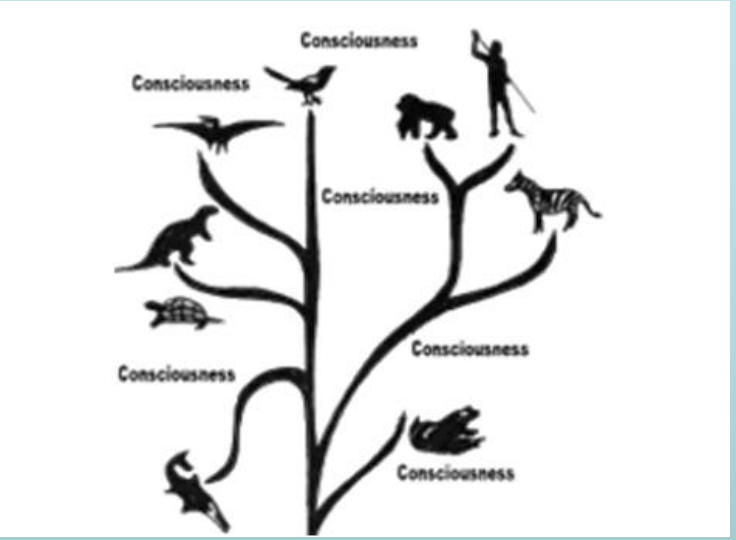

O-glycosylation and protein evolution: the case of the LHβ to CGβ development

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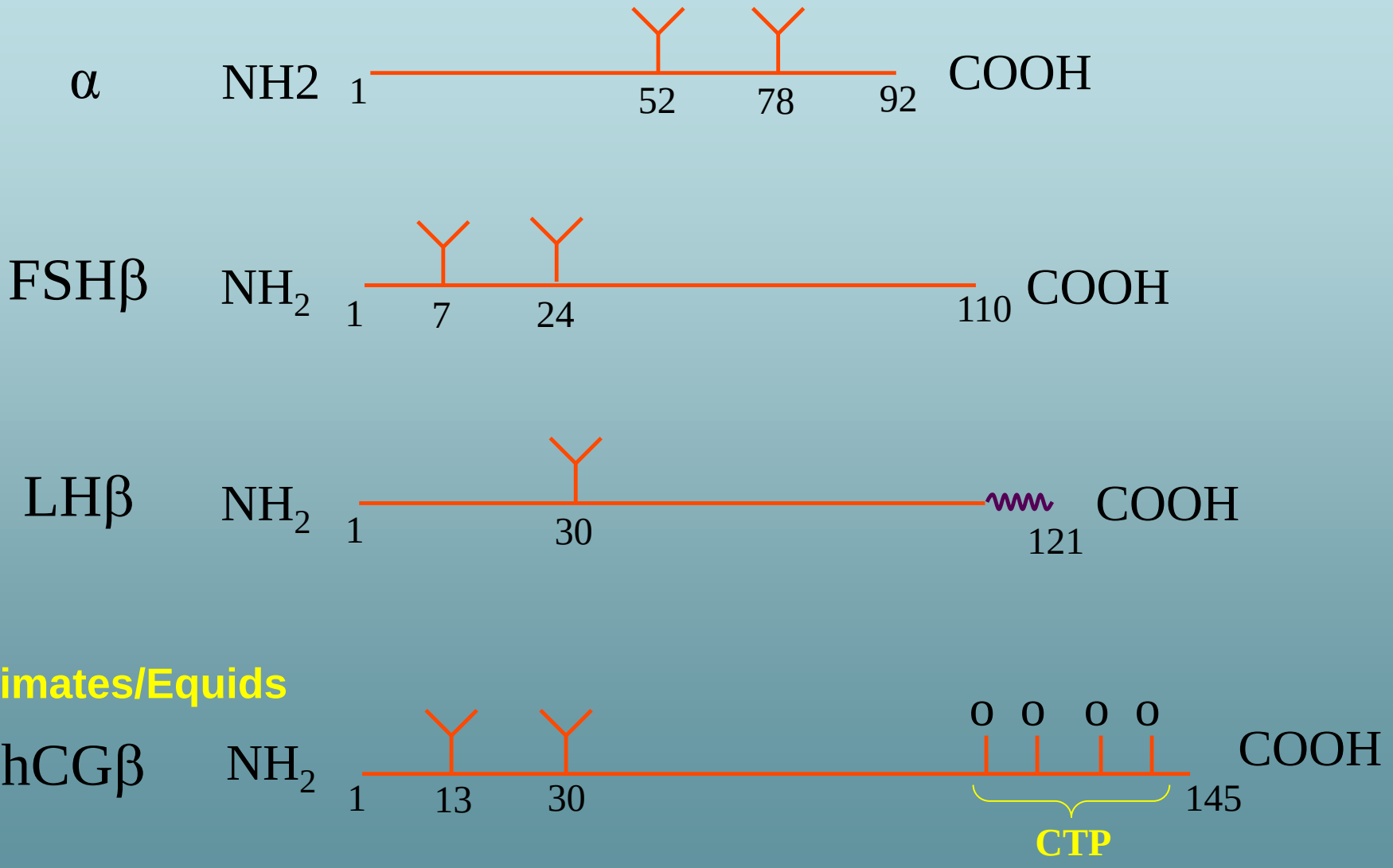
Structure-Function of the Gonadotropins; members of the glycoprotein hormone family

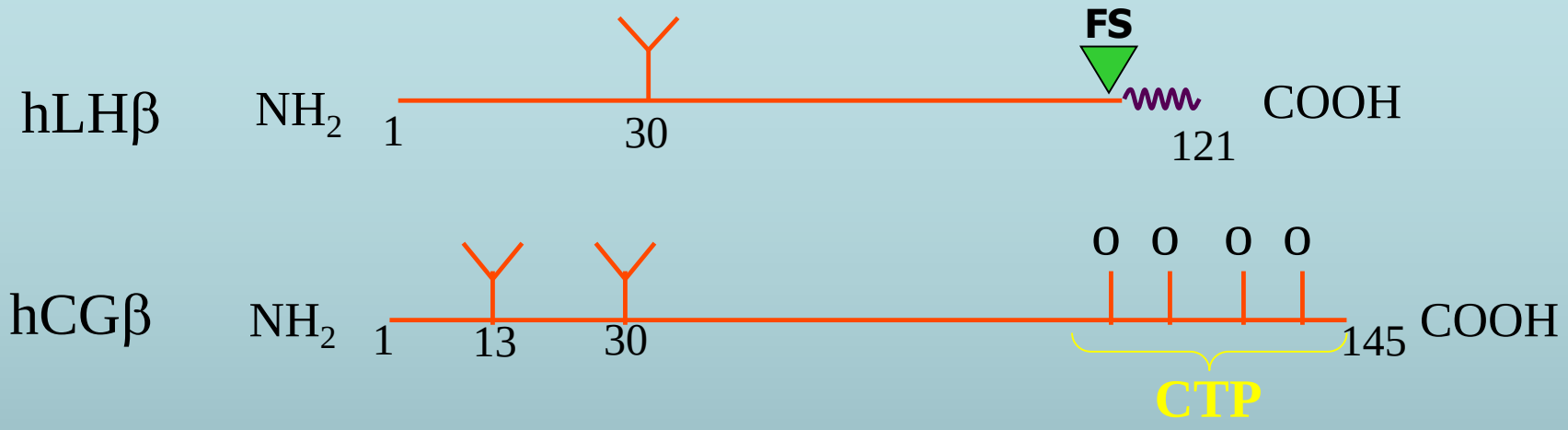
- Lutropin (LH), follitropin (FSH) are expressed in the pituitary and Choriogonadotropin (CG) is synthesized in the placenta of primates and equids

- Non-covalent heterodimers composed of a common α subunit and a hormone-specific β subunit. Only dimers are active; monomeric subunits do not bind to the cognate receptor. Both LH and CG activate the LH/CG receptor (LHR)

The Gonadotropin Subunits



The LHβ to CGβ subunit development; Carboxy Terminal Peptide extention (CTP) characteristics



- The CGβ gene presumably evolved from the ancestral LHβ gene
- Ser/Thr/Pro rich domain, multiple O-glycans attached to the CTP (4-12)
- Prolongs circulatory survival compared to LH
- Orient secretion of hCG from the apical side of placental trophoblasts into the maternal circulation to delay luteolysis in primates

Why the CTP domain is not wide-spread in the animal kingdom?

This is intriguing because the LHβ gene is conserved among mammals, few mutations localized to a small region and the gain of new hormonal properties

Whether the LHβ genes in species other than primates and equids contain an untranslated CTP-like sequence? Yes, a CTP-like sequence is cryptic in the LHβ gene of several mammals but not in birds, amphibians and fishes

Whether the incorporation of the cryptic CTP sequence in the bovine LHβ reading frame will result in misfolding and degradation or allow the expression of the extended subunit?

bLHβ: 110 CDHPPLPDILFL121

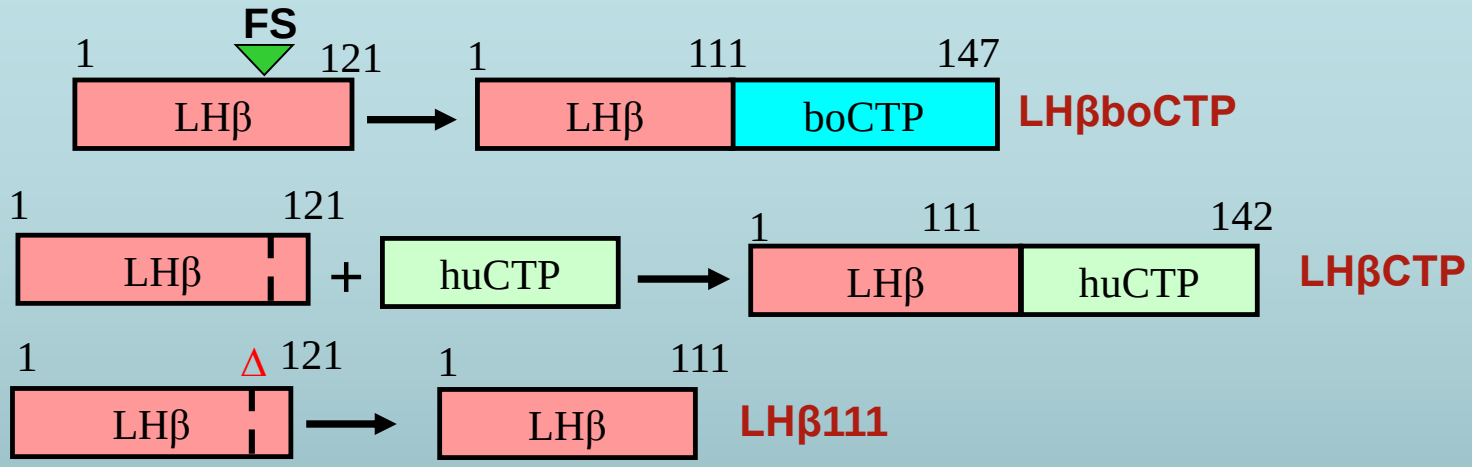
bLHβboCTP: 110CD....P…QTSSSSKDAPLQP...PMPILTLQTSRHSS PPFPIKTS147

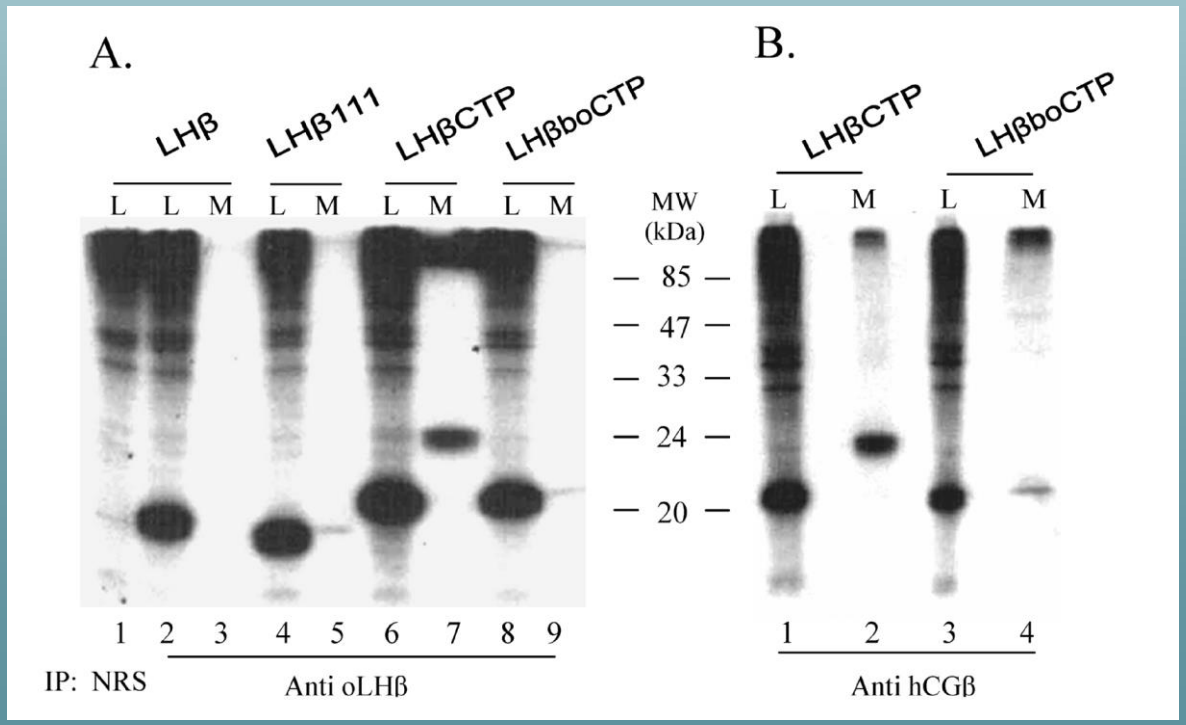
eLH/CGβ: 110CA....P…QASSSSKDPPSQPLTSTSTPTPGASRRSSHPLPIKTS149

hCGβ: 110CDDPRFQASSSSKAPPP...SLPSPSRL...PGPSDTPILPQ145

Nakav et al., 2005

Expression and secretion of the bovine elongated LHβboCTP subunit in transfected CHO cells





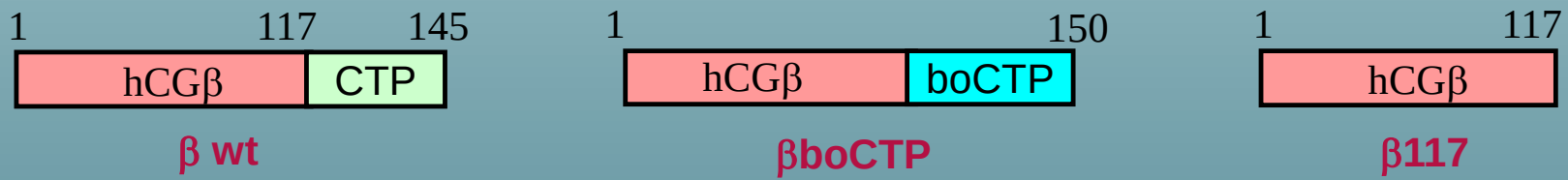
Nakav et al., 2005

Structure and Function of the boCTP Domain

hLHβ:	^{110}CDHPQLSGLLFL^{121}
hCGβ (βwt):	^{110}CDDPRFQA<u>SSSS</u>**S**KAPPP**S**LPSP**S**RLPGP**S**DTPILPQ^{145}
hCGβboCTP (βboCTP)	^{110}CDDPRFQA<u>SSSS</u>KDAPLQPPMPILTLQTSRHSSPPFPIKTS^{150}
hCGβ117 (β117)	^{110}CDDPRFQA^{117}

Cloned in PM^2 and stably transfected into CHO cells

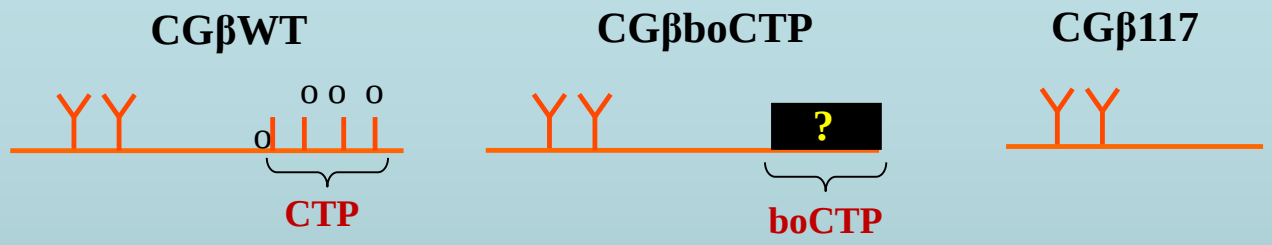
Secretion kinetics: Pulse Chase analysis

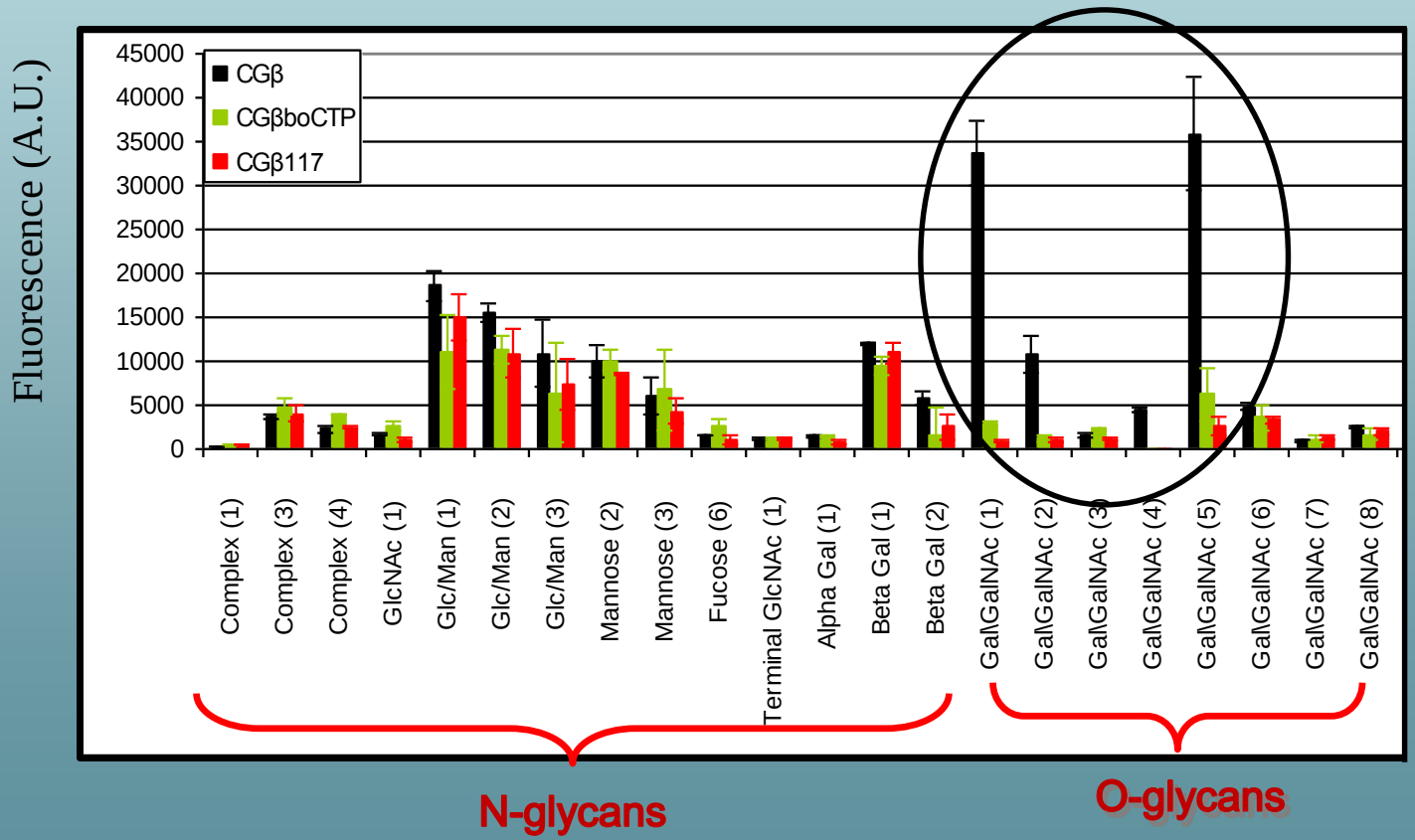


	β wt	βboCTP	β117
Recovery (%):	**65 ± 5**	**65 ± 5**	**50 ± 5**
t½ (min):	**80 ± 5**	**115 ± 10**	**90 ± 5**

Nakav et al., 2005

Lectin array analysis of the secreted chimeric subunit; absence of mucin type O-glycans

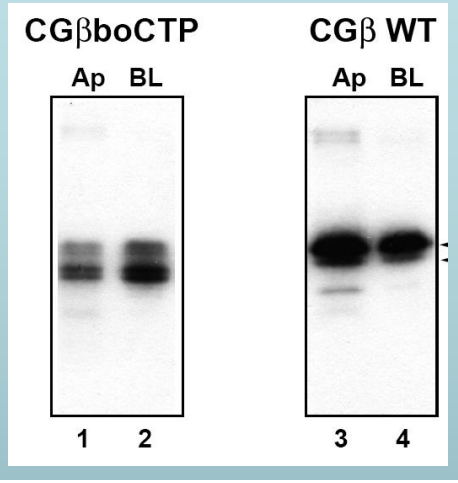




Gabay et al., 2014

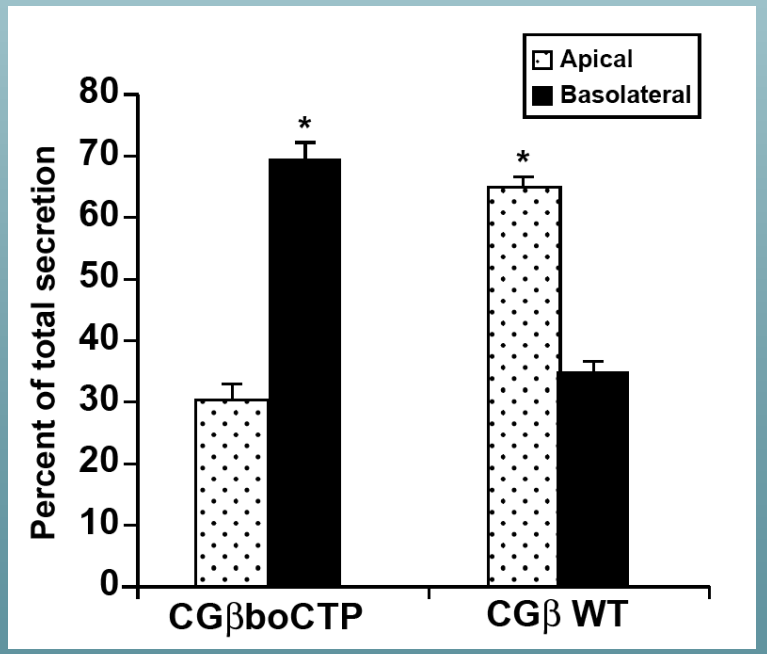
Basolateral secretion of the CGβboCTP chimera from polarized MDCK cells

	Apical (%)	Basolateral **(%)**
CGβboCTP 	30	70



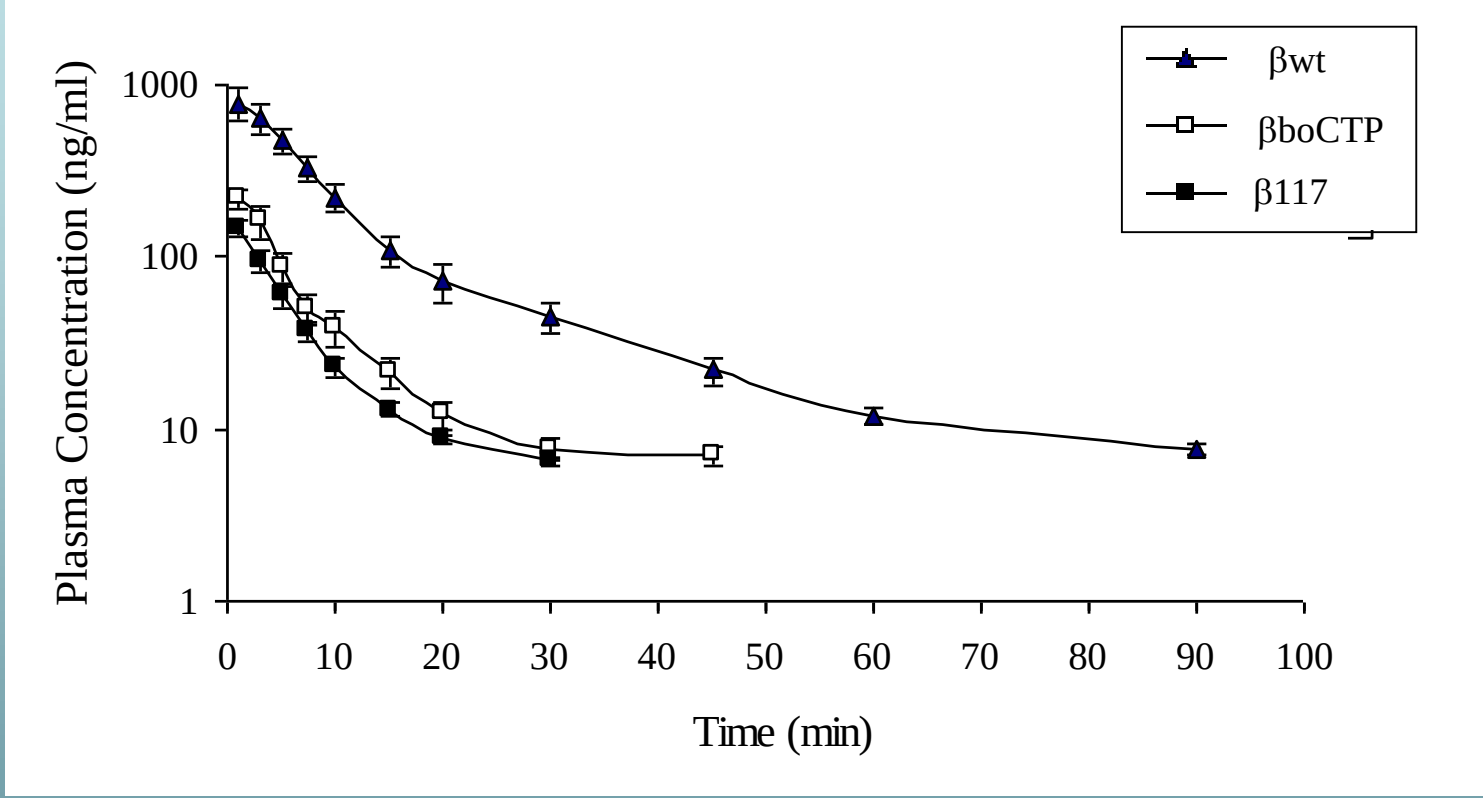
Boime and his colleagues

	Apical (%)	Basolateral (%)
CGβ 	65	35
LHβ 	25	75
CGβ - Odg 	20	80



Nakav et al., 2005

Pharmacokinetics of the CGβboCTP chimera; reduced circulatory survival compared to the WT subunit (that has the natural CTP)



Parameter	βwt	βboCTP	β117
C_0 (ng/ml)	875 ± 200[a]	265 ± 40[b]	185 ± 20[b]
AUC (ng.min/ml)	8125 ± 1360[a]	1560±250[b]	970 ± 80[b]
$t_{1/2}$ (min)	47.2 ± 1.8[a]	24.6 ± 0.7[b]	17.6 ± 1.0[c]

(different letters P<0.01)

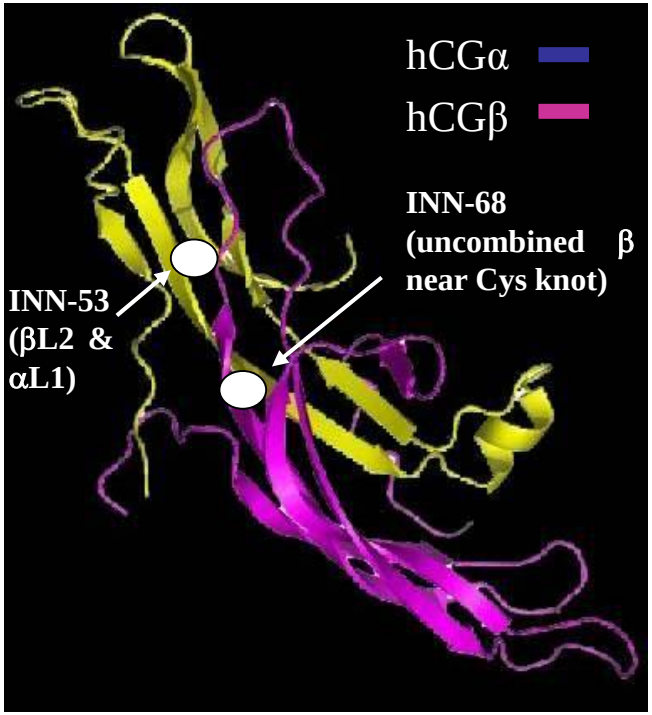
Gabay et al., 2014

Association of the CGβ variants with the human α subunit in transfected CHO cells to form heterodimers; Conformation-sensitive epitopes on heterodimers and monomeric subunit variants

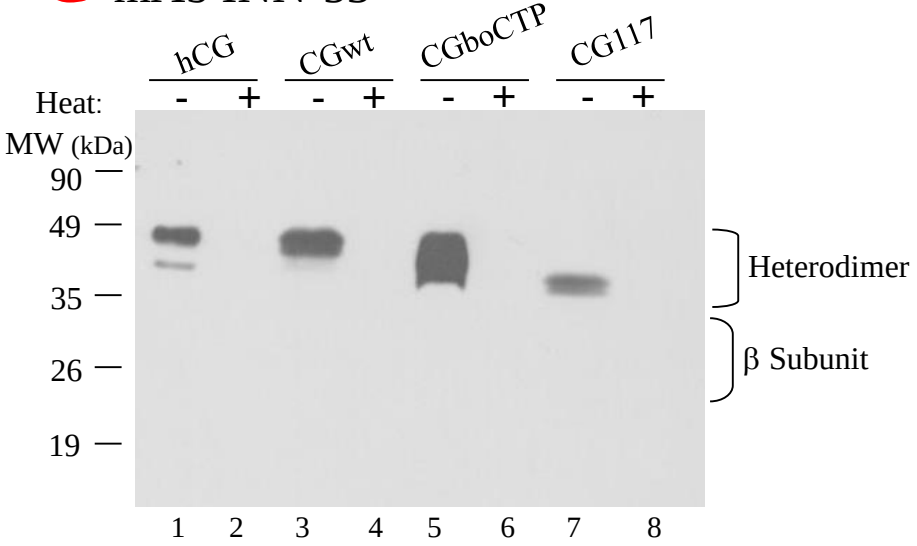
A Heterodimer assembly

CGwt	CGboCT P
55 ±7 %	54±6 %

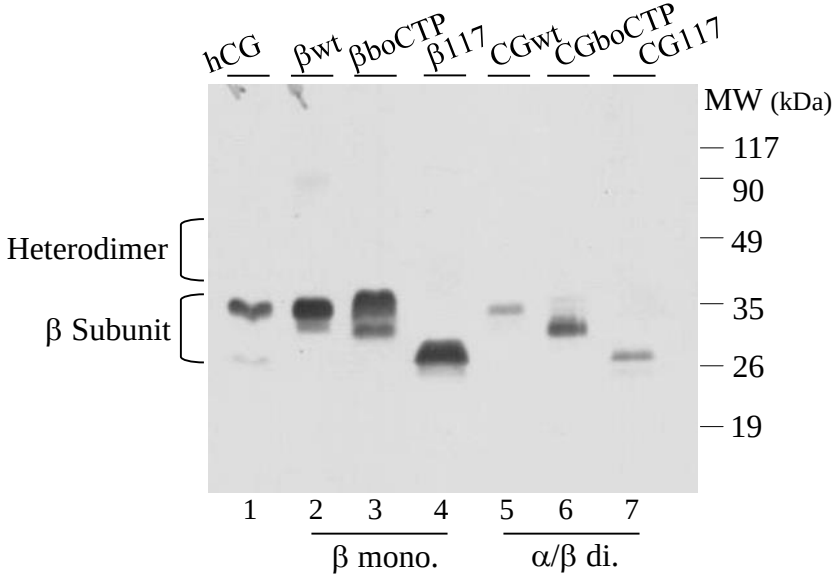
B Heterodimeric-like conformation



C mAb INN-53

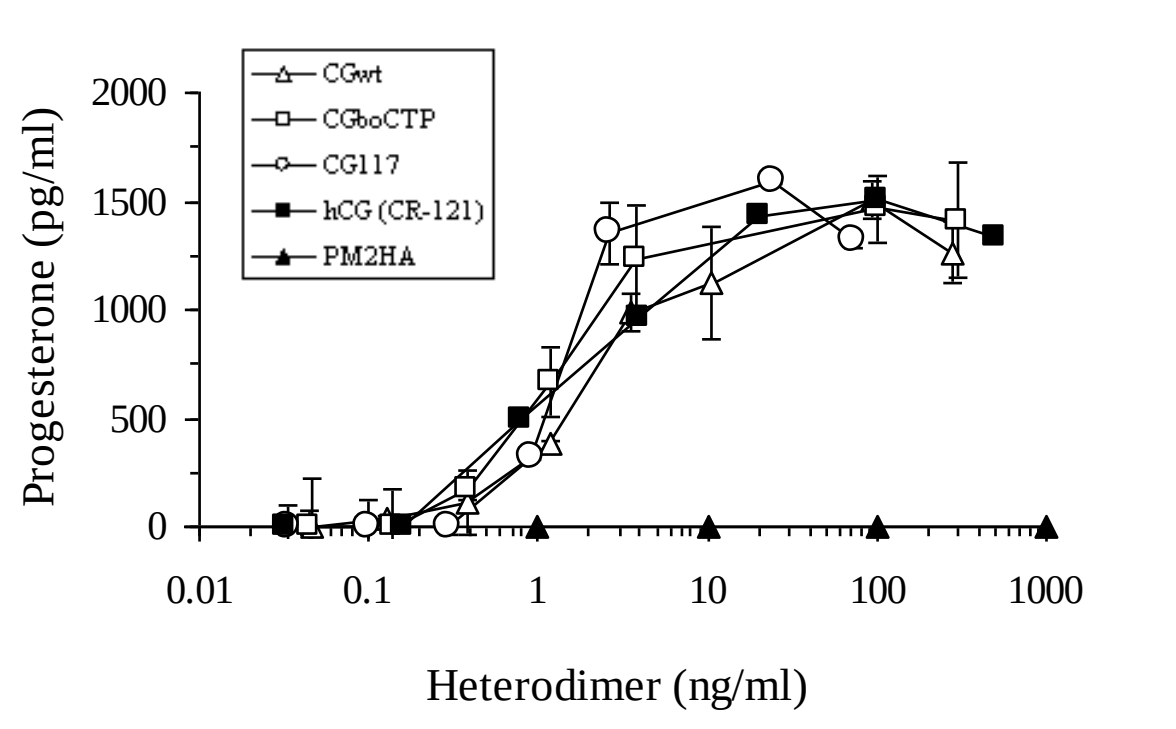


D mAb INN-68



Gabay et al., 2014

Bioactivity of the of the CGboCTP heterodimer; immortalyzed rat granulosa cell bioassay



Heterodimer	**Max. Progesetrone (pg/ml)**	**EC_{50} (ng/ml)**
CGwt	1515 ± 210	1.5 ± 0.5
CGboCTP	1555 ± 205	1.5 ± 0.4
CG117	1570 ± 255	1 ± 0.3

Gabay et al., 2014

How the intracellular behavior of the equine LH/CGβ subunits fulfill the needs for biosynthesis both in the pituitary and placenta?

- A single gene encodes the LHβ and CGβ subunits in equids in these two organs (known in the horse as eLH/CGβ; no CTP lacking lutropic subunit)
- Together with the α subunit, the eLH/CGβ gene is expressed in the pituitary to synthesize eLH and in the placenta to produce eCG (also known as PMSG) as part of reproduction endocrinology in mares
- The pituitary eLHβ and placental eCGβ subunits share the same amino acid composition and both have a O-glycosylated CTP

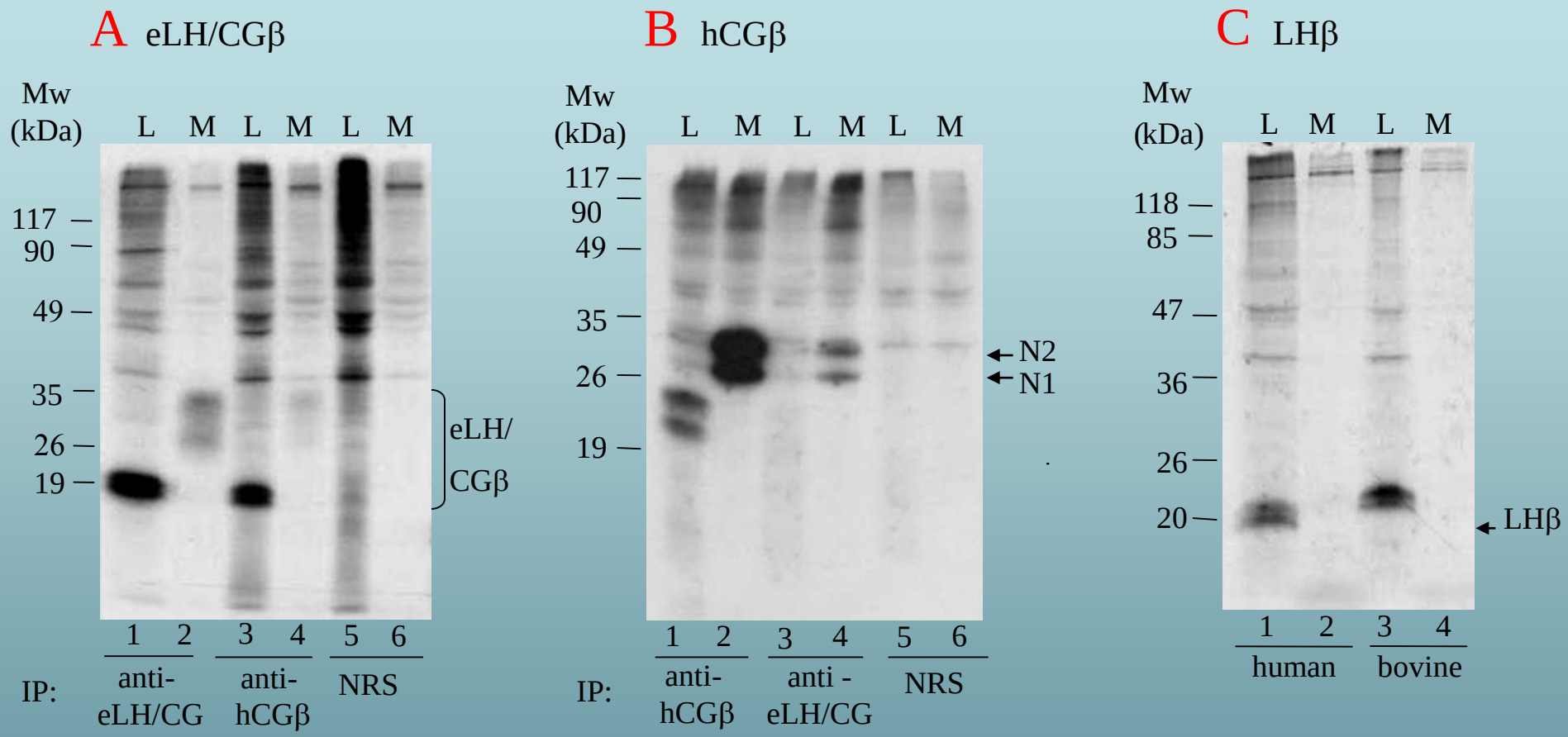
Whether the secretion kinetics and routing of the eLH/CGβ subunit from transfected cells are strictly hLHβ- or hCGβ-like, or combines characteristics of both?

Differences in the intracellular behavior of the human LHβ and CGβ subunits

- In primates, the LHβ and CGβ subunits are products of different genes which are efficiently expressed in the gonadotropes and trophoblasts, respectively
- Despite the similarities between the human LH and CG β subunits, the storage and secretion profiles of the heterodimers differ. Whereas The secretion of the hLHβ subunit is slow and inefficient, that of the hCGβ subunit is fast and quantitative
- Differences in the secretion from MDCK cells (hLHβ- basolateral; hCGβ apical)

Whether the secretion kinetics and routing of the eLH/CGβ subunit from transfected cells are strictly hLHβ- or hCGβ-like, or combines characteristics of both?

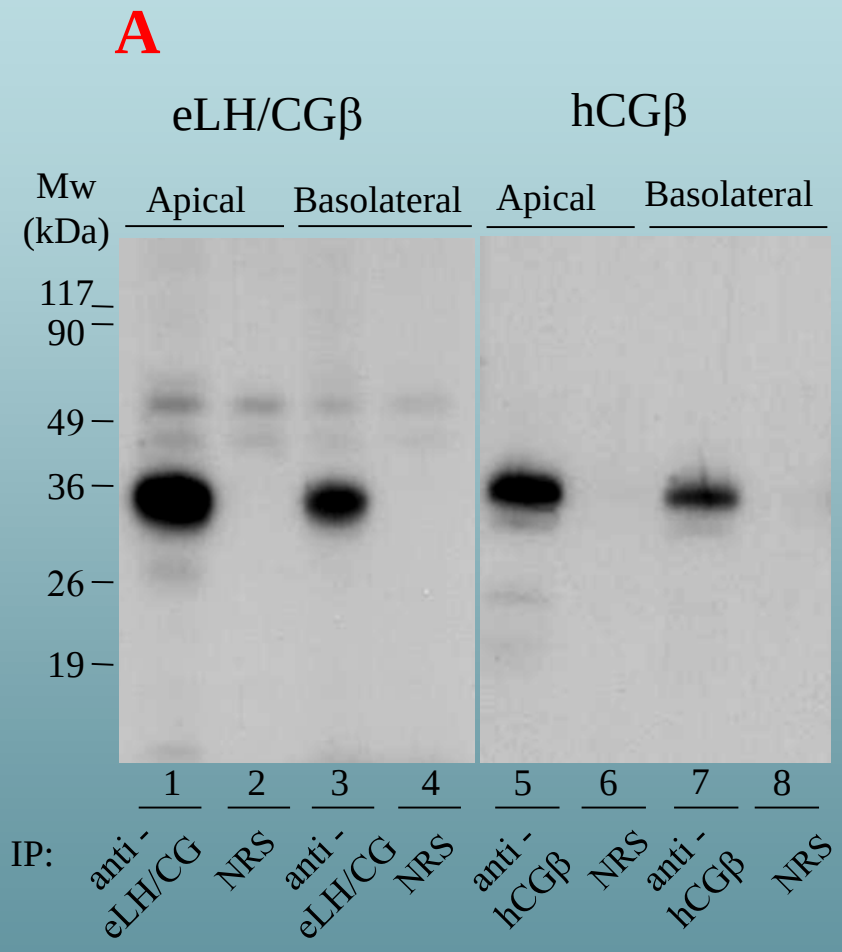
Expression and secretion of the eLH/CGβ, hCGβ and LHβ subunits in transfected CHO cells

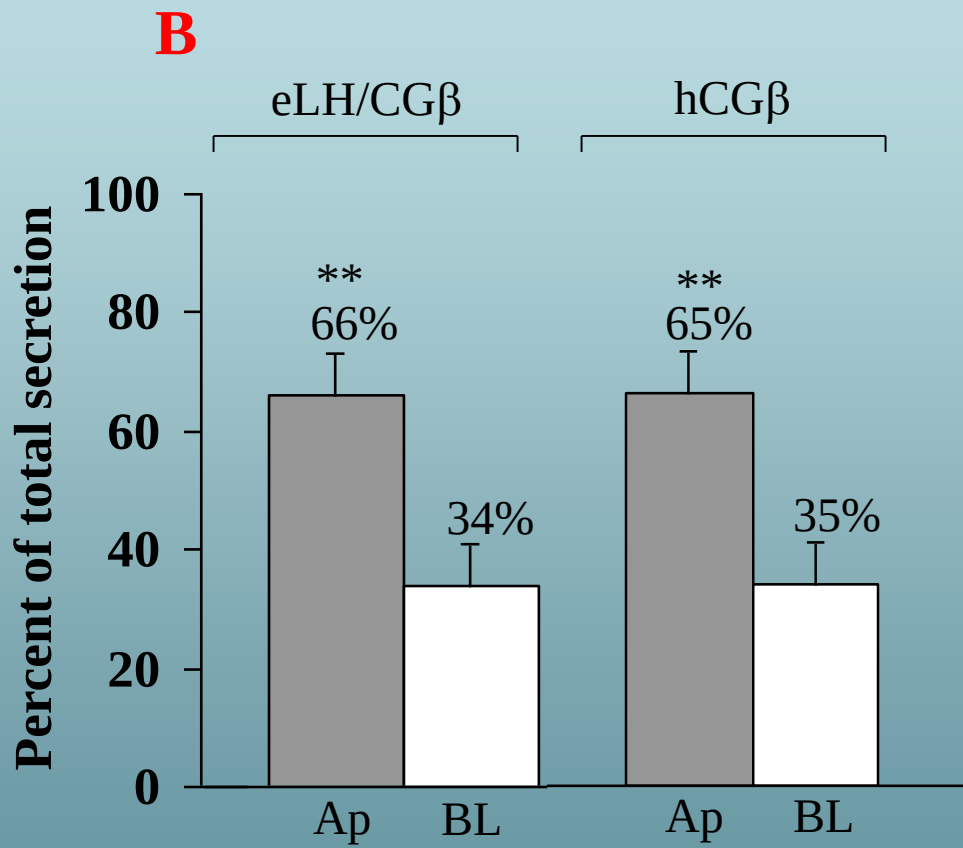


Media Recovery (%):	**25.6** ± 7.0 **MDCK 17.3** ± 4.4	**82.6** ± 6.0 **MDCK 81.6** ± 5.5	**<10%**
Kinetics: (Pulse chase)	$t_{1/2}$ (hr) = **6.6 ± 0.2**	$t_{1/2}$ (hr) = **1.5 ± 0.2**	
	Recovery (%) = **16 ± 2**	Recovery (%) = **63 ± 4**	

Cohen et al., 2015

Apical secretion of the eLH/CGβ and hCGβ subunits from polarized MDCK cells





Cohen et al., 2015

Summary (a): A role of the Carboxy-Terminal-Peptide O-glycosylation in the LHβ to CGβ evolution

•The LHβ to CGβ gene conversion is potentially wide-spread

•When translated, the cryptic boCTP stretch does not prevent crucial aspects of hormone biosynthesis (the assembly of the heterodimer, formation of conformational-sensitive epitopes and the activation of the cognate receptor). However, this domain is missing the set of O-linked glycans and lacks the hallmark function of prolonging the circulatory survival and determinants for apical secretion which are typical to the naturally expressed O-glycosylated CTP domain

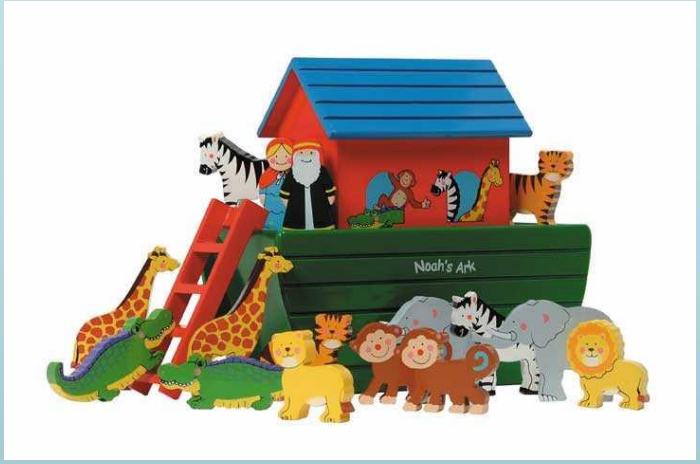
•The absence of extensive O-glycosylation and the associated failure to gain new hormonal properties provides an explanation as to why LH did not evolve into CG in ruminants, and possibly in additional species, that apply different strategies to delay luteolysis at the early stages of gestation

Summary (b): The production of the LH/CGβ subunit in equids

- The equine LH/CGβ subunit combines intracellular traits that diverged in the case of the human LHβ and CGβ subunits

- We propose that the distinguished intracellular behavior of the equine gonadotropin subunit evolved in association with the needs for biosynthesis in the pituitary and placenta

Acknowledgments:



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