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Sandrine Arbogast, Ana Ferreiro

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# Selenoproteins and Protection against Oxidative Stress: Selenoprotein N as a Novel Player at the Crossroads of Redox Signaling and Calcium Homeostasis

Sandrine Arbogast[1,2] and Ana Ferreiro[1–4]

**Abstract**

Healthy cells continually produce low levels of reactive oxygen species (ROS), which are buffered by multiple antioxidant systems. Imbalance between ROS production and elimination results in oxidative stress, which has been implicated in aging and in numerous human diseases, including cancer and diabetes. Selenoproteins are a family of proteins that contain the amino acid selenocysteine, encoded by an in-frame UGA. Those selenoproteins whose function is identified are catalytically active in redox processes, representing one of the main enzymatic antioxidant systems and important mediators of the beneficial role of selenium in human health. Nevertheless, the function of most selenoproteins remains unknown; this included Selenoprotein N (SelN), the only selenoprotein directly associated with a human genetic disease. Mutations of the SelN gene cause SEPN1-related myopathy, a particular early-onset muscle disorder. Recent studies have identified SelN as a key protein in cell protection against oxidative stress and redox-related calcium homeostasis. Furthermore, an effective *ex vivo* treatment of SelN deficiency has been identified, paving the way to a clinical therapy. In this review we discuss the physiological and pathophysiological role of SelN and the interest of SEPN1-related myopathy as a model paradigm to understand and target therapeutically other selenoproteins involved in human health and disease.

## Introduction

Living cells continually produce low levels of reactive oxygen species (ROS) and nitric oxide (NO) derivatives. Both ROS and NO influence cellular function by modulating excitation–contraction coupling (1, 82, 89, 101), glucose uptake (46), mitochondrial respiration (56), and gene expression (63). Furthermore, it has been recently demonstrated that ROS are part of the intracellular signaling cascade (45). The net activity of ROS or NO depends on the overall balance between synthesis and buffering; a correct balance is necessary to keep cells in a state of redox equilibrium and thus preserve normal functioning. When the equilibrium between ROS production and removal is disrupted, oxidative stress occurs. Increasing understanding of the importance of redox signaling pathways in the last years has modified the definition of oxidative stress, from 'imbalance between oxidants and antioxidants' to the more comprehensive 'disruption of redox signaling and control' (98). Oxidative stress has multiple targets, can cause cellular dysfunction or death, and is accompanied by numerous biochemical indexes: lipid peroxidation, protein carbonyl formation, glutathione oxidation or depletion, and even DNA damage. Its involvement in the pathogenesis of various chronic diseases, including muscle and cardiovascular disorders, diabetes, neurological diseases, and cancer, is increasingly documented (11).

To neutralize these reactive species, cells possess several antioxidant systems with different chemistries and intracellular localizations (84, 107). Nonenzymatic antioxidants such as glutathione, vitamin E, ascorbate, and carotenoids, directly neutralize (scavenge) ROS, being destroyed upon oxidation. The main enzymatic antioxidant systems include catalase, the mitochondrial and cytosolic isoforms of superoxide dismutase (MnSOD and CuZnSOD, respectively) and, importantly, selenoproteins; indeed, the selenoenzymes glutathione peroxidases (GPxs) are one of the major cell protective systems (84). Superoxide dismutase (SOD) dismutes superoxide anions to $H_2O_2$, which is rapidly converted by catalase into $H_2O$ and

[1]Inserm, U787, Institut de Myologie, Paris, France.
[2]UPMC Univ Paris 06, UMR_S787, IFR14, Paris, France.
[3]AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Centre de Référence des Maladies Neuromusculaires Paris-Est, Paris, France.
[4]AP-HP, Hôpital Raymond Poincaré, Service de Pédiatrie, Centre de Référence des Maladies Neuromusculaires GNMH, Paris, France.

molecular oxygen; selenoproteins such as glutathione peroxidase and thioredoxin reductase contribute, among others, to regenerate glutathione and thioredoxin oxidized by free radicals. A schematic overview of oxidant sources and main cell antioxidant systems is depicted in Fig. 1.

The human selenoproteome includes 25 characterized selenoproteins; each of them contains at least one selenocysteine, the biological form of selenium, encoded by a UGA codon and cotranslationally incorporated in response to a complex mechanism (9, 81) (reviewed by Donovan *et al*. in this issue). Selenoproteins are essential for mammals, as demonstrated by the fact that deletion of the selenocysteine tRNA gene, which controls the expression of the entire selenoproteome, induces early embryonic lethality in mice (15). Although the function of most selenoproteins remains unknown, the few which have been fully characterized are enzymes with oxidoreductase functions (81). Their catalytic group within the active site includes the selenocysteine residue, whose biochemical properties render it more reactive than thiols in nucleophilic reactions at physiological pH (43). Thus, selenocysteine is more actively oxidized than cysteine (79) and has the potential to repair oxidative damage in proteins by reducing tyrosyl radicals with higher efficiency (99). Selenium is a key factor in regulation of selenoprotein biosynthesis; selenium depletion in food supply has revealed a hierarchy in expression among different selenoproteins and tissues, GPx-1 being one of the first selenoproteins whose enzymatic activity and protein level decrease during selenium deprivation (17).

In the last years, significant progress has been made towards elucidating the functional importance of specific selenoproteins and their implication in human diseases, and large-scale selenium supplementation trials have been launched (64). However, only one selenoprotein, selenoprotein N (SelN), has been directly associated with a genetic disorder. This review will provide a synthetic overview of the antioxidant and redox signaling properties of known human selenoproteins, and will then focus on SelN, whose physiological and pathophysiological role has been recently elucidated, paving the way to the development of a pharmacological therapy for an infantile inherited disease.

## Antioxidant Properties of Human Selenoproteins

The human selenoproteome consists of 17 selenoprotein families. Three major classes, the glutathione peroxidases (GPxs), thioredoxin reductases (TrxRs), and iodothyronine deiodinases (DIOs), were the first discovered and are extensively studied. DIOs are involved in thyroid hormone metabolism (8). GPxs and TrxRs have an antioxidant role and, glutathione and thioredoxin being involved in the cellular redox pathways, they modulate cell function by modifying redox status (104). Regulatory and functional interactions between both families of selenoenzymes have been observed (Table 1).

### *Glutathione peroxidases*

Reduced glutathione (GSH) is the most abundant antioxidant; in muscle cells GSH is present in millimolar concentra-

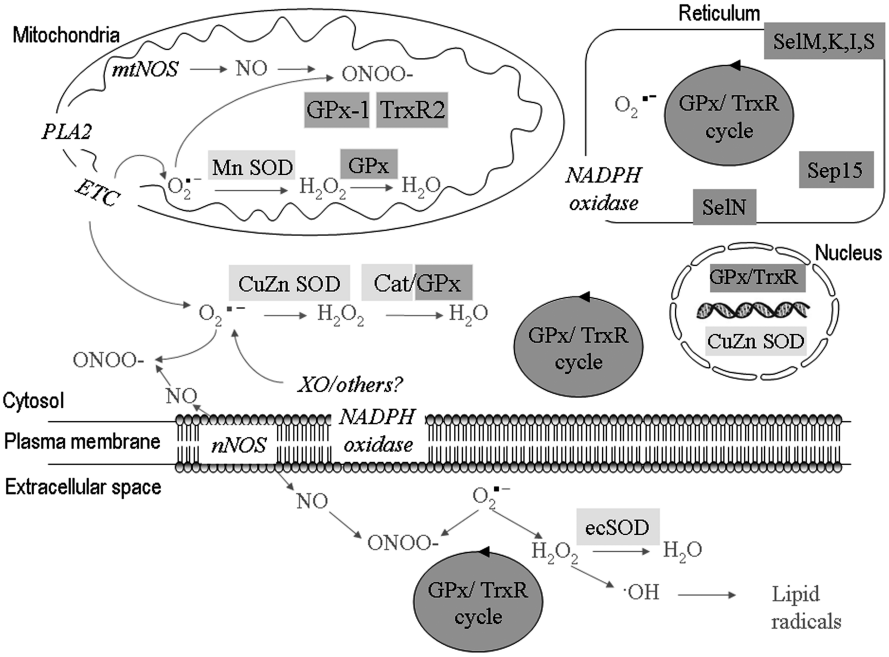



**FIG. 1. Schematic diagram depicting the different sites of ROS and NO generation by cells (italic font) and the main antioxidant enzymes present in a majority of human cell types (shadowed).** Superoxide anions ($O_2^{\bullet-}$), generated by mitochondria, NAD(P)H oxidase, 5-lipoxygenase, cyclooxygenase, or xanthine oxidase, are the parent molecule of the ROS cascade (*gray*), giving rise to oxygen-based derivatives including hydrogen peroxide ($H_2O_2$), and hydroxyl radical (OH). Antioxidant enzymes are shadowed in *dark gray* (selenoproteins) or *light gray* (nonselenoprotein enzymes). Cat, catalase; CuZn SOD, copper/zinc superoxide dismutase; ecSOD, extracellular superoxide dismutase; ETC, electron transport chain; Mn SOD, manganese superoxide dismutase; mt NOS, mitochondrial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; PLA2, phospholipase A2; reticulum, endoplasmic/sarcoplasmic reticulum; XO, xanthine oxidase; GPx, glutathione peroxidase; TrxR, thioredoxin reductase.

Table 1. Overview of the Experimentally-Documented Functions of Human Selenoproteins

| | |
|---|---|
| Maintenance of selenoproteins | |
| Transport and storage of Se | SeIP |
| Selenocysteine synthesis | SPS2 |
| Modulation of GPx activity and expression | SeIH |
| Thyroid hormone metabolism | DIO 1-3 |
| Transcription factor | SeIH |
| Ethanolamine phosphotransferase | SeII |
| Development | GPx-4, TrxR-1, TrxR-2, SeIW, SeIN? |
| Cell proliferation growth factor | TrxR-1 |
| Inflammatory response | SPS1 |
| Calcium homeostasis | SeIW, SeIT, SeIN |
| Redox signaling | |
| Regulation of transcription factors (NF-kB, AP-1, p53) | TrxR-1 |
| Detoxification; antioxidant | |
| Reduction of $H_2O_2$ | GPx-1 and 3, TrxRs, SeIW, SeIK, SeIN? |
| Reduction of various organic peroxides | GPxs |
| Protein reduction | |
| Methionine sulfoxide reduction | TrxR-1, TrxR-2, SeIR |
| Thiol disulfide isomerase | TrxR-3, Sep15, SeIM |
| Reduction of sulfoxymethyl group | SeIR |
| Unknown enzymatic activity (TrxR-like?) | SeIN |
| Protein folding | Sep15, SeIM, SelS, SeIN? |
| Apoptosis | TrxR-1, TrxR-2 |

Probable SelN functions requiring further investigation are indicated by a question mark.

tions and is the most important biologically (88). GSH reacts nonspecifically with various oxidants to yield an oxidized glutathione dimer (GSSG) in a reaction facilitated by the selenoenzyme glutathione peroxidase (GPx). Glutathione is recycled from GSSG by a second enzyme, glutathione reductase; the ratio of GSH and GSSG indicates the cellular redox status.

There are at least five selenocysteine-containing glutathione peroxidases (GPx-1 to -4 and GPx-6) in mammalian cells. Human GPxs are selenoenzymes with different substrate specificity and tissue-specific expression (4). GPx-1 and the phospholipid hydroperoxide GPx-4 are ubiquitously expressed proteins, GPx-3 is secreted to plasma, GPx-2 is specific of the gastrointestinal epithelium, and GPx-6 is found in the olfactory epithelium and embryonic tissues (81). All except GPx-3 are localized in the cytosol; GPx-4 presents also in mitochondrial and nuclear isoforms. Although each GPx is a distinct selenoprotein, they all have in common a catalytic site formed by selenocysteine, glutamine, and tryptophan residues in their active center (67). Their antioxidant role is based on their capacity to reduce potentially damaging ROS (such as hydrogen peroxide and organic hydroperoxides) to harmless products like $H_2O$ and alcohols, coupling their reduction with the oxidation of GSH (17).

*In vitro* and *in vivo* approaches have provided increasing proof of the importance of GPxs in cell homeostasis and survival. Cells overexpressing GPx-1 or GPx-4 are more resistant to oxidative stress elicited by different triggers of cell death (72, 111). These observations were corroborated using transgenic mice. Overexpression of either GPx-1 or GPx-4 resulted in increased survival of mice to the prooxidants paraquat and diquat (26, 86). Paradoxically, GPx-1 had a conflicting role in diquat- and peroxinitrite-induced oxidative injury, protecting against diquat-induced oxidative stress (attenuating NADH, NADPH, protein, and lipid oxidation) but promoting nitrosative stress under peroxinitrite (41). This raises interesting questions about the specificity of oxidant and antioxidant actions; antioxidant protection might not be a general property for a given enzyme such as GPx-1, but rather depend on the specific nature of oxidants (26, 40, 41). Although initial observations did not show any phenotype (48), genetically modified mice lacking GPx-1 have later been shown to have growth retardation, increased mitochondrial oxidative damage (32) and susceptibility to toxicity following administration of pro-oxidants (60). GPx-1 also is associated with protection against viral infection pathogenicity. Thus, selenium and particularly GPx-1 deficiency favor redox-related mutations of benign coxsackievirus, rendering it pathogenic and leading to the viral-induced myocardial lesions characteristic of Keshan disease (7, 59). On the other hand, GPx-4 is a key component of the mammalian antioxidant network (93, 112) and has an indispensable role in cell differentiation during mouse development (49).

Although less well known, other GPxs have also antioxidant capacities. Thus, GPx-2 plays a critical role in protecting mammal gastrointestinal tract from toxicity by ROS and from cancer development, and GPx-3 is an efficient plasma antioxidant (17, 81).

### *Thioredoxin reductases (TrxRs)*

TrxRs, thioredoxin (Trx), and NADPH form the thioredoxin system, a major cellular redox signaling in almost every living cell. TrxRs are involved in protection against reactive oxygen species through control of the redox state of the central molecule thioredoxin, using NADPH as a reducing agent. This system can also directly reduce numerous substrates, including hydroperoxides (13) or ubiquinone-10 (110). Thus, many cellular processes rely on the activity of these enzymes, including notably regulation of gene expression via redox control of transcription factors such as NF-kB or AP-1

(81). The three human TrxR isoenzymes contain one selenocysteine residue in their C-terminus. TrxR-1 is ubiquitously expressed in the cytosol (91, 94); TrxR-2 is localized in mitochondria and involved in control of mitochondrial redox processes; TrxR-3 is the testis-specific thioredoxin-glutathione reductase (44, 81). Like GPx-4, TrxR-1 and TrxR-2 are essential for mouse embryogenesis (28, 50). TrxR-1 has also the capacity of regulating the induction of the antioxidant enzyme heme oxygenase 1 (HO-1) (106).

### *Selenoprotein R (SelR)*

Selenoprotein R (SelR), also known as MsrB1 or selenoprotein X (SelX), is a member of the methionine sulfoxide reductases family, one of the rare systems able to repair mammalian oxidized proteins by reducing methionine sulfoxide residues (22, 54). SelR, located in the cytosol and nucleus, plays a major role in protein homeostasis during aging (22), thus contributing to delaying the aging process. Overexpression of SelR and other Msr activities leads to an increase in resistance to oxidative stress and life span (96). Furthermore, SelR and cysteine-containing isoforms have been demonstrated to have an important role in lens cell viability and protection against induced oxidative stress (70), potentially playing a role in cataract formation. A recent KO mouse model showed the strongest phenotype of SelR reduction and oxidative stress markers in liver and kidney, establishing an important contribution of SelR to the redox control in these organs (39).

### *Selenoprotein P*

SelP (selenoprotein P), identified early through biochemical studies, is primarily secreted from liver cells and acts as transporter protein, supplying extrahepatic tissues with selenium (20, 78, 95). However, it also acts as a ROS-detoxifying enzyme (21). SelP is one of the key effectors of redox regulation and cell viability in myofibroblasts (53), reduces phospholipid hydroperoxide, provides protection against *Trypanosoma congolense* infection through its N-terminal (redox) domain (16, 92), and protects human astrocytes from induced oxidative stress (77, 97, 98).

### *Other selenoproteins*

Other selenoproteins have been less extensively investigated, although experimental data support their antioxidant capacity. **Selenoprotein W (SelW**) (108) shares the redox motif with the GPx family and has a glutathione-dependant antioxidant activity (10, 51, 65). Its expression is closely modulated by selenium; nutritional selenium deprivation leads to severely reduced SelW expression as observed in white muscle disease (108). SelW is highly and preferentially expressed in proliferating myoblasts, protecting this and other cell types from oxidative stress (65). This selenoprotein has been proposed as a good marker to monitor immediate oxidation and toxic stress (81). **Selenoproteins localized in the ER** such as SelS, H, K, and Sep15 appear to be involved in regulation of intracellular ROS levels and protection from oxidative stress in cultured mammalian cells (for review, see Shchedrina, *et al*. in this issue). **SelS**, known for increasing production of inflammatory cytokines, is induced by ER stress and protects this cell compartment from oxidative stress (42). **SelH** significantly reduces superoxide production in neuronal cells challenged with UVB irradiation (12). Furthermore, SelH may act as a transcription factor, upregulating expression of other selenoproteins in response to stress and increasing glutathione levels and GPx activity (12). **Sep15** is a 15 kda selenoprotein characterized by a glutathione peroxidase-like enzymatic activity (57). Furthermore, in physiological conditions, Sep15 and **SelM** act as thiol-disulfide oxidoreductases (34). Both proteins, localized in the ER, are also involved in protein folding (34). Other ER proteins such as selenoproteins K, S, and T, have largely unknown functions. Only for **SelK** have antioxidant properties in cardiomyocytes been proposed (66).

## Selenoprotein N, a Novel Key Player in Antioxidant Protection of Human Cells

### *Selenoprotein N is the only selenoprotein implicated so far in a human genetic disorder*

In 1998, a peculiar and reportedly very rare form of congenital muscular dystrophy with early rigidity of the spine (rigid spine muscular dystrophy or RSMD1) was linked to chromosome 1p35-36 (38, 74). A few months later, *SEPN1,* the selenoprotein N gene localized at 1p36, was identified *in silico* (61). The previously described association between selenium deficiency and striated muscle dysfunction in livestock (25, 62) and humans (6, 14, 19, 59) immediately pointed *SEPN1* as a positional candidate for RSMD1. Indeed, homozygous or compound heterozygous *SEPN1* mutations were rapidly identified in most (75) although not all (76) the typical RSMD1 patients.

Surprisingly, mutations of the same gene, and often the same precise mutations (37), have thereafter been associated with three other early-onset muscle disorders, including two forms of congenital myopathy (the classical form of multiminicore disease (37) and rare cases of congenital fibre type disproportion (27)) and a myopathy with protein aggregates (desmin-related myopathy with Mallory body-like inclusions (35)). Actually, careful retrospective reassessment showed that these four autosomal recessive muscle conditions share so much clinical and molecular identity that they are best understood as the same unique disorder, now termed SEPN1-related myopathy (SEPN1-RM) (35, 37, 68, 103). Independently of the initial diagnosis, all patients carrying *SEPN1* mutations share a very homogeneous clinical phenotype. This phenotype is marked by severe weakness of neck and trunk muscles from infancy, which leads to major scoliosis and life-threatening respiratory insufficiency in childhood or adolescence and contrasts with relatively preserved limb strength and ambulation. Conversely, the pathological presentation of the disease is unusually heterogeneous. Most SEPN1-RM muscle biopsies show small focal areas of mitochondria depletion and sarcomere disorganization (short core lesions or "minicores") in muscle fibers; necrosis and regeneration and/or protein aggregates are less frequent but may also be present. Since muscle disorders are classically defined according with their pathological manifestations, the large myopathological spectrum of SEPN1-RM explained the unexpected overlap between different categories of myopathies, led to a revision of the established classification in the field and suggested a novel, polymorphic pathophysiological mechanism.

Currently, more than 180 patients with *SEPN1* mutations have been identified worldwide, for the most part in Europe (AF, personal communication), suggesting that this emerging disease is not as rare as once thought. The *SEPN1* gene contains 13 exons and produces a 4.5-kb transcript with an open reading frame encoding a 590-amino acid protein; the selenocysteine residue with predicted catalytic activity is encoded by exon 10 (75). Mutations are distributed along the whole gene, excepting exon 3 which corresponds to an Alu cassette, contains a second in-frame selenocysteine codon and is mostly spliced out in humans (83). Most of them are nonsense mutations, microdeletions or insertions leading to frameshifts, as well as splice-site mutations leading to aberrant pre-mRNA splicing (reviewed in (62)). This includes a nonsense mutation which converts the UGA selenocysteine (Sec) codon into a UAA stop codon, generating a premature termination codon and drastic mRNA decay (75). Missense mutations are relatively more common around or at the potential catalytic site. Interestingly, several mutations affect the *cis* sequences required for selenocysteine insertion. Thus, a homozygous point mutation in the 3′ UTR SECIS element abolishes binding of the SBP2 factor, thereby preventing redefinition of the UGA Sec and leading to a premature termination codon and severe *SEPN1* mRNA reduction (87). One of the mutations localized in the Sec codon redefinition element (SRE) induces a mismatch in the conserved SRE stem-loop structure, weakens the mRNA structure and reduces Sec insertion efficiency (68). Notably, all the heterozygous carriers of a *SEPN1* mutation are healthy, attesting that 50% normal SelN suffices to ensure its function.

The lack of function phenotype associated to SelN has focused attention on this protein. Yet its functional characterization has lagged behind, hindered among others by SelN weak expression in postnatal tissues and the lack of specific technical tools. Thus, no biochemical activity has been attributed to SelN yet, the pathogenesis of SEPN1-RM remained undetermined for a long time and no specific treatment was available for this potentially lethal disorder. However, recent studies have shed light on SelN and brought exciting progress both on the scientific and clinical fields.

### *SelN is an ER glycoprotein with an early developmental expression and a putative but unclear role in muscle development*

SelN is a 65-kDa transmembrane glycoprotein localized within the endoplasmic reticulum and containing a transmembrane addressing site close to an EF-hand motif (83). The topology of the protein has been determined recently: the N-terminal extremity is facing the cytoplasm, whilst the majority of the protein, including the predicted catalytic site and the C-terminal end, is located in the lumen of the ER (62).

Whilst the phenotype in SEPN1-RM involves exclusively skeletal muscles, human SelN is a ubiquitous protein (61) whose mRNA is detected in most fetal and, at a lower level, adult tissues (61, 75). Its expression is increased in proliferating cells such as fibroblasts and myoblasts, progressively decreasing during differentiation from myoblasts to myotubes (83).

An early developmental expression pattern of *SEPN1* was observed in zebrafish embryos in somites and notochord, which are the precursors of muscle and spinal cord, respectively (105). Recent studies have confirmed that the murine *Sepn1* transcript is also expressed early in somites, appears restricted to the myotome, the subectodermal mesenchyme and the dorsal root ganglia at mid-gestation (with levels peaking at E12.5) and then strongly decreases until birth. An additional fall in protein levels, not correlated with transcript modifications, was observed in the perinatal period, suggesting that post-transcriptional mechanisms contribute to SelN regulation (24).

This embryonic pattern of expression evoked a potential role of SelN in muscle and spinal cord development. The consequences of SelN absence on muscle development have been analyzed in zebrafish embryos (29, 52) and in *Sepn1* deficient mouse embryos (Rederstorff *et al.*, unpublished observations; 24), throwing interesting although somewhat conflicting results. In zebrafish embryos, inhibition of the *SEPN1* gene by injection of antisense morpholinos did not prevent muscle formation but caused reduced embryo motility (29). Muscle ultrastructure disclosed defects in the sarcomeric organization (29, 52) and/or myofibril attachment (29), suggesting a role of SelN at early steps of zebrafish embryogenesis and in muscle maintenance. In contrast, *Sepn1* deficiency in mouse embryos did not alter somitogenesis nor expression of myogenic factors, suggesting that SelN is dispensable for these processes in this mammal model currently under validation (24). The relatively normal morphological appearance of skeletal muscles at birth in most SEPN1-RM patients suggests that the latter conclusion may also apply to humans. No study has yet reported data on a potential implication of SelN in muscle regeneration or in cell proliferation, a key point that merits to be addressed.

### *SelN cellular function: At the crossroads of redox signaling, cell stress, and calcium homeostasis*

**SelN structure predicts a putative although uncharacterized enzymatic activity.** The biochemical activity of SelN remains unknown. To some extent, selenoproteins activity can be deduced from the sequence context of the selenocysteine residue, which constitutes a landmark of the catalytic center. SelN harbors a SCUG predicted catalytic site, reminiscent of the thioredoxin reductase GCUG motif (61), which suggests a putative reductase activity. However, classical thioredoxin reductases contain two other functionally important domains, the FAD and the NADPH-binding domains, which are absent in SelN, although this might be compensated by interactions with yet unidentified SelN partners (61). In addition, the highly accessible localization of the selenolate active site at the C-terminus of the thioredoxin reductases, supposed to confer them a broad range of substrates, contrasts with the localization of the active site in the central part of SelN; this lesser accessibility might reflect a higher selectivity of SelN for its substrate(s) (61).

**SEPN1 expression is potentially regulated by cell stress signals.** Analysis of the *SEPN1* promoter region predicts regulation of its expression by stress signals, supporting a potential implication of SelN in cell stress response pathways. NF-kB is one of the main transcription factors involved in cellular responses to exogenous insults such as free radicals or cytokines. Stoytcheva *et al.* revealed that all 25 human selenoprotein genes contain putative NF-kB response elements,

although only for DIO2, GPx-4, and SelS has regulation by NF-kB been experimentally proven (100). Using the MatInspector software (Genomatix Software Gmbh., Munich, Germany) (23), we identified five potential NF-kB binding sites in the promoter region (2500 basepairs upstream of the transcription start site) of *SEPN1*, as well as a putative ERSE (ER stress response) (113) element (-572/-554, CCAATCCCCA TAACCCATG) (SA and AF, unpublished results). In addition, the *SEPN1* promoter contains one putative CpG island (5, 100), suggesting a possible role of methylation in expression of this gene (Fig. 2). Putative binding sites for the redox-sensitive transcription factor AP-1 have also been found in *SEPN1* (100). Presence of binding sites for multiple redox-sensitive transcription factors in the *SEPN1* promoter suggests that SelN might be regulated by different cell stressors (ROS, ER stress, and/or cytokines) in a cooperative or synergistic manner. Interestingly, the predicted sequences of the ERSE element and of the first NF-kB putative binding site overlap (Fig. 2), suggesting a mutually exclusive, balanced regulation of *SEPN1* expression at this site. Experimental studies of *SEPN1* regulation are necessary to clarify this interesting point.

SelN and the pathophysiology of muscle disorders: SelN absence is associated with protein oxidation, $Ca^{2+}$ handling abnormalities, and increased susceptibility to oxidative stress. Muscle disorders associated with selenoprotein N deficiency constitute a valuable disease model, providing further indications of a potential relation between SelN and redox homeostasis. Thus, axial muscles and especially the diaphragm, which are predominantly affected in SEPN1-related myopathy, are particularly sensitive to oxidative stress (3, 80). Moreover, there is a morphological and in some cases clinical overlap between SEPN1-RM and core myopathy associated with *RYR1* mutations (36). *RYR1* encodes the ryanodine receptor type 1 (RyR1), a sarcoplasmic reticulum (SR) $Ca^{2+}$ release channel which plays a key role in excitation contraction (EC) coupling. Some primary *RYR1* mutations causing core myopathies have been associated with $Ca^{2+}$ leaking (30, 31). Since both *RYR1* and *SEPN1* mutations are associated to minicore lesions, one can hypothesize that the SR proteins encoded by these two genes might be implicated

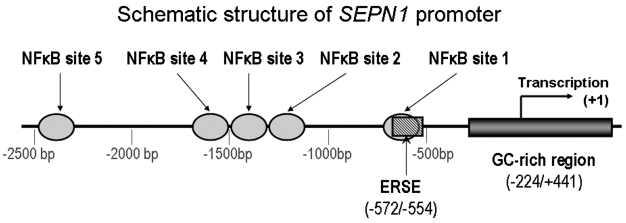



**FIG. 2. Schematic structure of the SelN promoter showing the positions of five putative NF-kB binding sites, the ERSE element, and the GC-rich region.** The numbers indicate the nucleotide positions relative to the transcription start site.

in common physiopathological pathways. The most obvious one would be redox homeostasis, since RyR1 is emerging as a paradigm of redox-sensor ion channel (71). Due to its important number of reactive cysteines (*i.e.*, thiols susceptible to redox-based modifications), RyRs are directly modulated by intracellular oxidants. NO and derivatives, via the nitrosylation of determined RyR thiol groups (33), may induce $Ca^{2+}$ release from the SR into the cytosol. Along these lines, in livestock muscle disorders due to selenium deficiency (white muscle disease, rigid lamb syndrome), altered ability of the SR membranes to retain calcium has been described (25, 62). Conversely, $Ca^{2+}$ overload and/or leaking could lead to oxidative/nitrosative stress (55) by activating calcium-dependant oxidant sources.

Recently, analysis of an *ex vivo* model of SelN deficiency allowed us to confirm that SelN plays an important role in cell defence against oxidative stress and $Ca^{2+}$ homeostasis in human skeletal muscle cells (3). Primary cultured myotubes from patients with *SEPN1* null mutations showed a significant increase in basal intracellular oxidant activity compared to controls, with a significant contribution of NO; in contrast, mutant fibroblast oxidant activity remained unaltered. Oxidized protein content, a hallmark of oxidative stress, was increased both in SelN-devoid fibroblasts and myotubes; in the latter, excessive oxidation of the contractile proteins actin and myosin heavy chain II was observed, potentially contributing to mechanical dysfunction (3, 58). Furthermore, SelN-devoid human myotubes had an increased resting cytosolic $Ca^{2+}$ concentration, together with reduced SR $Ca^{2+}$ load and caffeine-induced $Ca^{2+}$ release. This suggests that, in the absence of SelN, the ROS/NO generated by muscle cells regulate intracellular $Ca^{2+}$ concentration via modulation of $Ca^{2+}$ channels (RyR1 and/or SR $Ca^{2+}$ ATPase), favoring $Ca^{2+}$ release or leaking. Interestingly, independent studies using C2C12 myotubes knocked-down for *SEPN1* showed consistent results, namely increase of oxidant activity and protein carbonylation (73). In addition, zebrafish studies support the role of SelN as a RyR modifier. In zebrafish, SelN is physically associated with the ryanodine receptors; both types of proteins are required for the same cellular differentiation events and are needed for normal calcium fluxes in the embryo (52). Furthermore, in the absence of SelN, ryanodine receptors from zebrafish embryos or human diseased muscle have altered biochemical properties and have lost their normal sensitivity to redox conditions (52). Remarkably, *RYR* mutant and *SEPN1* mutant zebrafish have in common the disruption of slow muscle fiber formation and show a significant decrease in slow muscle fibers (29, 52). This contrasts with the human situation, stressing the important differences in redox signaling between zebrafish and mammals; indeed, in SEPN1-RM slow (type 1) muscle fibers are typically more abundant than fast fibers.

On the other hand, there is evidence to suggest that SelN is not exclusively a "redox chaperone" of the ryanodine receptors, but is also involved in modulation of redox defense and cell survival pathways. In the *ex vivo* cell model of SelN deficiency mentioned above, fibroblasts and muscle cells devoid of SelN showed a higher susceptibility to $H_2O_2$-induced oxidative stress, manifested by a severely increased cell death rate (3). Interestingly, no compensatory upregulation of MnSOD or catalase was observed either in basal conditions (despite high oxidant activity) or after exogenous oxidant

challenge, suggesting that SelN absence is associated with permanent abnormalities in antioxidant defense capacity (3). It has been previously reported that addition of $H_2O_2$ to mammalian cultured cells activates a series of growth arrest, cell death, and survival signaling pathways (63). In SelN-devoid cells treated with $H_2O_2$, cell death pathways (*e.g.*, p53 and NF-kB) might be more active than cell survival pathways (such as Akt, ERK, JAK/STAT, or phospholipase C-g1). Further studies are needed to dissect further these pathways, which could point towards potential therapeutic targets for selenoprotein N defects and other related disorders.

The current knowledge and our working model of SelN antioxidant role in human muscle cells (3) is summarized in Fig. 3. Absence of SelN is associated with oxidative/nitrosative stress, suggesting that this selenoprotein is involved in buffering and/or preventing overproduction of oxidants. Presence of the SelN selenocysteine in the lumen of the SR could protect proteins from excessive and irreversible oxidation/nitrosylation in at least two ways. First, since selenocysteine is more reactive than cysteine (62), SelN could react passively with oxidants more quickly than other cysteine-containing SR proteins (including the most cysteine-rich RyRs). Second, SelN could enzymatically exchange hydroxyl groups with oxidized proteins, hence allowing these to return to the reduced state and to pursue normal trafficking through the SR. Thus, SelN potentially protects general maturation and trafficking of proteins, preventing excessive protein carbonylation and degradation. In addition, SelN can protect specific target proteins of particular relevance for muscle function. This is the case of several contractile proteins whose function is known to be affected by oxidation [such as myosin heavy chain (2, 18), myosin light chain (102), actin (47, 58), troponin (85), or tropomyosin (109)]. Also, by limiting nitrosylation of RyR1, SelN would prevent excessive $Ca^{2+}$ leakage from the SR into the cytosol. In the absence of SelN, $Ca^{2+}$ leak reduces the amount of SR $Ca^{2+}$ available for EC coupling (potentially contributing to contractile dysfunction and weakness) and leads to cytosolic $Ca^{2+}$ overload, which in turn can increase oxidative/nitrosative stress (55) by activating calcium-dependant oxidant sources. Finally, SelN can contribute to activate a network of redox-sensitive transcription factors that modulate the expression of antioxidant enzymes (predictedly including SelN itself) and determine the fate of the cell between survival and death.

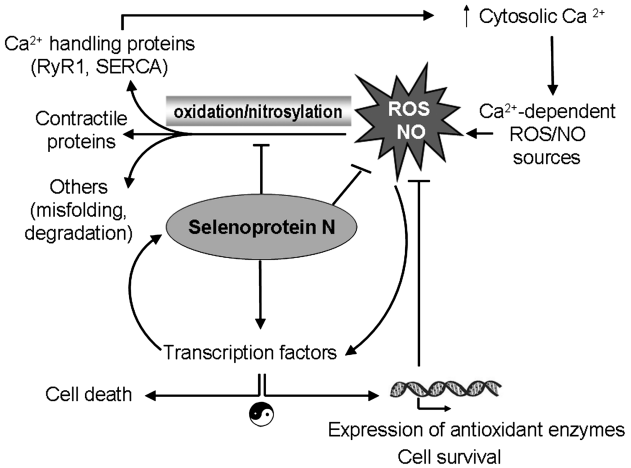



**FIG. 3. Putative role of SelN in redox homeostasis of human muscle cells.**

This model would account for the variety of lesions in muscles from SEPN1-RM patients ($Ca^{2+}$-related core lesions, protein aggregates, and/or necrosis and regeneration). More importantly, it shows SelN as a key protein at the crossroads of cell stress, redox signalling, and $Ca^{2+}$ homeostasis pathways.

### *Antioxidants as a therapeutic approach to selenoprotein defects: Lessons from SEPN1-RM*

SEPN1-RM is the only inherited disease due to a selenoprotein defect and the only structural myopathy primarily due to oxidative stress and antioxidant protection failure. As such, it represents an useful model paradigm for more complex, multifactorial disorders (such as cancer, cardiovascular disorders, diabetes, or aging) in which selenoproteins implication has been suggested (11). Therefore, finding a specific pharmacological treatment for this potentially-lethal, early-onset disease could also open interesting perspectives to human health at large.

The pathophysiological data described above suggest that the degree of muscle dysfunction in SEPN1-RM could be both free radical- and calcium-dependent; therefore, both pathophysiological pathways represent potential therapeutic targets for this disorder. Primary cultured cells from patients with *SEPN1* mutations show a quantifiable susceptibility to $H_2O_2$-induced oxidative stress, manifested by a significantly increased cell death rate (3). We used this cell phenotype to evaluate the *ex vivo* effect of antioxidant drugs targeting the primary pathogenic mechanism of the disease. Pretreatment of these SelN-devoid cells with the flavonoid fisetin or the carotenoid astaxanthin prior to $H_2O_2$ exposure had a partial or null protective effect. Interestingly, only pretreatment with N-acetyl cysteine (NAC) rescued this cell phenotype, significantly improving myoblast survival and rendering the cell death rate of SelN-devoid fibroblasts identical to that of control unchallenged cells. In addition, NAC normalized the levels of oxidized proteins in SEPN1-mutant myotubes (3). These findings demonstrate that NAC is an effective *ex vivo* treatment of SelN deficiency. Remarkably, they also establish that not all types of antioxidant drugs have the potential for therapeutic replacement of a particular selenoprotein function.

The specificity of NAC consists in its cysteine-donor activity, which could partially replace the hypothetical thiol exchanger activity of the selenocysteine in SelN. But this molecule has also other numerous biological roles (Fig. 4). NAC can act as a direct ROS scavenger and is a precursor for glutathione (GSH), an important nonenzymatic antioxidant. In addition, NAC increases the expression of gluthatione peroxidase, the enzymatic antioxidant that oxidizes glutathione (GSH) to glutathione-disulfide (GSSH), thereby reducing $H_2O_2$ to $H_2O$ and protecting cell components from oxidative damage. It has also been proposed that NAC downregulates the expression of NF-kB, which is a major mediator of inflammatory responses and controls expression of a large variety of genes (63). Therefore, NAC multifactorial antioxidant effect can potentially and specifically address at several levels the pathological consequences of SelN deficiency, and we propose that this antioxidant might be of particular interest to target defects of other selenoproteins in humans.

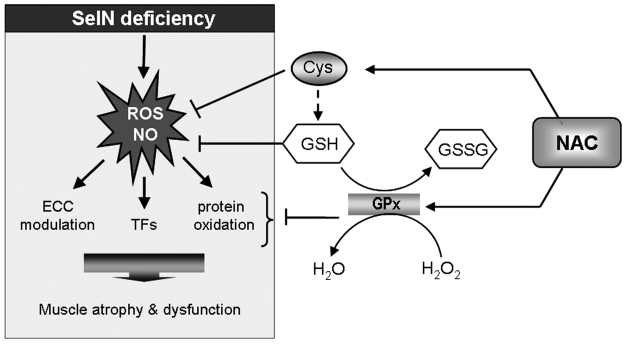



**FIG. 4. Mechanisms of action and pathophysiological targets of N-acetyl cysteine (NAC) in SEPN1-related myopathy.** ECC: excitation-contraction coupling; GPx: glutathione peroxidase; TFs: transcription factors.

NAC is one of the rare antioxidant drugs approved for human use, has been shown to inhibit muscle fatigue in healthy adults, and has no serious side effects when administered at the established dosage (69, 90). This, together with its *ex vivo* efficiency on SelN-devoid cells, paves the way to a first therapeutic trial of SEPN1-RM in human patients, which is currently under preparation.

## Conclusions, Bottlenecks, and Open Roads

In conclusion, important progress has been made in understanding the complex relation between selenoproteins and oxidative stress. Contribution of this particular protein family to different major cellular pathways—protection against oxidative stress, intracellular signaling, regulation of calcium homeostasis, or links with ER stress—represents an emerging and exciting field of research. With regards to SelN, recent results demonstrate that this selenoprotein plays a significant role in human cell defense against oxidative stress and is implicated in redox control of several key processes in skeletal muscle cells, including EC coupling and possibly transcriptional control of antioxidant enzymes. Thus, SEPN1-RM represents the first structural muscle disorder primarily related with oxidative/nitrosative stress, and can be used as a model paradigm to study other disorders in which oxidative stress or selenoproteins are involved.

Despite these significant advances, many central questions remain to be answered. The function of most selenoproteins is unknown; even for those which have been more extensively characterized, our understanding of their multiple actions, partners, and potential roles in human pathogenesis is still incomplete. The complex mechanisms involved in modulation of expression, localization, and splicing of selenoproteins are far from fully understood. Some selenoproteins have a potential dual oxidant/antioxidant action that must be carefully investigated prior to considering therapeutic interventions. Along these lines, identification or development of compounds that could modify either the whole selenoproteome or specific selenoproteins would be a valuable tool for therapeutic studies. In the case of SelN, two key points that need to be addressed regard its enzymatic function and its protein interactions, particularly whether RyR is its sole substrate. Contrary to RyR1, RyR3 shows a developmental expression pattern similar to that of *SEPN1* (52); although this RyR isoform is not thought to be relevant in postnatal skeletal muscle, its potential interaction with SelN deserves further investigation.

In the last years, the development and characterization of cellular or animal models, which allow connecting the molecular selenoprotein dysfunction and the physiological defects, have represented a significant contribution to the field. On the other hand, the differences in basal oxidant activity observed in different SelN-devoid human cell types (fibroblasts *versus* muscle cells) stress the complexity of antioxidant defense mechanisms that show species, time, and tissue specificities. Redox homeostasis and signaling are certainly not identical in mice and men; therefore, research using human material can provide invaluable information regarding human health and disease. Most importantly, characterization of these pathways has opened avenues of investigation for the design of therapeutic approaches, of which SEPN1-RM is a good example. Further studies are in progress to determine a safe dosage of NAC, to establish its clinical efficiency in this condition, and to identify other drugs. Because in this unique condition antioxidants target the primary pathogenic defect, the planned trial with NAC would represent the first specific pharmacological treatment for a congenital myopathy and provide an important proof-of-concept regarding the efficiency of antioxidant drugs in a genetic disorder.

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Address correspondence to:
*Dr. Ana Ferreiro*
*INSERM U787*
*GH Pitié-Salpêtrière*
*105 Bd. de l'Hôpital*
*75651 Paris*
*France*

*E-mail:* ana.b.ferreiro@gmail.com

**Abbreviations Used**

Cat = catalase
Cu/Zn SOD = copper/zinc superoxide dismutase
DIOs = iodothyronine deiodinases
ecSOD = extracellular superoxide dismutase
ERSE = endoplasmic reticulum response element
GPxs = glutathione peroxidases
Mn SOD = manganese superoxide dismutase
mt NOS = mitochondrial nitric oxide synthase
nNOS = neuronal nitric oxide synthase
NO = nitric oxide
PLA = phospholipase A2
ROS = reactive oxygen species
SECIS = selenocysteine insertion sequence
SEPN1-RM = SEPN1-related myopathy
SOD = superoxide dismutase
SR = sarcoplasmic reticulum
SRE = Sec codon redefinition element
TrxRs = thioredoxin reductases
XO = xanthine oxidase