科技部補助專題研究計畫成果報告
期末報告

建立演化上保留之蛋白質精胺酸甲基化網絡與研究平台

計 畫 類 別 ：個別型計畫
計 畫 編 號 ：NSC 101-2320-B-040-004-
執 行 期 間 ：101 年 08 月 01 日至 102 年 12 月 31 日
執 行 單 位 ：中山醫學大學生物醫學科學學系（所）

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處 理 方 式 ：
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2.「本研究」是否已有嚴重損及公共利益之發現：否
3.「本報告」是否建議提供政府單位施政參考：否

中 華 民 國 103 年 03 月 27 日

中 文 摘 要 ： 轉譯後修飾大幅增加蛋白質的複雜度，且能調節或微調所修飾蛋白質之活性、穩定度及交互作用。蛋白質精胺酸甲基化為一由蛋白質精胺酸甲基轉移酶(PRMT)家族成員所催化的轉譯後修飾，蛋白質精胺酸甲基轉移酶扮演關鍵生理角色也和許多疾病生成相關。蛋白質精胺酸甲基化網絡包含精胺酸甲基轉移酶、可能的去甲基酶、甲基化受質如組蛋白及hnRNP、以及能被徵召至特殊甲基位點以形成新的蛋白質或RNA 交互作用的’ 甲基讀者’ ，乃至於和精胺酸甲基化可能合作或競爭的其他轉譯後修飾。因此在本計畫中我們在不同的模式生物中對精胺酸甲基轉移酶進行系統性生物資訊分析， 延續於斑馬魚系統中有關 PRMT8 的研究以及細胞株中酵素甲基化受質分析，並建立大腸桿菌特定酵素/受質表達系統之精胺酸甲基化分析工具及平台。我們透過生物資訊資料挖掘及斑馬魚系統獲得關鍵資訊，更仔細描繪出蛋白質精胺酸甲基化網絡並且比較演化上的變遷，還可提供了解此網絡之關鍵資訊，以在未來對精胺酸甲基化異常相關疾病的治療與預防作出貢獻。

中文關鍵詞： 蛋白質精胺酸甲基化； 蛋白質精胺酸甲基轉移酶；系統性生物資訊分析

英 文 摘 要 ： Posttranslational modifications (PTMs) greatly increase the complexity of proteins and can regulate or fine-tune the activity, stability as well as interaction of the modified proteins. Of these modifications, protein arginine methylation is catalyzed by members of the protein arginine methyltransferase (PRMT) family. PRMTs play critical roles and are involved in many diseases. The partners involved in arginine methylation include the PRMTs, putative demethylases, the substrates such as histones and many hnRNPs, as well as the ’ methylarginine readers’ that can be specifically recruited to the arginine methylation sites for new sets of protein-protein or protein/nucleic acid interactions, and other PTM systems that might cooperate or compete with arginine methylation. Therefore, in this project we illustrated the network by conducting systematic bioinformatic analyses of the evolutionarily conserved PRMTs in different model systems. We continued the studies of methylaccepting substrates

in cell culture system, and developed better analyzing tools and platforms for the analyses of methylarginine containing proteins such as the E. coli expression system for specific enzyme/substrate pair. We obtained critical information through bioinformatic data mining and can outline a more detailed arginine methylation network and compare the network through evolution. We thus provide the insights of critical points in the network and contribute to further prevention and treatment of diseases related to the abnormal arginine methylation.

英文關鍵詞： Protein arginine methyltransferase； protein arginine methylation； systematic bioinformatic analyses

I. Introduction

In the post-genomic era, posttranslational modification (PTM) of proteins becomes an important issue for understanding gene expression, epigenetic regulation as well as the pathogenesis of many diseases. PTMs greatly increase the complexity of proteins and can regulate or fine-tune the activity, stability as well as interaction of the modified proteins. Of these modifications, protein arginine methylation is catalyzed by members of the protein arginine methyltransferase (PRMT) family (Krause et al., 2007). More and more evidences show that the PRMTs play critical roles physiologically and are involved in many diseases. The partners involved in arginine methylation not only include the PRMTs, putative demethylases, the substrates such as histones and many hnRNPs, but also the "methylarginine readers" that can be specifically recruited to the arginine methylation sites and initiate new sets of protein-protein or protein/nucleic acid interactions, and other PTM systems that might cooperate or compete with arginine methylation. Therefore, in this project we would like to illustrate the network by conducting systematic bioinformatic analyses of the evolutionarily conserved PRMT, continuing the studies of specific PRMTs, mostly PRMT6 and PRMT8 in our zebrafish system, and developing better analyzing tools and establishing platforms for the analyses of methylarginine containing protein.

The introduction of protein arginine methyltransferase family and protein arginine methylation

Protein arginine methylation has been shown to play roles in signal transduction, transcriptional and epigenetic regulation, protein trafficking, RNA processing and DNA repair (Bedford and Clarke, 2009; Pahlich et al., 2006). Arginine residues within glycine-arginine rich (GAR) motifs, RGG boxes or in the RXR sequences are the canonical sites of methylation by PRMTs (Bedford, 2007; Bedford and Richard, 2005; Pahlich et al., 2006). Most substrates of protein arginine methyltransferases (PRMTs) are nucleic acid binding proteins, among them are histones and many hnRNP proteins. Existence of PRMTs and the arginine methylation modification in eukaryotes seem to be closely related to the histone-rapped chromatin and complicated RNA processing. PRMTs can affect gene expression through it coactivator/corepressor activity to modify histones and regulate transcription and thus are critical players for epigenetic regulation (Fig. 1). PRMTs can also exert its impact by changing the substrates through the modification for different interactions, localization, function, or signaling pathways (Bedford and Clarke, 2009). After the identification of PRMT1 (Lin et al., 1996), the PRMT genes were designated by sequence homology and numbered according to the identification order. The PRMTs containing an *S*-adenosylmethionine (AdoMet) binding domain are illustrated in Fig. 1. PRMTs are divided into different groups according to the attachment of methyl groups to specific guanidino nitrogen atoms of arginines: type I catalyzes the formation of asymmetric ω-N^G, N^G dimethylarginine (aDMA), type II symmetric ω-N^G, $N^{G'}$ dimethylarginine (sDMA) and type III ω- N^G monomethylarginine (MMA). The type IV

activity that catalyzes the formation of δ- N^G methylarginine has only been reported for yeast RMT2 (Fig. 2) (Bedford and Clarke, 2009; Wang and Li, 2012). PRMT1, 2, 3, 4, 6 and 8 belong to the type I while PRMT5 to the type II PRMT. Type II and type III activity have been reported for PRMT7.

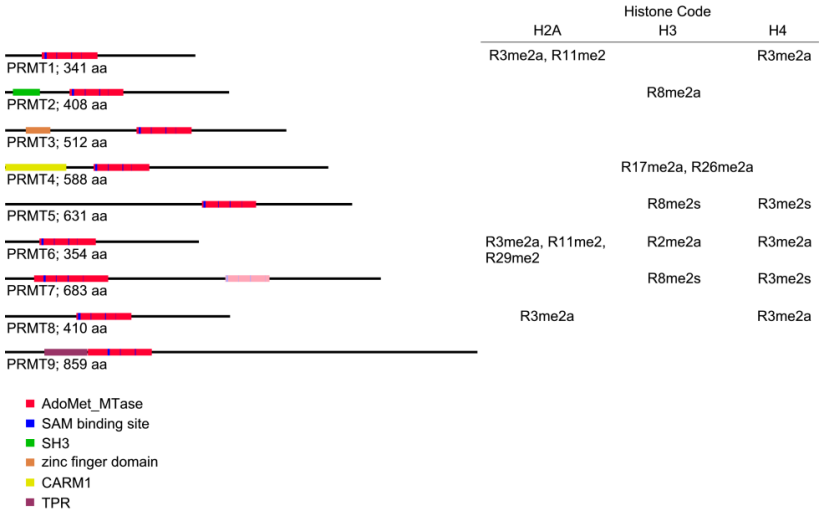

Fig. 1. The protein arginine methyltransferase family and histone arginine methylation. (from Wang and Li, 2012)

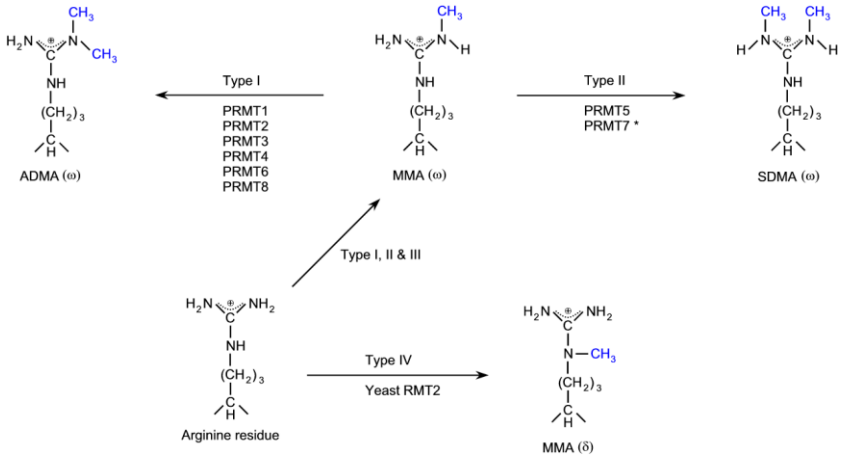

Fig. 2. Protein arginine methylation and types of protein arginine methyltransferases. (from Wang and Li, 2012)

The distribution of protein arginine methyltransferase family

We used nine PRMTs (PRMT1-PRMT8 and PRMT9(4q31)) identified in human as the templates to survey the homologous PRMTs in other biological systems. The distribution and sequence homologies of each PRMT are indicated in Fig. 3. The presence of specific PRMTs in these species and the sequence identities with their human homologues are shown for the distribution and conservations of PRMTs in the animal kingdom.

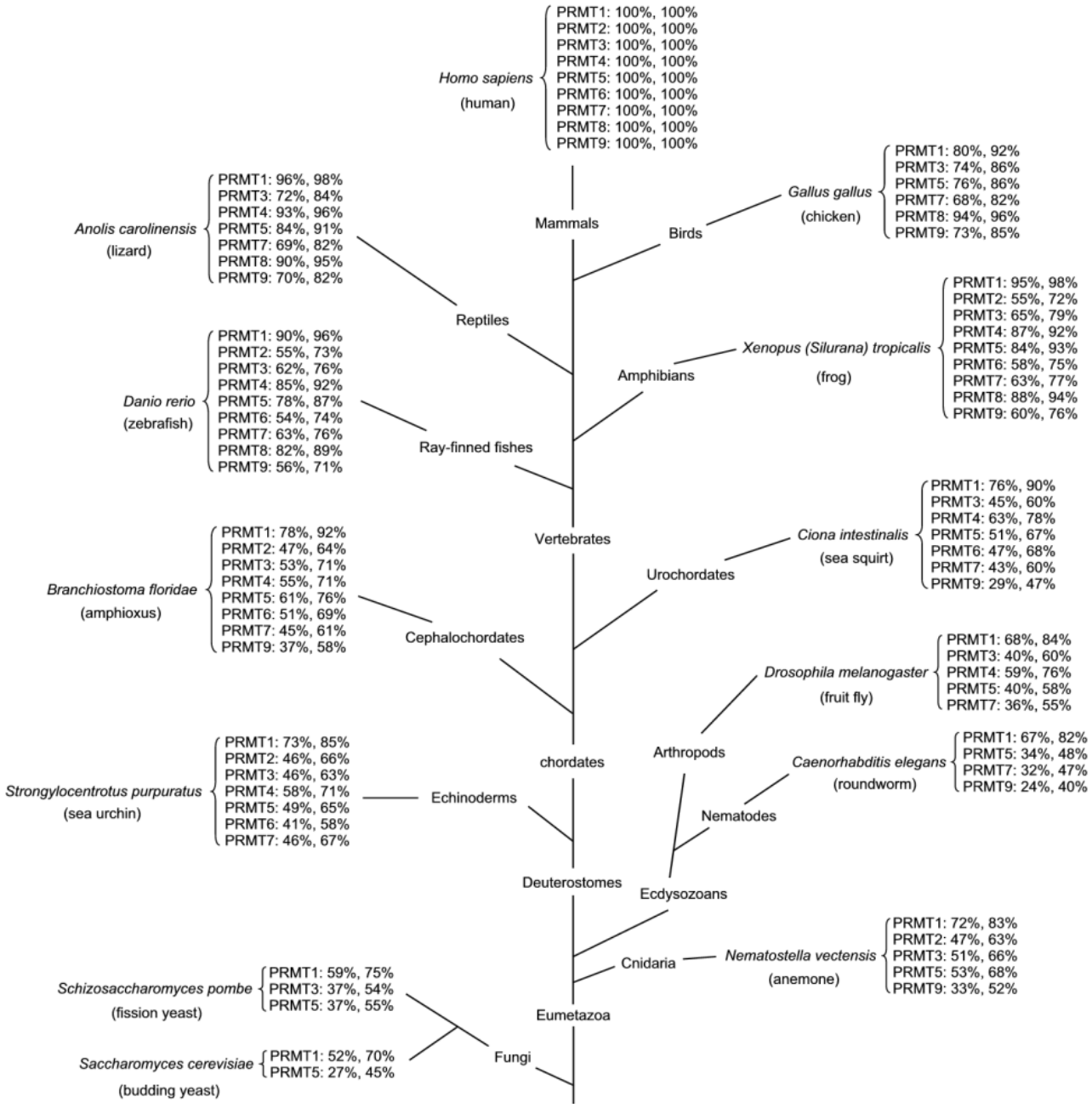

Fig. 3. Distribution and sequence homology of PRMTs in animals. (from Wang and Li, 2012)

PRMT1 and PRMT5 are the most broadly distributed type I and type II PRMTs present in all species. PRMT1 is the most conserved PRMT with sequence similarity higher than 90% in vertebrates and higher than 70% between human and budding yeast. Its paralogue PRMT8 is present only in vertebrates with higher than or close to 90% sequence similarity. It is interesting to note that PRMT2 and PRMT6 are absent in chicken and lizard even though their orthologues could be identified in amphibians and fish with higher than 50/70% sequence identity/similarity. PRMT3 which can be detected in fission yeast is widely distributed in almost all animal species we investigated with higher than 60% sequence similarities, but is absent in *C. elegans*. PRMT4 is conserved in vertebrate with higher than 90% sequence

similarity and in chordates, echinoderms and anthropods with higher than 70% similarities. PRMT5 and PRMT7 are generally distributed in the non-mammalian species we investigated. PRMT5 and 7 orthologues show higher than 85 and 75% similarities respectively in vertebrates, and both about 55% similarity in anthropods. PRMT9(4q31) orthologues shares 70% sequence similarities in vertebrates and about 50% in invertebrate chordates.

As shown above, all nine PRMTs (PRMT1-PRMT8 and PRMT9(4q31)) identified in human can find their homologous genes in zebrafish. PRMT1-8 identified in puffer fish, *Fugue rubripes*, and zebrafish, *Danio rerio*, were shown to have conserved amino acid sequences as well as gene structures (Hung and Li, 2004). Zebrafish thus would be a very nice model system for studying the PRMT functions.

Function of protein arginine methylation and the implication in human diseases

Protein arginine has been shown to play roles in signal transduction, transcriptional and epigenetic regulation, protein trafficking, RNA processing and DNA repair (Bedford and Clarke, 2009; Pahlich et al., 2006). The list of substrates increases with a steady rate and broad specificity. Arginine residues within glycine-arginine rich (GAR) motifs, RGG boxes or in the RXR sequences are the canonical sites of arginine methylation by PRMTs (Bedford, 2007; Bedford and Richard, 2005; Pahlich et al., 2006). These sites might overlap with the sites modified by other PTM systems. The Akt consensus phosphorylation sequence RxRxxS/T, for example, can be putative modification sites of PRMT1 (Sakamaki et al., 2011). Arginine methylation can thus crosstalk with other PTMs and increases the dimension of the regulation by this modification.

It is clear that numerous proteins involved in cell growth and tumor formation or other disease formation can be arginine methylated and the modification is related to its function. For example, p53, the most well studies tumor suppressor, can be modified by PRMT5 at R333, R335 and R337. The modification alters p53 recruitment to target genes and inhibit p53 oligomerization. Mutations in R337 destabilize p53 and occurs in Li Fraumeni syndrome, a cancer predeposition syndrome, and other tumors (Jansson et al., 2008). Furthermore, PRMT1 or its substrate Sam68 lead to oncogenesis when directly fused to the oncogene MLL (Cheung et al., 2007). PRMT1 mRNA level is elevated in a number of breast cancer cell lines than in normal controls (Goulet et al., 2007).

PRMTs appear to be involved in immune response signalings including STAT1/PAIS1, interferone α, and TCR signaling. Many type I or type II substrates such as fibrillarin, several hnRNPs, myelin basic protein and SmD1 and D3 are known to be autoantigens of different autoimmune diseases (van Boekel and van Venrooij, 2003). Ten different autoantisera recognize only the sDMA peptide of SmD1 and D3 but not unmethylated or aDMA peptides (Brahms et al., 2000), indicating methylarginine modification can be important for autoantibody recognition. Furthermore, peptides with aDMA modification were identified as natural MHC class I ligand, indicating that specific cytotoxic T-cell response against cells

presenting aDMA modified cells can be elicited (Yague et al., 2000). Abnormal arginine methylation thus can be correlated with the formation of autoimmune disease.

Arginine methylation also involves in cardiovascular disease. Free aDMA or MMA produced by proteolysis of arginine methylated proteins are inhibitor of nitric oxide synthase (NOS). Imbalanced NO signaling can increase the cardiovascular risk (Bulau et al., 2006).

II. Results and Discussion

(1) phylogenetic studies of the PRMTs through bioinformatic analyses

We conducted phylogenetic analyses of the nine PRMT through the method developed by the co-PI Dr. Wang that combines pair-wise sequence alignment and Pearson's correlation coefficient. The method can clearly separate of all PRMT sequences to different PRMT members in the phylogenetic tree. The tree is shown in Fig. 4.

Manuscript based on the results of this part of work is under preparation.

(2) Study of a PRMT substrate SERBP1. This part of work has been published in the FEBS Journal titled "Localization of SERBP1 in stress granules and nucleoli" (Lee et al., 2014) and thus will not be described in the report.

(3) Establishment of a system to overexpress mammalian arginine methylated proteins in Escherichia coli. This part of work has been thoroughly described Part II of the master thesis of Hung-Ming Wei (Wei, 2013; 第二部分: 建構可生產已受甲基化修飾的甲基 接受蛋白之*E. coli*表現系統 Establishment of a system to overexpress mammalian arginine methylated proteins in Escherichia coli) and thus will not be described in the report. Manuscript based on the results of this part of work is under preparation.

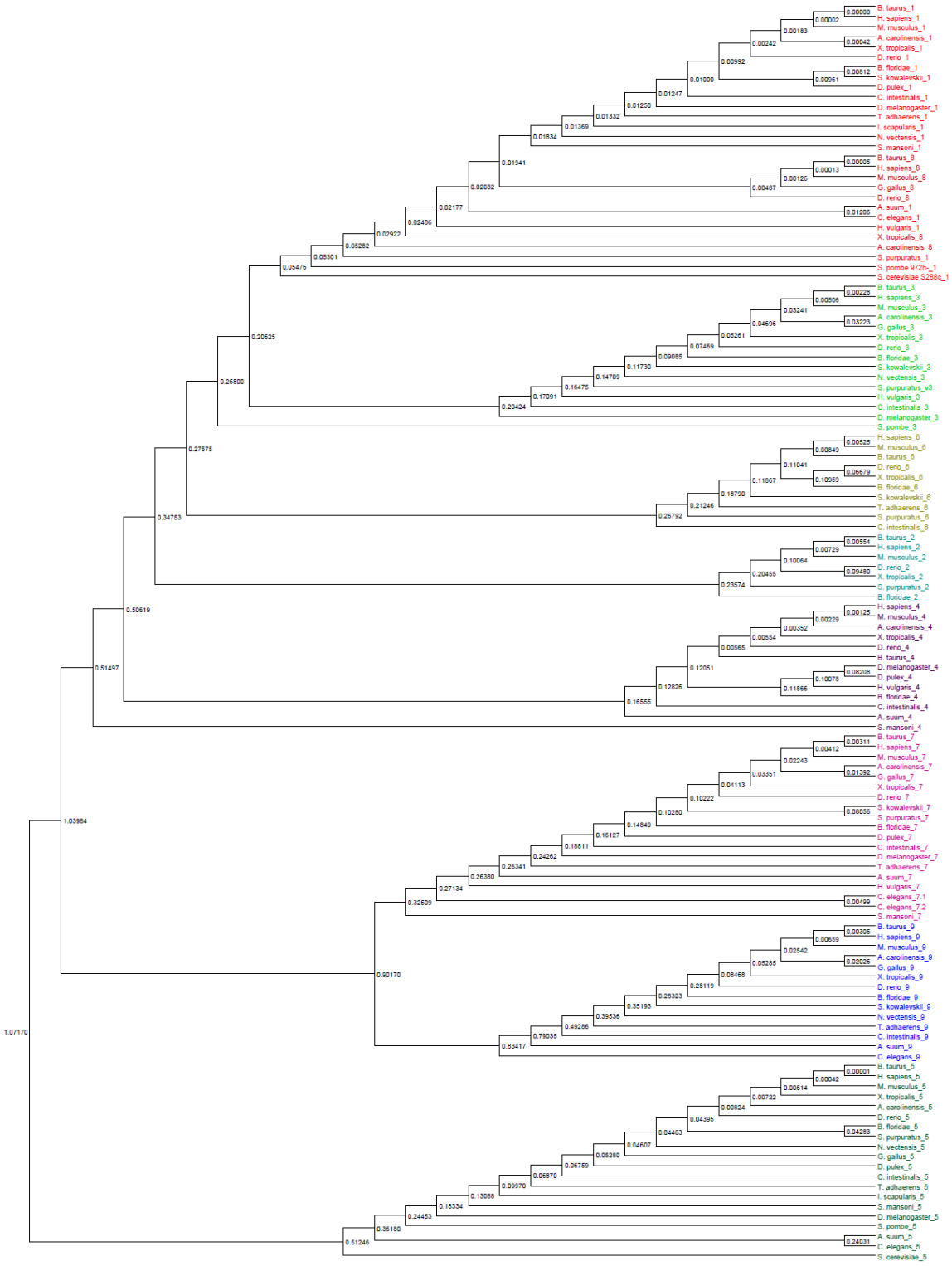

Fig. 4. phylogenetic tree of PRMTs homologues to mammalian PRMT members in different animal species.

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國科會補助計畫衍生研發成果推廣資料表

日期:2014/02/10

國科會補助計畫	計畫名稱: 建立演化上保留之蛋白質精胺酸甲基化網絡與研究平台
	計畫主持人: 李娟
	計畫編號: 101-2320-B-040-004- 學門領域: 醫學之生化及分子生物

無研發成果推廣資料

101 年度專題研究計畫研究成果彙整表

計畫主持人：李娟	計畫編號：101-2320-B-040-004-
計畫名稱：建立演化上保留之蛋白質精胺酸甲基化網絡與研究平台	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	2	2	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	
		博士生	1	1	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	2	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
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	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估 ■達成目標 □未達成目標（請說明，以 100 字為限） □實驗失敗 □因故實驗中斷 □其他原因 說明：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：■已發表 □未發表之文稿 □撰寫中 □無 專利：□已獲得 □申請中 ■無 技轉：□已技轉 □洽談中 ■無 其他：(以 100 字為限)
3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)