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No accelerated evolution of 3′UTR region in human for brain-expressed genes☆

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Abstract

The difference in cognitive skills between humans and nonhuman primates is one of the major characters that define our own species. It was previously hypothesized that this divergence might be attributable to genetic differences at gene expression level, and the *cis*-regulating elements of gene 3′UTRs might play important roles in the post-transcriptional regulation of gene expression. In this study, we constructed a cDNA library from the prefrontal cortex of rhesus monkey and generated a total of 754 high-quality ESTs. Using rhesus macaque as outgroup, we calculated the evolutionary rates of the 3′UTRs of 52 brain-expressed genes in humans and chimpanzees in order to dissect the role of natural selection during primate brain evolution. Comparison of 52 orthologous gene sequences of human and chimpanzee indicated that the mean substitution rates at nonsynonymous sites (K_a), synonymous sites (K_s) and 3′UTRs (K_{3u}) are 0.0024, 0.0116 and 0.0117, respectively. Relative rate tests and acceleration index tests demonstrated that only a few genes had significant rate divergence between human and chimpanzee. The 3′UTRs of the brain-expressed genes in primates has a similar evolutionary rate with the synonymous sites of the gene coding region, indicating a neutral evolution of the 3′UTR sequences in human.

Keywords: Gene expression; Rhesus monkey; Substitution rates; Neutral evolution

1. Introduction

There are marked differences in morphological, behavioral and especially cognitive aspects between humans and nonhuman primates though the genome sequence divergence between them is small. For example, there is only about 1.2% sequence divergence between human and chimpanzee genomes (Goodman et al., 1998; Chen and Li, 2001; Ebersberger et al., 2002; Fujiyama et al., 2002), and the sequence difference between human and rhesus monkey is only about 5%–7% through a 20–25 million-year divergence (Savatier et al., 1987; Kawamura et al., 1991; Osada et al., 2002; Wang et al., 2003). It was speculated that the phenotypic divergence between human and chimpanzee is attributable more to expression difference than to difference in gene coding regions (King and Wilson, 1975; Enard et al., 2002). Recently, using microarray technology, by comparing the expression patterns in brain, liver and leukocytes from humans, chimpanzees and other primates, Enard et al. concluded that species-specific differences in the overall gene expression patterns were particularly pronounced in the human brain due to the up-regulation of the human genes (Enard et al., 2002). Reanalyses with more robust statistical method based on the above microarray data confirmed the observation (Gu and Gu, 2003). Another study by Caceres et al. also indicated that genes involved in expression changes of the human cortex predominantly increased expression level, and that many of the genes up-regulated in humans could be related to higher levels of neuronal activities (Caceres et al., 2003).

The observed divergence of gene expression between humans and nonhuman primates can be caused by *cis*- and/or *trans*-regulatory changes (Wittkopp et al., 2004). It was

Abbreviations: 3′UTRs, 3′untranslated regions of genes; PFC, prefrontal cortex; ESTs, expressed sequence tags.

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reported that the between-species *cis*-regulatory changes are more common than the *trans*-regulatory changes based on the study of *Drosophila* (Wittkopp et al., 2004). It is well known that 3′UTRs (3′untranslated regions of genes) play key roles in the post-transcriptional regulation of gene expression (Bashirullah et al., 2001). They could modulate mRNA stability (Mignone et al., 2002), subcellular localization (Jansen, 2001), translation efficiency and transportation of mRNA out of the nucleus (Van Der Velden and Thomas, 1999). These regulations are important steps in cell proliferation, cell differentiation, embryogenesis, neurogenesis, sex determination and erythroporesis (Kuersten and Goodwin, 2003). The stability of mRNA directly affects the level of gene expression as well as the translation of proteins (Ewa et al., 2001). These modulating forms are carried out by the interaction of RNA-binding proteins and the *cis*-acting regions in 3′UTRs. Hence, mutations in the 3′UTR *cis*-regulating sequences may affect the interaction of mRNA and the regulatory proteins, and eventually alter the level of gene expression and protein production.

Previous study based on the comparison of human/mouse gene sequences showed that compared to the coding regions, the 3′UTRs are rarely conserved (Duret and Mouchiroud, 2000). However, the mutation patterns of 3′UTRs in humans and nonhuman primates were poorly investigated though distinctive expression profiles between them were observed. A recent study, by comparing 5055 expressed sequence tags between humans and chimpanzees concluded that the 3′UTRs are more variable than the 5′UTRs, which is consistent with the comparison of human/mouse gene sequences (Duret and Mouchiroud, 2000; Hellmann et al., 2003). However, without a close relative species as an outgroup, it is difficult to conduct lineage-specific analysis, which are critical to an in-depth understanding of the evolution patterns of gene expression during primate evolution.

In this study, by constructing a cDNA library from the prefrontal cortex (PFC) of rhesus monkey brain, we obtained 754 3′UTR sequences (ESTs), representing 61 genes expressed within the PFC. Using the rhesus monkey as the outgroup, we calculated the evolutionary rates of the 3′UTRs of human and chimpanzee. Our results showed that only a few genes showed rate differences between human and chimpanzee, and the substitution rates of the 3′UTRs (K_{3u}) did not show significant difference from the synonymous substitution rate (K_s) in the gene coding regions, which supports a neutral evolution of the 3′UTRs in primates.

2. Materials and methods

2.1. Construction of rhesus monkey PFC cDNA library and sequencing of cDNA clones

Brain PFC tissue was from an adult male rhesus monkey (*Macaca mulatta*) which was sacrificed for neurosurgical experiments. The animal protocol was approved by the Internal Review Board of Kunming Institute of Zoology, CAS. Fresh PFC tissue from rhesus monkey was dissected under anaesthesia and immediately placed into liquid nitrogen for storage. The total RNA was isolated from the PFC tissue with Trizol (*Invitrogen*) by standard protocol of RNA extraction. The mRNA was purified from total RNA by *Rneasy Mini Kit* (*QIAGEN*). High-quality mRNA was used to construct the cDNA library in a modified pBluescript II SK(+) vector with cDNA library construction kit (*Invitrigen*) according to manufacturer's instructions. The cDNA clones were randomly picked for sequencing with T3 primer and *BigDy2.0* kit (*ABI*) on an *ABI* 3100 automated sequencer.

2.2. Sequence alignment and blast search

After discarding short sequences (less than 100 bp), the 754 ESTs were aligned into 61 contigs by DNAstar SeqMan. With the use of the 61 cDNA contigs as query sequences, we performed blast search in the human and mouse RefSeq database (Pruitt et al., 2003) and chimpanzee RefSeq in Ensembl Genome Browser database at a cutoff *E*-value of $<10^{-45}$ and Scor >100. If several blasted sequences of the same species have identical *E*-value and score, they were all considered as orthologous genes. After the human's orthologous genes were identified, we used them (including the coding regions) as query sequences to blast orthologous genes of chimpanzee and mouse.

These orthologous sequences from human, chimpanzee, rhesus monkey and mouse were aligned by ClustalW (Thompson et al., 1994) in DAMBE software package (Xia and Xie, 2001) with the default parameters and the gaps removed before rate analysis. The alignments were checked by eyes to correct errors.

2.3. Data analysis

In coding region, the number of substitutions per nonsynonymous site (K_a), the number of substitutions per synonymous site (K_s) (Li, 1993; Pamilo and Bianchi, 1993) and the number of substitutions per site (K_{3u}) in 3′UTRs were calculated by *Mega2.0* program (Kumar et al., 2001) using Kimura 2-parameter model (Kimura, 1980). We used the *Fisher's Exact test* to test whether K_{3u} is significantly different from K_s. The relative rate tests of 3′UTRs of human and chimpanzee were carried out by Tajima's method (Tajima, 1993). An acceleration index for the human lineage (branch 1, Fig. 2) were defined following the method by Zhang et al. (2002). For the 3′UTRs, the numbers of nucleotide substitutions in branch 1, 2, 3+4 were h, c, and r which were derived from branch length estimates of the tree containing human, chimpanzee, and rhesus monkey genes (Fig. 2). The substitution numbers were calculated with *p*-distance. The divergence time between species followed previous studies with ~23million years between human and rhesus monkey (Goodman et al., 1998; Kumar and Hedges, 1998; Springer et al., 2003) and ~5.5 million years between human and chimpanzee (Goodman et al., 1998; Chen and Li, 2001; Stauffer et al., 2002). The acceleration index of human lineage (branch 1) comparing to rhesus lineage (branch3+4) is defined by $\lambda=(h/5.5)/[r/(2*23-5.5)=7.364h/r$ and that of the chimpanzee lineage (branch2) comparing to rhesus lineage (branch3+4) is defined by $\kappa=(c/5.5)/[r/(2*23-5.5)=7.364c/r$. When h, c and r are given, statistical significance [$p(\lambda)$ or $p(\kappa)$] of rate enhancement in the human lineage or the chimpanzee lineage were tested by the tail probability in a binomial distribution of ***B***

($h+r$, 0.1196) or ***B***($c+r$, 0.1196). Here, 0.1196=5.5/46, the time span of branch 1 comparing to that of branch 1+3+4 (Zhang et al., 2002).

3. Result

3.1. cDNA library construction, blast search and sequence alignment

A cDNA library was constructed from the prefrontal cortex (PFC) of rhesus monkey brain and a total of 754 high-quality ESTs were obtained by sequencing the 3′UTR regions of the genes. The 754 ESTs were aligned into 61 unique EST contigs representing 61 unique genes. Among the 61 genes, the longest is 842 bp, the shortest is 101 bp and the average length is 441 bp. By blast search against the public database with the 61 genes as query sequences, we identified 52 and 42 orthologous sequences in human/chimpanzee and mouse respectively. By comparison of these orthologous gene sequences with ClustalW (Thompson et al., 1994), we obtained 52 pairs of orthologous gene sequences of human/chimpanzee, 52 pairs of human/rhesus and 42 pairs of human/mouse. The coding region sequences of human, chimpanzee and mouse were obtained from the public database.

3.2. Expression patterns

Of the 52 human genes, 4 genes are neuro-specific (7.7%). There are 9 genes (17%) with unknown function and 9 genes without homologs in the human genome, and the other genes were considered housekeeping genes based on their ubiquitous expression pattern. The majority of the housekeeping genes are involved in basic metabolism of the brain, such as cell division and differentiation, energy metabolism, signal transduction etc. According to the relative abundance of clones, the highest expressed gene is dehydrogenase/reductase (SDR family) member 8 (108 clones), and there are 13 genes with single clones. The expression level of the four neuro-specific genes are relatively low (1, 2, 5, 46 clones respectively) (supplementary material).

3.3. Substitution rates and divergence of coding regions and 3′ UTRs

The previous report showed that the mean substitution rates of genes between human and chimpanzee are K_a=0.0017, K_s=0.0106 and K_{3u}=0.0073 (Hellmann et al., 2003). For the 52 brain-expressed genes in this study, the rates are similar (K_a=0.0024, K_s=0.0116 and K_{3u}=0.0117 (Table 1 and Fig. 1). We also calculated the rates between human and mouse, which are also consistent with the previous report (K_a=0.0432, K_s=0.3597 and K_{3u}=0.3175) (Duret and Mouchiroud, 2000).

Table 1
Estimation of evolutionary rates of the nonsynonymous (K_a), synonymous (K_s) and 3′UTR (K_{3u}) sites

Comparison of orthologous sequences	K_a	K_{3u}	K_s	K_a/K_s	K_{3u}/K_s
Human/chimpanzee	0.0024	0.0117	0.0116	0.2032	1.0085
Human/rhesus monkey	/	0.0644	/	/	/
Human/mouse	0.0432	0.3175	0.3597	0.1200	0.8828

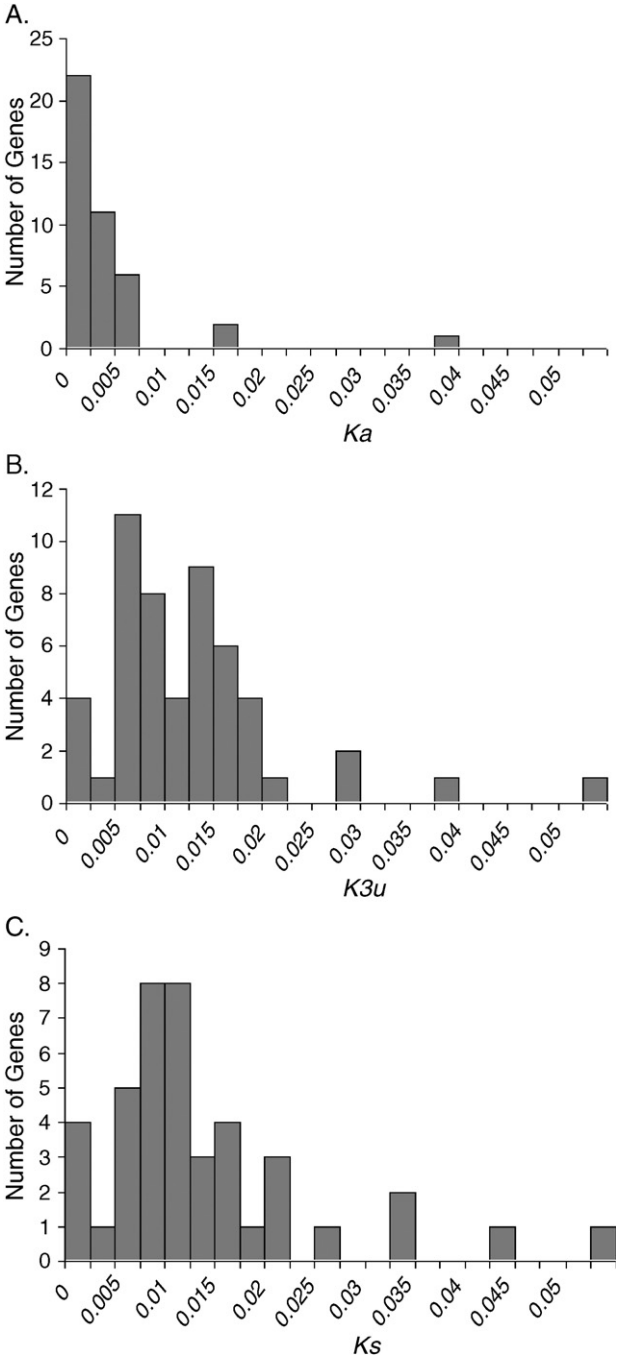

Fig. 1. The distribution of K_a (A), K_{3u} (B) and K_s (C) of the 52 human/chimpanzee orthologous genes.

For the coding regions, the average K_a/K_s ratio of human/chimpanzee is 0.2032, a strong signature of functional constraint (or negative selection) on these genes. Interestingly, the human/chimpanzee K_a/K_s ratio is higher than that of human/mouse (K_a/K_s=0.1200) (Table 1), implying that the brain-expressed genes in rodents are subject to stronger functional constraint compared with primates, which is consistent with the

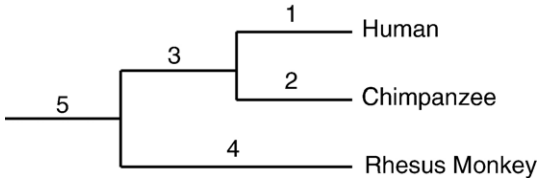

Fig. 2. The gene tree used in testing lineage-specific evolutionary rate divergence. The branches are labeled by numbers.

previous report (Dorus et al., 2004). The K_a/K_s ratio has been widely used in testing deviations from neutral expectation ($K_a/K_s=1$), in which a ratio smaller than one indicates functional constraint while a ratio larger than one indicates positive Darwinian selection. Similar logic can be used to test the 3′UTRs. As shown above, the human/chimpanzee K_{3u} is similar with K_s ($K_{3u}/K_s=0.6887$) (Fig. 1), a clear indication of neutral evolution of the 3′UTRs in human and chimpanzee.

3.4. Relative rate test and acceleration index test

To detect whether there are genes showing lineage differences between human and chimpanzee in the 3′UTRs, using rhesus monkey as the outgroup (Fig. 2), we conducted the relative rate test of the 52 human/chimpanzee sequence pairs by Tajima's method (Tajima, 1993). The results indicated that for the majority (48 genes) of the genes, the 3′UTRs do not have significant difference of evolution rates between human and chimpanzee. Only four genes showed rate differences with two of them faster in human and the other two in chimpanzee (supplementary material). We also conducted the acceleration index test developed by Zhang et al. (2002). Similar result was obtained with only five genes showing rate differences between human and chimpanzee though only one gene was overlapped with the result of the relative rate test (supplementary material). This pattern indicates that the majority of the 3′UTRs were under neutral evolution. In the genes showing rate differences between human and chimpanzee, the SLC1A2 gene showed accelerated evolution in the human lineage, which was confirmed by both the relative rate test and the acceleration index test. It is a neuro-specific gene functioning as a glial high affinity glutamate transporter (Arriza et al., 1994). The rapid evolution of the 3′UTR of SLC1A2 in the human lineage implies its functional significance during the evolution of the human brain.

4. Discussion

Among the 61 genes obtained from the rhesus monkey PFC cDNA library, there are 9 genes showing no orthologous sequences in the human genome, which could be due to either the incompleteness of the current human genome sequence database or the relatively rapid sequence substitution rate of the 3′UTRs as compared with the coding region (Li, 1997; Makalowski and Boguski, 1998). The less number of orthologous sequences in mouse (42 versus 52) reflected the influence of deep sequence divergence in identifying homologous sequences of the 3′UTRs in distantly related species (Yu et al., in press).

Because of only about 1.2% sequence divergence between human and chimpanzee (Goodman et al., 1998; Chen and Li, 2001; Ebersberger et al., 2002; Fujiyama et al., 2002), it was speculated that the cognitive divergence of human and chimpanzee was attributable more to gene expression difference than to gene coding region difference (King and Wilson, 1975; Enard et al., 2002). Our data and the previous reports (Duret and Mouchiroud, 2000; Hellmann et al., 2003; Zhang and Li, 2004) showed that the genes expressed in the primate brain have very small K_a/K_s values, implying that the coding regions of these genes have been under strong selective constraints during primate evolution.

Enard et al. (2002) reported that there is a significant expression divergence at the genome level within the brain between human and chimpanzee (Enard et al., 2002). Previous studies indicated that the *cis*-regulation elements existing in 3′ UTRs are involved in regulation of transcription and translation (Bashirullah et al., 2001; Ewa et al., 2001; Kuersten and Goodwin, 2003). According to these evidences, we may expect a relatively large sequence divergence of 3′UTRs of genes expressed in the brain, which could explain the observed expression divergence between human and nonhuman primates. However, from the data we generated in this study, we did not observe rapid evolution of the 3′UTR of brain-expressed genes in primates and a majority of the genes studied showed similar evolutionary rates as compared with the synonymous site rates, implying that in general, the evolution of the 3′UTRs of brain-expressed genes is neutral, consistent with previous studies (Duret and Mouchiroud, 2000; Hellmann et al., 2003; Zhang and Li, 2004). It should be noted that our observation might be subject to sampling bias considering that most of the genes are housekeeping genes and the number of genes studied is limited.

On the other hand, according to previous studies, the average length of 3′UTRs is 2 kb, but the length of most of the *cis*-regulation elements is usually short, about 10–100 bp (Gavis et al., 1996; Ewa et al., 2001; Goldberg-Cohen et al., 2002;Osada et al., 2005), which makes the detection of natural selection of the 3′UTRs less sensitive using the K_{3u}/K_s method since it is an average value. In addition, though the general evolutionary pattern of 3′UTR of brain-expressed genes supports neutral evolution, it is still possible that a small faction of the neuro-function related genes evolved rapidly during primate evolution and have a major contribution to the brain functional divergence between human and nonhuman primates since a regulatory gene acting on the upstream of a pathway could influence the expression of a lot of downstream genes. Hence, further studies on the genes showing 3′UTR rate divergence between human and chimpanzee will shed more light on our understanding of brain functional divergence during primate evolution and human origin.

Our results indicated that the 3′UTRs of brain-expressed genes in primates are in general under neutral evolution. The evolutionary rate of 3′UTRs is similar with that of the synonymous sites of the gene coding regions. Among the 52 brain-expressed genes compared between human and chimpanzee, only a few genes showed rate difference between them, implying that only a small fraction of the 3′UTRs of brain-

expressed genes were subject to positive Darwinian selection and may contribute to the brain functional divergence between human and nonhuman primates.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gene.2006.06.024.

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