbonyl formation [174].

The best characterized oxidative modification of peripherin is nitration, which has been described upon NGF-induced differentiation in PC12 cells, and occurs at the head (Y17) and tail (Y376) domains [175]. Apparently, the ratio between nitration levels and peripherin expression before and after NGF addition increases during differentiation. Remarkably, nitrated peripherin is only present in the insoluble cytoskeletal fraction. Heavy tyrosine nitration and phosphorylation of peripherin have been observed in ALS, in association with disruption of filament association and appearance of inclusions or aggregates [169].

5. Interplay between PTMs in type III IFs

In any given situation, proteins are subjected to multiple PTMs that can occur on the same or on different protein molecules in different combinations, giving rise to structurally and functionally different proteoforms. Type III IFs and, in particular, their conserved cysteine residues, are emerging as hot spots for modification by oxidants and electrophiles. Indeed, the studies summarized above highlight the susceptibility of these cysteine residues to a plethora of modifications including nitrosation, disulfide formation, sulfenylation and addition of various electrophilic lipids including HNE, MDA and cyPG. Interestingly, a complex interplay between modifications can take place both by direct and indirect mechanisms (Fig. 5). Reversible cysteine modifications, for instance, glutathionylation, could prevent irreversible oxidations or adduct formation; however, some oxidative modifications can increase or decrease the reactivity of the affected residues [176].

Phosphorylation is probably the most studied modification regulating protein function. Many oxidant species act on signaling proteins, for instance, kinases and phosphatases, triggering additional modifications of IFs. Vimentin is a target for phosphorylation by numerous kinases, including PKA and AKT, which are directly and/or indirectly regulated by redox mechanisms [177]. The impact of oxidative modifications on kinase activity can lead to activation or inhibition. In turn, tyrosine phosphatases are generally inhibited by oxidative stress, whereas oxidation of serine/threonine protein phosphatases can result in activation or inhibition. Interplay of nitric oxide with vimentin phosphorylation has been reported through modulation of PP2A activity [178]. Phosphatase inhibition would result in vimentin hyperphosphorylation, which could contribute to network disruption by oxidative modifications. In this context, we have recently observed that the phosphatase inhibitor calyculin A, an inhibitor of PP2A and PP1 phosphatases, potentiated the fragmentation of vimentin network induced by diamide, whereas a phosphorylation-deficient vimentin mutant "SA mutant", in which 11 phoshorylatable residues of the N-terminal domain had been substituted by alanines, was partially protected from the diamide-disruptive effect [91].

A complex interplay can also occur with the protein degradation machinery. Certain oxidative insults or other types of stress can lead to the degradation of vimentin and GFAP by activating proteases [179], although some ubiquitin ligases and subunits of the proteasome can be inhibited by lipoxidation [180], which, as stated above, would result in

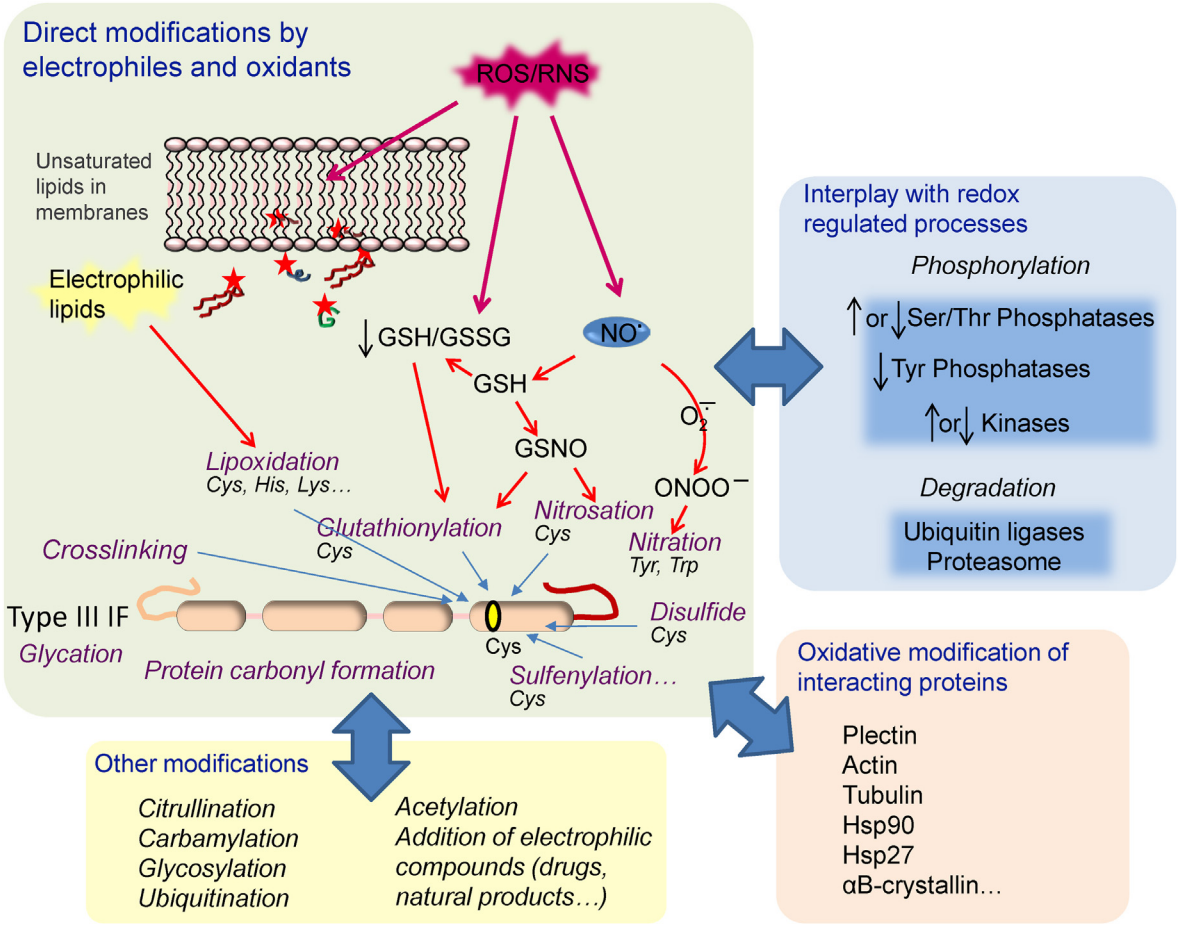

Fig. 5. Interplay between posttranslational modifications in type III intermediate filaments. Under oxidative stress, several oxidants are generated that can react with unsaturated lipids in membranes giving rise to the formation of electrophilic lipid species. These can directly form adducts with proteins in a process called lipoxidation. Oxidative stress also diminishes the ratio GSH/GSSG and favors the formation of disulfide bonds between glutathione and thiol groups in proteins (glutathionylation). This modification can also be mediated by nitrosoglutathione, which can also induce S-nitrosation. Oxidative modifications of cysteine residues can also lead to the formation of sulfenic, sulfinic and sulfonic acids. Other oxidative modifications include protein carbonyl formation, nitration or formation of protein crosslinks (see Ref. [41] for review). Other PTMs that are under redox control, such as phosphorylation and protein degradation will be modulated in situations of oxidative stress, thus contributing to the diversity and complexity of proteoforms arising under these conditions. The function of type III IFs will be also affected by oxidative modifications of interacting proteins, such as those described for plectin, actin, and several chaperones. Importantly, interplay can also take place with other PTMs, including those listed in the lower panel, which can occur on the same or nearby residues, affecting their availability or accessibility to modification.

accumulation of damaged proteins.

Interestingly, due to their polyelectrolyte properties, IFs interact with cations such as Ca^{2+}, Mg^{2+} or Zn^{2+} which, depending on their size and concentration, influence IF assembly [13,92,181]. Importantly, Zn^{2+} availability can influence the occurrence of certain PTMs on vimentin [13], which could occur through direct interaction or through its capacity to elicit antioxidant or pro-oxidant effects [182,183]. Although Zn^{2+} is a redox-inert ion, it can influence cellular redox status because it is necessary for the activity of enzymes involved in the antioxidant defense, whereas its deficiency or excess cause oxidative stress [183].

In addition, modification of interacting proteins can have an important impact on the regulation of IFs localization and function. The linker protein plectin has been reported to be nitrosated, affecting vimentin distribution [184], whereas actin and tubulin are targets for various oxidative and lipoxidative modifications [56]. Moreover, certain modifications can compete for the same site, for instance, carbamoylation, resulting from modification by isocyanic acid [185], can occur at citrullination sites, and tyrosine nitration could preclude phosphorylation. Modification of a given residue can also affect that of nearby amino acids, either by altering their reactivity or their accessibility. Therefore, bulky moieties can shield nearby residues, but certain modifications can cause unfolding and exposure of additional amino acids for modification. Additionally, many conditions associated with oxidative stress and oxidative modifications trigger important changes in gene expression, including the antioxidant stress response [57], contributing to the complexity of the effects.

6. Vimentin and other IFs as drug targets

Given their implication in pathological processes, IFs in general, and those belonging to the type III family in particular, are becoming important drug targets. Indeed, hypothesis-driven, as well as virtual and pharmacological screening approaches are being used to find small molecule modulators of these proteins. High expression levels of type III IF proteins have been considered as pathogenic factors in several diseases. Therefore, efforts have been devoted to find strategies to modulate their levels or organization in cells [186,187]. Nevertheless, this section will focus on approaches related to PTMs and more precisely on drugs that directly bind to these proteins and preferentially interact with their conserved cysteine residue.

As vimentin C328 and the equivalent cysteine residues in the other members of the family seem to be highly susceptible to modification by oxidant and electrophilic species, their modification by cysteine-reactive drugs is worth exploring. Among the first compounds reported to target vimentin C328 is the withanolide natural product withaferin A (WFA). This molecule was studied based on its antitumor and anti-angiogenic properties and was reported to covalently bind to C328 and induce vimentin aggregation in vitro and filament disruption in cells [188]. In addition, WFA caused vimentin downregulation in cells and showed in vivo antiangiogenic effects, reportedly mediated by vimentin [188]. It was later described that this molecule could bind to the single GFAP cysteine residue, C294 [189]. Moreover, it was shown that WFA reduced the levels of both vimentin and GFAP in primary astrocytes as well as in mouse retina in vivo [189]. Therefore, these WFA actions could be beneficial in situations associated with increased expression of these proteins, such as reactive gliosis. Thus, WFA has become a popular tool employed to target and disrupt the vimentin network in several experimental models [190]. However, being a cysteine reactive molecule, WFA could interact with other cellular targets. In fact, several works have called attention to its lack of specificity [191]. Indeed, WFA has recently been reported to covalently bind the heterogeneous nuclear ribonucleoprotein K (hnRNP-K) [192], and tubulin, and induce the degradation of the latter [193]. Moreover a vimentin mutant lacking C328 appears to respond to WFA as well [191]. Recent molecular dynamics simulations indicate that WFA binds in the vicinity of C328, but not directly to this residue [192]. Therefore, although WFA may be useful to disrupt vimentin and other type III IF proteins, its use as a selective target should be performed cautiously. Interestingly, the compound arylquin 1 also binds to vimentin at a region close to the cysteine residue. In this case, interaction of arylquins with vimentin displaced the binding of Par4, an apoptosis-inducing protein, with consequences for cell viability [194].

Additional evidence of the importance of C328 as a drug target has risen from the garlic compound ajoene [195], which has been shown to form a disulfide bond with this residue. This modification is associated with condensation of vimentin filaments and with an impairment of several vimentin-related processes, including cell migration and invasion of cancer cells. These effects were attenuated in cells in which vimentin expression had been transiently knocked down, thus pointing to vimentin importance as mediator of ajoene actions. Notably, another garlic defense substance, allicin, has been shown to induce S-thioallylation of several cytoskeletal proteins, including vimentin, in Jurkat cells [196]. In addition, phenyl vinyl sulfones, mechanism-based inhibitors of protein tyrosine phosphatase, covalently bind vimentin C328 in cells, although the significance of this finding is still not known [197,198].

A number of proteomic studies searching for protein targets of drugs or natural products have found vimentin [199], although the precise site of interaction or the functional consequences have not always been elucidated. Additional compounds found to bind vimentin include two quinone methide metabolites of the phenolic antioxidant butylated hydroxytoluene. These metabolites are believed to be responsible for promoting lung tumor formation in mice [200]. In contrast, the green tea polyphenol epigallocatechin gallate binds to vimentin and inhibits its phosphorylation in cells, which is associated with an antiproliferative effect [201].

7. Concluding remarks

Type III IF proteins are arising as integrators of basic cellular processes and as sensors of stress. These proteins, and in particular their conserved cysteine residue, have been proposed as hot spots for PTMs. Modification of this cysteine residue leads to marked reorganization of type III IFs in cells, which is supported by the lack of response of cysteine-deficient mutants. Remarkably, the nature of the reorganization appears to depend on the extent and the structure of the modification, illustrating an exceptional versatility of type III IFs remodeling. Moreover, this suggests that the conserved cysteine residue of type III IFs functions as a hub that can accept different types of modifying moieties and transduces them into distinct cytoskeletal responses, either per se or in cooperation with other PTMs, thus acting as a sensor for various signals. The position of the conserved cysteine residue in the structure of the protein could be critical for the functional outcome of the modifications, affecting its organization in filaments. Nevertheless, many aspects of structure and function of type III IFs still need investigation. Elucidation of the structure of filaments and of their various arrangements in cells will be key to understand the basis of their response to stress. Moreover, the downstream consequences of the diverse reorganizations are still poorly understood. The modification of type III IFs by oxidant and electrophilic species could impact on many cellular processes, but it also could represent a defensive mechanism by which these proteins would act as decoys or scavengers of reactive species. Therefore, further identification and characterization of the PTMs occurring in vivo, both structurally and quantitatively, will be necessary to elucidate their role in physiological IF regulation and their potentially protective or pathogenic effects under pathophysiological conditions. Finally, certain oxidative PTMs of type III IFs can be considered as disease biomarkers, whereas their modulation could become a therapeutic strategy in a variety of illnesses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101582.

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