

Selenium and cellular immunity. Evidence that selenoproteins may be encoded in the +1 reading frame overlapping the human CD4, CD8, and HLA-DR genes

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Abstract:

Selenium deficiency can lead to impaired immune function and reduced T-cell counts, as well as various specific disorders. Significantly, in ARC and AIDS patients, a progressive decline in plasma Se, paralleling T-cell loss, has been widely documented. Since evidence now suggests that there is an extremely high turnover of CD4⁺ T-cells in AIDS patients, with billions of new cells lost and replaced daily, any exceptional requirement for Se in lymphocytes could contribute to this progressive Se depletion. Thus, it may be significant that, over-lapping the known genes in the +1 reading frame, the mRNAs of several T-cell associated genes (CD4, CD8, HLA-DR p33) have open reading frames (ORFs) with as many as 10 in-frame UGA codons (CD4, p33), a clustering that is highly improbable by chance alone, and reminiscent of selenoprotein P, the predominant plasma form of Se. The presence of these ORFs, along with potential stem-loop RNA structures displaying consensus selenocysteine insertion sequences, AUG(N)_mAAA(N)_nUGR, suggests that these mRNAs may encode selenoproteins, in addition to the known T-cell glycoproteins. If so, the roles of Se in the immune system may be more diverse than previously suspected.

Keywords: Selenium deficiency | cellular immunity | selenoproteins | T-cell associated genes

Article:

Although the mechanisms involved have yet to be fully elucidated, it is well established that dietary selenium is important for a healthy immune response. The effects of selenium deficiency can include reduced T-cell counts, as well as impaired lymphocyte proliferation and responsiveness (1-7). These immunological effects are in addition to various specific disorders that have been associated with Se deficiency (8). Given this importance of Se for cellular immunity in particular, it is of considerable interest that a progressive decline in plasma Se has been widely documented in ARC and AIDS patients (9-17). This decline approximately parallels

T-cell loss or stage of HIV infection, but seems to be particularly noticeable in the terminal stages of AIDS, where Se deficiency is one of the hallmarks of the disease.

These observations are all the more intriguing in the light of recent evidence suggesting an extremely high turnover of CD4 + T-cells in AIDS patients (18,19), with billions of new cells lost and replaced daily. With such a rate of T-cell loss, and the constant formation of new cells to replace them, it is possible that any exceptional requirement for Se in lymphocytes might contribute to the documented progressive Se depletion, unless Se recycling mechanisms were 100% efficient, which is unlikely.

The clinical evidence for a progressive depletion of Se and the selenoprotein glutathione peroxidase (GPx) in the plasma and tissues of AIDS patients was recently reviewed by Dworkin (16). He points out that "selenium deficiency may be associated with myopathy, cardiomyopathy and immune dysfunction including oral candidiasis, impaired phagocytic function and decreased CD4 T cells" (16). Significantly, most of those are characteristic symptoms of AIDS. Dworkin also reports the results of his clinical studies showing that the reduced plasma Se and GPx levels in ARC and AIDS patients are "significantly correlated with total lymphocyte counts" but that this appears to be "irrespective of the presence or absence of diarrhea or gastrointestinal malabsorption" (16). This suggests that the decline in Se levels parallels the progression of HIV disease (decline in T-cell levels) in a way that cannot be entirely ascribed to nutrient malabsorption. Such observations challenge the common assumption that the decline in plasma Se is exclusively owing to malabsorption, and thus merely a consequence of the wasting syndrome characteristic of AIDS.

Over half of the Se in plasma is in the form of selenoprotein P; its mRNA has 10 UGA selenocysteine (SeC) codons, mostly concentrated in the C-terminal 125 amino acids (20). It has been suggested to serve as an antioxidant and possibly also as a Se transport/storage protein, but it is known to attach to various cell types via a specific receptor, and may also have other functions. Only a few mammalian selenoproteins have well understood roles (8); these include several forms of glutathione peroxidase (GPx) and the type I 5'-iodothyronine deiodinase involved in conversion of T4 thyroid hormone to T3. Thus, Se is critical in humans for the maintenance of glutathione-dependent antioxidant status, and thyroid T3 hormone levels, both of which appear to be impaired frequently in AIDS patients (reviewed in ref. 21).

Whatever its root causes, to rectify the apparent Se deficit in AIDS, Se supplementation therapy has been recommended repeatedly by those aware of these important biochemical functions of Se. In several very brief studies, Se supplementation has been reported to lead to some symptomatic improvements in HIV patients (12,14,17). It was also demonstrated recently that, in cell culture, Se supplementation inhibits both the activation of HIV-1 by oxidative stress, and the activation of NF- κ B, an important cellular transactivator of HIV-1 (22). By providing in vitro evidence of an antiviral effect, these observations substantiate previous claims that Se supplementation can be beneficial in slowing the progression of HIV disease (17).

In order to elucidate fully the molecular basis of the immunopotentiating actions of Se, and thus any possible role for Se in the pathogenesis AIDS, certain fundamental questions must be answered. Perhaps foremost among these is the question of whether the role of Se in leukocytes

In eukaryotes, efficient selenocysteine incorporation at UGA codons depends on the presence of a cellular protein factor (equivalent to the bacterial *selB* protein) and a structural signal in the mRNA 3'-untranslated region (UTR), consisting of a selenocysteine insertion sequence (SECIS) in a characteristic stem-loop structure (23,24). A possibly related stem-loop structure is required in bacteria, but must immediately follow the UGA codon (25). Berry et al. have shown that a SECIS can also function when placed in a 5'-UTR, or even in *trans*, when cotransfected on a separate plasmid; however, the efficiency of SeC insertion is highest when the SECIS is in a downstream 3'-UTR location (23). These results suggest that in-frame UGA codons can be translated as SeC with reasonable efficiency as long as there is an upstream or downstream SECIS in the mRNA. The potential SECIS associated with the UGA-containing ORFs of CD4, CD8, and the p33 invariant chain of HLA-DR (Fig. 2) are either in the coding regions (CD4, CD8) or in a 3'-UTR relative to the ORF encoding the hypothetical selenoprotein (p33). It must also be noted that there is an additional 1.2 kb of the human CD4 3'-UTR that has never been sequenced, and it is quite possible that one or more additional SECIS may be found in that region.

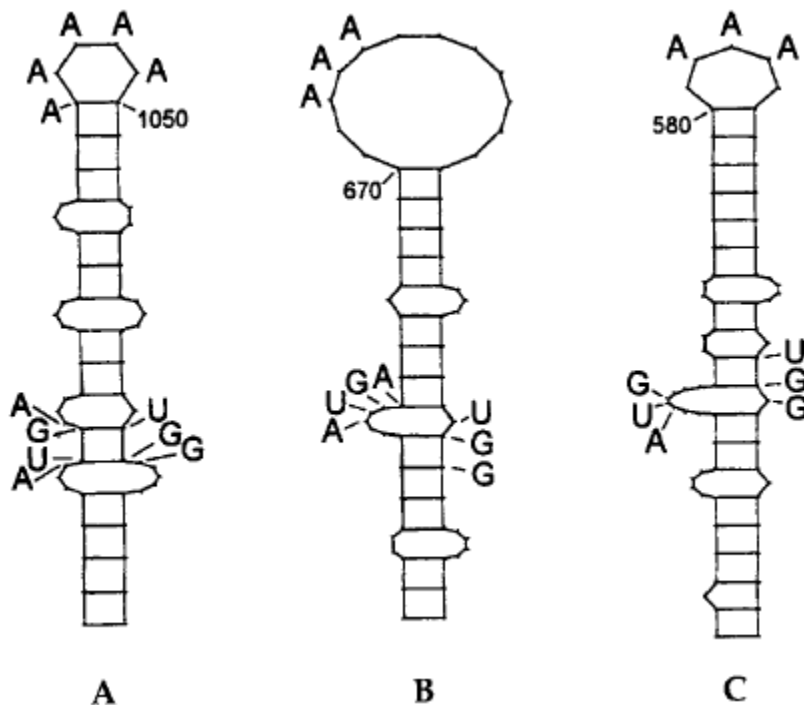


Figure 2. Potential stem-loop structures displaying consensus selenocysteine insertion sequences (SECIS elements) (23,24) found in the following mRNAs: **A.** CD4, bases 1017-1077 in GenBank #m35160; **B.** CD8, bases 647-706 in GenBank #m36712; **C.** HLADR invariant chain (p33), bases 544-662 in GenBank #m14765. The potential secondary structures of RNA regions containing the SECIS consensus features (AUG ... AAA ...UGR) were predicted using the FOLD program (37) with updated energy parameters as implemented in the GCG software package.

These potential stem-loop RNA structures (Fig. 2) have all the essential features determined from a comparison of known SECIS elements previously identified in mammalian selenoprotein genes: an AUG unpaired or partially paired in a bulge on the 5' arm of the stem, the unpaired adenine triad on the loop, and UGA or UGG in a bulge on the 3' arm of the stem (24). These

structures also have a near-ideal distance (10-12 bp) between the adenine loop and the AUG/UGR consensus bulge regions; the 9-bp distance in the CD8 SECIS is compensated by a larger adenine loop.

In all three cases, the start codon for the hypothetical selenoprotein is downstream from that of the "main" ORF encoding the known gene. Thus, selenoprotein synthesis could only be initiated on the relatively rare occasions when ribosomal scanning missed the first Met codon, an event which does happen and indeed has to happen for many viral genes to be expressed in mammalian cells. This inefficient initiation would in fact be desirable in this case, since the ability of cells to synthesize selenoproteins is inherently limited owing to the low abundance of tRNA_{Sec}.

In the case of CD4, the UGA-rich ORF in the +1 frame completely overlaps the C-terminal CD4 coding region. The predicted coding sequence (Table 1) is between 947 and 1315 (GenBank #m35160). It is also possible that this selenoprotein is translated from an alternatively spliced RNA, because there is a potential splice acceptor (SA) site (CCCTGCCCCAG/G) about 10 nucleotides upstream from its initiation codon. In a spliced RNA, this Met would be much closer to the 5' cap, where it could be initiated more efficiently. If this SA site were spliced to a potential donor (CAG/GT) at position 35, which is upstream from the CD4 start codon, the Met of the CD4-SP ORF would become the first start codon at the 5' end of the spliced mRNA.

Table 1. Translation of UGA-Rich Open Reading Frames, +1 to the Main Coding Region, in CD4, CD8 beta, and HLA-DR p33

CD4	
1	MLALETSPWP LKRKQESCIR <u>K</u> CTW <u>W</u> <u>C</u> CEPL SSRKI <u>C</u> PVRC GDPPPLS <u>C</u> CC
51	<u>A</u> CNWRTRRQR SRSGRRCGC <u>C</u> TLRRGCGSV <u>C</u> VTRDRSCW NPTSRFCHG
101	PPRCSQWP <u>C</u> L CWGASPASCF SLG
CD8	
1	MQAGSFSISQ <u>A</u> CSRKTVA <u>S</u> T <u>S</u> ACSSGAP <u>S</u> C PSGRELS <u>C</u> VW LISFPPLPSP
51	PRSPPSRREC AGYPGQRPRR AHFVAPSPLA CWWLASWFCW FPWEWPSTCA
101	AGGGEPGFVS <u>C</u> NNFTNKQRI RFWCPATKRH RSVMSTMWKN ERRDTFNPGE
151	FNGC <u>C</u> SCLLF TAARPFVC <u>C</u> AWEQLVRGSS GILGRRFHCP QGTSQSVLED
201	<u>C</u> VRNAAHATA SGSCAFPELG PLVVAI
HLA-DR p33	
1	MTSATLSPTM SNCPCWAGAL GPRRASAAAE PCTQAFPSW <u>C</u> LCSSLARPPP
51	PTSCTSSRAG WTN <u>C</u> QSPPRT CSWRTCA <u>C</u> SF PSLPSL <u>C</u> ARC AWPPRC <u>C</u> CRR
101	CPWEPCPRGP CRMPPSMAT <u>C</u> QRTM <u>C</u> CTCSR MLTP <u>C</u> CRTRH <u>C</u> RGASRRT <u>C</u> D
151	TLRTPWRP

UGA codons, potentially encoding selenocysteine, are indicated by C.

In the CD8- β mRNA, the potential selenoprotein is also encoded in the +1 frame, between 329 and 1006 (GenBank #m36712). It overlaps all but the first 94 N-terminal amino acids of CD8, and extends past its C-terminal by 110 amino acids. Thus, the predicted 226-residue product (Table 1), with 8 UGA codons and 11 Cys, is slightly larger than CD8 itself.

In the HLA-DR p33 mRNA, the UGA-rich ORF in the +1 frame completely overlaps the N-terminal half of the p-33 gene in the zero frame; the predicted coding sequence is between 60 and 533 (Gen Bank #m14765). The start codon is only 4 nucleotides past that of the p33 gene in the zero frame, and the hypothetical selenoprotein has 10 UGA codons and 15 Cys within its 158 residues (Table 1).

The only precedent for a gene having anything close to that number of in-frame UGA codons is the mammalian selenoprotein P, which also has 10 (20). It may be significant that the Cys and SeC-rich C-terminal region of selenoprotein P has a strong similarity to another protein module found in some cell adhesion molecules: the β chain of integrins and certain leukocyte adhesion proteins like CD18 (both in the top 10 matches, at > 6 SD significance in a PIR database search with this region of selenoprotein P as a probe; Taylor, unpublished). This could be a basis for the observed specific binding of selenoprotein P to cells via an unknown type of receptor (26). Given that β integrin chains are components of a number of leukocyte adhesion proteins and receptors, this potential integrin/selenoprotein P homology suggests a possible evolutionary relationship between selenoproteins and such molecules. This may provide some precedent for the possibility that divergent evolution may link the CD4 gene and known selenoproteins (e.g., Fig. 1).

Because CD4, CD8, MHC proteins, and other members of the immunoglobulin superfamily typically share common modules, it is also probable that similar UGA-rich ORFs will be found overlapping other genes that are important in the immune system.

If these ORFs do encode selenoproteins, their functional roles may be of considerable importance. The analysis suggests that, as well as functioning as independent proteins, they may be used as alternate modules that can be attached to part of the protein encoded in the main ORF by alternative RNA splicing or frameshifting. For example, there is a potential programmed -1 frameshift in the CD8-SP (Fig. 3), with a near-ideal heptameric shift sequence (G GGA AGG) and a potential pseudoknot that could permit a frameshift from the +10RF into the main ORF just before the membrane proximal domain of CD8. A frameshift here would lead to the formation of an alternate form of CD8 with the usual extracellular domain replaced by a selenoprotein module, which could have a totally different receptor binding specificity. Although this potential shift sequence differs from the "ideal" heptameric pattern (X XXY YYZ) in the sixth position (27), it is a variant that (like the ideal pattern) would still permit two out of three Watson-Crick base pairs in the codon-anticodon interactions of both tRNAs after slippage (Fig. 3). The distance intervening between the shift sequence and the pseudoknot is 4 nucleotides, well within the range observed in known frameshift sites (0-12 bases), and close to the optimal distance of 6 or 7 bases.

A possible advantage to the cell for such selenoprotein modules might be the potential for redox-regulated rearrangement of disulfide/diselenide/selenosulfide bonds, and consequent

Significantly, Taylor et al. recently pointed out the existence of novel ORFs overlapping known genes in HIV, potentially encoding selenoproteins expressed by ribosomal frameshifting (21,31). Several UGA codons in the -1 reading frame relative to the known coding regions are highly conserved in HIV-1. We also reported similar findings (31) in coxsackie B virus (CVB), which has been implicated as a cofactor in Keshan disease, a cardiomyopathy associated with severe Se deficiency. In a mouse model, the dependence of CVB3 cardiovirulence on vitamin E and Se deficiency has been demonstrated by Beck and coworkers (32,33).

It was these HIV findings that prompted the investigation of the CD4 gene for UGA-rich ORFs, because one of the potential selenoprotein genes reported in HIV-1 overlaps the gp41 region of the HIV-1 *env* gene (21), corresponding precisely to a region which has been previously suggested to represent a retroviral acquisition of a cellular gene, owing to a different base composition from the rest of HIV-1 (34). Furthermore, previous work has demonstrated possible evolutionary relationships between regions of HIV-1 *env* and both HLA molecules (35) and CD4 itself (36). The discovery of potential selenoprotein genes overlapping HLA-DR and CD4, as well as the HIV-1 *env* gene (21), is certainly consistent with that possibility.

If, as the current findings suggest, T-cells require significant quantities of Se for other important functions in addition to antioxidant protection via GPx, this could help explain how the proposed competition for Se between viral and cellular selenoprotein synthesis (21) might further impair T-cell function in AIDS. In addition, as the downward spiral of T-cell loss progresses, the recently demonstrated high turnover rate of T-cells in HIV-infected subjects (18,19) could significantly contribute to the observed decline in plasma Se levels (9-17). This might further compromise both helper and killer T-cell functions by inhibiting the formation of the hypothetical CD4, CD8, and MHC-encoded selenoproteins.

In summary, the hypothesis that these UGA-rich ORFs in T-cell associated genes encode selenoproteins is strongly supported by the implications of a number of independent observations:

1. a highly significant clustering of 8-10 UGA codons in these ORFs, always in the +1 frame relative to the known gene;
2. the presence of start codons upstream of the first UGA in all three cases;
3. at least for the CD4-SP ORF, significant sequence similarities to known selenoproteins (e.g., Fig. 1); and
4. the presence of consensus SeC insertion sequences in appropriate potential stem-loop RNA structures in all cases (Fig. 2).

Furthermore, this hypothesis is also consistent with existing literature data on the immunopotentiating effects of Se (1-3,5-7,28-30), and correlations between CD4+ T-cell counts and plasma Se levels that have been documented in animals (7), the elderly (4), and HW-infected patients (9-17).

If these UGA-rich ORFs do encode selenoproteins, the fact that they overlap extensively with the known genes in a different reading frame would be contrary to the conventionally held views

that human mRNAs are always monocistronic transcripts, and that our genes (almost) never overlap. However, such coding density is typical of retroviruses, which in humans just happen to preferentially infect T-cells. Rather than being a mere coincidence, the possibility that virus-like genetic processes may be going on in cellular genes in T-cells could be a reflection of the fact that retroviruses and their animal hosts have had plenty of time to learn each other's tricks during the millions of years that they have been evolving together.

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