REGULATION AND FUNCTION OF VAULT RNAS

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ABSTRACT

The function of vault RNAs remains unclear. The human genome encodes four vault RNAs: vtRNA1-1, 1-2, 1-3 and 2-1. Previous literature suggests that vault RNAs protect cells from apoptosis, regulate autophagy, and stimulate the immune response during viral infection. However, studies have shown that at least some of these functions are contingent on the presence of a 5' triphosphate moiety. DUSP11 (dual specificity phosphatase 11) is an RNA triphosphatase that removes the gamma and beta phosphates from the 5' end of RNAs. Regulation of phosphorylation status is not fully characterized and may impact downstream pathways that vault RNAs are involved in. Here, I hypothesize that DUSP11 plays a key role in regulating vault RNA levels and consequently, impacts the function of vault RNAs. I demonstrate that DUSP11 knockout results in an increased abundance of vault RNAs. Moreover, results indicate that DUSP11 mediated removal of the triphosphate on vault RNAs results in their degradation over time. Preliminary data suggests that DUSP11 is an important regulator of vault RNAs.

INTRODUCTION

Little is known about vault RNAs, although they were discovered over thirty years ago. Vault RNAs are a class of non-coding RNAs transcribed by RNA polymerase III and are 88-100nt long (Horos *et al.,* 2019). The human genome encodes four vault RNA paralogs: vtRNA1-1, vtRNA1-2, vtRNA1-3, and vtRNA2-1 (Buscher, Horos, & Hentze, 2020). Vault RNAs are named for their association with vault proteins which together, form large ribonucleoprotein complexes (Buscher *et al.,* 2020). These ribonucleoproteins, simply called 'vaults', are the largest known ribonucleoprotein complexes and are highly conserved (Buscher *et al.,* 2020). Some suggest that vaults play a role in transport between the nucleus and cytoplasm, drug resistance, apoptosis,

DNA damage repair, and more (Amort *et al.,* 2015). However, 95% of vault RNAs do not associate with these vault particles and are instead found in the cytoplasm, suggesting that they may have additional, unknown functions (Amort *et al.,* 2015). These functions for the most part remain unclear, but some studies suggest that vault RNAs play a role in the regulation of autophagy and the stimulation of an immune response during viral infection (Horos *et al.,* 2019).

The Role of Vault RNAs in Autophagy and Apoptosis

Autophagy, which translates to 'eating of self', is a process that is essential for maintaining homeostasis in response to stress and in times in development (Glick, Barth, & Macleod, 2010). Autophagy involves the recognition, removal and degradation of misfolded or aggregated proteins, damaged organelles, and some pathogens (Glick *et al.*, 2010; Horos *et al.,* 2019). Autophagy begins with a phagophore, also called the isolation membrane, which is a double-membraned structure derived from lipid bilayer (Glick *et al.*, 2010). The phagophore expands to engulf cargo in the cytoplasm, enclosing it in an autophagosome, a double-membraned vesicle (Glick *et al.*, 2010). The autophagosome fuses with the lysosome, causing degradation its components (Glick *et al.,* 2010; Carlsson & Simonsen, 2015). Autophagy can be thought of as cellular 'recycling.' Transporter proteins export leftover amino acids and by-products of degradation to the cytoplasm, where they can be reused in other cellular processes (Glick *et al.*, 2010). In this way, the cell maximizes efficiency in times of stress.

A recent discovery suggests that vtRNA1-1 plays a role in regulating p62-dependent autophagy (Horos *et al.,* 2019). Autophagy was long thought to be a non-selective process, with autophagosomes randomly engulfing intracellular components within the cytoplasm (Horos *et al.,* 2019). The discovery of autophagic receptors, however, indicated that the formation of autophagosomes is not random at all but is in fact a highly regulated process (Horos *et al.,* 2019).

Among these autophagic receptors is p62, also called sequestosome-1, which binds ubiquitinated intracellular aggregates with its C-terminal ubiquitin binding domain (Horos *et al.,* 2019). p62 also contains an LC3-interaction motif which allows it to co-localize with LC3-positive autophagosomes (Horos *et al.,* 2019). With its ubiquitin binding domain and LC3-interaction motif, p62 facilitates the degradation of intracellular pathogens and protein aggregates in the cytoplasm, thus promoting autophagy (Horos *et al.,* 2019).

In order to function properly, p62-dependent autophagy requires oligomerization of p62 (Horos *et al.,* 2019). Oligomerization allows p62 to associate with LC3-positive autophagosomes with higher affinity (Horos *et al.,* 2019). Understanding regulation of p62 oligomerization may provide a better understanding of how autophagy is regulated overall. A recent study showed that vtRNA1-1 inhibits oligomerization of p62 by binding to the receptor (Horos *et al.,* 2019). Through iCLIP, a method of identifying protein-RNA interactions, researchers discovered that p62 preferentially binds all four vault RNAs relative to other RNA Pol III transcripts (Horos *et al.,* 2019). This finding suggests that p62 is an RNA-binding protein and that vault RNAs may play a role in regulating autophagy.

Although all four vtRNAs preferentially bind p62, the relationship between vtRNA1-1 and p62 was chosen for further analysis (Horos *et al.,* 2019). Previous literature suggested that vtRNA1-1 may prevent cells from undergoing apoptosis and was therefore especially of interest in this study (Amort *et al.*, 2015). To further understand the interaction between vtRNA1-1 and p62, researchers studied how serum starvation affected vtRNA1-1 levels (Horos *et al.,* 2019). To 'starve' the cells, cells were grown in media lacking amino acids and serum (Horos *et al.,* 2019). After six hours, vtRNA1-1 levels were measured (Horos *et al.,* 2019). Results indicated that

vtRNA1-1 levels dropped significantly relative to the initial time point, suggesting that vtRNA1-1 levels are dependent on amino acid and serum starvation (Horos *et al.,* 2019).

Moreover, results indicated that vtRNA1-1 overexpression restricted autophagic flux overall (Horos *et al.*, 2019). Autophagic flux was measured by quantifying LC3B conjugation from LC3B1 to LC3BII. LB3BII has been shown to localize to autophagosomes, making it a good marker for studying autophagy (Tanida *et al.*, 2004). Increased levels of LC3BII therefore indicate increased levels of autophagic flux. Overexpression of vtRNA1-1 in HuH-7 cells resulted in reduced LC3B conjugation, indicating decreased autophagic flux (Horos *et al.*, 2019). These results suggest that vtRNA1-1 regulates autophagic flux through its association with p62.

Vault RNAs Stimulate an Immune Response

Cells have evolved clever ways to stimulate an immune response during viral infection. One way in which a cell can alert neighboring cells of the presence of a virus and trigger an immune response is through the interferon response. Type I interferons (IFN-I), named for their ability to 'interfere' with viral replication, stimulate the immune response by upregulating the expression of interferon-stimulated genes (ISGs) (McNab *et al.,* 2015). These genes promote an antiviral state in non-immune cells and trigger the adaptive immune response (Murira & Lamarre, 2016). Interferon induction typically occurs when proteins known as pattern recognition receptors (PRRs) recognize foreign nucleic acids (McNab *et al,* 2015).

RIG-I-like-receptors (RLRs) are PRRs that recognize self from non-self RNA (Zhao, Ye, Dunker, Song, & Karijolich, 2018). Retinoic acid-inducible gene-I (RIG-I), a member of the RLR family, recognizes and binds short 5' tri and diphosphorylated double stranded RNAs in the cytoplasm (Zhao *et al.*, 2018). Bioinformatics analyses revealed that only host RNAs bind to

RIG-I during KSHV (Kaposi's sarcoma-associated herpesvirus) lytic reactivation (Zhao *et al.*, 2018). Quantitative reverse transcription PCR (RT-qPCR), a method used to analyze relative RNA expression, indicated that vault RNAs were the most highly enriched RIG-I bound RNAs (Zhao *et al.*, 2018). Specifically, vtRNAs 1-1, 1-2, and 1-3 were identified as RIG-I substrates (Zhao *et al.*, 2018). This data suggests that vault RNAs may play an important role in stimulating an immune response during viral infection.

To test whether or not vtRNAs elicit an immune response and whether this process is 5'triphosphate dependent, vtRNAs were transfected into cells (Zhao *et al.*, 2018). A portion of these vtRNAs were CIP-treated, resulting in removal of their 5'triphosphate moieties. Only 5' triphosphorylated vault RNAs stimulated the ISG54-luciferase reporter, meaning that the 5' triphosphate is required for interferon stimulation via RIG-I (Zhao *et al.,* 2018). These findings highlight the importance of the triphosphate status of vault RNAs and the potential impact it may have on the immune response.

Understanding the mechanism by which vault RNA triphosphate status is regulated may provide better understanding of the function of vault RNAs. DUSP11 (dual specificity phosphatase 11) was identified as an RNA triphosphatase involved in altering the phosphorylation status of vault RNAs (Burke, Kincaid, Nottingham, Lambowitz, & Sullivan, 2016). RNA sequencing indicated that vault RNA expression levels doubled in the absence of DUSP11 (Burke *et al.,* 2016). This result was verified by Northern blot analysis, a method of quantifying relative RNA expression, which showed that endogenous vault RNA levels (vtRNA1-1, 1-2, and 1-3) increased when DUSP11 was knocked (Burke *et al.,* 2016). These results indicate that DUSP11 is a key modulator of vault RNA triphosphate status.

Here, I hypothesize that DUSP11 regulates the expression of vault RNAs and that DUSP11-mediated removal of the triphosphate moiety directly impacts the proposed functions and/or stability of vault RNAs.

METHODS

Plasmids

To explore how DUSP11 controls vault RNA levels, I constructed vault RNA-expressing plasmids. Each vault RNA was amplified from genomic DNA using specific PCR primers. Gel electrophoresis was used to verify the identity of the PCR amplicons. Each vault RNA (vtRNA1-1, 1-2, 1-3, and 2-1) was cloned into the Xba1/Kpn1 restriction of puc19, a cloning vector. DH5α competent E. coli cells were transformed with the plasmid to ensure proper cloning. Finally, plasmid samples were sequenced to confirm whether or not each vault RNA was correctly inserted into the backbone. I confirmed that the plasmids functioned properly *in vitro* by conducting Northern blot analysis.

Cell Culture

A549 wild-type and DUSP11 knockout cells were grown and maintained in DMEM containing 10% FBS. Transfections were done using Lipofectamine 2000 (Invitrogen). Cells were discarded after no more than 10 passages.

Northern Blot Analysis

To determine whether or not DUSP11 regulates vault RNA levels, vault RNA-expressing plasmids were transfected into A549 wild-type and DUSP11 knockout cells. After 24 hours, I harvested the RNA using TRIzol. Northern blot analysis was used to measure relative expression of vault RNAs between wild-type and DUSP11 KO cells. Northern blot analysis consists of

RNA extraction, fractionation on a PAGE-urea gel, and transfer to a membrane. I then probed the membrane using radiolabeled DNA oligos (complementary in sequence to vault RNA of interest) overnight. Membranes were then exposed to a phosphor screen which could then be visualized. Northern blot results were analyzed using an application called Image Studio. All relative expressions were normalized to 5S rRNA, a ribosomal RNA with a protected 5' end. 5S rRNA has shown no specific interaction with DUSP11 and is therefore an appropriate loading control to use in this set of experiments.

RNA Decay Assay

An RNA decay assay was conducted to measure endogenous vault RNA levels over time. This was done by plating A549 wild-type cells in a 12-well. Once cells were roughly 60-70% confluent, either siNC (mock) or siDUSP11 was transfected into the cells. After 24 hours, total RNA was collected from the first pair of wells (containing wild-type or DUSP11 knockout cells) using TRIzol. I then added actinomycin D, a transcription inhibitor, to the remaining wells and collected RNA in 40-minute intervals. After RNA was collected and isolated, Northern blot analysis was used to measure relative RNA expression at each time point. To strip the membrane, I placed it in boiling 0.1% SDS for 10 minutes, 3 times. Once stripped, the membrane could be re-probed with another radiolabeled oligo. Image Studio was used to analyze the data.

Real Time PCR

I transfected the vault-RNA-expressing plasmids into A549 wild-type and DUSP11 knock-down cells. DUSP11 was knocked down using RNAi. After 24 hours, total RNA was collected using TRIzol. RNA was isolated and real time PCR was conducted using Phusion polymerase. Real

time PCR provides data for relative expression of various genes. IFNβ-1 and ISG15 levels were analyzed with real time PCR.

RESULTS

DUSP11 knockout results in increased vtRNA1-1 expression

To understand the role of DUSP11 in regulating the abundance of vault RNAs, I transfected the vtRNA1-1-expressing plasmid into A549 wild-type and DUSP11KO cells. Based on previous literature, I expected that vtRNA1-1 levels would be approximately doubled in A549 DUSP11 knockout cells (Burke *et al.,* 2016). In our results however, Northern blot analysis showed an approximately 1.5-fold difference in vtRNA1-1 levels between A549 wild-type and DUSP11 knockout cell lines (Figure 1A/1B). Expression of vtRNA1-1 was normalized to 5s rRNA expression, a ribosomal RNA found abundantly in the cell.

DUSP11 knockout may or may not affect vtRNA2-1 expression

The same protocol described above was repeated with vtRNA2-1-expressing plasmid to overexpress vtRNA2-1. To my surprise, there was no significant difference in vtRNA2-1 levels between the wild-type and DUSP11KO cell lines (Figure 2). Expression of vtRNA2-1 was normalized to 5S rRNA expression.

However, in the data collected in the following experiment (below), there does seem to be a difference in vtRNA2-1 levels when DUSP11 is knocked down (figure 3C). In that experiment, however, cells expressed endogenous vtRNA levels. In other words, no vtRNA-expressing plasmid was transfected into the cells prior to Northern blot analysis. Results showed a roughly 1.6-fold difference between wild-type and DUSP11 knock-down cells (Figure 3C), which mirrors our expectations.

Whether knockout was less efficient than knock-down of DUSP11 is unclear. Moreover, why there would be a difference between studying endogenous or overexpressed vtRNA2-1 levels is unclear. This experiment should be replicated several times under both a knockout and knock-down background to confirm whether or not DUSP11 regulates vtRNA2-1 levels.

DUSP11 mediates vtRNA1-1 and 2-1 decay

To explore decay rates of vault RNAs in the presence and absence of DUSP11, I conducted an RNA decay assay. I collected total RNA mock-transfected (siNC) and siDUSP11-transfected A549 cells in 40-minute intervals and measured relative expression of both vtRNA1-1 and 2-1. The mock transfection served as the wild-type condition while siDUSP11 was used to knock down DUSP11. Northern blot analysis allowed for quantification of relative vtRNA1-1 (Figure 3A).

Our results indicate that the rate of vault RNA decay differs depending on whether or not DUSP11 is present. In the wild-type condition, vtRNA1-1 levels steadily decline over time, ultimately reaching a level about half that of its initial concentration (Figure 3B). In the DUSP11 knock-down (KD) cells, vtRNA1-1 levels were initially (at the 0-minute time point) about 1.5-fold higher relative to the mock transfected cells (Figure 3B). I expected this based on the results discussed previously. After 120 minutes, vtRNA1-1 levels only slightly declined. The concentration of vtRNA1-1 after 120 minutes in the DUSP11 KD cells was still higher than the initial concentration in the wild-type condition.

I stripped the membrane and re-probed with a radiolabeled oligo complementary to vtRNA2-1. Similar to vtRNA1-1, vtRNA2-1 was more abundant in DUSP11 KD cells at the initial time point than in mock-transfected cells. As expected, vtRNA2-1 levels declined after

120 minutes, reaching a concentration about half that of its initial concentration. In the DUSP11 KD condition, no significant decay of vtRNA2-1 was observed over the course of 120 minutes.

Vault RNA 1-1 stimulates ISG15

Previous studies suggest that vault RNAs stimulate the immune response through their interaction with RIG-I. To test this hypothesis, I explored the relationship between vtRNA1-1 concentration and IFNβ-I and ISG15 expression. Relative quantities of IFNβ-I and ISG15 were measured using real time PCR. Although preliminary, the data suggests that vtRNA1-1 may play a role in stimulating the immune response. These findings confirm the hypothesis that increasing vtRNA1-1 concentrations in the cell results in an increased interferon response.

IFNβ-I increased modestly with increasing concentration of vtRNA1-1 (Figure 4A/4B). This finding may not be significant however, because no clear trend could be observed. ISG15 increased approximately two-fold when vtRNA1-1 increased from 0 ng to 500 ng (Figure 4B). It is unclear why ISG15 increased more relative to IFNβ-I, as both are thought to be in the same pathway. This data suggests that vtRNA1-1 stimulates ISG15.

To further explore the relationship between vtRNA1-1 and ISG15 and whether or not DUSP11 plays a role in this interaction, I conducted real time PCR. In this experiment, I transfected siNC (mock transfection) or siDUSP11 (DUSP11 KD) into A549 cells. After 24 hours, increasing concentrations of vtRNA1-1-expressing plasmid were transfected into each well (0 ng, 300 ng, 700 ng, and 1000 ng). After another 24 hours, I collected total RNA from the cell and conducted a real time PCR to measure ISG15 expression. Unexpectedly, results showed no relationship between increasing vtRNA1-1 concentrations and ISG15 expression and no relationship between DUSP11 and ISG15 (Figure 4C). While results from the previous

experiment suggested a potential role of vtRNA1-1 in immune stimulation, this experiment did not do the same. In fact, there appeared to be a slight inverse correlation between vtRNA1-1 and ISG15 concentrations. This was true for both the siNC and siDUSP11 conditions. It is unclear why the same trend did not hold in the second experiment.

DISCUSSION

The results of this study demonstrate that DUSP11 plays a role in regulating vault RNA levels (vtRNA1-1 and 1-2). For the first experiment, overexpression of each vault RNA using an expression plasmid allowed for better visualization of the differences between wild-type and DUSP11 KO cells. Northern blot analysis revealed that vtRNA1-1 levels increased when DUSP11 was knocked out. This was not true for vtRNA2-1 however. There are several potential explanations for this. First, vtRNA2-1 is encoded by a different locus in the genome and is the largest of the four vault RNAs. These differences could suggest different interactions with DUSP11. Second, these results may be due to factors like poor RNA quality or poor transfection efficiency. Based on the results from the RNA decay assay, vtRNA2-1 does seem to be regulated by DUSP11. To confirm these findings, this experiment should be repeated.

These results suggest that when the triphosphate moiety is reduced to a monophosphate, vault RNAs are more susceptible to degradation. When DUSP11 is knocked out, vault RNAs retain the 5’ triphosphate and as a result, are more abundant in the cell. The 5’ triphosphate has a protective effect on the RNA. This implies that degradation of vault RNAs occurs in a monophosphate-dependent manner.

Results from the RNA decay assay further the idea that degradation occurs in a monophosphate-dependent manner. In cells lacking DUSP11 (DUSP11 knockdown), vault

RNAs degraded more slowly relative to vault RNAs in cells with wild-type DUSP11. This was true for vtRNA1-1 and vtRNA2-1. Again, the results seem to suggest that the 5' triphosphate moiety has a protective effect on vault RNAs, preventing them from being degraded. Based on our knockout study, it seems that DUSP11 is responsible for removing the triphosphate.

To explore whether immune stimulation by vault RNAs is affected by the presence DUSP11, I conducted real time PCR to measure ISG15 expression in cells transfected with different amount of vtRNA1-1. Data from real time PCR is preliminary and should be replicated. The first set of results (Figure 4A) suggest that vault RNA stimulates ISG15, the second dataset indicates the opposite. Previous literature has suggested that vault RNAs stimulate interferon and interferon-inducible genes, though our results do not confirm this. These results may be due to a low starting concentration of total RNA in the real time PCR. Further experimentation should be done to explain these results.

FUTURE DIRECTIONS

Identifying a monophosphate-dependent exonuclease

Results demonstrate that 5'monophosphorylated vault RNAs are turned over more rapidly than 5' triphosphorylated vault RNAs. The next step should be to identify the exonuclease responsible for vault RNA turnover. Although not tested, we speculate that the exonuclease responsible for degrading vault RNAs is XRN1 or XRN2. Our proposed model can be visualized in Figure 5. XRN1 targets substrates with a 5' monophosphate and has been suggested to promote attack on viral transcripts in the absence of DUSP11 (Kincaid, Lam, Chirayil, Randall, & Sullivan, 2018).

To study whether or not XRN (1 or 2) turns over monophosphorylated vault RNAs, XRN can be knocked down with RNAi in A549 wild-type cells. If our hypothesis is correct, vtRNA

levels should be greater relative to the wild-type condition. To confirm whether DUSP11 regulates vault RNAs via XRN-mediated decay, XRN1 can be knocked down in DUSP11 KO cells. If XRN and DUSP11 are truly involved in the same pathway, the amount of vault RNA in this condition should equal that of the wild-type condition. XRN upregulation with an expression plasmid can also be used to see if the opposite is true. In this case, based on the hypothesis, vtRNA levels would reduce in wild-type but not DUSP11 KO cells.

Exploring the role of DUSP11 in autophagy

Previous studies suggest that vtRNA1-1 acts as a riboregulator of autophagy (Horos *et al.,* 2019). Our work demonstrates that vault RNA levels are regulated by DUSP11. This brings up some interesting questions. How does the triphosphate status of vtRNA1-1 relate to its role in autophagy? Does the presence or absence of DUSP11 impact autophagy? This can be tested by overexpressing vtRNA1-1 in cells treated with siNC or siDUSP11. Western blot analysis can be used to examine differences in autophagy markers, including p62 and LC3.

CONCLUSIONS

Though preliminary, results indicate that DUSP11 may play a role in regulating vault RNAs. The future may reveal a regulatory role of DUSP11 in autophagy, apoptosis, interferon response, and more. Moreover, understanding how DUSP11 plays a role in these diverse biological processes may have therapeutic implications.

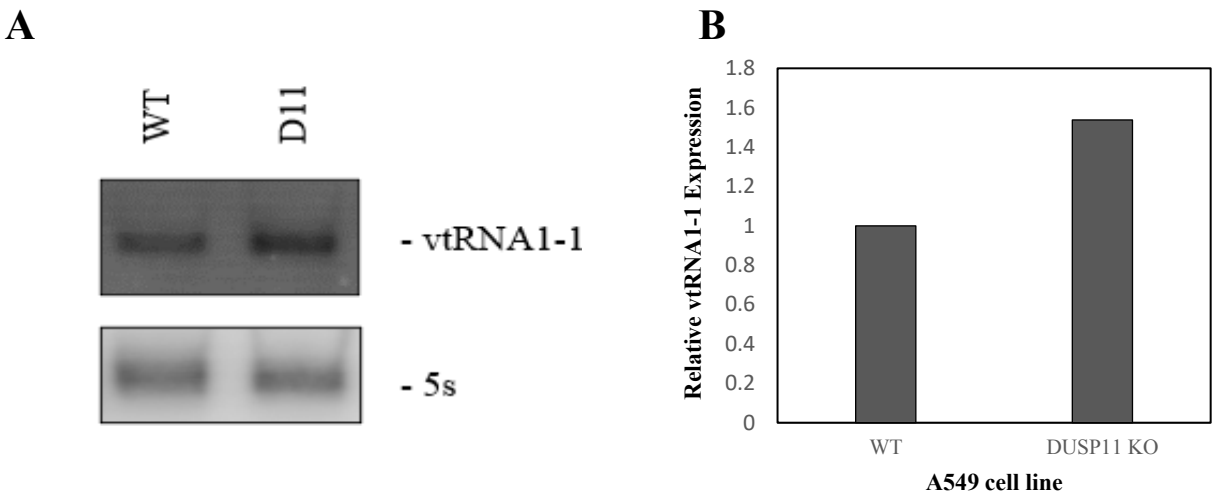


Figure 1 (A) Results from Northern blot analysis of vtRNA1-1 in A549 wild-type and DUSP11 knockout cells. 5S rRNA is provided as a loading control. (B) A graphical representation of vtRNA1-1 expression in wild-type and DUSP11 knockout cells. vtRNA1-1 expression increased approximately 1.5-fold in DUSP11 knockout cells.

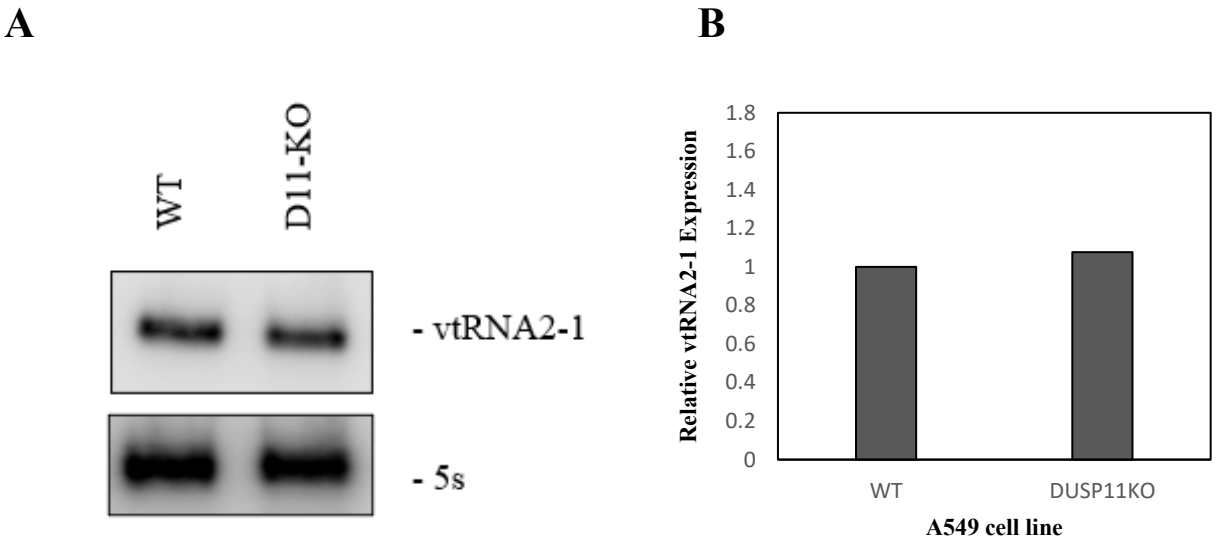


Figure 2 (A) Results from Northern blot analysis of vtRNA2-1 in A549 wild-type and DUSP11 knockout cells. 5S rRNA is provided as a loading control. (B) A graphical representation of vtRNA2-1 expression in wild-type and DUSP11 knockout cells. vtRNA 2-1 expression did not increase in knockout cells.

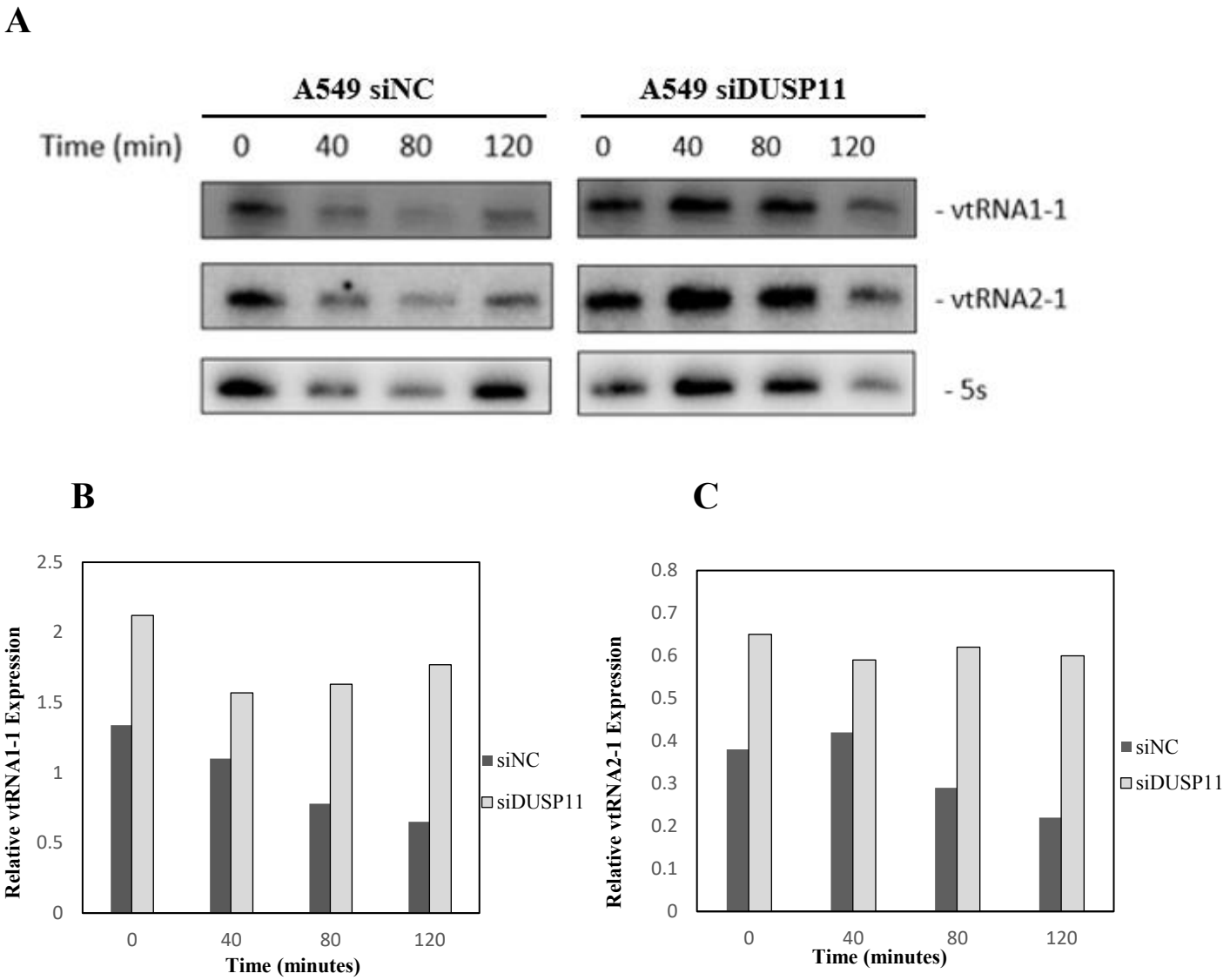
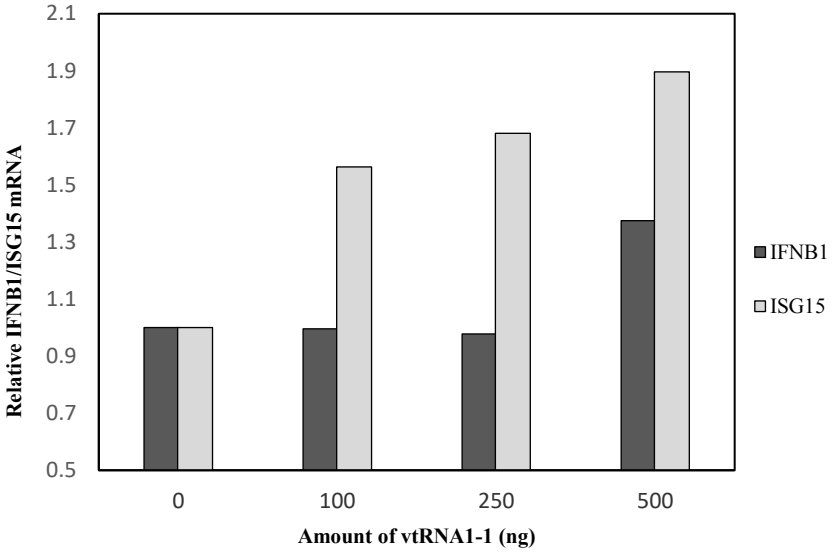


Figure 3 (A) Northern blot analysis of vtRNA1-1 and vtRNA2-1 in siNC and siDUSP11 transfected A549 cells. Loading was uneven, therefore 5S rRNA is used as a loading control. Each band represents vault RNA in the cell at a different time point. (B) vtRNA1-1 levels in siNC-transfected cells steadily decreased over 120 minutes. vtRNA1-1 levels in DUSP11 knockdown cells decreased modestly, but remained higher than siNC transfected cells. (C) vtRNA2-1 levels in siNC-transfected cells decreased over 120 minutes. VtRNA2-1 levels in DUSP11 knockdown cells remained constant over 120 minutes and was higher than vtRNA2-1 expression in the siNC transfected cells.

A

vtRNA1-1 quantity (ng)	Target	Fold Change
0	IFNB1	1
100	IFNB1	0.996
250	IFNB1	0.978
500	IFNB1	1.375
0	ISG15	1
100	ISG15	1.563
250	ISG15	1.681
500	ISG15	1.896

B



C

Sample	Target	RQ	Cт
siNC VTRNA-0	ISG15	1	33.046
siNC VTRNA-300	ISG15	0.942	29.650
siNC VTRNA-700	ISG15	0.731	29.328
siNC VTRNA-1000	ISG15	0.843	29.276
siD11 VTRNA-0	ISG15	2.979	27.633
siD11 VTRNA-300	ISG15	3.806	27.130
siD11 VTRNA-700	ISG15	2.692	26.732
siD11 VTRNA-1000	ISG15	1.214	27.804

Figure 4 (A) A table of values from real time PCR. The table demonstrates relative quantities of IFNB1 and ISG15 at different concentrations of vtRNA1-1. CT value indicates how many cycles were required for the signal to cross a certain threshold and can indicate how much RNA there was to start with. Smaller values indicate a greater abundance of RNA. (B) A graphical representation of real time PCR data. IFNB1 increased modestly when vtRNA1-1 increased. ISG15 increased approximately 1.9-fold when vtRNA1-1 increased from 0 ng to 500 ng. (C) A table of values from a second real time PCR reaction. This table indicates that there is no relationship or even an inverse relationship between ISG15, DUSP11, and vtRNA. These results are opposite that of the results found in 4A.

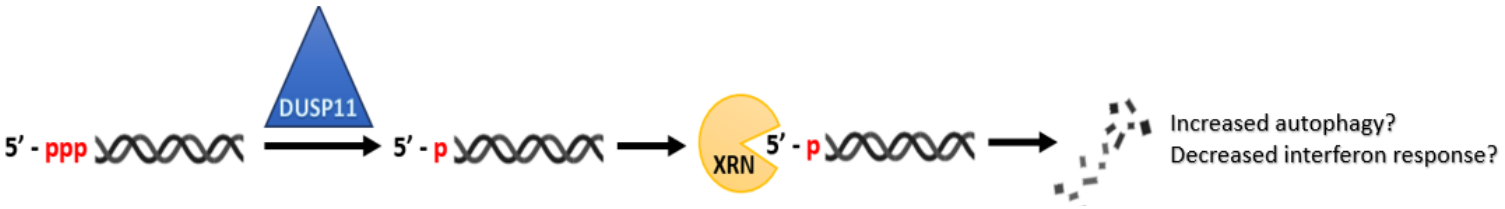


Figure 5 This diagram depicts the proposed mechanism of XRN mediated decay of vault RNAs. 5' triphosphorylated vault RNAs undergo dephosphorylation via DUSP11. Now monophosphorylated on their 5' end, these vault RNAs are susceptible to XRN-mediated decay. Downstream pathways, including autophagy and the immune response, may be affected by vtRNA decay.

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