Chapter 18
Uncovering the Importance of Selenium in Muscle Disease

Alain Lescure, Mireille Baltzinger, and Ester Zito

Abstract A connection between selenium bioavailability and development of muscular disorders both in humans and livestock has been established for a long time. With the development of genomics, the function of several selenoproteins was shown to be involved in muscle activity, including SELENON, which was linked to an inherited form of myopathy. Development of animal models has helped to dissect the physiological dysfunction due to mutation in the *SELENON* gene; however the molecular activity remains elusive and only recent analysis using both in vivo and in vitro experiment provided hints toward its function in oxidative stress defence and calcium transport control. This review sets out to summarise most recent findings for the importance of selenium in muscle function and the contribution of this information to the design of strategies to cure the diseases.

Keywords Selenium · Selenoproteins · Muscle disease · Oxidative stress · Calcium transport · RYR · SERCA

Introduction

Two decades ago, priority in the selenium field was the identification of the exhaustive list of selenium-containing proteins or selenoproteins, believed to be the main biological active form of selenium. Discovery of the selenoproteome based on the peculiarity of the selenocysteine translation machinery was an important milestone showing that selenium is present in a limited number of proteins, 25 in humans,

A. Lescure (✉) · M. Baltzinger
Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, IBMC-15, Strasbourg, France
e-mail: a.lescure@ibmc-cnrs.unistra.fr

E. Zito (✉)
Dulbecco Telethon Institute at IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy
e-mail: ester.zito@marionegri.it

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reflecting its specialised function in living organisms. To gain further insight into selenium's biological role, many laboratories have been involved in unveiling the function of the newly discovered proteins, contributing to the description of their localisation, expression, structure, or cellular partners. However, though our understanding of the biochemistry and catalysis of the principal members of the selenoprotein family has made considerable progresses, membrane-bound selenoproteins still lag behind (Liu and Rozovsky 2015). Among these, the selenoprotein N or SELENON appeared as a priority, since it was shown shortly after its discovery that mutations in its gene were the causative condition for a class of inherited muscle diseases, referred as SELENON-related myopathy. This indicated the first connection between a gene translated in a selenoprotein and an inherited disease and pointed to many observations indicating an important link between selenium and muscle activity or maintenance. SELENON was identified among a group of endoplasmic reticulum-resident selenoproteins controlling calcium signalling and oxidoreductive homeostasis (Pitts and Hoffmann 2017).

Selenium-Related Muscle Disorders in Human and Livestock

Among the diseases related to selenium, selenium deficiency was identified as a risk factor for different muscular malfunctions both in humans and animals. In animals, nutritional muscular dystrophy is an acute condition affecting cardiac and skeletal muscles, caused by limited selenium supply, also sometimes associated with vitamin E deficiency. These muscle diseases have been seen to be epidemically abundant in young animals reared in places with selenium-deficient soil (Oldfield 2002; Yao et al. 2013; Thompson and Scott 1969; Delesalle et al. 2017). These disorders have been characterised in different species, including lambs, pigs or chicken, with different presentations and so-called nutritional myopathy, rigid syndrome, white muscle disease or exudative diathesis. Skeletal muscle degeneration linked to muscle weakness or stiffness, postural instability or walking disability is usually accompanied by a pale discoloration of the muscle tissue, intramuscular oedema and white streaks corresponding to bands of coagulation necrosis, fibrosis and calcification (Hefnawy and Tortora-Perez 2010; Thompson and Scott 1969). Cardiac muscle necrosis was associated with respiratory distress, cardiac arrhythmia and ultimately sudden death.

Selenium and vitamin E supplementation appeared to be sufficient to prevent the symptoms and the progression of the disease, reducing myocardial degeneration and skeletal muscle wasting (Değer et al. 2008; Beytut et al. 2002; Streeter et al. 2012; Sharp et al. 1972; Combs et al. 1975). However, the pathogenic mechanism remains elusive, and it is not clear whether the muscle degeneration is caused by increased oxidative stress due to a reduced expression of selenoproteins in general or low activity of a subset of selenoenzymes.

Selenoprotein expression was examined in a selenium-deficient broiler chicks model, where several selenoprotein genes were down-regulated in muscle and liver tissues, promoting increased oxidative stress and inducing activation of a p53- and p38-dependent MAPK/JNK/ERK signalling pathway (Yao et al. 2013; Huang et al.

2011, 2015). Selenium supply has been proposed as beneficial for mitigating oxidative muscle damage in exercised horses. The cooperative effect of selenium and vitamin E supplementation to alleviate the muscle disorders suggests convergence in a common antioxidant process that reduces lipid peroxidation (White et al. 2016). Accordingly, a study by Fujihara and Orden (2014) showed that higher consumption of vitamin E resulted in lower selenium accumulation in various organs in rats, indicating the involvement of the two compounds in a common or similar process.

In humans, Keshan disease is an endemic cardiomyopathy that develops in individuals with low selenium status in different areas of eastern China (Beck et al. 2003; Bor et al. 1999; Manar et al. 2001). Necrotic lesions, inflammatory areas and calcification throughout the myocardium are characteristics of this disease (Gu et al. 1983; Burke and Opeskin 2002). A mouse model revealed a dual aetiology of the pathological mechanism of the disease where there is a combination of lack in selenium and infection by the enterovirus *Coxsackie*. The primary infectious virus strain is not harmful per se, but increased oxidative stress in the selenium-deficient host can introduce mutations in the viral genome, increasing its virulence and resulting in the cardiac disease (Beck et al. 1995).

A relation between selenium deprivation and muscle pain or weakness was initially described in patients under prolonged parenteral nutrition (Brown et al. 1986; Kelly et al. 1988; van Rij et al. 1979; Baptista et al. 1984). The characteristic features in the myocardial muscle of these patients are reminiscent of Keshan disease. The hypothesis of selenium as a contributing or aggravating factor in other inherited forms of muscular dystrophies, such as myotonic or Duchenne muscular dystrophies, was therefore investigated. However, no evidence for beneficial effects of selenium could be found (Orndahl et al. 1994; Backman and Henriksson 1990; Gamstorp et al. 1986; Backman et al. 1988; Jackson et al. 1989). Several studies reported a significantly lower serum level in selenium in elderly patients with sarcopenia, a muscle disorder causing muscle weakness and loss of skeletal muscle mass (Lauretani et al. 2007; Beck et al. 2007; Chen et al. 2014), but no supplementation trial was conducted to confirm the importance of selenium in this condition.

A definitive connection between selenium's role and muscle formation and maintenance was finally provided by the identification of mutations in one selenoprotein, the SELENON coding gene being the cause of several congenital muscle disorders.

Selenoproteins in Muscle Disorders

Selenium is incorporated into proteins as part of its main biological active form, the organic amino acid selenocysteine (Sec). The proteins containing selenium in the form of Sec are called selenoproteins. The selenoprotein N or SELENON (previously called SELN or SEPN) is the first member of this protein family to be characterised as the origin of an inherited disorder; in two different studies, genomic screening in patients presenting clinical manifestations of either rigid spine

muscular dystrophy (RSMD) or classic forms of multiminicore disease (MmD) detected mutations in the *SELENON* gene at multiple loci (Moghadaszadeh et al. 2001; Ferreiro et al. 2002a, b). Later reassessment of the nosological classification of these disorders, besides the linkage of *SELENON* to desmin-related myopathy with Mallory body-like inclusions (MB-DRM) (Ferreiro et al. 2004) and congenital fibre-type disproportion (CFTD) (Clarke et al. 2006), led to their redefinition under a novel broader entity named *SELENON*-related myopathy (*SELENON*-RM).

SELENON-RM is a congenital disorder originating from heterogeneous mutations in the *SELENON* gene; it affects the muscular system with a wide spectrum of phenotypes and different onset of clinical manifestations, all characterised by muscle weakness mainly affecting neck and trunk muscles, and general muscle atrophy, leading to spine rigidity, severe scoliosis and life-threatening respiratory malfunction requiring assisted ventilation, with relative preservation of limb muscles and ambulation (Cagliani et al. 2011; Scoto et al. 2011). Insulin resistance was also reported in several patients (Clarke et al. 2006), but more systematic analyses are required to address whether this is a general feature of the disease. The clinical pattern, combined with muscle magnetic resonance imaging, provides the basis for the diagnosis of *SELENON*-RM. The histological description of the abnormalities associated with the disease is unusually broad, including enlarged endomysial extracellular matrix, changes in the number and size of muscle fibre types and intracellular lesions such as disorganised bands of sarcomeres, also called minicores, or aggregates of proteins. Since these microscopic features are not systematically observed, they are likely to represent different cellular evolution states of the diseases. A list of nonsense and missense mutations has been identified to date in the *SELENON* gene that cause loss of function of the protein and result in myopathies with an autosomal recessive pattern (see Castets et al. 2012). No direct genotype-phenotype correlation has been established between the mutation and the myopathic phenotype.

Myopathic symptoms with fatigue and weakness affecting neck, proximal and axial muscles were reported in several patients with mutations in the gene coding for the Sec-specific translation factor SECISBP2 (reviewed in Schoenmakers et al. 2016). Patients with mutations in the *SECISBP2* gene present a multi-systemic disorder with abnormal metabolism of thyroid hormones, secondary to loss of activity of the three Sec-containing deiodinase enzymes, together with low plasma selenium. Therefore many *SECISBP2* cases have different degrees of developmental delay affecting mainly the musculoskeletal system. The muscular phenotype described in a subgroup of *SECISBP2* patients is predicted to be related to SELENON deficiency (Schoenmakers et al. 2010). At the mechanistic level, *SECISBP2* mutations result in impaired synthesis and low expression of many selenoproteins. However, some expression is preserved, as functional activity of selenoproteins is still detectable (Schoenmakers et al. 2016). The nature of the mutation occurring in *SECISBP2* is expected to differently affect the set of non-translated selenoproteins that may or may not include SELENON, related to the severity of the muscular symptoms. However, perturbed thyroid hormone metabolism has been recognised as having severe adverse effects on muscle function as well (reviewed in

Marsili et al. 2016). Recent data show that both the local inactivation of thyroid hormone by deiodinase type 3, induced by muscle injury, and deiodinase 2-mediated T4 activation during muscle regeneration control satellite cell proliferation and differentiation, two successive steps essential for proper muscle repair (Dentice et al. 2010, 2014).

Selenoprotein W or SELENOW activity was originally linked to a muscular disorder, since its expression was reduced in muscle from animals with white muscle disease (WMD) (Whanger 2000). The sarcoplasmic reticulum of WMD animals is defective in calcium sequestration, resulting in calcification of both cardiac and skeletal muscle. SELENOW is a cytoplasmic selenoprotein, particularly abundant in skeletal muscle and brain. Based on sequence alignment and NMR studies, SELENOW together with five other selenoproteins (SELENOM, SELENOF, SELENOV, SELENOT and SELENOH), was seen to be part of the Rdx family of proteins. Rdx proteins display a thioredoxin-like fold structure and a conserved CxxC or UxxC (C stands for cysteine and U for selenocysteine) motif located in an exposed loop similar to the redox-active site in thioredoxin (Dikiy et al. 2007; Aachmann et al. 2007). SELENOW was shown to be highly expressed in proliferating myoblasts, and to induce cell proliferation-differentiation transition in skeletal muscle cells (Jeon et al. 2014). SELENOW interacts with the signalling protein 14-3-3, preventing its interaction with other cellular target proteins, such as the cell cycle progression/differentiation controllers CDC25B, Rictor and TAZ. Binding of SELENOW to 14-3-3 protein promotes the translocation of TAZ from the cytoplasm to the nucleus, which is required for its interaction with several transcription factors including MyoD, a key regulator for muscle cell differentiation. In addition, the Sec residue of SELENOW is directly involved in its interaction with 14-3-3 and this complex formation is sensitive to the intracellular redox environment (Jeon et al. 2016).

Molecular Description of SELENON Gene and Protein: Expression and Cell Localisation

SELENON was first identified by a bioinformatic screen then characterised by an in vitro assay; this study showed that *SELENON* mRNA contains the common features for grouping in the selenoprotein family, including a SECIS element within the 3'-UTR that redefines an in-frame UGA codon into a Sec residue (Lescure et al. 1999). The human *SELENON* gene was mapped to chromosome 1 p36-11 and is composed of 13 exons (Fig. 18.1). Genomic studies predicted that *SELENON* gene encodes for two RNA isoforms differing in the alternative splicing of exon 3. However, this additional exon 3 appeared to be primate specific and later analyses indicated that the second isoform, in which exon 3 is spliced out, is the most abundant in cells and the only one to be translated into protein (Petit et al. 2003). Screening for *SELENON* mRNA revealed its expression in many tissues, including

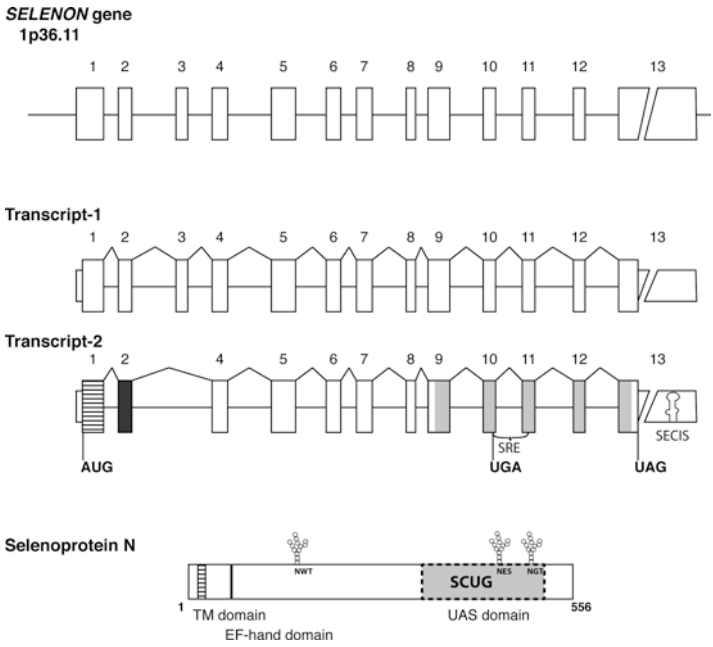

Fig. 18.1 Schematic representation of the human SELENON gene, the two SelenoN transcripts and the SELENON protein. The human *SELENON* gene spans an 18 kb region on the short (p) arm of chromosome 1 at position 36.11. It contains 13 exons (white boxes, upper panel). Exons 3, which corresponds to a primate-specific Alu sequence, is alternatively spliced, giving rise to two different mRNAs in human and other primates (middle panel). Only transcript 2 is translated into a protein. Exons 1 and 13 of transcript 2 contain the 5′- and 3'-UTR of the mRNA, respectively (small white boxes at both ends). Exons 10–11 and exon 13 contain the SRE and SECIS elements, two RNA motifs required for reprograming and selenocysteine insertion at the UGA codon. SELENON is a 556 amino acid long protein (lower panel) and is schematically represented with its functional transmembrane domain at the N-terminal end, allowing it to insert into the membrane of the endoplasmic reticulum (hatched box). Bioinformatic inspection by BLAST of the amino acid sequence identified an EF-hand domain (black line), a compact helix-loop-helix structural motif predicted to bind calcium. In the C-terminal part, a UAS conserved domain (grey box) includes the predicted catalytic site illustrated by the SCUG sequence (U = selenocysteine). SELENON is glycosylated at three different sites (NWT, NES and NGT) depicted in the scheme

skeletal and cardiac muscles, though at different levels (Lescure et al. 1999; Castets et al. 2009; Zhang et al. 2012).

The *SELENON* gene encodes for a 556-amino acid protein with a predicted mass of 62 kDa. However, when fractionated on a SDS-PAGE, SELENON migrated at a higher position, suggesting post-translational modifications, and glycosylation of the protein was experimentally validated (Petit et al. 2003). Inspection of the amino acid sequence revealed a stretch of hydrophobic residues at the N-terminus corresponding to a transmembrane domain (Fig. 18.1).

The cellular localisation of SELENON showed that it is an endoplasmic reticulum (ER)-resident protein, and that the sequence of the first exon was sufficient to

address the protein in the ER compartment. Protease protection assay showed that the protein contains a single N-terminal transmembrane domain and that most of the protein, including the catalytic site—predicted from the position of the Sec residue—is located within the lumen of the ER. Amino acid sequence homology identified a classical EF-hand motif, a calcium-binding domain in the N-terminal part of SELENON and a UAS domain in the C-terminal part (Fig. 18.1). UAS is a domain of unknown function conserved in a subgroup of the UBXD protein family: UBXD7, UBXD8 (FAF2) and UBXD12 (FAF1). UBXD proteins are mainly associated with the ER and contribute to the positive or negative regulation of the ER-associated degradation machinery or ERAD (Rezvani 2016). The UAS domain of UBXD8 and UBXD12 binds long-chain unsaturated fatty acids and mediates polymerisation of the proteins (Kim et al. 2013). The Sec residue of SELENON, which is a landmark for the catalytic centre, is located within the UAS domain and is present in a SCUG motif that resembles the active GCUG sequence of thioredoxin reductases (TXNRD), suggesting oxido-reductase activity. In the three TXNRD, this motif, located at the C-terminal of the proteins, is directly involved in reduction of the thioredoxin substrate (Arnér and Holmgren 2000).

Deciphering SELENON Activity

Both zebrafish and mouse animal models were designed to decipher the underlying molecular and physiological dysfunctions arising from *SELENON* gene mutations. The data collected from these models addressing the role of SELENON activity in muscle development and maintenance, as well as during muscle regeneration, were reviewed elsewhere (Castets et al. 2012; Lescure et al. 2016). Here we are focusing on the analysis and combination of information resulting from studies of in vitro, ex vivo and in vivo models.

Defence Against Oxidative Stress in the Endoplasmic Reticulum (ER)

The biochemical activity of SELENON is still unknown, but there are many clues pointing to its important role in the defence against hyperoxidation in the ER, namely the ER stress response. A putative ER stress-responsive element (ERSE) was identified in the promoter region of the *SELENON* gene (Arbogast and Ferreiro 2010), and *SELENON* induction was detected after in vitro treatment with ER stress inducers such as tunicamycin and thapsigargin (Marino et al. 2015). Furthermore, cells devoid of SELENON displayed higher levels of oxidised glutathione (Marino et al. 2015) and were more susceptible to H_2O_2 treatment (Arbogast et al. 2009). This increased susceptibility to oxidative species was rescued by pretreatment with

the reductant N-acetyl-cysteine (NAC). Altogether, these data indicate that *SELENON* expression is responsive to ER stress and that SELENON activity is involved in ER defence against oxidative insults.

These two characteristics of SELENON recall a connection with the homeostatic unfolded protein response (UPR), a mechanism which aims at restoring ER fitness after an ER hyperoxidation-induced stress (Ron and Walter 2007; Walter and Ron 2011). The UPR effector, ER oxidoreductin 1 (ERO1), is the main ER protein disulphide oxidase involved in oxidative protein folding, but since its activity is related to production of H_2O_2, it also burdens the ER cell compartment with potentially toxic reactive oxygen species (ROS) (Tu and Weissman 2002; Gross et al. 2004; Zito 2015). The relationship between oxidative protein folding in the ER and oxidative stress is supported by the fact that UPR activity, together with ERO1, also activates genes that combat oxidative stress. Protein kinase R(PKR)-like endoplasmic reticulum kinase (PERK) signalling accounts for a substantial part of this gene expression programme, and compromising signalling in this branch of the UPR markedly increases ROS levels in ER-stressed cells (Harding et al. 2003). A similar process may also exist in yeast, in which unusual high levels of ER stress are coupled with increased oxidative stress (Haynes et al. 2004). Experiments conducted in *C. elegans* confirmed that ERO1 activity contributes to ROS production as knockdown of *ero-1* reduced the levels of endogenous peroxides in ER-stressed tissues (Harding et al. 2003). These findings were consistent with the simple hypothesis that the combination of an ERO1 up-regulation and an inefficient antioxidant response during UPR may compromise cell viability in yeast. In mammalian cells, SELENON expression level paralleled that of ERO1, and *SELENON* hypomorphic myoblasts were hypersensitive to ERO1 overexpression. These two observations suggested the co-regulation of ERO1 and SELENON expression, and that SELENON may have evolved to be part of an UPR-dependent antioxidant response in higher eukaryotes (Marino et al. 2015).

Due to its high metabolic activity, skeletal muscle tissue has a propensity to be exposed to ER stress and produce ROS; therefore it may need to mount an adequate antioxidative response. The UPR is one of the primary processes triggered in skeletal muscle by environmental challenges such as long-distance running or dietary alteration. This signalling activates the skeletal muscle ER-stress pathway and generates ROS; ROS are also produced by skeletal muscle contraction and long periods of muscle immobilisation (Powers et al. 2011; Wu et al. 2011). Clues for an alteration of this signalling pathway in SELENON-deficient individuals were provided by the study of animal models. Unlike in humans and in a zebrafish model, in which *SELENON* loss of function gave rise to an overt muscle phenotype (Deniziak et al. 2007; Jurynec et al. 2008), *SelenoN* knockout (KO) mouse limb muscles were somehow protected and showed no major alterations in their histological or physiological presentation (Rederstorff et al. 2011; Moghadaszadeh et al. 2013). This protection may be provided by the activity of redundant pathways controlling redox balance, and/or the limited muscle activity restraining ER stress and oxidative insults (Rayavarapu 2012). In line with this, *SelenoN* KO mice compared to wild type showed a significant reduction in normalised muscle force after ERO1 overex-

pression in the gastrocnemius muscle, a symptom that recalls the intrinsic muscle fibre abnormalities and muscle weakness associated with the human phenotype of *SELENON*-related myopathies (Pozzer et al. 2017). These data support the hypothesis that SELENON plays a role in protecting skeletal muscle from the negative consequences of ERO1 overexpression.

In support with the hypothesis that SELENON plays a role in defending skeletal muscle against hyperoxidation, studies showed that removing or reducing the dietary intake of the antioxidants vitamin E or vitamin C worsens the muscle phenotype in *SelenoN* KO mice. Studies from Beggs' laboratory showed that muscle exercise combined with restricted dietary vitamin E led to extensive accumulation of core lesions in the muscle fibre that are reminiscent of the dense minicores described in patients carrying *SELENON* mutations (Moghadaszadeh et al. 2013). Interestingly, zebrafish depleted in SELENON activity by Morpholino injection (Deniziak et al. 2007) showed an overt muscle phenotype and, like humans, zebrafish are auxotroph for vitamin C or ascorbic acid (AA) (Toyohara et al. 1996). AA reductant activity and ER localisation may be perfectly suited to cope with the lack of SELENON. Mice producing their own AA may be more protected against a pathological muscle mechanism. On the basis of these observations, Zito's laboratory has generated a *SelenoN* KO mice model that is dependent on exogenous AA supply, and studied the possible connection between muscle AA levels and a pathological *SELNON*-related muscle phenotype. Supplying a high AA dose in drinking water (corresponding to an average of 20 ng/mg of muscle, a concentration comparable with the average AA level in wild-type or *SelenoN* KO muscles) did not lead to any histologically or physiologically detectable muscle dysfunction, but providing a medium dose (corresponding to an average of 8 ng/mg of muscle) led to muscle ER stress, as well as a myopathic reduction in normalised muscle force and appearance of minicores. Furthermore, a low dose (corresponding to an average of 4 ng/mg of muscle) led to muscle ER stress, more severe muscle atrophy and a reduction in absolute muscle force. In brief, the severity of the progressive muscle defect was inversely proportional to the muscle concentration of AA, suggesting a protective role for AA in the *SelenoN* KO mouse muscle (Pozzer et al. 2017).

SELENON and Calcium Transport: RYR1 and SERCA2

Aside of newly synthetised protein folding, another important ER function controlled by redox status is the flux of calcium between the ER compartment and the cytosol (Appenzeller-Herzog and Simmen 2016). Several transporters determine the homeostatic cytosolic calcium concentration that is essential to control skeletal muscle excitation/contraction coupling. Regulation of calcium handling by these channels is conferred by modification of redox-sensitive cysteines located on both sides of the ER membrane.

Ryanodine receptors (RyRs) are calcium channels located in the membrane of the sarcoplasmic/endoplasmic reticulum (SR/ER), where they mediate the release

of calcium ions in order to tune cytosolic calcium concentrations (Lanner et al. 2010). Most of the RyR1 mutations studied so far show a gain-of-function phenotype in which channel opening is facilitated, with a consequent leakage of calcium to the cytoplasm (Dirksen et al. 2002; Ducreux et al. 2006). The overlapping of some histological and clinical signs between patients affected by *SELENON*-related myopathies and the core myopathies associated with RYR1 mutations fuelled studies on a functional link between SELENON and RyRs (Ferreiro et al. 2002a, b).

SELENON protein was immunoprecipitated from rabbit muscle homogenate with an antiserum that recognises all of the RyR isoforms, thus indicating that SELENON and RyRs are present in the same complex. In addition, a functional analysis of equilibrium binding indicated diminished RyR-ryanodine binding capacity in SELENON-depleted tissue, which suggested that SELENON may modulate RyR activity. More strikingly, the reintroduction of SELENON into SELENON-depleted tissue restored the ability of ryanodine binding to respond to changes in redox potential, thus suggesting that SELENON modulates RyR channel behaviour at the level of redox-sensitive residues (Jurynec et al. 2008). However, further functional redox studies aimed at identifying RyR1 cysteines that are sensitive to redox-dependent SELENON activity were hampered by the large number of cysteines represented in RyRs (more than 80 cysteines/protomer) (Xu et al. 1998). More recently, the finding of a characteristic redox-active CU, motif similar to the catalytic site of thioredoxin reductases (TXNRD) on the ER side of the SELENON sequence, suggests an ER reductase activity for SELENON (Castets et al. 2012). Given the electron transfer mechanism of the active catalytic site of TXNRD formed by the C and U residues, it was hypothesised that SELENON may attack and form a covalent bond with an oxidised proteinaceous target via its nucleophilic U.

An unbiased mass spectrometry approach was subsequently used to identify redox proteinaceous interactors of SELENON, among which the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2) was prominently identified (Marino et al. 2015). SERCAs are multipass transmembrane proteins of the ER/SR importing calcium from the cytosol to the ER; their activity determines the resting cytosolic calcium concentration that is crucial for skeletal muscle excitation/contraction coupling. The importance of SERCA function in skeletal muscle diseases is exemplified by the fact that SERCA1 loss-of-function mutations give rise to Brody myopathy (Stammers et al. 2015; Guglielmi et al. 2013). Two isoforms of SERCA2 have been described: SERCA2a is expressed in the slow-twitch fibres of skeletal muscle and in cardiac muscle, whereas its C-terminally extended isoform, SERCA2b, is ubiquitously expressed in muscle and non-muscle tissues (Baba-Aissa et al. 1998). Redox-active SELENON was shown to co-immunoprecipitate both SERCA2a and SERCA2b.

Early studies suggested the presence of two regulatory cysteines on the ER side of the SERCA2b pump whose redox state influences pump activity (Li and Camacho 2004) and, on the basis of this model, it was shown that the interaction between SELENON and SERCA2 depends on the two redox-active CU amino acids of SELENON and the two cysteines in the L4 domain of SERCA2. Functional experiments investigating the ER calcium concentration in primary *SelenoN* KO mouse

embryonic fibroblasts (MEFs) showed reduced calcium levels not only at steady state, but also during maximum calcium uptake, a status in which calcium exit and buffering are negligible and ER calcium level mainly depends on SERCA activity. This result indicated a less active SERCAs in *SelenoN*-depleted MEFs, and normal activity was restored by expression of active SELENON including the selenocysteine (Marino et al. 2015). In contrary to the study of Jurynec et al. (2008), there was no difference in ER calcium exit, thus raising the question as to whether SELENON influences calcium exit from the ER by modulating ER calcium channels/receptors. However, no RyRs were expressed in the analysed cells, and calcium exit was mainly controlled by inositol triphosphate (IP3) receptors.

Very recently, the laboratories of Zito and Blaauw measured SERCA and RyR activity in the more physiological environment of muscle fibres extracted from the flexor digitorum brevis (FDB) of *SelenoN* KO mice. SERCA activity was evaluated by measuring relaxation time after an electrical input, and RYR activity was tested after challenging the muscle fibres with caffeine, a RyR agonist. The relaxation time of *SelenoN* KO fibres was longer, which is consistent with reduced SERCA activity. However, no difference was observed between caffeine-treated wild-type and *SelenoN* KO fibres, indicating once again that SELENON controls ER calcium entry rather than exit. The same laboratories also compared the FDB muscle fibres of *SelenoN* KO mice with those extracted from mice carrying a RyR1-Y522S mutation, a mutation leading to RyR1 leakage. These analyses confirmed that the absence of SELENON influences ER calcium entry, whereas the RyR1 mutant affects calcium exit.

In conclusion, this is a new mammal model based on the functional interaction between SELENON and SERCA2 in which SELENON modulates the SERCA calcium pumping activity into the ER. However, although the interaction between SELENON and the SERCA2a isoform could explain the selective hypotrophy of slow-twitch muscle fibres described in the SELENON loss-of-function zebrafish model (Jurynec et al. 2008), it is still to early to exclude the possibility of interactions between SELENON and other SERCA isoforms.

Other Possible Function for SELENON

It is worth mentioning that SELENON expression is not restricted to muscle tissue, actually being expressed in most tissues. In addition, phylogenetic studies identified an orthologue of the *SELENON* gene in many animal species, both vertebrate and invertebrate; it was shown that *SELENON* is more ancient than previously appreciated and that this gene is already part of the ancestral parazoa and eumetazoa gene repertoire, including primitive organisms such as sponges or cnidarians, which are lacking organised muscle structures. Therefore, it is likely that SELENON original function may have been unrelated to muscle differentiation and maintenance, and that it may have additional functions that remain to be characterised. Interestingly, studies investigating the function of the microRNA miR-193-3p, a microRNA

suppressor of breast cancer cell proliferation, identified *SELENON* mRNA as one the five targeted mRNAs (Tsai et al. 2016). Real-time PCR experiments confirmed the down-regulation of *SELENON* gene expression in miR193-3p mimic transfected cells, and knockdown of *SELENON*-suppressed cell growth similar to miR193-3p overexpression in breast cancer cells. In agreement with this observation, SELENON was shown to be highly expressed in proliferative fibroblast, but to be down-regulated during the differentiation into myoblast (Petit et al. 2003). These results point to a function of SELENON in cell proliferation control.

Exploring Therapeutic Strategies for SELENON-Related Myopathies: NAC and Vitamin C

From the therapeutic point of view, more studies are needed in order to clarify whether SERCA hypoactivity is the only factor that accounts for the *SELENON* KO muscle phenotype or whether SELENON has multiple targets, which would mean that rescuing reduced SERCA activity may only partially recover the muscle function.

The function of SELENON has not yet been fully characterised, but there are many indications that it is related to redox reactions and oxidative stress defence. It is therefore not surprising that the therapeutic approaches to *SELENON*-related myopathies have so far been based on this assumption.

Three antioxidants (the flavonoid fisetin, the carotenoid astaxanthin and the glutathione precursor NAC) were tested in the ex vivo pretreated cells taken from patients with *SELENON*-related myopathies in order to evaluate their protective effect against the detrimental consequences of oxidative H_2O_2 (Arbogast et al. 2009), but only NAC was found to improve the fitness of H_2O_2-challenged cells. In addition, it has been found that NAC improves the muscle histology of relatively relaxed (ryr) zebrafish, although no improvement in the muscle contractile properties was detected (Dowling et al. 2012). In another study, NAC was also shown to prevent mutated desmin aggregation in an in vitro cellular model for the desmin-related myopathy (Segard et al. 2013).

The existence of a significant clinical overlap with RYR1-related myopathies, and the fact that NAC, which had already been approved for human use, can counteract muscle fatigue without giving rise to any serious side effects (Reid et al. 1994) represented the proof of concept for the first, and still ongoing, NAC therapeutic trial in patients with *SELENON*-related myopathies. However, in vivo evidences for NAC protective benefits are still missing.

Working along the same lines, ex vivo *SELENON* KO MEFs were treated with low concentrations of EN460, an inhibitor of ERO1 (Blais et al. 2010) that has been shown to protect cells from the deleterious effects of the ER stressor tunicamycin, and may therefore inhibit ERO1 activity in SELENON-devoid cells during conditions of ER stress (Marino et al. 2015). Indeed, SELENON KO cells challenged

with EN460 are protected from cell death induced by ER stress. However, the use of EN460 in *SELENON* KO animal models is hampered by its toxicity, and chemical modification studies are currently under way in order to make ERO1 inhibition more selective and to reduce the toxic effects.

As an abundant ER-resident reductant (Lamande et al. 1999), AA stands out among the normally present cellular antioxidants that may counteract the lack of SELENON. A pulse-chase experiment showed that it may be involved in oxidative stress defence in SELENON-deficient cells (Pozzer et al. 2017). Taken together with the fact that reduced AA concentrations in *SELENON* KO skeletal muscle aggravate the pathological *SELENON*-related muscle phenotype, the relative innocuity of AA suggests that it deserves evaluation in a therapeutic trial for *SELENON*-related myopathy. However, it is worth pointing out that, although some positive results have been obtained in mouse models for several human diseases, many clinical trials using AA failed to show any clear improvement (Noto 2015; Passage et al. 2004). Bearing in mind that unlike humans who rely on dietary intake mice produce their own AA, this situation may reflect differences in tissue concentration for this reductant between the two species. It is also important to take into account the differences in the pharmacokinetics of orally or intravenously administered AA: oral administration leads to tightly controlled plasma concentrations, whereas only intravenous administration can lead to the high plasma concentrations that may provide supra-nutritional health effects (Padayatty et al. 2004), which indicates the need to test intravenous AA in any clinical trial.

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