Computational genomic analysis of hemorrhagic fever viruses: Viral selenoproteins as a potential factor in pathogenesis

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Abstract:

A number of distinct viruses are known as hemorrhagic fever viruses based on a shared ability to induce hemorrhage by poorly understood mechanisms, typically involving the formation of blood clots ("disseminated intravascular coagulation"). It is well documented that selenium plays a significant role in the regulation of blood clotting via its effects on the thromboxane/prostacyclin ratio, and effects on the complement system. Selenium has an anticlotting effect, whereas selenium deficiency has a proclotting or thrombotic effect. It is also well documented that extreme dietary selenium deficiency, which is almost never seen in humans, has been associated with hemorrhagic effects in animals. Thus, the possibility that viral selenoprotein synthesis might contribute to hemorrhagic symptoms merits further consideration. Computational genomic analysis of certain hemorrhagic fever viruses reveals the presence of potential protein coding regions (PPCRs) containing large numbers of in-frame UGA codons, particularly in the −1 reading frame. In some cases, these clusterings of UGA codons are very unlikely to have arisen by chance, suggesting that these UGAs may have some function other than being a stop codon, such as encoding selenocysteine. For this to be possible, a downstream selenocysteine insertion element (SECIS) is required. Ebola Zaire, the most notorious hemorrhagic fever virus, has a PPCR with 17 UGA codons, and several potential SECIS elements can be identified in the viral genome. One potential viral selenoprotein may contain up to 16 selenium atoms per molecule. Biosynthesis of this protein could impose an unprecedented selenium demand on the host, potentially leading to severe lipid peroxidation and cell membrane destruction, and contributing to hemorrhagic symptoms. Alternatively, even in the absence of programmed selenoprotein synthesis, it is possible that random slippage errors would lead to increased encounters with UGA codons in overlapping reading frames, and thus potentially to nonspecific depletion of SeC in the host.

Keywords: Blood clotting | Ebola virus | genomic analysis | hemorrhagic fever | RNA viruses | selenium | selenoproteins

Article:

INTRODUCTION

Hemorrhagic manifestations are the main pathophysiological features of all hemorrhagic fever diseases. The viruses causing hemorrhagic fevers in humans belong to the following groups: togavirus (Chikungunya), flavivirus (Dengue, Yellow fever, Kyansanu forest disease, Omsk hemorrhagic fever), arenavirus (Argentinean hemorrhagic fever, Bolivian hemorrhagic fever, Lassa fever), filovirus (Ebola, Marburg), phlebovirus (Rift Valley fever), nairovirus (Crimian-Congo Hemorrhagic fever) and hantavirus (hemorrhagic fever with renal syndrome, nephropathic epidemia) *(1).* Filovirus infections have several pathological features common with other severe viral hemorrhagic fevers *(2).* Among these viruses, filoviruses cause one of the highest fatality rates and the most severe hemorrhagic manifestations *(3,4).* The pathophysiological events that make the filovirus infections of humans so devastating are still obscure. The viruses are pantropic, but no single organ shows sufficient damage to account for either the onset of the severe shock syndrome or the bleeding conditions *(5).* Ebola virus (Zaire strain), the most lethal filovirus, will be studied in this communication. Since hemorrhagic conditions are the main feature, an analysis of the various mechanism by which hemorrhage can occur can give some clue to the viral pathogenesis.

MECHANISMS OF HEMORRHAGE FORMATION

Hemorrhage can occur by several mechanisms, which may be interrelated. One is the dysfunction and damage of endothelial cells, which form the inner surface of blood vessels and play a role in the regulation of blood pressure, homeostasis, and antithrombogenicity. Endothelial cell lysis is observed in the development of shock lung syndrome often associated with disseminated intravascular coagulation (DIC) *(6,7).* It has been shown that Marburg virus replicates in endothelial cells, but there is no direct experimental evidence that links the hemorrhagic manifestations to the vitally induced damage to the endothelial cells *(8).* Hantaan virus has also been shown to replicate in endothelial cells *(9).* Another mechanism is hemorrhagic conditions induced by complement activation *(10).* Extensive complement activation precedes onset of shock in Dengue patients *(11).*

Another fundamental mechanism in the production of DIC is via the formation of blood clots *(12).* The severe hemorrhaging produced by Ebola virus is essentially owing to the formation of blood clots--the "coagulation" of DIC--which leads to the obstruction and rupture of small blood capillaries. Thus, the hemorrhagic symptoms may be in large part a consequence of clot formation.

SELENIUM AND HEMORRHAGIC CONDITIONS

Significantly, it is well documented that selenium plays a major role in the regulation of blood clotting via its effects on the thromboxane and prostacyclin ratio *(13,14).* Prostacyclin (PG12) and thromboxane (TXA2) are eicosanoids metabolites of arachidonic acid, and are involved in controlling platelet activity and vascular tone. PG12 is synthesized by both endothelial and vascular smooth muscle cells, and is both a potent vasodilator and an endogenous inhibitor of

platelet aggregation *(15-17).* Conversely, TXA2, produced mainly by blood platelets, behaves as a vasoconstrictor and promotes platelet aggregation *(18,19).* The equilibrium between the production of arterial wall PG12 and platelet TXA2 has been suggested to be an important factor in platelet activity and aggregation. Thus, an increase in the PG12/TXA2 ratio will tend to inhibit clotting.

Selenium enhances glutathione peroxidase (GSH-Px) activity and prostacyclin release in cultured human endothelial cells, the very cell types that have been implicated in the pathogenesis of some hemorrhagic fevers *(13).* It has been reported that there is an increased PG12 production with selenium supplementation, whereas there is decreased aortic synthesis of prostacyclin-like compounds in selenium-deficient rats *(20,21).* An increase in TXA2 synthesis by rat lung neutrophils was observed during selenium deficiency *(22).* Increased bleeding time resulting from selenium supplementation has been observed owing to the increased GSH-Px activity, favoring PG12 over TXA2 formation *(20).* In a human study, selenium supplementation inhibited in vitro and in vivo production of TXA2, whereas PG12 synthesis was unaffected *(23).* Clearly, the net effect of selenium supplementation is an increased PG12/TXA2 ratio, which will inhibit clot formation. Thus, one would expect selenium to tend to reduce DIC, and the associated hemorrhagic symptoms. Conversely, any virally induced selenium depletion (at this point hypothetical) could favor hemorrhagic conditions.

This mechanism is supported by extensive documentation of hemorrhagic symptoms associated with extreme selenium deficiency. Pathological examination of pigs fed on a selenium-deficient diet showed liver necrosis, exudative diathesis, hemorrhages, and ulcers of the stomach, with hemorrhages and inflammation of the mucosa of the colon *(24).* Mulberry heart disease, which is characterized by hemorrhagic changes particularly in the myocardium and epicardium, was observed in a group of piglets with a severe selenium deficiency *(25).* Pathological examination of chicks revealed hemorrhagic conditions with large amounts of blue-greenish fluid under the skin *(26).* Similar hemorrhagic conditions have been seen in goats, elk, and lamb when fed on a selenium-deficient diet *(27-29).* Thus, both biochemical and in vivo studies show that selenium deficiency or selenium depletion can lead to hemorrhagic manifestations, probably via a proclotting mechanism.

These observations suggest that selenium might also be involved in the hemorrhagic manifestations seen in hemorrhagic viral diseases. If so, selenium could act by various mechanisms, either by exerting its effects in endothelial cells, on complement activation, or by affecting the PG12/TXA2 ratio and thus blood clotting. A significant role for selenium appears to be strongly supported by the results of Hou and coworkers, who treated victims of an Asian outbreak of viral hemorrhagic fever with high-dose oral sodium selenite, obtaining dramatic reductions in mortality *(30).* Although Hou et al. focused on complement activation as a probable mechanism of action, the possibility that this treatment also helped to counter a virally induced selenium depletion merits consideration in the light of the viral selenoprotein theory *(31)* and our recent demonstration of UGA-rich potential protein coding regions (PPCRs) in Ebola virus *(32).*

POSSIBLE MECHANISMS FOR VIRALLY INDUCED SELENIUM DEPLETION

As discussed in the accompanying article *(31),* there are at least two possible mechanisms by which selenium might be incorporated into viral proteins, and both are still hypothetical at the present time. The first is nonspecific selenoprotein formation associated with random translational slippage into UGA-rich overlapping reading frames, and the second is the possibility of specific virally encoded selenoproteins biosynthesized under the direction of RNA stem-loop structures.

Viruses are known to frameshift randomly at low frequency, leading to erroneous translational slippage into other frames. Rima has suggested that it may be advantageous for viruses to have a high density of stop codons in the overlapping frames, so that a ribosome that had slipped erroneously would stop quickly, conserving cellular resources *(33).* However, instead of a random distribution of stop codons, he noted a bias in the distribution of stop codons in RNA viruses. In paramyxoviruses, there are up to three times the expected number of UGA codons in -1 frame, and up to 1.5 times the expected number of UAG codons in the +1 frame in some genes *(33).* Consistent with Rima's observations, in many RNA viruses, we have noted a bias in favor of UGA codons in the overlapping reading frames, particularly the -1 frame, exemplified here by our analyses of rabbit hemorrhagic disease virus, Hantaan virus, and Ebola (Figs. 1 and 2). The rationale for how random slippage errors would lead to increased encounters with UGA codons, and thus potentially to nonspecific incorporation of selenocysteine (SeC), is discussed eleswhere *(31).* By this mechanism, even though the virus is not programmed to make selenoproteins, it is possible that under specific conditions, probably related to the severity of the infection, this process might lead to a nonspecific depletion of selenium in the host.

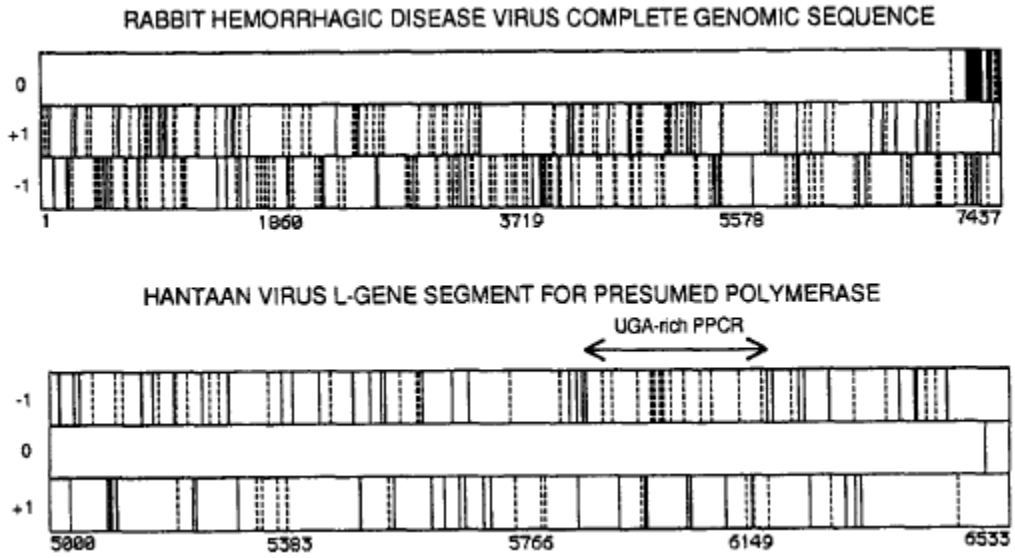

Fig. 1. Schematic figure of the complete genome of rabbit hemorrhagic fever virus (RHFV) (Genbank accession Z29514) and Haantan virus polymerse gene (Genbank accession X55901), translated in all three reading frames. The dotted lines represent UGA codons and the solid vertical lines represent non-UGA stop codons (UAA and UAG). This analysis is the first step in the general computational strategy for prediction of potential selenoprotein genes. Note the exceptionally high content of UGA codons relative to non-UGA stop codons in both overlapping frames for RHFV, and in the -1 frame for Hantaan virus *(see text* for discussion).

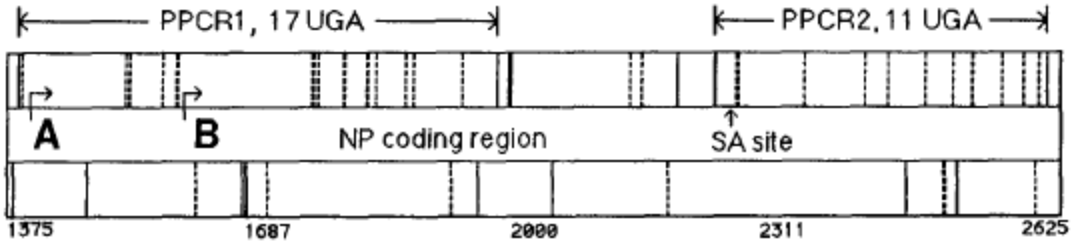

Fig. 2. Presence of UGA-rich PPCRs overlapping the Ebola Zaire nucleoprotein (NP) coding region. The figure shows a schematic of the three reading frames for a portion of the NP gene *(see* legend to Fig. 1). The dotted lines are UGA stop codons, which can potentially encode selenocysteine. There are two UGA-rich PPCRs -1 to the main NP reading flame, PPCR1 with 17, and PPCR2 with 11 in-frame UGA codons. Neither PPCR1 nor PPCR2 has a start codon. PPCR1 could be expressed as an NP fusion protein containing a selenoprotein module, by means of a frameshift at either one of two potential -1 frameshift sites, shown with an arrow symbol as A and B (shown in detail in Fig. 3). The alternate possibility of random nonspecific selenocysteine incorporation consequent to erroneous translation frameshifts into these regions is discussed in the text.

Alternatively, we find that in some cases these UGA-rich overlapping reading frames are associated with potential -1 frameshift sites *(31,32,34),* including potential RNA pseudoknots that are typically required for the enhancement of frameshifting efficiency *(35).* This suggests that there could be programmed synthesis of specific selenoproteins in such cases, which would require some type of SeC insertion element, an RNA stem-loop structure, somewhere in the viral mRNA *(36).*

As detailed in the accompanying paper *(31),* selenoproteins are formed by the translation of UGA as SeC, rather than its normal role as a stop codon. The possibility that UGA codons can be translated as SeC thus creates new PPCRs, which would be neglected in a conventional protein translation.

We have previously demonstrated the potential for selenoproteins to be encoded in regions overlapping known genes in HIV-1 *(31,34,37,38),* Coxsackie virus B3 *(31,38),* and Ebola virus *(32).* In each case, the link between Se deficiency and the associated viral diseases (AIDS, viral myocarditis, and hemorrhagic conditions, respectively) is supported by an extensive body of literature *(34,39-41).*

COMPUTATIONAL APPROACH FOR THE IDENTIFICATION OF POTENTIAL SELENOPROTEIN CODING REGIONS

A viral genomic sequence is translated in all the three frames using a modified translation table to distinguish the various stop codons. The PPCRs rich in UGA codons are analyzed for the presence of a start codon. If there is no start codon, it is possible that there could be a frameshift from the main frame into the UGA-rich frame (usually a -1 shift). This requires a frameshift sequence, ideally an XXXYYYZ heptamer, but reasonable deviations from this or tetramers suitable for P-site slippage may also function. If such a suitably located site is found, there should be an RNA stem-loop or pseudoknot (PK) just downstream of the frameshift site.

Theoretically, a novel PPCR can also be expressed by being linked in-frame to an expressed ORF by splicing. For splicing to play a role, transcription in the nucleus would have to occur, and appropriately located splice donor and splice acceptor sites identified in the sequence. The virus can also potentially switch between frames via RNA editing; the sequence signals required for this are not very well characterized, and may be difficult to identify. Finally, for a UGA codon to be translated as SeC, a characteristic step-loop structure called a SeC insertion sequence or an SECIS element is required *(36).* Candidate sequences having the consensus sequences features of an SECIS element are examined for potential to form the necessary RNA stem-loop structures, using the Zuker FOLD program *(42).*

If candidate selenoprotein or other genes are identified, the hypothetical protein sequence can be compared to known sequences in a protein data base in order to find potentially homologous known proteins. The data base search can be done using a program, such as FASTA, e.g., as implemented in the GCG software package (Program Manual for the Wisconsin Package, Ver. 8, September 1994, Genetics Computer Group, 575 Science Drive, Madison, WI 53711).

COMPUTATIONAL GENOMIC ANALYSIS OF EBOLA VIRUS

We recently reported theoretical molecular evidence that the highly pathogenic Zaire strain of the Ebola virus may be dependent on selenium owing to the presence of several PPCRs in the Ebola genome containing clusters of up to 17 in-frame UGA codons *(32).* We will illustrate the computational genomic analysis approach by reviewing the key findings on Ebola virus.

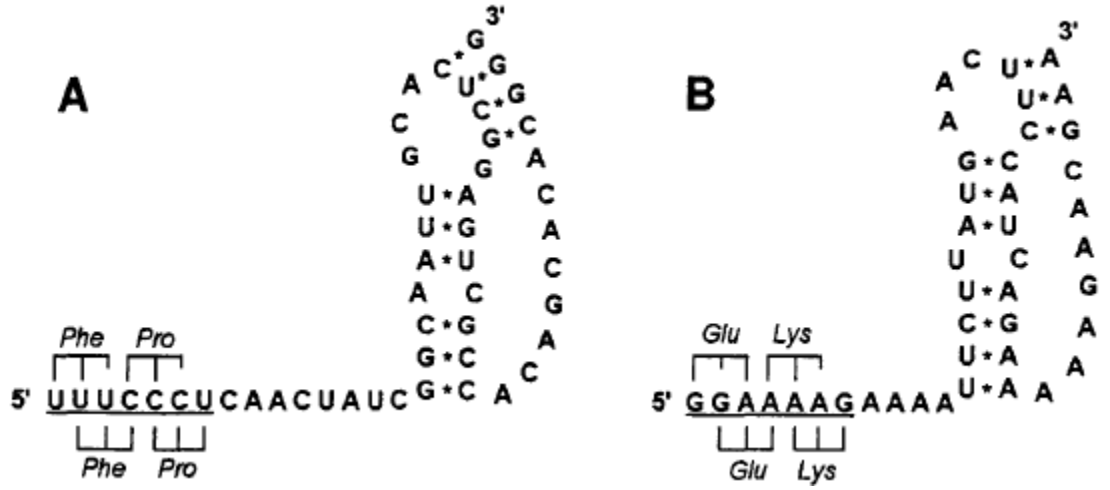

Fig. 3. Potential -1 frameshift sites near the beginning of the major UGA-rich PPCR in the Ebola Zaire nucleoprotein (NP) mRNA, consisting of slippery sequences (underlined) and potential RNA PKs. The locations of these sites are indicated in Fig. 2 by A (beginning at position 1405 in GenBank #Ll1365) and B (beginning at position 1582). Codon-anticodon interactions of the P- and A-site tRNAs are shown schematically both before (below sequence) and after slippage (above sequence). Note that slippage on runs of C bases as in site A is known to occur in measles virus *(33).*

Analysis of the Ebola sequence revealed two PPCRs in the -1 reading frame overlapping the major nucleoprotein NP gene, containing 17 and 11 UGA codons, respectively (Fig. 2). The first

PPCR has excellent potential to be expressed by a ribosomal frameshift from the NP coding region owing to the presence of an "ideal" heptameric shift sequence (UUUCCCU, at site A in Fig. 2) and an RNA PK 8 bases downstream (Fig. 3A). Note that slippage on a run of Cs has been shown in measle virus *(33).* This potential frameshift site A comes very near the beginning of the PPCR, and could permit the formation of a fusion protein consisting of the N-terminal 314 residues of NP fused to a 181 residue C-terminal module potentially containing 16 SeC residues, encoded in the -1 frame (bases 1411 to 1953 in GenBank #Ll1365; subsequent numbering refers to the same sequence).

Downstream from the first site, there is a second near-ideal frameshift site (B in Fig. 2) and potential PK, also in the NP coding region beginning at position 1582 (Fig. 3B). This second site follows the sixth UGA codon in the PPCR, so a frameshift here would yield a potential selenoprotein module with only 11 SeC residues. These redundant frameshift sites could provide for either an increased probability of translating the selenoprotein module, or for two alternate forms of the NP fusion protein.

Significantly, it has been shown that a pyrimidine base following a UGA codon (i.e., UGAY) will favor readthrough (e.g., by SeC insertion), whereas a purine base following a UGA codon (UGAR) will favor its function as a stop codon *(43).* Of the 17 UGA codons in PPCR1, all except for three near the 5'-end are followed by a pyrimidine base. Specifically, all 11 of the UGAs downstream of potential frameshift site B are UGAY, apparently programmed for readthrough, strongly favoring the possibility that this is a true selenoprotein gene. In this regard, it must be noted that the presence of a purine following a UGA does not completely rule out efficient translation as SeC, because in some cases, the requirement for a specific amino acid in the protein will dictate the necessity of a purine in this position, e.g., in GSH-Px, where there is a UGAG because there is a Gly following SeC in the protein sequence.

Encoded between bases 2212 and 2598, there is a second UGA-rich PPCR overlapping the Ebola NP gene. It has 11 UGA codons over 129 residues (PPCR2 in Fig. 2). This PPCR lacks a start codon, but could be expressed from an edited or spliced RNA. RNA editing would be the more likely possibility in this case, because RNA editing is known to occur in at least one location in Ebola virus *(44).* Since there are no reports of Ebola replication and transcription in the nuclei of infected cells, RNA splicing seems improbable in Ebola. However, perhaps this should not be entirely ruled out, because there is precedent for nuclear replication/transcription and RNA splicing in a negative nonsegmented single-stranded RNA virus, in the case of Borna disease virus *(45).* Whether it is functional or not, there is a potential splice acceptor site very near the beginning of PPCR2, consisting of a CAGA sequence preceded by a pyrimidine-rich sequence and an upstream "CURAY" sequence (CUGAC). There are various potential splice donors in the large NP mRNA that could bring this region in-frame to the main NP PPCR or the upstream selenoprotein PPCR with 16 UGAs. Thus, we cannot rule out the possibility that nuclear transcription and splicing of this Ebola mRNA could occur under special circumstances, possibly in the unknown "reservoir" species that is the natural host for Ebola virus.

Although the PPCRs overlapping the Ebola NP gene are the most striking owing to their high content of UGA codons, the analysis revealed several additional potential selenoprotein PPCRs overlapping other genes in the Ebola virus, including the vp24, vp30, vp35, and vp40 coding

regions, all of which have potential SeC insertion sequences in their mRNAs (shown for vp30 and vp35 in Fig. 4). Furthermore, on the Ebola minus strand genomic RNA, there are also potential SeC insertion sequences and several UGA-rich PPCRs (with up to nine UGAs), some with start codons in the context of Kozak-like sequences, and some potentially expressed from spliced genomic RNAs. Although we think it is rather improbable that any of these PPCRs on the minus strand are functional, expression of a protein encoded in a minus strand ORF has been proven in the case of HIV-1 *(46)*.

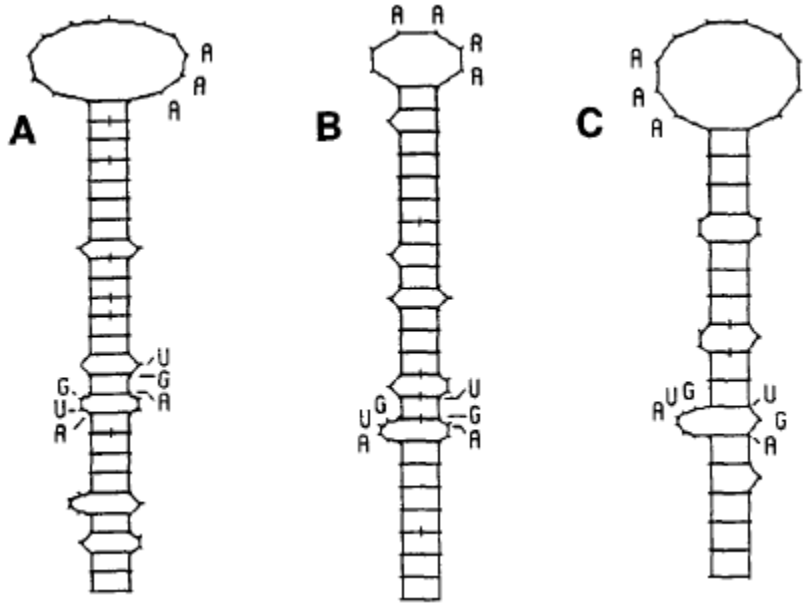

Fig. 4. Predicted RNA secondary structures for potential selenocysteine insertion sequences (AUG ... AAA ... UGA) in the Ebola virus RNA with potential to form the required stem-loop structures. A: In the 3'-untranslated region of the nucleoprotein mRNA, bases 2758-2836 in GenBank #L11365; E = -10.1 kcal/mol. B: At the 3'-end of the vp35 mRNA, bases 4094-4160; E = -13.4 kcal/mol. C: At the 3'-end of the vp30 mRNA, bases 9029-9087; E = -9.4 kcal/mol. Base pairs (shown as ladder rungs) marked by a slash are GU base pairs. Preliminary experimental results indicate that structure A is inactive in a standard assay for eukaryotic SECIS elements. Structures B and C have not yet been tested.

If any of these potential UGA-rich PPCRs are expressed by specific SeC insertion mechanisms, then functional SECIS elements or something similar must exist in the viral RNA. We have identified a number of candidate genomic regions having the consensus sequence features of an SECIS element, as well as the potential to form the necessary RNA stem-loop structures; several of these are shown in Fig. 4. Of these, only candidate SECIS A has been tested; it is inactive in regard to ability to direct SeC insertion at a UGA codon in the human deiodinase gene (Nadimpalli et al., unpublished data).

If no potential viral elements can be found capable of directing SeC insertion in known mammalian selenoprotein genes, several conclusions might be drawn about these UGA-rich overlapping PPCRs:

1. They are nonfunctional artifacts, possibly evolved as Rima has suggested to cause termination of erroneously frameshifted protein chains *(33).* If so, they still might lead to low-level nonspecific SeC insertion as previously discussed *(31).*
2. They are genuine selenoprotein genes that can be expressed in mammalian hosts, but a virally encoded cofactor is required for SeC insertion to take place. Elucidation of this possibility would require experimentation with live virus.
3. They might be genuine selenoprotein genes that are expressed in a nonmammalian host (e.g., some insect) that may have a somewhat divergent SeC insertion apparatus that does not function in mammals. In that case, they would not be significant for pathogenesis in humans, unless they could still contribute to low-level nonspecific SeC insertion in some manner.

These possibilities will require careful research to be eliminated from consideration.

PATHOGENICITY AND POTENTIAL SELENOPROTEINS IN EBOLA

One argument in favor of the possibility that these overlapping UGA-rich PPCRs may be significant for pathogenicity in humans involves differences between the Ebola Zaire and Ebola Reston strains. In the Ebola Reston strain, which was devoid of pathogenicity in the three humans that were infected, the major potential selenoprotein gene overlapping the NP gene in Ebola Zaire (PPCR1 in Fig. 2) appears to be truncated and is almost certainly inactive. In the Ebola Reston NP mRNA, the UGA-rich PPCRs are disrupted by non-UGA stop codons, there are fewer UGA codons, no analogous frameshift sites or PKs, and no candidate SECIS element in the 3'-UTR. Thus, there is no way that this potential selenoprotein gene could be specifically expressed in Ebola Reston. This is a definite major difference at the gene level between these strains, which have previously been considered to be very close genetically. This potential NP-associated selenoprotein gene is also absent in Marburg virus, which also has a lower mortality rate than Ebola Zaire. However, both Marburg and Ebola Reston do have higher than expected densities of UGA codons in overlapping frames, suggesting that the nonspecific selenoprotein synthesis mechanism we have proposed might still contribute to Se depletion, blood clotting, and hemorrhagic symptoms (Ebola Reston was pathogenic in monkeys).

CONCLUSIONS

Selenium deficiency can weaken the immune system's ability to fight viral infection, permitting increased replication, rapid mutation, and facilitating the emergence of more virulent strains, as Beck et al. have suggested in the case of Coxsackie virus *(40).* In addition, virally encoded selenoproteins may be a novel contributing factor to increased viral pathogenicity under conditions of Se deficiency *(31).* Since any hypothetical selenoprotein genes would depend on the bioavailability of Se, a rare trace element, it is possible that the presence and activity of such genes in a virus would vary with geographical areas and thus be strain-specific *(38).* Specifically, long-term selenium deficiency in a host population could lead to the inactivation and loss of such genes. Although it would be difficult to predict the effects of such gene loss on the virulence of the strains involved, it could potentially be a novel factor contributing to differences in virulence between different viral strains.

In the case of hemorrhagic fever viruses, the theoretical prediction of hypothetical selenoprotein genes is more convincing in light of the known role of Se in regulation of blood clotting discussed previously. The fact that hemorrhagic conditions are often associated with highly Se-deficient diets in various species is also supportive of the hypothesis that Se might be involved in manifestations of hemorrhagic viral diseases. The possibility of nonspecific depletion of selenium is supported by the bias toward the usage of UGA codons in the -1 frame and the tendency of the virus to frameshift erroneously leading to translational slippage to other frames *(33).* Alternatively, as our anlysis of Ebola suggests, the prescence of UGA-rich PPCRs and the potential SECIS elements may indicate depletion of host Se by the programmed synthesis of specific selenoproteins.

These hypothetical proteins, if present, may provide some type of antioxidant protection to the Ebola virions in a rapidly degenerating cellular environment. Because these PPCRs overlap the Ebola Zaire NP gene, they could only be expressed as an NP fusion protein. Therefore, it is possible that this Se module could be formed as an NP variant comprising a small fraction of the total NP present in virions. This is precisely equivalent to the possibility that some Coxsackie virus strains, like CVB3, may express a viral GSH-Px homolog as a fusion protein to the vp3 capsid protein, permitting attachment of a GSH-Px module to the virion surface *(31).* In the same way that we proposed for CVB3 *(31),* attachment of an antioxidant module to the Ebola virion or its release as a soluble factor in the cell may provide various benefits to the virus, including defense against oxidative attack by the immune system. If this Ebola NP fusion selenoprotein is formed, incorporated Se may be detectable in Ebola Zaire virions in early infections before cellular stores of SeC become depleted.

In conclusion, our analysis suggests that severe infection by Ebola and some other hemorrhagic fever viruses could produce an artificial and extreme Se depletion, resulting in extensive cellular damage owing to lipid peroxidation, combined with enhanced thrombosis. This suggests that indicators of Se status and lipid peroxidation ought to be examined in Ebola patients. This hypothetical disease mechanism is also consistent with the previously mentioned results of Hou et al. in which Se was used to treat an infectious viral hemorrhagic fever successfully *(30).*

As Lavander and Beck and coworkers have proposed, there is considerable merit to the idea that factors like low Se in certain geographic regions may be contributing to the emergence of new and more virulent viral diseases *(40).* Our theoretical findings in regard to hemorrhagic fever viruses suggest that a viral requirement or utilization of Se, whether specific or nonspecific, may be a significant mechanism contributing to increased viral pathogenicity under conditions of Se depletion in human and animal populations.

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