Among the 22 amino acids used for protein synthesis, cysteine (Cys) and methionine (Met) are the only two that contain a sulfur atom in their side chain. Cysteine can be synthesized by humans and other mammals, whereas Met is an essential amino acid that is produced by microorganisms and plants and provided to mammals with food [1]. Methionine plays major roles in a variety of cellular processes, serving as a central factor in sulfur metabolism and a precursor for several important compounds, such as taurine, carnitine, and S-adenosylmethionine. Methionine is also fundamental for protein translation as an initiator residue in protein synthesis. In cells, approximately 99% of Met is present in the form of proteins [2]. One of the least abundant residues, accounting for 2.4% of amino acids in proteins [3], Met is a nonpolar residue, typically buried in the hydrophobic core of proteins [3]. It is also enriched in the hydrophobic areas involved in protein–protein interactions [4]. The presence of sulfur in their side chains renders Cys and Met sensitive to oxidation. The conversion of Cys to the cystine form leads to the formation of intra- or intermolecular disulfide bonds, many of which are required for protein structure and function. Reactions of Cys with reactive oxygen species can also result in the generation of oxidized derivatives, such as sulfenic acid (Cys-SOH), sulfinic acid (Cys-SO$_2$H), or sulfonic acid (Cys-SO$_3$H), which participate in catalytic processes and regulation of enzymes, such as peroxiredoxins [5] and protein-tyrosine phosphatases [6].

The reaction of Met with an oxidant leads to the formation of two diastereomers, the R and S forms, of methionine sulfoxide (MetO). Methionine sulfoxide reductases A (MSRA) and B (MSRB) reduce MetO back to Met in a stereospecific manner, acting on the S and R forms, respectively. A third MSR type, fRMSR, reduces the R form of free MetO. MSRA and MSRB are spread across the three domains of life, whereas fRMSR is restricted to bacteria and unicellular eukaryotes. These enzymes protect against abiotic and biotic stresses and regulate lifespan. MSRs are thiol oxidoreductases containing catalytic redox-active cysteine or selenocysteine residues, which become oxidized by the substrate, requiring regeneration for the next catalytic cycle. These enzymes can be classified according to the number of redox-active cysteines (selenocysteines) and the strategies to regenerate their active forms by thioredoxin and glutaredoxin systems. For each MSR type, we review catalytic parameters for the reduction of free MetO, low molecular weight MetO-containing compounds, and oxidized proteins. Analysis of these data reinforces the concept that MSRA reduce various types of MetO-containing substrates with similar efficiency, whereas MSRBs are specialized for the reduction of MetO in proteins.

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vastly nothing is known about the proportion of each diastereomer in oxidized cellular proteins. The oxidation of Met in MetO can be reversed by the catalytic action of methionine sulfoxide reductases (MSRs) A (MSRA) and B (MSRB), which are specific for the S- and R-diastereomers, respectively [8-10]. The first evidence of MetO reduction activity was obtained in 1960 by fractionation of Saccharomyces cerevisiae cells. The authors demonstrated that three cellular fractions were required for the reduction of both R- and S-diastereomers of MetO at the expense of NADPH, and that the mechanism involved disulfide exchange [11]. In 1979, the in vivo reduction of MetO was shown in an Escherichia coli strain auxotrophic for Met, which grew in media containing MetO as a sole source of Met [12]. MSRA was then isolated from E. coli [8], whereas MSRB was discovered 20 years later in bacteria [9] and eukaryotes [10]. MSR genes have been found in almost all organisms with the exception of some parasites and hyperthermophiles [10, 13-15]. More recently, a third class of MSRs was discovered, rFMSR, which is specific for the reduction of the R-diastereomer of free MetO. It is present only in prokaryotes and lower eukaryotes, such as the yeast S. cerevisiae [16, 17]. In addition, it was shown that bacterial biotin sulfoxide reductase BisC can reduce specifically the S-diastereomer of free MetO [18].

The use of genetically modified organisms with knocked-out or overexpressed MSR genes allowed defining the two main roles of MSRs in cells, i.e. protection against oxidative stress and regulation of lifespan. However, because of the lack of clearly identified target proteins for either MSRA or MSRB, the specific cellular processes involved remain unclear. Due to its early discovery, MSRA received much more attention than other MSRs. It was found that knockout of MSR genes increased susceptibility to oxidative stress in bacteria [19-21], yeast [22, 23], Caenorhabditis elegans [24], mice [25], and plants [26, 27]. Conversely, overexpression of MSRA increased resistance to oxidative stress in Drosophila [28], mammalian cells [29, 30], and plants [26]. Deletion of MSRA often reduces lifespan, whereas its overexpression was shown to increase lifespan of fruit flies by 70% [28]. The involvement of MSRBs in protection against oxidative stress was similarly shown in knockout plants [31, 32] or human cells overexpressing the enzyme [33], but their roles remain somewhat less clear due to weaker phenotypes. For example, deletion of MSRA in yeast results in a stronger sensitivity to hydrogen peroxide than the deletion of MSRB [17, 22, 34], and MSRB overexpression did not affect the lifespan of fruit flies [35]. Moreover, deletion of MSRB did not noticeably affect yeast lifespan, although strains deficient in both MSRA and MSRB exhibited a greater reduction in lifespan compared to cells deficient in MSRA only [22, 34]. A role in the host/pathogen interaction was also identified for MSRs, whose genes were highly expressed during pathogen invasion in both the host and the pathogen in response to the high levels of reactive oxygen species produced by both organisms [21, 36-38].

DIVERSITY OF MSRS AND THEIR REGENERATION MECHANISMS

MSRs belong to the family of thiol oxidoreductases, which possess redox-active Cys and/or selenocysteines (Sec) residues. Although both MSRA and MSRB catalyze the reduction of MetO, they do not share sequence similarity [39]. Interestingly, determination of protein structures from various organisms indicates a mirror-like relationship between the MSRA and MSRB active sites [39], in which a Trp faces the catalytic Cys and allows the docking of the substrate in the optimal position for its reduction (Figs. 1a and 1b). MSRA has a G[C/U]FW motif, located in the N-terminal part of the protein, that includes the catalytic residue (Fig. 1c). The great majority of known MSRAs possess a catalytic Cys, with a few Sec-containing protein forms found in ticks, spiders, some marine organisms, and certain unicellular algae such as Chlamydomonas reinhardtii [15, 40]. Extensive characterization of bacterial MSRAs [41-43] as well as determination of three-dimensional structures of prokaryotic and eukaryotic enzymes [44-48] yielded a mechanism of MetO reduction wherein a sulfenic acid is formed on the catalytic Cys after the formation of a sulfuran-type transition state [42, 43]. Although the existence of the sulfenic acid was established for E. coli [41], P. trichocarpa [47], and mouse enzymes [49], the mechanism leading to its formation is still unclear. Indeed, it was proposed that the oxygen atom forming the sulfenic acid comes directly from a water molecule [49].

The majority of MSRBs possess a catalytic Cys as part of the RxxCxxN motif located in the C-terminal region. Mammals express a Sec-containing form of MSRB displaying a slightly different active site in which the asparagine conserved in all identified Cys-containing isoforms is replaced with phenylalanine [50] (Fig. 1d). As in the case of MSRA, theoretical and biochemical studies give conflicting models for the MetO reduction step catalyzed by MSRB. Both models agree on the fact that a sulfonium cation is the initial intermediate, but an in silico analysis indicates that the formation of a sulfenic acid is not enzymatically feasible [51-53]. On the other hand, biochemical analyses using sulfenic acid-specific reagents and mass spectrometry demonstrated the formation of a sulfenic acid in archaeal [54], plant [55, 56], and fruit fly [57] MSRBs, although the mechanism leading to its formation was not addressed.

In the case of both MSRA and MSRB forms containing Sec, the formation of a selenenic acid was pro-