Reactive oxygen and nitrogen species (ROS and RNS, respectively) are part of any aerobic lifestyle/metabolism: low (i.e., subtoxic) concentrations of selected ROS and RNS are continuously produced inside and outside the cell by a number of pathways, either accidentally or purposefully. In all eukaryotic cells, the mitochondrial electronic transport chain is the main endogenous source during cellular respiration. Activated phagocytes also release significant amounts of various ROS and RNS by NADPH oxidase, myeloperoxidase, and nitric oxide synthase activity while attacking microorganisms or damaged host cells. Other biologically significant sources of ROS and/or RNS include ionizing radiation, cytochrome p450 activity, the enzymatic system of hypoxanthine/xanthine oxidase, especially in ischemia/reperfusion, metal catalyzed reactions, osmotic stress, and chemotherapeutic drugs (Halliwell & Gutteridge, 2007; Jomova et al., 2010).

Due to their high chemical reactivity, ROS and RNS can modify and oxidize various biological molecules, often altering their biological function, such as unsaturated lipids, carbohydrates, nucleic acids, but mostly, because of their high abundance, proteins. These oxidized (and often damaged) cellular molecules can cause toxicity as such and/or may degrade to form further toxic products, such as reactive carbonyl species (RCS) generated by peroxidation of polyunsaturated fatty acids (PUFAs). For example, highly reactive α,β-unsaturated aldehydes/hydroxyl-alkenals such as 4-hydroxy-2-nonenal and 4-hydroxy-2-hexenal derive from the degradation of peroxidized n–6 and n–3 PUFAs (Català, 2009; Fritz & Petersen, 2011; Fritz & Petersen, 2013). RCS can, in turn, react with the nucleophilic sites of proteins, binding to the sulphydryl group of cysteine, the ε-amino group of lysine or the imidazole group of histidine residues to form Michael or Schiff base protein adducts, known as advanced lipoxidation end-products (ALEs), whose deleterious role is emerging in the etiology and/or progression of several human chronic diseases, such as cardiovascular, neurodegenerative, and liver diseases (Perluigi, Coccia, & Butterfield, 2012; Fritz & Petersen, 2013; Colzani, Aldini, & Carini, 2013). ROS/RNS and some related reactive species are not only injurious but also essential participants in cell signaling and regulation. Indeed, under physiological (basal) conditions, some specific ROS and RNS as well as some of the reactive products, including RCS, resulting from their oxidizing activity can act as signaling molecules (second messengers) in many signaling pathways (redox signaling) (Calabrese et al., 2007; Català, 2009; Rudolph & Freeman, 2009; Forman, Maiorino, & Ursini, 2010; Zhang et al., 2011) involved in a variety of different cellular processes including (but not limited to) apoptosis, cell proliferation and differentiation, autophagy, and aging.

Because of the potential toxicity of these reactive species, a number of cellular and extracellular antioxidant defenses, both enzymatic and non-enzymatic, are designed to remove/neutralize ROS, RNS, and RCS directly, to minimize the extent of either their production or damage they can provoke, or to repair oxidative/nitrosative damage caused by them. Therefore, under normal physiological conditions, an efficient antioxidant defense system constantly buffers the oxidative action of ROS, RNS, and RCS, thus minimizing oxidative/nitrosative damage, while allowing some reactive species to perform useful functions (Halliwell, 2011). However, excessive production of ROS/RNS and/or impairment or decrease of antioxidant defenses, as a whole resulting in an imbalance among pro-oxidant and antioxidant systems, cause a pathophysiological condition known as oxidative stress, leading to alteration of cellular redox homeostasis, altered redox signaling, and regulation and/or molecular damage (Sies & Jones, 2007). Oxidative/nitrosative damage appears to be linked to the pathogenesis and/or progression of many human diseases, including cardiovascular and neurodegenerative diseases, atherosclerosis, diabetes mellitus, respiratory diseases such as chronic obstructive pulmonary disease (COPD), and many types of cancer, and is also involved in physiological aging and degenerative processes occurring in age-related diseases.

As noted above, by virtue of their high overall abundance in biological systems, proteins are major targets of oxidants and can undergo reversible redox reactions, which also can be part of their normal function, thus supporting dynamic regulation of structure and function, or irreversible oxidative damage at various amino acids (mainly cysteine, histidine, tyrosine, tryptophan, lysine, proline, and threonine), which is generally associated with loss of function (Butterfield & Stadtman, 1997; Dalle-Donne et al., 2005, 2006a; Dalle-Donne, Scalon, & Butterfield, 2006c; Bachi, Dalle-Donne, & Scaloni, 2013). Although the side chain of virtually every amino acid may experience oxidative damage, the thiol group of the amino acid cysteine is a primary target for oxidation reactions. The other sulfur-containing amino acid methionine and the aromatic amino acids tyrosine and tryptophan as well are main targets for oxidation though generally to a lesser extent than cysteine. Reversible modifications, occurring at cysteine or methionine residues, may have a dual role: protection from irreversible damage and modulation of protein function (redox regulation). Redox reversible protein modifications, particularly thiol...
oxidation, may thus be an early cellular response to mild oxidative stress and may also play an important role in redox signaling pathways (Dalle-Donne et al., 2007, 2009; D’Autreux & Toledano, 2007; Brandes, Schmitt, & Jakob, 2009; Rudolph & Freeman, 2009; Zhang et al., 2011; Higdon et al., 2012). Irreversible protein modifications induced by ROS, RNS, or RCS, such as protein–protein cross-linking due to dityrosine formation, tyrosine halogenation and nitration, and protein carbonylation, are generally associated with loss of function (e.g., Butterfield & Stadtman, 1997; Dalle-Donne et al., 2001, 2006b; Liebler, 2008; Codreanu et al., 2009; Butterfield, Reed, & Sultana, 2011; Castro et al., 2013) and may lead to either misfolding or unfolding and proteolytic removal of the damaged proteins by the proteasomal system or by the lysosome-macrophagy pathway (Kiffin et al., 2004; Kaushik & Cuervo, 2006; Jung & Grune, 2008; Aiken et al., 2011; Dewaele et al., 2011; Di Domenico et al., 2012), or to their progressive accumulation into cytoplasmic or extracellular toxic aggregates, as observed in most age-related neurodegenerative disorders (Butterfield & Kanski, 2001; Bossy-Wetzel, Schwarzenbacher, & Lipton, 2004; Martinez et al., 2010; Grimm et al., 2011; Castro et al., 2012).

By influencing protein structure and function, post-translational (oxidative/nitrosative) modification of proteins leads to a proliferation of proteome diversity. The term proteome defines the entire protein supply in a given cell organelle, cell, tissue or even organism. Proteins take part in most (if not all) biological functions/processes and can therefore be considered the most functionally important biological macromolecules. In addition, protein expression and specific activity, post-translational modifications, and association with other proteins or other types of molecules are fundamental to understand how cells work and interact with each other in a given biological system and under a defined (patho)physiological condition. Consequently, the proteome is not a static expression of a genome, but a dynamic, complex network that can be altered by various endogenous and/or exogenous factors such as particular physiological or pathological states and multiple stress conditions. Proteomics—the systematic identification and characterization of proteins—is thus a powerful tool for a full understanding of a biological system or process including a biochemical pathway or disease state (Aebersold, 2003).

Proteomics, more specifically redox proteomics, is the best suited approach for identification of ROS/RNS/RCSevoked protein modifications both in redox signaling and under oxidative/nitrosative stress conditions (Dalle-Donne et al., 2005; Dalle-Donne, Scaloni, & Butterfield, 2006c; Sultana et al., 2010; Sultana & Butterfield, 2011; Butterfield & Dalle-Donne, 2012; Butterfield et al., 2012; Held & Gibson, 2012; Sheehan et al., 2012; Sultana, Perluigi, & Butterfield, 2012; Colombo et al., 2012a,b; Bachi, Dalle-Donne, & Scaloni, 2013; Keeney et al., 2013). Indeed, many redox proteomic studies have identified specific oxidized proteins in various human diseases related to oxidative/nitrosative stress, such as neurodegenerative disorders, diabetes mellitus, and ocular or cardiovascular pathologies as well as in aging, providing some mechanistic information on their development and insights into the pathways involved in their pathogenesis as well as into downstream functional consequences (for recent and comprehensive reviews, see Bachi, Dalle-Donne, & Scaloni, 2013; Butterfield et al., 2012). The most recent advances in redox proteomic technologies are contributing to define the functional impact of ROS/RNS/RCScorrelated signaling pathways and to establish functional relationships between disease hallmarks and protein structural and/or functional changes, promising a significant contribution also in the discovery of new diagnostic biomarkers enabling early detection of various diseases and in the identification of new drug targets. Progress in the field of redox proteomics (e.g., Dalle-Donne, Scaloni, & Butterfield, 2006; Madian & Regnier, 2010; Sheehan, McDonagh, & Barcena, 2010; Charles, Jayawardhana, & Eaton, 2013; Kumar et al., 2013) devoted, in particular, to advancements of methods and means to selectively detect and quantify, with both high sensitivity and fidelity, discrete protein oxidative modifications, of not only individual proteins but also of redox-sensitive residues within those proteins, is expected to advance our knowledge of regulatory mechanisms that involve protein oxidation. In particular, cysteine residues are typically involved in these processes and lead to the elucidation of function of protein (cysteine) oxidation in physiological and oxidative stress-related pathological conditions as well as of redox-based modifications relevant to cell signaling pathways. In this way, knowledge of fundamental biological processes is improved and accumulated.

In the current special issue of *Mass Spectrometry Reviews*, both methodological and applicative aspects of redox proteomics research are presented in a collection of 13 review articles contributed by some of the leaders in this field. Progress in analytical technology, in particular tandem mass spectrometry techniques and development of specific gel-based methods, labeling and enrichment procedures for better identification of specific oxidative modifications, which allowed the progress in understanding of the role of protein oxidative modifications both in normal cell signaling and regulation and under oxidative/nitrosative stress conditions, are addressed in most of the articles included in this special issue. Considerations on the methodological limits and pitfalls emerging from current redox proteomic studies and the possible future development in the field of redox proteomics in health and disease are also provided in most of the following review articles.

The review article by Butterfield et al. (2013) highlights many of the gel-based and non-gel based redox proteomics techniques that are currently available for analyzing several oxidative post-translational modifications. In addition, the authors provide many examples of redox proteomics applications, from their laboratory and others, as a tool to understand disease pathogenesis in neurodegenerative disorders, such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and Down syndrome, and others such as cancer, diabetes, kidney, and heart diseases. An up-to-date overview of the different gel-based and gel-free proteomic techniques that have been used to investigate tobacco smoke-induced protein damage in cell cultures, animal models, and humans is presented by Isabella Dalle-Donne and coworkers (Colombo et al., 2013). They report on both changes in protein expression and specific oxidative modifications of proteins induced by exposure to cigarette smoke as evidenced by means of, respectively, quantitative and redox proteomic approaches. This paper also provides a through survey of the pathophysiologi
As discussed above, there is much evidence that protein thiol oxidation occurs not only as a consequence of oxidative/nitrosative stress conditions, but this modification plays a crucial role in redox signaling pathways in the healthy cell. Amongst the different post-translational oxidative modifications that can occur at the cysteine thiol group, cysteine sulfenic acid (CySOH) is a key player in redox regulation of protein functions under both physiological and oxidative stress conditions and can mediate the transduction of the intracellular signal, hydrogen peroxide, acting as a second messenger, into a biological response (Poole & Nelson, 2008; Haskew-Laytona et al., 2010; Roos & Messens, 2011). However, relatively few molecular details of how this oxidant acts to regulate protein function are currently understood. Furdui and Poole (2013) describe in detail primarily classical and emerging chemical tools and approaches that can be applied to study protein sulfenylation in biological systems, also providing some of the biologically meaningful data that have been collected using such approaches, including demonstration of CySOH formation in IQGAP, a VEGF receptor binding scaffold protein involved in ROS-dependent endothelial cell migration and post-ischemic angiogenesis. S-Nitrosylation of proteins as well, that is, the addition of an NO group to a Cys thiol to form an S-nitrosoprotein, plays a regulatory role, mediating many of nitric oxide actions and participating in both physiological and pathophysiological processes (Murphy et al., 2012; Piantadosi, 2012; Maron, Tang, & Loscalzo, 2013; Nakamura et al., 2013). In this special issue, López-Sánchez, López-Pedrera, & Rodríguez-Ariz (2013) exhaustively present up-to-date advances in proteomic methods that are providing researchers with improved tools for exploring protein S-nitrosylation. In addition, they also review some recent studies of the S-nitrosoproteome in pathology, focusing on how these novel technologies will advance our current knowledge of the role of deregulated S-nitrosylation in disease, including neurodegenerative, hepatic, and cardiovascular diseases.

Protein cysteine residues can also be covalently modified through Michael addition by cyclopentenone prostaglandins (cyPG), endogenous lipid mediators involved in the resolution of inflammation and the regulation of cell proliferation and cellular redox status (Uchida & Shibata, 2008). The valuable contribution provided by proteomic approaches to the comprehension of numerous cellular processes elicited by cyPGs is highlighted in the review by Oeste and Pérez-Sala (2013). Their review focuses on protein targets of cyPG that play an important role in or are regulated by cellular redox status and also describes cyPG interaction with low molecular weight thiols, such as glutathione, and hydrogen sulfide anion. The comprehensive review by Claudia Maier and coworkers (Vasil’ev et al., 2013) complements the analysis of protein modification by electrophilic lipoxidation products, focusing on those with α,β-unsaturated keto/aldehyde moiety. The authors first provide a survey of chemistry and biological relevance of the protein addition by electrophilic lipoxidation products and then provide an overview of recently developed tandem mass spectrometry approaches for the analysis of protein modifications by electrophilic lipid oxidation products. They also present a lot of new, previously unpublished data from their own group and underline some shortcomings of currently employed techniques and search engines that hinder detection and identification of protein lipid adducts in highly complex biological specimens. The review by Frizzell and coworkers (Merkley et al., 2013) provides an excellent and up-to-date overview on succination, a non-enzymatic chemical modification of protein cysteine by the Krebs cycle intermediate, fumarate, via Michael addition reaction, yielding S-(2-succinyl)cysteine. This acid-stable, physiologically irreversible, thioether derivative of protein is a relatively novel post-translational cysteine modification that was originally reported by the same research group (Alderson et al., 2006). This detailed article also discusses the possible roles of succination in regulatory biology, as succination is a relatively specific reflection of mitochondrial dysfunction and also interfaces with oxidative and endoplasmic reticulum stress. This paper outlines some of the challenges associated with measuring this post-translational modification of proteins (the “succinated proteome”) by mass spectrometric techniques.

Like cysteine, methionine is one of the most readily oxidized amino acids by most ROS/RNS, owing to the presence of the sulfur atom in this amino acid. Mild oxidizing conditions determine in vivo generation of methionine sulfoxide, which can be reversed by the action of ubiquitous methionine sulfoxide reductases or can be further oxidized to methionine sulfone under stronger oxidizing conditions (Kim, Weiss, & Levine, 2013). Methionine oxidation may determine an altered protein conformation or function and increases with aging and age-related diseases (Bachi, Dalle-Donne, & Scaloni, 2013; Kim, Weiss, & Levine, 2013). Additionally, oxidation of certain methionine residues may also impact on cellular signaling, participating in the redox regulation of enzyme activity (Erickson et al., 2008). Mass spectrometry based and proteomics methods available for characterizing in vivo methionine oxidation are reviewed by Ghesquière and Gevaert (2013), who specifically highlight the suitability and versatility of COFRA-DIC technology for the study of methionine oxidation on a proteome-wide scale.

Protein tyrosine nitration, that is, post-translational modification of Tyr residues to 3-nitrotyrosine, occurs via multiple pathways and is usually a result of the combined, simultaneous production of various ROS and RNS (Souza, Peluffo, & Radi, 2008; Ischiropoulos, 2009). Formation of 3-nitrotyrosine alters protein structure and function and has been causally linked to a number of diseases and conditions, including cardiovascular and neurodegenerative diseases, some types of cancer, and aging (Sultana et al., 2006; Souza, Peluffo, & Radi, 2008; Ischiropoulos, 2009; Reed et al., 2009; Butterfield, Reed, & Sultana, 2011; Reyes et al., 2011; Feeney & Schöneich, 2012; Murakami et al., 2012; Zhan, Wang, & Desiderio, 2013). Notwithstanding that this type of post-translational modification has been known for over a century and is ubiquitous in biological tissues and fluids, its accurate and artifact-free detection and analysis is still very challenging. The comprehensive review article of Tsikas and Duncan (2013) is just devoted to highlight all the analytical challenges incidental to the analysis of free and peptide- or protein-bound 3-nitrotyrosine and discusses the approaches adopted to address them, with a special focus on stable-isotope labeled 3-nitrotyrosine analogs.

Protein carbonylation is generally defined as an irreversible oxidative modification that results from the addition of reactive carbonyl functional groups on proteins, often leading to a loss of protein function (Dalle-Donne et al., 2001, 2003, 2006b; Martínez et al., 2010; Sultana et al., 2010; Castro et al., 2013). Carbonylation is considered as the most general type of protein oxidation, because many of the over 30 oxidized amino acid...
products involve some form of carbonylation, even though different structures are associated with carbonyl groups, with the most reactive and common of these being in the form of lipid peroxidation-derived aldehydes. Carbonyl groups may be introduced within the protein primary structure at different sites and by different mechanisms (Stadtman & Berlett, 1998). Direct protein carbonylation can take place through a variety of reactions, with the majority of these reactions occurring with lysine, arginine, threonine, and proline residues. Alternatively, protein carbonylation can result from indirect mechanisms, by which RCS react with lysine, arginine, and cysteine residues leading to the formation of advanced glycation or lipoxidation end-products (AGEs or ALEs, respectively) (Stadtman & Berlett, 1998; Bachi, Dalle-Donne, & Scaloni, 2013). Although both carbonyl reductase and aldehyde reductase catalyze the NADPH-dependent reduction of a variety of free carbonyl compounds, including highly reactive lipid aldehydes and α-dicarbonyl compounds produced during the Maillard reaction, thus detoxifying them before they can form AGEs or ALEs, currently no established enzymatic repair machinery of cells has been shown to reverse protein carbonylation. Protein carbonylation is therefore considered a widespread indicator of severe oxidative damage as well as aging- and disease-derived protein dysfunction (Levine & Stadtman, 2001; Levine, 2002; Dalle-Donne et al., 2003, 2006a,b; Barreiro & Hussain, 2010; Sultana et al., 2010; Sultana & Butterfield, 2011; Castro et al., 2013).

Since protein carbonyls are the most commonly used markers of protein oxidation and of oxidative stress extent, a wide variety of methods have been developed for the detection and quantification of carbonylated proteins. The current biochemical and analytical methods for protein carbonyl detection are reviewed in detail by Hoffmann and coworkers (Fedorova, Bollini, & Hoffmann, 2013). The authors discuss biochemical and immunological techniques, which provide global information on the modified proteins and carbonylation levels, spectrophotometric and chromatographic assays to determine the total protein carbonyl content and, specifically, focus on mass spectrometry-based techniques for identification of the modified proteins, including modification sites, and relative quantification of protein-bound carbonyls. The authors also overview mechanisms and biological aspects, such as cell signaling activation, of protein carbonylation based on recent high-throughput proteomics data. The complementary article by Arena et al. (2013) provides an up-dated overview of the mass spectrometry-based techniques specifically developed for the identification of glycation/glycoxidation protein targets as well as for the characterization of the corresponding adducts, also emphasizing their limits compared to current proteomic approaches for the analysis of other post-translational modifications.

The relationship between protein carbonylation and aging, in different aging models from bacteria to humans, is specifically addressed in the review article by Ros and coworkers (Cabisco, Tamarit, & Ros, 2013), in which the authors also review the most commonly used proteomic tools for the identification of carbonylated proteins. Interestingly, the article also discusses those characteristics that seem to favor selectivity of protein carbonylation in target proteins versus indiscriminate oxidative damage. The review article by Esther Barreiro (Barreiro, 2013) provides a nice and detailed overview of protein carbonylation in skeletal muscles under several pathological conditions, including COPD, cancer cachexia muscle atrophy, and sepsis as well as in aging, reporting data from both humans and animal models of disease. In particular, she focuses on different studies in which the biological and functional significance of protein carbonylation has been explored so far in the skeletal muscle dysfunction associated with chronic pathophysiological conditions.

It is now clear that redox-dependent post-translational modifications of proteins, mediated by endogenously generated or environmental ROS and RNS, are not only an irreversible process primarily due to oxidative/nitrosative stress conditions, but reversible oxidative and nitrosative protein modifications play an important role in redox signaling pathways. Whenever ascertained, oxidative/nitrosative modifications may allow establishing a putative relationship between structural and/or functional protein alterations and pathological conditions or the redox regulation of a variety of cellular physiological processes. During the last decade, the relatively novel but expanding field of redox proteomics (Dalle-Donne, Scaloni, & Butterfield, 2006c; Butterfield et al., 2012; Butterfield & Dalle-Donne, 2012; Bachi, Dalle-Donne, & Scaloni, 2013) has allowed the identification of specific redox-sensitive proteins as fundamental effectors of cell physiology and biology and as molecular signposts of oxidative/nitrosative damage under different pathological conditions, highlighting some correlation between protein oxidation and human disease, although solving whether oxidation is a result or a primary cause of pathology remains a challenge. The reviews in this special issue of Mass Spectrometry Reviews well illustrate the recent progress in this field. It is hoped that continued application of more advanced redox proteomics technologies will yield a still better understanding of oxidative/nitrosative modifications to proteins and their altered function both under physiological conditions and under oxidative/nitrosative stress.

REFERENCES


