

# COMMENTARY

## Duplicate growth hormone genes in sheep and goat

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### INTRODUCTION

In most mammals a single gene codes for pituitary growth hormone (GH) and is not associated with closely-related genes. An exception occurs in higher primates. In man a cluster of five genes on chromosome 17 codes for GH-like proteins; one of these (at the 5' end of the cluster) codes for pituitary GH, while the other four code for genes expressed in the placenta, including two genes for placental lactogen, one for a placental lactogen-like protein, and one for a GH variant (Chen *et al.* 1989). In rhesus monkey, studies on cDNA indicate that there are at least five genes that code for GH-like proteins, one expressed in the pituitary and four in the placenta, although these may not be exactly equivalent to the human GH-like genes (Golos *et al.* 1993). There is also evidence for multiple GH-like genes in a new world monkey (O C Wallis & M Wallis, unpublished observations). These clusters of GH-like genes have presumably arisen as the consequence of duplications of the single GH gene found in most mammals (and other tetrapods), followed by divergent evolution. It is notable that evolution of GH in primates involved a burst of rapid change (contrasting with general conservation of GH structure seen in most mammals) which appears to have occurred before the gene duplications that gave rise to the gene cluster(s) (Wallis 1994, 1996).

Until recently it appeared that duplication of the GH gene in mammals was confined to primates. Placental lactogens are found in some other mammalian groups (ruminants, rodents) but appear to have arisen independently of those found in primates, by duplication of the prolactin gene (Schuler & Kessler 1992, Wallis 1992). However, a number of reports have now appeared indicating that there are duplicate GH genes in at least some

caprine ruminants (goats and sheep). These have interesting implications for the evolution of the GH gene in ruminants.

### DUPLICATION OF GH GENES IN CAPRINE RUMINANTS

On the basis of studies on a restriction fragment length polymorphism (RFLP), Valinsky *et al.* (1990) concluded that there are two alleles at the GH gene locus in sheep and goats. In sheep, in one allele (Gh1), the GH gene is represented by a single copy (*GH1* gene), while in the other (Gh2) the GH gene is duplicated (*GH2-N* (5') and *GH2-Z* (3') genes). Restriction maps of the sheep Gh1 and Gh2 loci indicated that the *GH1*, *GH2-N* and *GH2-Z* genes are all very similar (Fig. 1). The sequences flanking the 5' ends of the *GH1* and *GH2-N* genes are similar, but differ from that flanking the *GH2-Z* gene. Individual animals were homozygous for Gh1 (i.e. possessed 2 GH-like genes) or Gh2 (4 GH-like genes) or heterozygous, with one copy of Gh1 and one of Gh2 (3 GH-like genes). The frequency of the Gh2 allele was greater than that of the Gh1 allele. Subsequently, Gootwine *et al.* (1993) showed that the *GH2-Z* gene contains a PvuII polymorphism in the second intron and Gootwine *et al.* (1996) concluded that, in an animal homozygous for the Gh2 allele, only the *GH2-N* gene was expressed in the sheep pituitary. Sequence differences between the sheep *GH2-N* and *GH2-Z* genes have now been demonstrated by Ofir & Gootwine (1997) (see below). Lacroix *et al.* (1996) have shown that two forms of GH are expressed at low level in the sheep placenta, one of which is probably identical to pituitary GH while the other differs from it in sequence and is probably the product of the *GH2-Z* gene.

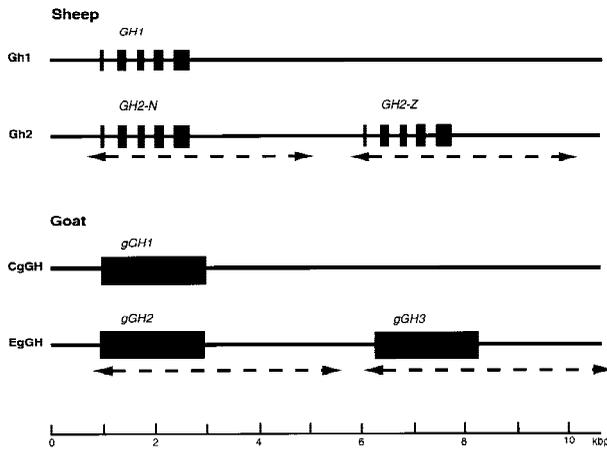


FIGURE 1. Organisation of the growth hormone-related genes in sheep and goat. Gh1 and Gh2 are the two alleles in sheep (based on Valinsky *et al.* 1990), CgGH and EgGH the two in goat (based on Yamano *et al.* 1991). Intron/exon structure is indicated for the sheep genes; it is likely to be very similar for the goat genes, but has not yet been formally demonstrated in each case. The dashed arrows indicate the apparent duplicate regions, based on sequence and restriction mapping (sheep) and restriction mapping and distribution of repetitive sequences (goat).

Yamano *et al.* (1991) investigated GH genes in a goat genomic library and found two types of fragment, one containing a single GH gene (*gGH1*) and the other containing two genes arranged in tandem (*gGH2* and *gGH3*). Again this appears to be due to allelic polymorphism, with the allele containing duplicate GH genes predominating. The tandem arrangement of the *gGH2* and *gGH3* genes is similar to that seen for the ovine *GH2-N* and *GH2-Z* genes (Fig. 1) but it is not yet clear whether the situations in goat and sheep are exactly equivalent.

## SEQUENCE STUDIES

Alignments of the various sheep and goat GH gene sequences discussed below have been prepared and are available on the World Wide Web at the following address: [http://www.biols.susx.ac.uk/Home/Mike\\_Wallis/GHAlign](http://www.biols.susx.ac.uk/Home/Mike_Wallis/GHAlign).

The sequence of cDNA corresponding to sheep pituitary GH was reported by Warwick & Wallis (1984) and subsequently confirmed (Warwick *et al.* 1989, Lacroix *et al.* 1996). The protein sequence predicted from this agrees with the known sequence for sheep GH. Orian *et al.* (1988) presented a sequence for the sheep GH gene; the coding

sequence predicted from this corresponds to the cDNA sequence for pituitary GH, and this gene presumably codes for the hormone expressed in the pituitary. This was confirmed by Ofir & Gootwine (1997) who showed that the sequence of the single *GH1* gene (Gh1 allele) is identical to the sequence described by Orian *et al.* (1988) and that the (partial) sequence of the *GH2-N* gene is also very similar to this, although there is some variation between strains of sheep. Lacroix *et al.* (1996) described sequences for three GH-related cDNAs derived from sheep placenta. One of these, coding for a protein identical to pituitary preGH, corresponds to the product of the *GH1* or *GH2-N* gene. The other two are very similar and code for a protein differing from pituitary preGH at three amino acid residues, which appears to correspond to the product of the *GH2-Z* gene. Byrne *et al.* (1987) provided a rather different sequence for a GH-like gene in sheep, which differs at about 44 nucleotides from the sequence of Orian *et al.* (1988); in some respects this resembles the *GH2-Z* gene, but the match is far from complete. This may reflect variation between sheep strains of the kind noted by Ofir & Gootwine (1997). Neither Orian *et al.* (1988) nor Byrne *et al.* (1987) reported the presence of two GH genes in the gene libraries that they screened. However, it is notable that Southern blotting of DNA from several individual animals led Byrne *et al.* (1987) to detect a GH-linked RFLP very similar to that which led Valinsky *et al.* (1990) to conclude that duplicated GH genes are present in some sheep.

The situation in goat is less clear. The sequence of goat pituitary GH cDNA was reported by Yamano *et al.* (1988) and Yato *et al.* (1988) and the GH gene sequence was described by Kioka *et al.* (1989). The coding sequence predicted from the gene sequence corresponds to the goat GH cDNA sequence and this gene was subsequently shown to be the *gGH1* gene. Sequence information for the *gGH2* and *gGH3* genes (i.e. the duplicate genes in the second allele) is not yet available.

## STRUCTURE-FUNCTION ASPECTS

The sequence of the second GH expressed in the sheep placenta (probably the product of the *GH2-Z* gene), codes for a protein that differs from pituitary GH at three sites, one (Pro→Leu at position -7) in the signal peptide, and two (Gly→Arg at position 9 and Gly→Ser at position 63) in mature GH (Lacroix *et al.* 1996) (the numbering is based on N-terminal Ala as residue 1). The substitution at residue 9 is in the second receptor-binding site on

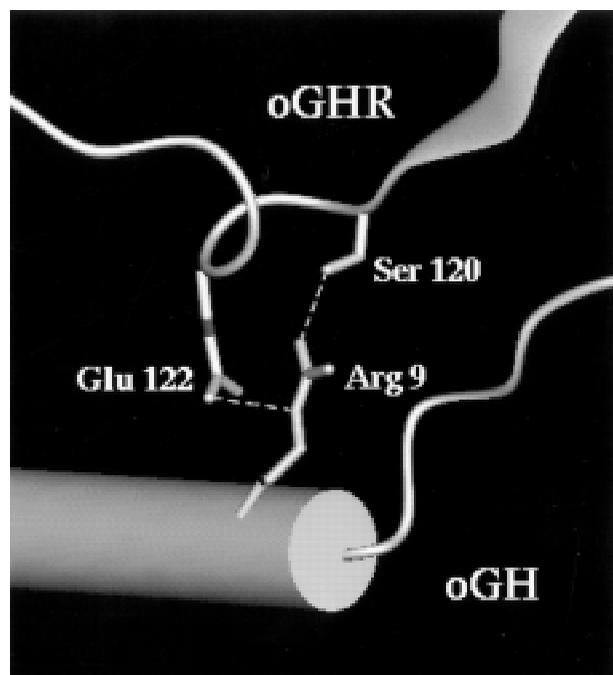


FIGURE 2. Growth hormone–receptor interactions in sheep. A model of part of the site 2 hormone–receptor interface is shown for the complex between the placental ovine GH (oGH) variant and the GH receptor (oGHR). The ovine GH–receptor complex (GH(GHR)<sub>2</sub>) was constructed by homology modelling using the QUANTA software package (Molecular Simulations Inc., San Diego, CA, USA) and the CHARMM field for energy minimisation in a Silicon Graphics computer, and starting coordinates based on the human GH(GHR)<sub>2</sub> complex (de Vos *et al.* 1992). Residues Gly<sup>9</sup> and Gly<sup>63</sup> were then mutated computatively into Arg and Ser respectively for the placental oGH variant, and the side chains of Arg<sup>9</sup> and Ser<sup>63</sup> and neighbouring residues were allowed to adopt optimal conformations after minimisation. In binding site 2, hydrogen bonds are predicted to form (see dashed lines) between Arg<sup>9</sup> (oGH variant) and Glu<sup>122</sup> (oGHR) and between Arg<sup>9</sup> (oGH variant) and Ser<sup>120</sup> (oGHR).

GH as defined by Cunningham *et al.* (1991) for human GH. Conversion of this residue (Arg) in human GH to Ala led to some lowering of receptor binding (Cunningham *et al.* 1991), so the Gly→Arg conversion in the sheep placental GH may lead to enhanced affinity for the receptor at the second site. This is supported by modelling studies (A Lioupis, unpublished observations) on ovine GH – introduction of Arg at position 9 would allow formation of a salt bridge and hydrogen bond to Glu<sup>122</sup> and Ser<sup>120</sup> respectively on the receptor, with enhanced binding (Fig. 2).

Residue 63 (Gly→Ser substitution in the placental GH) is located in binding site 1 in human GH (Cunningham & Wells 1989, de Vos *et al.* 1992). The corresponding residue in human GH is Asn, and conversion of this to Ala (Cunningham & Wells 1989) led to some decrease in receptor binding. The effect of the Gly→Ser substitution is difficult to predict, and modelling studies suggest that it would not be great. However, it should be noted that the substitution at residue 63 might introduce a glycosylation site into the protein (a Ser residue in a serine-rich loop); this would accord with the observation of Lacroix *et al.* (1996) that the second placental GH-like protein that they observed was substantially larger than normal pituitary GH. Introduction of a bulky carbohydrate moiety in the region of binding site 1 might be expected to interfere with receptor binding. Combining this with enhanced binding affinity at binding site 2 might give a GH variant which could form non-productive hormone–receptor complexes – in effect an inhibitor. There is clearly a need for studies on the biological properties of this placental protein.

## EVOLUTIONARY SIGNIFICANCE

How widespread is the GH gene duplication in ruminants? On the whole it seems likely that the duplication occurred during the evolution of the caprine ruminants after divergence of these from other bovid groups. Gene sequences have been reported for GH from ox (Woychik *et al.* 1982, Gordon *et al.* 1983) and red deer (Lioupis *et al.* 1997); in neither case was a second gene isolated, although Southern blotting of restriction digests of bovine genomic DNA did provide some evidence for a second GH-related gene (Woychik *et al.* 1982, Gordon *et al.* 1983). It is notable that the sheep *GH2-N* and *GH2-Z* genes are very similar – much more similar in non-coding sequence than the sheep *GH2-N* (or *GH1*) gene and the bovine GH gene – suggesting that the duplication arose rather recently, although gene conversion may have homogenised the two diverging gene sequences as is thought to have happened in the case of human placental lactogens (Chen *et al.* 1989).

One of the most intriguing aspects of the GH gene duplication in both goat and sheep is that the duplicate genes exist as an allelic polymorphism with the single (presumably ancestral) form; in neither species has the allele corresponding to the duplication completely replaced that corresponding to the single gene. It seems likely that the two GH genes arose as the consequence of a single

gene-duplication event, predating the divergence of sheep and goat. The polymorphism seen in sheep and goat must therefore also have arisen before the divergence of these two species and have been sustained through speciation and during the following 5–7 million years. Such long-lived polymorphism is not completely unprecedented—for example it has been shown that polymorphism of MHC proteins extends across mouse and rat species (Figuroa *et al.* 1988)—but it is certainly unusual. It implies the existence of strong selective forces, presumably involving heterozygote advantage, to maintain it. The nature of such selective forces remains to be elucidated—as yet major physiological differences between sheep of Gh1/Gh1, Gh1/Gh2 and Gh2/Gh2 genotypes have not been detected. Ram lambs with these three genotypes showed no differences with regard to body weight, body composition or most aspects of GH expression, storage or secretion, although subtle differences were found with respect to pituitary GH content and responsiveness to GH releasing hormone (Fleming *et al.* 1997, Gootwine *et al.* 1997). Detailed physiological studies on goat, pregnant sheep and wild animals remain to be reported. Increased understanding of the basis of this GH polymorphism may have relevance both for our understanding of molecular evolution in general and for animal husbandry/production.

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