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Evolution of the SEC1 gene in New World monkey lineages (Primates, Platyrrhini)

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ABSTRACT. The structure and evolution of the SEC1 gene were examined for the first time in New World primates of the genera *Alouatta*, *Aotus*, *Ateles*, *Brachyteles*, *Callicebus*, *Callithrix*, *Cebus*, *Chiropotes*, *Lagothrix*, *Leontopithecus*, *Pithecia*, *Saguinus*, and *Saimiri*. This gene has a high CG content (63.8%) and an estimated heterogeneous size ranging from 795 (*Callithrix*) to 1041 bp (*Pithecia*), due to numerous indel events. Similar to other fucosyltransferases, three conserved regions are shared by these primates, except for the callitrichines, *Aotus* and *Pithecia*, in which indel events resulted in premature stop codons that are related to the production of a supposedly non-functional protein. Phylogenetic analysis of the SEC1 gene, transition/transversion rates, and nucleotide sequence alignment support the hypothesis that primate SEC1 evolved by divergent evolution, and that the lack of activity in some lineages occurred independently at least twice in New World primates, once in the *Aotus*-*Cebus*-Callitrichinae group and again in *Pithecia*. Likelihood-based inference of ancestral states for the activity of SEC1 leads us to suppose that inactivation of SEC1 in the Callitrichinae was a result of a more complex series of events than in *Pithecia*.

Key words: Fucosyltransferases; Divergent evolution; Molecular inactivation; New World monkeys

INTRODUCTION

The α1,2 fucosyltransferases (α1,2 FUT) are a family of similarly structured type II transmembrane proteins that have a short NH_2-terminal cytoplasmic tail, a signal membrane anchor domain, a stem region and a globular COOH-terminal catalytic domain within the luminal trans-Golgi compartment (Paulson and Colley, 1989). They are coded by FUT1 (H), FUT2 (SE) and SEC1 genes, which are grouped in the long arm of human chromosome 19 (19q 13.3) (Reguigne-Arnould et al., 1995, 1996).

Although differing in genomic organization and specificities of encoding enzymes, all α1,2 FUT share some characteristics, including i) a monoexonic coding sequence, ii) a complex tissue- and cell type-specific expression pattern regulated by several 5' untranslated exons, and iii) encode an enzyme that catalyses the final step in fucosylated glycan synthesis (Costache et al., 1997; Saunier et al., 2001; Javaud et al., 2003).

In humans, tissue-specific expression patterns of FUT1 and FUT2 genes are well known; FUT1 is expressed in vascular endothelium and erythrocytes, while FUT2 is expressed in epithelial cells and body fluids, such as saliva. Conversely, SEC1 is inactive due to the deletion of two nucleotides, resulting in a truncate polypeptide with 246 amino acids. This gene also presents some characteristics of a non-autonomous retrotransposon, such as association with Alu-like elements and presence of a poly-A tail (Kelly et al., 1995; Saunier et al., 2001). On the other hand, Hitoshi et al. (1995) and Barreaud et al. (2000) showed that these three genes are fully activated in bovines and rabbits.

SEC1 was investigated in non-human primates by Apoil et al. (2000). They proposed that it is inactive in chimpanzees, gorillas and marmosets, due to nonsense mutations in apes and several deletion events in marmosets. They also suggested that the SEC1 gene is active in other Neotropical primate genera (e.g., *Saimiri*), due to the absence of known mutations and indel events that could cause a premature stop codon (Hitoshi et al., 1995, 1996; Apoil et al., 2000).

We compared SEC1 DNA sequences from 13 New World monkey genera and analyzed their evolution in primates.

MATERIAL AND METHODS

Isolation, amplification and DNA sequencing

DNA was obtained from 19 samples of 13 New World monkey genera (from the primate blood sample bank of Laboratório de Biologia Molecular, Universidade Federal do Pará) with the phenolic extraction protocol (Sambrook et al., 1989). The coding region was amplified using the primers designed by Apoil et al. (2000) specifically for New World monkeys. The polymerase chain reaction contained 1X reaction buffer, 100 ng DNA, 0.4 mM of each primer, 0.03 U/μL Taq DNA polymerase, 1.4 mM $MgCl_2$, 0.1 μg/μL BSA and 10 mM of each dNTP. The following conditions were applied (repeated 35 times): 94°C for 50 s, 60°C for 50 s and 72°C for 1.5 min. Amplified fragments were purified with the Wizard® PCR Preps kit (Promega), and sequenced by the dideoxyterminal method (Sanger et al., 1977), using an ABI 377 (Applied Biosystems) automatic sequencer.

Databases and sequence alignment

The nucleotide database was composed of the sequences obtained as described above (Table 1), with addition of others from the literature (GenBank accession codes within parentheses): *Callithrix jacchus* (AF111938), *Gorilla gorilla* (AB006611), *Homo sapiens* (U17895), *Hylobates agilis* (AB006609), *Macaca fascicularis* (AF112475), *Macaca mulatta* (AF080608), *Pan troglodytes* (AB006612), *Pongo pygmaeus* (AB006610), and *Saimiri sciureus* ssp (AF111937).

Sequences from *Mus musculus* (NM019934), *Bos taurus* (AF187851) and *Oryctolagus cuniculus* (X80225) were included in the amino acid database to compare their functional proteins with the protein sequences obtained from New World monkeys. Both nucleotide and amino acid sequences were aligned automatically by the software BioEdit 5.0.9 (Hall, 1999), using the default options, and then refined manually. Mutational saturation (transitions and transversions over divergence) was tested with the software DAMBE 4.2.13 (Xia and Xie, 2001), using Kimura's distance (Kimura, 1980), and no saturation was observed in these New World monkeys.

Evolutionary analyses

Transition and transversion rates among nucleotides, relative nucleotide and amino acid frequencies, codon usage, number of synonymous (dS) and nonsynonymous (dN) substitutions per site were calculated by the modified Nei-Gojobori method (Zhang et al., 1998) and computed using MEGA 3.0 (Kumar et al., 2004). Positive selection was assessed by testing the hypothesis H_1: dN > dS against a null hypothesis H_0: dN = dS using the one-tailed Z-test for selection (Nei and Kumar, 2000), implemented in MEGA; the standard error was calculated by the bootstrap method, using 1000 replicates; $P < 0.05$ was considered to be statistically significant.

The evolutionary rate of the gene (r) was calculated as K/2T, where K is the distance between the lineages analyzed and T is their divergence time (Li, 1997). To check if all sequences evolved at similar rates, a relative rate test was performed with the software HyPhy (Pond et al., 2005) using HKY85 (Hasegawa et al., 1985) model parameters.

Phylogenetic analysis was performed using the software PAUP*4b10 (Swofford, 2003), with parsimony criterion and application of successive weighting (Farris, 1969). Gaps were coded as a "fifth base" in order to preserve their information. Initially, 16 fundamental most-parsimonious trees were obtained through the parsimony-ratchet search strategy (Nixon, 1999), implemented via the accessory software PaupRat (Sikes and Lewis, 2001), using the "tree bisection and reconnection" branch-swapping algorithm and 200 ratchet iterations. Characters were then re-weighted based on their rescaled consistency index, and heuristic searches (iterations) were performed using the "tree bisection and reconnection" with 300 replicates of random sequence addition until the weights stabilized for two consecutive runs (Kitching et al., 1998). The analysis resulted in a single most parsimonious tree (Figure 1). The root was placed *a posteriori* following Nixon and Carpenter (1993), assuming *Gorilla*, *Homo, Hylobates*, *Macaca*, *Pan*, and *Pongo* as outgroups. Nodal support was assessed by 1000 bootstrap replicates (Felsenstein, 1985).

Divergence times among the sequences were estimated in order to date the split events within the New World monkey SEC1 lineages. First, model parameters of unequal nucleotide substitution, transition/transversion rate and heterogeneity rate among sites were obtained with the software BASEML, included in PAML 3.15 (Yang, 1997), using the likelihood criterion, following Rutschmann (2005). Next, branch lengths for the phylogenetic tree were estimated with the software ESTBRANCHES from the MULTIDISTRIBUTE package (see Thorne et al., 1998); these were used for Bayesian molecular clock inferences (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002), performed with the MULTDIVTIME software, also included in aforementioned package. The Markov Chain Monte Carlo was run for 1,000,000 cycles before taking the first sample, and then sampled 10,000 times with 100-cycle intervals.

Time constraints were specified for three tree nodes, corresponding to data from Opazo et al. (2006). The nodes represent the Cebidae divergence (20.2-22.8 million years ago, MYA), the divergence of the callitrichine lineage (14.8-16 MYA) and the Atelidae-Pitheciidae divergence (23-24.3 MYA). The molecular clock inferences were repeated three times to check the consistency of the results (Rutschmann, 2005).

Finally, to trace the evolution of SEC1 activity through the ancestral nodes, its condition in extant species was treated as a binary character, 0 being the "inactive" state and 1 the "active" state, and their distribution was plotted against the terminal taxons of the parsimony tree (Coddington, 1988; Carpenter, 1989; de Queiroz, 2000). The ancestral states on internal nodes were inferred using the software Mesquite 2.01 (Maddison and Maddison, 2007) by the likelihood criterion (Schluter et al., 1997; Pagel, 1999), using the previously obtained branch lengths. The "Asymmetrical Markov k-state 2 parameter" model of character evolution was adopted, with rates of forward and backward transformations of 8.87 and 8.07, respectively. The root state frequencies were set to equilibrium, so they are assumed to be consistent with the model's rates, and the threshold for decision among the two states at each tree node was set to 1.0. Relative proportional likelihoods for each character state are depicted as small pie diagrams on the tree nodes, accompanied by the numeric value for the most likely state.

RESULTS AND DISCUSSION

Partial sequences were obtained for all samples, with estimated gene size ranging from 795 bp in *Callithrix*, to 1041 bp in *Pithecia* (Table 1). This variation is due to indel events, some of them shared within lineages, such as the callitrichines. The sequences of *Callicebus brunneus*, *Brachyteles arachnoides*, and *Saimiri sciureus* spp (Apoil et al., 2000) were also excluded from phylogenetic analysis due to the short fragments obtained; however, the latter two were used in the analysis of protein structure.

The nucleotide composition of SEC1 is marked by a high frequency of pyrimidines (54.6%). The average GC content was 63.8% and the GC:AT proportion was 1.76. These values are similar to those described for the FUT1 gene (Borges and Harada, 2004) and suggest that SEC1 is located in a GC-rich isochore, similar to that proposed by Sharp et al. (1995) for humans. The analysis of nucleotide substitution patterns revealed a predominance of transitions over transversions, with an average ratio of 2.73. Pairwise comparisons showed a slight predominance of C↔T transitions, 1.14 times higher than A↔G.

Table 1. Monkey samples, their respective GenBank accession numbers, sample origins, and number of base pairs sequenced.

Primate group/species	Accession number	Origin[1]	Base pairs sequenced
Family Atelidae			
Alouatta belzebul	DQ166183	Unknown	608
Ateles belzebuth	DQ166185	CENP, Brazil	979
Ateles paniscus	DQ166186	Rio Trombetas, Pará, Brazil	991
Brachyteles arachnoides	DQ166187	CPRJ, Brazil	490
Lagothrix lagotricha	DQ166194	Unknown	967
Family Pitheciidae			
Callicebus brunneus	DQ166188	Rio Jamari, Rondônia, Brazil	990
Callicebus brunneus	DQ166189	Rio Jamari, Rondônia, Brazil	704
Chiropotes satanas	DQ166192	Unknown	986
Chiropotes albinasus	DQ166193	Unknown	975
Pithecia irrorata	DQ166196	Rio Jamari, Rondônia, Brazil	1018
Pithecia irrorata	DQ166197	Rio Jamari, Rondônia, Brazil	1005
Family Cebidae			
Aotus azarae	DQ166184	Rio Jamari, Rondônia, Brazil	991
Cebus olivaceus	DQ166191	Rio Grande do Sul, Brazil	990
Saimiri boliviensis boliviensis	DQ166200	Bolivia	995
Subfamily Callitrichinae			
Callithrix humeralifer	DQ166190	Unknown	745
Saguinus fuscicollis weddelli	DQ166198	Rio Jamari, Rondônia, Brazil	979
Saguinus mystax	DQ166199	CPRJ, Brazil	989
Leontopithecus chrysomelas	DQ166195	Unknown	968

[1]CENP = Centro Nacional de Primatas; CPRJ = Centro de Primatologia do Rio de Janeiro.

Comparisons among the dN/dS gene ratios were made for the following primate groups (Schneider et al., 2001): Cebidae (Callitrichinae), Pitheciidae, Atelidae, and Platyrrhini. In all cases, the dN/dS ratio was higher than 1, ranging from 1.18 in Atelidae to 1.58 in Pitheciidae (Table 2), slightly higher than those obtained in humans and Old World monkeys. The dN/dS ratio was also compared in different regions of SEC1: N-terminal region, transmembrane domain and C-terminal region. We observed that the dN/dS ratio in the C-terminal region, where all protein domains were located, was similar to that described for the whole coding region (Table 2). The Z-test indicated positive selection, which can be interpreted as evidence of weak selective pressure in key regions for protein function, as proposed by Breton et al. (1998) and Martinez-Duncker et al. (2003).

Table 2. Rates of synonymous (dS) and nonsynonymous (dN) substitutions per site of the SEC1 gene for the different primate groups.

Group	dN / dS			
	Whole region	Coding N-terminal region	Transmembrane domain	C-terminal region
Primates	1.13	0.46	3.90	1.11
HOM	0.91	0.26	0	0.91
OWM	1.14	0	0	1.00
NWM	1.32	0	0.37	1.32
Atelidae	1.18	n/c	0	1.18
Cebidae	1.22	0	3.46	1.20
Pitheciidae	1.58	n/c	0.73	1.58

HOM = Apes and humans; OWM = Old World monkeys; NWM = New World monkeys. n/c = not computed.

The alignment of nucleotide sequences showed two deletions (nucleotides 278-301 and 411-621) in *Callithrix humeralifer*. These deletions were previously described in *Callithrix jacchus* by Apoil et al. (2000). Other callitrichine genera also present deletion events. *Saguinus* sequences show a single 2-bp deletion at positions 408-409, and in *Leontopithecus* there is a 1-bp deletion at position 566 and a 4-bp deletion at position 1095-1098. Insertion events were observed in *Pithecia irrorata* at position 121-141 (21 bp) and 354-357 (4 bp); in *Callithrix humeralifer* at 1022-1023 (2 bp), 1036-1041 (6 bp) and 699. The latter also occurs in *Aotus azarae.*

The predicted protein of these species (Table 3) lacks similarity with other fucosyltransferases from residues 90 in *Callithrix jacchus*, 93 in *Callithrix humeralifer*, 119 in *Pithecia irrorata*, 135 in *Saguinus*, 188 in *Leontopithecus* and 232 in *Aotus azarae*. Consequently, they lack the three conserved domains proposed by Oriol et al. (1999) for all α1,2 FUT, except for that of *Callithrix*. Histidine and arginine residues (positions 188 and 191 in Neotropical primates), related by Takahashi et al. (2000) as important for α1,2 and α1,6 FUT enzymatic activity, are also not conserved in these species. In addition, these species have multiple stop codons, except for *Callithrix humeralifer.* These results strongly suggest that the predicted protein has no activity. In the remaining primate sequences, the domains are relatively conserved, suggesting that, despite dN/dS results indicating positive selection, SEC1 in all other New World monkeys produces a fully active protein.

Our results indicate that the proposed inactivation of SEC1 in callitrichines, *Aotus* and *Pithecia*, as well as in apes (*Homo*, *Pan* and *Gorilla*), may have evolved by independent transformations. Also, *Pithecia irrorata*, *Saguinus* and *Aotus* show a high similarity in the carboxy portion of the human and gorilla protein, including a stop codon at position 276 of the alignment.

The α1,2 FUT are widely expressed in vertebrates, invertebrates, plants, and bacteria. They are involved in the final stages of the synthesis of human H histo-blood group antigens associated with the ABO system and in a variety of pathological processes as many parasites, bacteria and viruses are know to bind to carbohydrate structures in their effort to initiate the infection process (Costache et al., 1997; Ma et al., 2006).

During the evolutionary process, the α1,2 FUT showed a progress from endodermal to ectodermal tissues. In humans, the erythrocytes (mesodermal origin) were the last cells to acquire the histo-blood group ABH antigens (Barreaud et al., 2000).

In Catarrhini, only two α1,2 FUT (FUT1 and FUT2) are expressed, with the SEC1 gene inactive due to an accumulation of mutations, like deletions and stop codons (Apoil et al., 2000). However, this non-functional gene does not alter the expression of the H antigens, because the other two are responsible for protein expression in secretions and cell surfaces (Mollicone et al., 1995; Costache et al., 1997).

The Platyrrhini species possess the three α1,2 FUT genes, and it is known that their expression is quite different from that observed in the Catarrhini, the H antigen being present only in secretions; it is replaced in red blood cells by the α-Gal antigen (Oriol et al., 1992; Apoil et al., 2000). Nevertheless, no mutation was found in the FUT1 gene that could explain the lack of expression (Borges and Harada, 2004). Apoil et al. (2000) suggest that an Alu-like element inserted in a region crucial for regulation is responsible for expression of the H enzyme in human and ape erythroid lineages. The same was proposed for bovines and humans, in which SEC1 gene expression was detected only in intestinal tract cells (Barreaud et al., 2000).

Table 3. Amino acid sequence of the SEC1 gene.

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                                        10        20        30        40        50
                                ....|....|....|....|....|....|....|....|....|....|
Bos taurus                      MWDMRAVAPQRPAAGHPRAGWPRKLKTAATRFWATCPSSST-------VC
Mus musculus                                  MPSDSCLLSL.VLQ.LR.I..PL..-------FY
Oryctolagus cuniculus                        MRFAPDYVLCPP..TR.LR..H..V..-------IY
Homo sapiens                                       MSSL.P..VKG....R..F..-------FY
Pan troglodytes                                    MSSL.P..VKG....H..F..-------FY
Gorilla gorilla                                    MSSL.P..VKG....R..F..-------FY
Hylobates agilis                                   MSSL.PA.VKG.......F..-------FY
Pongo pygmaeus                                     MSSL.PA.VKG....R..F..-------FY
Macaca fascicularis                                XXXXXXA.VKG..T....F..-------FY
Macaca mulatta                                     MSSL.P..VKG..T....F..-------FY
Saimiri sciureus ssp                               XXXXXXA.IKGY.T.R..F..-------IY
Saimiri boliviensis boliviensis                    XXXXXXX.IKGY.T.R..F..-------IY
Cebus olivaceus                                    XXXXXXXXXXKG..T.R..F..-------.Y
Aotus azarae                                       XXXXXXXXXXKG..TSR..F..-------.Y
Callithrix jacchus                                 XXXXXXA.IKG..T.R..F..-------.Y
Callithrix humeralifer                             XXXXXXXXXXXX.T.R.FF..-------.Y
Saguinus mystax                                    XXXXXXXXXXKG..T....F..-------.Y
Saguinus fuscicollis weddelli                      XXXXXXXXXXXX.T.R..F..-------.Y
Leontopithecus chrysomelas                         XXXXXXXXXXXXXXXR..F..-------IY
Pithecia irrorata Pi2                              XXXXXXXXIKG..T.H..F..THPSFST.Y
Pithecia irrorata Pi43                             XXXXXXXXXXXX.T.H..F..THPSFST.Y
Callicebus brunneus Cb44                           XXXXXXXXXKG..T.R..F..-------IY
Chiropotes satanas                                 XXXXXXXXXX.I.T.H..F..-------.Y
Alouatta belzebul                                  XXXXXXXXXXG..T.R..F..-------.Y
Lagothrix lagotricha                               XXXXXXXXXXXXXXXR..F.I-------.Y
Brachyteles arachnoides                            XXXXXXXXXXXXXXXXXXXXXXXXXXXX.Y
Ateles paniscus                                    XXXXXXXXXKGL.T....F..-------.Y
Ateles belzebuth                                   XXXXXXXXXXXXT.R..F..-------GY
                                       60        70        80        90       100
                                ....|....|....|....|....|....|....|....|....|....|
Bos taurus                      FLFVIFAVSTVFHCHRRLALVPAPWAYAGHVVLFPRHLPRGGVFTINAKG
Mus musculus                    LF....V...I.......G.......SPSL..FP...M..E.M...RV..
Oryctolagus cuniculus           ...T..V........Q...........SAR..VV.G....E.MW....M.
Homo sapiens                    .V.A..V...I....QH..........SAR...A......EDL....S..
Pan troglodytes                 .V.A..V...I....Q...........SA....A......E.L....S..
Gorilla gorilla                 .V.A..V...I....Q...........SAR...A......E.L....S..
Hylobates agilis                .V.A..V...I....Q...........SAR...A......E.L....S..
Pongo pygmaeus                  .I.A..V...I....Q...........SAR...A......E.L....S..
Macaca fascicularis             ...A..V...I....Q...........SA....A......E.L....S..
Macaca mulatta                  ...A..V...I....Q...........SA....A......E.L....S..
Saimiri sciureus ssp            ...A..V........Q...........SAR...A......E.L....S..
Saimiri boliviensis boliviensis ...A..V........Q...........SAR...A......E.L....S..
Cebus olivaceus                 ...A..V........Q...........SAR...A.....QE.L....S..
Aotus azarae                    ...A..V..A.....Q...Q..T....SAR...A.....QE.L....S..
Callithrix jacchus              ...A..V........QC...A..T...SAR...A.DTS.----------
Callithrix humeralifer          ...A..V........QC...A..T...SAR...A..Y..WE.--------
Saguinus mystax                 ...A...........QH....L.T...SAC...A.....WEVL....S..
Saguinus fuscicollis weddelli   ...A..V........QH....L.T...SAR...A.....WEVL....S..
Leontopithecus chrysomelas      ...A..V........Q.......T...SAC...A.....WE.L....S..
Pithecia irrorata Pi2           ...A..V..A.....Q......T....SA....A......E.L....S..
Pithecia irrorata Pi43          ...A..V..A.....Q......T....SA....A....L.E.L....S..
Callicebus brunneus Cb44        ...A..V........Q......T....SAR...A......E.L....S.S
Chiropotes satanas              ...A..V...I....Q......T....SAR...A......E.L....S..
Alouatta belzebul               ...A..V........Q...........SAR...A......E.L....S..
Lagothrix lagotricha            ...A..V........Q...........SAR...A.....QE.L....S..
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Table 3. Continued.

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Brachyteles arachnoides          ...A..V........Q...........SAR...A......E.L....S..
Ateles paniscus                  ...A..V........Q...........SAR...A......E.L....S..
Ateles belzebuth                 ...A..EEA......Q...........SAR...A......E.L....S..

                                         110       120       130       140       150
                                 ....|....|....|....|....|....|....|....|....|....|
Bos taurus                       RLGNQMGEYATLYALAKMNGRAAFIPPQMHSTLAPIFRITLPVLHDATAR
Mus musculus                     ............F...R....L....AS...........S.....SD..K
Oryctolagus cuniculus            ................E...P.Y..A...........S.....SS..S
Homo sapiens                     ....................P....A....RV...........S...S
Pan troglodytes                  ....................P....A.................S...S
Gorilla gorilla                  ....................P....A.............L...S...S
Hylobates agilis                 ....................P....A.................SV..S
Pongo pygmaeus                   ....................P....A.................S...S
Macaca fascicularis              ....................P....V.................S...S
Macaca mulatta                   ....................P....V.................S...S
Saimiri sciureus ssp             ................L...P....A....A............SS..S
Saimiri boliviensis boliviensis  ................L...P....A....A............SS..S
Cebus olivaceus                  ................L...P....A....A............ST..S
Aotus azarae                     ................L...P....A....V...V.........ST..S
Callithrix jacchus               ------------------....WG----------------TRWVS.PH
Callithrix humeralifer           ---------------------RLG----------------NQMGEY.T
Saguinus mystax                  ............T...L..QS....A.......--HLQNH.AGAAQHHG
Saguinus fuscicollis weddelli    ................L...P....A....A..--HLQNH.AGAAQHHG
Leontopithecus chrysomelas       H...........T...L...P....A....A..A..........ST..S
Pithecia irrorata Pi2            .........T.......LGE...CLH.SP.AQRPLPHLQNH.AGAAQHHS
Pithecia irrorata Pi43           ................LGE...CLH.SP.AQRPLPHLQNH.AGAAQHHS
Callicebus brunneus Cb44         ................L...P....A....A............ST..S
Chiropotes satanas               ................L...P....A....A............ST..S
Alouatta belzebul                ................L...P....A....A............ST..S
Lagothrix lagotricha             L...............L...P....A....S............ST..S
Brachyteles arachnoides          ................L...P....A....A............ST..S
Ateles paniscus                  ................L...P....A....A............ST..S
Ateles belzebuth                 ................L...P....A....A............ST..S

                                         160       170       180       190       200
                                 ....|....|....|....|....|....|....|....|....|....|
Bos taurus                       SVPWQNYHLNDWMEEQYRHIPGEYVRLTGYPCSWTFYHHLRAEILQEFTL
Mus musculus                     RI............R......H...F.............P...K....
Oryctolagus cuniculus            R.............R.....VP................H...R....
Homo sapiens                     RI............E......RC.H..............Q........
Pan troglodytes                  RI............E......R.................Q........
Gorilla gorilla                  RI............E......R..............Y...Q........
Hylobates agilis                 RI............E.H....R..C.M....T.......CH........
Pongo pygmaeus                   RI............E......R.................H........
Macaca fascicularis              RI........N....K......R.................H........
Macaca mulatta                   RI........N....K......R.................H........
Saimiri sciureus ssp             RI............E.H....R........S.........H........
Saimiri boliviensis boliviensis   RI............E......R........S.........H........
Cebus olivaceus                  RI............E......R........S.........H........
Aotus azarae                     RI...........K.E......H..C.....S.........Q........
Callithrix jacchus               CM..P-------------------S*MG.L.S.RPRCTAP.----------
Callithrix humeralifer           LYALA-------------------KLNGRPAFIPAQI.STT---------
Saguinus mystax                  QQDPLAELPPERLDGRGIPPHPGALCPPHGLPQLLDLLPPPPPGDPPGVH
Saguinus fuscicollis weddelli    QQDPLVELPPERLDGRGVPPHPRALCPPHGLPQLLDLLPPPPPGDPPGVH
Leontopithecus chrysomelas       RI............E.H..L.R..H.....S.....TTSARRSSRSSPC
Pithecia irrorata Pi2            QQDPLAELPPERLDGRGVPPHPGALCSPHGLPQLLDLLPPPPPRDPPGVH
Pithecia irrorata Pi43           QQDPLAELPPERLDGRGVPPHPGALCSPHGLPQLLDLLPPPSPRDSPGVH
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Table 3. Continued.

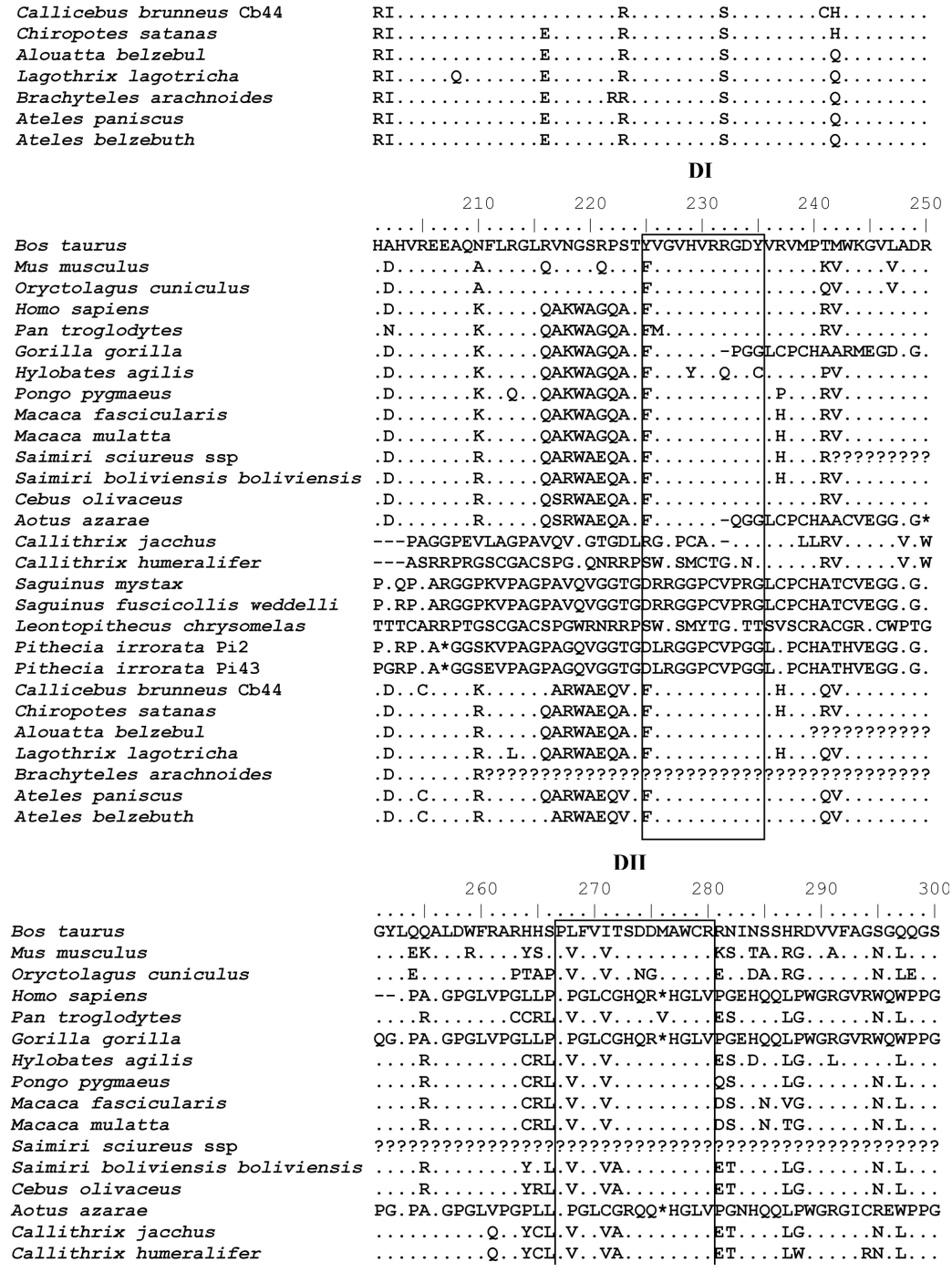

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Table 3. Continued.

```
Saguinus mystax                 PG.PA.GPGLVPGPLP.PSLCGRQR*HGLVPGNRQQLPWGRGFCREWPPG
Saguinus fuscicollis weddelli   PG.PA.GPGLVPGPLP.PSLCGRQR*HGLVPGNHQQLPWGRGFCREWPPG
Leontopithecus chrysomelas      ATCSGPWTGSGPVTT.WSLWSPAMTWPGAGKPSTAPSGMWFLP.MASRAH
Pithecia irrorata Pi2           PG.PA.GPGLVPGPLP.PSLCGHQR*RGLVPGNYQQLPRGCGVRREWPPG
Pithecia irrorata Pi43          PG.PA.GPGLVPGPLP.PSLCGHQR*HGLVPGNYQQLPRGCGVRREWPPG
Callicebus brunneus Cb44        ....R........YRL.V..VA........ET....LG......N.LK..
Chiropotes satanas              ....R........YRL.V..VA........ET....LG......D.L...
Alouatta belzebul               ????????????????????????????????????????????????
Lagothrix lagotricha            ....R........YRL.V..VA........ET....LG......N.LK..
Brachyteles arachnoides         ????????????????????????????????????????????????
Ateles paniscus                 ....R........Y.L.V..VA........ET....LG......N.L...
Ateles belzebuth                ....R........YRL.V..VA........ET....LG......N.L...
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DIII

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                                   310        320        330        340        350
                                   ....|....|....|....|....|....|....|....|....|....|
Bos taurus                      PARDFALLTQCNHTVITVGTFGIWAAYLAGGSTVYLANFTLPGSRFRMIF
Mus musculus                    ..K.I...M........L..........T..D........Q.N.P.HTV.
Oryctolagus cuniculus           ..K............M.I....F.....T..D......Y.A.D.P.HLV.
Homo sapiens                    LTCQGLRTAHTVQPHHHHRGHLRGLGRVPR.RGHC.PGQLH.AQLPFQRG
Pan troglodytes                 ..K...........I.........V.........D..........S.P.NVV.
Gorilla gorilla                 LTCQGLRTAHTVQPHHHHCGHLRGLGRVPR.RGHCQPGQLH.AQLPFQRG
Hylobates agilis                ..K...........I.........F.........D..........N.P.DVV.
Pongo pygmaeus                  ..K...........I.........V.....V..D..........N.P.DVV.
Macaca fascicularis             ..K...........I.........V.........D.....K....N.P.N.V.
Macaca mulatta                  ..K...........I.........V.........D..........N.P.N.V.
Saimiri sciureus ssp            ????????????????????????????????????????????????
Saimiri boliviensis boliviensis ..............I.........T.....T..D.I......R.H.P.NLV.
Cebus olivaceus                 ..............I.........T.....T..D.I......R.TPPSTWSL
Aotus azarae                    LTCQGLCTADTGQPHHHHCGHLWNLGC.PH.WGHH.PGQLHTAALPFRPG
Callithrix jacchus              ..................M....T.S...M..D.I..V...R.H.P.NLV.
Callithrix humeralifer          ....................T.S...T..D.I..V.LSTSH.HTPPST
Saguinus mystax                 LTCQGLCTADTVQPHCHHRGHLWNLGC.PH.RGHH.PGQLHTAALPLQPG
Saguinus fuscicollis weddelli   LTCQGLCTADTVQPHCHHRGHLWNLGC.PH.RGHH.PGQLHTAALPLQPG
Leontopithecus chrysomelas      LPGTLHC*HSATTPSSLWAPLEPGPPTSRA.TPSTWPTSHSHTPPSTWSL
Pithecia irrorata Pi2           LTCQGLCTSDTVQPHHHHRGHLWNLGR.PHRWGHH.PGQLH.APLPLRPG
Pithecia irrorata Pi43          LTCQGLCTSDTVQPHHHHRGHLWNLGR.PHRWGHH.PGQLH.APLPLRPG
Callicebus brunneus Cb44        ..............I.........T.....T.RD.I......W.H.P.DLV.
Chiropotes satanas              ..............I.........T.....T..D.I......R.H.P.DLV.
Alouatta belzebul               ????????????????????????????????????????????????
Lagothrix lagotricha            ..............I.........T.....T..D.I......R.H.P.DLV.
Brachyteles arachnoides         ????????????????????????????????????????????????
Ateles paniscus                 ..............I.........T.....T..D.I......R.H.P.DLV.
Ateles belzebuth                ..............I.........T.....T..D.I......R.R.P.CLV.

                                          360        370        380        390
                                          ....|....|....|....|....|....|....|....|..
Bos taurus                      KPQAAFLPEWVGIAANLGQARESHP*
Mus musculus                    ..E..Y.........D...PNTVGSGHASARAPKRHWGALL*
Oryctolagus cuniculus           ..E..........T..M.R.LW.GL*
Homo sapiens                    L*AVSG..AR..GPCG*PWTGWTERPL
Pan troglodytes                 R.*.........L..D....GQNSL*
Gorilla gorilla                 L*AVSG..AR..GPCG*PWTGWTEWPL
Hylobates agilis                R...........L..D....GQNGL*
Pongo pygmaeus                  R...........L..D....GQNGL*
Macaca fascicularis             R...V..........????????????????????????????????
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Continued on next page

Table 3. Continued.

Macaca mulatta	R.............D....GQNGL*
Saimiri sciureus ssp	??
Saimiri boliviensis boliviensis	R..........M..????????????????????????????
Cebus olivaceus	GRKQPSCQSGWAWRL????????????????????????????
Aotus azarae	L*ATSSF.AT.RGHGG*
Callithrix jacchus	R..........M..????????????????????????????
Callithrix humeralifer	LSLGHKQ.SCQSGW????????????????????????????
Saguinus mystax	L*ATSS..AR..GHGG*
Saguinus fuscicollis weddelli	L*ATSS..AR..GHGG*
Leontopithecus chrysomelas	GHKQLSCQSGH.G*
Pithecia irrorata Pi2	LWTTSS..AR..GHGG*
Pithecia irrorata Pi43	LWTTSS..AR..GHGG*
Callicebus brunneus Cb44	R..........M..????????????????????????????
Chiropotes satanas	R..........M..????????????????????????????
Alouatta belzebul	??
Lagothrix lagotricha	R..........M.????????????????????????????
Brachyteles arachnoides	??
Ateles paniscus	R.........M.M..????????????????????????????
Ateles belzebuth	R..........M..????????????????????????????

Dots represent identity, dashes represent amino acid deletions, "X" represents unknown residues, asterisks (*) indicate stop codons, and question marks (?) indicate missing data. Boxes indicate the three domains present in all α1,2 fucosyltransferases. Residue numbers are indicated above the reference sequences for the alignment. DI = conserved domain I, DII = conserved domain II; DIII = conserved domain III.

Comparison of SEC1 gene evolutionary rates among Atelidae, Cebidae and Pitheciidae families showed variation ranging from 0.791 x 10^{-9} (Atelidae) to 1.314 x 10^{-9} (Cebidae) (Table 4), while in all New World monkeys the overall ratio was 1.074 x 10^{-9}. This difference could be explained by several changes in the nucleotide sequences (indels), especially in callitrichines, which may increase the rate in the cebids compared to other groups. This is supported by the results of the relative rate test, which indicated significant differences in the evolutionary rates ($P < 0.05$) in *Aotus*, callitrichines and *Pithecia* when compared to other primates.

Table 4. Estimates of the rates of evolution (r = K/2T) of the SEC1 gene in the different groups of primates.

Group	K	T	Substitutions/site/year (r)
Primates	0.0659	63	0.523 x 10^{-9}
HOM	0.0332	18	0.922 x 10^{-9}
OWM	0.0247	14	0.881 x 10^{-9}
NWM	0.0537	26	1.033 x 10^{-9}
Atelidae	0.0253	15.8	0.800 x 10^{-9}
Cebidae	0.0578	20.2	1.431 x 10^{-9}
Callitrichinae	0.0625	14.2	2.200 x 10^{-9}
Pitheciidae	0.0404	21.3	0.948 x 10^{-9}

Divergence times (T) are given in millions of years ago, according to Opazo et al. (2006), for New World monkeys and Goodman et al. (1998) for other primates. HOM = Apes and humans; OWM = Old World monkeys; NWM = New World monkeys.

Except in Cebidae, in which evolutionary rates described here are closer to those reported for ψβ-globin (2.0 x 10^{-9}, in primates) and ψη-globin (1.9 x 10^{-9} in *Aotus*) (Li et al., 1981; Li and Gojobori, 1983; Goodman et al., 1984; Harris et al., 1984), all other values were closer to that reported for FUT1 (0.739 x 10^{-9}) in these primate groups, which is relatively

lower than the evolutionary rates of pseudogenes (Borges and Harada, 2004).

The SEC1-like family presents higher evolutionary rates than the FUT2-like family due to accumulation of mutations, such as premature stop codons, in the SEC1 pseudogene (Apoil et al., 2000). These mutations are also observed in some Old and New World monkeys, but until now no SEC1 transcript has been found in these species or in healthy human tissues. Besides the mutations described, probably a DNA rearrangement at the SEC1 locus occurred during and/or after a retrotransposition event in humans. It is widely known that subsequent mutations are facilitated by the previous gene-inactivating rearrangement (Saunier et al., 2001).

The phylogenetic tree obtained for the SEC1 gene (Figure 1) is basically consistent with published arrangements, except for *Callicebus*, which grouped within the Atelidae (bootstrap support = 76%), instead of within the Pitheciidae (e.g., Harada et al., 1995; Porter et al., 1997; Goodman et al., 1998; Schneider, 2000; Schneider et al., 1993, 1996, 2001; Schrago, 2007).

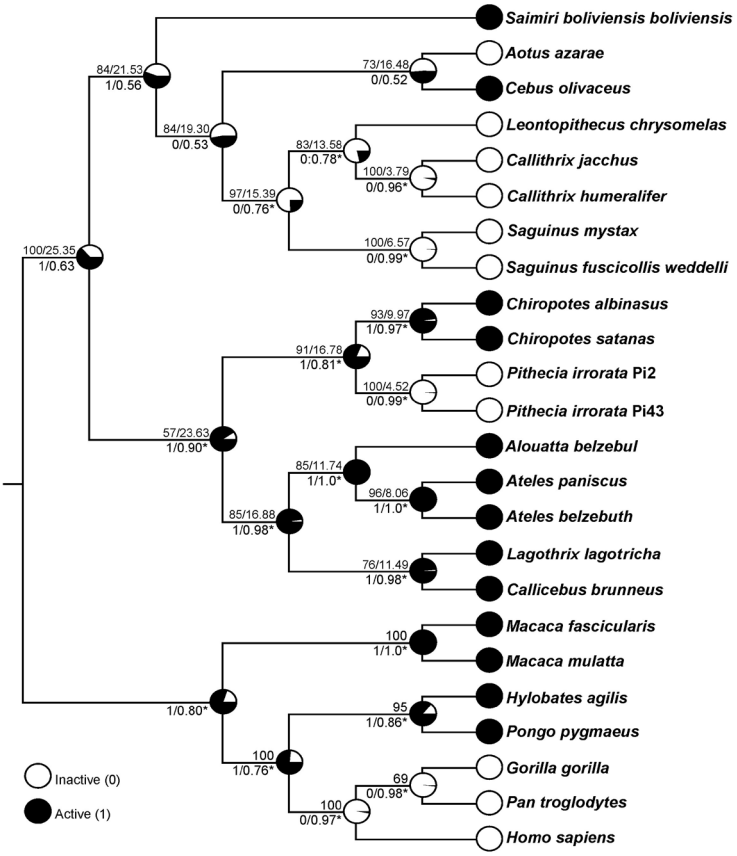

Figure 1. Phylogenetic tree of the SEC1 gene inferred through the maximum parsimony method. The pie diagrams at the nodes denote the proportional likelihoods for each state of the binary character "SEC1 gene activity". The numbers associated with the internal branches correspond to character state/character state likelihood. Bootstrap values/divergence times are above the branches. Asterisks denote unambiguous choice of ancestral states, according to the decision threshold. Ancestral states inference -logL = 13.864273495.

The transition/transversion rates and nucleotide sequence alignment support the hypothesis that primate SEC1 evolved by divergent evolution, as proposed for the origin of fucosyltransferases (Breton et al., 1998; Oriol et al., 1999; Barreaud et al., 2000; Bureau et al., 2001; Borges and Harada, 2004). In contrast, multiple stop codons and amino acid changes present in different species suggest convergent inactivation events in apes, Callitrichinae, *Aotus* and *Pithecia*.

Considering the stop codon detected in SEC1 gene of callitrichines, *Aotus* and *Pithecia*, an enzyme that lacks at least 131 amino acids is produced in comparison with the functional ones, suggesting the non-functionality. This hypothesis is supported by the description of reduced activity of different fucosyltransferases induced by deletion of one or more amino acids in the carboxy-terminal region (Xu et al., 1996).

Based on Bayesian analysis of divergence times (Table 5), the differentiation of the Platyrrhini SEC1 lineage occurred at approximately 25.35 MYA. This is consistent with reported values for New World monkey radiation, which range from 20 to 35 MYA (Goodman et al., 1998; Schneider, 2000; Schneider et al., 1993, 2001; Figueiredo, 2006; Schrago, 2007). Likelihood inference of ancestral states for the activity of SEC1 gene in New World monkeys (Figure 1) indicated that the inactive condition evolved independently at least twice in the Platyrrhini.

Table 5. Divergence times of SEC1 in Platyrrhini (in millions of years ago) and those proposed by other authors, with their respective 95% of credibility intervals, when available.

Lineage	Present study	Goodman et al. (1998)	Schneider et al. (2001)	Figueiredo (2006)	Opazo et al. (2006)	Schrago (2007)
Platyrrhini	25.35 (23.89-28.8)	25	26	30.4 (24.2-42.2)	26	20.1 (15.6-28.3)
Atelidae	16.88 (11.27-22.49)	16	15	21.1 (17.1-29.5)	16.75	12.4 (9.1-18.6)
Cebidae	21.53 (20.28-22.73)	22	23	26.4 (20.7-36.6)	22.75	16.9 (12.7-23.3)
Callitrichinae	15.39 (14.83-15.96)	13	16	18.0 (13.8-25.4)	16	11.8 (8.3-17.4)
Chiropotes-Pithecia	16.78 (10.99-22.25)	10	11	15.6 (11.7-22.0)	11.96	9.1 (6.4-13.4)

The inactive condition of SEC1 proposed for *Pithecia irrorata* could not be traced back to the last common ancestor with *Chiropotes*. Consequently, it probably evolved in the *Pithecia* lineage, at about 4.62 MYA. In the *Aotus*-*Cebus*-Callitrichinae group, it is not evident whether SEC1 inactivation occurred independently in *Aotus* and Callitrichinae lineages or in the last common ancestor of the group. In this latter case, the active condition in *Cebus* would be a reversion (Figure 1). Despite the inactive state observed in the last common ancestor of this group, the nucleotide changes leading to this condition seem to be independent with different indel events responsible for gene inactivation in *Aotus*, *Leontopithecus*, *Saguinus* (with considerable changes in the open reading frame) and in *Callithrix*.

The similarity of the carboxy-portion of the amino acid sequences, especially at domains II and III (Table 3) amongst *Callithrix* and hypothetically active in the New World monkeys, suggests that a great deletion event occurred at domain II, followed by other events (including a premature stop codon mutation in *Callithrix jacchus*), which would be responsible for the inactivation of the protein. In our tree, one of the main differences in relation to other phylogenies is the arrangement of the Cebidae. In other molecular phylogenies of primates, the position of *Aotus*, *Cebus* and *Saimiri* species varies in relation to other Callitrichinae (e.g., Harada et al., 1995; Porter et al., 1997; Goodman et al., 1998; Schneider, 2000; Schneider et al., 1996, 2001; Opazo et al., 2006; Schrago, 2007). This could be a result of the rapid divergence events that occurred in the Cebidae, which would decrease the resolution

within this group. Although in our tree, *Aotus* grouped with *Cebus*, both *Cebus* and *Saimiri* share an active state for SEC1.

In conclusion, our proposition is the inactivity of SEC1 in some New World monkey lineages, a feature that could have evolved independently more than once. Further analyses of protein expression and tertiary structure, as well as new DNA sequences that include promoter regions will help to clarify this subject.

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