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Evolutionary Analysis of the *Translin- Associated Protein X* (*TraX*) Gene Family in Eukaryotes

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Abstract: *TraX is a protein that interacts with Translin, which has been proposed to be involved in mRNA transport and translation, chromosomal translocation and DNA recombination. Although numerous studies have focused on the functional identification of TraX, limited information is known about its molecular evolution in eukaryotes. In this study, I identified 89 TraX genes in 85 selected eukaryotes species and performed a comprehensive analysis to explore the evolutionary patterns of these TraX genes. The results showed that the conserved gene structure existed in each group, indicative of their functional conservation. Some dynamic and conserved evolutionary characteristics of the TraX gene family were also investigated through synteny and karyotypes analyses. In addition, adaptive evolution analysis also indicated some positive selection sites. The results will contribute to further function research in further.*

Key words: *TraX, evolution, synteny analysis, selection pressure*

1. INTRODUCTION

TraX encodes Translin- associated 33 kDa proteins partner which was first discovered in a yeast two-hybrid screen of a human cDNA library using Translin as bait (Aoki *et al.*, 1997). These two sequences have about 28% identity with each other. *TraX* orthologs and *Translin* usually exist in the same species, indicating that they are likely to play a biological role of fundamental importance (Li et al., 2008; Jaendling and McFarlane, 2010). Previous researches indicated that the Translin can mediate the combination of the Translin/TraX complex and DNA, and TraX has no measurable independent nucleic- acid- binding capability (Wu *et al*., 1998; Chennathukuzhi *et al*., 2001; Wang *et al*., 2004; Gupta *et al*., 2005; Lluis *et al*., 2010).

In some species, such as yeast, *Drosophila* and mouse, deletion of *Translin* usually leads to the loss of TraX protein, but does not affect *TraX* mRNA levels, suggesting that the presence of Translin enhancer the stability of TraX protein (Chennathukuzhi *et al*., 2003; Yang *et al*., 2004; Claussen *et al*., 2006). On the contrary, deletion of the *Drosophila TraX* does not cause the loss of Translin protein (Jaendling *et al*., 2008). Structurally, a NLS (nuclear localization signal) and a functional NES (nuclear export signal) exist in the TraX and Translin, respectively. These signals can operate the Translin/TraX complex to shuttle between the cytoplasm and nucleus and mediate the dynamic changes of the heteromeric complex in their subcellular localizations (Cho *et al*., 2004).

Several lines of evidence have demonstrated that this complex functions in a broad spectrum of biological activities. Such as, it mediates RNA trafficking in neurons (Severt *et al*., 1999; Finkenstadt

et al., 2000; Wang *et al*., 2005). Co transfection of Translin or TraX with SF- 1 (steroidogenic factor-1) transcription factor can enhance its ability to activate transcription (Mellon *et al*., 2007). Research by Sun *et al*. (2006) demonstrates that TraX can down- regulate the activation of A2A receptor activation by binding to its intracellular tail in PC12 cells. In addition, Translin/TraX is also involved in double- strand DNA damage response. DNA damaging agents (such as γ- irradiation etc.) induce the interaction between TraX and nuclear matrix protein C1D, which is essential for DNA repair (Erdemir *et al*., 2002). TraX interacts with Translin or C1D in a mutually exclusive fashion; this might be the reason why mutants of *Drosophila Translin* and *TraX* did not show an enhanced sensitivity for DNA double- strand breaks (Claussen *et al*., 2006). Maybe *Translin* and *TraX* are redundant with *C1D* gene in this DNA break and repair pathway. In addition, the Translin/TraX complex can function as a ribonuclease in tRNA processing (Li *et al*., 2012).

Except Translin, TraX is also detected to combine with other proteins and presents additional functions. A kinesin family protein, KIF2Aβ, was identified as a TraX- interacting protein in a yeast two- hybride screen. And they were shown co- localize the perinuclear region. Thus, TraX may play a part in macromolecular movement in germ line cells (Bray *et al*., 2002 and 2004). TraX also associates with male- enhanced antigen 2 (Mea2), which might be involved in spermatogenesis (Matsuda *et al*., 2004). TraX coexpresses with growth- associated protein GAP- 43 and regulates its transcription and regeneration (Schröer *et al*., 2007). Moreover, some G- protein- regulated enzyme, such as phospholipase Cβ1 (PLCβ1), is likely involved in the RNA interference through TraX (Philip *et al*., 2012).

Despite the fact that some functions of TraX or Translin/TraX complex have been investigated, very little information about the *TraX* gene evolution can be obtained in eukaryotes (Gupta *et al*., 2012). In this study, genome- wide analysis of the *TraX* gene family was performed in eukaryotes.

2. MATERIALS AND METHODS

2.1. Sequence Retrieval And Characterization Analysis

Multiple database searches were performed to identify potential members of the *TraX* gene family in eukaryotes. Human TraX sequence (Meng *et al*., 2000) was used as queries in BLAST searches against the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov), JGI Genome Portal (http://genome.jgi.doe.gov) and the *Cyanidioschyzon merolae* Genome Project (http://merolae.biol.s.u- tokyo.ac.jp). Compute pI/Mw (http://web.expasy.org/compute_ pi/) was used to estimate the molecular weights and isoelectric points of TraXs. And CD- Search analyses (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchler- Bauer *et al*., 2011) were also used to examine potential domains for their authenticity. Protein subcellular localization and DNA- binding site were predicted using the WoLF PSORT (http://wolfpsort.org) (Horton *et al*., 2007) and DP- Bind web- server (http://lcg.rit.albany.edu/dp–bind/) (Hwang *et al*., 2007), respectively.

2.2. Phylogenetic and synteny analyses of the *TraX* gene family in eukaryotes

Firstly, MUSCLE 3.52 (Edgar, 2004) was used to perform the multiple sequence alignments of full-length protein sequences. And then, MEGA 5 (Tamura *et al*., 2011) was used to carry out phylogenetic analyses of the TraX proteins based on amino acid sequences with maximum likelihood (ML) method. ML analyses were done using a partial deletion of gaps (95%), uniform rates among sites and Poisson substitution model. Support for each node was also tested with 1000 bootstrap replicates. Genomicus (http://www.dyogen.ens.fr/genomicus) (Muffato *et al*., 2010) was used to compare different animals to determine and refine the orthologous gene sets of human *TraX* gene.

2.3. Exon- intron structure, intron phase and conserved motifs analyses

Gene organization was deduced after merging the genomic sequences with CDSs and ESTs. Phase 0, 1, and 2 intron was defined as it falls before the first, second, and third bases of a codon, respectively (Sharp, 1981). The MEME program (http://meme.sdsc.edu) (Bailey *et al*., 2006) was used to identify potential motifs in the TraX proteins with the parameters: maximum number of motifs = 15, number of repetitions = any, and with optimum motif widths constrained to between 6 and 50 residues.

2.4. Positive selection assessment of the TraX genes in different evolutionary branches

Selecton Server (http://selecton.tau.ac.il/) (Stern *et al*., 2007) was used to calculate and identify site-specific positive and purifying selection. In this study, I used five evolutionary models (M8, M8a, M7, M5 and MEC) to test the data. Each model uses different biological hypotheses to account for heterogeneous K_a/K_s values among sites (Stern *et al*., 2007).

3. RESULTS AND DISCUSSION

3.1. Identification and characterization of the TraX gene family in eukaryotes

In this study, 89 TraX sequences from 85 selected species were obtained in eukaryotes (Table S1). TraX genes in eukaryotes encoded for polypeptides ranging from 93 to 395 amino acids in length, with predicted an isoelectric point (pI) ranging from 4.38 to 9.79. Protein subcellular localization was also predicted using the WoLF PSORT (http://wolfpsort.org/) software (Horton et al., 2007). It was found that most candidates TraXs identified in our study are likely to be localized in the cytoplasm, nucleus or extracellular regions (Table S1). Next, I also identified some candidate DNA- binding residues based on the consensus sites detected by the DP- Bind web server (http://lcg.rit.albany.edu/dp–bind/) (Hwang et al., 2007) in TraX proteins. The results found that one to six motifs were predicted to be present in TraX sequences. About 43.8% members possessed four DNA- binding motifs. TraX of rice was predicted to hold one DNA- binding residue, while TraXs in mouse and turkey constitute the largest number of DNA- binding motifs, each containing six motifs (Table S1).

3.2. Gene organization and motif distribution of the TraX members in eukaryotes

Phylogenetic analyses of the TraX protein sequences were also performed based on the maximum likelihood (ML) method of using MEGA 5 (Tamura et al., 2011). In general, some species- special groups were appeared, such as TraXs in all of plants were clustered in one subclass, while TraXs in most insects were also clustered in another subclass. Gene organization can provide some clues for evolutionary relationships. To examine the mechanisms of structural evolution of TraX orthologs, I compared the exon- intron organization of the TraX genes in eukaryotes. A detailed illustration about intron distribution of each TraX ortholog is shown in Fig. 1. It suggests that the phases and positions of some introns are conserved in the orthologous genes, whereas others are group- specific. From Fig. 1, I also found that most of TraX members in fungi and protists hold fewer introns. While TraXs in plants and vertebrates possessed more introns, suggesting that intron insertions (or gains) have occurred in these two species. Similarly, numerous intron gains also occurred in some lower animals TraX genes, such as in Monosiga brevicollis and Strongylocentrotus prupuratus. Interestingly, intron gains do not occur in insects TraX genes, that is, most of the insects TraX genes possessed similar number of introns as them in fungi and protists. In a word, the gene organization of the TraX family is dynamic, implying that numerous intron losses or gains exist in the evolution of the TraX genes. Introns are important components of eukaryotic genes, and their gains and losses can produce

organizational complexity, which is the basis of multiple gene family evolution (Cao et al., 2010; Cao and Li, 2015). In addition, several researchers have indicated significant roles of introns in genetic evolution (Castillo- Davis et al., 2002). Divergent exon- intron organization between different TraX branches might have some evolutionary functions. While, similar exon- intron structures in the same group strongly support their close evolutionary relationship.

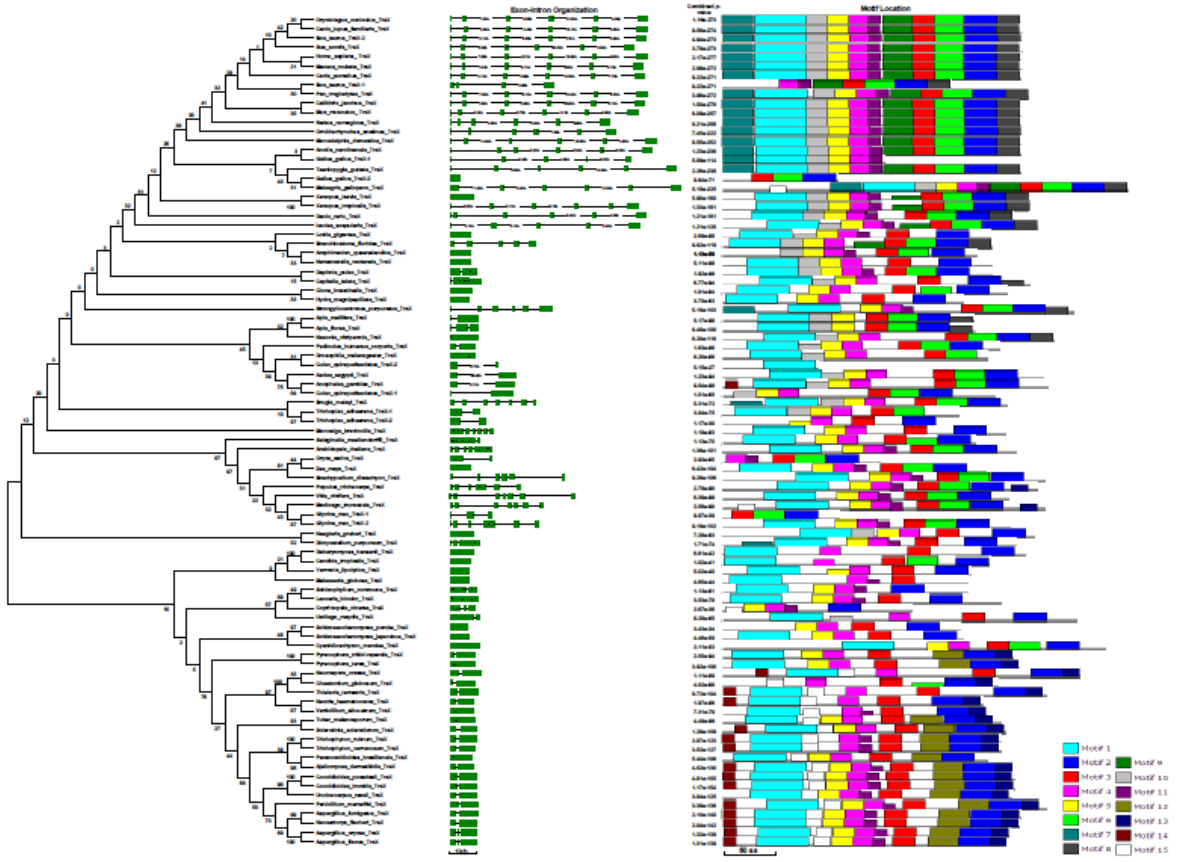

Fig1. *Phylogenetic relationship, gene organization and motif composition. Phylogeny tree was constructed using full- length TraX protein sequences in the left panel. Gene organization of the TraX genes is shown in the middle panel. Green boxes stand for exons and black lines for introns. A motif composition in each TraX protein is displayed in the right panel.*

CD- Search analyses (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchler- Bauer *et al.*, 2011) were used to identify the major domains of TraX proteins in eukaryotes. And all TraX proteins possessed a structurally conserved translin domain that is essential for DNA binding activity (Gupta and Kumar, 2012). However, some smaller motifs can not be recognized by this tool. Therefore, the MEME program (http://meme.sdsc.edu) (Bailey *et al.*, 2006) was used for further analysis and fifteen distinct motifs were identified in the eukaryotic TraX proteins (Fig. 1) (Table 1). Interestingly, I also found that most of the TraX members between the similar species usually have similar motif compositions (Fig. 1). Most members of vertebrates groups possess 11 motifs, while most members of plants group have 7 motifs. All TraX proteins shared five motifs (Motif 1, 2, 3, 4 and 5). While I also found some specific motifs existing in specific groups, such as Motif 7 is specific to the vertebrates group, and Motif 12 and Motif 13 are specific to the fungi and protests group. Whether motifs that are peculiar to some groups have unique functional roles remain to be further investigated.

Table 1. *Motif sequences identified by MEME tools.*

Motif	Multilevel consensus sequence
1	SS[PA][VI]MxAFKS[FY][RQ]QELD[EA][RH]HD[KR][RY]ER[LIV][VI]K[LA]SRD[IV]T[IA][EL]SK[RK][IT]IFLLHRITSA[NP]
2	P[YKR][ED][VL]S[KR]K[LM][YE][VT][LM][KR]QS[LV][AE]K[VI]ENACY[GAT][LI]KVRGSE
3	DYLLG[VLI][AF]DLTGELMR[FLM][CA]I[NT]SV
4	[LI]QE[YF][VI]EA[VL][ST]FQH[FY][LI][KE]T[RGQ][ST]L
5	Q[IV]A[QK]ELxGEDM[YWH][QR][YF]HR[AQ][YI][ST]PG
6	[GS]NG[DE]I[DE]T[PA]FE[VIL][SC]QF[LV]R[QD][VI]Y[DR][GE][FL][ST][FL]I[GV]NTG
7	MS[GNS]KEGSGGFRKRKHDNFPH[NG]QRRE[GE]KD[VN]
8	PKHMLADVFSVK[TA]EMIDQE[ED]G
9	E[DE][NS]G[KE]ENKTP[SP]SDAQDKQ[FL][GV]TW[RS]L[KR][VI]TPV
10	[DN][MK]E[ED][IV]L[TN]E[SA]EIKLD[GA]VRQKI[LKF]
11	I[ST][LM][DE]E[IV]NKQLI
12	[GP]x[SE][QV]AG[IV][LV]VDLR[EQ][LM]R[AS]M[FL]EKL[NDS]VPR[GR]H[SN]L
13	RP[KS]GW[VMI]PD[LM]S[SAG]AA[AD][EGV]
14	M[AS]G[NT]KR[SD][WR][DE]G[NK]
15	IAKE[TN]D[DS]R[FL]AQI[QS][KT]L

Numbers correspond to the motifs described in Supplementary Figure S1.

3.3. Conserved synteny analyses and karyotypes evolution near TraX in some vertebrates

Synteny analysis can establish the relationship between orthologous genes (Chen et al., 2014). In this study, the Genomicus (http://www.dyogen.ens.fr/genomicus) (Muffato et al., 2010) was used to search the conserved synteny regions between different genomes. I found that gene clusters near TraX show conserved synteny in most identified vertebrates, indicating partially conservation near the TraX region between fishes,birds and mammal(Fig.2A)

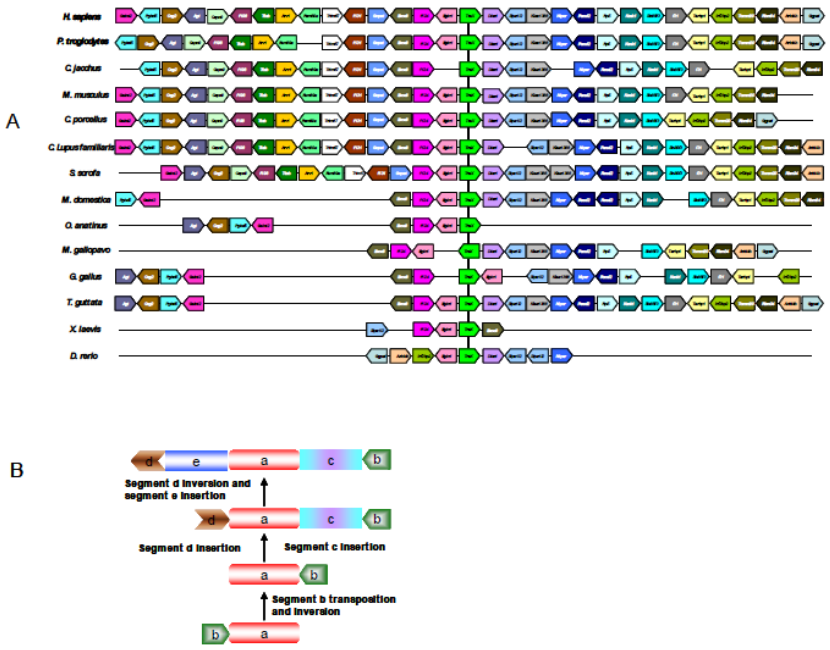

Fig2. *Conserved synteny around TraX genes. Genomicus (http://www.dyogen.ens.fr/genomicus) was used to determine the synteny groups. Orthologous genes of the TraXs are shown in light green over a vertical line. Other orthologues are also shown with the same color.*

Next, I also reconstructed ancestral karyotypes near the TraX loci based on the conserved synteny

analyses as described above. As Fig. 2B shown, the ancestral karyotypes were predicted to solely consist of segment b (Ggps1- Arid4b- Irf2bp2 gene clusters) and segment a (Egln1- TraX- Disc1- Sipa1i2- Ntpc1 gene clusters). Before Birds appearing, at least three segmental rearrangement events have been occurred. i: segment b transposed and inversed into the other side of the segment a; ii: segment c (Pcnxl2- Rp5- Kcnk1- Slc35f3- f31- Tarbp1 gene clusters) inserted between segments a and b; iii: segment d inserted near segment a. This genetic structure near TraX loci continued to the appearing of Marsupialia species about 148 million years ago. After that, two more segmental rearrangement events have been occurred on the other mammals. One event is that segment d (Agt- Cog2- Pgbd5- Galnt2 gene clusters) inversed. The other one is that segment e (Capn9- f198- Ttcb- Arv1- Fam89a- Trim67- f131- Gnpat gene clusters) inserted between segments a and d. This structure evolution was relatively conserved in vertebrate's genomes with some exceptions. For instance, some gene duplication events (Sipa1i2 gene in Danio rerio; Kiaa1388 gene in Sus scrofa) have been occurred. Further gene loss (Egln1 gene in Callithrix jacchus; f31 gene in Cavia porcellus; Pgbd5 and Exoc8 genes in Sus scrofa) has been similarly appeared. Intriguingly, a larger segmental lost (containing segment b, c and most of the segment a) has been occurred in Ornithorhynchus anatinus. These changes might be attributed to comparatively recent segmental duplication or loss events, showing different patterns of genomic evolution between species.

3.4. Site- specific selective pressures analysis

Ka/Ks ratio stands for the selection pressure. A Ka/Ks ratio less than 1 suggests purifying selection and a ratio greater than 1 suggests positive selection (Yang and Bielarski, 2000; Hurst, 2002). In protein evolution, different residues are expected to undergo different selective pressures, which is a useful means for detecting functional residues changes (Morgan et al., 2010). The Selecton Server (http://selecton.tau.ac.il) (Stern et al., 2007) was used to analyze selection pressure of different residues in full- length TraX protein sequences among Vertebrates, Arthropods, Lower Animals, Plants, and Fungi- Protists branches. The results showed that the Ka/Ks ratios of the sequences from different evolutionary branches were significantly different (Table 2). I used five evolution models (M8, M8a, M7, M5 and MEC) to check these data and found that M8a, M7 and M5 models do not detect any positively selection sites, whereas MEC model detected some consequences for these branches. Except one positively selected site was predicted in Plants, M8 model does not identify any positively selection sites among other evolutionary branches (Table 2). Fig. 3 showed different selection pressure on each amino acid residue of Plants TraX proteins predicted by the MEC model. A total of 4 positively selected sites (sites 4, 18, 68 and 191) were identified. Interestingly, some strong purifying selection sites are predominantly located in the alpha helix- 4, - 5, - 6 and - 9, suggesting the conservation within these regions and importance for the TraX function.

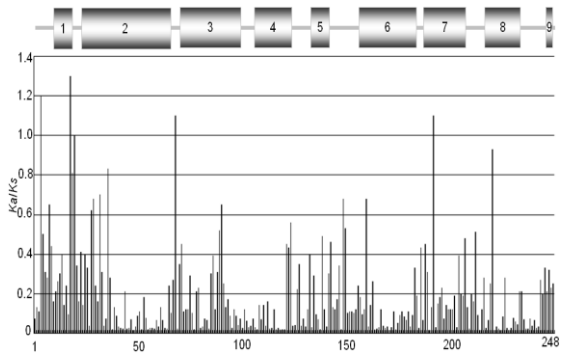

Fig3. *Site specific profiles for evolutionary rate changes in the Plants TraX protein. A histogram of the K_a/K_s ratios for each amino acid residue of TraX protein in Plants is shown below. The predicted secondary structure of Plants TraX protein is shown above. Nine alpha helixes are also marked from 1 to 9.*

Table2. *Selection pressure assessment of the TraX gene in different evolutionary branches*

Branches	Selection model	*Ka/Ks**	Log-likehood	Positive selection sites
	M8(beta+w>=1)	0.1295	-7977.04	Not Found
	M8a(beta+w=1)	0.1316	-7972.39	Not Found
	M7(beta)	0.1277	-7977.13	Not Found
	M5(gamma)	0.1384	-7973.21	Not Found
Vertebrates	MEC	0.1389	-7855.01	174,184,
	M8(beta+w>=1)	0.2345	-8503.69	Not Found
	M8a(beta+w=1)	0.2313	-8500.91	Not Found
	M7(beta)	0.2222	-8500.67	Not Found
	M5(gamma)	0.2769	-8511.09	Not Found
Arthropods	MEC	0.2090	-8270.16	252,
	M8(beta+w>=1)	0.3489	-11450.6	Not Found
	M8a(beta+w=1)	0.3489	-11449.2	Not Found
	M7(beta)	0.3426	-11450.2	Not Found
	M5(gamma)	0.3954	-11457	Not Found
Lower Animals	MEC	0.3506	-11180	51,59,95,232,243,
	M8(beta+w>=1)	0.2093	-5780.23	**18**,
	M8a(beta+w=1)	0.2104	-5780.09	Not Found
	M7(beta)	0.1961	-5780.77	Not Found
	M5(gamma)	0.2138	-5779.21	Not Found
Plants	MEC	0.1861	-5656.8	4,**18**,68,191,
	M8(beta+w>=1)	0.3039	-24829.6	Not Found
	M8a(beta+w=1)	0.2966	-24821.5	Not Found
	M7(beta)	0.2848	-24833.5	Not Found
	M5(gamma)	0.3304	-24865.5	Not Found
Fungi and Protists	MEC	0.2414	-24193.6	137,138,181,184,187,190,222,

**Ka/Ks ratio is an average over all sites of gene branch alignments. Bold sites indicate codons that were at least identified with two methods.*

4. CONCLUSION

A comparative genomic analysis addressing gene structure, synteny and karyotypes analyses, selective pressures and functional networks of the eukaryotes TraX gene family was provided in this study. The highly conserved gene organization in each group suggests functional conservation. The synteny analyses and karyo types evolution of the TraX in some mammals implied conserved and dynamic evolution characteristics of this gene family and genome segments located by TraX. An additional adaptive evolution analyses identified some site- specific selective change in several TraX orghologs. This study will provide useful information for further functional investigations of this gene family in future.

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REFERENCES

[1] Aoki K, Ishida R, Kasai M (1997). Isolation and characterization of a cDNA encoding a Translin-like protein, TRAX. FEBS Lett. 401: 109- 112.

[2] Bailey TL, Williams N, Misleh C, Li WW (2006). MEME: discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Res. 34: W369- 373.

[3] Bray JD, Chennathukuzhi VM, Hecht NB (2002). Identification and characterization of cDNAs encoding four novel proteins that interact with translin associated factor- X. Genomics. 79: 799-808.

[4] Bray JD, Chennathukuzhi VM, Hecht NB (2004). KIF2Aβ: a kinesin family member enriched in mouse male germ cells, interacts with translin associated factor- X (TRAX). Mol Reprod Dev. 69: 387- 396.

[5] Cao J, Li X (2015). Identification and phylogenetic analysis of late embryogenesis abundant proteins family in tomato (*Solanum lycopersicum*). Planta. 241: 757- 772.

[6] Cao J, Shi F, Liu X, Huang G, Zhou M (2010). Phylogenetic analysis and evolution of aromatic amino acid hydroxylase. FEBS Lett. 584: 4775- 4782.

[7] Castillo- Davis CI, Mekhedov SL, Hartl DL, Koonin EV, Kondrashov FA (2002). Selection for short introns in highly expressed genes. Nat Genet. 31: 415- 418.

[8] Chen Y, Hao X, Cao J (2014). Small auxin upregulated RNA (*SAUR*) gene family in maize: Identification, evolution, and its phylogenetic comparison in *Arabidopsis*, rice, and sorghum. J Integr Plant Biol. 56: 133- 150.

[9] Chennathukuzhi V, Stein JM, Abel T, Donlon S, Yang S, Miller JP, Allman DM, Simmons RA, Hecht NB (2003). Mice deficient for testis–brain RNA- binding protein exhibit a coordinate loss of TRAX, reduced fertility, altered gene expression in the brain, and behavioral changes. Mol Cell Biol. 23: 6419- 6434.

[10] Chennathukuzhi VM, Kurihara Y, Bray JD, Hech NB (2001). Trax (translin- associated factor X), a primarily cytoplasmic protein, inhibits the binding of TB- RBP (translin) to RNA. J Biol Chem. 276: 13256- 13263.

[11] Cho YS, Chennathukuzhi VM, Handel MA, Eppig J, Hecht NB (2004). The relative levels of Translin- associated factor X (TRAX) and testis brain RNA- binding protein determine their nucleocytoplasmic distribution in male germ cells. J Biol Chem. 279: 31514- 31523.

[12] Claussen M, Koch R, Jin ZY, Suter B (2006). Functional characterization of Drosophila translin and Trax. Genetics. 174: 1337- 1347.

[13] Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32: 1792- 1797.

[14] ErdemirT, BilicanB, OncelD, GodingCR,YavuzerU(2002)DNA damage- dependent interaction of the nuclear matrix protein C1D with Translin- associated factor X (TRAX). J Cell Sci. 115: 207-216.

[15] Finkenstadt PM, Kang WS, Jeon M, Tang W, Baraban JM (2000). Somatodendritic localization of Translin, a component of the Translin/Trax RNA binding complex. J Neurochem. 75: 1754-1762.

[16] Gupta GD, Kale A, Kumar V (2012). Molecular evolution of translin superfamily proteins within the genomes of eubacteria, archaea and eukaryotes. J Mol Evol. 75: 155- 167.

[17] Gupta GD, Makde RD, Kamdar RP, D'Souza JS, Kulkarni MG, Kumar V, Rao BJ (2005). Co-

expressed recombinant human Translin–Trax complex binds DNA. FEBS Lett. 579: 3141- 3146.

[18] GuptaGD, KumarV(2012).Identification of nucleic acid binding sites on translin-associated factor X (TRAX) protein. PLoS One. 7(3): e33035.

[19] Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams- Collier CJ, Nakai K (2007). WoLF PSORT: protein localization predictor. Nucleic Acids Res. 35: W585–587.

[20] Hurst LD (2002). The *Ka*/*Ks* ratio: diagnosing the form of sequence evolution. Trends Genet. 18: 486–487.

[21] Hwang S, Gou Z, Kuznetsov IB (2007). DP- Bind: a web server for sequence- based prediction of DNA- binding residues in DNA- binding proteins. Bioinformatics. 23: 634- 636.

[22] JaendlingA,McFarlaneRJ(2010)Biological roles of translin and translin- associated factor-X: RNA metabolism comes to the fore. Biochem J. 429: 225- 234.

[23] Jaendling A, Ramayah S, Pryce DW, McFarlane RJ (2008). Functional characterisation of the *Schizosaccharomyces pombe* homologue of the leukaemia- associated translocation breakpoint binding protein translin and its binding partner, TRAX. Biochim Biophys Acta. 1783: 203- 213.

[24] Li L, Gu W, Liang C, Liu Q, Mello CC, Liu Y (2012). The translin- TRAX complex (C3PO) is a rebonuclease in tRNA processing. Nat Struct Mol Biol. 19(8): 824- 830.

[25] Li Z, Wu Y, Baraban JM (2008). The Translin/Trax RNA binding complex: clues to function in the nervous system. Biochim Biophys Acta. 1779: 479- 485.

[26] Lluis M, Hoe W, Schleit J, Robertus J (2010). Analysis of nucleic acid binding by a recombinant translin- TRAX complex. Biochem Biophys Res Commun. 396: 709- 713.

[27] Marchler- Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese- Scott C, Fong JH, Geer LY, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Lu F, Marchler GH, Mullokandov M, Omelchenko MV, Robertson CL, Song JS, Thanki N, Yamashita RA, Zhang D, Zhang N, Zheng C, Bryant SH (2011). CDD: A conserved domain database for the functional annotation of proteins. Nucleic Acids Res. 39: D225- 229.

[28] Matsuda M, Kondo M, Kashiwabara S, Yoshihara M, Sutou S, Matsukuma S (2004). Co-localization of Trax and Mea2 in Golgi complex of pachytene spermatocytes in the mouse. J Histochem Cytochem. 52: 1245- 1248.

[29] Mellon SH, Bair SR, Depoix C, Vigne JL, Hecht NB, Brake PB (2007). Translin coactivates steroidogenic factor- 1- stimulated transcription. Mol Endocrinol. 21: 89- 105.

[30] Meng G, Aoki K, Tokura K, Nakahara K, Inazawa J, Kasai M (2000). Genomic structure and chromosomal localization of the gene encoding TRAX, a Translin- associated factor *X*. J Hum Genet. 45: 305- 308.

[31] Morgan CC, Loughran NB, Walsh TA, Harrison AJ, O'Connell MJ (2010). Positive selection neighboring functionally essential sites and disease- implicated regions of mammalian reproductive proteins. BMC Evol Biol. 10: 39.

[32] Muffato M, Louis A, Poisnel CE, Roest Crollius H (2010). Genomicus: a database and a browser to study gene synteny in modern andancestral genomes. Bioinformatics. 26: 1119-1121.

[33] Philip F, Guo Y, Aisiku O, Scarlata S (2012). Phospholipase Cβ1 is linked to RNA interference of specific genes through translin- associated factor X. FASEB J. 26: 4903- 4913.

[34] Schröer U, Volk GF, Liedtke T, Thanos S (2007). Translin- associated factor- X (Trax) is a molecular switch of growth- associated protein (GAP)- 43 that controls axonal regeneration. Eur

J Neurosci. 26: 2169- 2178.

[35] Severt WL, Biber TU, Wu X, Hecht NB, DeLorenzo RJ, Jakoi ER (1999). The suppression of testis- brain RNA binding protein and kinesin heavy chain disrupts mRNA sorting in dendrites. J Cell Sci. 112: 3691- 3702.

[36] Sharp PA (1981). Speculations on RNA spicing. Cell. 23: 643- 646.

[37] Stern A, Doron- Faigenboim A, Erez E, Martz E, Bacharach E, Pupko T (2007). Selecton 2007: advanced models for detecting positive and purifying selection using a Bayesian inference approach. Nucleic Acids Res. 35: W506- 511.

[38] Sun CN, Cheng HC, Chou JL, Lee SY, Lin YW, Lai HL, Chen HM, Chern Y (2006). Rescue of p53 blockage by the A2a adenosine receptor via a novel interacting protein, translin- associated protein X. Mol Pharmacol. 70: 454- 466.

[39] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol. 28: 2731- 2739.

[40] Wang H, Iacoangeli A, Lin A, Williams K, Denman RB, Hellen CU, Tiedge H (2005). Dendritic BC1 RNA in translational controlmechanisms. J Cell Biol. 171: 811- 821.

[41] Wang J, Boja ES, Oubrahim H, Chock PB (2004). Testis brain ribonucleic acid- binding protein/translin possesses both single- stranded and double- stranded ribonuclease activities. Biochemistry. 43: 13424- 13431.

[42] Wu XQ, Xu L, Hecht NB (1998). Dimerization of the testis brain RNA- binding protein (translin) is mediated through its C- terminus and is required for DNA- and RNA- binding. Nucleic Acids Res. 26: 1675- 1680.

[43] Yang S, Cho YS, Chennathukuzhi VM, Underkoffler LA, Loomes K, Hecht NB (2004). Translin-associated factor X is post- transcriptionally regulated by its partner protein TB- RBP, and both are essential for normal cell proliferation. J Biol Chem. 279: 12605- 12614.

[44] Yang Z, Bielawski JP (2000). Statistical methods for detecting molecular adaptation. Trends Ecol Evol. 15: 496–503.