

Sequential Loss of Two Neighboring Exons of the Tropoelastin Gene During Primate Evolution

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Abstract. Previous evidence has demonstrated the absence of exons 34 and 35 within the 3' end of the human tropoelastin (ELN) gene. These exons encode conserved polypeptide domains within tropoelastin and are found in the ELN gene in vertebrate species ranging from chickens to rats to cows. We have analyzed the ELN gene in a variety of primate species to determine whether the absence of exons 34 and 35 in humans either is due to allelic variation within the human population or is a general characteristic of the Primates order. An analysis of the 3' end of the ELN gene in several nonhuman primates and in 546 chromosomes from humans of varying ethnic background demonstrated a sequential loss of exons 34 and 35 during primate evolution. The loss of exon 35 occurred at least 35-45 million years ago, when Catarrhines diverged from Platyrrhines (New World monkeys). Exon 34 loss, in contrast, occurred only about 6-8 million years ago, when Homo separated from the common ancestor shared with chimpanzees and gorillas. Loss of both exons was probably facilitated by Alu-mediated recombination events and possibly conferred a functional evolutionary advantage in elastic tissue.

Key words: Tropoelastin gene — Alu repeat sequences — Gene evolution

Introduction

Elastic fibers are extracellular matrix structures that confer the property of resilience and elastic recoil to all elastic tissues (Sandberg et al. 1969). Elastin is the major protein component of elastic fibers (Rucker et al. 1973). Extensively cross-linked and highly insoluble, elastin is assembled from a family of precursor proteins collectively referred to as tropoelastin (Sandberg et al. 1969; Rucker et al. 1973; Foster et al. 1975). Tropoelastins (ELN) are multidomained proteins composed predominantly of several hydrophobic regions rich in glycine, valine, and proline (Foster et al. 1973a) and multiple lysine-containing alanine-rich domains (Foster et al. 1973b) that serve as a substrate for the catalysis of lysine-derived cross-links by the enzyme lysyl oxidase (Kagan and Trackman 1991). A single-copy multiexon gene is responsible for the synthesis of multiple isoforms of tropoelastin by extensive alternate usage of several exons encoding both hydrophobic domains and lysinecontaining cross-link regions (Boyd et al. 1993).

In recent years, a number of investigators have char-

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acterized the structure of the gene coding for tropoelastin from several species including humans, cows, and rats (Bashir et al. 1989; Raju and Anwar 1987; Pierce et al. 1990). This analysis has revealed a gene that, in most species, is composed of 36 exons distributed throughout approximately 40,000 bp of genomic DNA, has an intron:exon ratio of almost 19:1, and has an unusually high frequency of repetitive DNA sequences within many introns, particularly at the 3' end (Indik et al. 1987).

In contrast to the bovine and rat ELN genes, the human ELN gene has only 34 exons. Several years ago Rosenbloom and co-workers reported (Bashir et al. 1989; Indik et al. 1987) the absence of two exons at the 3' end of the human ELN gene (exons 34 and 35). These authors suggested that the abundance of Alu repetitive DNA sequence elements surrounding exons 33 and 36 may have been responsible for the loss of exons 34 and 35 in the human ELN gene. These two exons are present within the tropoelastin gene in all vertebrate species analyzed to date and also encode highly conserved crosslink and hydrophobic regions (Boyd et al. 1991). To explore the possibility that Alu-mediated recombination events may indeed have contributed to the loss of exons 34 and 35, either within the human population or elsewhere within the primate order, we have analyzed the 3'end of the ELN gene within the human population and in several other primate species. Here we report the sequential loss of exons 34 and 35 within the tropoelastin gene during primate evolution and suggest that these evolutionary changes within the ELN gene may be functionally important within primate phylogeny.

Materials and Methods

Isolation of the Baboon Tropoelastin cDNA and Genomic DNA Recombinants

A λ gt11 cDNA library (kindly provided by Dr. A.W. Clowes, Department of Surgery, University of Washington, Seattle) was constructed using poly(A⁺) RNA from cultured baboon smooth muscle cells. Plaque forming units (pfus; 5 × 10⁴) were screened with a previously characterized human tropoelastin cDNA, H-11 (Olson et al. 1995), using a standard screening procedure (Sambrook et al. 1989). DNA from autoradiographically positive plaque recombinants, obtained after screening primary and secondary positives, was subject to dideoxy DNA sequence analysis.

A baboon tropoelastin cDNA (BEL8) was used to screen a partial *Sau*3AI baboon genomic DNA library prepared using the λ DASH II cloning vector and supplied by Clonetech. pfus (5 × 10⁴) were screened and autoradiographically positive recombinants were also characterized by restriction enzyme analysis and DNA sequencing.

Human and Primate Genomic DNA Preparations

Genomic DNA was isolated from aliquots of blood from 53 unrelated primates using a previously described procedure (Sambrook et al. 1989). Aliquots of blood were provided by the San Diego Zoo and were obtained from the following species (the number of individual primates from which blood was obtained is indicated in parentheses): *Pan troglodytes*—chimpanzee (3); *Gorilla gorilla*—gorilla (2); *Pongo pygmaeus*—orangutan (2); *Hylobates lar*—gibbon (3); *Papio cynocephalus anubis*—olive baboon (12); *Papio cynocephalus cynocephalus* yellow baboon (12); *Papio cynocephalus hamadryas*—hamadryas baboon (12); *Madrillus sphinx*—mandrill (3); *Macacus rhesus*—rhesus monkey (3); and *Macacus cyanomegalus*—cyanomegalus monkey (2).

Human genomic DNA was isolated from aliquots of blood from 273 unrelated individuals representing the following ethnic and geographical groups (the number of individuals from which blood was obtained is also indicated in parentheses): African Americans (50), Hispanic Americans (50), Caucasian Americans (76), Hungarians (46), Zaire pygmies (10), Central African Republic pygmies (7), Indonesian Rotis (1), Indonesian Ternates (4), Indonesian Hiris (5), Papua New Guinea—highland region (10), and Papua New Guinea—coastal region (14).

PCR Analysis of the 3' End of the Human and Primate Tropoelastin Genes

PCR analysis of human genomic DNA was carried out in a 25- μ l volume containing intron specific primers derived from intron 33 (5' AAC ACA GGG AAC ATT TGC TTT 3') and intron 36 (5' CCT CTC AGT TTC GGC TCT AAT 3'), 67 mM Tris–HCl (pH 8.3), 16.6 mM (NH₄)₂SO₂, 10 mM 2-mercaptoethanol, 1.5 mM MgCl₂, 10% DMSO, 0.625 U AmpliTaq polymerase, and 100 ng genomic DNA. Following a 3-min incubation at 94°C, reactions were incubated for 34 cycles, each cycle consisting of a denaturation step at 94°C for 30 s, an annealing step at 61°C for 30 s, and an extension step at 72°C for 75 s.

PCR analysis of primate genomic DNA was carried out using similar incubation conditions and primers derived from exon 33 (5' GGC TTC GGA TTG TCT CCC AT 3') and exon 34 (5' CCA ACT CCC AAG CCT CCA GC 3') of the baboon tropoelastin gene. The reaction conditions, following a preincubation at 94°C for 3 min, involved 35 cycles, and each cycle consisted of an incubation at 94°C for 30 s, 63°C for 30 s, and 72°C for 15 s. PCR products from both human and primate DNA samples were analyzed by electrophoresis through 1.2% agarose and visualized by staining with 0.5 μ g/ml ethidium bromide.

Results

The Sequence Analysis of Baboon Tropoelastin cDNAs

Six positive individual recombinants (four of which were identical) were obtained following screening of a baboon smooth muscle cell cDNA library with a human tropoelastin cDNA. Analysis of these recombinants provided DNA sequence from the domains corresponding to exon 23 of the human ELN gene to the first polyadenylation consensus sequence in the 3' untranslated region within exon 36. The nucleotide sequence corresponding to the coding domain, the derived amino acid sequence, and a comparison to the derived amino acid sequences from human, rat, and chicken are presented in Fig. 1. The baboon tropoelastin cDNA sequence, as one would expect, shows the most identity to the human sequence. Exon 34, which does not exist in human, encodes a hydrophobic domain that is identical in sequence between baboon and rat. The polypeptide domains encoded by exons 27 and 33 are identical in baboon and human;

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Chicken		C	A			X	T	G	X		X X	X	GGT	GCC	GCT	GTT	GGA	gg a	OTC	CC Y	OGC	GCT	oto	CCY	C
Baboon	A	λ	X	K	λ	λ	A	ĸ	λ	λ	Q	F	-	-	-	-	-	-	-	-	-	-	-	-	G
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Bovine	GCT	GC G	с	A	т				c	т				G			T 	A	С	G	ACA		T	С	
Baboon		v	ъ.		v	~	v			~	_	_	_	v		v	2	ъ.				v		ъ	_
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Baboon Human Rat Bovine Chicken Baboon Human	GCA TG CC A	GCT C A	GCC C A	ала K	GCC А	слс Q	CTC TA T T	CGA A G R	GСТ А	GCA T T A	GСТ А	GGG G	СТТ L	GGT CCC G	GCT C A	GGC T T G	АТС G Т I	сст Р	ссл с	CTT C	GGA G G	GTT G V	GGT C G	GTC CT CT A	GGT C A G
Baboon Human Rat Bovine Chicken Baboon Human Rat	GCA TG CC A V	GCT C A	GCC C A	R K	GCC	слс Q	CTC TA T L Y	cga a g r	GСТ А	GCA T T	сст λ	GGG C G	СТТ L	GGT CCC G	GCT C 	GGC T T G	ATC G T I V	сст Р	GGA G G	CTT C	GGA G G	GTT G V	GGT C G	GTC CT CT A V	GGT C A G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GCA TG CC A V	GCT C A P	GCC C A P	R K	GCC A	сло Q 	CTC TA T L Y F F	cga a g R	ст л	GCA T T A	сст д	GGG C G	CTT L	GGT CCC G P -	GCT C A	GGC T T G	ATC G T I V V -	сст р -	GGA G G	CTT C	GGA G G	GTT G V 	GGT C G	GTC CT CT A V	GGT C A G
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Baboon Human Rat Bovine Chicken Baboon Human Baboon Human	GCA TG CC A V GTT	GCT C A P CCT	GCC C A P GGA	R R CTT	GCC А GGA	слс Q стт	CTC TA T L Y F F GGT	CGA A G R - GCT	GCT A GGC	GCA T T A A GTT	сст сст	666 G 600	CTT L CTT	GGT CCC G P GGA	GCT C A GTT	GGC T T G G GGT	ATC G T I V V GCT	CCT P GGT	GGA G G G TT	CTT C L CCT	GGA G G G G G G G G G C	GTT G V - TTC	GGT G 	GTC CT CT A V -	GGT C G
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Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GCA TG G C C V C C C C C C C C C C C C C C C	GCT C C P CCT C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C	R CTT T GGA	GCC GGA G G C C T A G C T A	CAG Q 	CTC TA T T L Y F F GOT G G G G C C C C	CGA A G C C C C C C C A C C C C C C C C	GCT A GGC T T T G G G C T T C C 	GCA T T T A OTT C V A A AAA	GCT A GG G GCA T	666 C G 6666 A A C C 6 C C C T 	CTT L CTT T L F AAAA G G	GGT G CCC G F G G G G G G G C C TAT C TC 	GCT C A - GTT C C - - V A - 7 128 GGA 	66C T T G G G G G G G G G G G C A	ATC G T I V V GCT C C C A GCA	P GGT GGT	GGA G G G G TT C C C V C C V C C C C C C C C C C C	CCT L P CCT	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC TTC F F GGG	667 C G G G G G G C C C C C C C C C C C C C	GTC CT CT A V GCA T A GGA	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Baboon Human Rat Bovine Chicken Baboon Human Rat Baboon Human Rat Bovine Chicken	GCA TG G CC V GTT C C C V - 2 GCA	GCT C C P C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C P C C C P C C A C P C C A C P C C A C C A C C A C C C C	K CTT T GGA G	GCC A G G G C T A A A G G A 	CAG Q 	CTC TA T T L Y F F GGT G G C C C A	CGA A G C C C C C C C C C C C C C C C C	GCT A GGCC T T T T G G C T T C A	GCA ТТ А 	GCT A GG GG GCA T 	666 <u>C</u> G - 600 A C C - G C C C T - A	CTT L CTT T L F AAAA G K	GGT G G G G G G G G G G G C C C T T T T	GCT C A C C C C C C V A - 7 [28 GGA G	GGC T T G G G G G G G G G G G G G G G G	ATC G T I V V GCT C GCT A 	CCT P GGT GGT 	GGA G G G TT C C C V V 	CCT L CCT P CCT	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC TTC F F	667 C G G A A G C C G 	GTC CT A V 	GGT C A G G GGA
Baboon Human Rat Bovine Chicken Baboon Human Rat Baboon Human Rat Bovine Chicken Saboon Human Rat Baboon Human Rat Baboon Human Rat Baboon Human	GCA TG G CC V GTT C C C V GCA	GCT C R P CCT C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C C C C C C C C C A C C C A C	K CTT T GGA G	GCC A - GGA G GCC T A S	CAG Q G TT G CTG L	CTC TA T T GGT G GCT G C C A	CGA A G C R G C C C C A G C C A A A A A	GCT A GGCC T T T T G G C T T C A S	GCA ТТ Т ОТТ С ОТТ С А АААА К	GCT A GG GG GCA T A	GGG C G G G G G G G G C C T A	CTT L CTT T L F AAAA G C K	GGT G G G G G G G G G G G C C C T C T C	GCT C A GTT C C C C C C C C C C C C C C C C C C	GGC T T G G G G G G G G G G C A	ATC G T I V V GCT C C C A GCA T A	CCT P GGT G G G G G G G	GGA G G G TT C C C C V C C C C C C C C C C C C C	CCT P CCT	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC CCT F GGG G	GGT G G G G G G G G G G G G C T C T T P	GTC CT CT A V - GCA T A - - - - - - - - - - - - -	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Chicken	GCA TG G C C C C C C C C C C C C C C C C C	GCT C R P CCT C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C T P C C T C C A	AAA K CTTT T L F GGA G	GCC A GGA G G G G C C T A A S T - A	CAG Q GTT 	CTC TA T T GGT G GCT G C A	CGA A G C C C C C C C C C C C C C C C C	GCT A G G G G A S A	GCA ТТ Т ОТТ С ОТТ С ОТТ С А АААА К	GCT A GG GCA T A A	GGG C G G G G G G G C C C C C C C C C C	CTT L CTT T L F - AAAA G K	GGT G G G G G G G G G G G C C C C C C C	GCT C A C C C V A - 7 28 GGA G GA	GGC T T G G G G G G G G G G C A T T T T T T T T T T T T T T T T T T	ATC G T I V V V GCT A GCA - A - A	CCT P GGT G G G G G G	GGA G G G T T C C C C C C C C C C C C C C C	CCTT L CCTT P P	GGA G G G G G G G G G G G G G G G G G	GTT G V TTC TTC F F G G G G	GGT G G G G G G G G G C C C C C C C C C	GTC CT CT A V GCA T GCA T GCA	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GCA TG G C C C C C C C C C C C C C C C C C	GCT C P CCT C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C T P C C T C C C A P GGA	AAA K CTT T L F - GGA G -	GCC A - GGA G G G - - - - - - - - - - -	CAG Q Q OTT 	CTC TA T T L Y F F GOT C C C C C C A	CGA A G C R C C C C C C C C C C C C C C C C C	GCT A G G G G G A S A	GCA Т Т Т А ОТТ С ОТТ С А А А А С С А А С С А А С С А А С С А А С С А А С С А А А С С А А А А А А А А А А А А А	GCT A G G P G G CA T A A A 	GGG C G G G G G G G C C T G C T G C C T A 	CTT L CTT T L F F AAAA G G K 	GGT G G G G G G G G G G G C C C C C C C	GCT C A V A - 7128 GGA G G	GGC T T G G G G G G G G G G C A C G G C A C G C A C C C C	ATC G T I V V GCT GCT A - - A - - A	CCT P GGT G G G G G G G G G G G G	GGA G G G TT C C C C V C C C C C C C C C C C C C	CCTT L CCCT P CCTT L L L L L	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC TTC F F GGG G G G	GGT G G G G G G G G G C C T C C T C C T C C C C	GTC CT CT CT GCA T GCA T A GCA GCA	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon	GCA TG CC CC A V C C C C C C C C C C C C C C C	GCT C C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C C C C C C C A P C C A P C GGA C C C A C C A C C C A C C C C A C C C A C	AAA K CTTT T L F - GGA G -	GCC A G G G G C T A S T - - A S T - - - - - - - - - - - - -	CAG Q GTT CTG L 	CTC TA T T L Y F F GOT C C C C C C C C C C C C C C C C C C C	CGA A G G C C C C C C C C C C C C C C C	GCT A 	GCA T T T A GTT C V A K K GCC	GCT	GGG C G G G G G G G C C T G C T G G C C C C	CTT L CTT T L F - AAAA G G K - GGG	GGT G G G G G G G G G G G C C C C C C C	GCT C GTT C C C V A - 7 128 GGA G G 000	GGC T T G G G G G G G G G G G G G G G G	ATC G T I V V GCT GCA 	CCT P GGT A G G G G G G G G G G G G G G G G G	GGA G G G T T C C C C C C C C C C C C C C C	CTT L P P CTT L GTA	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC CCT F F GGG G GGC	GGT G G G G G G G G G G C C T C C C C C	GTC CT CT CT GCA T GCA T GGA CCA	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Rat Baboon Human Rat Bovine Chicken Baboon Human Rat Baboon Human Rat Baboon Human Rat Baboon	GCA TG G C C C C C C C C C C C C C C C C C	GCT C C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C C C C C C C A P C GGA C C C A C C A C C C A C C C C C C	E CTT T GGA G CT	GCC A GGA G G G C T A S T - G G C C C C C C C C C C C C C	CAG Q GTT CTG L GGA	CTC TA T T L Y F F F GGT G G C C A GTG C T	CGA A G C C C C C C C C C C C C C	GCT A G G C A S G G G T T T T T T T T T T T T T	GCA T T T T T T T T T T T T T T T T T T T	GCT A GG P G GCA T A CTT C	GGG C G G G G G G G C C T G G C T G G A C C C C C C C C C C C C C C C C C	CTT L CTT T L F AAAA G G K - GGGG A	GGT G G G G G G G G G G G G C C C C C C	GCT C GTT C C V A - 7128 GGA G G G G G G T	GGC TT G G G G G G G G G G G G G G G G G	ATC G T I V V GCT C C A C C C C C C C C C C C C C	CCT P GGT A G G G G G G G G G G G G G G G G G	GGA G G G T C C C C C C C C C C C C C C C C	CTT L CCT P CTT CTT CTT	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC CCT F F GGG G GGC T	GGT G G G G G G G G C C T C C T C G G C C C C	GTC CT CT A V GCA T GCA T GGA CCA T	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GCA TG G C C C C C C C C C C C C C C C C C	GCT C R P CCT C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C C C C C C A P GGA C C A P GGA C C A C C A C C A C C C A C C C A C C C A C C C C A C	E CTT T GGA G CTT CTT CCT	GCC GGA G G GCC T A S T - GCA G G C - - - - - - - - - - - - -	CAG Q G TT CTG L GGA GGA	CTC TA T T L Y F F F GGT G G C C C C C C C C C C C C C C C C	CGA A G C C C C C C C A C C C A C C C A C C C A C	GCT A - GGC T T T T T T T T T T T T T	GCA T T T T T T T T T T T T T T T T T T T	GCT A - CCT GG P G - - GCA T A - CTT C -	GGG C G G G G G G G C C C C C C C C C C C C C	CTT L CTT T L F - AAAA G G G G G G G G G G G G G G	GGT G G G G G G G G G G C C C C C C C C	GCT C A - GTT C C V A - 7]28 GGA GGGG GGGG T A 	GGC T G G G G G G G G G G C T T T T T T	ATC G T I V V GCT C C C C C C C C C C C C C	CCT P GGT A G G G G G G 	GGA G G G TT C C C C C C C C C C C C C C C	CTT L CCT P CTT L L CTT CTT CTT CTT CTT CTT	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC TTC TT C C T T C C T T G G G G G G	GGT G G G G G G G G C C T C C T C C T C C G G T T C C C C	GTC CT CT A V GCA T A CCA T CCA T	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GCA TG G CC C C C C C C C C C C C C C C C C	GCT C C A P CCT C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C C C C C C C A P GGA C C A C C A C C A C C A C C C A C C C A C C C C A C	R CTT T GGA G CCT CCT	GCC A - GGA G G G G G G G G G G G 	CAG Q GTT G G T C C G G G G G G G G G G G	CTC TA T T L Y F F F GGT G G C C C C C C C C C C C C C C C C	CGA A G G C C C C C A C C C A C C C A C C C A C C C A C	GCT A GGCC T T T T C G G G C T C C C C C T C C C C	GCA T T T T T T T T T T T T T	GCT A - CCT GG P G - GCA T A - CTT C L	GGG - - - - - - - - - - - - -	CTT L CTT T L F - AAAA G G G G G G G G G G G G	GGT G G G G G G G G G G G C C C C C C C	GCT C A - GTT C C C V A - - GGA G GGG GGG GGG GGG GGG GG	GGC T G G G G G G G G G G C T T T T T T	ATC G T I V V GCT C C C C C C C C C C C C C	CCT P GGT G G G G G G G G G G G G G	GGA G G G T C C C C C C C C C C C C C C C C	CTT L CCT P P CTT CTT CTT CTT CTT V	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC TTC TT C C T F F G G G G G G G G G G G G G G G G G	GGT G G G G G G G G G G G G T T T I	GTC CT CT A V GCA T A GGA GGA CCA T P	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Baboon Human Rat Bovine Baboon Human Baboon Ba	GCA TG G C C C C C C C C C C C C C C C C C	GCT C C A P CCT C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C C C C C C C A C C A C C A C C C A C	R CTT T GGA G CCT CCT	GCC A - GGA G G G G G G - G - - - - - - - - - - -	CAG Q GTT G G C T G G G G A G G A	CTC TA T T L Y F F F GGT G G C C C C C C C C C C C C C C C C	CGA A G G C C C C C A C C C A C C C A C C C A C	GCT A GGCC T T T T C G G G C T C C C C C T C C C C	GCA TT T C GTT C C C C C C C C C C C C C C	GCT A - CCT GG P G - - GCA T A - CTT C L P	GGGG 	CTT L CTT T L F - AAAA G G G G G G G G G G	GGT G G G G G G G G G G G C C C C C C C	GCT C A - GTT C C C C V A - - GGA G G G G G G G G G G G G	GGC T G G G G G G G G G G C T T T T T T	ATC G T I V V GCT C C C C C C C C C C C C C	CCT P GGT G G G G G G G G G G G G G G G G	GGA G G G T C C C C C C C C C C C C C C C C	CTT L CCT P CTT L CTT CTT CTT CTT CTT C CTT C C C C	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC TTC TT C C T T C C T T G G G G G G	GGT G G G G G G G G G G G G T T T I V	GTC CT CT A V GCA T A A C GGA T CCA T CCA	GGT C A G G G G G G G G G G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GCA TG G CC C C C C C C C C C C C C C C C C	GCT C C A P CCT C C C C C C C C C C C C C C C C C	GCC C A P GGA C C C C C C C C C C C C C C C C A C C A C C A C C A C C C A C C C A C C C C A C	R CTT T GGA G G CCTA G G F P P	GCC GGA G A G A G A G A G A G A 	CAG Q GTT G G C T G G C T G G G A G G A	CTC TA T T L Y F F F GGT G G C C C C C C C C C C C C C C C C	CGA A G G C C C C C A C C C A C C C A C C C A C C C C A C	GCT A GGC T T T T G G G G G G G G G G G G G G	GCA TT T GTT C GTT C V A A A A A C C K GCC GC C C C A V G C C A A C A A A A A A A A C A C A C A	GCT A - CCT GG P G - - GCA T A - CTT C L P	GGG - - - - - - - - - - - - -	CTT L CTT T L F - AAAA G G G G G G G G G	GGT G G G G G G G G G G G C C C C C C C	GCT C A - GTT C C C G GGA G GGG G G	GGC T G G G G G G G G G G G C T G G G C T T T T	ATC G T I V V GCT C C C C C C C C C C C C C	CCT P GGT A G G G G G G G G G G G 	GGA G G G T C C C C C C C C C C C C C C C C	CTT L CCT P P CTT CTT L CTT CTT CTT CTT L L C CTT C C T L L L C C C C	GGA G G G G G G G G G G G G G G G G G G	GTT G V TTC TTC TT C C T T C C T T G G G G G G	GGT G G G G G G G G G G G G T C C T G G G G	GTC CT CT A V GCA T A CCA T CCA T P	GGT C A G G G G G G G G G G G G G G G G G G

Fig. 1. Continued.

Baboon	GGT	GTG	GTG	GGA	GCC	GGA	CCG	GCC	GCC	GCC	GCT	GCC	GCC		YYY	GCT	GCT	GCC	AAA	GCC	GCC	CAG	TTT	GGC	CTA
Human			C 2		ርሞ	c	C T	Ŧ	TP.	Ŧ		Ŧ	A	GCC			c	ጥ	a	π			AC	т	т
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Chicken																								T	
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Human	•	•	•	•		•	-							λ								-			
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Chicken	-	-	<u>.</u>	-	ž	_	-	_	-	-	-	-	_	-	-	_	_	_	- -	_	_	-	-		-
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Bovine	-		-	-		v	-	-	-	-	-	-	-											-	-
Chicken	v	₽	G			v	P			G	G	I	P										-	-	-
Baboon																						GLC	CCA		
Human																				003	ana		~		
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Chicken																							GGT	GGC	CTT
Baboon	-	-	-	-	-	-	-	-	-	-	-	_	_	_	_	_	_	_	_	-	_	v	р	_	_
Human	-	_	-	-	-	-	-	_	_	_	-	-	-	-	_	-	-	-	-	-	-	•	-	-	-
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Baboon Human Rat	ggt ggt	GTC GTC	ста ста	ggg gga	GGT GCC	GCC	ggg Agg	CAG CCA	TTC TTC	CCA CCA	CTT GGC	GGA GGA	-GA G G	GTG	gca	GCG A A	AGA	CCC T T	GGC G	TTC	GGA	TTG C T		T	С
Baboon Human Rat Bovine Chicken	ggt ggt ggt	GTC GTC GTC	CTA CTA CTA	ggg gga gga	GGT GCC GCT	GCC GGC	GGG AGG CAG	CAG CCA CG	TTC TTC TTC	CCA CCA CCA	CTT GGC ATT	gga gga gga	-GA G G G T CT	ere c	GCA TT	GCG A A A GA	AGA G GTT	CCC T T	GGC G T	TTC T	GGA T	TTG C T C C G	A	T T G	C A
Baboon Human Rat Bovine Chicken	ggt ggt ggt ggt	GTC GTC GTC	СТА СТА СТА	ggg gga gga 	GGT GCC GCT	GCC	GGG AGG CAG	CAG CCA CG	TTC TTC TTC	CCA CCA CCA	CTT GGC ATT	gga gga gga	-GA G G T CT	GTG G	gca TT	GCG A A GA	AGA G GTT	CCC T T	GGC G T	TTC T	GGA T	TTG C T C C G	А	T T G	с л
Baboon Human Rat Bovine Chicken Baboon	GGT GGT GGT	GTC GTC GTC	CTA CTA CTA	GGG GGA GGA	GGT GCC GCT	GCC	GGG AGG CAG	CAG CCA CG	TTC TTC TTC	CCA CCA CCA	CTT GGC ATT	GGA GGA GGA	-GA G G T CT	GTG G V	GCA TT A	GCG A A GA GA	AGA G GTT R	CCC T T	GGC G T G	TTC T F	GGA T G	TTG CT CC G	A S	T T G P	C A I
Baboon Human Rat Bovine Chicken Baboon Human Rat	GGT GGT G G	GTC GTC GTC V V	CTA CTA CTA 	GGQ GGA G G	GGT GCC GCT G	GCC GGC	GGG AGG CAG G R	CAG CCA CG Q P	TTC TTC TTC F F	CCA CCA CCA 	CTT GGC ATT L G	GGA GGA GGA G G	-GA G G T CT G G	GTG G V	GCA TT A	GCG A A A GA A	AGA G GTT R	CCC T T	GGC G T G	TTC T F	GGA T G	TTG C T C C G L	A S	T T G P	C A I
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine	GGT GGT GGT - G G G G	 GTC GTC V V V	CTA CTA CTA L L L L	GGG GGA GGA G G G	GGT GCC GCT G A A	GCC GGC A G	GGG AGG CAG G R Q	CAG CCA CG P P	TTC TTC TTC F F	CCA CCA CCA P P P	CTT GGC ATT L G I	GGA GGA GGA G G G	-GA G G T C T G G G G G G	GTG G V	GCA TT A	GCG A A A GA A	AGA G GTT R	CCC T T	GGC G T G	TTC T F	GGA T G	TTG CT CC G L	A S	T T G P	c A I
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT - G G G G	GTC GTC GTC V V V V	CTA CTA CTA L L L	GGG GGA GGA G G G G	GGT GCC GCT G A A	GCC GGC A G G	GGG AGG CAG G R Q -	CAG CCA CG P P -	TTC TTC TTC F F F	CCA CCA CCA - P P P P	CTT GGC ATT L G I -	GGA GGA GGA G G G G	-GA G G T CT - G G G A	GTG G V G	GCA TT A V	GCG A A GA GA G	AGA G GTT R V	CCC T T	GGC G T G	TTC T F	GGA T G	TTG CT CC G L V	A S S	T G P	C A I
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT G G G G G	GTC GTC GTC V V V V - 3	CTA CTA CTA L L L L S	GGG GGA GGA G G G G G G G	GGT GCC GCT G A A -	GCC GGC A G G	GGG AGG CAG G R Q -	CAG CCA CG Q P P -	TTC TTC TTC TTC F F F	CCA CCA CCA P P P P	CTT GGC ATT L G I -	GGA GGA GGA G G G 	-GA G G T CT - G G G A	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V	GCCG A A GA GA G	AGA GTT R V	CCC T T	GGC G T G	TTC T F	GGA T G	TTG CT CC G L V	д 8 8	T G P	C A I
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon	GGT GGT GGT - G G G - TTC	GTC GTC GTC V V V - 3 CCA	CTA CTA CTA L L L L S GGT	GGG GGA GGA G G G G G G G G G G G C	GGT GCC GCT - G A A A - GGC	GCC GGC - A G G GCT	GGG AGG CAG G R Q - GGA	CAG CCA CG P P 	TTC TTC TTC F F F	CCA CCA CCA CCA P P P TTG	CTT GGC ATT L G I - G	GGA GGA GGA G G G GTT	-GA G G T CT - G G G A	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V	GCCG A A GA G G G	AGA GTT R V	CCC T T	GGC G T G	TTC T F	GGA T G	TTG CT CC U L V	д 8 8	T G P	с л 1
Baboon Human Rat Bovine Chicken Baboon Human Baboon Human Pat	GGT GGT GGT - G G G G C TTC	GTC GTC GTC V V V V - 3 CCA	CTA CTA CTA - L L L J GGT	GGG GGA GGA G G G G G G G C - - - - - - - - - -	GGT GCC GCT G A A G G C C G C C T 	GCC GGC 	GGG AGG CAG - G R Q - GGA	CAG CCA CG Q P P P 	TTC TTC TTC F F F 	CCA CCA CCA CCA P P P P TTG	CTT GGC ATT L G I G G I G GA	GGA GGA GGA G G G G G G T T G T T	-GA G G T CT G G G G A CT	GTG G V G G G G G G G A	GCA TT A V	GCG A A GA G G G	AGA GTT R V	CCC T T	GGC G T G	TTC T F	GGA T G	TTG C T C C G L V	а s s 	T G P	
Baboon Human Rat Bovine Chicken Baboon Human Rat Baboon Human Rat Baboon Human Rat Baboon	GGT GGT GGT - G G G G TTC A	GTC GTC GTC V V V V V S CCA	CTA CTA CTA L L L S J 34 GGT	GGG GGA GGA G G G G G G G G G G G C - - - - - - - -	GGT GCC GCT - G A A A - GGC - T	GCC GGC A G G G G C T	GGG GGG GGA GGA GGA GGA GGA	CAG CCA CG P P GGC	TTC TTC TTC F F F F	CCA CCA CCA P P P P C TTG	CTT GGC ATT L G I - GGA	GGA GGA GGA G G G G T T 	-GA G T CT G CT G CT G G G T G CT G G G T G CT	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V	GCG A A GA A G G CCC CCT	AGA GTT R V	CCC T T P	GGC G T G	TTC T F	GGA T G	TTG C T C C G L V V	A S S GCC GCC	T G P	C A I GGA GGA
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT - G G G - TTC A T	GTC GTC GTC V V V V - 3 CCA	CTA CTA CTA L L L L GGT	GGG GGA GGA G G G G G G G G G G G G G G	GGT GCC GCT G A A A GGC T	GCC GGC G G G C T C	GGG AGG CAG G R Q G GGA GGA C	CAG CCA CG P P - GGC 	TTC TTC TTC F F F F CAG	CCA CCA CCA CCA P P P P C TTG C C C	CTT GGC ATT L G I -	GGA GGA GGA G G G T T C	-GA G T C C C C C C C C C C C C C C C C C C C	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V	GCG A A GA A G G G	AGA GTT R V CCG CCC	P P	GGC G T G	TTC T F TAT TAT TAC	GGA T G	TTG C T C C G L V V GGA GGG GGA	A S S GCC GCC GCC	T G P CTT CTTG CTG	I GGA GGA CCC
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT - G G G C - TTC A T	GTC GTC GTC V V V V S CCA	CTA CTA CTA L L L L GGT	GGG GGA GGA G G G G G G G G G G G G G G	GGT GCC GCT G A A - GGC T 	GCC GGC - A - G - - - - - - - - - - - - - - - -	GGG AGG CAG CAG C G G G G G G G G G G G	CAG CCA CG - Q P P P C C C C C	TTC TTC TTC F F F CAG	CCA CCA CCA CCA TTG P P P P C C C C	GGA	GGA GGA GGA G G G T T C V	-GA G G T C T G G G T G G G A - G G G G G G G G G G G G G G G G G G G	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V AAA AAA AAA AAG	GCG A A GA G G G G CCC CCC CCC CCC	AGA GTT R V CCG CCC CCC	P P AAG AAG	GGC G T G C C C C C C C C C C C C C C C	TTC T F TAT TTC TAC	GGA T G G G G G G G G G G G G G G G G G	TTG C T C C G L V C G G G G G G G G G G G G G G G G G G	A S S GCC GCC GCC	T G P CTTG CTG CTG	I GGA GCC
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Baboon Human	GGT GGT GGT - G G G C - TTTC A T	GTC GTC GTC V V V - 3 CCA	CTA CTA CTA L L L J GGT GGT	GGG GGA GGA - G G G G G G G G G G	GGT GCC GCT - G A A A - GGC - T - - - - - - - - - - - - - - - - -	GCC GCC A G G G C T C A -	GGG AGG CAG CAG C G G G G G G G G G G G	CAG CCA CGA CG P P P P C GGC C G G	TTC TTC F F P 	CCA CCA CCA CCA CCA TTG C C C C C C C C	GGC GGC ATT GGC GGA GGA	GGA GGA GGA G G G T T C V 	-GA G G G T C T C T G G G G G G G G G G G G	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V AAA AAA AAA AAA -	GCG A A GA A G G G CCC CCT CCCC	AGA GTT R V CCG CCC CCC	P P AAG AAG AAG	GGC G T G G	TTC T F TAT TTC TAC TAC	GGA T G G G G G G G G G G G G G G G G G	TTG C T C C G L V C C C C C C C C C C C C C C C C C C	A S S GCC GCC GCC	T G P CTTG CTG C C TG	C A I GGA GGA CCC
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Rat Baboon Human Rat Baboon Human Rat Baboon Human Rat Baboon Human Rat Rat Baboon Human Rat Rat Baboon Human Rat Rat Rat Rat Rat Rat Rat Rat Rat Rat	GGT GGT GGT GGT G G G G TTC A T F Y	GTC GTC GTC V V V - 3 CCA	CTA CTA CTA L L L L GGT G G	GGG GGA GGA GG G G G G G G G G G G	GGT GCC GCT G A A A T T G G C	GCC GGC A G G T C A -	GGG AGG AGG CAG - G R Q - GGA - G G G C G G	 CAG CCA CG P P P GGC C G 	TTCC TTCC TTCC F F F F CAG	CCA CCA CCA CCA P P P P P C C C C C	GGC ATT	GGA GGA GGA GGA G G G G G T C V -	-GA G G G T C T C G G G G G G G G G G C T C T	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V S AAAA AAAG - K	GCG A A A GA GA G G G CCC CCC CCC CCC	AGA G GTT R V CCGG CCCC CCCC	CCCC T T P P AAG AAG AAG AAG	GGC G T G C C C C C C C C C C C C C C C	TTC T F TAT TAT TAC - Y	GGA T G GGA GGA GGA GGA	TTG C T C C G L V V GGA GGG GGA	A S S GCC GCC GCC	TTG P P CTTG CTG L	C A I GGA GGA CCC C C C
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT GGT G G G G C T T T C A T F Y	GTC GTC GTC V V V - 3 CCA	CTA CTA CTA L L L L GGT GGT	GGG GGA GGA G G G G G G G G G G	GGT GCC GCT G A A A C GGC T T T T C G G	GCC GGC - A - G - C - TC A - V	GGG AGG CAG CAG G R Q G G G G G G G G G	 CAG CCA CG CG P P P GGC C G 	TTCC TTCC TTCC F F F F CAG	CCA CCA CCA CCA P P P P P C C C C C C	GGA GGA	GGA GGA GGA GG G G G G G T C T T C V F	-GA G G G T C T C G G G G G G G G G G G G G	GTG C G V G G G G G G G G G G G G G G G G	GCA TT A V V AAAA AAAG - K K K	GCG A A A GA GA GA G CCC CCC CCC CCC CCC	AGA G GTT R V CCCG CCC CCC CCC	CCCC T T P P AAG AAG AAG AAG K K K	GGC G T G C C C C C C C C C C C C C C C	TTC T F TAT TAT TAC Y F Y	GGA T G GGA GGA GGA GGA G G G G G G G	TTG C T C C G G L V G G G G G G G G G G G G G G	A S S GCC GCC GCC GCC	TTG P CTTGG CTTGG	C A I GGA GGA CCC - - G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT G G G C TTC A T F Y	GTC GTC GTC V V V - 3 CCA	CTA CTA CTA L L L L GGT G G	GGG GGA GGA G G G G G G G G G	GGT GCC GCT G A A A C GGC T T T T	GCC GGC - - - - - - - - - - - - - - - -	GGG AGG CAG G G G G G G G G G G G G G G	CAG CCA CG P P P GGC C G G	TTC TTC TTC F F F CAG	- CCA CCA CCA CCA - P P P P P P - C C C C C C L	GGC GGC G G G G G G G G G G	GGA GGA GGA GG G G G G G T C T T C V P	-GA G G G T C T G G G G G G G G G G G G G G	GTG C G V G G G G G G G G G G G G G G G G	GCA TT A V C AAAA AAAA AAAG C K K K	GCG A A GA GA G G G CCC CCC CCC CCC CCC	AGA G GTT R V CCG CCC CCC CCC	CCC T T P P AAG AAG AAG C K K K K	GGC G T G G CCC CCC CCC CCC CCC ACC	TTC T F TAT TAT TAC TAC P F Y	GGA T G G GGA GGA GGA G G G G G G G	TTG C T C C G G G G G G G G G G G G G G G G G G	A S GCC GCC GCC GCC	T G P P CTTG CTG CTG CTG L L L	C A I GGA GGA CCCC - G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT G G G G T T T C A T F Y	GTC GTC GTC V V V J CCA	CTA CTA CTA L L L L GGT G G	GGG GGA GGA G G G G G G G G G G G G	GGT GCC GCT 	GCC GGC GGC GCT TC A S 5136	GGG AGG CAG G CAG G R Q Q GGA G G G G G G G G	CAG CCA CG - Q P P - GGC C G -	TTC TTC TTC F F F F CAG	- CCA CCA CCA CCA - P P P P P P - C C C C C C L -	GGC GGC G G G G G G G G G G C	GGA GGA GGA G G G G G G T T C V T F	-GA G G T CT G G T CT G G G G G G G G G G G G G G G G G G	GTG C G V V G G G G G G G G G G G G G G G	GCA TT A V C AAAA AAAA AAAA AAAA K K K K	GCG A A GA GA A G G CCC CCC CCC CCC CCC	AGA G GTT R V CCCG CCCC CCCC CCCC	CCCC T T P P AAG AAG AAG AAG K K K K	GGC G T G G G CCC CCC CCC CCC CCC CCC CC	TTC T F TAT TATC TATC TAC - - - Y F Y	GGA T G G G G G G G G G G G G G G G G G	TTG C T C C G G L V G G G G G G G G G G G G G G G G	A S S GCC GCC GCC GCC	T T G P P CTTC CTG CTG CTG CTG L L L	C A I GGA GGA CCCC - G G G G
Baboon Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT G G G G G G C T T C X T F Y	GTC GTC GTC V V V J CCA	CTA CTA CTA L L L GGT G G	GGG GGA GGA G G G G G G G G G G G G	GGT GCC GCT 	GCC GGC GGC GGC TC A S5136 GGT	GGG AGG CAG G G R Q G G G G G G G G G G G G G G G	CAG CCA CG Q P P P C G G C C G 	TTC TTC TTC F F F F F CAG	CCA CCA CCA CCA CCA CCA CCA CCA CCA CCA	GGA GGA TGC	GGA GGA GGA G G G G G T T C V F F	-GA G G G T C T G G G G G G G G G G G G C T G G G G	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V V G AAA AAA AAA K K K K	GCG A A GA GA G G G CCC CCC CCC CCC CCC	AGA GTT R V CCC CCC CCC CCC CCC T P P P TGT	CCC T T P P AAG AAG AAG AAG K K K K	GGC G T G G CCC ACC P P T CGG	TTC T F TAT TAT TAC TAC TAC Y Y Y Y AAG	GGA T G G G G G G G G G G G G G G G G G	TTG C T C C G L V G G G G G G G G G G G G G G G G G	A S S GCC GCC GCC GCC GCC GCC GCC GCC GCC	T T G P P CTTT CTG CTG CTG CTG L L L	C A I GGA GGA CCC G G G G G G G G G G G G G G
Baboon Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT G G G G G G T T T C A T F Y	GTC GTC GTC GTC V V V V C CA	CTA CTA CTA L L L GGT G GGT	GGG GGA GGA GGA G G G G G G G G G G G G	GGC GCC GCT - G G A A A - - - - - - - - - - - - - -	GCC GGC A G G G T C A S S S 36 G G G T	GGG AGG CAG G G G G G G G G G G G G G G	CAG CCA CG CG P P P C C C G G C C C C	TTC TTC TTC F F F F F CAG	CCA CCA CCA CCA CCA CCA CCA CCA CCA CCA	GGA GGA TGC	GGA GGA GGA G G G G G T C V F F	-GA G G G T C T G G G G G G G G G G G G G G	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V V AAA AAA AAA C K K K K K	GCG A A A GA A GA G G CCC CCC CCC CCC CC	AGA GTT R V CCCC CCCC CCCC CCCC CCCC T TGT	CCC T T P P AAG AAG AAG AAG K K K K K	GGC G T G G C C C C C C C C C C C C C C	TTC T F TAT TAT TAT TAC Y F Y X	GGA T G G G G G G G G G G G G G G G G G	TTG C T C C G G G G G G G G G G G G G G G G G G	A S S GCC GCC GCC GCC GCC GCC GCC GCC GCC	T T G P P CTTC CTCC CTCC CTCC CTCC CTCC	C A I GGA GGA CCCC G G G G G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT GGT G G G G G G C TTC A T T F Y	GTC GTC GTC GTC V V V V V CCA	GGA GGA GGA	GGG GGA GGA GGA G G G G G G G G G G G G	GGC GCC GCC GC G G G G G G G G C CAA	GCC GGC - - - - - - - - - - - - - - - -	GGG AGG CAG CAG G R Q - G G G G G G G G G G G G G G	CAG CCA CG Q P P P C C G G C	TTC TTC TTC F F F CAG GCG GCG	CCA CCA CCA P P P P P C C C C C C C C C	GGA GGA GGA GGA GGA	GGA GGA GGA G G G G G G G T T C V F F	-GA G G G T C T G G G G G G G G G G G G G G	GTG G V G G G G G G G G G G G G G G G G	GCA TT A A AAA AAAA K K K K K X AAAA	GCG A A A GA A GA A G G CCC CCC CCC CCC	AGA GTT R V CCC CCC CCC CCC CCC T TGT	CCC T T P P AAG AAG AAG K K K K K	GGC G T G G CCCC CCCC CCCC CCCC CCCC CC	TTC T F TAT TAT TAT TAC Y F Y Y AAG	GGA T G G G G G G G G G G G G C G C C C C C	TTG C T C C G G G G G G G G G G G G G G G G G G	A S GCC GCC GCC GCC GCC T J A A A TGA	T T G P P CTTC CTCC CTCC CTCC CTCC CTCC	C A I GGA GGA CCC G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken	GGT GGT G G G G G G T T C A T F Y	GTC GTC GTC GTC V V V CCA P P	GGA GGA GGC	GGG GGA GGA G G G G G G G G G G T T T T	GGT GCT GCT G A A A GGC T T T C CAA CCA AGA	GCC GGC GGC GCT TC A S S S S S GGT	GGG AGG CAG G G G G G G G G G G G G G G	CAG CCA CG Q P P P - C C G G C C G T	TTC TTC TTC F F F F CAG GCG GCG	CCA CCA CCA CCA P P P P P C C C C C C C	GGA GGA TGC	GGA GGA GGA G G G G G G G G G T T C V P P G G A	-GA G G G T C T G G G G G G G G G G C T T T T	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V V AAAA AAAG - K K K K X AAAA	GCG A A A GA GA GA G CCC CCC CCC CCC CCC	AGA GTT R V V CCC CCC CCC CCC CCC T TGT	CCC T T P P AAG AAG AAG C K K K GGC G	GGC G T G CCC CCC CCC CCC CCC CCC CCC CC	TTC T F TAT TAT TAC TAC Y Y Y X	GGA T G G G G G G G G G G C G C G C C G	TTG C T C C G G G G G G G G G G G G G G G G G G	A S GCC GCC GCC GCC T A A A TGA	T T G P P CTTC CTCC CTCC CTCC CTCC L L L	C A I GGA GGA CCC G G G G G
Baboon Human Rat Bovine Chicken Baboon Human Baboon Human Human Baboon Human Baboon Human Haboon Human Haboon Human Haboon Human Haboon Human Haboon Human Haboon Human Haboon Human Haboon Haboon Human Haboon Human Haboon Human Haboon Human Haboon Human Haboon Haboon Human Haboon Human Haboon Human Haboon Human Haboon Human Haboon Human Haboon Haboon Human Haboon Human Haboon Human Haboon H	GGT GGT GGT G G G G G T T T C A T F Y	GTC GTC GTC GTC V V V V CCA P P	GGA GGA GGC GGA GGC GGA	GGG GGA GGA G G G G G G G G G G G T T T T	GGT GCT GCT G A A A GGC G G G C T T C CAA CCA AGA	GCC GGC A G G TC A S S S S G G G G G G G G G G G G G G G	GGG AGG CAG G R Q G G G G G G G G G G G G G G G G	CAG CCA CG CCA CG C C C C C C C C C C C	TTC TTC TTC TTC TTC TTC TTC TTC TTC TC T	CCA CCA CCA CCA F P P P P C C C C C C C C C C C C C C A	GGA GGA GGA C	GGA GGA GGA GGA G G G G G G T C V F F GCA	-GA G G G T C T G G G G G G G G G G G G G G	GTG G V G G G G G G G G G G G G G G G G	GCA TT A V C C C C C C C C C C C C C C C C C C	GCG AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AGA GTT R V V CCCC CCC CCC CCC CCC CCC CCC CCC	CCC T T P P AAG AAG AAG C K K K K GGC G G	GGC G G G G CCC CCC ACC P P P T CCCC ACC	TTC T F TAT TAT TAC TAC Y F Y ANG	GGA T G GGA GGA GGA GGA C G R	TTG C T C C C C C C C C C C C C C C C C C C	A S S GCC GCC GCC GCC GCC GCC GCC GCC GCC	T T G P P CTTG CTG CTG CTG L L L	C A I GGA GGA CCC G G G G G G G
Baboon Human Rat Bovine Chicken Human Rat Bovine Chicken Human Rat Bovine Chicken Baboon Human Rat Bovine Chicken Human Rat Bovine Chicken Human Rat Bovine Chicken Human Rat Bovine Chicken Human Rat Bovine Chicken Rat Rath Rath Rath Rath Rath Rath Rath	GGT GGT GGT G G G G G C T T T T T Y S C C C G G G C T	GTC GTC GTC V V V V - 3 CCA P P	GGA GGC GGC GGC GGC GGC	GGGG GGA GGA G G G G G G G G G G G G T T T T	GGT GCT GCT G G G G G G G C C A A C C A A G G C C C C	GCC GGC - A G G TC A - V S5136 GGT G	GGGA GGGA GGGA GGGG G G G G G G G G G G	CAG CCA CG CCA CG C C C C C C C C C C C	TTC TTC TTC TTC TTC TTC TTC TTC TTC TTC	CCA CCA CCA CCA CCA CCA CCA CCA CCA CCA	GGA GGA GGA GGA CCC CCC	GGA GGA GGA G G G G T C V P P GCA	-GA G G G T C T G G G G G G G G G G G G G G	GTG G G G G G G G G G G G G G G G G G G	GCA TT A V C C C C C K K K K K K K K	GCG A A A GA GA GA GA CCCC CCCC CCCC CC	AGA GTT R V CCCG CCCC CCCC CCCC CCCC CCCC TGT TGT	CCC T T P P AAG AAG AAG K K K K GGC G G	GGC G G G CCCC CCCC ACC C CCCC ACC C CCCC ACC C C CCCC ACC C C C C C C C C C C C C C C C C C C C	TTC T F TTTT TTC TAC TAC TAC TAC TAC TAC	GGA T G GGA GGA GGA GGA C G G G C G R	TTG C T C C G G G G G G G G G G G G G G G G G G	A S GCC GCC GCC GCC GCC GCC GCC GCC GCC G	T T G P P CTTG CTG CTG CTG L L L L	C A I GGA CCCC - G G G G G G G G G G G G G G G G
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Fig. 1. Multiple alignment of the nucleic acid and the translated amino acid sequences of the ELN gene corresponding to exons 23 through 36 from different vertebrate species. Nucleic acid alignments for the five species alternate with the corresponding amino acid alignments. *Bars* above the nucleic acid sequence indicate exon boundaries and the numbers on either side of these bars identify the particular exons. The exon boundaries for the chicken ELN gene are inferred by comparing the chicken tropoelastin amino acid sequence to the amino acid sequence for rat, bovine, and human ELN. Sequence conservation within the exon-encoded domains known in rat, bovine, and human

permitted the inferred assignment of exon-intron boundaries in the chicken ELN gene. Only the nucleic acid sequence from the baboon ELN gene is presented in full; in the comparison of this sequence to the other mammalian sequences, no designation represents an identical sequence and *dashes* represent lack of sequence. Note the high degree of conservation of sequence from exon 33 through exon 36 that is evident in the comparison of these domains in chicken, rat, and cow. Exon 35 is absent in the baboon sequence. Exons 34 and 35 are absent in the human sequence.

Poly A Kpn I Smal Hinc II EcoRI EcoRV Sma I EcoRI Ex 33 Ex 34 Ex 36 pGB3-1 pGB3-2 pGB3-3 pGB3-5 pGB3-4

Fig. 2. Restriction map and subcloning strategy for the λ genomic clone GB3. *Filled boxes* represent coding sequences within individual exons; the *hatched area* in exon 36 is the 3' untranslated region (UTR). *Arrows* indicate unique restriction enzyme recognition sites as well as

there is only a single valine-to-alanine change between human and baboon in the sequence encoded by exon 26. The sequence encoded by exon 29 in baboon lacks a single alanine preceding the first lysine compared to the human sequence. Exons 24, 25, and 30 differ the most in sequence. Similarly to humans, the elastin receptor binding hexapeptide repeating element VGVAPG is encoded seven times in exon 24 in baboons. In addition, there is a leucine-to-valine substitution in the fourth repeat and a valine and glycine insertion in the baboon sequence encoded at the end of exon 24. Five additional amino acids (GVGAP) are encoded in exon 25 in baboons and an additional alanine-to-glycine substitution creates a pentapeptide VGAPG, very similar to the repeating receptorbinding VGVAPG found in other mammals. A polar threonine also substitutes a hydrophobic alanine in the domain encoded by exon 25. The domain encoded by the baboon exon 30 contains a valine-to-glycine and an alanine-to-proline substitution, in addition to an insertion of glycine, valine, and alanine. A valine-serine dipeptide replaces an isoleucine and proline following the first amino acid residue in exon 31. Exon 36 contains an alanine insertion and a serine-to-alanine substitution, making the 3' end of the baboon tropoelastin cDNA more polar by comparison to the human sequence.

The 3' End of the Baboon Tropoelastin Gene

Baboon tropoelastin cDNA recombinants B8 and B16 were used to obtain five recombinants from a baboon genomic DNA library. Restriction and PCR analysis combined with DNA sequencing permitted the overlapping alignment of the insert DNA from these recombinants and the identification of exon and intron sequence corresponding to the 3' end of the baboon tropoelastin gene. The results are summarized in Fig. 2. The 5' overlapping DNA fragments contained 5942 bp of genomic DNA sequence. The 5' end of this sequence was located within intron 32. The 3' end of this sequence was located immediately downstream of a second polyadenylation site within exon 36. Within this genomic DNA sequence, we were able to identify successfully intact exons 33, 34, and 36. Extensive sequence analysis revealed no evidence of an intact exon 35 within the intron separating

two putative polyadenylation sites within exon 36. The genomic clone GB3 was divided into five overlapping plasmid subclones, which are represented as *shaded horizontal bars* and referred to as pGB recombinants.



3' end of the human ELN gene

Fig. 3. A comparison of exon and intron sequence within the 3' end of the human and baboon ELN genes. The 3' end of the human gene and the baboon ELN gene are graphically represented and drawn to scale on the abscissa and the ordinate, respectively. *Filled boxes* represent coding sequences, 3' UTR is represented by a *hatched box*. Identical nucleic acid sequences are indicated in this comparison as *single dots*. The *dotplot chart* was produced by the program Compare from the GCG software package. *Diagonal lines* designate matching sequences. The *boxed region* between the two longest diagonal lines, corresponding to 506 bp in the human and 2943 bp in the baboon ELN gene, represents a domain of little sequence identity. This domain contains Alu repeat elements which are represented in this comparison as *short, multiple parallel lines*.

exons 34 and 36. At sequence positions 2636–2671 a potential vestige of exon 35 was identified (AGG-GAAACCCCCCAGCGCGTGCAAGGTGCCTGGGC), but the absence of a canonical acceptor site precluded the possibility that this intron sequence could be used as a functional exon sequence.



Comparison of DNA sequences from intron 32 to the 3' end of exon 35 from both human and baboon ELN genes revealed the expected and extensive identity between exon 33 and exon 36 (Fig. 3). Moreover, considerable identity was also noted within the intron sequence surrounding exon 33 and the 5' region of exon 36 between both orthologues. This similarity in intron sequence ended 5' of exon 34 in the baboon gene and 5' of exon 36 in both the baboon and the human gene, directly within an Alu repeat sequence (Fig. 4). Between these Alu repeats, 2943 bp of DNA in the baboon gene shared only a 40% identity with the corresponding 506 bp of the human sequence, as opposed to 80% identity for the rest of the 3' end. The 506-bp sequence corresponded to a complex Alu repeat structure composed of a complete Alu repeat in the reverse orientation and a right monomer in the 5'-3' orientation. The putative breakpoints themselves fall in the right half of the surrounding Alu element.

PCR Analysis of the 3' End of the Primate and Human ELN Genes

PCR primers derived from the 3' end of exon 33 and the 5' end of exon 36 in the human tropoelastin gene were used to generate a 1655-bp amplimer that represented the arrangement of intron 33 as previously characterized in the human gene and is presented in Fig. 5A. PCR analysis, using these intron 33 primers, of genomic DNA from 546 alleles that represented 11 diverse ethnic groups revealed no size variation in the recovery of this 1655-bp PCR fragment (Table 1). Representative examples of the recovery of PCR-derived DNA from these human genomic samples are presented in Fig. 5B.

Similarly, a PCR assay was established from exon 33 to exon 34 in the baboon tropoelastin gene that yielded a PCR amplimer of 880 bp (Table 1). Analysis of genomic DNA from 53 unrelated primates, ranging from gorillas to baboons to rhesus monkeys revealed a consistent recovery of a PCR derived DNA fragment of 880 bp. A representative example of this result is shown in Fig. 5B.

Fig. 4. A comparison of the 3' end of the baboon (Papio cyanocephalus) and human (Homo sapiens) tropoelastin genes. Regions containing a high percentage of identical sequences are indicated as shaded areas, exons are represented as *filled boxes*. The 3' UTR in exon 36 is marked as a cross-hatched box. Distances between exons and other domains are shown as basepairs. A vestige of exon 35 is marked as () in the middle of intron 34 of the baboon ELN gene. (Alu \rightarrow) Alu repeat, $(\leftarrow Alu)$ Alu repeat in reverse orientation, $(Alu^c \rightarrow)$ complementary sequences to the Alu repeat.

Discussion

The vertebrate ELN gene has been analyzed in species ranging from chicken to human. In comparing the sequence divergence within functionally defined exons in the rat and human tropoelastin genes, it is clear that exons encoding hydrophobic domains diverge more rapidly than exons encoding cross-link domains (Boyd et al. 1991). Exon 34 encodes a hydrophobic domain; exon 35 encodes a potential cross-link domain. Both these exon domains are relatively conserved in sequence between bovine and rat tropoelastin. Significantly, however, both these exon-defined domains are present in all nonprimate vertebrate species analyzed, including chicken, suggesting that they predate both primate and mammalian radiation. The evolutionary radiation of mammals occurred about 200 million years ago (Macdonald 1993). Therefore, for over 200 million years, sequences defined by exons 34 and 35 were maintained in vertebrate tropoelastin genes. The conservation of this hydrophobic sequence and potential cross-link domain were therefore clearly important to the function of tropoelastin within vertebrate elastic tissues.

In contrast, exons 34 and 35 show little evolutionary stability during primate phylogeny. Nonhuman primates and human all diverged during the last 70 million years (Martin 1993). During this relatively short evolutionary period, two exons that had been maintained for over 200 million years were sequentially lost through what appears to be a series of Alu element-mediated recombination events. From the remnant of exon 35 detected in baboon genomic DNA, it would seem that mutational events resulted in the loss of exon 35 through the disappearance of constitutive splicing recognition signals. Continued recombination events at the 3' end of the tropoelastin gene in the primate lineage that eventually developed into modern humans resulted in the additional removal of exon 34.

One can only speculate as to the functional and evolutionary significance of the sequential loss of exons 34 and 35 during the development of primates. In several patients with a heritable vascular disorder, supravalvular



Fig. 5. Detection of species-specific configurations of the 3' end of the ELN gene in human and other nonhuman primate genomic DNA. A The upper part depicts the PCR strategy for the detection of exon 33, intron 33, and exon 34 in nonhuman primate genomic DNA. Arrows denote the primers (33E and 34E) used for PCR, and the size of the expected PCR fragment is indicated as base pairs. The sequence of these primers and PCR reaction conditions are described under Materials and Methods. The lower part depicts the PCR strategy for the detection of intron 33 as characterized in human genomic DNA. The arrows represent the positions of the primers (33I and I36). The size of the expected PCR product is indicated as base pairs. B Representative examples of the recovery of PCR-derived DNAs from primates and different human populations. Cauc., Caucasian; Am., American; Afr., African; Hisp., Hispanic; Hun., Hungarian; Pyg., pygmy; Ind., Indonesian; Ter., Ternate; Gui., Papua New Guinea; CAR, Central African Republic; Chimp., chimpanzee; Orang., orangutan.

Table 1. A summary of the PCR screening of human and nonhuman primate DNA for the detection of size variations of the 3' end of the ELN gene^a

DNA source	Number of individuals	Presence of exon 34	Presence of exon 35
chimpanzee	3	+	_
mandrill	2	+	_
gorilla	2	+	_
orangutan	2	+	_
gibbon	3	+	_
baboon	36	+	_
Rhesus monkey	3	+	_
Cyanomegalus	2	+	_
Total Primates	53		
Caucasian American	76	_	_
African American	50	_	_
Hispanic American	50	_	_
Hungarian	46	_	_
Zaire pygmy	10	_	_
C.A.R. pygmy	7	_	_
Indonesian	10	_	_
New Guinean	24	_	_
Total Humans	273		

^a The lack of any size variation demonstrates that all nonhuman primates contained exon 34 but lacked exon 35, while all human DNA samples analyzed revealed the lack of both exon 34 and exon 35.

aortic stenosis (SVAS), it has been shown that Alu element-mediated recombination events led to deletion mutations either within or involving the tropoelastin gene (Olson et al. 1995). While other elastic tissues such skin are affected by these tropoelastin gene deletions (Urbán and Csiszár, manuscript submitted), the overwhelming phenotype is a vascular disorder. In considering therefore selective advantages conferred through an Alu mediated loss of exons 34 and 35 during primate evolution, it is not unreasonable to speculate that perhaps alterations in vascular (particularly arterial) wall function, coincident with the evolution of primates, may have provided the selective advantage that led to the rapid loss of these two 3' exons.

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