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Ribosomal Protein S4 Genes in Macaca fuscata: Sequence, Evolution, and Phylogeny

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Abstract The cDNA encoding ribosomal protein S4(RPS 4) from an ovary cDNA library of the Japanese monkey (*Macaca fuscata*) was cloned and sequenced. The RPS4X gene from monkey X chromosome encodes a deduced protein of 263 amino acids and share 99.1% cDNA sequence similarity and 100% amino acid sequence identity with the human RPS4X. Rate of synonymous substitution was higher in RPS4Y than in RPS4X in comparison to the monkey and human. The ratio of synonymous and nonsynonymous substitutions per site indicated that directional selection has not occurred in RPS4 genes. Phylogenetic analysis using the neighbor-joining method revealed that X and Y-linked RPS4 genes have evolved independently.

Key words: evolution, Japanese monkey, phylogeny, RPS 4X cDNA, Y chromosome

Introduction

The eukaryotic ribosome is a complex of four RNA molecules and about eighty distinct proteins [1]. It has been suggested that there are multiple processed pseudogenes and one functional gene for mammalian ribosomal protein [2]. Human ribosomal protein S4, RPS4X (Xq13.1) and RPS4Y (Yp11.3), has been suggested as candidates for the genes responsible for Turner syndrome [3]. Both genes have been expressed in various human tissues. The X-linked gene, RPS 4X, encodes 263 amino acids was identical in sequence to mouse Rps4 [4]. The human and mouse ribosomal proteins are also almost identical to their homologues in chicken [5]. A *Drosophila* Rps4 containing 260 amino acids has been shown to be 75% homologous to human RPS4X [6]. The transcript of 1.0 kb in length of *Drosophila* Rps4 has been detected throughout development. Partial sequences of Rps4 gene from several species, Japanese monkey, cat, cattle, dog, horse, and pig, were investigated to understand the evolution of Rps4 genes [7]. In order to elucidate the molecular evolution of ribosomal protein gene, the nucleotide sequences of primate RPS4Y (chimpanzee, bonobo, gorilla, and orangutan) have been determined and analysed with those of other species, suggesting that a transposition event of ancestral primate RPS4X to the Y chromosome prior to the divergence of *Prosimii* [8]. In this study, the full-length of the RPS4X cDNA was isolated from ovary cDNA library of the Japanese monkey and analysed with other RPS4 genes.

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Materials and methods

Isolation of total RNA from monkey tissues

Tissue was collected from adult female of Japanese monkey (*Macaca fuscata*) in accordance with the guidelines of the Primate Research Institute, Kyoto University. Total RNA was extracted by TRIZOL reagent (Gibco BRL).

cDNA cloning and library screening

Polyadenylated mRNA (poly (A) mRNA) was purified using the Poly(A)Track mRNA isolation system (Promega). Double-stranded cDNA was prepared using a cDNA synthesis kit (Boehringer Mannheim). After methylation of the internal *Eco*RI sites and addition of *Eco*RI linkers, the cDNA was fractionated according to size by agarose gel electrophoresis. The cDNA ranging from 0.7 kb to 1.5 kb in length were ligated into the *Eco*RI site of λgt10. The phages were packaged, and the recombinants were selected by plating on *E.coli* C600Hfl. Screening for cDNA of ribosomal protein S4 from monkey ovary was performed with pDP1278 probe. The *Eco*RI inserts of hybridizing phages were cloned into the pUC18 plasmid.

Sequencing and data analyses

Plasmid DNAs were extracted by an automatic plasmid isolation system (Pharmacia). Nucleotide sequences were determined on both strands of plasmid DNA using the dideoxy chain termination method [9] with T7 and M13 reverse primers (Pharmacia). Sequence analyses of cDNA and deduced

amino acids were performed using the GENETYX system (Ver. 3.2, SDC) and GCG program (University of Wisconsin). Sequence homology of ribosomal protein S4 gene was retrieved from the GenBank database with the aid of BLAST network server [10]. The neighbor-joining phylogenetic analysis [11] was performed with the CLUSTAL W and MEGA programs [12,13]. The number of synonymous and nonsynonymous nucleotide substitutions obtained by the MEGA program (Ver.1.01, USA).

Results and discussion

Cloning of monkey RPS4X/Y cDNA

By screening 37000 plaques for ovary cDNA library from the Japanese monkey, two positive cDNA clones were identified. Those clones were subcloned and sequenced. They contained 853 nucleotides for monkey RPS4X, and compared to monkey RPS4Y that identified from testis cDNA library (Fig. 1). Both cDNA encoded a protein of 263 amino acids in length. One of the molecular features of these proteins was hydrophilic nature as shown in Fig. 2. The nucleotide and amino acid sequences of ORF had 83 and 92.8% similarity between monkey RPS4X and RPS4Y (Table 1,2). Monkey RPS4X differed from monkey RPS4Y at 19 of 263 amino acid residues (Fig. 1).

Sequence comparison of RPS4X/Y

Monkey RPS4X was aligned with those of the human, mouse, chicken, and *Drosophila* (Fig. 3). When full-length amino acid sequences for ribosomal protein S4 of human, monkey, mouse, and chicken were deduced from the nucleotide sequences of cDNAs, all the amino acid sequences revealed to be very similar. The nucleotide and amino acid sequences of monkey RPS4Y had 95.4 and 95.4% similarity with human RPS4Y [3], 81.7 and 92.8% with mouse Rps4 [4], and 81.6 and 94.3% with chicken Rps4 [5], whereas the nucleotide and amino acid sequences of monkey RPS4X had 99.1 and 100% similarity with human RPS4X, 91.4 and 100% with mouse Rps4, and 83.8 and 98.5% with chicken Rps4, respectively (Table 1,2). The amino acid sequence of

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        M  A  R  G  P  K  K  H  L  K  R  V  A  A  P  K  H  W  M  L
JM-RPS4Y ATGGCCCGGGGCCCCAAGAAGCACTTGAAGCGTGTTGCAGCGCCGAAGCATTGGATGCTT  60
JM-RPS4X ATGGCTCGTGGTCCCAAGAAGCATCTGAAGCGGGTAGCAGCTCCAAAGCATTGGATGCTG
        M  A  R  G  P  K  K  H  L  K  R  V  A  A  P  K  H  W  M  L

        D  K  L  T  G  V  F  A  P  R  P  S  T  G  P  H  K  L  R  E
JM-RPS4Y GACAAACTAACGGGTGTATTTGCACCTCGTCCATCGACAGGTCCCCACAAGCTGAGGGAA 120
JM-RPS4X GATAAATTGACCGGTGTGTTTGCTCCTCGTCCATCCACCGGTCCCCACAAGTTGAGAGAG
        D  K  L  T  G  V  F  A  P  R  P  S  T  G  P  H  K  L  R  E

        C  L  P  L  I  I  F  L  R  N  R  L  K  Y  A  L  T  G  D  E
JM-RPS4Y TGTCTTCCCCTGATCATCTTCCTCAGGAATAGACTCAAGTATGCGTTGACTGGAGATGAG 180
JM-RPS4X TGTCTCCCCCTCATCATCTTCCTAAGGAACAGACTTAAGTATGCCCTGACAGGAGATGAA
        C  L  P  L  I  I  F  L  R  N  R  L  K  Y  A  L  T  G  D  E

        V  K  K  I  C  M  Q  R  F  I  K  I  D  G  K  V  R  V  D  V
JM-RPS4Y GTAAAGAAGATATGTATGCAACGCTTCATCAAAATTGATGGCAAGGTTCGAGTGGATGTC 240
JM-RPS4X GTAAAGAAGATTTGCATGCAGCGGTTCATTAAGATCGATGGCAAGGTCCGAACTGATATA
        V  K  K  I  C  M  Q  R  F  I  K  I  D  G  K  V  R  T  D  I

        T  Y  P  A  G  F  M  D  V  I  S  I  E  K  T  G  E  H  F  R
JM-RPS4Y ACATACCCTGCTGGATTCATGGATGTCATCAGCATCGAGAAGACAGGTGAACATTTCCGC 300
JM-RPS4X ACCTACCCTGCTGGATTCATGGATGTCATCAGCATTGACAAGACGGGAGAGAATTTCCGT
        T  Y  P  A  G  F  M  D  V  I  S  I  D  K  T  G  E  N  F  R

        L  V  Y  D  T  K  G  R  F  A  V  H  R  I  T  A  E  E  A  K
JM-RPS4Y CTGGTCTATGACACCAAGGGCCGTTTTGCTGTTCATCGTATCACAGCGGAAGAGGCAAAG 360
JM-RPS4X CTGATCTATGACACCAAGGGTCGCTTTGCTGTACATCGTATTACACCTGAGGAGGCCAAG
        L  I  Y  D  T  K  G  R  F  A  V  H  R  I  T  P  E  E  A  K

        Y  K  L  C  K  V  R  K  I  T  V  G  T  K  G  I  P  H  L  V
JM-RPS4Y TACAAGTTGTGCAAAGTGAGGAAGATTACTGTGGGGACAAAGGGAATTCCACACCTGGTG 420
JM-RPS4X TACAAGTTATGCAAAGTGAGAAAGATCTTTGTGGGCACAAAAGGAATCCCTCATCTGGTG
        Y  K  L  C  K  V  R  K  I  F  V  G  T  K  G  I  P  H  L  V

        T  H  D  A  R  T  I  R  Y  P  D  P  V  I  K  V  N  D  T  V
JM-RPS4Y ACTCATGATGCTCGAACCATTCGCTACCCAGATCCTGTCATCAAAGTGAATGATACTGTC 480
JM-RPS4X ACTCATGATGCCCGCACCATCCGCTACCCCGATCCCCTCATCAAGGTGAATGATACCATT
        T  H  D  A  R  T  I  R  Y  P  D  P  L  I  K  V  N  D  T  I

        R  I  D  L  G  S  G  K  I  T  S  F  I  K  F  D  T  G  N  V
JM-RPS4Y CGGATTGATTTAGGGAGTGGCAAGATAACCAGCTTTATCAAATTTGACACAGGCAATGTG 540
JM-RPS4X CAGATTGATTTGGAGACTGGCAAGATTACTGATTTCATCAAGTTCGACACTGGTAACCTG
        Q  I  D  L  E  T  G  K  I  T  D  F  I  K  F  D  T  G  N  L

        C  M  V  I  G  G  A  N  L  G  R  V  G  V  I  T  N  R  E  R
JM-RPS4Y TGTATGGTGATTGGTGGAGCTAACCTCGGTCGTGTTGGTGTGATCACAAACAGGGAAAGA 600
JM-RPS4X TGTATGGTGACTGGAGGTGCTAACCTGGGAAGAATTGGTGTGATCACCAACAGAGAGAGG
        C  M  V  T  G  G  A  N  L  G  R  I  G  V  I  T  N  R  E  R

        H  P  G  S  F  D  V  V  H  V  K  D  A  S  G  N  S  F  A  T
JM-RPS4Y CATCCTGGTTCTTTCGATGTGGTGCATGTGAAGGATGCCAGTGGCAACAGCTTTGCCACA 660
JM-RPS4X CATCCTGGATCTTTTGACGTGGTTCACGTGAAAGATGCCAATGGCAACAGCTTTGCCACT
        H  P  G  S  F  D  V  V  H  V  K  D  A  N  G  N  S  F  A  T

        R  I  S  N  I  F  V  I  G  N  G  N  K  P  W  I  S  L  P  R
JM-RPS4Y AGGATTTCCAACATTTTTGTCATTGGCAATGGTAATAAACCTTGGATTTCCCTGCCCAGG 720
JM-RPS4X CGACTTTCCAACATTTTTGTTATTGGCAAGGGCAACAAACCATGGATTTCTCTTCCCCGA
        R  L  S  N  I  F  V  I  G  K  G  N  K  P  W  I  S  L  P  R

        G  K  G  I  R  L  T  I  A  E  E  R  D  K  R  L  A  A  K  Q
JM-RPS4Y GGAAAGGGCATCCGACTTACTATTGCTGAAGAGAGAGACAAGAGACTGGCTGCCAAACAG 780
JM-RPS4X GGAAAGGGTATCCGCCTCACCATTGCTGAAGAGAGAGACAAAAGACTGGCGGCCAAACAG
        G  K  G  I  R  L  T  I  A  E  E  R  D  K  R  L  A  A  K  Q

        S  S  G  *
JM-RPS4Y AGCAGTGGCTAA----GTTACAGTAGTGGCATATTTTTTCTCTTTGCACAAATAAACAGT 840
JM-RPS4X AGCAGTGGGTGAAATGGGTCCCTGGGTGCCATGTCAGACCTTTGTACGTAATTAAAAATA
        S  S  G  *

JM-RPS4X TTGTGGCAGGATT 853
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Fig. 1. Sequences of RPS4X and RPS4Y cDNAs from the Japanese monkey together with the translation of their open reading frame (ORF). Amino acids are aligned with the first nucleotide of each codon. The deduced amino acid sequence is also presented in Fig. 3. The cDNA sequence data of the monkey RPS4X will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with accession number AB024285.

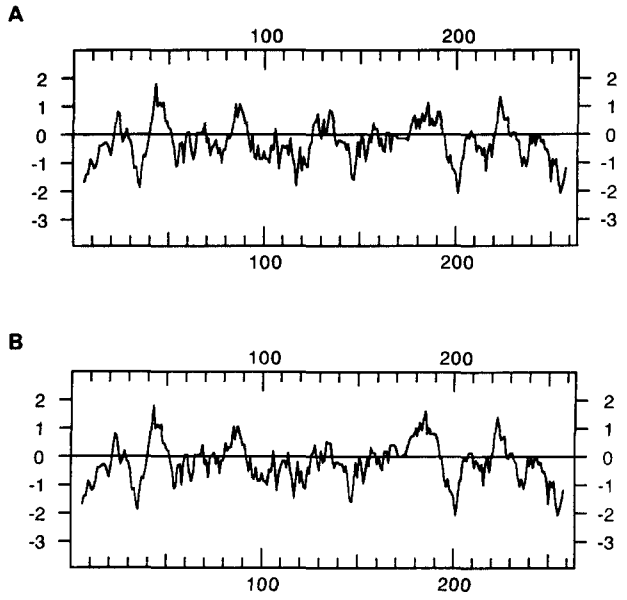

Fig. 2. Hydrophilicity and hydrophobicity plot [14] of monkey RPS4X (A) and RPS4Y (B).

the monkey RPS4X and RPS4Y shared 75.3 and 74.5% identity with that of *Drosophila* Rps4, respectively [6]. Human X-linked gene RPS4X was identical to monkey RPS 4X and mouse Rps4. The sequence of the human RPS4X, monkey RPS4X, and mouse Rps4 differed from that of chicken Rps4 by four amino acid substitutions (Fig. 3). The Y-linked RPS4Y gene has evolved rapidly in the human and monkey. The sex-linked zinc finger genes (ZFX/Y) also showed that ZFX has been well conserved in both the primate and the rodent lineages, but ZFY has evolved rapidly in the rat lineage and even faster in the mouse lineage [19]. Comparing with testis-specifc protein Y (TSPY) gene on Y chromosome between monkey and human [15], ribosomal protein S4 (RPS4) gene on sex chromosome revealed that the similarity of nucleotide and amino acid sequences were higher than that of TSPY gene.

Phylogeny and evolution rate

Phylogenetic analysis has been shown as a powerful tool to understand human evolution using nucleotide sequences from mtDNA [16,17], globin gene [18], ribosomal RNA gene [19], and PABY/X genes [20]. Recently, Y chromosome-linked genes, TSPY [21], SRY [22], and RPS4Y [8,23], have been used to investigate the human evolution because mutation rate is much higher in the male germ line than in the female germ line. It was estimated that mutation rate of genes on the Y chromosome has evolved approximately twice faster than that on the X chromosome [24].

To understand evolutionary relationship among ribosomal protein S4 genes, a phylogenetic tree was constructed with the neighbor-joining method using the nucleotide sequences of ORF from the Japanese monkey with the human [3], mouse [4], chicken [5], and *Drosophila* [6] (Fig. 4). Monkey RPS4X and human RPS4X were found to be closely related to each other than either of them was to the mouse Rps4. Monkey RPS4Y and human RPS4Y showed the sister relationship. This result indicates that X and Y- linked RPS4 genes have evolved independently.

In order to determine the evolutionary forces, synonymous and nonsynonymous substitutions were analyzed using the cDNA sequences of RPS4 genes among the human, monkey, and mouse (Table 3). As nonsynonymous substitutions per site (Ka) are more strongly influenced by selective pressure than synonymous substitutions per site (Ks), their ratio is a good indicator of selection. Comparing the monkey Y-human Y and monkey X-human X, the values of Ks were

Table 1. Nucleotide sequence similarity in coding region of RPS4 genes

Species	HU-Y	HU-X	JM-Y	JM-X	MOU	CHI	DRO
Human-Y(HU-Y)	100						
Human-X(HU-X)	82.2	100					
J.monkey-Y(JM-Y)	95.4	83.0	100				
J.monkey-X(JM-X)	82.2	99.1	83.0	100			
Mouse(MOU)	81.1	91.0	81.7	91.4	100		
Chicken(CHI)	81.7	83.9	81.6	83.8	83.8	100	
Drosophila(DRO)	67.9	68.9	67.5	69.3	68.7	72.0	100

Table 2. Amino acid sequence identity in RPS4 genes

Species	HU-Y	HU-X	JM-Y	JM-X	MOU	CHI	DRO
Human-Y(HU-Y)	100						
Human-X(HU-X)	92.8	100					
J.monkey-Y(JM-Y)	95.4	92.8	100				
J.monkey-X(JM-X)	92.8	100	92.8	100			
Mouse(MOU)	92.8	100	92.8	100	100		
Chicken(CHI)	93.9	98.5	94.3	98.5	98.5	100	
Drosophila(DRO)	73.7	75.3	74.5	75.3	75.3	76.4	100

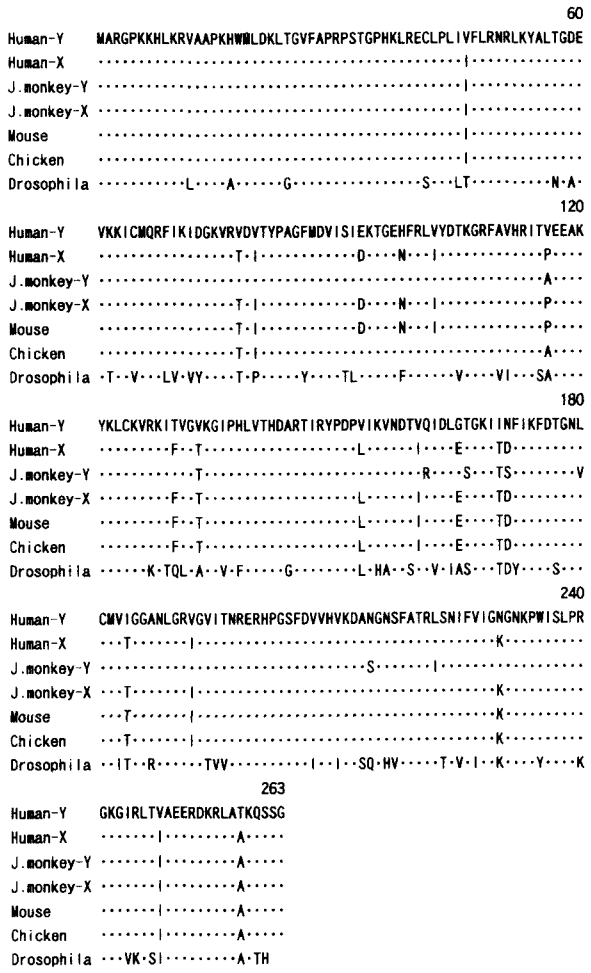

Fig. 3. Sequence alignment of amino acids of ribosomal protein S4 (RPS4) genes. Dots indicate no change to the human RPS4Y. The accession numbers of the RPS4 genes, obtained from the GenBank databases, are as follows: Human-Y (M58459), Human-X (M58458), Monkey-Y (AB024286), Mouse (M73436), Chicken (L24368), *Drosophila* (D16257).

0.13 and 0.04, while those of Ka/Ks were 0.15 and 0, respectively. The rate of synonymous substitution was higher in RPS4Y than in RPS4X from the human and monkey.

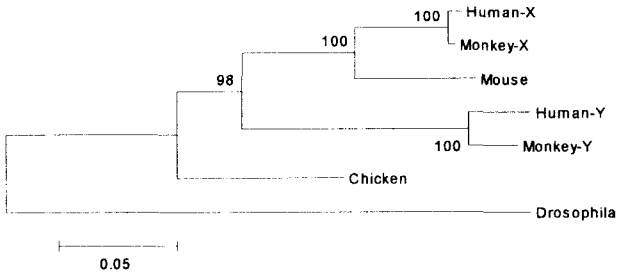

Fig. 4. Phylogenetic tree comparing RPS4 genes from several species.

A similar phenomenon has been shown in ZFX/Y genes [24]. Comparing the RPS4X/Y and TSPY gene in the monkey and human [15], the values of Ka/Ks of RPS4X/Y gene were obviously lower than that of TSPY gene. The values of Ks between monkey Y-mouse and monkey X-mouse were 1.42 and 0.50, respectively. Therefore, the negative selection has occurred in RPS4 genes.

In summary, the full-length monkey RPS4 cDNA was isolated from an ovary cDNA library. The cDNA sequences shared a high degree of similarity with the human, mouse, and chicken, suggesting that there is strong evolutionary conservation at the RPS4 locus. Phylogenetic analysis revealed that X and Y-linked ribosomal protein S4 genes have evolved independently. The ratio of synonymous and nonsynonymous substitutions per site indicated that directional selection has not occurred in RPS4 genes.

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Table 3. Synonymous substitutions per site (Ks), nonsynonymous substitutions per site (Ka), and their ratio (Ka/Ks) in the RPS4 genes

Species pair	Ks	Ka	Ka/Ks
Human-Y and Human-X	1.29 ± 0.20	0.04 ± 0.01	0.03
Human-Y and J.monkey-Y	0.13 ± 0.03	0.02 ± 0.01	0.15
Human-Y and J.monkey-X	1.29 ± 0.20	0.04 ± 0.01	0.03
Human-Y and Mouse	1.60 ± 0.29	0.04 ± 0.01	0.03
Human-X and J.monkey-Y	1.17 ± 0.17	0.04 ± 0.01	0.03
Human-X and J.monkey-X	0.04 ± 0.01	0 ± 0	0
Human-X and Mouse	0.53 ± 0.07	0 ± 0	0
J.monkey-Y and J.monkey-X	1.17 ± 0.17	0.04 ± 0.01	0.03
J.monkey-Y and Mouse	1.42 ± 0.23	0.04 ± 0.01	0.03
J.monkey-X and Mouse	0.50 ± 0.07	0 ± 0	0

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