The roles of the exoribonucleases DIS3L2 and XRN1 in human disease.

Amy L. Pashler[1], Ben Towler[1], Christopher I. Jones[2] and Sarah F. Newbury[*]
[1]Brighton and Sussex Medical School, Medical Research building, University of Sussex, Falmer, Brighton, BN1 9PS, U.K.
[2]Brighton and Sussex Medical School, Department of Primary Care and Public Health, University of Brighton, Falmer, Brighton, BN1 9PH, U.K.

[*]Corresponding author: s.newbury@bsms.ac.uk +44 (0)1273 877874

Abstract

RNA degradation is a vital post-transcriptional process which ensures that transcripts are maintained at the correct level within the cell. DIS3L2 and XRN1 are conserved exoribonucleases which are critical for the degradation of cytoplasmic RNAs. Although the molecular mechanisms of RNA degradation by DIS3L2 and XRN1 have been well studied, less is known about their specific roles in development of multicellular organisms or human disease. This review focusses on the roles of DIS3L2 and XRN1 in the pathogenesis of human disease, particularly in relation to phenotypes seen in model organisms. The known diseases associated with loss of activity of DIS3L2 and XRN1 are discussed, together with possible mechanisms and cellular pathways leading to these disease conditions.

Key words: RNA stability, RNA degradation, human disease, virus-host interactions, XRN1, DIS3L2

Abbreviations used: UTR, untranslated region; HCV, Hepatitis C virus; sfRNA, small flaviviral non-coding RNA; mRNA, messenger RNA; miRNA, microRNA.

Introduction

Ribonucleases are key enzymes involved in the control of mRNA stability, which is crucially important in maintaining RNA transcripts at the correct levels within cells. Studies have shown that 40-50% of changes in gene expression occur at the level of RNA stability [1] which means that this process is highly important in regulating gene expression. In multi-cellular organisms, it is increasingly evident that degradation of specific mRNAs is critical for the regulation of many cellular processes, including early development, infection and inflammation, apoptosis, and ageing [2-7]. Although progress has been made in identifying the targets regulated by these ribonucleases [8-11], the mechanisms whereby they affect cellular processes leading to disease are largely unknown. This review focusses on two highly conserved cytoplasmic exoribonucleases, DIS3L2 and XRN1, and how dysregulation of these enzymes is implicated in human diseases. The association of the related exoribonuclease DIS3 with human disease has recently been reviewed elsewhere [12].

An mRNA transcript is usually protected from degradation by the 5’ methylguanosine cap and the 3’ poly-A tail. mRNAs have been shown to be held in a circular conformation by interactions of protein factors such as eIF4E (the cap-binding protein), eIF4G and the poly-A binding protein. Degradation usually begins with decircularisation of the RNA and the removal of the poly-A tail. Following this process, degradation can proceed in two ways: in the 3’-5’ direction or in the 5’-3’ direction. In some circumstances (such as during nonsense-mediated decay or miRNA induced degradation), internal cleavage occurs prior to degradation of the mRNA in both the 3’-5’ or 5’-3’ directions [2, 4, 5] (Figure 1).

During 3’-5’ degradation, deadenylases first digest away the poly-A tail leaving an exposed 3’ end. This facilitates 3’-5’ degradation by a family of ribonucleases [9, 12], including DIS3 and DIS3L2. DIS3L2 is a 3'-5' exoribonuclease which is conserved from bacteria to humans. It acts independently of the 3'-5' exoribonuclease-exosome complex and degrades non-coding RNAs as well as mRNAs [13, 14]. In humans and the yeast *S. pombe,* this is often achieved by addition of a polyuridine (polyU) tract to the 3' end of the transcript which provides an ideal landing pad for DIS3L2 to initiate/reinitiate 3'-5' decay [14-16]. As well as mRNAs, miRNAs such as *pre-let-7* have been shown to be poly-uridylated and then degraded by DIS3L2 in human cells and mouse embryonic stem cells [17, 18].

In contrast, there is only one known exoribonuclease involved in cytoplasmic 5’-3’ degradation; XRN1. After deadenylation by the Ccr4/Not deadenylase complex [19] the Lsm1-7 complex is recruited to the 3' end of the RNA, together with XRN1 and decapping proteins such as Dcp1 and Dcp2 [20]. This complex activates the decapping enzymes which cleave off the 5’ methyl-guanosine cap allowing the threading of the exposed 5’ end through to the active site of XRN1 where it is digested. Although RNA degradation by XRN1 is likely to be cotranslational [21], XRN1 is also found in cytoplasmic foci, termed processing bodies (P-bodies) [22, 23], which constitute a concentration of mRNA decapping machinery, messenger ribonucleoproteins and ribonucleases [24]. Here, mRNAs can also be stored for re-entry into translation at a later time. Being the only cytoplasmic exoribonuclease of its kind, this places XRN1 at a pivotal position in the RNA degradation pathway, with any modulation to this enzyme having the potential to have significant effects on cellular function.

Recent work using human tissue culture cells and model organisms shows that DIS3L2 and XRN1 affect important cellular processes such as proliferation and apoptosis. Experiments using human HeLa cells have demonstrated that knockdown of DIS3L2 results in increased proliferation [25] and reduced apoptosis [16]. Knockdown of DIS3L2 in HeLa cells was also shown to result in mitotic errors and affect the expression of key cell cycle proteins including Cyclin D and Aurora B kinase [25], suggesting that it has a role in regulating the cell cycle. In multicellular organisms, XRN1 is involved in a number of developmental processes [26, 27] but more recently has been shown to be a key player in controlling apoptosis [8, 9]. We have recently demonstrated that null mutations in the *Drosophila* XRN1 homologue *pacman* result in wing imaginal discs that are 45% the size of wild-type controls as well as resulting in lethality during pupation. Mutant wing discs show an increase in the number of cells undergoing apoptosis, especially in the wing pouch area. The *pacman* mutant imaginal discs also undergo compensatory proliferation, but this is not sufficient to compensate for the large amount of apoptosis occurring in these discs. The pro-apoptotic RNAs *hid* and *reaper* increase at the post-transcriptional level in *pacman* mutants, suggesting that they are targets of Pacman [8]. Additionally, RNA-seq experiments have shown that a potential target of Pacman is *dilp8*, a *Drosophila* insulin-like peptide with similarity to the insulin-like peptides IGF-1 and IGF-2 in humans [9].

Human diseases associated with DIS3L2

The clinical importance of DIS3L2 is demonstrated by its association with Perlman syndrome, which is a congenital overgrowth syndrome inherited in an autosomal recessive manner [28]. Affected children display foetal gigantism, abnormal enlargement of organs (e.g. kidneys), macrocephaly, facial abnormalities, neurodevelopmental delay and high neonatal mortality. Histological examinations reveal nephroblastomatosis, which is an important precursor for Wilms' Tumour. Germline mutations in these children include deletions of either exon 6, 9 and/or 19 which are consistent with a loss of function of DIS3L2 [25, 29].

Mutations in *DIS3L2* have also been associated with sporadic occurrence of Wilms' Tumour, a kidney cancer also referred to as a nephroblastoma [25, 28, 29]. Wilms' Tumour results from the failure of groups of kidney cells to mature and differentiate, instead undergoing continuous proliferation. DIS3L2 is also likely to be important in sporadic Wilms' Tumour as 30% of these tumours (6/20) show partial or complete *DIS3L2* deletion [25]. This tumour is not only associated with aberrations in *DIS3L2*, but is also linked to mutations in the *DROSHA* gene, leading to aberrant expression of oncogenic miRNAs [30], such as *miR-562* [31], which promotes deregulation of *EYA1*. *EYA1* is involved in the retinal determination gene network, and has been associated with driving various malignancies (such as breast cancer) through the cell cycle, leading to extensive proliferation, whilst also inhibiting apoptosis [32]. This is interesting because it suggests that Wilms' Tumour is partially driven by extensive loss of the RNA degradation machinery itself, highlighting the importance of this pathway.

Other aberrations in the *DIS3L2* gene have been associated with a Marfan-like syndrome with skeletal overgrowth [33]. Three patients carrying a chromosomal translocation at the chromosomal region 2q37.1 were shown to have a breakpoint within the *DIS3L2* gene at intron 5 (2 patients) and intron 6 (1 patient) [33-35]. The symptoms of these patients included skeletal overgrowth and malformations, leading to long, slender fingers, curvature of the hands and feet

(arachnodactyly), lack of apoptosis between digits (mild syndactyly), and abnormal curvature of the spine (severe scoliosis). However, for these patients, it cannot be ruled out that these phenotypes are also affected by mis-expression of the neighbouring gene *NPPC* (encoding C-type natriuretic peptide) or deletion of the gene encoding *hsa-miR-562*, which is located within intron 9 of *DIS3L2* [33].

Human diseases associated with XRN1

Wilms' Tumour is not the only tumour which has been associated with defects in RNA degradation. Reduced XRN1 mRNA expression has been demonstrated in many osteosarcoma cell lines as well as osteosarcoma patient samples [36]. Osteosarcoma is the third most common cancer affecting adolescents with a second smaller peak occurring between 60 to 80 years, particularly in people affected by Paget's disease. Overall, osteosarcoma has a moderate incidence rate, with 10 to 26 per million new cases worldwide each year [37]. Osteosarcoma often develops at either end of the long bones of the arms or legs, although tumours can also occur in the skull and pelvis. Tumours typically arise from osteoid-producing neoplastic cells adjacent to the long bone growth plate. The main cause of death in these patients is the spread of cancer cells to the lungs; these secondary tumours are present in 10-20% of cases at diagnosis [37].

In osteosarcoma cells, XRN1 is often down-regulated, suggesting that lower levels of XRN1 promote proliferation. However, null mutations and hypomorphic mutations of this conserved exoribonuclease in *Drosophila* result in apoptosis [8]. This apparent contradiction can be explained by hypothesising that apoptosis is blocked in osteosarcoma cells, meaning that the down-regulation of XRN1 causes an increase in proliferation of the tumour cells without the associated apoptosis. By this means, it may be that reduction of XRN1 confers a growth advantage on the tumour cells. Alternatively, downregulation of XRN1 may lead to increased expression of specific miRNAs and/or protein-coding RNAs which then promote the cancerous phenotype. Possible candidates are IGF-1, which is known to induce proliferation of osteoblasts [38-40] and/or pro-inflammatory RNAs such as FOS and MYC which are widely known to be involved in cancer progression.

XRN1 and the host response to viruses

XRN1 also appears to play a pivotal role during the host response to viral infection and has been implicated in the pathogenesis of a number of viral families. Recent studies have shown that the activity of XRN1 is specifically inhibited by certain *Flaviviruses,* which include Dengue fever virus, West Nile virus [41], Yellow fever virus [42], Japanese Encephalitis virus [11] and, perhaps more notably at present, the Zika virus. Flaviviruses are single-stranded (+) sense arthropod-borne viruses which are usually transmitted to humans through mosquitos but can also be transmitted through ticks [43]. Encephalitis is symptomatic of each of these infections, alongside hemorrhagic fevers. The activity of XRN1 is also repressed in a similar manner by Hepatitis C (HCV) virus which primarily infects the liver, and can lay asymptomatic for many years. During this time, the virus can cause liver scarring, potentially leading to cirrhosis, liver cancer and liver failure [44]. HCV, along with Hepatitis B, has also been tenuously linked with pancreatic cancer and leukaemia/lymphoma [45-47]. The targeting of XRN1 by these viruses indicates that XRN1 is normally vitally important in protecting the cell from these viral pathogens.

A series of recent publications have elucidated the molecular mechanisms whereby these viruses inhibit XRN1 as well as shedding light on the disease mechanisms. In the case of Dengue and West Nile virus (Kunjin strain), viral transcripts are able to inhibit the 5'-3' RNA degradation pathway by stalling XRN1 within the 3' UTR of the viral RNA (Figure 2A). The formation of RNA pseudoknots ("slipknots") [48], as well as other RNA structures within the 3'UTR, stall the progression of XRN1 by blocking entry of the RNA into the active site [41]. XRN1 degrades the viral RNAs from the 5' end of the transcript, until it reaches these structures, leaving small flaviviral non-coding RNAs (sfRNAs), which accumulate in the cytoplasm. These small, structured RNAs bind to and sequester XRN1 thus reducing 5'-3' degradation of other cellular RNAs [49] (Figure 2A). For HCV, similar highly structured regions within the 5'UTRs of their transcripts stall and repress the activity of XRN1 (Figure 2B). Using tissue culture cells infected with HCV, it was shown that the disruption of the 5'-3' mRNA decay pathway results in stabilisation of short-lived RNAs such as transcription factors involved in oncogenesis (e.g. *MYC*, *FOS* and *JUN*) and angiogenesis (e.g. *VEGFA, HIF1A* and *CXCL2*) [50]. Since HCV infection is associated with the development of hepatocellular carcinoma, these factors might contribute to the development of the diseased state [49]. This study also showed an increase in levels of capped and presumably functional RNAs suggesting that repression of XRN1 leads to shut down of the entire degradation pathway, possibly by sequestration of other decay components [50]. In the case of Flaviviruses, the available evidence suggests that viral infection disproportionately affects short-lived transcripts encoding cytokines and factors involved in innate immunity leading to the inflammatory symptoms observed [49].

The importance of XRN1 in limiting the pathogenesis of HCV is illustrated by the fact that this virus has evolved two ways to inhibit the action of XRN1. Recent publications show that 5'-3' degradation of HCV transcripts is stalled by expression of *miR-122* in the host cell, which binds via its miRNA seed sequence to the 5'UTR of the viral transcript (Figure 2C). The association of *miR-122/*RISC, including Ago2 [51], at two sites in the 5' UTR, protects it from degradation by XRN1 [52]. *miR-122* is a major determinant of viral RNA replication, as it inhibits XRN1 from degrading the viral RNA, allowing accumulation, increased replication, and increased translation of viral RNA. Studies have shown that *miR-122* only needs to be bound at one of the two sites for it to have this inhibitory effect, and binding to both sites maintains a co-operative effect, rather than increasing the effectiveness [52]. In addition to the *miR-122* binding site, the 5'UTR HCV viral RNA includes four stem loops of which three (loops 2-4) appear to function as a highly structured internal ribosome entry site, allowing translation initiation of the viral RNA [50]. Translation of the HCV core protein has been shown to activate the proto-oncogene, *MYC,* allowing the progression of hepatocellular carcinomas [53]. While Loops 1 and 2 are involved in viral replication, they also inhibit the activity of XRN1 by presenting structures which stall the progress of XRN1 as described above.

Another way in which viruses can inhibit the 5'-3' RNA degradation pathway is by disruption of P-bodies, affecting the localisation of XRN1. Rotavirus infection is understood to disrupt P-bodies, the sites of concentration of the RNA degradation machinery. Rotavirus is an RNA virus which causes acute diarrhoea across many developing countries, mainly affecting young children, with its spread being attributable to poor sanitation and hygiene [54]. This virus is a double-stranded, non-enveloped virus which utilises rotavirus protein NSP1 to degrade the deadenylase, PAN3, in the P-bodies, resulting in transcript stabilisation, an effect also seen in Poliovirus [49]. Rotavirus also causes the relocalisation of XRN1 from the P-bodies to the nucleus, thereby inhibiting the degradation of viral RNA. However the molecular details of this are unknown. Viral proteins

specifically prevent degradation of six structural transcripts and six non-structural transcripts, which are either translated into protein, or serve as templates for replication of viral progeny [55].

Concluding remarks

When DIS3L2 and XRN1 were first identified, it could not have been envisaged that investigation of these apparent 'housekeeping" exoribonucleases would lead to such interesting and fundamental insights into the molecular mechanisms of RNA decay. As described above, recent work on DIS3L2 and XRN1 is now revealing their important roles in cellular function, viability and human disease. Finding a way to combat disease by engineering the RNA degradation machinery to alter patterns of RNA regulation observed during pathogenesis offers a new hope to many people in the future. This is especially true in the cancer field. For example, if DIS3L2 or XRN1 is proven to facilitate overgrowth syndromes or the progression of osteosarcoma there is potential to develop novel therapies for these diseases. The data implicating XRN1 as a key enzyme in the control of Flavivirus and Hepatitis C viral infection also presents an opportunity for the development of broad spectrum antiviral treatments. This is particularly important in the wake of new viral epidemics such as the outbreak of the Zika virus in South America. It is clear that there is still much work to be done to fully understand the ways in which DIS3L2 and XRN1 regulate cellular pathways resulting in disease progression. A good starting point for this would be to pinpoint the exact RNA transcripts which are being targeted by specific exoribonucleases in these pathways to elucidate how changes in RNA levels could contribute to pathogenesis. From starting at the level of basic science, this could have profound implications in the medical world in the future.

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Figure Legends:

Figure 1: Cartoon depicting deadenylation dependent RNA degradation pathways. Bulk eukaryotic mRNA degradation is deadenylation dependent, which is predominantly carried out by the Ccr4-Not complex. (A) Cleavage can occur during nonsense mediated decay or cleaved internally by endonucleases leaving unprotected 3’ and 5’ ends which can be degraded in either the 5’ or 3’ direction by XRN1 (5’) or the exosome complex (3’). (B) Following deadenylation [56-58], RNA 5'-3' degradation occurs, after the binding of the Lsm1/Pat1 complex at the 3'end activating the decapping complex. The unprotected 5’ ends are then degraded by XRN1. (C) Alternatively, degradation can occur in the 3' - 5' direction by the exosome complex, of which the catalytic domain is DIS3. (D) Deadenylated mRNAs can also be uridylated by uridyl transferases (TUTases) which then target the RNA for degradation by the exosome independent 3'-5' exoribonuclease DIS3L2.

Figure 2: Inhibition of XRN1 in the 5' and 3' UTRs of viral transcripts. (A) XRN1 is stalled at the site of the Flaviviral 3' UTR due to the presence of pseudoknots or stem loops. It is blocked from continuing degradation, allowing the accumulation of RNA secondary structures which constitute the 3'UTR of the flaviviral transcript. **(B)** XRN1 is stalled at the 5'UTR of Hepatitis C viral RNA due to the presence of stem loops and pseudoknots. In the case of A and B, XRN1 is bound to and sequestered by these strong secondary structures. **(C)** XRN1 is stalled at the 5' UTR due to binding of *hsa-miR-122* (green) at the seed sequence site and the presence of stem loops (1-4), during HCV infection.

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