Human RNA lariat debranching enzyme protein 1 – A surveillant for branch RNAs for degradation

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Received: August 13, 2015
Published online: September 02, 2015

Splicing is a process to remove introns from precursor of mRNAs (pre-mRNAs). Introns are excised as a lariat form, and they should be debranched before degradation. Debranching reaction is conferred by a RNA lariat debranching enzyme 1 (Dbr1) protein. The Dbr1 protein is evolutionarily conserved among many species and shares GNHE motif for debranching activity that is identical to protein phosphatase activity center. The human Dbr1 protein has a bipartite type nuclear localization signal, and it shuttles between the nucleus and the cytoplasm, which suggests novel function(s) in the cytoplasm. The human Dbr1 protein interacts with two proteins, Xab2 and hDrn1. Since Xab2 is involved in both splicing and transcription-coupled DNA repair (TCR), hDbr1 may also have a role in TCR. Although the function of hDrn1 is not known yet, this protein specifically interact with carboxy terminal of hDbr1 and it is also a nucleo-cytoplasmic shuttling protein. A heterodimer of hDbr1-hDrn1 may have role(s) not only in the nucleus but also in the cytoplasm of human cells.

Keywords: Splicing; debranching; Dbr1; lariat intron; nucleo-cytoplasmic shuttling

To cite this article: Naoyuki Kataoka. Human RNA lariat debranching enzyme protein 1 – A surveillant for branch RNAs for degradation. RNA Dis 2015; 2: e963. doi: 10.14800/rd.963.
well conserved among many species and this protein shares a protein phosphatase catalytic motif, GNHE motif, as a catalytic center (Figure 1A)\(^{[13,14]}\). Dbr1 gene is not essential in *Saccharomyces cerevisiae* for cell viability, although Dbr1 mutant exhibits accumulation of lariat RNAs\(^{[17]}\). However, in fission yeast, *Schizosaccharomyces pombe*, Dbr1 null mutant cells have severe growth defect phenotype with elongated cell shape in addition to the accumulation of RNA lariat. Interestingly, Dbr1 is also unessential for its viability in fission yeast despite of those phenotypes\(^{[16]}\). In a plant, *Arabidopsis thaliana*, null mutant is embryonic lethal\(^{[19]}\) and knockout of human Dbr1 gene by CRISPR system revealed human Dbr1 protein is essential for cell survival\(^{[20]}\). It is highly likely that rapid and correct intron turnover processes including RNA lariat debranching reaction is critical for higher eukaryotes. In addition to a catalytic GNHE motif,
human Dbr1 (hDbr1) protein has a bipartite type nuclear localization signal (NLS) (Figure 1A) and it is a nucleo-cytoplasmic protein (Figure 1B), while the steady state subcellular localization of hDbr1 is in the nucleoplasm. This finding strongly suggests that hDbr1 has role(s) in both the nucleus and in the cytoplasm.

One possible function of hDbr1 in the cytoplasm is to debranch introns in the cytoplasm. It was reported that several introns in certain genes could be detected in the cytoplasm of the cells, too [21-23], and it was also demonstrated that the splice-defective lariat intron with 3’ exon was debranched in the cytoplasm by yeast Dbr1p and degraded by cytoplasmic exosome and the cytoplasmic 5’ to 3’ exonuclease Xrn1p in budding yeast [24]. Furthermore, genome-wide screenings for loss of TDP-43 toxicity in Amyotrophic lateral sclerosis (ALS) using yeast cells identified deletion of Dbr1 as the strongest suppressor activity [25]. When Dbr1 activity is reduced, lariat introns accumulate in the cytoplasm and it is likely they serve as decoys for TDP-43 to prevent aggregates formation with RNAs and other essential RNA binding proteins [25]. These results imply that the lariat intron RNAs can be exported to the cytoplasm possibly by unknown mechanism and hDbr1 linearizes them in the cytoplasm (Figure 1B). Continuous nucleo-cytoplasmic shuttling of hDbr1 may contribute to survey the lariat-introns and to rapidly debranch them to degrade in the cytoplasm. Other than lariat RNAs, a subset of circular RNAs (circRNAs) was recently identified as a large class of regulatory RNAs, and subcellular localization of some circRNAs are in the cytoplasm [26-30]. Since it was demonstrated that circRNA biogenesis requires both canonical splice site sequences and their flanking intronic sequences, hDbr1 may participate in circRNA degradation pathway.

Another possibility is Dbr1 protein has a role in the cDNA synthesis by reverse transcription. Originally, genetic screening to identify host factors essential for transposition of Ty1 retrotransposon was carried out with a budding yeast screening to identify host factors essential for transposition synthesis by reverse transcription. Originally, genetic pathway.

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Another possibility is Dbr1 protein has a role in the cDNA synthesis by reverse transcription. Originally, genetic screening to identify host factors essential for transposition of Ty1 retrotransposon was carried out with a budding yeast and the Dbr1 gene was isolated [17]. Since low level of Ty1 cDNA was found in the Dbr1 mutant strains, it was suggested that Dbr1p participates in Ty1 mRNA reverse transcription [31-33]. It was also demonstrated that knockdown of hDbr1 by siRNA inhibited Human Immunodeficiency Virus-1 (HIV-1) replication by reducing both viral cDNA and protein production [34, 35]. These papers suggested that debranching activity of hDbr1 was essential for reverse transcription reaction. Most recently it was demonstrated that HIV-1 reverse transcription starts in the cytoplasm but completion of this step occurs in or on the surface of the nucleus. This ‘nuclear-associated’ reverse transcription depends on the human Dbr1 activity, while cytoplasmic reaction is independent of this enzyme.

Two protein factors were demonstrated to specifically interact with hDbr1. Our group found that xeroderma pigmentosum, complementation group A (XPA)-binding protein 2 (Xab2) can interact with hDbr1 both in vivo and in vitro [36]. Xab2 is a splicing factor and it is also a protein component of Intron Large (IL) complex, an intermediate complex for intron degradation pathway in human [37]. This protein was originally identified as a specific interactor of XPA protein, which is involved in Transcription coupled DNA repair (TCR) [38-40]. One interesting explanation of Xab2-hDbr1 interaction is that Xab2 inhibits the access of hDbr1 to the lariat-introns in IL complex to prevent from debranching. Another interesting possibility of this interaction is that hDbr1 is involved in TCR. Xab2 forms a ‘nineteen complex’ with hPrp19 and other nineteen complex proteins to take part in splicing reaction [41-43]. The hPrp19 protein is also a TCR factor, and we have detected co-precipitation of hDbr1 and hPrp19 with Xab2 (unpublished observations). The hDbr1 protein may be involved in TCR in addition to intron turnover. It would be interesting to test whether Dbr1 knockout yeast cells and hDbr1 knockdown HeLa cells show defects in TCR or not.

The other specific interactor to Dbr1 protein was recently reported with yeast and human. This protein is debranching enzyme-associated ribonuclease 1 (Drn1), a homolog of RNA lariat debranching enzyme 1 in Saccharomyces cerevisiae [44]. Drn1 is a nuclear protein and it was shown that hDrn1 associate with the splicing products that contain lariat intron and modulate Dbr1 mediated lariat intron turnover [44]. The human Drn1 protein, originally called as Cwf19L1 in the database, was also identified as a specific interactor to hDbr1 by co-precipitation [36]. This protein is localized in the nucleoplasm of HeLa cells, and it binds to the carboxy-terminal region of hDbr1 (Figure 1A). So far the function of hDrn1 was not identified yet, since it could not pull down splicing products produced by in vitro splicing reaction, and hDrn1 could not show any effects on RNA lariat debranching reaction catalyzed by hDbr1 in vitro, either. hDrn1 may have role(s) in the cytoplasm, as hDbr1, since this protein is a nucleo-cytoplasmic shuttling protein, as well as hDbr1 [36]. It should be determined whether these two proteins

In summary, human Dbr1 protein is likely a surveillant for branch RNAs to debranch them for degradation. It would be interesting to determine hDbr1-associated RNAs from both nucleus and cytoplasm. This may uncover novel role(s) of hDbr1 and unidentified cellular processes that branched RNA molecules participate. Since inhibition of hDbr1
activity reduces HIV-1 replication and TDP-43 toxicity in ALS neurons, this protein can be a good candidate for drug development.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research on Innovative Areas “Diversity and asymmetry achieved by RNA program” (No. 23112706) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

Financial Conflicting interests

The author declares no financial conflicting interests.

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