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# Selenocysteine incorporation: A trump card in the game of mRNA decay

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## Abstract

The incorporation of the 21st amino acid, selenocysteine (Sec), occurs on mRNAs that harbor in-frame stop codons because the Sec-tRNA$^{Sec}$ recognizes a UGA codon. This sets up an intriguing interplay between translation elongation, translation termination and the complex machinery that marks mRNAs that contain premature termination codons for degradation, leading to nonsense mediated mRNA decay (NMD). In this review we discuss the intricate and complex relationship between this key quality control mechanism and the process of Sec incorporation in mammals.

## 1.1 Introduction

The utilization of the trace element selenium is primarily, if not solely, directed toward the synthesis and incorporation of the "21st" amino acid, selenocysteine (Sec). Importantly, this process requires the redefinition of a UGA codon from Stop to Sec. Figure 1 shows a diagram of the factors required for Sec incorporation. These include the selenocysteine-specific tRNA (Sec-tRNA$^{Sec}$), the Sec-specific elongation factor (eEFSec) and an essential 3′ UTR binding protein (SBP2). While the function of an eEFSec/Sec-tRNA$^{Sec}$ complex is clear, its access to the cognate UGA codons is strictly regulated by sequences in selenoprotein mRNA 3′ UTRs called Sec insertion sequences (SECIS). As a member of the kink-turn family of RNA structures, the SECIS element must interact with SBP2 in order to alter UGA codons from Stop to Sec. Ostensibly, this process is not relevant to the quality control of protein synthesis, but the fact that Sec is encoded by a stop codon means that many quality control pathways must either be avoided or regulated for efficient Sec incorporation. As such, there are two major areas that relate to translational quality control. First, eEF1A must be prevented from binding the Sec-tRNA$^{Sec}$. Second, the UGA Sec codon must not be interpreted as a premature termination codon (PTC) and induce nonsense mediated decay (NMD).

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## 1.2 Elongation factor specificity

Because the Sec-tRNA$^{Sec}$ harbors a cognate UCA anticodon, it is imperative that it not be bound by eEF1A and incorporated at UGA stop codons. The tRNA$^{Sec}$ is unique not only because of its UCA anticodon, but in its biosynthesis as well. Newly transcribed and processed tRNA$^{Sec}$ is first serylated by the Ser aminoacyl tRNA synthetase (RS), then the Ser residue is phosphorylated by a specific phosphoseryl tRNA kinase (PSTK) to generate O-phosphoserine (Sep), and finally the Sep-tRNA$^{Sec}$ is converted to Sec by Sec synthetase (SepSecS). In E coli, experiments with aminoacylatable minihelices demonstrated that three base pairs in the acceptor arm of the tRNA$^{Sec}$ are necessary and sufficient for preventing elongation factor binding (Rudinger et al., 1996). In *Trypanosoma brucei*, strong indirect evidence also implicated the acceptor stem as the primary antideterminant for eEF1A binding. In this case it was found that a mutation at the unique U:U pair at the base of the acceptor stem allowed import of the Sec-tRNA$^{Sec}$ into the mitochondrion, which along with other evidence, supported the idea that eEF1A directly imports the tRNAs required for mitochondrial translation (Bouzaidi-Tiali et al., 2007). From this they concluded that a point mutation eliminating this base pair was sufficient to prevent eEF1A binding. Despite this evidence for a strong antideterminant, one of the original studies that identified the tRNA$^{Sec}$ as a serylated UGA suppressor tRNA showed that this Ser-tRNA$^{Sec}$ precursor species could suppress UGA codons during in vitro translation (Diamond et al., 1981), suggesting that eEF1A was able to deliver this tRNA to the ribosome. This raises the question as to whether the sequence of tRNA$^{Sec}$ is sufficient or whether a specific binding factor may help prevent eEF1A access. It is entirely possible, of course, that the Sec-tRNA$^{Sec}$ synthetic machinery itself is sufficient to make sure that it is only loaded onto its cognate elongation factor and not eEF1A. Until detailed studies are performed that establish the in vivo ratios of all of the factors, it will not be possible to sort out whether eEF1A must be actively prevented from Sec-tRNA$^{Sec}$ binding or whether there simply is never an opportunity for binding due to the stoichiometry of factors. Along those lines, a recent study shows that the endogenous SepSecS enzyme that converts Ser to Sec after serine phosphorylation is present as a more than 3-fold molar excess over total tRNA$^{Sec}$ in human hepatoma cells, thus supporting the idea that Sec-tRNA$^{Sec}$ may be “channeled” onto eEFSec by virtue of its complex synthetic pathway (French et al., 2014).

## 1.3 Interplay with nonsense mediated decay (NMD)

As mentioned above, the link between selenoprotein synthesis and translation quality control is the Sec codon, which must avoid detection as a PTC by the NMD surveillance pathway. Under conditions of adequate dietary selenium, when Sec-tRNA$^{Sec}$ levels are high, NMD presumably does not occur because Sec incorporation is efficiently out-competing translation termination. This idea is somewhat challenged by the observation that several selenoprotein mRNAs were found to be incompletely loaded by ribosomes, suggesting inefficient translation elongation (Fletcher et al., 2000; Martin and Berry, 2001). In addition, more recent ribosomal profiling data indicates that pausing at the UGA codon appears to occur on a subset of selenoprotein mRNAs even in the presence of adequate selenium, suggesting that there may be multiple mechanisms by which NMD is evaded (Howard et al., 2013). Thus, although it has not been proven, it is possible that the active Sec incorporation

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complex may provide certain selenoprotein mRNAs additional protection from NMD. One such example of protection from NMD is evident from the ApoB 100 mRNA, which is edited by the APOBEC1-ACF editing complex to yield a premature termination codon. This RNA editing event allows the production of the ApoB48 truncated protein, which is an isoform that is required for fat absorption and synthesis of chylomicrons. Decay of the ApoB100 transcript containing the PTC by the NMD pathway is prevented by the APOBEC1-ACF editing complex bound to the transcript (Chester et al., 2003). Moreover, it has also been proposed that the Sec-tRNA$^{Sec}$ synthesis and Sec incorporation machinery may exist in a large complex that may influence the efficiency of Sec incorporation at the UGA codon (Small-Howard et al., 2006). This complex would channel efficient delivery of Sec into the ribosomal A -site thereby enhancing UGA readthrough (Small-Howard et al., 2006; Stapulionis and Deutscher, 1995). In fact, very recently selenium supplementation was demonstrated to enhance multiple but not single Sec incorporation events, further implicating a role for active aminoacylation and channeling of reactants (Shetty et al., 2014). Overall, therefore, it appears that the process of Sec incorporation is sufficient to prevent activation of the NMD pathway.

Although the Sec incorporation machinery is capable of efficient UGA codon redefinition and evades the NMD quality control pathway, Se depletion results in dramatically reduced mRNA levels for a select few selenoprotein mRNAs (Sunde et al., 2009). Presumably, insufficient selenium affects Sec-tRNA$^{Sec}$ levels thereby affecting Sec incorporation efficiency. Thus, in the absence of a complete Sec incorporation complex, a translating ribosome on a selenoprotein mRNA is forced to prematurely terminate at the Sec codon, likely triggering the NMD pathway. The best studied example of selenoprotein mRNA down-regulation during low selenium is that encoding glutathione peroxidase 1 (*Gpx1*) (Lei et al., 1995; Weiss et al., 1996; Weiss and Sunde, 1997). In both animals and cultured mammalian cells, selenium deprivation causes *Gpx1* mRNA to fall to ~ 10% of that in selenium adequate conditions (Saedi et al., 1988). This is not surprising since the *Gpx1* transcript fulfills the basic requirements of an NMD target. As described elsewhere in this issue, detection of transcripts as targets of classical NMD for degradation requires two main features: 1) the presence of a ribosome stalling at a premature termination codon (PTC) >50-55 nucleotides from the exon-exon junction; 2) an exon junction complex located downstream of the PTC (reviewed in Maquat 2004). Typically the *Gpx1* Sec codon always resides in the first exon and it also fits the >50-55 nucleotides rule of NMD, and several lines of evidence strongly support the role of NMD in causing *Gpx1* mRNA decay under conditions of low selenium. First, *Gpx1* mRNA decay is intron-dependent since *Gpx1* mRNA from transfected cDNA did not trigger NMD (Weiss and Sunde, 1998). Moreover, the cysteine mutant of *Gpx1* (in which the Sec is mutated to cysteine) is very stable irrespective of selenium conditions (Sun et al., 2000; Sun et al., 2001). In addition, recent work showing the association of *Gpx1* mRNA with the key NMD factor UPF1 in low selenium conditions as well as the up-regulation of *Gpx1* transcript levels upon knockdown of another essential NMD factor (*Smg-1*) further illustrates NMD regulation (Seyedali and Berry, 2014). It is important to note, however, that Gpx1 is the only selenoprotein mRNA that has mechanistically been shown to be a target of NMD. By extension it is often assumed that lower selenoprotein mRNA levels during selenium deprivation is the product of

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activated NMD, but it has never been ruled out that concomitant transcriptional regulation does not also play a role. From a mechanistic standpoint, therefore, the more salient question is why many of the selenoprotein mRNAs remain unaffected by NMD even during selenium depletion.

A systematic study conducted on the complete mouse selenoprotein transcriptome shows the sharp contrast between the selenoprotein transcripts that are predicted to be ideal NMD targets versus their in vivo regulation by NMD (Sunde et al., 2009). Table 1 reproduces data from this and another study that used SBP2 knockout mice to study the effects of reduced selenoprotein production by an alternative means (Seeher et al., 2014). In the case of selenium depletion, generally more selenoprotein mRNA loss occurred in liver compared to kidney, but three mRNAs were dramatically reduced in both tissues (Gpx1, Selh and Sepw1). Interestingly, similar results were obtained upon loss of SBP2, but there were three mRNAs that were found to be retained during selenium depletion but reduced by more that 40% during SBP2 depletion (Gpx4, Sels and Sep15). As the authors point out, this may implicate SBP2 in directly playing a role in mRNA stabilization, but further work will be required to determine the extent to which this is the case. Although not directly comparable, it was previously shown that the reduction of SBP2 in cultured cells by shRNA treatment resulted in a very different pattern of mRNA changes (Squires et al 2007), suggesting that there may exist cell-type specific regulatory networks. Similarly, the brain-specific reduction of SBP2 in mice showed a unique pattern of selenoprotein mRNA concentrations, further implicating SBP2 in regulating mRNA abundance (Seeher et al 2014a). Overall, the striking finding from these studies is the number of selenoprotein mRNAs that are unaffected by the lack of translation. Considering that there is no known mechanism to keep non-translated PTC-containing mRNAs out of the NMD pathway, this serves once again to focus attention on the mRNAs that remain stable under these conditions.

The selective protection of certain selenoprotein transcripts against NMD during inadequate selenium may be necessary to preserve the two levels of hierarchy on selenium utilization observed in vivo: 1) Upon prolonged selenium deprivation, some tissues such as testes, thyroid gland and brain remain favorably sustained (Behne and Höfer-Bosse, 1984; Behne et al., 1988); 2) specific selenoprotein expression within a tissue, such as *Gpx4* and *Sepp1,* is prioritized in low selenium conditions, indicating that selenium is selectively channeled into some of the selenoproteins over others (Yang et al., 1989; Gross et al., 1995). The need for hierarchy among the selenoprotein transcripts is assumed to allow for expression of essential proteins, referred to as "housekeeping" selenoproteins, versus non-essential or stress-related selenoproteins. Thus, limited selenium is available exclusively for a subset of essential selenoproteins that maintain their Sec incorporation efficiency and thus evade NMD regulation. For instance *Gpx4*, which is abundant in almost all tissues, is essential for survival and remains NMD resistant even in low selenium conditions. Similarly, *Sepp1*, which is required for male fertility and neurological health, is moderately regulated by selenium responsive NMD (Renko et al., 2008). Mice deficient in *Gpx1*, however, develop normally (Ho et al., 1997). While the need to maintain essential selenoproteins can explain differential selenium utilization and thereby NMD regulation for some of the selenoproteins, not all NMD-protected selenoprotein transcripts are essential for survival (e.g. deiodinase 1

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[Dio1] and selenoprotein T [SelT]). In addition, *Gpx4* mRNA levels remain steady even when their protein levels are reduced dramatically, reiterating the fact that mere Sec incorporation efficiency is not sufficient for NMD evasion. One of the major limitations in identifying factors that provide NMD protection to selected selenoprotein transcripts is the model system that is used. NMD regulation of selenoprotein transcripts in cultured cells versus whole animal studies is discordant and this is most apparent for *Gpx4*. In cultured mouse fibroblasts and rat hepatocytes both endogenous and transiently transfected *Gpx4* is regulated via NMD in low levels of selenium, however in whole animal studies, liver and testes *Gpx4* evaded NMD decay (Sun et al., 2001). Overall, the mRNA stability landscape for selenoprotein mRNAs is highly variable, likely due to the varying demands placed on the individual pathways that require selenoprotein function. In addition, it is likely that other mRNA surveillance pathways intersect with NMD to regulate selenoprotein mRNA levels. Specifically, the process of "No-Go Decay" (NGD) identifies ribosomes that are stalled on the transcript due to stable RNA secondary structures or clusters of rare codons (reviewed in Harigaya et al 2010). The core components of NGD are distinct from those required for NMD and include Dom34 (Pelota in humans) and Hbs1. Whether selenoprotein transcripts are regulated by NMD only or by both NMD and NGD is still unresolved due to lack of studies analyzing effects of NGD components on selenoprotein expression.

## 1.4. Potential mechanisms for NMD evasion

Several factors have been implicated in the differential stabilization of selenoprotein transcripts against NMD when selenium levels are inadequate. However, these factors may employ different mechanisms such as 1) influencing Sec incorporation efficiency; 2) recruiting *trans* factors that interfere with UPF1 or other NMD factor access and/or 3) by adopting RNA conformations that prevent stable UPF1 association or enhance UPF1 dissociation. Independently or together these factors may influence NMD regulation of different selenoprotein transcripts at multiple levels in a selenium-dependent manner. The following section deals with each of these potential regulatory systems in the context of the Sec incorporation process.

While all selenoproteins utilize the common Sec incorporation machinery for Sec insertion, additional factors include UGA codon context and cis elements located in the coding region or untranslated region. Alteration of the bases immediately following Sec codons has been shown to influence Sec incorporation efficiency. For instance, DIO1 activity increased significantly when the base following its UGA codon was mutated from its natural C to either A or G (McCaughan et al., 1995). In addition, rare codons or secondary structures in the coding region have been shown to influence ribosome stalling and elongation rates (Wolin and Walter, 1988; Ikemura and Ozeki, 1983). Thus, UGA codon contexts, e.g. being surrounded by rare codons or secondary structures, may further contribute to selenoprotein transcript stability. For instance, recent ribosome profiling data from a mouse liver transcriptome identified four selenoproteins that showed pausing at Sec codons, two of which are known targets of NMD (*Gpx1* and *Sepw1*). Interestingly, ribosome stalling occurs even when there is adequate selenium suggesting a role for the UGA codon context (Howard et al., 2013). A study using luciferase reporter constructs constituting a portion of the UGA codon native context (up to 12 bases on both sides), implicated that UGA codon contexts

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may form a cis-element that regulate efficient and specific Sec incorporation (Gupta 2007). This is true especially in the case of selenoprotein N, wherein a cis-element located in the coding region known as the Sec codon redefinition element (SRE) stimulates Sec incorporation efficiency, and a point mutation in the SRE greatly affects RNA stability (Maiti et al., 2008; Howard et al., 2007). Very recently, the 3′ UTR of selenoprotein S was also shown to contain sequences that regulated Sec incorporation efficiency both upstream and downstream of the SECIS in the 3′ UTR (Bubenik et al., 2013). Thus the varying UGA codon environment among selenoprotein transcripts and potential structural elements in the coding and untranslated region may modulate UPF1 interaction and thus NMD activation.

Over the last two decades, many SECIS binding proteins such as SBP2, nucleolin, L30, SBP2L and eIF4A3 have been identified, of which only SBP2 has been found to be essential for Sec incorporation. Differential binding of SBP2 to SECIS elements has led to the proposal that SBP2 could be a major determinant in modulating Sec incorporation efficiency, which may allow the evasion of NMD (Squires et al., 2007). Indeed, SBP2 has been shown to bind isolated SECIS elements with varying affinities (Donovan and Copeland, 2012), but no direct correlation between the in vitro obtained binding constants and in vivo Sec incorporation has been identified so far. This implicates other variables such as the SECIS form, its neighboring sequence and the transcript coding sequence in potentially regulating SBP2 binding affinity and consequently Sec incorporation efficiency. The differential binding affinity may in turn confer varying levels of protection against NMD among selenoprotein transcripts. In fact, several selenoprotein mRNAs such as *Sps2*, *Gpx4* and *SelX* (Table 1) that are NMD resistant in selenium deficiency are unstable in SBP2 knockdown indirectly suggesting that SBP2 may confer protection to these transcripts, albeit perhaps from a non NMD-related degradation pathway (Squires et al 2007). Another potential regulator that has thus far remained unexplored is the SBP2 paralog, SECISBP2L. This protein also has been shown to bind to all human SECIS elements, albeit with generally lower affinity, but reduction by shRNA treatment does not affect selenoprotein expression in cultured cells (Donovan and Copeland, 2012), its role in regulating mRNA stability remains untested.

eIF4A3 is a DEAD box helicase and a crucial member of the exon junction complex that has also been shown to bind the *Gpx1* SECIS element specifically. In vitro, eIF4A3 selectively modulates Sec incorporation into GPX1 (and to a lesser extent SELR) by competing with SBP2 in a selenium and SECIS-dependent manner (Budiman et al., 2009; Budiman et al., 2011). Furthermore under selenium deficient conditions in cultured rat liver cells, eIF4A3 efficiently distinguishes between SECIS forms of *Gpx1* (form 1) and *Gpx4* (form 2) and preferentially binds to *Gpx1* and regulates its expression. In addition, an eIF4A3 mutant lacking helicase activity regulates *Gpx1* expression in vitro, thus ruling out SECIS unwinding or reorganization as the mechanism of regulation. Specific binding of the *Gpx1* SECIS by eIF4A3 may be due to the SECIS sequence context. Thus, in selenium limiting conditions eIF4A3 may down-regulate some of the non-essential selenoprotein expression, especially *Gpx1,* to make available the Sec incorporation machinery for essential selenoproteins. Although the mechanism of eIF4A3 regulation of *Gpx1* is still unknown, it was speculated that since SBP2 was unable to displace eIF4A3 from the Gpx1 SECIS

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element that it is primarily the concentration of cytoplasmic eIF4A3 that determines the level of regulation (Budiman et al, 2009). It should be noted, however, that in this study, Gpx1 mRNA levels did not increase upon selenium supplementation but protein levels did, suggesting that in this case translational regulation was the primary mechanism at play. Any role that eIF4A3 may play in regulating mRNA stability has yet to be determined.

In summary, the regulation of selenoprotein mRNA concentration in a cell cannot be explained by a single event and is clearly governed by many factors. While the role of mRNA degradation in establishing part of the hierarchy of selenoprotein synthesis is clear, the mechanism by which essential selenoprotein mRNAs are able to evade the NMD pathway has yet to be determined. As such, the next challenge for this field will be to determine precisely how this is accomplished and whether such a mechanism exists for any non-selenoprotein mRNAs as well.

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## Highlights

1. The utilization of selenium as selenocysteine is regulated at the level of transcription, translation and mRNA stability.
2. The fact that the selenocysteine tRNA recognizes a UGA stop codon means that all selenoprotein mRNAs contain premature termination codons.
3. One of the key pathways for regulation is that of nonsense mediated mRNA decay (NMD), which degrades mRNAs possessing premature termination codons.
4. Most selenoprotein mRNAs are resistant to NMD by mechanisms that are not yet known.

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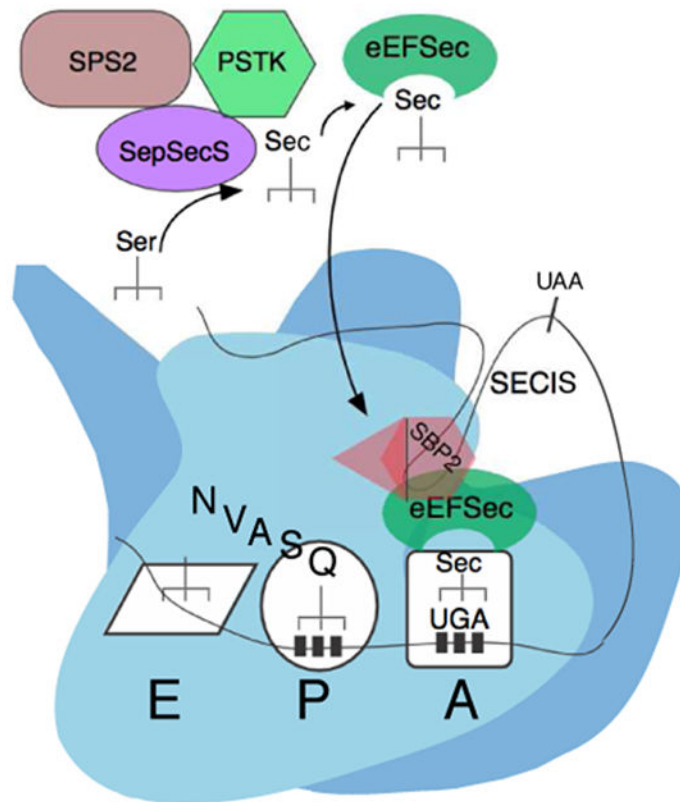


**Figure 1.**
Diagram of the factors required for selenocysteine incorporation in eukaryotes.

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## Table 1

**Differential selenoprotein sensitivity to NMD in low Se and SBP2 knockout conditions**

qRT-PCR data from two independent studies that analyzed mouse selenoproteins in either high/low selenium conditions or in a SBP2 liver-specific knockout. The mRNA decay data for selenium response in liver and kidney is reproduced from Sunde et al. (2009). The mRNA decay data for the liver-specific SBP2 knockout is reproduced from Seeher et al. (2014).

| Selenoprotein mRNA[1] | UGA exon[2] | Predicted NMD decay | mRNA loss in Liver[3] | mRNA loss in Kidney[3] | mRNA loss in Liver (SBP2 KO)[3] |
|---|---|---|---|---|---|
| *Dio1* (SR) | 2nd of 4 | Yes | Low | Low | High |
| *Dio2* (HK) | 2nd of 2 | No | Absent | nd | Absent |
| *Gpx1* (SR) | 1st of 2 | Yes | High | High | High |
| *Gpx3*[4] | 2nd of 5 | Yes | Absent | Moderate | Absent |
| *Gpx4* (HK) | 3rd of 7 | Yes | Low | Low | Moderate |
| *Selh* | 2nd of 6 | Yes | High | High | High |
| *Sepw1* (SR)[4] | 2nd of 6 | Yes | High | High | High |
| *Selk* (SR)[4] | 4th of 5 | Yes | Moderate | Low | No change |
| *Selm*[4] | 2nd of 5 | Yes | Moderate | Moderate | Moderate |
| *Sepp1* (HK)[4] | 2nd of 5 | Yes | Moderate | Low | High |
| *Selt* (SR) | 2nd of 6 | Yes | Low | Low | Low |
| *Selo* | 9th of 9 | No | nd | Low | No change |
| *H47/Sels* | 6th of 6 | No | Low | Low | Moderate |
| *Sephs2* | 1st of 1 | No | nd | nd | High |
| *Ept1/Seli* | 10th of 10 | No | nd | nd | No change |
| *Sep15* (HK)[4] | 3rd of 5 | Yes | Low | Low | Moderate |
| *Msrb1/Sepx1/Selr* (SR)[4] | 3rd of 5 | Yes | Low | Low | low |
| *Txnrd1* (HK) | 15th of 15 | No | Moderate | Low | No change |
| *Txnrd2* (HK) | 17th of 18 | Yes | Moderate | Low | nd |
| *Txnrd3* (HK) | 16th of 16 | No | Absent | Low | Absent |

[1] Selenoproteins are annotated as to whether they are considered housekeeping (HK) or stress response (SR).

[2] exon position of the UGA Sec codon out of total

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[3] Level of decay compared to control. Low is less than 70% reduction, Moderate is 40-70% and High is more than 70% of selenium adequate animals, nd is not determined and Absent means that the transcript was not detectable in that tissue.

[4] Note that we have changed the susceptibility designation for these genes. They were reported to be not susceptible to NMD in the cited works, but according to the classical definition of NMD, any downstream exon junction is sufficient to trigger mRNA decay.