Yeast Dxo1 is required for 25S rRNA maturation and acts as a transcriptome-wide distributive exonuclease

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ABSTRACT

The Dxo1/Rai1/DXO family of decapping and exonuclease enzymes can catalyze the in vitro removal of chemically diverse 5′ ends from RNA. Specifically, these enzymes act poorly on RNAs with a canonical ^{7m}GpppN cap, but instead prefer RNAs with a triphosphate, monophosphate, hydroxyl, or nonconventional cap. In each case, these enzymes generate an RNA with a 5′ monophosphate, which is then thought to be further degraded by Rat1/Xrn1 5′ exoribonucleases. For most Dxo1/Rai1/DXO family members, it is not known which of these activities is most important in vivo. Here we describe the in vivo function of the poorly characterized cytoplasmic family member, yeast Dxo1. Using RNA-seq of 5′ monophosphate ends, we show that Dxo1 can act as a distributive exonuclease, removing a few nucleotides from endonuclease or decapping products. We also show that Dxo1 is required for the final 5′ end processing of 25S rRNA, and that this is the primary role of Dxo1. While Dxo1/Rai1/DXO members were expected to act upstream of Rat1/Xrn1, this order is reversed in 25S rRNA processing, with Dxo1 acting downstream from Rat1. Such a hand-off from a processive to a distributive exonuclease may be a general phenomenon in the precise maturation of RNA ends.

Keywords: 5′ exoribonuclease; degradome; rRNA processing

INTRODUCTION

The maturation and degradation of mRNAs and ncRNAs are multistep processes that are crucial throughout all forms of life. Eukaryotic pre-mRNAs must be spliced, polyadenylated, and capped before they can be utilized in translation. rRNA processing utilizes at least a dozen ribonucleases, including endoribonucleases that separate the 25S, 18S, and 5.8S rRNAs from their common precursor (Woolford and Baserga 2013; Henras et al. 2014). After this separation, each RNA molecule is further processed by other ribonucleases (endoribonucleases, 5′ exoribonucleases, and/or 3′ exoribonucleases) to produce mature ribosomes (Woolford and Baserga 2013; Henras et al. 2014; Tomecki et al. 2017). These ribonucleases often have multiple functions, although in many cases their in vivo functions have not been fully defined. For example, the 5′ exoribonuclease Rat1 and the 3′ exoribonuclease the RNA exosome are both required for rRNA maturation, but they also have many other substrates, including released mRNA introns and aberrant RNAs (Wasmuth and Lima 2012; Januszyk and Lima 2014; Dhoondia et al. 2021). Yeast has proven to be a powerful eukaryotic system to initially identify ribonuclease functions, most of which are conserved in other eukaryotes, including humans. Mutations in ribonucleases cause many human genetic diseases (Morton et al. 2018; van Dijk et al. 2018; Weskamp and Barmada 2018; Wolin and Maquat 2019), further underscoring the relevance of these enzymes and the overall importance of RNA processing.

The activity and structure of the Rai1/Dxo1/DXO family of enzymes has been well characterized in vitro and is suggested to be important for the removal of aberrant caps from mRNAs as well as for other pathways of RNA degradation. This family of enzymes can act on a variety of RNA ends, and removes pyrophosphates, NpN dinucleotides, and aberrant caps such as Gpp, NAD, FAD, and coA (Chang et al. 2012; Doamekpor et al. 2020b; Zhang et al. 2020). Importantly, aberrant caps cannot be removed by the conventional decapping enzyme, Dcp2, or its homologs. Some family members can also remove mononucleotides and thus act as 5′ exonucleases (Chang et al. 2012; Doamekpor et al. 2020a). In all the above cases, Rai1/Dxo1/DXO enzymes produce an RNA with a 5′ monophosphate that is further degraded by the nuclear

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5′ exoribonuclease Rat1 or its cytoplasmic homolog Xrn1. However, it is not fully understood which of these catalytic capacities of Rai1/Dxo1/DXO are important in vivo. The human genome only encodes one of these enzymes (DXO), while fungal genomes have been found to encode one or more of the family members. For example, *Schizosaccharomyces pombe* has a single gene (*RAI1*), while *Saccharomyces cerevisiae* and *Kluyveromyces lactis* each have two genes (*RAI1* and *DXO1*).

Mammalian DXO is one of the most well-studied family members. DXO has 5′ exonuclease activity, and thus can remove various cap structures and degrade uncapped transcripts (Jiao et al. 2013; Doamekpor et al. 2020b). DXO products may then be degraded by XRN1 or RAT1 as DXO has been reported to be either a nuclear or cytoplasmic protein (Zheng et al. 2011; Picard-Jean et al. 2018; Lynch 2019). One in vitro activity of human DXO is its ability to convert an RNA with a 5′ hydroxyl to one with a 5′ monophosphate by removing the first 2 nt (NpN) from the 5′ end of the RNA (Doamekpor et al. 2020a). This is particularly interesting because some endonucleases produce a 5′ hydroxyl that must be converted to a 5′ monophosphate in order for the RNA to be degraded by Xrn1 (Calvin and Li 2008; Cherry et al. 2019; Navickas et al. 2020). DXO could participate in this pathway, which would provide an explanation for how 5′ hydroxylated endonuclease cleavage products are eventually degraded by Xrn1 and/or Rat1 (Nagarajan et al. 2013; Peach et al. 2015).

Rai1 was initially identified as a Rat1 interacting and stabilizing protein, which allows for efficient 5′ to 3′ degradation by Rat1 in the nucleus (Xue et al. 2000). A key study ascertained the structure of Rai1 and noted a putative active site (Xiang et al. 2009). This observation led to the discovery that Rai1 has catalytic functions independent of stabilizing Rat1 (Xiang et al. 2009). The catalytic activities of Rai1 have most extensively been characterized for the *S. pombe* enzyme and include the removal of FAD, CoA, NAD, unmethylated, or incomplete caps (Jiao et al. 2010; Chang et al. 2012; Zhang et al. 2020).

In contrast to the nuclear Rai1, *S. cerevisiae* Dxo1 (ScDxo1) localizes to the cytoplasm and appears to act independently of other exonucleases, as ScDxo1 is unable to form a complex with either Rat1 or its cytoplasmic equivalent, Xrn1 (Huh et al. 2003; Chang et al. 2012; Wang et al. 2015). The catalytic activities of ScDxo1 have not been characterized, but Dxo1 from *K. lactis* (KlDxo1) has increased exonuclease activity in vitro compared to Rai1 and DXO (Wang et al. 2015). This increased exonuclease activity is due to several amino acid substitutions near the active site (Chang et al. 2012). These same mutations also reduced the decapping activity of Dxo1, indicating a tradeoff between exonuclease and decapping activities. The in vivo function of ScDxo1 has been partially characterized. Dxo1 has been identified as a minor contributor to 5′ exonuclease activity in No-Go Decay and the unfolded protein response (Cherry et al. 2019; Navickas et al. 2020). During No-Go decay, ribosomes stall on an RNA transcript resulting in cleavage of the trapped mRNA. Similarly, during the unfolded protein response (UPR), the *HAC1* intron is removed by Ire1-mediated endoribonucleolytic cleavage. In both cases, Xrn1 is primarily responsible for degradation of the cleaved mRNA/intron. However, when Xrn1 is deleted, Dxo1 can less efficiently degrade at least a few bases of the mRNA/Hac1 intron (Cherry et al. 2019; Navickas et al. 2020). It remains unclear whether these Dxo1 functions in No-Go decay and the UPR reflect a more global role.

In this paper, we seek to understand the roles of ScDxo1 more completely. Because all the biochemical activities of Dxo1 produce similar 5′ monophosphate RNAs that are degraded by Xrn1, we reason that 5′ monophosphate RNAs that accumulate in an *xrn1Δ* strain but are absent in *xrn1Δdxo1Δ* are likely the products of Dxo1. We used Parallel Analysis of RNA Ends (PARE), an RNA sequencing strategy specific for 5′ monophosphate ends (Addo-Quaye et al. 2008; German et al. 2008), to identify such products of Dxo1. We found that Dxo1 acts as a transcriptome-wide distributive exonuclease, consistent with its known functions in No-Go decay and the UPR, but greatly expanding the scope of its exonuclease activity. Surprisingly, we also identified Dxo1 as the enzyme primarily responsible for the maturation of 25S rRNA from a 25S′ intermediate. The 25S′ to the 25S rRNA processing step is well described, but the enzyme responsible had not been convincingly identified (Geerlings et al. 2000; Oeffinger et al. 2009; Thomson and Tollervey 2010; Tomecki et al. 2017). Overall, this study provides the first global understanding of the function of Dxo1 in vivo.

RESULTS AND DISCUSSION

Sequence divergence of Dxo1 and Rai1 suggests functional differences

As a first step in understanding how ScDxo1 differs from its paralog ScRai1, we sought to determine when the duplication occurred and what sequence changes occurred when the two proteins diverged. We found two loci corresponding to Dxo1/Rai1 in the genomes of most Saccharomycetaceae and the very closely related Saccharomycodaceae. This includes species that diverged both before and after the well-characterized whole genome duplication in the *Saccharomyces* lineage (Fig. 1). In contrast, the more distant budding yeasts of the Phaffomycetaceae, Dipodascaceae, and Lipomycetaceae each contained a single gene, which we will refer to as Rai1/Dxo1. This suggests that Dxo1 and Rai1 arose by gene duplication in a shared ancestor of the Saccharomycetaceae and Saccharomycodaceae.

Next, we compared the sequences of Rai1 and Dxo1 by multiple sequence alignment to determine how the two proteins diverged (Supplemental Fig. S1). Importantly,

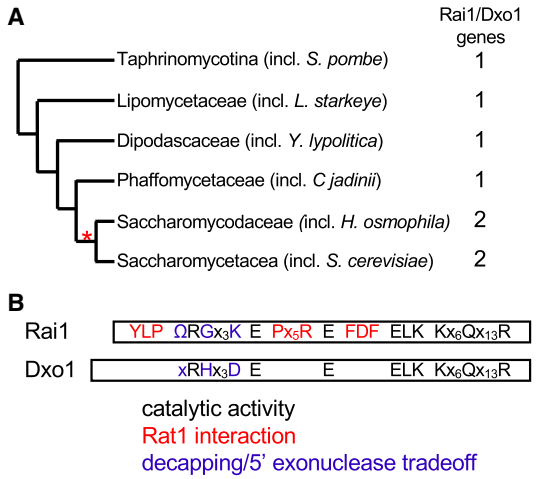

FIGURE 1. Dxo1 and Rai1 are duplicated genes that arose in a common ancestor of the Saccharomycetaceae and Saccharomycodaceae and subsequently diverged. (*A*) Evolutionary tree representing the relationship between different budding yeast families. The number on the *right* indicates the number of Rai1/Dxo1/DXO genes per genome. The red asterisk indicates the Rai1/Dxo1 duplication that predates the well-characterized whole genome duplication in a subset of Saccharomycetaceae. (*B*) Conserved sequence motifs of duplicated Rai1s and Dxo1s suggests functional differences between the two enzymes. Catalytic residues are retained in both and indicated in black letters. Residues important for Rat1 interaction are in red and are lost in Dxo1s. The Dxo1s also have a xRHx3D motif replacing the ΩRGx_3K motif of Rai1 in blue, with Ω being an aromatic residue. This change enhances the 5′ exonuclease activity and reduces decapping activity.

both proteins retain the catalytic residues of the active site (Fig. 1B, black letters). One noteworthy change is that amino acid residues known to be important for Rat1 interaction (Xiang et al. 2009) were lost from one copy (hereafter Dxo1 proteins; Fig. 1B, red letters). These Dxo1 proteins also gained a short amino-terminal extension that is absent from Rai1 and Rai1/Dxo1 proteins (Fig. 1B). This extension is predicted to be unstructured (www.pondr.com), consistent with the crystal structure of KlDxo1 (Chang et al. 2012). The Dxo1 proteins also consistently had a $xRHx_3D$ motif replacing the ΩRGx_3K motif (Ω being an aromatic residue) of Rai1 and Rai1/Dxo1 proteins (Fig. 1B, blue letters). This change was shown to enhance the 5′ exonuclease activity and reduce the decapping activity of KlDxo1 (Chang et al. 2012). Although the biochemical activities of ScDxo1 have not been directly characterized, the sequence conservation suggests to us that, like KlDxo1, it has robust 5′ exonuclease activity and reduced decapping activity.

Dxo1 can act as a distributive exonuclease on many different RNAs

Next we wanted to determine the in vivo function of ScDxo1. To investigate this, we performed PARE in *dxo1Δxrn1Δ* and *xrn1Δ* strains. We have previously used the same strategy to characterize the highly specific activity of the tRNA splicing endonuclease (TSEN). In contrast to the few changes in the PARE profile caused by TSEN inactivation, we saw thousands of PARE signals increase and decrease as a result of Dxo1 inactivation, indicating that Dxo1 has transcriptome-wide effects (Supplemental Fig. S2). This included Dxo1 activities downstream from known endonucleases (Cherry et al. 2019; Hurtig et al. 2021), decapping (Harigaya and Parker 2012), and spliceosome-mediated decay (Harigaya and Parker 2012; Volanakis et al. 2013).

We first examined one of the most well-studied examples of an endonucleolytic cut in an mRNA, Ire1-mediated cleavage of *HAC1* mRNA. The two cleavage sites in *HAC1* are precisely known, but PARE identified clear peaks in *xrn1Δ* that were shifted a few nucleotides 3′ of the actual cleavage sites (Fig. 2A). In contrast, in *xrn1Δdxo1Δ*, the peak at the actual cleavage site of *HAC1* became more prominent, while the peaks just 3′ disappeared (Fig. 2A). We observed this pattern at both cleavage sites, and both products are known to be degraded by Xrn1 (Cherry et al. 2019). Our results confirm that when Xrn1 is absent, these RNAs can be partially degraded by Dxo1 (Cherry et al. 2019).

We next looked at a *bona fide* TSEN target, *CBP1*. TSEN cleaves the *CBP1* mRNA, facilitating further degradation by Xrn1 (Tsuboi et al. 2015; Hurtig et al. 2021). We examined the peaks within *CBP1* and found a similar trend to that of *HAC1*: *xrn1Δ* showed three major peaks as we previously reported, with smaller peaks one or a few nucleotides downstream. In *xrn1Δdxo1Δ*, peaks 1 and 2 become sharper, and the peaks just downstream disappear (Fig. 2B). Interestingly, while peak 2 becomes more predominant, peak 3 (5 nt downstream from peak 2) almost completely disappears in *dxo1Δxrn1Δ*. We conclude that *CBP1* is cleaved in only two positions, and the sharper peaks 1 and 2 reflect the direct cleavage products. Peak 3 and smaller peaks downstream from peaks 1 and 2 result from Dxo1 digestion of these direct cleavage products. We previously reported that *CBP1* was cleaved by recombinant TSEN in vitro and were initially puzzled that only sites 1 and 2 are robustly cleaved by TSEN (Hurtig et al. 2021). The conclusion that site 3 is the product of Dxo1 and only an indirect product of TSEN provides a probable explanation for the discrepancy between our previous in vivo and in vitro sites.

Spliceosome-mediated mRNA degradation refers to a decay pathway initiated by the first step of splicing (Volanakis et al. 2013). Instead of being used in the second step of splicing, the lariat intermediate (containing the intron, exon 2, and the poly(A) tail) is debranched and degraded by Xrn1. A previous degradome study in yeast showed that this occurred in a subset of intron-containing mRNAs (Harigaya and Parker 2012), and we observed the

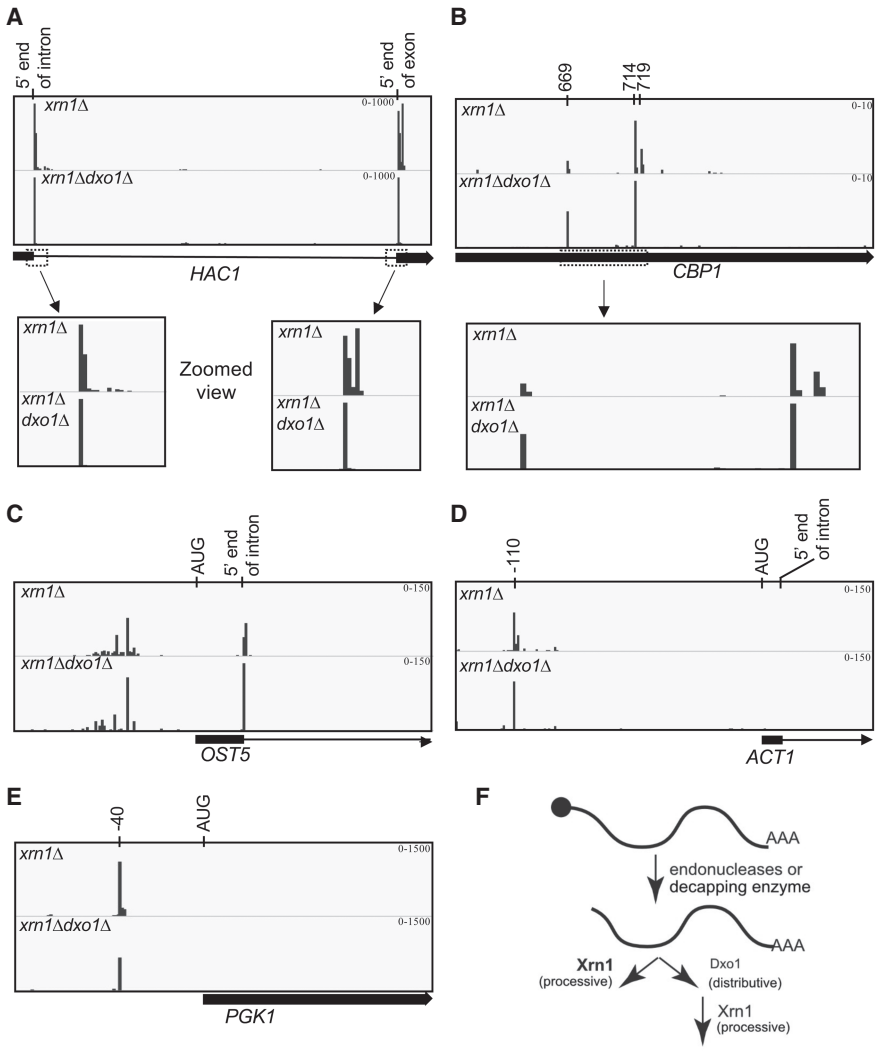

FIGURE 2. Dxo1 is a distributive exonuclease that acts downstream from decapping and endonuclease cleavage. (*A–E*) Read maps of PARE data on *xrn1Δ* and *xrn1Δdxo1Δ* yeast showing the accumulation of 5′ monophosphorylated RNA ends as peaks in the mRNAs *HAC1*, *CBP1*, *OST5*, *ACT1*, and *PGK1*. For each gene, a single representative PARE repeat is shown. The *y*-axes are linear with the range indicated in the *top right*. Each endonuclease site results in a single sharp peak in the *xrn1Δdxo1Δ* strain, but a cluster of peaks in xrn1Δ. This pattern is highlighted in the zoomed in areas in *A* and *B*. Decapping of *OST5*, *ACT1*, and *PGK1* also results in fewer peaks in *xrn1Δdxo1Δ* than in xrn1Δ, but a few peaks remain, which likely reflects multiple transcription start sites. The main capping sites for *ACT1* at −110 and *PGK1* at −40 are indicated *above* the graphs and the coding regions (boxes) and introns (lines) *below* the graphs. (*F*) Model showing the distributive activity of Dxo1. After endonuclease cleavage or decapping, Dxo1 can act as a distributive exonuclease before or in the absence of complete processive degradation by Xrn1.

same spliceosome-mediated decay targets in our data set. However, in both the previous study and our *xrn1Δ* strain, the 5′ monophosphate ends were often one to three nucleotides downstream from the known 5′ splice site. One example of this is *OST5*. When examining the *xrn1Δ* PARE data, we observed the same group of peaks downstream from the 5′ splice site as was reported previously (Harigaya and Parker 2012). In contrast, *xrn1Δdxo1Δ* accumulates 5′ monophosphate ends that precisely match the 5′ splice site (Fig. 2C). This indicates that Dxo1 not only processes introns from atypical splicing events like *HAC1*, but it also processes spliceosomal introns. Both our data and the degradome sequencing of Harigaya and Parker use poly(A) plus RNA, which enriches for the products of spliceosome-mediated decay. Conversely, when both steps of splicing are completed, the intron is released without a poly(A) tail and rapidly degraded by Rat1. *ACT1* is an example of an efficiently spliced transcript and, as such, neither our study nor the previous study detected abundant 5′ monophosphate ends on the intron (Fig. 2D). Overall, these results suggest that Dxo1 can act downstream from several endoribonucleases, including Ire1, TSEN, and the spliceosome.

The major cytoplasmic mRNA degradation pathway involves removal of the 5′ cap structure by Dcp2 and degradation of the decapped mRNA by Xrn1. Therefore, 5′

monophosphorylated decapped mRNAs accumulate in *xrn1Δ* strains, but not a *dcp2Δ xrn1Δ* strain (Harigaya and Parker 2012; Hurtig et al. 2021). In our PARE data, the products of Dcp2 are represented by peaks located at the beginning of the 5′ UTRs (e.g., *ACT1* and *PGK1*; Fig. 2D,E) in *xrn1Δ*. When only Xrn1 is deleted, we detect these decapped products as clusters of peaks, similar to what was seen for the endonuclease products. In *dxo1Δxrn1Δ*, these clusters of peaks are replaced by fewer, sharper peaks (Fig. 2D,E). For example, the *xrn1Δ* sample shows a prominent peak 110 nt upstream of the start codon of *ACT1*, and smaller peaks at −109 and −108. Similarly, there are peaks at −40, −39, and −38 of *PGK1*. In comparison, the *xrn1Δdxo1Δ* strain produced sharper peaks at −110 and −40, respectively. These *dxo1Δxrn1Δ* peaks match exactly those seen in previously published TL-seq, a genome wide-mapping method of capped 5′ ends (Arribere and Gilbert 2013), and are absent in previously published PARE data from a *dcp2Δxrn1Δ* strain (Harigaya and Parker 2012). The most likely explanation for these data is that Dxo1 acts on the Dcp2 product and removes 1 or 2 nt. A possible alternative explanation is that Dxo1-mediated removal of canonical 7mGpppN or aberrant (GpppN, NAD or FAD) caps would also produce peaks 1 nt downstream from the cap. However, the additional signal 2 nt downstream is not readily explained by such decapping activity. Though our data cannot completely rule out this alternative scenario, the homology studies of ScDxo1 also suggest this enzyme is a better exonuclease than decapping enzyme (Fig. 1).

Overall, based on the general pattern that *xrn1Δ* strains often have smaller peaks just downstream from a main peak and that these diffuse peaks sharpen to a single main peak in *xrn1Δdxo1Δ*, we conclude that in the absence of Xrn1, Dxo1 can act as a distributive exonuclease that removes a few nucleotides from the 5′ end of a wide variety of transcripts (Fig. 2F). All of the Dxo1 substrates mentioned above have previously been shown to be stabilized by *xrn1Δ*, suggesting that in a wild-type strain, Xrn1 is the major exoribonuclease while Dxo1 makes minor contributions. Importantly, by sharpening peaks, our *xrn1Δdxo1Δ* data set increases the mapping precision for decapping and cleavage sites, and thus serves as a useful resource that complements past and future degradome studies.

Dxo1 is required for 25S rRNA maturation

One of the most prominent differences we noticed in *xrn1Δdxo1Δ* was a shift in the 5′ end of 25S rRNA. In the *xrn1Δ* PARE data, the 25S rRNA 5′ end usually produces the most abundant signal. 25S rRNA has a 5′ monophosphate end and an internal A-rich sequence that causes incomplete depletion by oligo(dT) beads (Roy and Chanfreau 2020). In *xrn1Δdxo1Δ*, we saw prominent peaks two and 7 nt upstream of the normal 25S rRNA 5′ end, with an almost complete loss of the normal 5′ end, suggesting a defect in 25S 5′ end processing.

In the 5′ end processing pathway of 25S rRNA, Las1 cleaves the 35S precursor into a 27S intermediate (Schillewaert et al. 2012; Woolford and Baserga 2013; Henras et al. 2014; Gasse et al. 2015). Then, Rat1 processes the 27S intermediate to 25S′, which has a 5′ extension of several nucleotides (Geerlings et al. 2000; Woolford and Baserga 2013; Henras et al. 2014). The enzyme responsible for 25S′ to 25S processing has not been definitively assigned. It has been suggested that Rat1 may continue removing nucleotides when it reaches 25S′ to produce the 25S, though slower than in the previous step (Geerlings et al. 2000). Alternatively, it has been suggested that Rrp17 may be responsible for the final step in producing 25S rRNA. However, both *rat1* and *rrp17* mutants accumulate earlier rRNA processing intermediates, not specifically the 25S′ (Geerlings et al. 2000; Oeffinger et al. 2009). Therefore, previous data suggesting that either Rat1 or Rrp17 is responsible for 25S′ to 25S processing is inconclusive.

To further analyze the possible role of Dxo1 in rRNA processing, we also performed PARE in wild type (*XRN1+ DXO1+*) and *dxo1Δ* single mutants (Fig. 3A). In both the wild type and *xrn1Δ* single mutant, we observe a large peak at the mature 25S 5′ end. This indicates that Xrn1 is not required for 25S 5′ end processing. On the other hand, the *dxo1Δ* and *xrn1Δdxo1Δ* mutants show the accumulation of two peaks at −2 and −7 relative to the normal 25S 5′ end, which corresponds to the 25S′ intermediate (Fig. 3A). We did not see a similar shift in the mature 18S rRNA 5′ end (Fig. 3B). These findings indicate that Dxo1 is required for processing 25S′ to the mature 25S rRNA. The effect of Dxo1 on 25S rRNA seems to be highly specific since we observed no notable differences for other ncRNAs with 5′ monophosphate mature ends in the *dxo1Δ* PARE data. Thus, in the presence of Xrn1, Dxo1 does not appear to have transcriptome-wide effects, but rather is highly specific for 25S′ to 25S rRNA processing.

To confirm that Dxo1 is required for 25S 5′ end formation, we performed northern blot analysis with a probe designed to hybridize to 25S′ but not 25S rRNA. As shown in Figure 3C and quantitated in Figure 3D, we detected a large increase in 25S′ rRNA in *dxo1Δ* and *dxo1Δxrn1Δ*. Thus, *dxo1Δ* is the first mutant known to specifically accumulate 25S′ rRNA and almost completely lack the mature 25S rRNA. Remarkably, this processing defect does not cause a marked growth defect (see below), indicating that ribosomes with 25S′ rRNA are largely functional. This is similar to 3′ extended 5.8S and 5S rRNA that are largely functional under standard laboratory conditions (Briggs et al. 1998; van Hoof et al. 2000; Faber et al. 2002).

These observations suggest that the processing of 27S to 25S is initiated by Rat1 but completed by Dxo1 (Fig. 3E). Analogous cases of hand-off RNA processing reactions

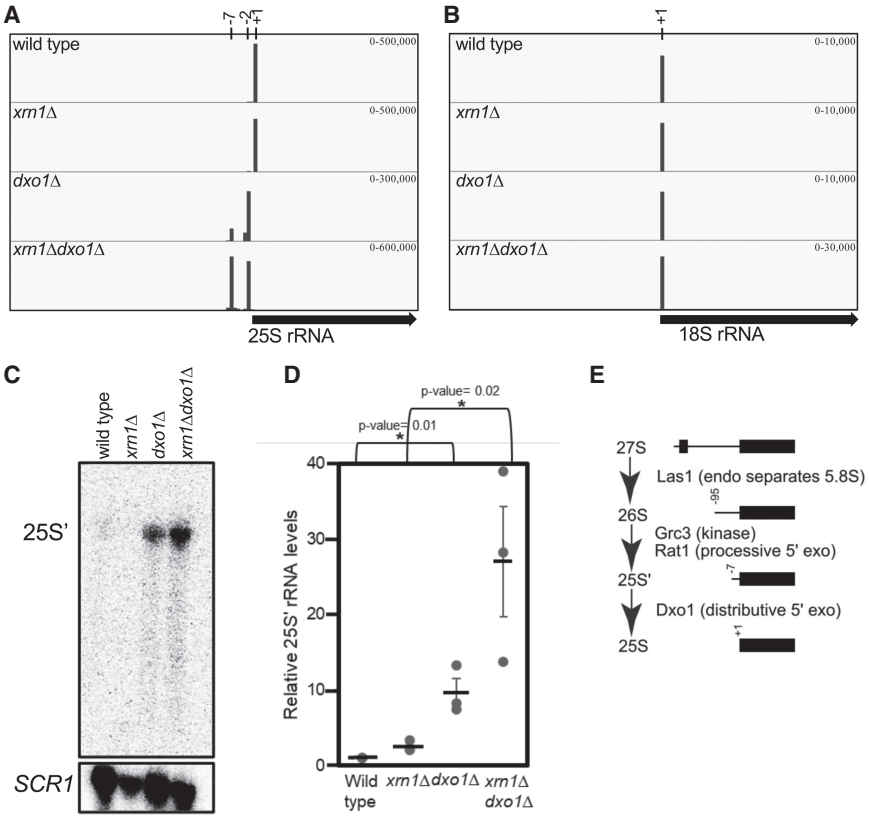

FIGURE 3. Dxo1 processes the 25S′ intermediate to 25S rRNA. (*A*,*B*) Read maps of PARE data from wild-type *xrn1Δ*, *dxo1Δ*, and *xrn1Δdxo1Δ* yeast showing the accumulation of 5′ monophosphorylated RNA ends as peaks in rRNA. The *y*-axes are linear with the range indicated in the *top right*. The scale of the *y*-axes is different between samples, reflecting some variability in the efficiency of rRNA removal. (*A*) When Dxo1 is deleted, the peak for mature 25S is replaced by peaks 2 and 7 nt upstream that correspond to the 25S′ intermediate. (*B*) The 18S rRNA 5′ end is not affected by Dxo1, demonstrating the specificity of Dxo1 to 25S′ processing. (*C*) Representative northern blot of the same strains measuring 25S′ accumulation. *SCR1* is used as a loading control. (*D*) Quantification of northern blots of three biological replicates normalized to the *SCR1* control and presented relative to the wild-type strain. Mean and standard error are indicated. Significance was determined by a two-tailed *t*-test. (*E*). Model showing rRNA processing steps including the new finding that Dxo1 processes the 25S′ to the 25S rRNA.

have been described for yeast 3′ exonucleases, including those involved in 5.8S rRNA 3′ end formation (van Hoof et al. 2000; Thomson and Tollervey 2010). We speculate that both Dxo1 and Rat1 are required for mature 25S formation due to the processivity/affinity of Rat1/Xrn1 enzymes (Jinek et al. 2011; Nagarajan et al. 2013). Processivity requires that the enzyme remain bound to the substrate between subsequent rounds of catalysis. An X-ray structure of Xrn1 bound to an RNA substrate indicates that the enzyme binds three nucleotides at a time, and in vitro assays show that Xrn1 activity requires a three-nucleotide single stranded overhang (Jinek et al. 2011; Nagarajan et al. 2013). While a short single stranded 5′ overhang is critical for processive 5′ nucleases, the mature 5′ end of 25S rRNA forms a double stranded structure with 5.8S rRNA. This effectively prevents final 5′ end maturation by Xrn1/Rat1 and necessitates hand-off to a distributive enzyme. Consistent with our findings of hand-off, in vitro studies of Rat1 have shown that it can process preribosomal subunits to 25S′ but not to the mature 25S rRNA (Gasse et al. 2015; Fromm et al. 2017). Because the processivity of Xrn1/Rat1 is highly conserved, we hypothesize that this cooperative processing of 25S rRNA may also be conserved. Indeed, human 28S rRNA processing produces an intermediate analogous to 25S′ (Mullineux and Lafontaine 2012).

As previously mentioned, Dxo1 is primarily cytoplasmic (Huh et al. 2003). Because 25S 5′ end maturation appears to be a major function of Dxo1, this implies that the final maturation of 25S rRNA occurs in the cytoplasm. While the initial steps of rRNA maturation are carried out (cotranscriptionally) in the nucleolus, the final steps in 5.8S and 18S rRNA maturation are cytoplasmic as well (Fatica et al. 2003, 2004; Thomson and Tollervey 2010). Furthermore, the 25S′ intermediate coimmunoprecipitates with Arx1, a factor that is coexported with the ribosomal subunit (Thomson and Tollervey 2010), which is consistent with cytoplasmic final maturation of 25S rRNA.

dxo1Δ suppresses the *xrn1Δ* growth defect

Because Xrn1 and Dxo1 both act as cytoplasmic 5′ exoribonucleases, we were curious whether the double mutant had a more severe growth defect than either single mutant. As previously reported (Larimer and Stevens 1990; Tishkoff et al. 1991), *xrn1Δ* causes a growth defect, while *dxo1Δ* does not have a notable effect on growth. However, contrary to our expectation, the *dxo1Δxrn1Δ* strain had improved growth compared to the single *xrn1Δ* (Fig. 4A). The growth of the *xrn1Δdxo1Δ* strain is not restored to wild-type levels but seems to present an intermediate phenotype. To confirm this observation, we introduced either a *DXO1* plasmid or empty vector control plasmid into the *dxo1Δxrn1Δ* and *xrn1Δ* strains. We then quantified the doubling time of these strains to more precisely assess their growth. Figure 4B shows that deleting *DXO1* from the *xrn1Δ* strain decreased the doubling time ~20% ($P = 0.01$), and reintroducing *DXO1* to *dxo1Δxrn1Δ* returned the doubling time to the level of

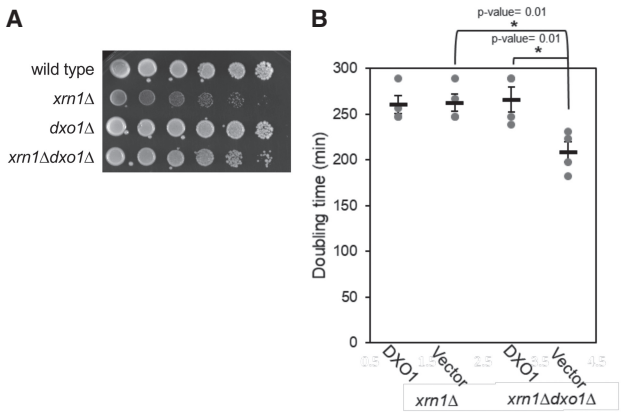

FIGURE 4. Deletion of Dxo1 partially restores the slow growth of *xrn1Δ*. (*A*) The indicated yeast strains were serially diluted for the growth assay and plated on YPD at 30°C for 3 d. (*B*) Doubling time of the indicated strains was determined in biological triplicates. Mean and standard error are indicated and significance was calculated using a two-tailed *t*-test.

xrn1Δ ($P = 0.01$), confirming that *dxo1Δ* suppresses the *xrn1Δ* growth defect.

It has long been unclear why *xrn1Δ* mutants grow slowly. Canonically, the main function of Xrn1 is to degrade largely untranslated mRNAs that are decapped and deadenylated (Johnson 1997; Nagarajan et al. 2013). It is not clear why the accumulation of these seemingly inert mRNAs would negatively affect cell growth. A novel hypothesis is that Dxo1 converts some unknown Xrn1 substrate into a toxic RNA, but in the absence of data we can only speculate on the identify of that toxic RNA. An alternative possibility is that *dxo1Δ* somehow affects the activity of the cytoplasmic RNA exosome. In the absence of Xrn1, the RNA exosome degrades mRNAs and thus becomes essential (Johnson and Kolodner 1995). Thus, if *dxo1Δ* increases cytoplasmic exosome activity or the accessibility of RNAs to the exosome, this would also explain the improved growth of *dxo1Δxrn1Δ*.

Conclusions

While the biochemical activity of KlDxo1 has been carefully characterized (Chang et al. 2012), neither the biochemical activity nor in vivo functions of ScDxo1 were well studied. Here we show that Dxo1 is a conserved enzyme that is related to, but distinct from, Rai1 and that it arose in a common ancestor of the Saccharomycetaceae and Saccharomycodaceae. This duplication may have allowed specialization, with Rai1 better suited to remove various aberrant caps in the nucleus and Dxo1 better suited for distributive exonuclease activity in the cytoplasm. In species that do not have a duplicated Rai1/Dxo1, one enzyme may have to carry out both decapping and exonuclease activity. Optimal decapping and exonuclease activity require mutually exclusive sequence motifs (Fig. 1; Chang et al. 2012), so such a bifunctional decapping and exonuclease enzyme is likely suboptimal for both. We expect that similar duplication may have occurred in other eukaryotes, but we have not extensively tested that possibility. We did notice that a subset of *Candida* species independently duplicated this gene, with orf19.8253 more closely resembling Dxo1 and orf19.13690 more closely resembling Rai1. More extremely, the *C. elegans* genome includes as many as nine Rai1/Dxo1/DXO genes.

In this study, we identify in vivo Dxo1 products by PARE and show that in the absence of Xrn1, Dxo1 can act as transcriptome-wide distributive 5′ exonuclease. However, in the presence of Xrn1, Dxo1 appears to be specifically required to convert 25S′ pre-rRNA to the mature 25S rRNA, a known step for which the enzyme had not been conclusively identified. Our results, combined with previous work (Thomson and Tollervey 2010), imply that the large ribosomal subunit is exported while containing 6S and 25S′ rRNA intermediates that are subsequently matured in the cytoplasm. While several examples of hand-off from one 3′ exoribonuclease to another have been reported (Allmang et al. 1999; van Hoof et al. 2000; Tucker et al. 2001; Faber et al. 2002; Zuo and Deutscher 2002), we expand this concept to 5′ exoribonucleases. We further speculate that distributive exonucleases may be needed to generate precise structured ends on a variety of ncRNAs.

Three types of reactions have now been ascribed to Dxo1: 25S rRNA processing, removal of aberrant cap structures, and distributive transcriptome wide 5′ exonuclease activity. Because aberrant caps are thought to be added in the same positions as the more abundant canonical caps, the PARE reads derived from aberrant decapping likely only form a minor fraction of the reads mapped to mRNA start sites and thus the aberrant decapping activity cannot be distinguished by PARE. These three functions raise the question: Which is the primary function of Dxo1? Dxo1 removes 7 nt from 25S′ for each of the 2000 ribosomes synthesized per minute in a growing yeast cell, which amounts to 14,000 catalytic cycles per minute per cell (Warner 1999). A rapidly growing yeast cell also produces approximately 1000 mRNAs per minute (Pelechano et al. 2010). Most of these receive a canonical cap, but an unknown fraction receives an aberrant cap. It appears unlikely that as much as 10% of the caps are aberrant, but even with this high estimate, the 100 aberrant decapping events per minute would be more than two orders of magnitude lower than the 14,000 catalytic cycles to form the mature 25S rRNA. Finally, while in the absence of Xrn1, a 5′ exonucleolytic role in mRNA decay becomes detectable, this role is limited in the presence of Xrn1 because *xrn1Δ* is known to stabilize most mRNAs. Thus we predict this mRNA decay role of Dxo1 is also minor when compared to 25S rRNA processing. Overall, we conclude that Dxo1 has several functions with its primary role being 25S rRNA processing.

MATERIALS AND METHODS

Sequence analysis

Homologs of ScDxo1 and ScRai1 were identified using BLAST at https://blast.ncbi.nlm.nih.gov/ and at https://yeastgenome.org. This was supplemented by sequences retrieved from the yeast gene order browser (http://ygob.ucd.ie/). This identified two homologs in most species of Saccharomycetaceae, including *S. cerevisiae*, *S. mikatae*, *S. kudriavzevii*, *S. uvarum*, *Candida glabrata*, *Naumovozyma castellii*, *N. dairenensis*, *Tetrapisispora blattae*, *T. phaffii*, *Vanderwaltozyma polyspora*, *Zygosaccharomyces rouxii*, *Torulaspora delbrueckii*, *Kluyveromyces lactis*, and *Lachancea kluyveri*. Exceptions include that *Eremothecium cymbalariae* has only one gene due to loss of *DXO1* and *Lachancea thermotolerans* has three genes due to an additional duplication of *DXO1*. Similarly, two homologs were identified in the Saccharomycodaceae *Hanseniaspora osmophila*. In contrast, we could only find one gene in the Phaffomycetaceae *Cyberlindnera jadinii*, *Komagataella phaffii*, and *Wickerhamomyces ciferrii*, the Dipodascaceae *Yarrowia lipolytica*, the Lipomycetaceae *Lipomyces starkeyi* and the Pichiaceae *Ogataea polymorpha*. Some species within the CUG clade also had one gene (e.g., *Candida auris, Clavispora lusitaniae*, and *Metschnikowia bicuspidata*), while others had two (e.g., *Candida albicans, C. dubliniensis*, and *C. parapsilosis*), suggesting an independent duplication within this clade. The retrieved sequences were then aligned, together with the biochemically and structurally characterized human DXO and *S. pombe* Rai1 proteins, with CLUSTAL omega (http://www.clustal.org/omega/).

Strains and plasmids

The wild type (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), *xrn1Δ::NEO* (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xrn1Δ::NEO*) and *dxo1Δ::NEO* (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dxo1Δ::NEO*) were obtained from the yeast knock out collection (Giaever and Nislow 2014). The *xrn1Δ::NEO* strain was converted to *xrn1Δ::HYG* (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xrn1Δ::HYG*) by transformation with pAG32 (Goldstein and McCusker 1999). The *xrn1Δ::HYG* was then crossed with *dxo1Δ::NEO* to obtain the double mutant (*MATa his3Δ1 leu2Δ0 met15Δ0 lys2Δ0 ura3Δ0 xrn1Δ::HYG dxo1Δ::NEO*).

DXO1 was PCR amplified from genomic DNA using oligos TACCGGGCCCCCCCTCGAGGAGAGGTTGTTAGTACCAAC and AGGGAACAAAAGCTGGAGCTCCTATACTATAAGTTTTGAAGG CCTGAAGACAACATGATATAA. pRS426 (Sikorski and Hieter 1989) was digested with SacI-HF and SalI-HF, and the amplified *DXO1* was inserted using Gibson assembly (NEBuilder; New England BioLabs) and confirmed by sequencing. Yeast strains *xrn1Δ::HYG* and *xrn1Δ::HYG dxo1Δ::NEO* were transformed as previously described (Gietz and Schiestl 2007) with either empty pRS426 or DXO1–pRS426. The transformed strains were then selected for on SC-URA plates.

RNA extraction, PARE, and northern blot

Yeast strains (wild type, *xrn1Δ::HYG*, *dxo1Δ::NEO* and *xrn1Δ::HYG dxo1Δ::NEO*) were grown overnight in YPD at 30°C and diluted the next day to an OD of 0.3. Yeast were then grown to mid-log phase at 30°C in YPD, and a hot phenol RNA extraction was performed as previously described (He et al. 2008). RNA was precipitated with isopropanol at room temperature and resuspended in 100 µL of RNase free water. PARE was performed by LCSciences as previously described (Hurtig et al. 2021). The sequencing data have been deposited in SRA with the ID PRJNA752382 and analyzed as described (Hurtig et al. 2021).

For northern blotting, 10 µg RNA samples were dissolved in NorthernMax Formaldehyde load dye (Ambion) and run on a 1.3% agarose formaldehyde gel with 1× MOPS running buffer. RNA was transferred to a Zetaprobe membrane, UV crosslinked, and probed with 5′ P^{32} end labeled oligos designed to anneal to the 25S′ rRNA (TTTGAGGTCAAACTTTAA) and *SCR1* RNA (GTCTAGCCGCGAGGAAGG). Blots were imaged with a Typhoon imager (GE Healthcare), and the signals were quantified using ImageQuant software.

Growth assays

For growth assays on plates, yeast strains (wild type, *xrn1Δ::HYG, dxo1Δ::NEO* and *xrn1Δ::HYG dxo1Δ::NEO*) were grown overnight in YPD and diluted to an OD of 0.2 the next day. The cells were then grown to an OD ~ 0.6, spun down, and resuspended in water. The yeast were then serially diluted and spotted onto YPD. Plates were incubated for 3 d at 30°C before imaging.

For growth assays in liquid cultures, yeast strains (*xrn1Δ::HYG* and *xrn1Δ::HYG dxo1Δ::NEO* transformed with either *DXO1* plasmid or empty vector) were grown in SC-URA liquid overnight, diluted to an OD of 0.2 the next day, and then grown until they doubled twice. The strains were again diluted to an OD of 0.2 in a 96-well plate with YPD. A plate reader was used to measure the absorbance change over 16 h of growth at 30°C, and doubling times were determined for the exponential growth phase.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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REFERENCES

Addo-Quaye C, Eshoo TW, Bartel DP, Axtell MJ. 2008. Endogenous siRNA and miRNA targets identified by sequencing of the *Arabidopsis* degradome. *Curr Biol* **18:** 758–762. doi:10.1016/j.cub.2008.04.042

Allmang C, Petfalski E, Podtelejnikov A, Mann M, Tollervey D, Mitchell P. 1999. The yeast exosome and human PM–Scl are related complexes of 3′→5′ exonucleases. *Genes Dev* **13:** 2148–2158. doi:10.1101/gad.13.16.2148

Arribere JA, Gilbert WV. 2013. Roles for transcript leaders in translation and mRNA decay revealed by transcript leader sequencing. *Genome Res* **23:** 977–987. doi:10.1101/gr.150342.112

Briggs MW, Burkard KT, Butler JS. 1998. Rrp6p, the yeast homologue of the human PM-Scl 100-kDa autoantigen, is essential for efficient 5.8 S rRNA 3′ end formation. *J Biol Chem* **273:** 13255–13263. doi:10.1074/jbc.273.21.13255

Calvin K, Li H. 2008. RNA-splicing endonuclease structure and function. *Cell Mol Life Sci* **65:** 1176–1185. doi:10.1007/s00018-008-7393-y

Chang JH, Jiao X, Chiba K, Oh C, Martin CE, Kiledjian M, Tong L. 2012. Dxo1 is a novel eukaryotic enzyme with both decapping and 5′-3′ exoribonuclease activity. *Nat Struct Mol Biol* **19:** 1011–1017. doi:10.1038/nsmb.2381

Cherry PD, Peach SE, Hesselberth JR. 2019. Multiple decay events target HAC1 mRNA during splicing to regulate the unfolded protein response. *Elife* **8:** e42262. doi:10.7554/eLife.42262

Dhoondia Z, Elewa H, Malik M, Arif Z, Pique-Regi R, Ansari A. 2021. A termination-independent role of Rat1 in cotranscriptional splicing. *Nucleic Acids Res* **49:** 5520–5536. doi:10.1093/nar/gkab339

Doamekpor SK, Gozdek A, Kwasnik A, Kufel J, Tong L. 2020a. A novel 5′-hydroxyl dinucleotide hydrolase activity for the DXO/Rai1 family of enzymes. *Nucleic Acids Res* **48:** 349–358. doi:10.1093/nar/gkz1107

Doamekpor SK, Grudzien-Nogalska E, Mlynarska-Cieslak A, Kowalska J, Kiledjian M, Tong L. 2020b. DXO/Rai1 enzymes remove 5′-end FAD and dephospho-CoA caps on RNAs. *Nucleic Acids Res* **48:** 6136–6148. doi:10.1093/nar/gkaa297

Faber AW, Van Dijk M, Raué HA, Vos JC. 2002. Ngl2p is a Ccr4p-like RNA nuclease essential for the final step in 3′-end processing of 5.8S rRNA in *Saccharomyces cerevisiae*. *RNA* **8:** 1095–1101. doi:10.1017/S1355838202021027

Fatica A, Oeffinger M, Dlakić M, Tollervey D. 2003. Nob1p is required for cleavage of the 3′ end of 18S rRNA. *Mol Cell Biol* **23:** 1798–1807. doi:10.1128/MCB.23.5.1798-1807.2003

Fatica A, Tollervey D, Dlakić M. 2004. PIN domain of Nob1p is required for D-site cleavage in 20S pre-rRNA. *RNA* **10:** 1698–1701. doi:10.1261/rna.7123504

Fromm L, Falk S, Flemming D, Schuller JM, Thoms M, Conti E, Hurt E. 2017. Reconstitution of the complete pathway of ITS2 processing at the pre-ribosome. *Nat Commun* **8:** 1787. doi:10.1038/s41467-017-01786-9

Gasse L, Flemming D, Hurt E. 2015. Coordinated ribosomal ITS2 RNA processing by the Las1 complex integrating endonuclease, polynucleotide kinase, and exonuclease activities. *Mol Cell* **60:** 808–815. doi:10.1016/j.molcel.2015.10.021

Geerlings TH, Vos JC, Raué HA. 2000. The final step in the formation of 25S rRNA in *Saccharomyces cerevisiae* is performed by 5′→3′ exonucleases. *RNA* **6:** 1698–1703. doi:10.1017/S1355838200001540

German MA, Pillay M, Jeong D-H, Hetawal A, Luo S, Janardhanan P, Kannan V, Rymarquis LA, Nobuta K, German R, et al. 2008. Global identification of microRNA-target RNA pairs by parallel analysis of RNA ends. *Nat Biotechnol* **26:** 941–946. doi:10.1038/nbt1417

Giaever G, Nislow C. 2014. The yeast deletion collection: a decade of functional genomics. *Genetics* **197:** 451–465. doi:10.1534/genetics.114.161620

Gietz RD, Schiestl RH. 2007. High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nat Protoc* **2:** 31–34. doi:10.1038/nprot.2007.13

Goldstein AL, McCusker JH. 1999. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* **15:** 1541–1553. doi:10.1002/(SICI)1097-0061(199910)15:14<1541::AID-YEA476>3.0.CO;2-K

Harigaya Y, Parker R. 2012. Global analysis of mRNA decay intermediates in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci* **109:** 11764–11769. doi:10.1073/pnas.1119741109

He F, Amrani N, Johansson MJO, Jacobson A. 2008. Chapter 6. Qualitative and quantitative assessment of the activity of the yeast nonsense-mediated mRNA decay pathway. *Methods Enzymol* **449:** 127–147. doi:10.1016/S0076-6879(08)02406-3

Henras A, Plisson-Chastang C, O'Donohue M-F, Chakraborty A, Gleizes PE. 2014. An overview of pre-ribosomal RNA processing in eukaryotes. *Wiley Interdiscip Rev RNA* **6:** 225–242. doi:10.1002/wrna.1269

Huh W-K, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK. 2003. Global analysis of protein localization in budding yeast. *Nature* **425:** 686–691. doi:10.1038/nature02026

Hurtig JE, Steiger MA, Nagarajan VK, Li T, Chao T-C, Tsai K-L, van Hoof A. 2021. Comparative parallel analysis of RNA ends identifies mRNA substrates of a tRNA splicing endonuclease-initiated mRNA decay pathway. *Proc Natl Acad Sci* **118:** e2020429118. doi:10.1073/pnas.2020429118

Januszyk K, Lima CD. 2014. The eukaryotic RNA exosome. *Curr Opin Struct Biol* **24:** 132–140. doi:10.1016/j.sbi.2014.01.011

Jiao X, Xiang S, Oh C, Martin CE, Tong L, Kiledjian M. 2010. Identification of a quality-control mechanism for mRNA 5′-end capping. *Nature* **467:** 608–611. doi:10.1038/nature09338

Jiao X, Chang JH, Kilic T, Tong L, Kiledjian M. 2013. A mammalian pre-mRNA 5′ end capping quality control mechanism and an unexpected link of capping to pre-mRNA processing. *Mol Cell* **50:** 104–115. doi:10.1016/j.molcel.2013.02.017

Jinek M, Coyle SM, Doudna JA. 2011. Coupled 5′ nucleotide recognition and processivity in Xrn1-mediated mRNA decay. *Mol Cell* **41:** 600–608. doi:10.1016/j.molcel.2011.02.004

Johnson AW. 1997. Rat1p and Xrn1p are functionally interchangeable exoribonucleases that are restricted to and required in the nucleus and cytoplasm, respectively. *Mol Cell Biol* **17:** 6122–6130. doi:10.1128/MCB.17.10.6122

Johnson AW, Kolodner RD. 1995. Synthetic lethality of *sep1* (*xrn1*) *ski2* and *sep1* (*xrn1*) *ski3* mutants of *Saccharomyces cerevisiae* is independent of killer virus and suggests a general role for these genes in translation control. *Mol Cell Biol* **15:** 2719–2727. doi:10.1128/MCB.15.5.2719

Larimer FW, Stevens A. 1990. Disruption of the gene *XRN1*, coding for a 5′→3′ exoribonuclease, restricts yeast cell growth. *Gene* **95:** 85–90. doi:10.1016/0378-1119(90)90417-P

Lynch ER. 2019. *The Dxo decapping exonuclease is a restriction factor for RNA viruses*. Master of Science Thesis. Colorado State University.

Morton DJ, Kuiper EG, Jones SK, Leung SW, Corbett AH, Fasken MB. 2018. The RNA exosome and RNA exosome-linked disease. *RNA* **24:** 127–142. doi:10.1261/rna.064626.117

Mullineux S-T, Lafontaine DLJ. 2012. Mapping the cleavage sites on mammalian pre-rRNAs: where do we stand? *Biochimie* **94:** 1521–1532. doi:10.1016/j.biochi.2012.02.001

Nagarajan VK, Jones CI, Newbury SF, Green PJ. 2013. XRN 5′→3′ exoribonucleases: structure, mechanisms and functions. *Biochim Biophys Acta* **1829:** 590–603. doi:10.1016/j.bbagrm.2013.03.005

Navickas A, Chamois S, Saint-Fort R, Henri J, Torchet C, Benard L. 2020. No-go decay mRNA cleavage in the ribosome exit tunnel produces 5′-OH ends phosphorylated by Trl1. *Nat Commun* **11:** 1–11. doi:10.1038/s41467-019-13991-9

Oeffinger M, Zenklusen D, Ferguson A, Wei KE, El Hage A, Tollervey D, Chait BT, Singer RH, Rout MP. 2009. Rrp17p is a eukaryotic exonuclease required for 5′ end processing of pre-60S ribosomal RNA. *Mol Cell* **36:** 768–781. doi:10.1016/j.molcel.2009.11.011

Peach SE, York K, Hesselberth JR. 2015. Global analysis of RNA cleavage by 5′-hydroxyl RNA sequencing. *Nucleic Acids Res* **43:** e108. doi:10.1093/nar/gkv536

Pelechano V, Chávez S, Pérez-Ortín JE. 2010. A complete set of nascent transcription rates for yeast genes. *PLoS One* **5:** e15442. doi:10.1371/journal.pone.0015442

Picard-Jean F, Brand C, Tremblay-Létourneau M, Allaire A, Beaudoin MC, Boudreault S, Duval C, Rainville-Sirois J, Robert F,

Pelletier J, et al. 2018. 2′-*O*-methylation of the mRNA cap protects RNAs from decapping and degradation by DXO. *PLoS One* **13:** e0193804. doi:10.1371/journal.pone.0193804

Roy KR, Chanfreau GF. 2020. Robust mapping of polyadenylated and non-polyadenylated RNA 3′ ends at nucleotide resolution by 3′-end sequencing. *Methods* **176:** 4–13. doi:10.1016/j.ymeth.2019.05.016

Schillewaert S, Wacheul L, Lhomme F, Lafontaine DLJ. 2012. The evolutionarily conserved protein LAS1 is required for pre-rRNA processing at both ends of ITS2. *Mol Cell Biol* **32:** 430–444. doi:10.1128/MCB.06019-11

Sikorski RS, Hieter P. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122:** 19–27. doi:10.1093/genetics/122.1.19

Thomson E, Tollervey D. 2010. The final step in 5.8S rRNA processing is cytoplasmic in *Saccharomyces cerevisiae*. *Mol Cell Biol* **30:** 976–984. doi:10.1128/MCB.01359-09

Tishkoff DX, Johnson AW, Kolodner RD. 1991. Molecular and genetic analysis of the gene encoding the *Saccharomyces cerevisiae* strand exchange protein Sep1. *Mol Cell Biol* **11:** 2593–2608. doi:10.1128/mcb.11.5.2593-2608.1991

Tomecki R, Sikorski PJ, Zakrzewska-Placzek M. 2017. Comparison of preribosomal RNA processing pathways in yeast, plant and human cells: focus on coordinated action of endo- and exoribonucleases. *FEBS Lett* **591:** 1801–1850. doi:10.1002/1873-3468.12682

Tsuboi T, Yamazaki R, Nobuta R, Ikeuchi K, Makino S, Ohtaki A, Suzuki Y, Yoshihisa T, Trotta C, Inada T. 2015. The tRNA splicing endonuclease complex cleaves the mitochondria-localized CBP1 mRNA. *J Biol Chem* **290:** 16021–16030. doi:10.1074/jbc.M114.634592

Tucker M, Valencia-Sanchez MA, Staples RR, Chen J, Denis CL, Parker R. 2001. The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in *Saccharomyces cerevisiae*. *Cell* **104:** 377–386. doi:10.1016/S0092-8674(01)00225-2

van Dijk T, Baas F, Barth PG, Poll-The BT. 2018. What's new in pontocerebellar hypoplasia? An update on genes and subtypes. *Orphanet J Rare Dis* **13:** 92. doi:10.1186/s13023-018-0826-2

van Hoof A, Lennertz P, Parker R. 2000. Three conserved members of the RNase D family have unique and overlapping functions in the processing of 5S, 5.8S, U4, U5, RNase MRP and RNase P RNAs in yeast. *EMBO J* **19:** 1357–1365. doi:10.1093/emboj/19.6.1357

Volanakis A, Passoni M, Hector RD, Shah S, Kilchert C, Granneman S, Vasiljeva L. 2013. Spliceosome-mediated decay (SMD) regulates expression of nonintronic genes in budding yeast. *Genes Dev* **27:** 2025–2038. doi:10.1101/gad.221960.113

Wang VY-F, Jiao X, Kiledjian M, Tong L. 2015. Structural and biochemical studies of the distinct activity profiles of Rai1 enzymes. *Nucleic Acids Res* **43:** 6596–6606. doi:10.1093/nar/gkv620

Warner JR. 1999. The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci* **24:** 437–440. doi:10.1016/S0968-0004(99)01460-7

Wasmuth EV, Lima CD. 2012. Structure and activities of the eukaryotic RNA exosome. *Enzymes* **31:** 53–75. doi:10.1016/B978-0-12-404740-2.00003-3

Weskamp K, Barmada SJ. 2018. RNA degradation in neurodegenerative disease. *Adv Neurobiol* **20:** 103–142. doi:10.1007/978-3-319-89689-2_5

Wolin SL, Maquat LE. 2019. Cellular RNA surveillance in health and disease. *Science* **366:** 822–827. doi:10.1126/science.aax2957

Woolford JL, Baserga SJ. 2013. Ribosome biogenesis in the yeast *Saccharomyces cerevisiae*. *Genetics* **195:** 643–681. doi:10.1534/genetics.113.153197

Xiang S, Cooper-Morgan A, Jiao X, Kiledjian M, Manley JL, Tong L. 2009. Structure and function of the 5′→3′ exoribonuclease Rat1 and its activating partner Rai1. *Nature* **458:** 784–788. doi:10.1038/nature07731

Xue Y, Bai X, Lee I, Kallstrom G, Ho J, Brown J, Stevens A, Johnson AW. 2000. *Saccharomyces cerevisiae RAI1* (YGL246c) is homologous to human *DOM3Z* and encodes a protein that binds the nuclear exoribonuclease Rat1p. *Mol Cell Biol* **20:** 4006–4015. doi:10.1128/MCB.20.11.4006-4015.2000

Zhang Y, Kuster D, Schmidt T, Kirrmaier D, Nübel G, Ibberson D, Benes V, Hombauer H, Knop M, Jäschke A. 2020. Extensive 5′-surveillance guards against non-canonical NAD-caps of nuclear mRNAs in yeast. *Nat Commun* **11:** 5508. doi:10.1038/s41467-020-19326-3

Zheng D, Chen C-YA, Shyu A-B. 2011. Unraveling regulation and new components of human P-bodies through a protein interaction framework and experimental validation. *RNA* **17:** 1619–1634. doi:10.1261/rna.2789611

Zuo Y, Deutscher MP. 2002. The physiological role of RNase T can be explained by its unusual substrate specificity. *J Biol Chem* **277:** 29654–29661. doi:10.1074/jbc.M204252200

MEET THE FIRST AUTHOR

Jennifer Hurtig

***Meet the First Author(s)* is a new editorial feature within *RNA*, in which the first author(s) of research-based papers in each issue have the opportunity to introduce themselves and their work to readers of *RNA* and the RNA research community. Jennifer Hurtig is the first author of this paper, "Yeast Dxo1 is required for 25S rRNA maturation and acts as a transcriptome-wide distributive exonuclease." Jennifer is a PhD candidate in the laboratory of Ambro van Hoof, PhD at The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences in the Microbiology and Infectious Disease program. Her research focuses on the targets of the tRNA splicing endonuclease (TSEN) and how these cleavage products are further degraded.**

Continued

What are the major results described in your paper and how do they impact this branch of the field?

The main findings of this paper define unexpected functions of Dxo1 in *S. cerevisiae*. We found Dxo1 acts as a distributive 5′ exoribonuclease that can "nibble" at RNA before processive degradation by Xrn1. This finding is especially helpful in our field for defining exact endonuclease sites. Our sequencing data of RNAs with 5′ monophosphate ends had several clustered peaks that could be mistaken for different endonuclease cleavage or decapping sites, but this paper now shows that deletion of Dxo1 can eliminate these shadow peaks and establish precise endonuclease cleavage sites. We also found Dxo1 processes the 25S′ rRNA to the 25S. The enzyme catalyzing this step has been missing from our knowledge of rRNA processing and in addition provides evidence that the 25S′ rRNA is exported to the cytoplasm before final maturation.

What led you to study RNA or this aspect of RNA science?

My interest in RNA began with my fascination of the central dogma. The diversity of life around us is dictated by the same four DNA bases which I still find beautiful and humbling. However, the further I studied biology the more it became apparent that RNA, which my high school textbooks always glossed over for simplicity, is another driving factor behind the complexity and diversity of life. Not only does mRNA encode proteins, but RNA can act in so many other ways which we are constantly discovering and refining. The sheer expanse and variety of RNA biology means there is always something new to learn and discover.

During the course of these experiments, were there any surprising results or particular difficulties that altered your thinking and subsequent focus?

We were looking for an enzyme that could convert the 5′ hydroxyl products of the tRNA splicing endonuclease into 5′ monophosphate Xrn1 substrates. The Dxo1/Rai1/DXO family of enzymes had been shown to be able to do this in vitro, so we tested Dxo1 as a candidate enzyme in yeast. The finding that its main function is to mature 25S′ into 25S rRNA was completely unexpected but was immediately obvious from the sequencing results.

What are some of the landmark moments that provoked your interest in science or your development as a scientist?

My interest in science grew when I was a college student. I participated in a summer research program in my current department and decided to come back for graduate school because of the people I met. The students were happy and excited about their research and the faculty were eager to help students succeed, and these qualities continue to allow the department to thrive.

What are your subsequent near- or long-term career plans?

I plan to finish my PhD in about a year's time. I would ultimately like to work as a scientist in industry identifying druggable targets of diseases and other preclinical work. I plan to leave Houston and pursue a biotech position or industry postdoc in Boston. I miss seasons and am not convinced that hot and hotter count as such despite the arguments of native Houstonians.