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Current Opinion in Cell Biology

The redox-responsive roles of intermediate filaments in cellular stress detection, integration and mitigation

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Abstract

Intermediate filaments are critical for cell and tissue homeostasis and for stress responses. Cytoplasmic intermediate filaments form versatile and dynamic assemblies that interconnect cellular organelles, participate in signaling and protect cells and tissues against stress. Here we have focused on their involvement in redox signaling and oxidative stress, which arises in numerous pathophysiological situations. We pay special attention to type III intermediate filaments, mainly vimentin, because it provides a physical interface for redox signaling, stress responses and mechanosensing. Vimentin possesses a single cysteine residue that is a target for multiple oxidants and electrophiles. This conserved residue fine tunes vimentin assembly, response to oxidative stress and crosstalk with other cellular structures. Here we integrate evidence from the intermediate filament and redox biology fields to propose intermediate filaments as redox sentinel networks of the cell. To support this, we appraise how vimentin detects and orchestrates cellular responses to oxidative and electrophilic stress.

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Current Opinion in Cell Biology 2024, **86:**102283

This review comes from a themed issue on **Cell Architecture (2023)**

Edited by **John Eriksson** and **Patrick Lusk**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.ceb.2023.102283

Keywords

Oxidative stress, Cysteine modification, Oxidation and lipoxidation, Vimentin, Redox signaling.

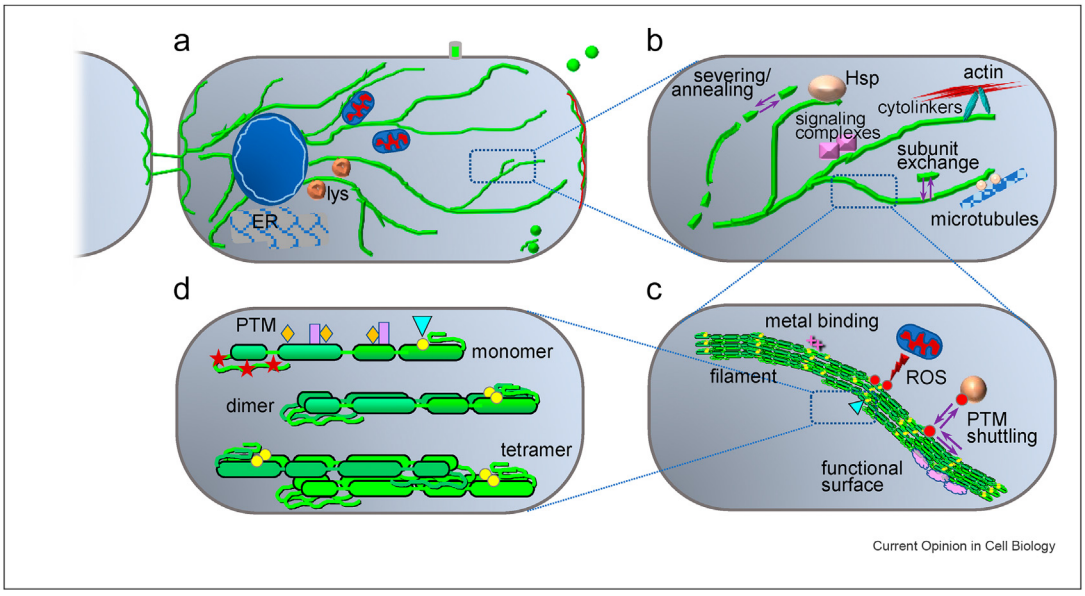
Abbreviations

ER, endoplasmic reticulum; GFAP, glial fibrillary acidic protein; MEF, mouse embryonic fibroblast; PTM, posttranslational modification; ROS, reactive oxygen species.

Introduction

The cytoskeleton of the cell comprises three main filament systems and a multitude of interacting and linking proteins. Each system plays its own specific roles, and at the same time integrates into a complex assemble of molecular machines [1,2]. Of the three filament systems, intermediate filaments afford the greatest diversity and possess unique properties, which are key to mitigating and coordinating aspects of the cellular stress response [3,4]. Specifically, they are non-polar filaments that can fuse, severe and exchange subunits along their length (Figure 1), and play functions in cell signaling beyond the biomechanical role classically attributed to them [5–8]. In contrast to the few building blocks constituting actin structures and microtubules, there are more than seventy different proteins, classified into six protein families, that form the cytoplasmic and nuclear intermediate filaments [9]. They possess a modular structure that includes intrinsically disordered head (N-) and tail (C-terminal) domains flanking a central rod domain, which is mostly α-helical (Figure 2a). Certain intermediate filaments, such as keratins and vimentin, are key components of the cell cortex [10,11], and connect the plasma membrane and the cell periphery to the nuclear compartment [2,12]. In the nucleus, lamins, line and shape the inner surface of the nuclear envelope, and intimately interact with chromatin to safeguard nuclear and genome integrity [13]. It is the trans-cellular network of cytoplasmic and nuclear intermediate filaments that physically interconnects different compartments, both within an individual cell and between cells in a tissue [2,14] (Figure 1). This interconnection underlies the mechanosensory roles of intermediate filaments, but to settle on this as their primary function, would imply to ignore their critical role(s) in the detection and management of heat, oxidative and chemical stresses [3,9,14–17]. In this review, we explore the mechanistic links between these various stresses and intermediate filaments as key players and integrators of cellular responses.

Figure 1



Features of intermediate filaments at the cellular and molecular levels. (**a**) Intermediate filaments form extended networks in the nuclear envelope, cytoplasm and cell cortex, providing interconnectivity and protection for numerous cellular organelles, including the nucleus, endolysosomes (lys), mitochondria and endoplasmic reticulum (ER). In addition, intermediate filaments appear in various cell surface or extracellular structures, which support intercellular communication, including plasma membrane, primary cilia, tunneling nanotubes and extracellular vesicles as well as other extracellular forms (represented as green cylinders and spheres). (**b**) Within the cytoplasm, taking vimentin as an example, intermediate filaments form versatile and dynamic filament structures which cannot only elongate at both ends and exchange subunits along their length, but sever and join sections enabling their rapid reorganization. Here, they interact with a plethora of proteins such as chaperones, cytolinkers, signaling proteins, etc. (**c**) Intermediate filaments are the target for numerous PTMs, including various cysteine modifications. These PTMs can occur along the length of the filament in different proportions and combinations, depending on the location, the proximity to sources of ROS, and protein–protein interactions. They can be shuttled within the filament and with interacting proteins and contribute to functionalize the surface of the filament, which can have multiple effects such as creating cell compartments. Cysteine residues are represented by yellow circles. Nevertheless, this scheme does not intend to accurately depict filament structure. (**d**) At the molecular level, intermediate filaments are formed by homodimers which form antiparallel tetramers and then associate head to tail. Precisely the head and tail domains of intermediate filaments are intrinsically disordered segments which establish labile interactions essential for proper filament assembly. This cartoon view depicts the monomer, dimer and tetramer species with the approximate position of the conserved cysteine residue of type III intermediate filaments highlighted by yellow circles. A monomer with several PTMs is schematically represented. Nevertheless, the number of modifications on a single proteoform could be dozens.

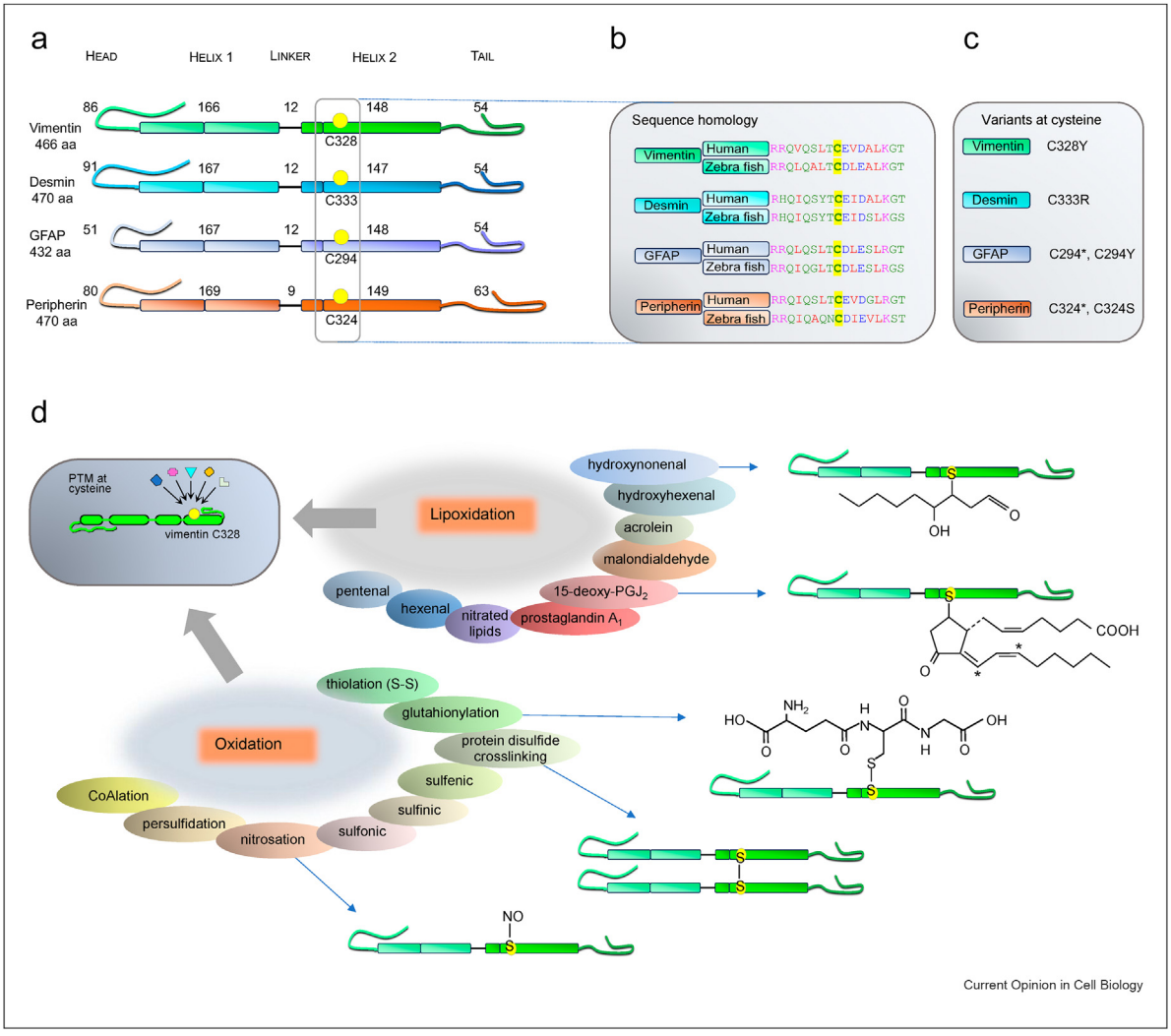
Intermediate filaments as key players in cellular stress responses

The concept of intermediate filaments as stress proteins is currently accepted, although they do not have either protein chaperone or enzymatic activities *per se* (see the study by Leube et al. [3] and citations therein). Nevertheless, intermediate filaments participate in the organization of the endoplasmic reticulum, modulate gene transcription, interact with and regulate stress kinases, and act as signaling platforms, which put them at the heart of the stress response [9,18–22]. Importantly, the physical network formed by intermediate filaments hardwires signaling and cellular responses, and is ideally placed to provide a structural, but reactive interface integrating redox signaling, stress responses and mechanosensing [8,23–25].

Recently, membraneless organelles formed by the phase separation of cellular macromolecules have been proven essential for the organization of certain cellular structures, and for fundamental cellular processes, and in particular for the heat shock response [26,27]. The key to such reversible phase separations in the cell is the involvement of polyanions, such as nucleic acid and protein polymers [26]. For proteins, the co-existence of structured domains and intrinsically disordered domains is crucial [28]. Intermediate filament proteins are prefect examples of this, with N- and C-terminal intrinsically disordered domains extending from the central, α-helical, rod domain [29,30] (Figures 1d and 2a). Specifically, sequences within the N-terminal domains of vimentin, desmin and certain neurofilaments appear critical to their phase separation properties [31].

Under certain stresses, such as heat shock, intermediate filaments undergo rapid reorganization (Figures 1b and 3). Now it is appreciated that these rearrangements, leading to a concentration of their associated chaperones [27], proteostatic machinery [4] and signaling molecules [5] constitute a tunable response to support cell survival. This rapid and targeted spatial and temporal response of intermediate filaments relies in their unique

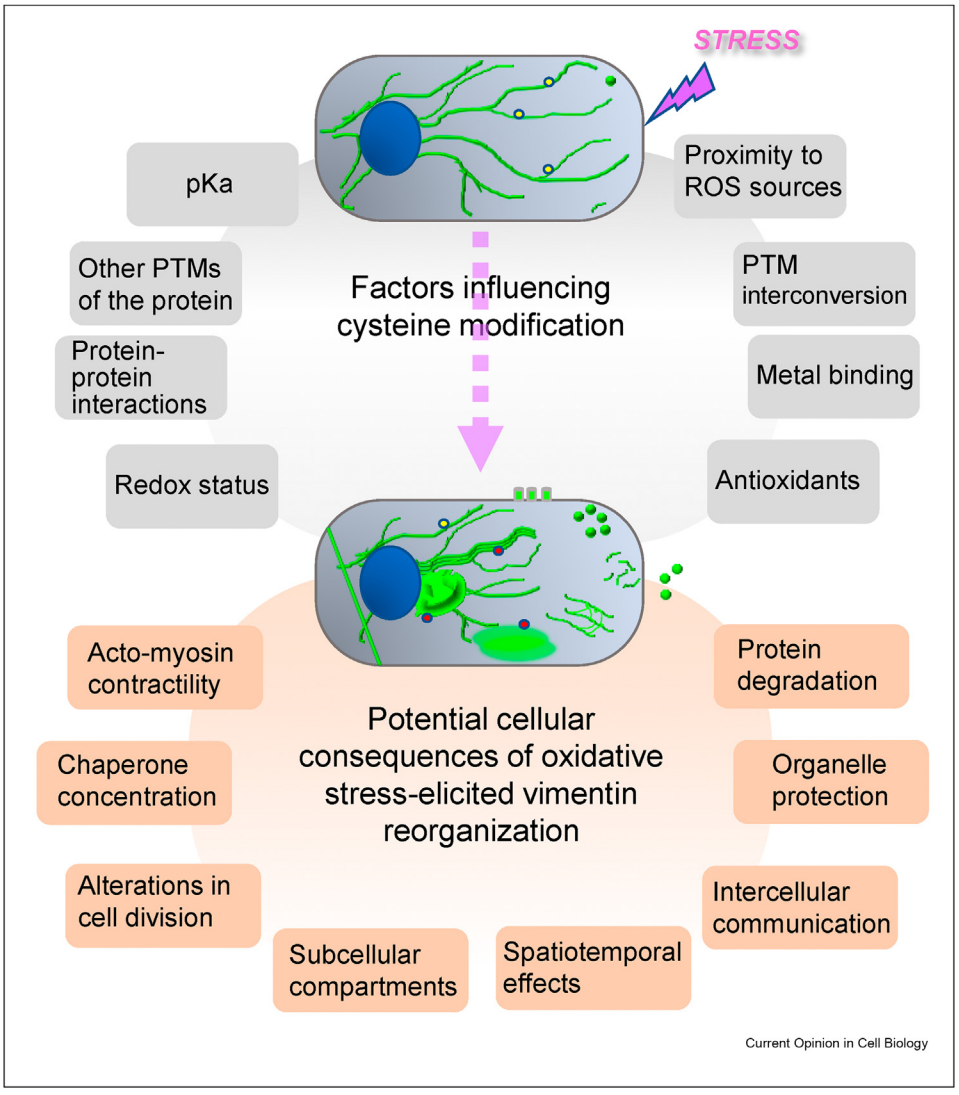
Figure 2



Main features of the conserved cysteine residue of type III intermediate filaments and the oxidative and lipoxidative modifications reported for vimentin C328. (**a**) Cartoon view of the structure of the monomers of the type III intermediate filament proteins, illustrating the head, rod (comprising the two main helical segments plus the linker) and tail domains, with their corresponding lengths in number of amino acids (aa), as well as the position of the conserved cysteine residue. Amino acid numbering corresponds to the human proteins. Nevertheless, the position of C328 of vimentin is conserved in rat and mouse. (**b**) Detail of the sequences surrounding the conserved cysteine residues in the four members of the type III intermediate filament class, both from human and from zebra fish, illustrating the high homology of this region (obtained with MUSCLE multiple sequence alignment). (**c**) Variants affecting the conserved cysteine residue identified in the four members of the class according to the Uniprot database, with accession numbers: vimentin, P08670; desmin, P17661; GFAP, P14136; and peripherin, P41219. (**d**) A considerable number of different PTMs of vimentin C328 have been reported. Studies in vitro indicate that structurally different modifications may exert distinct effects on filament assembly and/or morphology. Schematic examples of some of the modifications for which a functional correlation has been characterized in vitro are shown at the right. Non-overlapping vimentin network morphological patterns are observed in cells treated with diverse oxidants and electrophiles. However, these are likely the result of multiple modifications affecting not only C328 but other residues in vimentin as well as other cellular proteins. For details and references, please, see text and [16].

remodeling properties, which unlike microfilaments and microtubules, show dynamic assembly and disassembly at any point along their entire length [8,9,22] (Figure 1). These filament and subunit dynamics are powered by a complex array of posttranslational modifications (PTMs) (Figure 1c). Intermediate filaments can be viewed as a cellular compartment in its own right, providing a physical but tunable platform for signaling complexes, protein–protein interactions, organelle docking, and a hub for posttranslational modifications (PTMs) [32–34]. PTMs have the potential to affect the function, not only of the intermediate filaments, but also of their associated proteins, and in some cases, they can be exchanged between them (Figure 1c). Besides forming versatile intracellular arrays, certain intermediate filament proteins can be exposed at the cell surface or released into the extracellular medium, where they can behave as autocrine and paracrine signals for

Figure 3



Factors influencing cysteine modification and potential consequences of vimentin reorganization of vimentin under stress. Various kinds of stress induce vimentin remodeling, which can adopt different patterns, ranging from filament condensation, stiffening, bundling, aggregation, retraction from the cell periphery, fragmentation, disassembly, and even its release from cells to either bind to the cell surface (small cylinders) or be transported in various forms (extracellular spheres). Vimentin single cysteine residue (unmodified, yellow circle; modified, red circles) is necessary for full vimentin reorganization under oxidative and electrophilic stress. Several factors can affect the reactivity and accessibility of this cysteine residue, including protein–protein interactions, local ROS gradients, presence of metals, levels of antioxidants and/or occurrence of other PTMs. In turn, vimentin modification and/or disruption will influence other cellular processes, including protein degradation, organelle and protein localization, cytoskeletal interplay, nuclear integrity, cell division, intercellular communication, or the generation of subcellular compartments.

neighboring cells and tissues (Figure 1a), either propagating stress or providing protection against it [35,36], reviewed in the study by Ramos et al. [37].

Intermediate filaments are key managers of mechanical stress, caused by cell stretching, compression, bending and shear forces. Intermediate filaments are the most adaptable and resilient cytoskeletal elements. They have the ability to bend in response to stress and stiffen or soften in an "intelligent" manner to protect cellular structures [8]. For instance, vimentin filaments mediate a compression-elicited stiffening of cells that protects the nucleus [38]. In contrast, under osmotic stress, in particular during hypotonic shock, vimentin filaments undergo a fast, reversible disassembly, which constitutes the first cytoskeletal response [39,40]. Although this could suggest that vimentin yields to this kind of stress, it actually sustains its protective role, since vimentin depletion impairs cell resistance to hypotonic shock [40]. Vimentin also protects cells against proteostatic stress caused by extensive protein damage or accumulation of unfolded proteins, by forming cage-like structures that

enfold the damaged proteins, and helps localize them and the protein degradative machinery to the aggresomes [4], thus playing an important role in protein clearance. In addition, because of their multiple phosphorylation sites, a role for vimentin as a buffer for phosphorylation events has been proposed [9]. These observations evidence a strong correlation between intermediate filaments, and particularly vimentin, with stress resistance in cells. Although often overlooked, redox signaling and oxidative stress underlie the response of cells to various kinds of stress [41,42], and contribute to the modulation of intermediate filament function, which will be considered below.

Reactive oxygen species and electrophilic lipids in stress

Reactive oxygen species (ROS) are important biological signals, generated as a consequence of cell respiration, and through the action of various enzymes [43]. ROS are essential for processes such as cell metabolism, gene transcription, cell division and cell programming [44]. ROS are normally balanced by cellular antioxidant defenses, in the form of millimolar concentrations of small molecule anti-oxidants such as glutathione and ascorbate, and the presence of antioxidant proteins and enzyme systems such as thioredoxin, superoxide dismutase, catalase and glutathione peroxidase [44,45]. In a simplified view, when the generation or exposure to oxidants surpasses the cellular antioxidant defenses, oxidative stress arises. Although an increased generation of oxidants can evoke cellular defense or adaptation responses, uncontrolled oxidative stress is a pathogenic factor concurring and/or contributing to numerous disease conditions, including metabolic syndrome, inflammatory, neurodegenerative, proliferative and cardiovascular disease [43]. Oxidative stress is also an important factor in aging, and drives cellular senescence [43,46,47]. Importantly, the increased generation of reactive oxygen and/or nitrogen species elicits a chain reaction oxidizing cellular components, especially unsaturated lipids, which in turn give rise to other reactive species that propagate cellular damage of membranes, proteins and nucleic acids [47,48]. In particular, lipid peroxidation can yield hundreds of reactive species with non-overlapping properties and biochemical actions [49,50]. Modification of proteins by these varied electrophilic lipid species, known as protein lipoxidation, can affect protein function, conformation, subcellular localization, and/or biomolecular interactions [50]. Moreover, the simultaneous modification of many targets by reactive species, including enzymes involved in PTMs such as phosphorylation, dephosphorylation or ubiquitination, can activate numerous signaling cascades multiplying the complexity of the PTM landscape [32]. Importantly, heat, mechanical and osmotic stress all change the redox state of the cell, elicit the production of ROS and induce oxidative stress or increase the sensitivity to it [41,42], thus highlighting the role of ROS as signaling elements common to several kinds of stress. On the other side of the spectrum, the persistent depletion of ROS by the overproduction of antioxidants, leads to reductive stress, which can block cell differentiation and contribute to pathology as well [51].

Intermediate filaments interplay with redox regulation

Redox balance is therefore critical for cell and tissue homeostasis, and intermediate filaments are not passive elements in this complex scenario. Indeed, there is mounting evidence indicating that intermediate filaments are tightly regulated by redox-dependent processes. They are highly sensitive to oxidative stress, and constitute a framework for the transmission of redox alterations into cytoskeletal rearrangements [16,22,52,53]. Intermediate filaments are important targets for various kinds of reactive species, including oxygen, nitrogen and lipid reactive species. Modifications of intermediate filaments by reactive species occur mainly at nucleophilic residues, and can evolve dynamically and accumulate during aging and pathological processes [32,54]. Several intermediate filaments, including vimentin (Figure 2) and the nuclear lamins possess cysteine residues that are targets for oxidative modifications of regulatory importance, which will be considered in more detail below [15,55].

Reciprocally, intermediate filaments are also intimately involved in redox regulation, in part via their close interactions with mitochondria, but also because of their biomechanical signaling and stress protein roles. Mitochondria are a main source of ROS and their alterations produce oxidative stress, whilst intermediate filaments help maintain mitochondrial homeostasis. Vimentin, desmin and GFAP (glial fibrillary acidic protein) interact with and regulate the function, position and dynamics of mitochondria [56,57]. Both desmin and vimentin deficiency associate with mitochondrial dysfunction and oxidative stress in experimental models [58,59]. The impairment of mitochondrial function appears as a pathogenic factor in genetic diseases caused by mutations of the type III intermediate filaments desmin and GFAP [57,60] leading to increased oxidative stress. The aberrant accumulation of neurofilaments occurring in Giant Axonal Neuropathy, a genetic disease affecting the protein degradation machinery, prevents mitochondrial motility and increases oxidative stress [61]. Keratin 8 physically interacts with oxidative stress-damaged mitochondria, facilitating their fission-mediated mitophagy, and preserving mitochondrial health and cell viability [62]. Mutations in wound-repair-associated keratin genes KRT6A, KRT6B, KRT6C, KRT16 or KRT17, associate with increased oxidative stress and defective antioxidant response in keratoderma diseases [63]. On the other hand, certain lamin mutations cause

an aberrant antioxidant response and reductive stress [64]. Therefore, all the different protein classes within the intermediate filament family have at some point being linked to oxidative stress in human diseases, supporting a collective contribution to redox regulation.

The "thiol proteome" and its role in redox signaling and stress sensing

Redox regulation mainly relies in the modification of protein thiols [43]. Cysteine residues constitute a low proportion of the human proteome, but they are often highly conserved across species, and typically support important functional roles [44]. The cysteine redox regulated proteome, also called "redoxome," refers to the landscape of accessible reactive cysteines in all proteins that are susceptible to modulation by redox modifications in normal cell signaling or in response to pathophysiological stimuli [65]. Here, we will use the term "thiol proteome" to include other cysteine modifications, such as lipoxidation [50] and various enzymatic modifications. Taking into account the great structural and functional diversity of cysteine modifications, the thiol proteome constitutes a signaling constellation interconnected with multiple cellular pathways [66,67]. Reversible cysteine oxidations play a key role in redox signaling and can interexchange and evolve in a dynamic fashion depending on the balance of oxidants and antioxidants. They are involved in the regulation of mitochondrial ROS production, respiration, protein synthesis, and metabolism. Importantly, the redox states of protein cysteines in pathways of protein turnover and cytoskeletal dynamics can display both tissue-specific patterns and aging-dependent profiles [54,68], and can frequently constitute regulatory switches. Various reversible modifications, including thiolation, nitrosation, sulfenylation or persulfidation, are considered protective mechanisms that temporarily shield important cysteine residues from irreversible modifications [54]. Notably, some cysteine alkylations as well as certain types of lipoxidation can also be reversed, which increases their regulatory potential [16,50,69]. On the other hand, in abundant proteins such as the lens crystallins that can be present in millimolar concentrations, cysteine oxidation can function as a scavenger of reactive species and absorb and redistribute transient spikes until the redox balance is re-established [70].

Cysteine residues can also suffer modifications resulting in protein crosslinks or protein unfolding and amyloid formation and aggregation, as well as several irreversible modifications, usually associated with protein damage [71,72]. Conversely, under reductive stress, the excess of reducing equivalents can impair the formation of disulfide bonds important for protein folding and maturation with deleterious consequences, e.g., the unfolded protein response. Lastly, cysteine residues are also target for enzymatic modifications, such as palmitoylation and isoprenylation, and even phosphorylation, increasing the possibility of PTM crosstalk and signal transduction.

Interestingly, within the thiol proteome, certain proteins or protein families possess cysteine residues at precise locations, which constitute unique signatures and/or modification platforms, known as "cysteine codes." The 27 cysteine residues of Keap1, the protein that controls the degradation of the transcription factor Nrf2, which is the master regulator of the antioxidant response [73], constitute an example of cysteine code. Keap1 cysteine residues display "custom" reactivities towards various oxidants and/or electrophiles, thus ensuring that the system detects virtually any compound that should elicit a cellular antioxidant response [74]. This system is vital for epithelial keratinization that involves disulfide formation and filaggrin association of keratin intermediate filaments required for barrier formation [75], and is therefore very clearly linked to skin pathologies. The overexpression of Nrf2 in an Alexander Disease mouse model decreased both GFAP levels and pathology [76], suggesting a direct link between intermediate filaments and the Keap1-Nrf2 system. In proteins of the Ras superfamily, which are key regulators of intermediate filament organization, cysteine residues located at distinct spacings within precise sequence contexts of their C-terminal domains generate specific motifs for enzymatic and non-enzymatic modifications. The resulting PTM combinations determine protein subcellular localization, stability and/or interactions [77], illustrating the sophistication and versatility of the thiol proteome.

Cysteine residues in the regulation of intermediate filament organization: focus on vimentin

Cysteine residues in filaments and protein polymers arouse special interest because their quaternary structure imposes spatially defined intervals that can constitute specific cysteine codes. The cysteine content of intermediate filaments is highly variable, in particular in keratins, where it can range from no cysteines, in the case of keratins 8, 18 and 19, to over 30 in the case of keratins 31 and 81. Therefore, in most keratins, a kind of a cysteine code could be proposed since specific cysteine residues are involved in disulfide bonds that are force-responsive and important for stability of keratin fibers, and that rearrange upon mechanical stress [78]. The redox state of keratin cysteines impacts their assembly, organization and interactome, to influence epidermal homeostasis and barrier function [79]. This is likely a general property of intermediate filaments, given the extensive structural rearrangements that occur under mechanical stress (reviewed in the study by Sapra et al. [8]). Indeed, the polymeric nature of intermediate

filaments opens the possibility to "shuttling" cysteine modifications within the filament and perhaps to associated proteins (Figure 1c) in a redox cascade, as seen for crystallins [70]. This could provide additional signaling and structural diversity. In addition, within a filament, different PTMs may coexist and combine, providing mechanisms to fine tune the filament network in a spatiotemporal manner.

Importantly, type III intermediate filament proteins possess a reactive cysteine residue, which is the only one in three of the four members of this class, and is conserved across species (Figure 2a and b). This supports its functional importance as well as its potential role as a target for redox regulation. Early studies showed that oxidants elicited cysteine crosslinking associated with a drastic impairment of filament assembly [80–82], which was virtually prevented in a cysteine-deficient mutant [82]. This set the scene for later studies in cells that showed that the single cysteine residue of vimentin (C328 in human vimentin) is key, not only for its reorganization in response to oxidants and electrophiles, but also for the assembly and function of the protein under basal conditions (reviewed in the study by Viedma-Poyatos et al. [16]).

The location of the cysteine residue in type III intermediate filaments appears critical for filament assembly and also for the function of the network. Conservative mutations, such as C328S or C328A in vimentin, have little impact on filament morphology, but produce less functionally competent filament networks in cells [15]. Indeed, filament network formation is delayed when these mutants are transfected in cells deficient in the corresponding filament proteins [15,83] (reviewed in the study by Viedma-Poyatos et al. [16]). Although the roles of the conserved cysteine residue have been studied in more depth for vimentin, studies on GFAP and desmin indicate similar functions [83,84]. The high conservation of the sequence surrounding the cysteine residue also supports analogous properties in the different proteins (Figure 2b). Importantly, several genetic variants affecting the conserved cysteine residue of type III intermediate filaments have been identified, according to the Uniprot database (Figure 2c), although their functional outcomes and potential clinical consequences have yet to be investigated.

Modifications of vimentin cysteine and their functional consequences

Numerous biochemical studies as well as protein models indicate that the single cysteine residue of vimentin is solvent exposed in the filament and is reactive [22,52,82,85], suggesting that its modifications could change the filament surface. This is supported by a recent model generated from cryo-electron tomography of filament segments in mouse embryonic fibroblasts (MEF) [86]. At least fourteen different modifications of the vimentin solitary cysteine, C328, have been reported to date and the number is ever increasing (reviewed in the study by Viedma-Poyatos et al. [16]) (Figure 2d). Among the most recently identified, vimentin has appeared in proteomic analyses of persulfidation [87] and CoAlation targets [88]. In addition, C328 can be modified by various drugs and it has been proposed as a drug target. The accessibility and relatively low pKa of C328 may underlie its reactivity under physiological conditions [85], in a manner similar to other redox-responsive cysteine residues in proteins such as Ras, certain proteases or transcription factors. Moreover, C328 can bind zinc, and this can influence its susceptibility to modification, as well as filament bundling [15,85].

A functional correlate is available for some of the C328 PTMs identified. In vitro, C328 modifications cause distinct filament alterations that depend on the structure of the modifying agent, and can range from a mild impairment of filament assembly to more severe outcomes with the formation of shorter and thicker filaments, or even amorphous aggregates. Whereas glutathionylation inhibits filament elongation [52], constraining modifications such as disulfide crosslinking, affecting 30% or more of the protein, induce protein aggregation and impair assembly [22,82]. In turn, addition of 4-hydroxynonenal (HNE) results in shorter and wider filaments, while several electrophilic compounds cause filament bundling [22]. In contrast, incorporation of small moieties, as in nitrosation, are better tolerated [52]. On the other hand, although preformed filaments appear more resistant to structural changes caused by C328 modifications [22], quantitative glutathionylation can cause filament severing [52]. Thus, the proportion of cysteine residues modified in the filament is most likely important for the severity of filament disruption or remodeling. A particularly interesting modification is the formation of disulfide-bonded or chemically cysteine-crosslinked vimentin dimers [15,80–82]. The occurrence of these dimers suggests that at least in certain conformations, cysteine residues in different dimers/tetramers must be spatially close. Indeed, molecular dynamics indicates that dimers of the vimentin rod can associate in such a way that cysteine residues fall within close distance [85]. The recently published structure of vimentin filaments proposes the presence of five protofibrils per filament [86], and is compatible with the presence of several cysteine residues in close proximity within each protofibril. This putative "clustering" could amplify the impact of cysteine modifications on the organization of the filament and the network. Interestingly, in copolymers of vimentin with either desmin or GFAP the ratio of cysteine-crosslinked dimers varies, indicating that coassembly affects the molecular arrangement in the filament [80,81].

In cells, the highly dynamic and versatile vimentin structures undergo remodeling in response to stress or to cell requirements. Thus, vimentin can be found in filaments, in bundles, in particles, and be exposed at the cell surface or secreted in a vesicular or non-vesicular form [4,35,53,89] (Figure 1). The reorganization of the vimentin network in response to oxidants and electrophiles can adopt distinct morphological patterns depending on the agent. Strong oxidants can induce filament fragmentation into particles, whereas certain electrophiles can elicit bundling and retraction of filaments from the cell periphery or even alignment along actin stress fibers [22,25] (Figure 3). On the other hand, certain C328 modifications such glutathionylation [90] or addition of malondialdehyde [89], have been involved in membrane exposure or secretion of vimentin.

The characterization of C328 modifications and their functional consequences in cells is complicated by the fact that any particular oxidative or electrophilic stimulus can generate a cascade of reactive species, giving rise to multiple vimentin proteoforms. For instance, a single nitroxidative challenge elicits several structurally and functionally different C328 modifications in cells, including nitrosation, sulfonylation, glutathionylation and lipoxidation, the proportion of which evolves with time in a dynamic fashion [32]. This indicates that several distinct modifications, potentially with non-overlapping functional consequences, could coexist within a filament, which could contribute to the diversity of vimentin arrangements. Interestingly, cysteine crosslinking studies have revealed a variable proportion of vimentin homodimers, as well as heterodimers with GFAP in cells expressing both intermediate filament proteins, such as astrocytes [83]. Notably, this strategy also results in the detection of a number of proteins, including plectin, Hsp70 or Hsp90, which could potentially form mixed disulfides with vimentin C328 under oxidative stress [15].

In spite of this complexity, the functional relevance of C328 modification, both in vitro and in cells, is supported by the fact that vimentin cysteine mutants are highly resistant to reorganization by various oxidants and electrophiles, including H_2O_2, diamide, HNE or cyclopentenone prostaglandins [22,25,82]. Substituting C328 with less susceptible residues, attenuates vimentin reorganization by such broadly reactive agents. This highlights the key role of this residue within the context of not only the modification of other residues in vimentin but also of other cellular proteins [25]. On the other hand, the observation that introducing certain mutations at the site of C328 is sufficient to alter filament morphology also supports the importance of modifications at this site [25].

Challenges and limitations in the detection and tracking of cysteine modifications in intermediate filament proteins

The structural and functional characterization of intermediate filament reactive cysteine residues is technically challenging [91]. Cysteine reactive probes, in combination with classical biochemical methods, can be used to identify reactive cysteines both in vitro and in vivo [92]. Derivatives of reactive species targeting cysteine residues, including electrophilic lipids and glutathione, bearing various labels, are very useful in cellular models. Also, antibodies against certain types of cysteine modification are available [92]. Nevertheless, the gold standard for assessing cysteine PTM is mass spectrometry, which has been discussed in several reviews [92–94]. Ideally, structural characterization would need to be applied not only at the level of individual cells, but also for specific subcellular compartments.

Functional consequences of cysteine modification in intermediate filaments have been addressed in vitro mainly by monitoring filament assembly through electron and atomic force microscopy, or deuterium exchange [22,52,82]. In the cellular context, high resolution studies at the subcellular level combined with genetic strategies will be required to characterize the spatiotemporal dimension of vimentin C328 modifications, and their potential implications in the fine tuning of the filament network. Apprehending the functional importance of a given cysteine residue in the complex cellular landscape of protein oxidation and lipoxidation, also in vimentin, has relied heavily on the study of cysteine mutants, involving substitutions with non-redox sensitive residues such as alanine, or mimetics of the modifications [25,55,74]. Nevertheless, some of these mutants appear less competent to integrate stress responses since they do not position organelles appropriately and are less efficient at recruiting ubiquitinated proteins and forming cage-like structures at the aggresomes upon proteasome inhibition in vimentin-deficient cells [15].

In addition, various cellular factors influencing cysteine reactivity or accessibility pose challenges for these studies (Figure 3). Cysteine residues in different regions of the network could present distinct susceptibility to modification due to their proximity to diverse sources of oxidants, which will also determine the type of ROS acting on the protein, and consequently the nature of the modifications. Therefore, the local environment of the cysteine will influence the modification. Protein–protein interactions could compete for the modification or shield the target residues [44]. Local concentrations of cellular factors, such as antioxidants or metals could also affect the spatiotemporal regulation of the modification. In this respect, vimentin binds several

divalent cations, and in particular zinc, with high affinity [85]. Molecular dynamics predicts the binding of zinc ions at several carboxylic residues along the sequence of vimentin, and importantly, at C328 in its thiolate form, with E329 and D331 stabilizing zinc coordination [85]. In support of this, zinc supplementation protects C328 from modification by oxidants and electrophiles [85]. Conversely, oxidative modifications of the cysteine residue could release this metal. Lastly, PTMs at other residues in vimentin and other proteins, as well as PTM interconversions can affect C328 modifications and their cellular consequences (Figure 3).

PTM combinations arising under stress and their potential impact on vimentin networks

The complexity of the vimentin PTM landscape is greatly increased by the fact that C328 modifications act in concert with many other enzymatic and non-enzymatic PTMs. These include phosphorylations, oxidations or lipoxidations, which occur throughout the entire vimentin sequence under oxidative stress [32]. Indeed, reactive species can elicit vimentin modification at many other residues. For instance, in addition to C328, the reactive aldehyde HNE [95] has been found in adducts with lysine and histidine residues [32], consistent with its broad colocalization with vimentin filaments in a dermal fibroblast model of skin aging [47]. Under nitroxidative stress, a plethora of oxidative modifications have been detected at various vimentin residues, including proline, tryptophane, tyrosine, and, notably, at ten methionine residues [32], which could also contribute to ROS scavenging. Importantly, vimentin is highly regulated by phosphorylation at multiple sites [9,96], and is the substrate for several redox-sensitive kinases and phosphatases. Inhibition of protein phosphatases under oxidative stress could contribute to an increase in the levels of phosphorylated vimentin. Indeed, calyculin A, an inhibitor of PP1 and PP2A phosphatases [96], has been shown to potentiate the disruption of vimentin by oxidants, whereas a vimentin mutant lacking 11 phosphorylation sites in the head domain is partially protected from disruption by the oxidant diamide [22]. Remarkably, the single vimentin tryptophan residue (W290), and the adjacent tyrosine residue (Y291), which are conserved in type III filaments, are also target for various oxidative modifications [32]. Importantly, PTMs can cooperate or compete with others at the same or neighboring residues, and, in particular tyrosine oxidative modifications can preclude phosphorylation (reviewed in the study by Viedma-Poyatos et al. [16]). Whilst the functional consequences have yet to be elucidated, the decoration of filaments with multiple PTMs can change their physical properties and/or generate functionalized surfaces the could serve as selective docking sites for proteins/organelles (Figure 1) [97,98]. Indeed, besides inducing filament redistribution, electrophile modifications alter vimentin epitope accessibility at particular cellular locations, showing that the surface architecture responds to such PTMs [53]. These observations reinforce the hypothetical view of vimentin filaments as a subcellular compartment, providing interconnectivity that would help integrate cellular responses to stress, as well as spatial constraint, thus affording balance for stress managing. This behavior would be finely tuned by a sophisticated combination of PTMs, with those affecting C328 playing an important role.

Downstream consequences of stress-mediated vimentin reorganization

A central question is whether vimentin, and the other intermediate filament proteins, play an active role in the modulation of cellular responses to redox alterations, and ultimately in their mitigation [99]. Several hypotheses regarding a potential protective function of intermediate filaments against oxidative stress are suggested. In an early phase, intermediate filament cysteine residues could contribute to buffering and restoring redox balance. Curiously, glutathionylation of vimentin detected under basal conditions has been observed to decrease transiently upon exposure of cultured cells to reactive species [32]. Hypothetically, disulfide formation between certain glutathionylated proteins could release glutathione for the protection of other targets from irreversible oxidation. Later, as oxidative stress overwhelms the redox reservoir (glutathione/ascorbate) in the cell, the intermediate filament polymers could potentially act as a capacitor to store such "stress" so that once the redox balance is re-established, the filaments can be reduced. Evidence from studies on lamins indicate that oxidative damage to the nucleus is more severe in cells expressing cysteine-deficient lamins, which supports a protective role of cysteine-mediated intermediate filament regulation under stress, and/or an oxidant scavenger role [55].

Vimentin reorganization could help shield some cellular structures or organelles against oxidative stress and/or set the scene for cellular repair (Figure 3). As vimentin interacts with many protein chaperones, including small heat shock proteins, Hsp70 and Hsp90, the juxtanuclear condensation of vimentin upon electrophilic stress would bring together damaged proteins and the repair machinery, which has been proposed as an optimization of cell survival mechanisms [20]. However, failure of the repair mechanisms with persistence of lipoxidation-elicited juxtanuclear aggregates can cause alterations in cell division, leading to asymmetric partitions or mitotic catastrophe [10]. Remarkably, electrophile-elicited perinuclear "collapse" is attenuated in cysteine-deficient mutants of vimentin and other type III intermediate filaments [15,83,84]. Whether this hampers cellular recovery from stress needs further investigation. Nevertheless, recent evidence showing

that transduction of mice with cysteine-deficient desmin worsens the outcome of ischemia/reperfusion gives credence to this view [100].

Importantly, vimentin C328 modification also impacts other cytoskeletal structures [25]. In particular, expression of electrophile-resistant vimentin cysteine mutants also blunts the formation of actin stress fibers in response to these agents. Thus, the rearrangement of the vimentin network in a C328-dependent manner could be responsible for triggering other cytoskeletal responses or for releasing signaling complexes to effect such changes in the actin cytoskeleton (Figure 3). The mechanisms of cytoskeletal interplay under oxidative stress requires further investigation. Nevertheless, it is clear that the vimentin cysteine C328 behaves as a privileged target for oxidants and electrophiles, which could contribute to a role of vimentin and potentially other intermediate filaments as sentinel networks.

The consequences of vimentin response to oxidative and electrophilic stress in disease constitute an important but little explored field of research. Interestingly, there is a bias within the human genome for somatic mutations to introduce cysteine residues in proteins [101]. This is also the case for all type III intermediate filament proteins, associating in many cases, with a pathological outcome that is better characterized for desmin and GFAP [102,103]. In the case of vimentin, multiple missense variants have been recorded according to the Uniprot database that introduce cysteines at 19 different positions in the sequence, although their potential pathological impact needs to be studied. These pathologically acquired cysteine residues can also be the target for redox or electrophile modifications, altering the responses of intermediate filaments. Indeed, an R239C GFAP mutant, one of the many variants causing the rare neurodegenerative disease known as Alexander disease [103], shows increased susceptibility to oxidation and lipoxidation, and this associates with increased aggregation and poor cell survival upon stress [57].

In summary, intermediate filaments, as exemplified by vimentin, GFAP and desmin, with their single, highly conserved cysteine residues, are very important redox-sensitive targets or redox hubs, which could be compared to p53 or NF-κB [43]. We propose that the reactive cysteines of the intermediate filament networks constitute significant elements of the "redox sentinel cysteine redoxome," a phrase coined by Held [44]. The added value of the intermediate filament network is that it extends throughout the cell, interconnecting cellular organelles, interacting with and integrating signaling complexes and cellular machines. Intermediate filaments offer potential for spatiotemporal control as well as for either buffering or propagating ROS signaling. Therefore, intermediate filament networks are ideally placed to deliver a physical and responsive interface for redox signaling, stress responses and mechanosensing.

Conclusions and perspectives

A long journey into the biology of intermediate filaments together with exciting recent results is evidencing that these structures are not just essential elements of cellular architecture, but also key players in cell signaling, stress sensing and integration of defense responses. Intermediate filament networks possess unique assembly and network adjusting properties to deliver their mechanosensory and stress response roles, but here we have shown how they interface with ROS regulation, mitochondrial function and positioning, how they respond to redox stress and how their cysteine code can potentially buffer and protect the cell via their associated signaling, protein and organelle platforms. Much needs still to be learned to comprehend these roles. A summary of current concepts and future perspectives is provided in Table 1. Recent advances in

Table 1

Summary of accepted concepts, especial properties and perspectives for the study of intermediate filaments in the cellular responses to stress.

What is known	What intermediate filaments offer	Hypotheses to address
Dynamic filaments with a rapidly exchangeable soluble pool	Spatio-temporal control	Responses to localized ROS production
Stress proteins	A distinct, non-membranous compartment. Chaperones, aggresomes, and proteostasis	Protective role for diverse types of stress
ROS and free radical targets	Free radical buffering capacity and protection against oxidative stress	Sentinel function
Extensive and varied PTMs	Subcellular specific PTM localization	PTM signatures associated with different stresses
Function in organelle positioning and homeostasis	Mitochondrial position and function	ROS level correlation with intermediate filament assembly and network status
Mechanosensory function	Transcellular filament network and intra-tissue signalling	Stress-dependent signaling platform composition

microscopy and electron tomography techniques, structure prediction and understanding of membraneless compartments guarantees exciting discoveries in the near future. Among them, the long-sought vimentin structure is beginning to reveal unsuspected features, which will help understand the complexity of vimentin assembly and the mechanisms for cysteine dependent reorganization. Finally, from a biomedical point of view, "harnessing the power" of intermediate filaments and their versatility under stress will help understanding the mechanisms of disease and envisage novel therapeutic strategies for common and rare diseases.

Credit author statement

Conceptualization: DPS, RAQ. Writing: DPS, RAQ. Illustrations, DPS.

Funding

Work at DPS and RAQ groups has been supported by Grants RTI2018-097624-B-I00 and PID2021-126827OB-I00, funded by MCIN/AEI/10.13039/501100011033 and ERDF, A way of making Europe; Astromad, LCF/PR/HR21/52410002 from Fundación "la Caixa"; RETIC Aradyal RD16/0006/0021 from ISCIII, cofunded by ERDF; European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie Grant agreement no. 675132 "Masstrplan" to DPS. RAQ acknowledges the support of the Biophysical Sciences Institute, the Institute of Advanced Study (Durham University, Durham, UK) and the Department of Biological Structure (Univerity of Washington, Seattle, USA).

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.

Data availability

No data was used for the research described in the article.

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