

Analyses of gene structures and splicing variants of *prmt1* and its vertebrate paralogue *prmt8* suggest their molecular evolution

Yi-Chun Wang¹, Li-Ching Chuang¹, Yu-Jen Lee¹, Chien-Ping Chang¹ and Chuan Li^{1,2*}

¹ Department of Biomedical Sciences, Chung Shan Medical University. No.110, Sec.1, Jianguo N. Rd., Taichung 40201, Taiwan (R.O.C)

² Department of Medical Research, Chung Shan Medical University Hospital. No.110, Sec.1, Jianguo N. Rd., Taichung 40201, Taiwan (R.O.C)

Protein arginine methylation is a posttranslational modification involved in transcriptional regulation, RNA processing and many different cellular processes. There are nine different protein arginine methyltransferases (PRMTs) genes in mammals. PRMT1 is the most conserved and widely distributed PRMT in eukaryotes. PRMT8 is a vertebrate-specific paralogue of PRMT1 and the major difference between the two PRMTs is that PRMT8 contains an extra N-terminus of about 60-90 amino acids in different species. We collected the *prmt1* as well as *prmt8* genomic and cDNA sequences from typical vertebrate and animal species and constructed the gene structure diagram. A basic 10-exon configuration with exactly the same intron/exon junctions was observed for vertebrate *prmt1* and *prmt8* as well as some chordate *prmt1*. Multiple sequence alignments of the amino acid sequences of PRMT1 and PRMT8 clearly illustrated the major differences in N-terminal variable region through evolution. We also collected RNA variants of the two genes. Most of the *prmt1* variants in human and mice encode different N-termini due to alternative splicing. However, similar *prmt1* variants with missing sequences close to the C-terminus in human (variant 4) and mouse (variant 3) have not been reported in previous publications. A *prmt8* variant 2 in primates with an alternative exon 1 was identified in the data base and similar variants can be predicted in primates with conserved protein sequences and genomic localization. We also constructed phylogenetic tree of the *prmt1* and *prmt8* mRNAs and showed clear vertebrate duplication pattern. Our analyses of the genomic structures and the putative transcriptional variants of *prmt1* and *prmt8* will help to trace the evolution origin of *prmt8* and provide critical information for future experimental designs of these genes.

Key words: PRMT1, PRMT8, gene structures, splicing variants

Introduction

Post-translational modifications (PTMs) increase the complexity of expressed proteome for more subtle regulation of the activity, stability

and interaction of the modified proteins. Protein arginine methylation is a post-translational modification that was reported early in histones and many nucleic acid-binding proteins. Histone modifications that specify the histone code appear to be the major components of epigenetic regulation. Protein arginine methyltransferases (PRMTs) that catalyze of attachment of methyl groups to specific guanidino nitrogen atoms in

* Corresponding Author: Chuan Li

E-mail: cli@csmu.edu.tw

Tel: +886-4-24730022 ext. 12321

argininyl residues are among the major histone modifiers (Jahan and Davie 2015). By now there are nine different *prmt* genes identified in mammals by their sequence homology with the first identified *prmt1* and numbered according the identification order. PRMT1, 2, 4, 5, 6 and 7 are co-activators/repressors involving epigenetic controls via the modification of histones (Di Lorenzo and Bedford 2011).

Protein arginine methylation is a posttranslational modification not restricted to the histone-rapped chromatin but also reported in many nucleic acid binding proteins. Many proteins related to RNA processing are also modified by arginine methylation. The modification can be divided into type I, type II and type III according to the formation of asymmetric ω -N^G, N^G dimethylarginine (ADMA), symmetric ω -N^G, N^G dimethylarginine (SDMA) and ω -N^G monomethylarginine (MMA) respectively. The type IV activity to catalyze the formation of δ -N^G methylarginine has only been reported for yeast RMT2 (Bedford and Clarke 2009). The methyltransferase activities of most PRMTs have been characterized as PRMT1, 2, 3, 4, 6 and 8 as type I while PRMT5 as the type II PRMT. Recent studies confirmed the type III activity of PRMT7 (Zurita-Lopez et al. 2012) and type II activity of PRMT9 (Yang et al. 2015).

PRMT1 and PRMT5 are the most broadly distributed type I and II PRMTs in most of the typical eukaryotic species. Sequence similarity of PRMT1 is higher than 90% in vertebrates and higher than 70% between human PRMT1 and budding yeast (*Saccharomyces cerevisiae*) RMT1. PRMT1 appears to be the most conserved PRMT and is likely to be present in the early eukaryotic common ancestor. Its paralogue PRMT8 is present only in vertebrates with higher than or close to 90% sequence similarity. High degree of sequence conservation indicated that PRMT8 and PRMT1 evolve at a slower rate compared with other PRMTs (Wang and Li 2012).

Most studies of PRMTs are from the mammalian system. PRMT1 is the first identified and the most predominant protein arginine methyltransferase in mammals (Lin et al. 1996). It is well studied

with broad substrate spectrum and plays roles in various cellular processes. For example, it is implicated in RNA processing and some nucleic acid binding proteins such as hnRNP A1, EWS and Sam68 are among the earliest reported substrates of PRMT1 (Nicholson et al., 2009; Pahlich et al., 2006). Furthermore, asymmetric dimethylation of histone H4 Arg-3 (H4R3me2a) by PRMT1 is part of the epigenetic histone code and thus PRMT1 is a coactivator for some nuclear receptors as well as various transcription factors including p53 and YY1. Substrates involved in DNA repair and signal transduction have also been identified (Nicholson et al. 2009; Pahlich et al. 2006).

Homozygous mouse *prmt1* mutant embryos failed to develop shortly after implantation, consistent with a fundamental role in cellular metabolism. However, ES cell lines can be established from mutant blastocysts, indicating that PRMT1 is not required for cell viability (Pawlak et al. 2000). Further analyses showed that the loss of PRMT1 in MEFs leads to spontaneous DNA damage, cell cycle progression delay, checkpoint defects, aneuploidy, and polyploidy, indicating that PRMT1 is required for genome integrity and cell proliferation (Yu et al. 2009). We knocked down *prmt1* by antisense morpholino (AMO) injections in zebrafish embryos and showed defective convergence and extension during gastrulation. Biochemical analyses confirmed the reduced PRMT1 protein level, type I methyltransferase activity as well as histone H4R3 methylation, and thus the PRMT1-mediated modification is conserved from human to fish (Tsai et al. 2011).

All PRMTs express ubiquitously in mammalian tissues except PRMT8, which shows neuron-restricted expression (Lee et al. 2005). PRMT8 appears to be a paralogue of PRMT1 in vertebrates and is highly homologous to PRMT1 with about 90% sequence identity (Hung and Li 2004). The major difference between the two PRMTs is that PRMT8 contains an extra N-terminus of about 60~90 amino acids in different species. Plasma membrane localization through N-myristoylation of PRMT8 at the second glycine residue has been shown by transfection (Lee et al. 2005). However, dominant nuclear localization of PRMT8 was

observed in mouse central nervous neurons by a PRMT8-specific antibody (Kousaka et al. 2009). The N-terminus of PRMT8 suppressed the methyltransferase activity of human PRMT8 and the proline-rich sequence in this region can interact with the SH3 domain (Sayegh et al. 2007). Automethylation of two argininy residues in the N-terminus has been identified and can reduce PRMT8 activity by increasing the K_m of AdoMet (Dillon et al. 2013). We showed that *prmt8* plays important roles non-overlapping with *prmt1* in embryonic and neural development depending on its specific N-terminus (Lin et al. 2013).

In our previous study we proposed that the *prmt8* is likely to be duplicated from the *prmt1* gene with acquired exon 1 and the 5'-half of exon 2 encoding the N-terminal sequence along with sequence for the neuron-specificity at its 5' region such as the neuron-restrictive silencing elements (NRSEs) /repressor element 1 (RE-1) (Lin et al. 2013). Recently we developed a BLAST-Based Relative Distance (BBRD) method to reconstruct reliable phylogenies. We had collected protein sequences of PRMTs from twenty two representative species to construct the overall PRMT tree (Wang et al. 2015). PRMT1 isoforms with different N-termini due to alternative splicing have been reported in human and rodents (Goulet et al. 2007) and alternatively spliced PRMT1 isoforms can play different roles in cancer (Baldwin et al. 2012). To better understand the molecular evolution of PRMT1 and its vertebrate paralogue PRMT8, in this study we further collected their nucleotide sequences of the major mRNA variants to analyze their gene structure and phylogenetic relations.

Materials and Methods

Obtaining nucleotide or protein sequences from NCBI and Ensembl

Human *PRMT1* and *PRMT8* mRNA or protein were obtained from GenBank by key word searching. Then, these sequences were as queries to search against the GenBank or Ensembl to obtain corresponding homologues sequences of other animal species.

BLAST and MSA

The BLAST program we used was either the NCBI BLAST service (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) or the Ensembl BLAST service (<http://asia.ensembl.org/Multi/Tools/Blast?db=core>). For phylogenetic analysis, we used the stand alone NCBI BLAST+ 2.2.30. The MSA was performed by CLUSTAL W program (Thompson et al. 1994) and the result was adjusted manually.

Definition of Gene Structure

The gene structure was as suggested by the Ensembl or determined by NCBI BLAST to map the mRNA sequences to genomic DNA. The exon/intron structure was defined according to the BLAST result manually.

Phylogenetic Analysis

BLAST Based Relative Distance method (BBRD) was employed to reconstruct the phylogenetic relationship (Wang et al. 2015) of 34 mRNA sequences from 22 species. The steps of BBRD method was described briefly as follows:

1. Perform pairwise alignment of all sequences by BLAST.
2. Take all HSPs of a given sequence pair into account to build the BLAST total score matrix (BTSM)
3. Transform BTSM to sequence relative distance matrix (SRDM) by using Pearson's correlation coefficient.
4. Construct the Tree map from SRDM by single-linkage method.

The tree was rendered by Dendroscope (Huson and Scornavacca 2012).

Results

Data collection and curation

To trace the molecular evolution of *prmt1* and *prmt8*, we carefully conducted bioinformatic data mining for the cDNA and EST database for transcribed mRNA sequences of *prmt1* and *prmt8*. We have collected genomic DNA and mRNA/cDNA/EST sequences of *prmt1* and *prmt8* from

22 animal species as representatives of different phyla. For few species without such data from direct search, predicted mRNA sequences were recruited by BLAST and manual inspection. For apparent incomplete sequences, overlapping EST sequences were used to bridge the gap. For example, incomplete 5'-terminal sequence of *prmt1* from amphioxus, *Branchiostoma floridae*, and nematode *Caenorhabditis elegans*, was edited with overlapping ESTs to obtain a more complete sequence.

The sequences used in this study are shown in Table 1. Interestingly, we could not identify *prmt1* but only *prmt8* gene in chicken. Examination of other avian (mallard, collared flycatcher, and zebra finch) genome or mRNA/cDNA/EST sequences revealed similar results. We thus included only their *prmt8* sequences. We compared the mRNA/cDNA sequences with the genomic sequences to determine the exons. All exon/intron data have been compared with the NCBI/Ensembl sequences. We thus constructed the gene structures and splicing patterns of these genes.

The gene structures of *prmt1* and *prmt8*

As indicated before, PRMT1 and PRMT8 (*hrmt113*) are highly homologous proteins with similar gene structures (Hung and Li 2004). The amino acid sequences since the last 18-20 amino acid encoded by the 3'-part of exon (e) 2 until the end of the gene share high sequence identity for all vertebrate *prmt1* and even *prmt8*. Besides, all vertebrate *prmt1* and *prmt8* share exactly the same intron/exon junctions. The alignments of the sequences with the illustration of the exons of *prmt1* and *prmt8* are shown in Fig. 1. Including the alternative exons, basically the *prmt1* genes in vertebrates contain 10-12 exons. Alternatively spliced PRMT1 isoforms with different N-termini in human and rodents have been reported (Goulet et al. 2007). For the consistency for all vertebrate *prmt1* genes, we designated the two 5' alternative exons in mammals as a and b, the same as the e2 and e3 designation in Goulet et al (Goulet et al. 2007). It is clear that the exons from e3 to e10 are highly conserved not only in *prmt1*, but also in *prmt8*. Then we refer to the ten constitutive exon gene structure as 10-exon structure.

Outside the vertebrates, the amphioxus *Branchiostoma floridae*, a cephalochordate, and *Saccoglossus kowalevskii*, a hemichordate, both contain 10 exons for *prmt1*. However, another chordate, the ascidian *Ciona intestinalis*, contains 8 exons with the third exon equals to e3 and e4, and the 5th exon equals to e6 plus the 5'-half of e7 in vertebrates. The third exon of sea urchin *Strongylocentrotus purpuratus* equals to vertebrate e3-e5 thus contains 8 exons. *Drosophila* contains 4 exons with exon 2 equals to e2 plus the 5'-half of e3, exon 3 equals to the 3'-half of e3 and e4 with the 5'-half of e5, and the fourth exon equals to the 3'-half of e5 plus e6-e10 in vertebrate. Hydra *Hydra vulgaris* contains 6 exons with exon 2 equals to e2 to e4 and exon 3 equals to e5 to e7. Interestingly, another cnidarians sea anemones *Nematostella vectensis* also contain 10 exons with exact exon/intron structure as vertebrates. Even though the exon numbers of *prmt1* in non-vertebrate species vary greatly from 3 to 10, the sequences are still highly conserved.

prmt8 is only present in vertebrates and contains 10 exons as exactly the same intron/exon junctions as *prmt1*. Multiple sequence alignment of the amino acid sequences of PRMT1 and PRMT8 with the illustration of the exons are shown in Fig. 2A, B. The glutamine-rich sequences encoded by the 5'part of exon 2 in zebrafish was not detected in other fish species. Nevertheless, extra sequences of about 15-20 amino acids with scattered glutamines before the proline rich sequences highly conserved from mammals to amphibians can be detected in a few fish species.

The 10-exon gene structure of *prmt1* and 8 is present in all vertebrates as well as in representative species of cephalochordate and hemichordate, indicating that this 10-exon gene structure of *prmt1* is likely to be evolved in chordates before the common ancestor of vertebrates. The *prmt8* gene was likely to be evolved in the common ancestor of vertebrates with duplicated *prmt1* gene starting from the end of exon 2. The *prmt8* gene appears to obtain the neuron-specific transcriptional regulation together with its first exon and the 5'-part of exon2, and thus preserved the 10-exon gene structure.

Table 1. The GI number of sequences used in this study.

Species	Common name	cDNA	GI Number
Homo sapiens	Human	PRMT1 , transcript variant 1	359338974
		PRMT1 , transcript variant 2	359338976
		PRMT1 , transcript variant 3	359338975
		PRMT1 , transcript variant 4	359338973
		PRMT8 , transcript variant 1	374858038
		PRMT8 , transcript variant 2	374858039
Pan troglodytes	Chimpanzee	PRMT1	694976217
		PRMT8 , transcript variant X1	694948234
		PRMT1 , transcript variant 1	357197156
Mus musculus	Mouse	PRMT1 , transcript variant 2	357197157
		PRMT1 , transcript variant 3	357197159
		PRMT8	357527368
Sarcophilus harrisii	Tasmanian devil	PRMT8	821487478
Anolis carolinensis	Lizard	PRMT1	637336819
		PRMT8	637304292
Gallus gallus	Chicken	PRMT8 , transcript variant X5	513160928
Ficedula albicollis	Flycatcher	PRMT8 , transcript variant X1	525028212
Taeniopygia guttata	Zebra finch	PRMT8 , transcript variant X1	823453162
Anas platyrhynchos	Mallard	PRMT8 , transcript variant X3	874451615
Xenopus tropicalis	Western clawed frog	PRMT1	348041382
		PRMT8	847113108
Xenopus laevis	African clawed frog	PRMT1	34396085
Danio rerio	Zebrafish	PRMT1	347800729
		PRMT8 , transcript variant X1	688546998
Takifugu rubripes	Pufferfish	PRMT8	410918744
Oryzias latipes	Ricefish	PRMT8 , transcript variant X1	765159746
Branchiostoma floridae	Lancelet	PRMT1*	260826853
Ciona intestinalis	Sea squirt	PRMT1	699253178
Saccoglossus kowalevskii	Acorn worms	PRMT1	296434233
Strongylocentrotus purpuratus	Sea urchin	PRMT1	780186249
Caenorhabditis elegans	Nematode	PRMT1‡	392923167
Drosophila melanogaster	Fruit fly	PRMT1	665393775
Hydra vulgaris	Hydra	PRMT1	828210212
Nematostella vectensis	Sea anemone	PRMT1	156402591

* 5' Sequence was extended by catenating the overlapping *B. floridae* EST (gi: 66323047).

‡ 5' Sequence was extended by catenating the overlapping *C. elegans* EST (gi: 18249474).

Isoforms of PRMT1 in mammals

In this study we also included the RNA variants

of *prmt1* that are annotated in the NCBI database.

The schematic presentations of the splicing variants

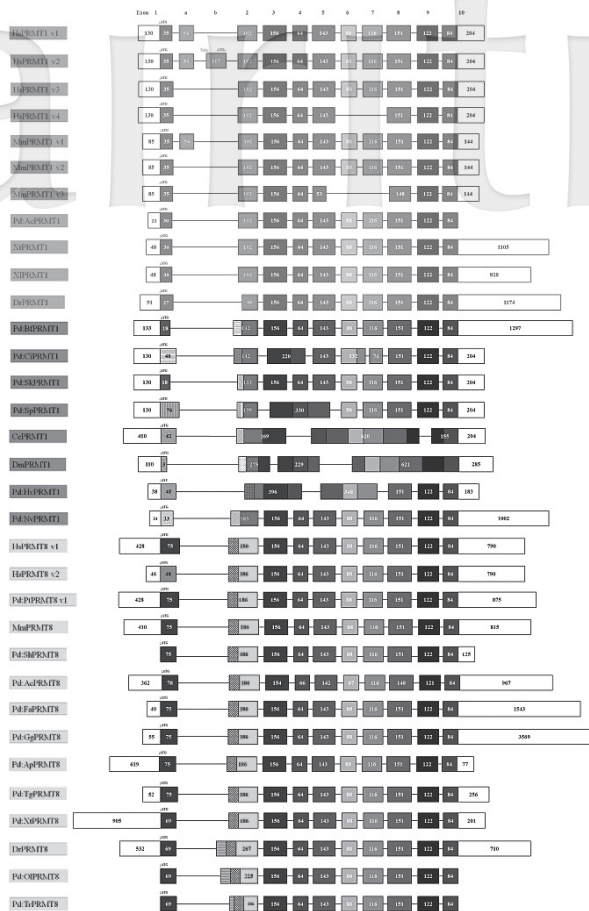


Fig. 1. The gene structure diagram of *PRMT1* and *PRMT8*.

Twenty-four different species are selected. The gene structure was constructed as described in the Materials and Methods. The forefront of Pd: representatives of the predicted mRNA sequence, Hs: *Homo sapiens*, Pt: *Pan troglodytes*, Mm: *Mus musculus*, Sh: *Sarcophilus harrisii*, Ac: *Anolis carolinensis*, Gg: *Gallus gallus*, Ap: *Anas platyrhynchos*, Fa: *Ficedula albicollis*, Tg: *Taeniopygia guttata*, Xt: *Xenopus tropicalis*, Xi: *Xenopus laevis*, Dr: *Danio rerio*, Tr: *Takifugu rubripes*, Ol: *Oryzias latipes*, Bf: *Branchiostoma floridae*, Ci: *Ciona intestinalis*, Sk: *Saccoglossus kowalevskii*, Sp: *Strongylocentrotus purpuratus*, Ce: *Caenorhabditis elegans*, Dm: *Drosophila melanogaster*, Hv: *Hydra vulgaris*, Nv: *Nematostella vectensis*. Exons are shown as boxes.

of *prmt1* are shown in Fig. 3A. All human *PRMT1* variants contain a short upstream open reading frame (uORF) with a translation start and an in-frame stop codon before the main ORF. Variant 2

that contains both alternative exons has another 5' uORF before the main ORF and is now designated as “long noncoding RNA” (NCBI gi: 150456457).

Even though various isoforms at the N-terminus of PRMT1 due to alternative splicing of exon a and b have been reported (Goulet et al., 2007), no isoforms with different C-terminal regions have been described. From data mining we encountered a specific mRNA variant 4 (GI:359338973, protein GI:333360913) without exon 6 and 7, which has not been reported in previous publications. BLAST against EST database identified few supports of the variant in neuroblastoma and prostate carcinoma (BX403078, BX352789 and BM043971).

The sequences in exon 6 and 7 encode the dimerization arm of PRMT1. Since dimer formation has been shown to be critical for the catalytic activity of PRMT1, it might be possible that the PRMT1 protein isoform encoded by variant 4 might interrupt dimer formation and has a dominant-negative effect. By now the ESTs that provide direct evidence of variant 4 is scarce compared with other variants.

However, a similar isoform v3 is present in mouse with different splicing acceptor and donor in exon 5 and 8 that leads to further deletions in exon 5 and 8 beside the loss of exon 6 and 7 (gi:357197160). An EST from mouse wolffian duct support the presence of the variant. Similar *prmt1* variant with even longer C-terminal deletions can be found in mouse database, indicating that similar splicing events of *prmt1* at both the 5' and middle part of the transcripts occur in mammals.

Novel isoforms of PRMT8 in primates

The schematic presentations of the splicing variants of *prmt8* are shown in Fig. 4. A new putative alternative 5'-exons of human *PRMT8* has been suggested. The 5'-exon of variant 2 is far upstream of the original 5'-exon of variant 1. The first 25 N-terminal amino acid sequence of isoform 1 (NP_062828) would be changed to a novel 16 amino acid sequence in the newly released isoform 2 (NP_001243465) without the initiating MG sequence as the N-myristoylation signal (Fig. 4A). *PRMT8* variant 2 can be predicted in primates with high sequence homology and similar genomic configuration as those in human

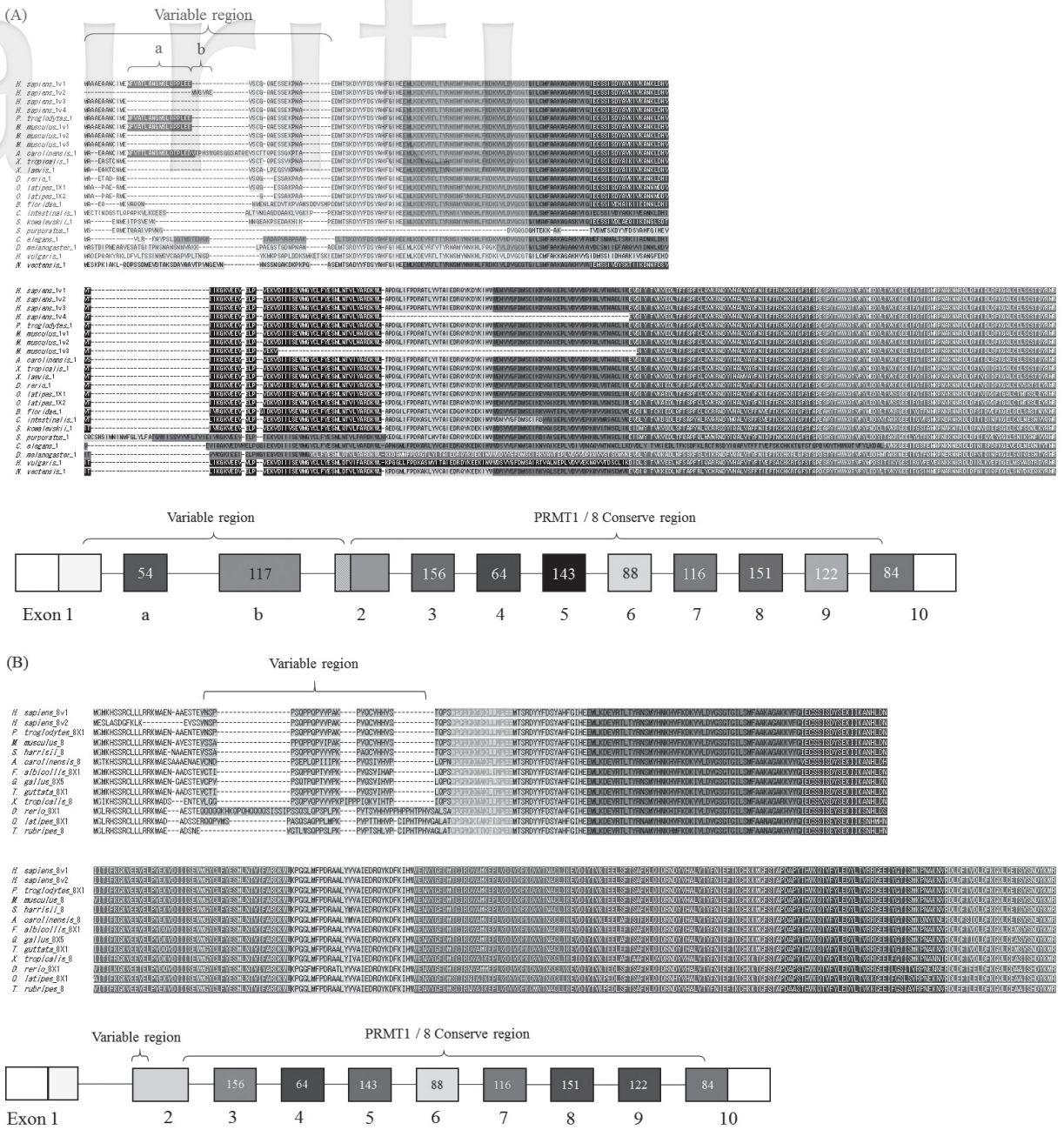


Fig.2. Multiple sequence alignment of the amino acid sequences of PRMT1s and PRMT8s with the illustration of the exons.

(A) The amino acid sequences of PRMT1 from different animal species are aligned with different colors highlight according to their encoding exons represented below. (B) The amino acid sequences of PRMT8 from different species in mammals, marsupials, birds, amphibians and fish are aligned with different color highlights according to their encoding exons represented below.

(Fig. 4B). BLAST with the first 300 nucleotide sequences of human *PRMT8* isoform v2 showed that there is only one mRNA (gi: 164695809, with an extra n at nucleotide 155) and one EST (gi:

82331749, SKNMC2 Homo sapiens cDNA clone SKNMC2002270 5') that matches.

The phylogenetic relationships of *prmt1* and *prmt8* genes

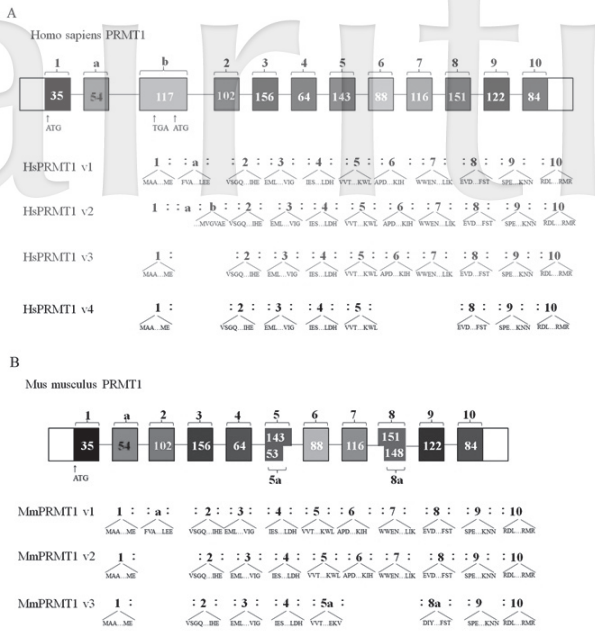


Fig.3. Different splicing patterns of *prmt1* and *prmt8*. Different *PRMT1* variants in human (A) and mice (B) that are annotated in the NCBI database are shown. Unreported human *PRMT1* variant 4 and mouse *prmt1* variant 3 with exon 8 and exon 9 missing identified from data mining are shown.

In previous study we had used the recently developed BBRD method to resolve 124 protein arginine methyltransferases (PRMTs). The method well separated all PRMTs, however, PRMT1 and 8 are distributed in one branch with some confusions. We thus used the mRNA sequences collected in this study to further analyze the phylogeny of *prmt1* and *prmt8* by BBRD. As shown in Fig. 5A, it is apparent that all *prmt8* were in one branch and then connected to *prmt1*. All of the vertebrates were clearly classified with zebrafish as the basal vertebral taxon for both *prmt1* and *prmt8*. The trees of either *prmt1* in animal kingdom or *prmt8* in vertebrates constructed by their mRNAs are relative close to the common taxonomy tree (Fig. 5B).

The three human *PRMT1* and the two mouse N-terminal variants grouped together respectively. The C-terminal v4 variants of human and the v3 variant of mouse were at the outside of mammals and then connected with reptile *prmt1*. It thus shows that the differences in the 5' variable

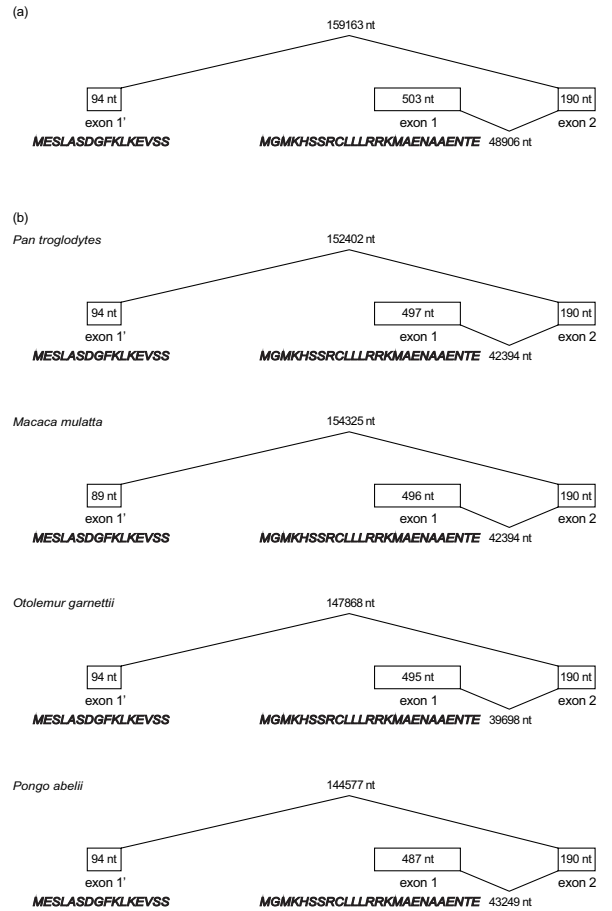


Fig.4. Illustration of putative *PRMT8* variants. (A) The schematic presentation of the putative 5'-alternative exon 1 of human *PRMT8*. The exon 1 (encoding variant 1) and exon 1' (encoding variant 2) are shown with their encoded amino acid sequences shown below the exon boxes. The lengths of the exons and introns are shown. (B) The schematic presentation of the predicted putative 5'-alternative exon 1 (encoding variant 1) and exon 1' (encoding variant 2) of *prmt8* in primates are shown with their encoded amino acid sequences shown below the exon boxes. The lengths of the predicted exons and introns are shown.

region within one species is less than the general differences of the whole *prmt1* gene in mammals. Deletion of about 200~300 nucleotides in *PRMT1* v4 in human and v3 in mouse still group the variants with mammalian PRMT that separates with reptiles by the BBRD analyses. The results indicate that BBRD has superior ability to group closely matched nucleotide sequences even if they

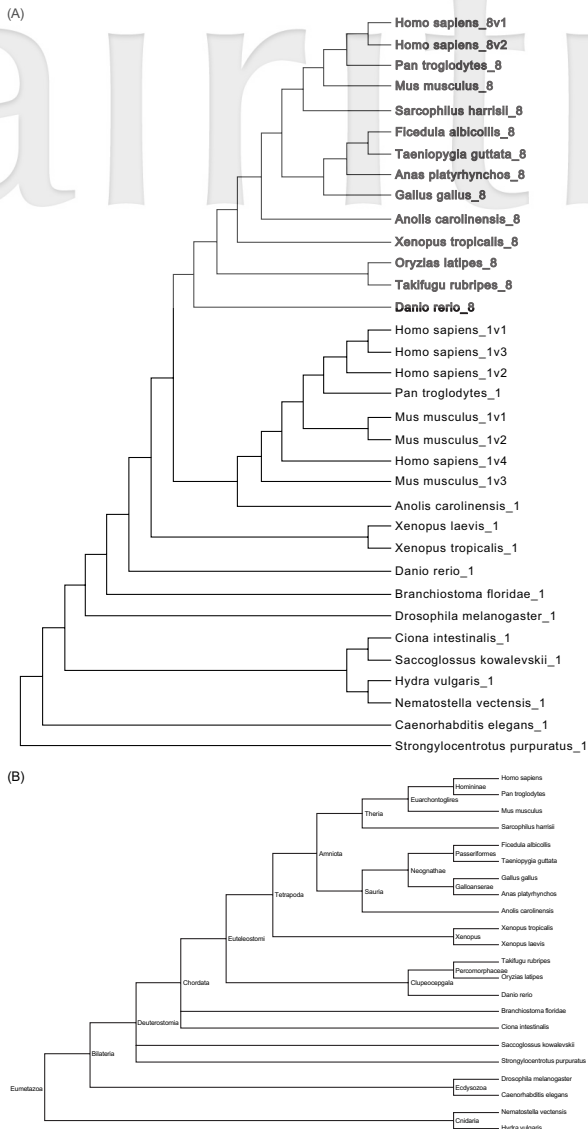


Fig.5. The evolutionary tree of *prmt1* and *prmt8*. (A) The evolutionary tree of *prmt1* and *prmt8* is calculated by BBRD and rendered by Dendroscope with *prmt1* in black and *prmt8* in blue. (B) The common tree of the species analyzed in BBRD.

contain some large fragment deletions.

The *prmt1* from two chordate *Saccoglossus kowalevskii* grouped with *Ciona intestinalis*. Two cnidarian hydra *Hydra vulgaris* and anemone *Nematostella vectensis* also grouped together. It is interesting that even though the *prmt1* of two cnidarian hydra and anemone were grouped together, their genome configuration are different. The results again showed that

the mRNA sequences are more conserved than the gene structures. Even though echinoderma *Strongylocentrotus purpuratus* has the PRMT systems close to chordates/vertebrates (Wang and Li, 2012), its *prmt1* sequence varies greatly with that in chordates/vertebrates and is located outside of these sequences. Similar sequence variations have been observed for other PRMTs in sea urchin (Wang and Li, 2012). Thus the evolution rate of PRMTs appears to be higher in this species.

Conclusions

In this study we collected 34 *prmt1* and *prmt8* cDNA sequences from 22 typical vertebrate and animal species and constructed the gene structure diagram. All vertebrate *prmt1* and *prmt8* as well as some chordate *prmt1* share the same 10-exon configuration with same intron/exon junctions. RNA variants of the two genes are detected in mammals. We described *prmt1* variants with missing sequences close to the C-terminus in human (variant 4) and mouse (variant 3) and a *prmt8* variant in primates with an alternative exon 1 not reported in previous publications. The phylogenetic tree of *prmt1* and *prmt8* cDNAs constructed by the BBRD method showed *prmt8* was like to be originated from a duplication event in early ancestors of vertebrates. Our analyses of *prmt1* and *prmt8* will help to trace the evolution origin of *prmt8* and provide critical information for future experimental designs of these genes.

Acknowledgement

This work was supported by the grants NSC 101-2320-B-040-004 from National Science Council, MOST 103-2320-B-040 -022-MY3 from Ministry of Science and Technology, and CSMU-INT-102-10 from Chung Shan Medical University to CL.

References

1. Baldwin RM, Morettin A, Paris G, Goulet I, Cote J (2012). Alternatively spliced protein arginine

- methyltransferase 1 isoform PRMT1v2 promotes the survival and invasiveness of breast cancer cells. *Cell Cycle* 11: 4597-4612. doi: 10.4161/cc.22871
2. Bedford MT, Clarke SG (2009). Protein Arginine Methylation in Mammals: Who, What, and Why. *Mol Cell* 33: 1-13.
 3. Di Lorenzo A, Bedford MT (2011). Histone arginine methylation. *FEBS Lett* 585, 2024-2031.
 4. Dillon MB, Rust HL, Thompson PR, Mowen KA (2013). Automethylation of protein arginine methyltransferase 8 (PRMT8) regulates activity by impeding S-adenosylmethionine sensitivity. *J Biol Chem* 288: 27872-27880.
 5. Goulet I, Gauvin G, Boisvenue S, Cote J (2007). Alternative splicing yields protein arginine methyltransferase 1 isoforms with distinct activity, substrate specificity, and subcellular localization. *J Biol Chem* 282: 33009-33021.
 6. Hung CM, Li C (2004). Identification and phylogenetic analyses of the protein arginine methyltransferase gene family in fish and ascidians. *Gene* 340: 179-187.
 7. Jahan S, Davie JR (2015). Protein arginine methyltransferases (PRMTs): role in chromatin organization. *Adv Biol Regul* 57: 173-184.
 8. Kousaka A, Mori Y, Koyama Y, Taneda T, Miyata S, Tohyama M (2009). The distribution and characterization of endogenous protein arginine N-methyltransferase 8 in mouse CNS. *Neuroscience* 163: 1146-1157.
 9. Le Romancer M, Treilleux I, Leconte N, Robin-Lespinasse Y, Sentis S, Bouchekioua-Bouzaghrou K, Goddard S, Gobert-Gosse S, Corbo L (2008). Regulation of Estrogen Rapid Signaling through Arginine Methylation by PRMT1. *Mol Cell* 31: 212-221.
 10. Lee J, Sayegh J, Daniel J, Clarke S, Bedford MT (2005). PRMT8, a new membrane-bound tissue-specific member of the protein arginine methyltransferase family. *J Biol Chem* 280: 32890-32896.
 11. Lin WJ, Gary JD, Yang MC, Clarke S, Herschman HR (1996). The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. *J Biol Chem* 271: 15034-15044.
 12. Lin YL, Tsai YJ, Liu YF, Cheng YC, Hung CM, Lee YJ, Pan H, Li C (2013) The critical role of protein arginine methyltransferase *prmt8* in zebrafish embryonic and neural development is non-redundant with its paralogue *prmt1*. *PLoS ONE* 8(3): e55221. doi: 10.1371/journal.pone.0055221.
 13. Nicholson TB, Chen T, Richard S (2009). The physiological and pathophysiological role of PRMT1-mediated protein arginine methylation. *Pharmacol Res* 60: 466-474.
 14. Pahlich S, Zakaryan RP, Gehring H (2006). Protein arginine methylation: Cellular functions and methods of analysis. *Biochimica et Biophysica Acta (BBA) - Proteins & Proteomics* 1764: 1890-1903.
 15. Pawlak MR, Scherer CA, Chen J, Roshon MJ, Ruley HE (2000). Arginine N-methyltransferase 1 is required for early postimplantation mouse development, but cells deficient in the enzyme are viable. *Mol Cell Biol* 20: 4859-4869.
 16. Sayegh J, Webb K, Cheng D, Bedford MT, Clarke SG (2007). Regulation of Protein Arginine Methyltransferase 8 (PRMT8) Activity by Its N-terminal Domain. *J Biol Chem* 282: 36444-36453.
 17. Tsai YJ, Pan H, Hung CM, Hou PT, Li YC, Lee YJ, Shen YT, Wu TT, Li C (2011). The predominant protein arginine methyltransferase PRMT1 is critical for zebrafish convergence and extension during gastrulation. *FEBS J* 278: 905-917.
 18. Wang YC, Li C (2012). Evolutionarily conserved protein arginine methyltransferases in non-mammalian animal systems. *FEBS J* 279: 932-945.
 19. Wang YC, Wang JD, Chen CH, Chen YW, Li C (2015). A novel BLAST-Based Relative Distance (BBRD) method can effectively group members of protein arginine methyltransferases and suggest their evolutionary relationship. *Mol Phylogenet Evol* 84: 101-111.
 20. Yang Y, Hadjikyriacou A, Xia Z, Gayatri

- S, Kim D, Zurita-Lopez C, Kelly R, Guo A, Li W, Clarke SG (2015). PRMT9 is a type II methyltransferase that methylates the splicing factor SAPI45. *Nature Commun* 6: 6428.
21. Yu Z, Chen T, Hebert J, Li E, Richard S (2009). A Mouse PRMT1 Null Allele Defines an Essential Role for Arginine Methylation in Genome Maintenance and Cell Proliferation. *Mol Cell Biol* 29: 2982-2996.
 22. Zurita-Lopez CI, Sandberg T, Kelly R, Clarke SG (2012). Human protein arginine methyltransferase 7 (PRMT7) is a type III enzyme forming omega-NG-monomethylated arginine residues. *J Biol Chem* 287: 7859-7870.
 23. Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.
 24. Huson DH, Scornavacca C (2012). Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Syst. Biol.* 61: 1061–1067.