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Mini-Review: new insights into the evolution of chorionic gonadotrophin

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1 **Abstract**

2

3 The glycoprotein hormones luteinizing hormone (LH) and chorionic gonadotrophin (CG) are

4 crucial for reproduction, as LH induces sex hormone production and ovulation, and CG is

5 essential for the establishment of pregnancy and fetal male sexual differentiation. Both consist

6 of two heterodimeric peptides of which the α -subunit is common to both hormones whereas

7 the β -subunit is hormone-specific. The CGB gene was derived from LHB by gene duplication

8 and frame shift mutation that led to a read-through into the formerly 3'-untranslated region,

9 giving rise to the carboxyl-terminal peptide. Owing to nucleotide changes within the 5'-region

10 of CGB, a new transcriptional start site and regulatory region was gained. These changes led

11 to the specific expression of CGB in the placenta and its decrease in the pituitary. Recent

12 findings on gonadotrophins led to an extended model for the sequence of events in the

13 evolution of the CGB gene in primates and its tissue-specific expression.

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1 **Gonadotrophin physiology**

2 Vertebrate reproduction is controlled by hormones of the hypothalamic-pituitary-gonadal
3 axis. The adenohypophysis synthesizes and releases the gonadotrophins luteinizing hormone
4 (LH) and follicle stimulating hormone (FSH), which act on the gonads and induce sex steroid
5 synthesis and gamete production and maturation in both sexes. FSH is necessary for follicle
6 maturation in the ovary and the stimulation of testicular Sertoli cells to support
7 spermatogenesis. LH triggers sex steroid production in the ovary, whereas LH peak levels
8 induce ovulation. In the testis, LH induces testosterone biosynthesis in Leydig cells.

9 The third gonadotrophin, chorionic gonadotrophin (CG), is the first biochemical signal from
10 the embryo to the maternal environment. It is produced by extraembryonic trophoblasts at the
11 beginning of a pregnancy and stimulates the corpus luteum to maintain progesterone synthesis
12 that represses further follicle maturation, thereby ensuring embryo implantation and
13 continuity of the pregnancy (Pierce and Parsons 1981). During pregnancy, CG is produced by
14 the fetal part of the placenta and is also crucial for male fetal sexual differentiation, as CG
15 stimulates the fetal testosterone synthesis in the testicular Leydig cells (Abramovich et al.
16 1974; Clements et al. 1976; Huhtaniemi et al. 1977; Tapanainen et al. 1981; Gromoll et al.
17 2000). Further biochemical and physiological details on LH and CG are compiled in Table 1.

18 Both LH and CG bind to the same receptor, the LH-/CG-receptor (LHR). Reports show that
19 the expression of LHR is not restricted to the gonads, but is also expressed in the
20 endometrium and other reproduction-associated tissues (Ziecik et al. 2007). It was therefore
21 speculated that the special structure of the primate placenta favoured CG function in
22 facilitating embryo implantation (Licht et al. 2007). Reports of CG's promotion of
23 angiogenesis support the hypothesis that the embryo fosters maternal blood vessel growth via
24 CG for better supply of nutrients and oxygen and easier release of CG and other factors
25 (Zygmunt et al. 2002; Herr et al. 2007). Furthermore, it was reported that CG is necessary for
26 the invasion of cytotrophoblasts into the endometrium during embryo implantation (Islami et

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1 al. 2001; Carver et al. 2003). One study demonstrated the association between recurrent
2 miscarriage and low serum CG values in patients; it was concluded that low serum CG
3 hinders embryo implantation (Rull and Laan 2005).

4

5 Biochemical studies have revealed that LH, FSH and CG consist of two subunits, of which
6 the α -subunit is identical in all hormones and the β -subunit confers hormone-specificity
7 (Bousfield and Ward 2006). Together with thyroid stimulating hormone (TSH) they form a
8 family of glycoprotein hormones and the resolution of their three-dimensional structure
9 classified them as part of the superfamily of cystine knot growth factors such as PDGF, NGF,
10 VEGF or TGF β (Lapthorn et al. 1994; Wu et al. 1994).

11

12 **Prepubertal gonadotrophin expression**

13 LH and FSH in serum are not only detectable in adults but also at very low serum
14 concentrations in prepubertal and pubertal children (Apter et al. 1989). In 5-6 year-old girls, a
15 pulsatile and diurnal LH pattern is present and levels rise clearly before the onset of puberty
16 (Dunkel et al. 1990a; Dunkel et al. 1990b; Dunkel et al. 1992; Demir et al. 1995; Clark et al.
17 1997; Mitamura et al. 2000). As found in man, a rise of LH and testosterone levels from the
18 prepubertal to the adult could be shown in the rhesus macaque (Meeran et al. 2003). Despite
19 these findings, the function, if any, of childhood gonadotrophins remains to be established.

20

21 **Human LH variants**

22 There are several reports on common variant LH (V-LH), which escaped detection by
23 conventional LH-antibodies (Pettersson et al. 1992). The investigation of the V-LHB gene
24 identified two new SNPs in protein-coding parts that result in two amino acid changes: Trp8
25 => Arg8 and Ile15 => Thr15 (Furui et al. 1994; Okuda et al. 1994; Pettersson et al. 1994).
26 Eight additional SNPs in the promoter of V-LHB lead to higher promoter activity compared

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1 with the normal LHB promoter (Jiang et al. 1999). A study of a homozygous individual
2 showed a higher activity of V-LH compared with normal LH but also a shorter half-life: 26
3 min in males and 44 min in females for V-LH versus 48 min in males and 53 min in females
4 for WT-LH (Haavisto et al. 1995).

5 V-LH is present in nearly all populations worldwide, posing an important genetic LHB
6 variant: among 30 different ethnicities, the carrier frequency had a mean \pm SD of 16.5 ± 10.8
7 %, ranging from 0 % to 54% (Nilsson et al. 1997; Nilsson et al. 1998; Ramanujam et al. 1998;
8 Elter et al. 1999; Ramanujam et al. 1999; Starka et al. 1999; Lamminen and Huhtaniemi
9 2001).

10

11 **Ectopic human CG**

12 Additional expression of CG was also found in the pituitary and at very low levels in the
13 serum of normal individuals, in whom CG levels were slightly elevated in elderly individuals
14 >50 years (Borkowski et al. 1984; Stenman et al. 1987; Hoermann et al. 1990; Odell et al.
15 1990; Alftan et al. 1992; Birken et al. 1996; Dirnhofer et al. 1996). Furthermore, it was
16 found that the CG levels were responsive to positive feedback via GnRH *in vivo* and *in vitro*
17 and to negative feedback via progesterone and oestrogen *in vivo* (Stenman et al. 1987; Henke
18 et al. 2007). Despite all these findings, so far no physiological function could be attributed to
19 the CG expression in the pituitary and resulting serum CG in non-pregnant individuals.

20

21 **A common evolutionary origin of gonadotrophin subunits**

22 The peptide sequences of the β -subunits of the gonadotrophins revealed a homology among
23 them and therefore a common evolutionary origin (Dayhoff 1976). Sequence comparison of
24 the human LHB and CGB mRNAs and genes displays high homology and a duplication of
25 CGB from LHB, confirming the concept of a common evolutionary origin (Talmadge et al.
26 1984). The LHB and CGB transcripts encompass 1.1 and 1.5 kilobases, respectively, and are

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1 therefore relatively small transcripts. The detailed structures and lengths of the LHB/CGB
2 genes and transcripts are given in Figure 1 and Table 2, respectively.

3

4 **The structure of the LHB and CGB genes**

5 Gene duplication, copy diversification and the acquisition of new function are important
6 mechanisms of gene evolution (Zhang 2003). In the case of primate gonadotrophin subunit
7 genes, LHB underwent a gene duplication, in which one copy was conserved as LHB, while
8 the other copy developed into CGB by a single nucleotide deletion in codon 114; this led to a
9 frame shift mutation, thereby elongating the open reading frame (ORF) into the previously 3'
10 untranslated region (Table 2). In addition, two nucleotides inserted in codon 138 for human
11 CGB led to a further frame-shift in the ORF. These changes resulted in eight different and 24
12 additional codons for CGB in comparison with LHB (Table 2).

13

14 **Primate LHB/CGB genes are organised in clusters**

15 It was found that human CGB is a multicopy gene, as six CGB gene copies and one LHB
16 gene copy form a cluster (Figure 1A) (Boorstein et al. 1982; Policastro et al. 1983; Talmadge
17 et al. 1983; Fiddes and Talmadge 1984). The cluster is located on chromosome 19 at
18 19q13.32 (Naylor et al. 1983; Brook et al. 1984; Julier et al. 1984). However, the exact map
19 of the cluster itself remained elusive until it was cloned completely as a 58 kb DNA fragment
20 by Policastro *et al.*, (Policastro et al. 1986) and confirmed by others (Jameson and Lindell
21 1988). The single genes are adjacent to each other in tandem and inverted repeats within the
22 gene cluster. A recent re-sequencing of this cluster in humans of different ethnicities revealed
23 a high rate of SNPs and a low linkage disequilibrium due to excessive gene conversions so
24 that this gene cluster represents a hot spot of genomic diversity (Hallast et al. 2005). CGB1
25 and CGB2 underwent further differentiation from canonical CGB owing to a DNA insertion
26 (736 bp for CGB1, 724 bp for CGB2) and a deletion of 52 bp plus 356 bp in the region

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1 serving as 5'UTR in the paralogous CGB3/5/7/8 genes. These rearrangements led to a novel
2 exon 1 (58 bp) and constitutive skipping of canonical exon 1, in which the donor splice site is
3 disrupted. The two known exons 2 and 3 are included in the ORF but are frame-shifted,
4 thereby leading to a hypothetical ORF of 132 codons with a new stop codon and elongated
5 3'UTR (Figure 1 and Table 2). Despite extensive descriptions of their mRNAs, no CGB1/2
6 protein product has been demonstrated yet (Bo and Boime 1992; Hollenberg et al. 1994;
7 Dirnhofer et al. 1996; Hallast et al. 2007).

8 A BLAST search for CGB gene copies in the chimpanzee genome (Chimpanzee Sequencing
9 and Analysis Consortium 2005) resulted in several hits within a contig allocated to
10 chromosome 19 (NW_001228244.1) but the hits were adjacent to gaps and did not allow an
11 allocation of human CGB homologues to the chimpanzee counterparts except for LHB. We
12 believe that a correct contig is not available yet.

13 A BLAST search in the rhesus macaque genome (Rhesus Macaque Genome Sequencing and
14 Analysis Consortium et al. 2007) resulted in four hits in the BAC CH250-325I15, available
15 under accession number AC202849.6. These four hits were identified as one LHB and three
16 CGB genes, which spanned approximately 30 kb and are shown as schematic cluster in Figure
17 1 B.

18 **Transcription of human CGB genes**

19 In the human, the placental syncytiotrophoblasts are the major expression site of CGB during
20 the first trimester of pregnancy. Gene copies CGB3/5/8 are expressed strongest whereas
21 CGB1/2/7 genes are expressed at very low levels. CGB7 was also detected in normal breast
22 tissue with an as yet unknown function (Talmadge et al. 1984; Bo and Boime 1992; Rull and
23 Laan 2005). Interestingly, human CGB isoforms 3,5,8 were also detected, albeit at low
24 expression, in the pituitary at the mRNA and protein levels (Hoermann et al. 1990; Odell et
25 al. 1990; Birken et al. 1996; Dirnhofer et al. 1996). It follows that among the different human
26 CGB gene copies there are differences in the strength of their promoters, dependent on the

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1 tissue (Rull et al. 2008). It is also well known that epithelial tumours of the urogenital system,
2 breast tumours and testis tumours can express *CGB* ectopically. Real time PCR of the mRNA
3 isoforms in breast cancer revealed an up-regulation of *CGB* isoforms 3, 5 and 8 but not 1, 2
4 and 7 (Bellet et al. 1997; Giovangrandi et al. 2001). However, the ultimate reason for ectopic
5 *CGB* expression in some tumours is unknown.

6

7 **New World monkey LH/CG**

8 A surprising plasticity of the LH/CG system was recently discovered in the common
9 marmoset (*Callithrix jacchus*), a New World monkey. It appears that LHB became a
10 pseudogene and its loss was compensated for by *CGB* (Muller et al. 2004; Scammell et al.
11 2008). The marmoset LHB and *CGB* genes have exon/intron structures homologous to the
12 human situation, however marmoset *CGB* transcripts of the pituitary have a remarkably short
13 5'UTR of only 7 bp, which is comparable with other mammalian LHB 5'UTRs that are 9 bp in
14 length (Jameson et al. 1986; Muller et al. 2004; Henke et al. 2007).

15

16 **Gonadotropins in equid species**

17 In the equine species horse (*Equus ferus caballus*), donkey (*Equus asinus*) and zebra (*Equus*
18 *burchelli*), a single copy gene gives rise to an LHB/*CGB*-like gonadotrophin peptide that also
19 harbours a CTP (Leigh and Stewart 1990; Sherman et al. 1992; Chopineau et al. 1995;
20 Chopineau et al. 1999). The horse CTP was derived from a 10 bp deletion in exon 3, which
21 was different from the single nucleotide deletion in the primate *CGB* gene. The same
22 promoter region of the horse LHB/*CGB* gene serves as promoter in the pituitary and placenta.
23 The main difference from other mammalian LHB promoters is a small insertion (50 bp) that
24 gave rise to a second TATA-box, leaving the remaining promoter largely unchanged
25 (Sherman et al. 1992). Owing to these characteristics it was concluded that this single copy

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1 gene evolved independently from the primate CGB, occurs only in equine species and
2 therefore demonstrates convergent evolution at the molecular level (Sherman et al. 1992).
3 This gene is expressed in both the pituitary and placenta of equine species and was confirmed
4 in several studies at the protein level (Bousfield et al. 1987; Sugino et al. 1987).
5 During gestation, equine CG is synthesised and secreted by the endometrial cups that arise
6 from the placental trophoblast chorionic girdle cells. In serum, CG appears from day 40 of
7 gestation onwards, increases until it reaches its peak level between days 55-70, decreases until
8 day 130 and then becomes undetectable (Moore et al. 1975; Spincemaille et al. 1975;
9 Wooding et al. 2001). In contrast, in humans, CG is detectable earlier in the pregnancy than in
10 horses and drops after a peak to a low, but still detectable, level, until parturition.
11 There are no reports on whether pregnant mare CG contributes to pregnancy recognition,
12 since in horses the conceptus signals its presence to the maternal environment *via*
13 prostaglandin F₂ α (PGF₂ α) in a paracrine manner. PGF₂ α normally induces luteolysis of the
14 *corpus luteum* and results in pregnancy loss, when embryos secrete too much PGF₂ α . (Allen
15 2001). This mechanism contrasts not only the primate CG mechanism of pregnancy
16 recognition but also the pregnancy recognition in ruminants *via* interferon tau (Roberts 2007;
17 Roberts et al. 2008). Unfortunately, there are no studies on the mechanism of pregnancy
18 recognition in prosimians and tarsier primate species that lack the CGB gene; otherwise it
19 might have been possible to track the switch from prostaglandin/interferon-tau to CG-
20 mediated pregnancy recognition.

21

22 **Glycosylation patterns and the role of the carboxy-terminal peptide (CTP) in CG**

23 The human LHB and CGB peptides are N-glycosylated at Asn13 and Asn 30. In addition, the
24 CTP of CGB prolongs the β -subunit peptide and enables extensive glycosylation of four novel
25 O-glycoslation sites at Ser121, Ser 127, Ser 132 and Ser 138 (Birken and Canfield 1977;
26 Keutmann and Williams 1977). CTP glycosylation leads to a prolonged half-life of 5-6 hours

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1 for CG compared with 40-60 minutes for LH and an increased molecular weight from 31 kDa
2 for LH holohormone to 36 kDa for CG holohormone (Wehmann and Nisula 1981; Storrington
3 al. 1988; Haavisto et al. 1995; le Cotonnec et al. 1998a; le Cotonnec et al. 1998b; le Cotonnec
4 et al. 1998c). The glycosylated CTP also results in an altered route of hormone secretion: LH
5 is secreted baso-laterally from gonadotrophic storage granules after GnRH-stimulation,
6 whereas CG is released constantly and apically by trophoblasts towards the maternal blood
7 circulation system (Table 2) (Childs et al. 1983; Handwerger et al. 1987; Lloyd and Childs
8 1988; Currie and McNeilly 1995; Jablonka-Shariff et al. 2002). *In vitro* experiments with
9 polarised Madin-Darby canine kidney cells demonstrated that monomeric CGB subunit is
10 released 30% baso-laterally and 70% apically, whereas the LHB subunit is released 30%
11 apically and 70% baso-laterally. When the CTP is removed from the CGB peptide, it is
12 released like LHB and thereby proving that the CTP determines the route of secretion
13 (Jablonka-Shariff et al. 2002). As in equine species a previously cryptic CTP became
14 expressed, and Nakav *et al.* (Nakav et al. 2005) investigated whether other species also
15 possess a cryptic CTP in the 3' region of their LHB genes. *In vitro*, bovine LHB was fused to
16 either bovine or human CTP. In both cases efficient glycosylation failed and therefore the
17 protein was secreted baso-laterally as found for LH. The absence of apical routing, which is
18 necessary for efficient placental release of CG into the maternal blood stream, provides an
19 explanation of why CGB did not emerge in ruminants (Nakav et al. 2005). Furthermore,
20 predominantly oviparous vertebrate taxa such as fishes, amphibians, reptiles and birds, do not
21 show *in silico* potential CTP sequences. Only mammals do so, and even marsupials (e.g.,
22 kangaroo and opossum) show a putative CTP, although its sequence deviates significantly
23 from CTP sequences found in the taxon Placentalia. Thus, genuine CTP-like sequences, either
24 encrypted or expressed, correlate well with the presence of a placenta (Nakav et al. 2005).
25 However, the glycosylation pattern of a potential CTP would not be beneficial in all species,

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1 with respect to the mode of secretion, and we speculate that this is one reason why CG is
2 restricted to primate and equine species.

3 The marmoset differs from the human with regard to the glycosylation pattern of CGB, as
4 marmoset CGB possesses one N-glycosylation and two O-glycosylation sites, and therefore
5 there are differences both with human CGB and LHB (see also Table1). Thus, marmoset CGB
6 represents a peptide like hCGB but with a glycosylation pattern intermediate between hLHB
7 and hCGB (Simula et al. 1995; Amato et al. 1998). These peculiar biochemical properties
8 lead to a disruption of clear CG pulses emanating from the pituitary, as the half-life of
9 marmoset CG appears to be intermediate between that of human CG and human LH
10 (Tannenbaum et al. 2007a; Tannenbaum et al. 2007b). The half-life of marmoset CG has not
11 been investigated yet, nor has its placental glycosylation pattern, which might differ from that
12 in the pituitary, since the exact composition of glycosylation depends upon the tissue-specific
13 enzymes expressed. For instance, there is a hyperglycosylated form of human CG (hCG-H)
14 that has a molecular weight of approximately 40 kDa. This form is produced in the first
15 trimester of pregnancy (O'Connor et al. 1998). Its biological role is the cytotrophoblast
16 invasion into the endometrium. Ectopic expression of hCG-H occurs in pregnancies with
17 Down-syndrome and in some malignant tumours. The more malignant the cells were, the
18 more CG was hyperglycosylated (Valmu et al. 2006; Cole and Khanlian 2007). Although the
19 reason for ectopic CGB expression in tumours is still unclear, it could be demonstrated that
20 CG can drive prostate carcinoma malignancy (Wu and Walker 2006).

21

22 **An extended model of primate CGB evolution**

23 The recent findings on gonadotrophins led us to propose a revised and extended version of
24 primate CGB gene evolution, covering the sequence of events such as duplication, mutation
25 and acquisition of new functions (Fiddes and Talmadge 1984).

26

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1 **1. Duplication of the LHB gene**

2 Approximately 55-50 million years ago (mya) the split of the tarsiers from the lineage of
3 Simiiformes occurred. The anthropoid line encompasses New World monkeys (NWM), Old
4 World monkeys (OWM), small apes, great apes and humans. The latter three groups form the
5 taxon Hominoidea. As there is no CGB or second LHB gene in prosimians and tarsiers, the
6 LHB gene duplication must have occurred before the NWM diverged from the OWM,
7 approximately 55-35 mya (see also Fig. 2) (Bailey et al. 1991; Schrago and Russo 2003;
8 Opazo et al. 2006; Schrago 2007). Because LHB and CGB have not only highly homologous
9 coding sequences, but also highly homologous promoters, we assume that, after the LHB gene
10 duplicated, both LHB copies were functionally expressed in the pituitary.

11

12 **2. The appearance of CGB**

13 In one LHB gene copy a single nucleotide was deleted in the 114th codon and led to a frame
14 shift mutation. This slipped the last seven codons out of frame compared with LHB, and led
15 to a read-through into the previous 3'UTR and an incorporation of this sequence into the
16 coding region. An insertion of the two nucleotides C and G in the 138th codon further
17 elongated the ORF, expanding the CGB subunit for 24 additional codons that encode the
18 CTP. These frame shifts resulted in a new stop codon, TAA, in the previous 3'UTR. It is part
19 of the polyadenylation signal and yields a very short 3'UTR of only 16 nucleotides (Talmadge
20 et al. 1984). However, it is not possible to elucidate which event occurred first; the single
21 nucleotide deletion or the insertion of the two bases.

22 This concept of events for the mechanism of CGB gene evolution by LHB gene duplication
23 and nucleotide modifications that led to the CTP, has already been proposed (Dayhoff 1976;
24 Fiddes and Goodman 1980; Fiddes and Talmadge 1984; Talmadge et al. 1984).

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1 Since CGB derived from LHB, including the promoter, and because CGB is still expressed at
2 very low levels in human pituitaries, we speculate that CGB was still expressed in the
3 pituitary in ancient primates at this stage of evolution.

4 **3. CG gains expression in the placenta**

5 Changes in the promoter region of CGB led to a shift in expression from the pituitary to the
6 placenta (Hollenberg et al. 1994). The hCGB transcriptional start site relocated to 365 bp
7 upstream of ATG, so that the 5' UTR of the mRNA comprises the region which serves as core
8 promoter and transcriptional start site for LHB transcription. Human CGB transcription is
9 mainly regulated by a TATA-less promoter, which is regulated by the transcription factors
10 (TF) cAMP element binding protein (CREB), v-ETS erythroblastosis virus E26 oncogene
11 homolog 2 (ETS2), activating protein 2 (AP2) and selective promoter factor 1 (SP1).
12 Although each of these factors is expressed in several different tissues, they form together a
13 trophoblast-specific combinatorial code for CGB gene transactivation (Jameson and
14 Hollenberg 1993; Hollenberg et al. 1994; Pestell et al. 1994; Johnson and Jameson 1999;
15 Johnson and Jameson 2000; Ghosh et al. 2003). At the blastula stage, CG is synthesised by
16 the trophectoderm but not by the embryonic stem cells in the inner cell mass. The
17 pluripotency marker Oct3/4 is expressed by the embryonic stem cells and acts as a
18 transcription factor. Oct3/4 is able to bind to the promoters of the glycoprotein hormone alpha
19 gene (GPHA) and CGB and thereby repress their transcription (Liu and Roberts 1996; Liu et
20 al. 1997). Thus, in the placenta, CGB expression is controlled tissue-specifically by a
21 completely different regulatory region and set of TFs compared with pituitary-expressed
22 LHB.

23 The glycoprotein α -subunit, which is necessary for full functional and biological activity of
24 the gonadotrophins, also gained expression in the placenta, otherwise no functional CG
25 holohormone could have formed. However, it remains to be clarified when exactly this
26 happened and which of the two subunit genes was expressed first in the placenta.

1

2 4. Differences of CG expression in pituitaries depend on taxonomic group

3 The LHB core promoter for mouse, rat, cattle and man comprises two transcription factor
4 binding sites for steroidogenic factor 1 (SF1), two for early response growth protein 1
5 (EGR1), one for pituitary homeobox factor 1 (PITX1) and a TATA box. SF1 is involved in
6 steroid synthesis in a wide spectrum of tissues (e.g. gonads and adrenals) and EGR1 is
7 expressed mainly during embryogenesis. PITX1 is a pituitary-specific transcription factor and
8 all three factors together form a gonadotrophe-specific, tripartite regulatory complex for LHB
9 activation (Kaiser et al. 2000; Jorgensen et al. 2004).

10 It was demonstrated that in human CGB only three nucleotide changes in transcription factor
11 binding sites (TFBS) were sufficient to cause CGB down-regulation in the pituitary and
12 reduced GnRH-responsiveness in comparison with LHB (Henke et al. 2007). In one TFBS for
13 EGR1, a C-T transition and a G-C transversion occurred at positions -119 and -112,
14 respectively, in relation to the start codon ATG. An A-G transition at -106 in the TFBS for
15 PITX1 decreased its protein binding ability, and all three nucleotide changes together
16 demonstrated synergistic effects in pituitary CGB down-regulation (Henke et al. 2007).

17

18 5. CGB gene duplications in OWM and Hominoidea and a new LH/CG system in NWM

19 In the lineages of OWM and Hominoidea, the CGB gene itself underwent several
20 duplications, leading to different copy numbers varying from three to six, as indicated in
21 Table 3 and Fig. 2. This was reported first for humans and later investigated systematically
22 for the order of primates (Boorstein et al. 1982; Talmadge et al. 1983; Maston and Ruvolo
23 2002). A recent study demonstrated that CGB1 is the most recent gene copy, as it evolved in
24 the last common ancestor of African great apes (gorilla, chimpanzee and human), after the
25 split from gibbons and orang utan, approximately 15 to 8 mya (Hallast et al. 2007).

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1 Originally both genes, LHB and CGB, must have been present in NWM. However, for yet
2 unknown reasons, LHB became non-functional in the common marmoset and its loss was
3 compensated for by CGB, which is a single copy gene, expressed in both pituitary and
4 placenta (Muller et al. 2004).

5 This is valid for the complete branch of NWM because of the conserved CGB promoter
6 architecture across all four NWM families (unpublished data from our laboratory), and the
7 lack of LHB and presence of CGB in pituitaries in other NWM species (Scammell et al.
8 2008). Further striking evidence comes from the similar exon architecture of the LHR type 2
9 (Gromoll et al. 2003; Gromoll et al. 2007). The LHR types 1 and 2 are G protein-coupled,
10 seven transmembrane receptors. In OWM and Hominoidea, the LHR type 1 is able to bind
11 and transduce signals from LH and CG equally well. However, in NWM, the LHR type 2
12 lacks exon 10 of 11 exons at the mRNA and protein levels, although it is present at the
13 genomic level (Gromoll et al. 2003; Gromoll et al. 2007). Exon 10 encodes the extra-cellular
14 hinge region of the receptor and its absence in LHR type 2 impairs LH signal transduction but
15 not CG signalling (Muller et al. 2003). Thus, the LHR type 2 of the NWM is able to
16 discriminate between LH and CG and transduces CG signalling only. Therefore Fig. 2
17 illustrates not only the CGB evolution in primates but also includes the LHR type 2 co-
18 evolution and important dates of primate speciation.

19

20 **Summary and Conclusion**

21 In this review we have discussed the evolutionary events that led to the rise of CGB in
22 primates and were able to approximate the time lines during which each event took place. In
23 brief, we propose the following sequence of events:

24 LHB gene duplication, emergence of the CTP, co-expression of CGB & GPH α in the placenta
25 and gain-of-function of CG in the placenta occurred approximately 55-35 mya, a relatively
26 short time in evolutionary terms.

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1 The events specific for NWM, such as emergence of LHR type 2 and LHB's becoming a
2 pseudogene, most likely happened 35-20 mya. In the lineage leading to OWM, small apes,
3 great apes and the human, CGB was duplicated in the period 35-25 mya. A further
4 duplication, which is specific for gorilla, chimpanzee and humans, occurred 15-8 mya.

5
6 The CGB gain-of-function, its gene copy number variance in OWM and Hominoidea, and the
7 strong pituitary CG expression in the NWMs demonstrate a remarkable plasticity of the
8 LH/CG hormone system not previously anticipated. Thus, the CGB gene cluster represents a
9 hallmark of primate evolution and is an excellent example of how a few changes in duplicated
10 DNA sequences can lead to a novel gene, characterised by a changed cellular expression,
11 peptide extension as well as a novel physiological role. Therefore this gene cluster gives
12 insight into currently ongoing evolution in primates, including the human.

13

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1 **Table 1: Main differences between human LH and CG.**

Characteristic	human LH	human CG
Major site of expression	pituitary	placenta
Time of physiological relevant expression	starts at adolescence	starts at pregnancy
Major function	ovulation & synthesis of sex steroids and INSL3	establishment of pregnancy in ♀ Fetal differentiation in ♂
Initial half-life in blood	40-60 min	5-6 h
Peptide length and of glycosylation pattern of mature β-subunit	121 amino acids 2 x N-glycan no O-glycan	145 amino acids 2x N-glycan 4x O-glycan
Secretion pattern	storage in vesicles; pulsatile release in baso-lateral direction	constitutive secretion in apical direction

2 The data are based on Pierce and Parsons 1981; Wehmann and Nisula 1981; Storrington et al.
3 1988; le Cotonnec et al. 1998a, b, c ; Jablonka-Shariff et al. 2002; Bousfield and Ward 2006.
4 INSL3 = insulin-like growth factor 3.

5

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1 **Table 2: Comparison of human gonadotrophin β -subunit transcripts.**

2

	LHB [bp]	CGB3/5/7/8 [bp]	CGB1/2 [bp]
5'UTR	9	365	174
Exon 1*	15	15	58
Intron 1	352	352 (CGB 3/5/7) 353 (CGB 8)	399
Exon 2	168	168	168
Intron 2	235	235	235
Exon 3*	240	312	170
3'UTR	91	20	162
Total	1110	1467	1366

3

4 * length of UTR sequence is given separately and therefore excluded from given exon size

5

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1 **Table 3: CGB copy numbers in different primate species.**

Primate group	Species	CGB copy numbers
Hominoidea	Human (<i>Homo sapiens</i>)	6
	Chimpanzee (<i>Pan troglodytes</i>)	5
	Orang utan (<i>Pongo pygmaeus</i>)	4
Cercopithecoidea (Old World monkeys)	Rhesus macaque (<i>Macaca mulatta</i>)	3
	Guereza monkey (<i>Colobus guereza</i>)	4
	Dusky leaf monkey (<i>Presbytis obscura</i>)	5
Platyrrhini (New World monkeys)	Owl monkey (<i>Aotus trivirgatus</i>)	1
	Dusk titi monkey (<i>Callicebus moloch</i>)	1
Prosimii	Ring-tailed lemur (<i>Lemur catta</i>)	0

2

3 Data are based on (Maston and Ruvolo 2002; Hallast et al. 2007).

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1 **Figure 1: The structure of LH/CGB gene clusters and their genes.**

2 A schematic overview of the (A) organisation of the human LHB/CGB gene cluster on
3 chromosome 19.3 with indications of the orientation of the genes and the intergenic
4 distances, (B) the organisation of the rhesus macaque's LHB/CGB gene cluster and
5 (C) details of the human genes themselves. The numbering of rhesus macaque CGB
6 genes is in numerical order and does not imply special homology to the accordingly
7 numbered human CGB genes. Black boxes indicate UTR, white boxes the exons
8 (Ex) and solid vertical lines introns (Int) and promoter regions. The original CGB
9 structure for CGB1/2 before deletions and insertions is indicated by grey shading.

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1 **Figure 2: Schematic evolution of CGB within the primates.**

2 This is a schematic overview of CGB evolution in primates. Prosimians and tarsiers
3 possess only LHB but not CGB. Solid arrows indicate when an event happened,
4 based on published time points. Dashed arrows indicate events for which the point of
5 time is unknown. The period in which the event most likely happened is explained in
6 the text. When more than one CGB copy number is present in a lineage, it is
7 indicated by (n+2) or (n+3). Mya, million years ago; NWM, New World monkeys;
8 OWM, Old World monkeys. Branching time points are according to Bailey et al. 1991;
9 Kumar and Hedges 1998; Schrago and Russo 2003; Poux et al. 2006; Rhesus
10 Macaque Genome Sequencing and Analysis Consortium et al. 2007 and Schrago
11 2007.

12

13

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