Chapter 4
SECIS-Binding Proteins Regulate the Expression of the Selenoproteome

Donna M. Driscoll and Jodi L. Bubenik

Abstract The incorporation of selenocysteine into selenoproteins during translation is dictated by a UGA codon in the mRNA. The recoding of UGA as selenocysteine instead of stop depends on a stem-loop structure in the 3′ untranslated region of the mRNA. This element acts as a platform for RNA-binding proteins, including components of the basal selenocysteine incorporation machinery (SECIS-Binding Protein 2 and ribosomal protein L30) and two newly discovered regulatory proteins that selectively modulate selenoprotein expression (eukaryotic initiation factor 4a3 and nucleolin). Thus, multiple RNA-binding proteins may act in a combinatorial manner to regulate the expression of the selenoproteome.

4.1 Introduction

The health benefits of selenium in humans are largely attributed to its presence as selenocysteine (Sec), which is found in a small but important subset of proteins, called selenoproteins. Sec is considered the 21st amino acid because it is encoded by the UGA stop codon and is incorporated into selenoproteins during translation.

D.M. Driscoll (✉)
Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine, Cleveland, OH, USA
e-mail: driscod@ccf.org

J.L. Bubenik
Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, USA

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The recoding of UGA as Sec depends on the Sec Insertion Sequence (SECIS) element, a stable stem-loop in the 3′ untranslated region (UTR) of the selenoprotein mRNA [1]. All eukaryotic SECIS elements share a similar structure composed of two stems separated by an internal loop. The apical region of SECIS contains either an apical loop (Type 1 SECIS) or apical bulge (Type 2 SECIS) [2, 3]. Two conserved motifs are required for Sec incorporation: the SECIS core and the AAR motif [4]. Over the last 10 years, significant advances have been made in defining the role of the SECIS element in the Sec incorporation mechanism. A number of proteins have been shown to bind to the SECIS element in vitro. This chapter will focus on four SECIS-binding proteins whose functions in selenoprotein synthesis have been established. SECIS-binding protein 2 (SBP2) [5] and ribosomal protein L30 (L30) [6] are components of the basal Sec incorporation machinery in eukaryotes, whereas eukaryotic initiation factor 4a3 (eIF4a3) [7] and nucleolin [8] are regulatory proteins that play opposing roles in modulating the expression of a subset of selenoproteins. SBP2, a low abundance protein, appears to be dedicated solely to selenoprotein synthesis. By contrast, L30, eIF4a3, and nucleolin are abundant multifunctional RNA-binding proteins that have been co-opted into the Sec incorporation pathway. These four proteins will be considered in the context of their SECIS-binding activities and distinct roles in dictating the expression of the selenoproteome.

4.2 SBP2

SBP2 is the best-characterized *trans*-acting protein in the selenoprotein biosynthetic pathway. SBP2 was initially detected in rat testis extracts as a 120 kDa protein that bound to SECIS elements in UV crosslinking assays [9]. This binding activity was purified by RNA affinity chromatography using a SECIS element as the ligand [10]. The purified protein was analyzed by mass spectrometry and the peptide sequence information was used to isolate a cDNA clone encoding the binding activity [5]. This powerful strategy of biochemical purification coupled with mass spectrometry was also successfully used to identify L30, nucleolin, and eIF4a3 as SECIS-binding proteins.

The full-length rat SBP2 cDNA encodes a novel protein of 846 amino acids [5]. Interestingly, the protein is not highly conserved across species, although there are local regions of high sequence conservation. SBP2 is a limiting and likely an essential factor for selenoprotein synthesis in mammalian cells [11]. The N-terminal half of the protein is not required for Sec incorporation and does not contain any known motifs [5]. Elucidating the function of this region has proved difficult in the absence of testable hypotheses. The C-terminal half of SBP2 performs several critical functions during Sec incorporation, including binding to the SECIS element, recruiting the Sec-specific elongation factor, EFSec, and interacting with the ribosome [12–14]. The importance of the last two activities to the Sec incorporation mechanism is discussed in Chaps. 3 and 5.

4.2.1 Expression of SBP2

Given the pivotal role of SBP2 in dictating the expression of the selenoproteome, it is important to elucidate the expression and regulation of this protein. SBP2 protein and activity levels vary between tissues and cell types [10, 15]. This variation is not explained by differences in SBP2 mRNA levels [5, 15], suggesting that the expression of SBP2 may be regulated at the translational level. Indeed the 3′ UTR of the SBP2 mRNA contains several highly conserved regions that interact with members of the Turnover and Translation Regulatory RNA-binding Protein family [15]. To add to this complexity, SBP2 has been shown to be a redox-sensitive protein. Oxidative stress resulted in the accumulation of SBP2 in the nucleus and a decrease in selenoprotein synthesis in cells [16]. Understanding how the expression and activity of SBP2 is regulated is an important direction for future research.

SBP2 protein is expressed at low levels in somatic tissues, which has implications for models of Sec incorporation. It has been proposed that SBP2 remains stably bound to SECIS elements in selenoprotein mRNAs through multiple rounds of translation [11]. Based on an analysis of relative transcript levels, the ratio of the SBP2 mRNA to the total pool of selenoprotein mRNAs ranges from 1:200 to greater than 1:1,000 in different mouse tissues [17]. Presumably, SBP2 is substoichiometric to the number of potential mRNA targets in a cell. Given the stoichiometry of the players involved, the proposed requirement for a stable SBP2:SECIS interaction seems unlikely, unless most selenoprotein mRNAs are not competent for translation. Thus, alternative models in which SBP2 acts transiently at SECIS should be considered.

4.2.2 SECIS-Binding Activity of SBP2

Based on mutagenesis studies, the binding of SBP2 to the SECIS element requires an intact SECIS core [5]. RNA footprinting studies revealed that SBP2 binds to both strands of the SECIS core and along the 5′ side of the internal loop and helix 1 [18]. A recent study identified additional determinants for SBP2 binding in helix 2 and showed that SBP2 preferentially binds to RNAs that contain a large internal loop [19]. Subtle differences in these determinants outside of the SECIS core may explain how SBP2 can distinguish among SECIS elements from different selenoprotein mRNAs.

The SECIS core contains two sheared tandem G-A pairs [20], which have been shown to undergo a kink-turn folding in other mRNAs [21, 22]. Several lines of indirect evidence suggest that the SECIS element can also undergo an open-to-kinked transition [6]. This hypothesis needs to be validated using biophysical approaches. Interestingly, SBP2 appears to bind to the open form of the SECIS element but not the kinked form [6]. The implications of this differential binding for the mechanism of Sec incorporation are discussed in Sect. 4.3.1.

4.2.3 RNA-Binding Domain of SBP2

SBP2 and L30 are both members of the L7Ae family of RNA-binding proteins. This family includes the archaeal ribosomal protein L7Ae, other eukaryotic ribosomal proteins, and proteins involved in RNA processing, ribonucleoprotein assembly, and termination of protein synthesis [23]. The family members share a similar RNA-binding domain, called the L7Ae motif, and they often bind to kink-turn motifs in RNA [24]. The L7Ae motif in SBP2 most closely resembles the domain found in the spliceosomal 15.5 kDa protein. Using this homology as a guide, the Krol laboratory identified amino acids in the SBP2 L7Ae motif that are critical for interacting with the SECIS core [25]. However, the L7Ae motif alone was not sufficient to mediate SECIS binding. Based on sequence conservation, mutagenesis studies, and secondary structure predictions, Bubenik and Driscoll [26] proposed that the SBP2 RNA-binding domain is bipartite. Both the L7Ae motif and a second noncontiguous region that is located N-terminal to this motif are required for the SECIS-binding activity of SBP2 [26]. Two subsequent studies also demonstrated the importance of this region, which has also been termed SID or K-rich [12, 27]. Whether this region directly contacts the RNA or induces a conformational change in the L7Ae motif to enhance SECIS binding is controversial.

4.2.4 SBP2 as an Essential Factor for Sec Incorporation

SBP2 has been shown to be an essential factor for Sec incorporation in a rabbit reticulocyte lysate in vitro translation system [5]. siRNA-mediated knockdown of SBP2 in mammalian cells led to a reduction in the expression of endogenous selenoproteins [16]. Whether SBP2 is required for selenoprotein synthesis in vivo remains to be established but the sentiment in the field is that an SBP2 knockout is likely to be lethal. Importantly, reducing SBP2 levels in cultured cells has biological consequences. The knockdown of SBP2 in mesothelioma and neuroblastoma cell lines led to a reduction in telomere length, possibly due to lesions induced by oxidative damage [28]. Papp et al. [29] showed that depletion of SBP2 caused an increase in reactive oxygen species, leading to oxidative stress and apoptosis. The effects of the SBP2 knockdown in these two studies were presumably due to the loss of selenoprotein production. However, the possibility that SBP2 plays other roles in the cell in addition to Sec incorporation cannot be excluded.

4.2.5 SBP2 as the Master Regulator of Selenoprotein Synthesis

An early study from the Berry lab showed that SBP2 is the limiting factor for Sec incorporation when selenoprotein mRNAs are overexpressed in transfected cells [11].

The overexpression of SBP2 also had a differential effect on the Sec incorporation activity of various SECIS elements. These results led the authors to propose that SBP2 dictates the efficiency of Sec incorporation and ultimately, the expression of the selenoproteome. A subsequent study used the converse approach of siRNA knockdown to show that reducing SBP2 levels had various effects on the levels of individual selenoprotein mRNAs [30]. Based on a co-immunoprecipitation approach, SBP2 was preferentially bound to certain endogenous selenoprotein mRNAs compared to others in cells [30]. The conclusion of this study was that SBP2 determines the expression of the selenoproteome by differentially regulating selenoprotein mRNA stability and/or translational efficiency.

The finding that SBP2 plays a critical role in selectively regulating the expression of individual selenoproteins raises the question as to how this selectivity is determined. The simplest model is that individual selenoprotein mRNAs contain SECIS elements that differ in their affinity for SBP2. The protein has been shown to have a selective SECIS-binding activity when it is expressed in transfected cells and in vitro translation assays but other cellular factors are present in these systems [26, 30]. Indeed, when purified recombinant SBP2 was tested in gel shift assays, the protein bound to different SECIS elements with similar affinity [18]. There are several possible explanations for the lack of selectivity observed in these experiments. First, the bacterially expressed SBP2 may have been improperly folded or lacking a critical posttranslational modification. Second, the SECIS-binding activity of the recombinant protein was tested using minimal SECIS elements [18]. Additional sequences in the 3′ UTR may influence the affinity of the SBP2:SECIS interaction. Finally there may be other proteins in the cell that modulate the affinity or selectivity of SBP2. This hypothesis is supported by the recent finding that eIF4a3 prevents the binding of SBP2 to a subset of selenoprotein mRNAs, as discussed in Sect. 4.4.

4.2.6 Importance of the SBP2:SECIS Interaction in Human Disease

An exciting area of research is the newly discovered link between mutations in the SBP2 gene and human disease. Several SBP2 mutations and their physiological consequences are summarized in a recent review [31]. The effects of these mutations specifically on thyroid hormone metabolism are discussed in more detail in Chap. 29. In the first report of a genetic defect in SBP2, mutations in the SBP2 gene in two families were linked to decreased iodothyronine deiodinase expression and thyroid hormone dysfunction [32]. This was the first demonstration of an inherited defect in deiodinase activity in humans. Despite normal mRNA levels, the activities of type 2 iodothyronine deiodinase (Dio2) and glutathione peroxidase 1 (GPx1) were reduced in these individuals. The expression of other selenoproteins appeared to be unaffected since the patients were otherwise healthy, suggesting a selective defect in SBP2 activity [32]. One family had a missense mutation (R→Q) in the first region of the bipartite SBP2 RNA-binding domain. This single amino acid change

altered the RNA-binding affinity of SBP2, so that the mutant protein did not stably interact with SECIS elements from certain selenoprotein mRNAs, including Dio2 and GPx1 [26]. This selective SECIS-binding defect would explain the mild phenotype of the patients since the essential selenoproteins would still be expressed, while others like Dio2 and GPx1 would be lost.

Since this initial report, the search for additional SBP2 mutations in the human population has intensified. Such mutations appear to be rare and result only in partial SBP2 deficiency, presumably because complete loss of SBP2 function would be lethal. Recent studies have identified several patients who are compound heterozygotes for unique SBP2 mutations that result in variable phenotypes, ranging from mild to severe [33–35]. An emerging theme in the field is that the extent of the defect of an individual mutant SBP2 protein dictates which subset of selenoproteins show impaired synthesis, thus determining the complexity of the phenotype.

4.3 Ribosomal Protein L30

The second SECIS-binding protein to be characterized in depth is ribosomal protein L30, which is a component of the large ribosomal subunit in eukaryotes. Unlike SBP2, L30 is an abundant protein that is ubiquitously expressed in mammalian tissues. Although L30 is primarily associated with the ribosome, a small fraction exists in other cellular compartments. L30 is found in the nucleolus where it is involved in rRNA processing, in the nucleus where it binds to the L30 pre-mRNA to inhibit splicing, and in the cytoplasm where it binds to the mature L30 mRNA to inhibit translation [36–38]. The existence of this autoregulatory feedback loop suggests that the expression levels of L30 need to be tightly controlled in mammalian cells.

4.3.1 SECIS-Binding Activity of L30

L30 has been shown to bind to SECIS elements both in vitro and in cells [6]. This interaction is specific as mutations that disrupted the SECIS core abrogated L30 binding. Based on mutagenesis studies, L30 and SBP2 have similar nucleotide requirements for binding to the SECIS element [6]. A major question that remains to be answered is whether L30 and SBP2 bind to identical or overlapping sites on SECIS elements. As discussed above, the SECIS element may undergo an open-to-kinked conformational transition. In vitro binding studies suggested that SBP2 only binds to the open form of SECIS elements. By contrast, L30 can interact with either the open or kinked conformer [6]. Interestingly, the affinity of L30 is much higher for an SBP2:SECIS complex than for the free SECIS alone, which suggests that SBP2 may remodel SECIS elements so that they become high-affinity targets for L30. As binding of L30 induces a kink in the L30 pre-mRNA [39], L30 may also induce the SECIS core to undergo a kink-turn folding. SBP2 may not be sufficiently flexible to do this, given that this protein has a much larger RNA-binding domain

than L30. These studies suggest a model in which the SECIS element acts as a molecular switch that undergoes conformational changes upon protein binding as discussed in [6].

4.3.2 Role of L30 in Sec Incorporation

In addition to the SECIS-binding activity of L30, there is also functional evidence that this protein plays a role in Sec incorporation. The overexpression of L30 in rat hepatoma cells enhanced the UGA recoding activity of a co-transfected reporter construct that detects translational readthrough in transfected cells [6]. Thus, L30 was limiting in this system in the presence of overexpressed reporter RNAs. However, definitive evidence that L30 is essential for endogenous selenoprotein synthesis is still needed. As L30 is an essential gene in yeast [40], targeted disruption of the L30 gene in mice is likely to be lethal. The alternative strategy of siRNA knockdown is likely to be a more fruitful line of investigation.

The identification of L30 as a component of the eukaryotic UGA recoding machinery led to new ideas about the mechanism of Sec incorporation. We proposed a specific order of events in which SBP2 binds to the SECIS element in an early targeting event, and L30 acts at the ribosome during Sec incorporation [6]. This model takes into consideration the observations that SBP2 is expressed at very low levels in most somatic tissues and binds SECIS elements with high specificity and affinity. By contrast, L30 is an abundant, ubiquitous protein that binds to the SBP2:SECIS complex with higher affinity than to the free SECIS element alone. The rationale and supporting evidence for this model are presented in [6]. An alternative and equally viable model in which SBP2 acts at the ribosome is discussed in [13].

A number of critical questions about the mechanism of action of L30 remain. Does L30 tether the SECIS element to the ribosome or does the protein leave the ribosome to bind to the SECIS element? There are several examples of other nonessential ribosomal proteins that perform noncanonical functions unrelated to polypeptide synthesis, including L13a, which leaves the ribosome to participate in the translational silencing of interferon-induced mRNAs in macrophages [41]. Does ribosome-associated L30 or the extra-ribosomal pool of L30 participate in the Sec incorporation mechanism? Finally, is the SECIS-binding activity of L30 sufficient to promote UGA recoding? It is intriguing to speculate that L30 may perform additional functions during this process, such as tethering the SECIS element to a specific site on the ribosome or suppressing termination at the UGA/Sec codon.

4.4 eIF4a3

When selenium becomes limiting, cells need to prioritize the utilization of this important micronutrient in order to ensure the proper expression of the selenoproteome. During selenium deficiency, there is a hierarchy of expression in which the synthesis

of essential selenoproteins is maintained while other less important selenoproteins are poorly expressed [42–45]. While SBP2 may contribute to the preferential translation of certain selenoprotein mRNAs, this protein is not regulated by selenium. A major advance in the field was the recent discovery that eIF4a3 serves as a link between selenium status and differential selenoprotein expression.

eIF4a3 belongs to the DEAD box family of RNA-dependent ATPases [46]. The protein, which is ubiquitously expressed in mammalian cells, is predominantly nuclear [47]. Despite sharing homology with other initiation factors, eIF4a3 has no known role in protein synthesis. The canonical function of eIF4a3 is to bind to spliced mRNAs during the formation of the exon junction complex [47, 48]. As part of the exon junction complex, eIF4a3 plays a role in nonsense-mediated decay, a surveillance pathway that eliminates mRNAs that contain premature stop codons [49, 50]. By contrast, eIF4a3 binds selectively to a subset of SECIS elements and regulates selenoprotein expression at the level of mRNA translation [7].

4.4.1 SECIS-Binding Activity of eIF4a3

The stable interaction of eIF4a3 with spliced mRNAs depends on other proteins in the exon junction complex [51]. However, the binding of eIF4a3 to the SECIS element is specific and selective. Purified eIF4a3 bound with high affinity to SECIS elements from GPx1 and Selenoprotein R (SelR), which are nonessential selenoproteins. By contrast, eIF4a3 bound with low affinity to the SECIS elements from two mRNAs that encode essential selenoproteins [7]. Thus, the selective eIF4a3:SECIS interaction must rely on a different mechanism than the sequence-independent binding of eIF4a3 to spliced transcripts.

Based on mutagenesis studies, the internal loop of the GPx1 SECIS is required for binding of eIF4a3. When this region was replaced with a different internal loop, eIF4a3 was still able to bind, suggesting that the protein recognizes additional determinants. Of the four SECIS elements tested, eIF4a3 interacted with two Type 1 elements that contained an apical loop, but not with two Type 2 elements that contained an apical bulge [7]. These results suggest that the apical loop may be part of the signature motif for eIF4a3. The identification of the nucleotide sequences and/or structures in SECIS elements that are required for binding of eIF4a3 will provide critical insight into how this protein discriminates among selenoprotein mRNAs.

4.4.2 eIF4a3 Is a Negative Regulator of Sec Incorporation

The binding of eIF4a3 to a SECIS element has functional consequences. eIF4a3 inhibited UGA recoding directed by the GPx1 and SelR SECIS elements in an in vitro translation system [7]. This effect was specific, as eIF4a3 had no effect when the UGA codon was replaced with UGU/Cys. Likewise the recoding activities

of SECIS elements that did not bind eIF4a3 were not inhibited when the protein was added to the assay. How does eIF4a3 inhibit UGA recoding? eIF4a3 has been shown to have a helicase activity in vitro. The simplest model is that eIF4a3 unwinds the SECIS element, thus eliminating the stem-loop structure. However, neither the helicase activity nor the ATPase activity of eIF4a3 was required for the protein to inhibit Sec incorporation. Instead, eIF4a3 competes with SBP2 for binding to the SECIS element because the two proteins share overlapping binding sites [7]. Furthermore, once eIF4a3 is bound to the SECIS element, it cannot be displaced by SBP2. Since eIF4a3 is predominantly nuclear in cells, the protein likely binds to the SECIS element before the GPx1 mRNA is exported to the cytoplasm for translation. The interaction of eIF4a3 with the SECIS element would mask the SBP2-binding site and consequently prevent Sec incorporation.

4.4.3 eIF4a3 Is Regulated by Selenium

Based on in vitro studies, eIF4a3 acts as a translational repressor for GPx1 and SelR. One might expect that the synthesis of these two selenoproteins would be compromised in normal cells given that eIF4a3 is widely expressed. Insight into this conundrum came from the discovery that eIF4a3 is regulated by selenium status [7]. It appears that there is sufficient eIF4a3 in selenium-adequate cells to carry out its canonical function of binding to spliced mRNAs in the exon junction complex. However, the amount of eIF4a3 is limiting with respect to binding to selenoprotein mRNAs. In response to selenium deficiency, eIF4a3 protein levels are upregulated several-fold. This increase in eIF4a3 is required for selective translational repression because siRNA knockdown of eIF4a3 rescued GPx1 expression in selenium-deficient cells [7]. Furthermore, the overexpression of eIF4a3 in selenium-adequate cells reduced GPx1 protein levels with no effect on mRNA levels [7]. Thus, eIF4a3 is necessary and sufficient to repress the synthesis of GPx1. eIF4a3-mediated translational repression is an attractive model for nutrient regulation of gene expression where rapid and transient changes in protein synthesis might be desired in response to dietary fluxes. These studies provided the first mechanistic insight into how the translation of nonessential selenoprotein mRNAs is selectively inhibited to ensure the synthesis of essential selenoproteins when selenium is limiting.

4.4.4 Additional Roles for eIF4a3 in Regulating Selenoprotein Synthesis?

In addition to inhibiting GPx1 synthesis, does eIF4a3 direct a translational regulon in which the synthesis of a cohort of selenoproteins is repressed in selenium-deficient cells? What happens when selenium status is restored? One interesting idea is that eIF4a3 may dissociate from the GPx1 SECIS element in response to an

event such as a selenium-dependent posttranslational modification. The mRNA would then be available for interaction with SBP2, rapidly restoring GPx1 synthesis when selenium becomes available. Finally, is there a role for the SECIS-bound eIF4a3 in mediating the degradation of the GPx1 mRNA? As discussed above, eIF4a3 and the exon junction complex participate in the nonsense-mediated decay pathway. It is intriguing to speculate that the binding of eIF4a3 to the SECIS element could lead to the formation of a pseudo-exon junction complex in an ectopic location, resulting in mRNA degradation.

4.5 Nucleolin

Nucleolin is best known for its classical role in facilitating ribosome biogenesis in the nucleolus. Over the last few years, it has become clear that the protein also performs a variety of unexpected functions in other cellular compartments [52]. Nucleolin regulates the expression of several cellular and viral transcripts at the posttranscriptional level by altering mRNA stability or translation in the cytoplasm, as discussed in [8]. A recent study established that nucleolin acts as a positive regulator for the translation of selenoprotein mRNAs that encode essential functions [8].

4.5.1 SECIS-Binding Activity of Nucleolin

Nucleolin was initially identified as a putative SECIS-binding protein by screening a bacterial expression library with a radiolabeled GPx1 SECIS probe [53]. This study did not determine whether nucleolin bound to other SECIS elements or whether the nucleolin:SECIS interaction was functionally important. A subsequent study showed that nucleolin is a selective SECIS-binding protein. Nucleolin bound with higher affinity to SECIS elements from selenoproteins that are preserved in selenium deficiency and/or exhibit a severe phenotype when deleted in mice [8]. Interestingly, nucleolin had a very low affinity for the SECIS element from the GPx1 mRNA, which encodes a nonessential selenoprotein. Thus, it is not clear why nucleolin was identified as a GPx1 SECIS-binding protein in the earlier ligand screening experiments.

Mutational analysis of SECIS elements revealed that the upper part of the basal stem is required for nucleolin binding [8]. This region may directly interact with nucleolin or it may be required to stabilize the SECIS structure so that nucleolin can bind elsewhere on the molecule. How does nucleolin discriminate among SECIS elements? A number of different nucleolin-binding sites have been identified in preribosomal RNAs and in the 5′ or 3′ UTRs of several cellular mRNAs [54–57]. To date, a consensus binding site common to the SECIS elements that are bound by nucleolin with high affinity has not been identified.

Defining the domains in nucleolin that are required for SECIS binding is another important area for investigation. The central region of nucleolin contains four non-identical RNA recognition motifs (RRM), which are found in many proteins that are involved in RNA processing and metabolism [58]. Some of these RNA-binding proteins contain multiple RRMs. In such a protein, the individual motifs or combinations of RRMs often have different binding specificities, allowing the protein to interact with more than one target mRNA sequence. In the case of nucleolin, studies on pre-ribosomal RNA found that binding of this protein to the nucleolin recognition element (NRE) requires only RRMs 1 and 2, whereas all four RRM domains are essential for binding to another sequence, the Evolutionary Conserved Motif [59, 60]. It will be of interest to identify the RRMs in nucleolin that mediate SECIS binding.

4.5.2 Role of Nucleolin in Selenoprotein Synthesis

Unlike eIF4a3, the expression and activity of nucleolin is not regulated by selenium. Even in selenium-adequate cells, SBP2 and L30 are both limiting factors for Sec incorporation. Thus, the limiting UGA recoding machinery may need to be preferentially recruited to a subset of transcripts, which encode selenoproteins that perform critical functions. The role of nucleolin in regulating selenoprotein expression was investigated using an siRNA strategy [8]. siRNA knockdown of nucleolin inhibited the synthesis of essential selenoproteins, with no effect on the expression of nonessential selenoproteins. Furthermore, the levels and the nuclear/cytoplasmic localization of selenoprotein mRNAs were not altered in the nucleolin-deficient cells. These results support the hypothesis that nucleolin is required for the optimal expression of a subset of selenoproteins, which encode essential functions.

There are a number of mechanisms by which nucleolin could enhance selenoprotein mRNA translation. The simplest model is that nucleolin converts a SECIS element into a more effective competitor for the UGA recoding machinery, which is limiting in cells. Nucleolin may bind to the SECIS element and recruit SBP2 or other factors in the Sec incorporation pathway through protein:protein interactions. Alternatively, nucleolin may stabilize the structure of the SECIS element or modify its conformation so that high affinity interactions with SBP2 can occur. Finally, it will be important to validate the physiological significance of these studies in an in vivo setting. Of particular interest is whether nucleolin plays a role in preserving the synthesis of essential selenoproteins in animal models of selenium deficiency.

4.6 Other SECIS-Binding Proteins

In addition to the four proteins discussed above, several other putative SECIS-binding proteins have been observed. Two groups reported discrepant results as to whether nuclease sensitive element-binding protein 1 (NSEP1), also known as

DNA-binding protein B, binds to the GPx1 SECIS element [61, 62]. A later study showed that the siRNA knockdown of NSEP1 led to a twofold decrease in GPx1 levels but the expression of other selenoproteins or control proteins other than actin was not analyzed [63]. Whether the reduction in GPx1 expression was mediated at the level of mRNA stability or translation was not investigated in this study. Proteins of 47.5 and 60–65 kDa in cell extracts have also been reported to bind to the GPx1 SECIS element [64, 65]. However, the identity of these proteins and the functional significance of these interactions have not been determined. Of note, the unknown 47.5 kDa SECIS-binding protein may have been eIF4a3, which has a similar molecular weight.

4.7 Conclusions

An exciting development in the field of selenoprotein biology is that the SECIS element interacts with a number of *trans*-acting factors. These SECIS-binding proteins have been shown to be involved either in the Sec incorporation mechanism or in the regulation of this pathway. Although SBP2 plays an important role in dictating the expression of the selenoproteome, eIF4a3 and nucleolin perform critical functions in determining which selenoproteins are synthesized. These recent discoveries shift the current paradigm from a simplified SBP2-centric view to a more complex model in which multiple SECIS-binding proteins combinatorially regulate the expression of individual selenoproteins or subsets of selenoproteins. In the future, we hope to identify polymorphisms or mutations in the L30, nucleolin, or eIF4a3 genes that impact selenoprotein expression and consequently human health.

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