RNA & DISEASE 2016; 3: e963. doi: 10.14800/rd.963; © 2016 by Naoyuki Kataoka
http://www.smartscitech.com/index.php/rd

REVIEW

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

Naoyuki Kataoka

Medical Innovation Center, Laboratory for Malignancy Control Research, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto 606-8507, Japan

Correspondence: Naoyuki Kataoka
E-mail: kataoka.naoyuki.6m@kyoto-u.ac.jp
Received: August 13, 2015
Published: May 02, 2016

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 2016; 3: e963. doi: 10.14800/rd.963.

In higher eukaryotes, most of nuclear-encoded genes are separated by introns [1]. Pre-mRNA splicing is to remove introns and connect exons, which is an essential step for gene expression. Introns are removed as a lariat form by a huge RNA-protein complex, spliceosome [2-5]. Excised introns are supposed to be restricted and get degraded in the nucleus. Although intron degradation pathway has not been well understood compared with the splicing reaction, this step is supposed to be very important especially in higher eukaryotes. For example, some of small nucleolar RNAs (snoRNAs) and microRNAs (miRNAs) are embedded in introns and the biogenesis of these noncoding RNAs is functionally associated with splicing reactions [6-12]. Splicing takes place in two catalytic steps [2-5]. As the first step, the 5' splice site is cleaved and the 5' end of the intron is linked to the branch point nucleotide by forming a 2'-5' phosphodiester bond (Figure 1A). This produces splicing intermediates, such as 5' exon and a lariat form intron RNA with 3' exon. Then they undergo the second step reaction that the cleavage at 3' splice site takes place and two exons are concurrently ligated to generate mRNA (Figure 1B). Upon the completion of the second step reaction, the spliceosome is dismantled into two RNA-protein complexes, spliced mRNA-protein complex and lariat-intron containing RNA-protein complex. The lariat-intron complex undergoes intron degradation processes. Before lariat introns are degraded, a 2'-5' phosphodiester bond between branch point residue and the guanosine which resides at the end of the 5' splice site has to be dissolved. This reaction is called as debranching reaction, and it is mediated by an RNA lariat debranching enzyme, Debranching enzyme protein 1 (Dbr1) (Figure 1A) [13-18]. Amino acid sequence of Dbr1 protein is

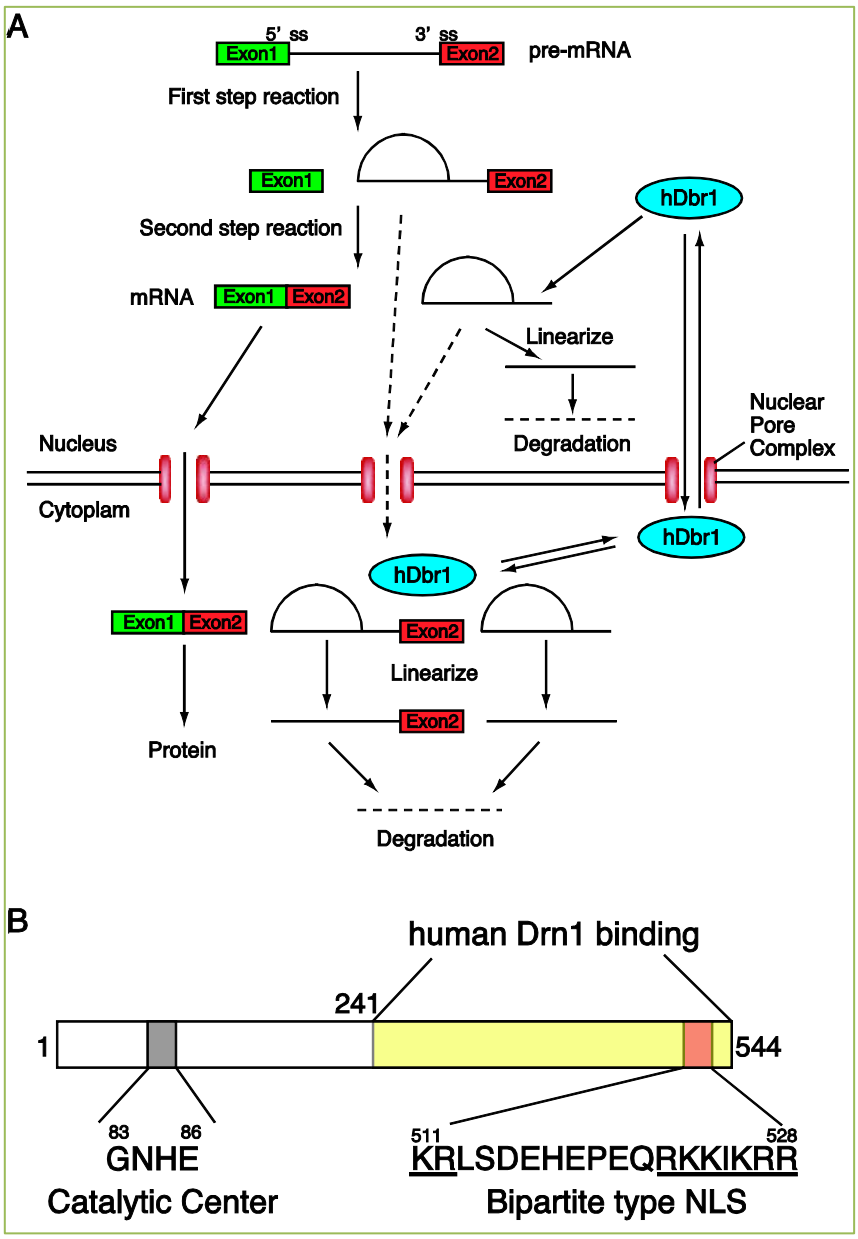

Figure 1. Schematic representation of mRNA splicing, post-splicing intron turnover process in human cells and human Dbr1 protein. (A) A scheme for mRNA splicing and intron turnover in human cells. Boxes indicate exons and lines show introns. Major pathways are shown in arrows, while minor pathways are demonstrated by dashed arrows. (B) A structure of human Debranching enzyme 1 protein. Numbers are amino-acid numbers of human Dbr1 protein. Amino-acid sequences for catalytic center and bipartite nuclear localization signal (NLS) are shown in one letter code. Critical amino-acids required for NLS activity are indicated by underlines.

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 [13]. This finding strongly suggests that hDbr1 has role(s) in both in 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 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.

References

1. International Human Genome Sequencing C. Finishing the euchromatic sequence of the human genome. Nature 2004; 431: 931-945.
2. Dreyfuss G, Kim VN, Kataoka N. Messenger-RNA-binding proteins and the messages they carry. Nat Rev Mol Cell Biol 2002; 3: 195-205.
3. Hastings ML, Krainer AR. Pre-mRNA splicing in the new millennium. Curr Opin Cell Biol 2001; 13: 302-309.
4. Jurica MS, Moore MJ. Pre-mRNA splicing: awash in a sea of proteins. Molecular cell 2003; 12: 5-14.
5. Maniatis T, Reed R. An extensive network of coupling among gene expression machines. Nature 2002; 416: 499-506.
6. Hirose T, Ideue T, Nagai M, Hagiwara M, Shu MD, Steitz JA. A spliceosomal intron binding protein, IBP160, links position-dependent assembly of intron-encoded box C/D snoRNP to pre-mRNA splicing. Mol Cell 2006; 23: 673-684.
7. Hirose T, Shu MD, Steitz JA. Splicing-dependent and -independent modes of assembly for intron-encoded box C/D snoRNPs in mammalian cells. Mol Cell 2003; 12: 113-123.
8. Hirose T, Steitz JA. Position within the host intron is critical for efficient processing of box C/D snoRNAs in mammalian cells. Proc Natl Acad Sci USA 2001; 98: 12914-12919.
9. Kataoka N, Fujita M, Ohno, M. Functional association of the Microprocessor complex with the spliceosome. Mol Cell Biol 2009; 29: 3243-3254.
10. Kim, YK, Kim, VN. Processing of intronic microRNAs. EMBO J; 2007, 26: 775-783.
11. Pawlicki, JM, Steitz, JA. Nuclear networking fashions pre-messenger RNA and primary microRNA transcripts for function. Trends Cell Biol 2010; 20: 52-61.
12. Okamura, K, Hagen, JW, Duan, H, Tyler, DM, Lai, EC. The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell 2007, 130: 89-100.
13. Kataoka, N, Dobashi, I, Hagiwara, M, Ohno, M. hDbr1 is a nucleocytoplasmic shuttling protein with a protein phosphatase-like motif essential for debranching activity. Sci Rep 2013; 3: 1090.
14. Kim, JW, Kim, HC, Kim, GM, Yang, JM, Boeke, JD, Nam, K. Human RNA lariat debranching enzyme cDNA complements the phenotypes of Saccharomyces cerevisiae dbr1 and Schizosaccharomyces pombe dbr1 mutants. Nucleic Acids Res 2000; 28: 3666-3673.
15. Moore, M. Nuclear RNA turnover. Cell 2002, 108: 431-434.
16. Nam, K, Lee, G, Trambley, J, Devine, SE, Boeke, JD. Severe growth defect in a Schizosaccharomyces pombe mutant defective in intron lariat degradation. Mol Cell Biol 1997; 17: 809-818.
17. Chapman, KB, Boeke, JD. Isolation and characterization of the gene encoding yeast debranching enzyme. Cell 1991; 65: 483-492.
18. Khalid, MF, Damha, MJ, Shuman, S, Schwer, B. Structure-function analysis of yeast RNA debranching enzyme (Dbr1), a manganese-dependent phosphodiesterase. Nucleic Acids Res 2005; 33: 6349-6360.
19. Wang, H, Hill, K, Perry, SE. An Arabidopsis RNA lariat debranching enzyme is essential for embryogenesis. J Biol Chem 2004; 279: 1468-1473.
20. Findlay, GM, Boyle, EA, Hause, RJ, Klein, JC, Shendure, J. Saturation editing of genomic regions by multiplex homology-directed repair. Nature 2014; 513: 120-123.
21. Clement, JQ, Maiti, S, Wilkinson, MF. Localization and stability of introns spliced from the Pem homeobox gene. J Biol Chem 2001; 276: 16919-16930.
22. Gardner, EJ, Nizami, ZF, Talbot, CC Jr, Gall, JG. Stable intronic sequence RNA (sisRNA), a new class of noncoding RNA from the oocyte nucleus of Xenopus tropicalis. Genes Dev. 2012, 26, 2550-2559.
23. Talhouarne, GJ, Gall, JG. Lariat intronic RNAs in the cytoplasm of Xenopus tropicalis oocytes. RNA 2014; 20: 1476-1487.
24. Hilleren, PJ, Parker, R. Cytoplasmic degradation of splice-defective pre-mRNAs and intermediates. Mol Cell 2003; 12: 1453-1465.
25. Armakola, M, Higgins, MJ, Figley, MD, Barmada, SJ, Scarborough, EA, Diaz, Z, *et al*. Inhibition of RNA lariat debranching enzyme suppresses TDP-43 toxicity in ALS disease models. Nat Genet 2012; 44: 1302-1309.
26. Ashwal-Fluss, R, Meyer, M, Pamudurti, NR, Ivanov, A, Bartok, O, Hanan, M, *et al*. circRNA biogenesis competes with pre-mRNA splicing. Mol Cell 2014; 56: 55-66.
27. Hansen, TB, Jensen, TI, Clausen, BH, Bramsen, JB, Finsen, B, Damgaard, CK, *et al*. Natural RNA circles function as efficient microRNA sponges. Nature 2013; 495: 384-388.
28. Ivanov, A., Memczak, S, Wyler, E, Torti, F, Porath, HT, Orejuela, MR, *et al*. Analysis of intron sequences reveals hallmarks of circular RNA biogenesis in animals. Cell Rep 2015; 10: 170-177.
29. Memczak, S, Jens, M, Elefsinioti, A, Torti, F, Krueger, J, Rybak, A, *et al*. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 2013; 495: 333-338.
30. Starke, S, Jost, I, Rossbach, O, Schneider, T, Schreiner, S, Hung, LH, *et al*. Exon circularization requires canonical splice signals. Cell Rep 2015; 10: 103-111.
31. Karst, SM, Rutz, ML, Menees, TM. The yeast retrotransposons Ty1 and Ty3 require the RNA Lariat debranching enzyme, Dbr1p,

for efficient accumulation of reverse transcripts. Biochem Biophys Res Commun 2000; 268: 112-117.

32. Roth, JF. The yeast Ty virus-like particles. Yeast 2000; 16: 785-795.
33. Wilhelm, FX, Wilhelm, M, Gabriel, A. Reverse transcriptase and integrase of the Saccharomyces cerevisiae Ty1 element. Cytogenet Genome Res 2005; 110: 269-287.
34. Ye, Y, De Leon, J, Yokoyama, N, Naidu, Y, Camerini, D. DBR1 siRNA inhibition of HIV-1 replication. Retrovirology 2005; 2: 63.
35. Galvis, AE, Fisher, HE, Nitta, T, Fan, H, Camerini, D. Impairment of HIV-1 cDNA synthesis by DBR1 knockdown. J Virol 2014; 88: 7054-7069.
36. Masaki, S, Yoshimoto, R, Kaida, D, Hata, A, Satoh, T, Ohno, M, *et al*. Identification of the specific interactors of the human lariat RNA debranching enzyme 1 protein. Int J Mol Sci 2015; 16: 3705-3721.
37. Yoshimoto, R, Kataoka, N, Okawa, K, Ohno, M. Isolation and characterization of post-splicing lariat-intron complexes. Nucleic Acids Res 2009; 37: 891-902.
38. Kuraoka, I, Ito, S, Wada, T, Hayashida, M, Lee, L, Saijo, M, *et al*. Isolation of XAB2 complex involved in pre-mRNA splicing, transcription, and transcription-coupled repair. J Biol Chem 2008; 283: 940-950.
39. Nakatsu, Y, Asahina, H, Citterio, E, Rademakers, S, Vermeulen, W, Kamiuchi, S, *et al*. XAB2, a novel tetratricopeptide repeat protein involved in transcription-coupled DNA repair and transcription. J Biol Chem 2000; 275: 34931-34937.
40. Yonemasu, R, Minami, M, Nakatsu, Y, Takeuchi, M, Kuraoka, I, Matsuda, Y, *et al*. Disruption of mouse XAB2 gene involved in pre-mRNA splicing, transcription and transcription-coupled DNA repair results in preimplantation lethality. DNA repair 2005; 4: 479-491.
41. Grey, M, Dusterhoft, A, Henriques, JA, Brendel, M. Allelism of PSO4 and PRP19 links pre-mRNA processing with recombination and error-prone DNA repair in Saccharomyces cerevisiae. Nucleic Acids Res 1996; 24: 4009-4014.
42. Mahajan, KN, Mitchell, BS. Role of human Pso4 in mammalian DNA repair and association with terminal deoxynucleotidyl transferase. Proc Natl Acad Sci USA 2003; 100: 10746-10751.
43. Revers, LF, Cardone, JM, Bonatto, D, Saffi, J, Grey, M, Feldmann, H, *et al*. Thermoconditional modulation of the pleiotropic sensitivity phenotype by the Saccharomyces cerevisiae PRP19 mutant allele pso4-1. Nucleic Acids Res 2002; 30: 4993-5003.
44. Garrey, SM, Katolik, A, Prekeris, M, Li, X, York, K, Bernards, S, *et al*. A homolog of lariat-debranching enzyme modulates turnover of branched RNA. RNA 2014; 20: 1337-1348.