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Genomic organization and evolution of immunoglobulin kappa gene enhancers and kappa deleting element in mammals

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Abstract

We have studied the genomic structure and evolutionary pattern of immunoglobulin kappa deleting element (KDE) and three kappa enhancers (KE5′, KE3′P, and KE3′D) in eleven mammalian genomic sequences. Our results show that the relative positions and the genomic organization of the KDE and the kappa enhancers are conserved in all mammals studied and have not been affected by the local rearrangements in the immunoglobulin kappa (IGK) light chain locus over a long evolutionary time (~120 million years of mammalian evolution). Our observations suggest that the sequence motifs in these regulatory elements have been conserved by purifying selection to achieve proper regulation of the expression of the IGK light chain genes. The conservation of the three enhancers in all mammals indicates that these species may use similar mechanisms to regulate IGK gene expression. However, some activities of the IGK enhancers might have evolved in the eutherian lineage. The presence of the three IGK enhancers, KDE, and other recombining elements (REs) in all mammals (including platypus) suggest that these genomic elements were in place before the mammalian radiation.

Keywords

Immunoglobulin kappa light chain; Kappa deleting element (KDE); Recombination signal sequence (RSS); Kappa enhancer; Transcription factor

1. Introduction

The expression of immunoglobulin kappa (*IGK*) light chain genes is restricted in the B-cell lineage. This cell-type specific expression is regulated by the interaction of DNA-binding proteins with specific promoter and enhancer sequences (Falkner and Zachau, 1984; Lenardo et al., 1987; Sen and Baltimore, 1986). Three enhancer elements have been described and

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Appendix A. Supplementary Table 1

functionally characterized in the IGK-encoding locus of humans and mice, but whether these sequences are conserved in other mammalian species remains unknown (Gimble and Max, 1987; Liu and Garrard, 2005; Meyer et al., 1990; Xiang and Garrard, 2008). The first enhancer, the KE5′, is located in the intronic region between the immunoglobulin kappa joining (*IGJK*) and constant (*IGCK*) genes. The other two enhancers are located at the proximal (KE3′P) and distal regions (KE3′D) of the 3′ end of the IGCK gene (Inlay et al., 2002; Lenardo et al., 1987; Xu et al., 1996). These enhancers contain specific nucleotide motifs that bind to specific transcription factors (Lenardo et al., 1987; Pongubala et al., 1992; Schanke and Van Ness, 1994).

During B-cell differentiation, the immunoglobulin light chain gene rearrangements occur in an orderly fashion starting with kappa chain gene rearrangements and proceeding to lambda chain gene rearrangement (Alt et al., 1980). Usually only one type of immunoglobulin light chain gene is expressed in a particular B-cell. In about 90 % of cases the lambda-encoding genes undergo rearrangements only when the recombination events of the kappa-encoding genes lead to nonfunctional products (Hieter et al., 1981; Korsmeyer et al., 1982; van der Burg et al., 2001). If the rearrangement between the immunoglobulin kappa variable region gene (*IGVK*) and *IGJK* produces a nonfunctional *IGVK-IGJK* product, the locus undergoes segmental deletion through a rearrangement with the kappa deleting element (KDE), which is located downstream of the *IGCK* gene (Siminovitch et al., 1985). The KDE has been described in humans and mice (Graninger et al., 1988; Langerak et al., 2004; Siminovitch et al., 1985), but whether these sequences are present in other mammals is currently unknown. Most of the KDE-mediated *IGK* gene rearrangements occur either via recombining element (RE) located in the *IGJK-IGCK* intron or via recombination signal sequence (RSS) located immediately 3′ end to the *IGVK* genes (Graninger et al., 1988; Langerak et al., 2004; Siminovitch et al., 1985). Recent studies have demonstrated that KDE can recombine to the RSS flanking *IGJK* genes (Seriu et al., 2000). An alternative recombination mechanism that can delete the entire *IGJK* cluster by means of a rearrangement between RSS of *IGVK* and intronic RE has also been reported (Feddersen et al., 1990).

The regulation of IGK expression has been mainly studied in humans and mice, and only a few studies have identified some of the regulatory elements in the rabbit and the horse IGK regions (Emorine et al., 1983; Ford et al., 1994; Schanke and Van Ness, 1994; Siminovitch et al., 1987; Xiang and Garrard, 2008). However, it is currently unknown whether all mammals use similar or different sequences to regulate IGK expression. In addition, when these regulatory elements evolved in mammalian IGK locus is not known. The multiple genomic sequences of different mammalian species currently present in the public databases provide an excellent opportunity to study the level of conservation of these regulatory regions. In the present study, we used comparative genomics and bioinformatics approaches to gain insights into the genomic structure and evolution of sequences that regulate the IGK expression.

2. Materials and methods

2.1. Genomic localization of the IGJK, IGCK and the ribose-5-phosphate isomerase (RPIA) gene

The genomic location of the *IGJK* and *IGCK* genes and the flanking non-IG gene *RPIA* (ribose-5-phosphate isomerase) were determined in a previous study (Das et al., 2008a) for eight mammalian species (i.e. human, mouse, rat, dog, cow, horse, Opossum, and platypus). To identify the *IGCK* and *RPIA* genes in the chimpanzee, orangutan, and macaque genome sequences we performed tBLASTn search using the human *IGCK* (accession no. AAI10395) and the *RPIA* (accession no. AAH15529) sequences as queries. To identify the *IGJK* genes, which are very short and cannot be detected by BLAST searches, we manually screened 7 kb upstream of the *IGCK* gene, taking into account the location of the RSS sequence at the 5′ of

the *IGJK* gene. We confirmed that the identified sequences are *IGJK* genes using the IGJK-specific molecular markers (Das et al., 2008a).

2.2. Identification of the kappa enhancers

We identified the kappa enhancer elements in 11 mammalian genomes (> 5× genome coverage) using a four step approach. The information of the genome assembly of the 11 mammalian species is given in Supplementary Table 1. We first performed BLASTn searches using the previously reported three enhancer sequences from humans and mice: the 5′ enhancer (KE5′), located at the 5′ end of the *IGCK* gene, the 3′ proximal enhancer (KE 3′P), located at the proximal region of the 3′ end of the *IGCK* gene, and the 3′ distal enhancer (KE3′D), located at the distal region of the 3′ end of the *IGCK* gene (Judde and Max, 1992; Liu et al., 2002; Schanke and Van Ness, 1994). This way we identified sequences homologous to the KE5′ enhancer in chimpanzee, orangutan, macaque, rat, and dog genomes and sequences homologous to the KE3′P and KE3′D enhancers in chimpanzee, orangutan, macaque, rat, dog, and horse genomes. In the second step, we aligned the identified enhancer sequences from primates and rodents using the ClustalW (Thompson et al., 1994) and DiAlign (available at www.genomatix.de) programs and searched for conserved motifs using CoreSearch (available at www.genomatix.de) and MEME (Version 3.5.7) (Bailey et al., 2006). We used the default parameters of these programs and inspected the alignments manually to maximize similarity. In the third step, the motifs identified in the previous step and the genomic organization of the *IGJK*, *IGCK*, and *RPIA* genes were used to scan the horse genomic sequence, to identify the KE5′ enhancer, and the cow, opossum, and platypus genomic sequences, to identify of all three enhancer elements. In the fourth step, we searched the retrieved sequences for common restriction sites and the motifs in which common transcription factors (TFs) can putatively bind. This way we confirmed that the identified sequences were homologous to the human and mouse κ enhancer elements. To find the common restriction sites we used the restriction site detection program in GEMS Launcher (available at www.genomatix.de). The programs P-Match (available at www.gene-regulation.com) and MatInspector (available at www.genomatix.de) were used to locate the DNA motifs for putative TF-binding sites. These two programs utilize the TRANSFAC database (Wingender et al., 1996) to identify match in the DNA sequences.

2.3. Identification of the KDE and the recombining element (RE) in the IGJK-IGCK intron

The RE in the *IGJK-IGCK* intron of the human κ encoding locus contains a palindromic heptamer signal sequence (CACAGTG) (Klobeck and Zachau, 1986; Siminovitch et al., 1985). To identify sequences in other mammalian genomes, which are homologous to the RE sequence of human, we first scanned the *IGJK-IGCK* intron sequences for the conserved CACAGTG motif. We then analyzed the level of sequence similarity between the reported human RE and all the other mammalian sequences using 50 bp upstream and 50 bp downstream sequences of the CACAGTG motif and finally we searched for common restriction sites. To identify the KDE sequence we performed BLASTn searches using the human (Klobeck and Zachau, 1986) and mouse (Siminovitch et al., 1987) KDE sequence as queries. Both of these sequences contain a heptamer signal sequence (CACTGTG) and a nonamer sequence (AGTTTCTGC) separated by a 23 bp spacer (Siminovitch et al., 1987). These searches identified potential homologous KDE sequences in all eutherian mammals. To identify KDE homologous sequences in noneutherian mammals we scanned the genomic sequences of opossum and platypus for the presence of the conserved CACT(A)GTG motif between the *IGCK* and *RPIA* genes and we analyzed the sequence similarities between eutherian and non-eutherian mammals using 50 bp upstream and 50 bp downstream sequences of the CACT(A)GTG motif. Additionally, we considered the relative positions of the heptamer and nonamer sequences as well as the relative positions of the KDE homologous sequences to the enhancer

sequences and the *RPIA* genes. Finally, we searched for common restriction sites in the 106 bp long sequence including the heptamer motif.

3. Results

3.1. Evolutionary conservation of the 5′ enhancer (KE5′) element

The cross-species comparison of ~ 400 bp sequences of the *IGJK-IGCK* introns revealed that a ~150 bp region is fairly conserved among all mammalian species under study (Fig. 1). In this region six common restriction sites are found in eutherian mammals, three of which are also conserved in non-eutherian mammals. To characterize the potential gene regulatory regions, we searched for putative TF-binding sites (see Materials and Methods). One class of such sites is the E-box, which contains the consensus sequence CANNTG (Murre et al., 1991;Yutzey and Konieczny, 1992). We identified three E-boxes (Schanke and Van Ness, 1994) in the 150 bp region of the *IGJK-IGCK* intron. Two (E1 and E2) of these E-boxes are conserved in all mammalian species examined and the third (E3) is conserved only in eutherian mammals (Fig. 1). The E-boxes are the potential binding sites for the twist subfamily of basic helix-loop-helix (bHLH) transcription factors (Virolle et al., 2002). We also identified a potential NFκB (Nuclear factor κB)-binding site. This site is located immediately upstream of the E-box (Fig. 1) proximal to the *IGJK* genes. This NFκB-binding site is conserved in all eutherian mammals studied (Schanke and Van Ness, 1994). However, no potential NFκB site was found in the homologous region in non-eutherian mammals.

3.2. Conserved motifs in 3′ enhancer elements (KE3′P and KE3′D)

Our analysis indicated that the two enhancer elements at the 3′-end of the *IGCK* gene (Inlay et al., 2002; Judde and Max, 1992; Xiang and Garrard, 2008), KE3′P and KE3′D, are conserved in all mammals studied (Fig.s 2 and 3). In both regions we identified several motifs that are conserved throughout mammalian evolution. In particular, we found that the KE3′P enhancer sequence contains two conserved E-box motifs. One E-box (CAACTG) is conserved between eutherian mammals and the other (CAT(C)CTG) is present in both eutherian and non-eutherian mammals (Fig. 2). The KE3′P region contains five putative TF-binding sites. Among them, the binding sites for the macrophage-specific factor (PU.1), the interferon regulatory factor (IRF), and the bHLH factor are conserved in all mammals studied whereas a potential binding site of the paired box (PAX) transcription factor is conserved only between eutherian mammals.

In the distal 3′ enhancer sequence (KE3′D), two E-boxes (CACCTG and CAGA(C)TG) and one NFκB site have been described in humans and mice (Liu et al., 2002). Our analysis suggests that all three sites are present in all 11 mammalian species under study (Fig. 3). Additionally, we identified another putative TF-binding site for a TF that belongs to the ETS family, which is conserved in all mammals.

3.3. Analysis of the KDE and the RE of IGJK-IGCK intron

We screened the downstream and upstream sequences of *IGCK* genes (see Materials and Methods) to identify the KDE and the intronic RE sequences. Our results suggest that the KDE contains a conserved nonamer sequence located at the 5′-end of a conserved heptamer sequence (CACTGTG) in all mammals studied (Fig. 4a). This configuration resembles the one previously reported for the human and mouse KDE (Siminovitch et al., 1987). In all mammals studied, the length of the spacer between heptamer and nonamer sequences is 23 bp. The only exception is the platypus KDE, which contains a 24 bp spacer between the heptamer and nonamer sequences. Whether the 24 bp spacer is characteristic of non-eutherian mammals cannot be deduced with certainty, because the opossum genomic sequence is incomplete in this region. A comparison of the restriction sites on KDE sequences showed that only TspRI

and Tsp4CI sites are common in all mammalian species studied, whereas BisI, CviJI, and MaeI restriction sites are common in eutherian mammals.

In order to determine the exact recombination site in *IGJK-IGCK* intron we used the 106 bp genomic region (i.e. 50 bp upstream and 50 bp downstream sequences of CACAGTG heptamer) containing human IGK intronic recombining element as a reference sequence. In humans, the IGK intronic RE contains a canonical heptamer sequence CACAGTG (Klobeck and Zachau 1986). Similar to the human sequence one CACAGTG motif can be identified in the *IGJK-IGCK* intron in all mammals studied (Fig. 4b). Sequence analysis showed that with the exception of the conserved heptamer sequence, the remaining sequence of the intron is highly diverged. Only two common restriction sites (TspRI and Tsp4CI sites) could be found in the heptamer signal sequence (Fig. 4b). No nonamer-like conserved sequence is found either 12 bp or 23 bp upstream and downstream from the conserved heptamer motif.

3.4. Conservation in the relative positions of KDE, RE and enhancer elements

The comparative analysis of IGK encoding locus revealed that the physical distance between RE-KE5′, KE5′-*IGCK*, *IGCK*- KE3′P, KE3′P-KE3′D, and KE3′D-KDE can little vary from species to species (Table 1). However, the relative positions and the orientation of the KDE, RE, three enhancer elements, *IGCK*, and *IGJK* genes are conserved in all mammalian species studied (Fig. 5).

4. Discussion

The immunoglobulin-encoding locus has been the subject of multiple gene rearrangements during the evolution of mammals and these rearrangements have resulted in variation in the total length of the locus, the number of component genes, and their orientation (Das, 2009; Das et al., 2008a; Das et al., 2008b). Our analysis also suggests that most of the non-coding sequences are highly diverged even in closely related species. In contrast, the relative positions and the genomic organization of the regulatory elements of the IGK-encoding locus (KDE, RE, and the three enhancers) are conserved in all mammals studied (Fig. 5). These observations suggest that although the non-coding sequences of the locus evolve more or less neutral and mutations accumulate in random (Nei, 2007), the motifs in these regulatory elements evolve under purifying selection due to functional constraints. These constraints are most probably related to the proper regulation of expression and rearrangements of the immunoglobulin genes.

The presence of all three enhancers and the conservation of specific TF-biding sites in all mammals suggest that these species may use similar mechanisms to regulate the expression of the *IGK* genes. However, some TF-binding sites can be detected only in eutherian mammals [e.g., the NFκB binding site and the distal E-box in the 5′ enhancer (KE 5′) (Fig. 1), and one E-box sequence and the PAX transcription factor binding site in the KE3′P enhancer (Fig. 2)]. These findings suggest that some activities of the κ enhancers might have evolved in the eutherian lineage.

The recombination signal sequences in immunoglobulin genes contain conserved heptamer and less conserved nonamer motifs, separated by either 12±1 or 23±1 bp spacer (Akira et al., 1987). The recombination generally takes place between one RSS with a 12 bp spacer and one with a 23 bp spacer (12/23 joining rule). The comparative analysis of KDE shows that like *IGJK* genes it is composed of conserved heptamer and nonamer sequences, separated by 23 bp spacer sequence. However, in platypus the length of the spacer is 24 bp. In contrast to canonical RSS, the RE in *IGJK-IGCK* intron is composed of an isolated conserved heptamer (CACAGTG) motif without an obvious nonamer sequence. The κ gene deletion is generally mediated by the site-specific recombination event either between KDE and RE of *IGJK-IGCK* intron or between KDE and RSS of *IGVK* gene (Beishuizen et al., 1997; Moore et al.,

1985), while only few κ gene deletions are mediated via an alternative deletion mechanism (Beishuizen et al., 1994; Feddersen et al., 1990). There is evidence that in some cases KDE can also recombine to the RSS of *IGJK* gene (Seriu et al., 2000). The possible recombination events in IGK locus are summarized in figure 6. Some of these recombination are atypical (cannot be formed directly either because of the inverted positions of their respective RSS or because their RSS spacer lengths do not obey the 12/23 joining rule) and occur in very low frequency (Langerak et al., 2004). However, the conservation of the KDE element and the RSSs with which it recombines suggest that all mammals may use all the different pathways of rearrangement to achieve proper selection of functional immunoglobulin light chain proteins.

The presence of the KDE, RE, and three enhancer elements (Fig. 5) in all mammalian IGK locus (including platypus) suggest that these genomic regions must have been shaped before the radiation of the mammalian lineages from their common ancestor. The conservation in the genomic organization of the regulatory sequences and the conservation in the location of the regulatory elements relative to transcription unit indicate that all mammalian species possibly use the similar molecular apparatus for the regulation of IGK light chain expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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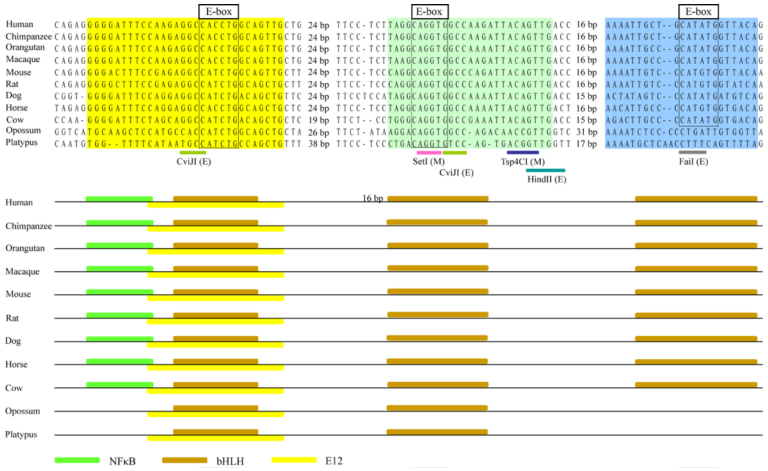

Fig. 1.

Alignments of the most conserved regions of the KE5′. Three conserved motifs are highlighted in colors. Common restriction sites and common putative transcription factor binding sites are shown by horizontal bars below the alignments. The common restriction sites for eutherian mammals are indicated by the letter E and the restriction sites common for both eutherian and non-eutherian mammals are indicated by the letter M. For putative TF-binding sites, the bars above and below the lines indicate their orientation. The spacing between the two conserved regions is given in base pairs. Three E-box sequences are marked as E1, E2 and E3.

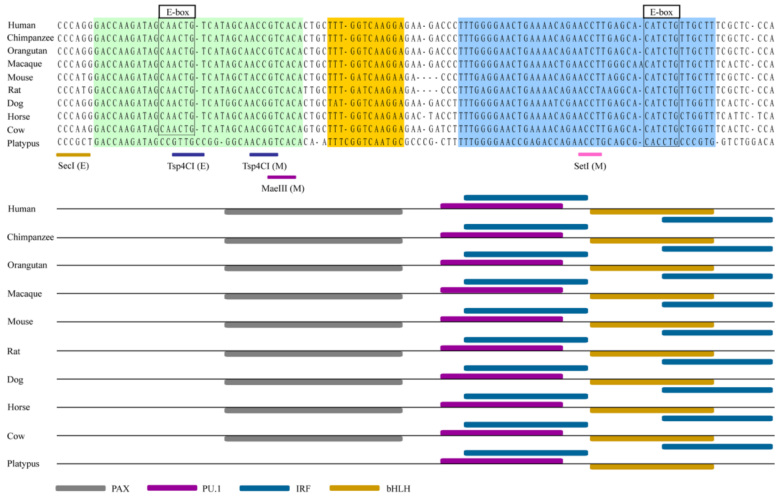

Fig. 2.

Alignments of the most conserved regions of the KE3'P. Three conserved motifs are highlighted with different colors. Common restriction sites and common putative transcription factor (TF)-binding sites are shown by horizontal bars below the alignments. The “M” in the parenthesis indicates that the restriction sites are common in both eutherian and non-eutherian mammals, whereas “E” indicates that the restriction sites are common in eutherian mammals only. For putative TF-binding sites, the bars above and below the lines indicate their orientation. The E-box sequences are shown with boxes. Due to the incompleteness of the opossum genome sequence project the KE3′P site could not be detected.

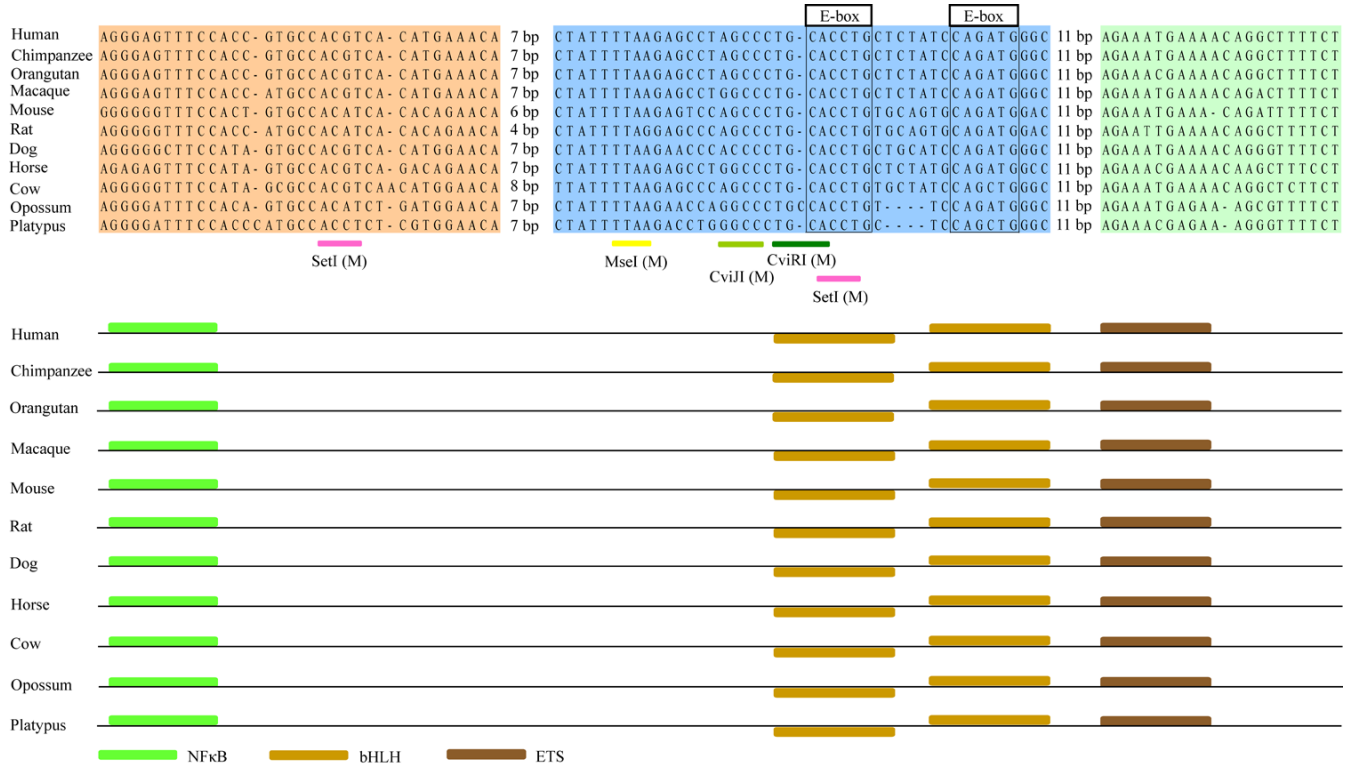

Fig. 3.

Alignments of three conserved motifs of the KE3′D. Common restriction sites and common putative TF-binding sites are shown by horizontal bars below the alignments. The “M” in the parenthesis indicates that the restriction sites are common in both eutherian and non-eutherian mammals, whereas “E” indicates that the restriction sites are common in eutherian mammals only. For putative TF-binding sites, the bars above and below the lines indicate their orientation. The spacing between the two conserved regions is given in base pares. The E-box sequences are shown in boxes.

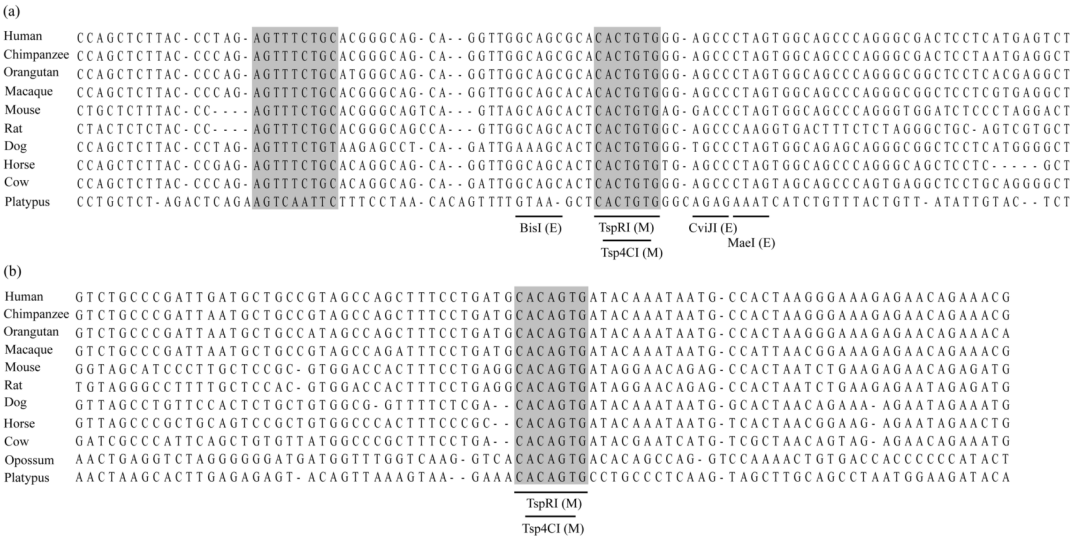

Fig. 4.

Sequence comparisons of the KDE and the *IGJK-IGCK* intronic RE. Common restriction sites are shown by horizontal bars below the alignments (M: common restriction sites for both eutherian and non-eutherian mammals; E: common restriction sites for eutherian mammals only). (a) Sequence alignments of the KDE region. The conserved heptamer and nonamer sequences are highlighted. Due to the incompleteness of the opossum genome sequence project the potential KDE could not be detected. (b) Sequence alignments of RE located in the *IGJK-IGCK* intron. The conserved heptamer sequences are highlighted. No nonamer-like conserved sequence is found either in the upstream or downstream of the conserved heptamer motif.

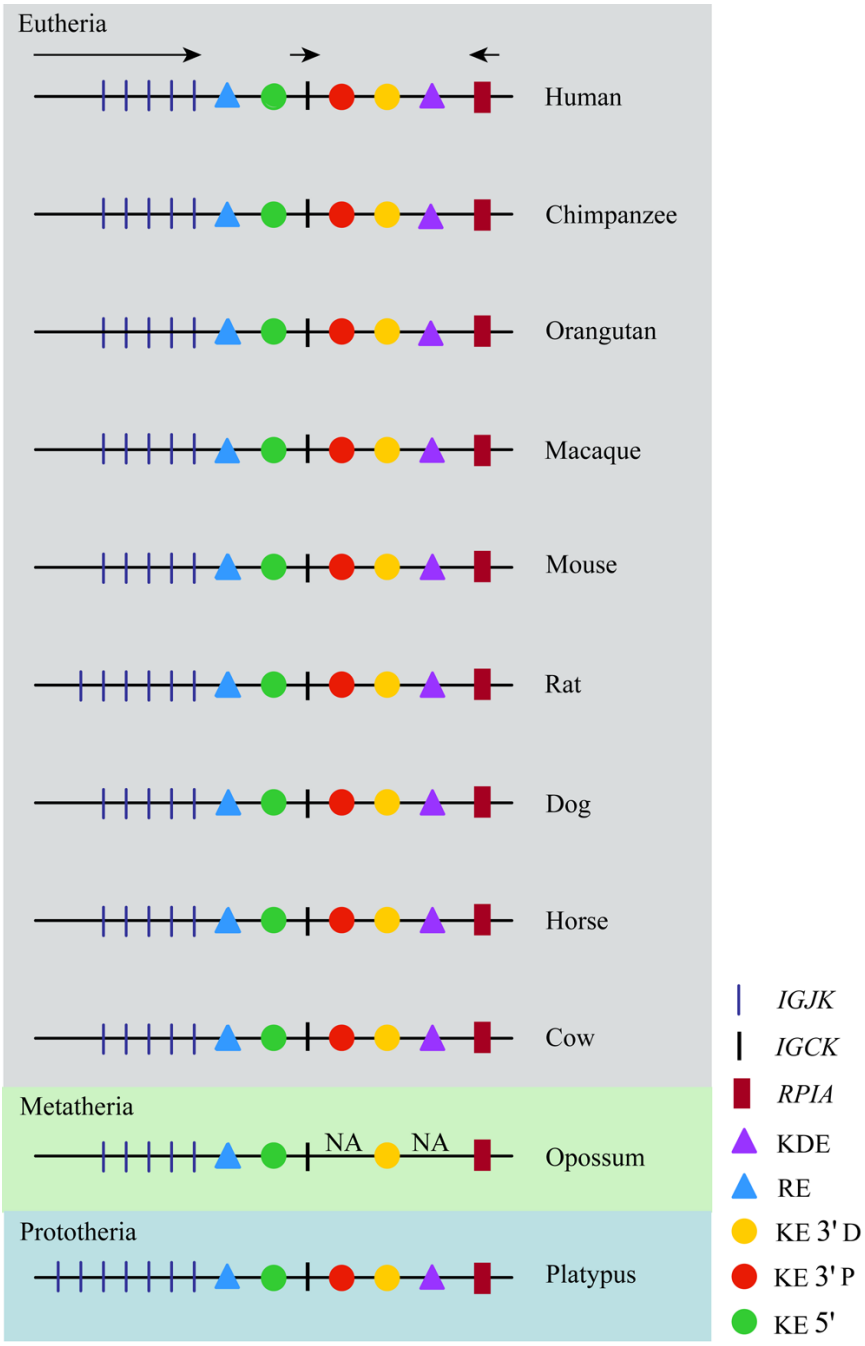

Fig. 5.
Relative positions of the KDE, RE, three enhancer elements, *IGCK*, and *IGJK*, genes. The 3′ end of the IGK-encoding locus is flanked by the non-IG gene *RPIA* (ribose-5-phosphate isomerase). Arrows indicate the transcription orientation of the genes.

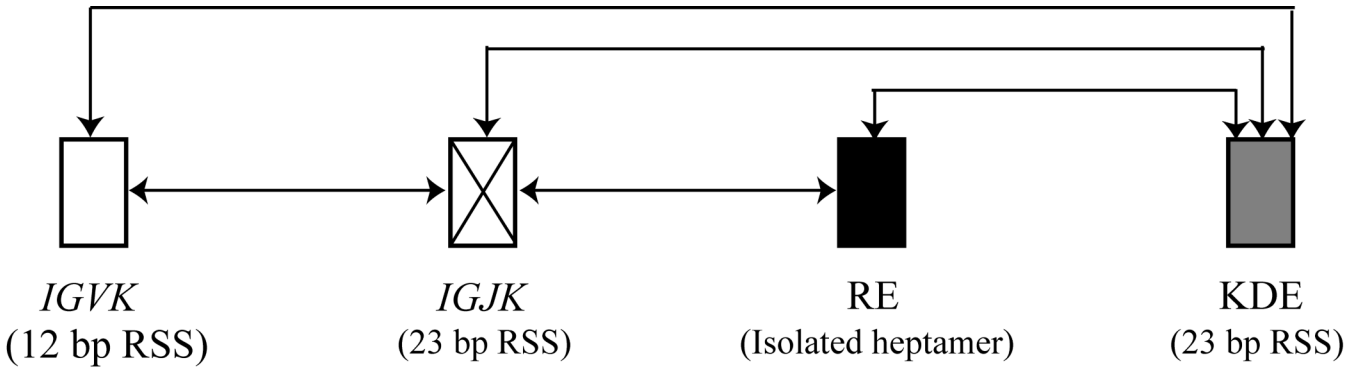

Fig. 6.
Schematic diagram of possible recombination events in IGK locus.

Table 1

Physical location of RE, KE5′, IGCK, KE3′P, KE3′D, and KDE in the genome of different mammalian species.

	Distance (Kb)				
Species	RE-KE5′	KE5′-IGCK	IGCK-KE3′P	KE3′P-KE3′D	KE3′D-KDE
Human	1.1	0.57	11.27	7.36	5.61
Chimpanzee	1.1	0.57	11.26	7.36	5.61
Orangutan	1.01	0.56	13.32	7.38	5.75
Macaque	1.11	0.56	12	8.16	5.61
Mouse	0.65	.055	8.88	8.25	7.4
Rat	0.66	0.55	8.9	8.7	6.23
Dog	1.06	0.58	5	8.61	6.22
Cow	1.19	0.95	11.17	11.66	5.22
Horse	1.11	0.63	10.82	7.72	5.25
Opossum	0.9	1.02	-	-	-
Platypus	0.74	0.98	12.3	5.05	5.59