of a major histocompatibility class I-presented peptide. *Proc. Natl Acad. Sci. USA* **94,** 10850–10855 (1997).
24. Rubin, D. M. *et al.* Identification of the *gal4* suppressor Sug1 as a subunit of the yeast 26S proteasome. *Nature* **379,** 655–657 (1996).
25. Otwinowski, Z. & Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276,** 307–326 (1997).
26. Collaborative Computing Project No. 4.. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr. D* **50,** 760–763 (1994).
27. Navaza, J. AMoRe: an automated package for molecular replacement. *Acta Crystallogr. A* **50,** 157–163 (1994).
28. Jones, T. A., Zou, J.-Y., Cowan, S. W. & Kjeldgaard, M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr. A* **47,** 110–119 (1991).
29. Cowtan, K. & Main, P. Miscellaneous algorithms for density modification. *Acta Crystallogr. D* **54,** 487–493 (1998).
30. Brünger, A. T. *X-PLOR Version 3.843, a System for X-ray Crystallography and NMR* (Yale Univ., New Haven, Connecticut, 1996).

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erratum

Production of gene-targeted sheep by nuclear transfer from cultured somatic cells

K. J. McCreath , J. Howcroft, K. H. S. Campbell, A. Colman, A. E. Schnieke & A. J. Kind

Nature **405,** 1066–1069 (2000).

An incomplete version of Figure 1 was printed in this Letter. The correct Figure is reproduced below. □

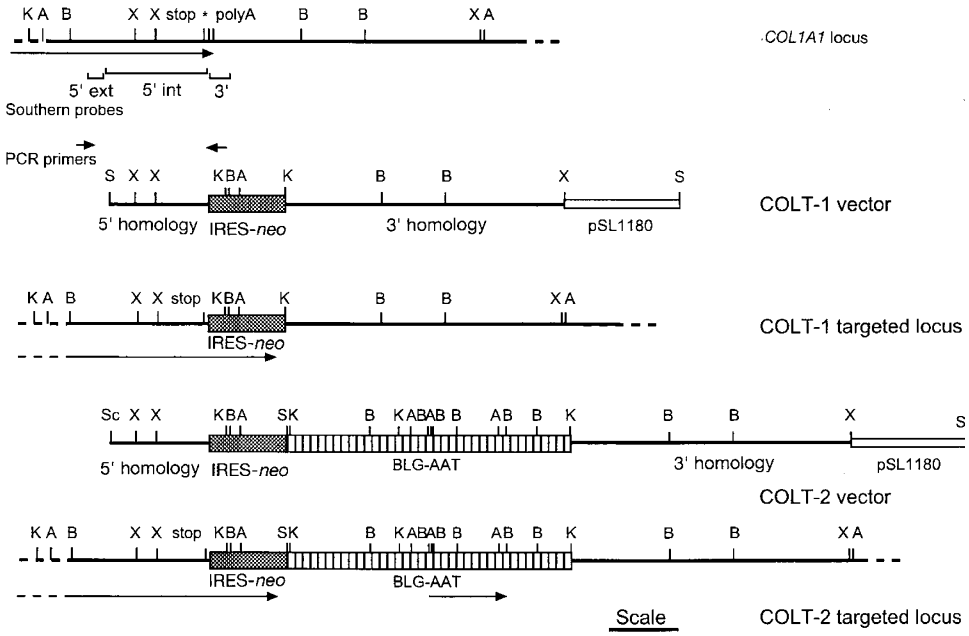

Figure 1 Diagrams of ovine *COL1A1*, COLT-1 and COLT-2 targeting vectors and targeted *COL1A1* locus. The map of ovine *COL1A1* shows the translational stop and polyadenylation sites, the direction of transcription and extent of the *COL1A1* mRNA is indicated by an arrow, an asterisk marks an *Ssp*1 site where the targeted gene insertions were made. Southern hybridization probes and PCR primers are indicated. The maps of the COLT-1 and COLT-2 vectors and the targeted *COL1A1* locus show the IRES–*neo* cassette as a shaded box, the BLG driven AAT transgene by a striped box, and the bacterial vector pSL1180 by an open box. The direction and predicted extent of the *COL1A1* / IRES–*neo* bicistronic mRNA and the AAT transgene mRNA are shown as arrows. The scale bar represents 2 kb. Restriction enzyme sites: A, *Asp*I; B, *Bam*HI; Sc, *Sac*II; S, *Sal*I; K, *Kpn*I; X, *Xho*I.

Production of gene-targeted sheep by nuclear transfer from cultured somatic cells

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It is over a decade since the first demonstration that mouse embryonic stem cells could be used to transfer a predetermined genetic modification to a whole animal[1]. The extension of this technique to other mammalian species, particularly livestock, might bring numerous biomedical benefits, for example, ablation of xenoreactive transplantation antigens, inactivation of genes responsible for neuropathogenic disease and precise placement of transgenes designed to produce proteins for human therapy. Gene targeting has not yet been achieved in mammals other than mice, however, because functional embryonic stem cells have not been derived. Nuclear transfer from cultured somatic cells provides an alternative means of cell-mediated transgenesis[2,3]. Here we describe efficient and reproducible gene targeting in fetal fibroblasts to place a therapeutic transgene at the ovine α1(I) procollagen (*COL1A1*) locus and the production of live sheep by nuclear transfer.

We previously showed that transfection of fetal fibroblasts with nuclear transfer offers an efficient and practical method of producing sheep carrying randomly integrated transgenes[3], and similar work has been reported in cattle[4]. Gene targeting is a more powerful method of genetic manipulation and requires essentially the same procedures of transfection and drug selection of cultured cells. Although experimental gene targeting was first carried out in somatic cell lines[5,6], the use of embryonic stem (ES) cells now predominates; however, there has been no definitive comparison of gene targeting efficiency in primary somatic cells and ES cells (but see ref. 7 for review). We wished to determine whether practically useful gene targeting could be achieved in primary ovine fetal fibroblasts, and whether these cells could produce viable animals by nuclear transfer. The ovine *COL1A1* gene represented a suitable target with which to establish gene targeting in fetal fibroblasts for three reasons. First, we expected that gene-targeting events would be very rare compared with random integrations. *COL1A1* is highly expressed in fibroblasts, allowing promoter-trap enrichment of gene-targeting events. Second, few ovine genes have been cloned and characterized. *COL1A1* is well studied and highly conserved in several species, facilitating molecular cloning and construction of ovine gene-targeting vectors. Third, mutations in *COL1A1* can cause connective tissue disorders in humans, for example, *osteogenesis imperfecta*[8,9]. The ability to generate models of human genetic disorders by gene targeting in animals other than mice might be valuable for clinical research; however, we chose to target a site that would not significantly affect type I collagen protein function or expression to avoid affecting fetal development.

We used two gene-targeting vectors to target ovine *COL1A1* (Fig. 1). Each vector incorporated two regions of *COL1A1* homology, derived from a contiguous fragment of Poll Dorset fetal fibroblast[3] (PDFF2) genomic DNA. COLT-1 was designed to insert a promoterless *neo* selectable marker between the *COL1A1* translational stop and polyadenylation signal, such that transcription of the targeted locus resulted in a bicistronic messenger RNA. An internal ribosomal entry site (IRES)[10] immediately 5′ of *neo* facilitated translation. COLT-2 had the same structure, but included a transgene as a separate transcription unit located 3′ of *neo*. This transgene, termed AATC2, comprised human α1-antitrypsin (AAT) complementary DNA within an ovine β-lactoglobulin (BLG) expression vector designed to direct expression in the lactating mammary gland[11].

We transfected COLT-1 DNA into early passage PDFF2 female and PDFF5 male ovine primary fetal fibroblasts; we transfected COLT-2 DNA into PDFF2 cells. Stable G418 resistant clones

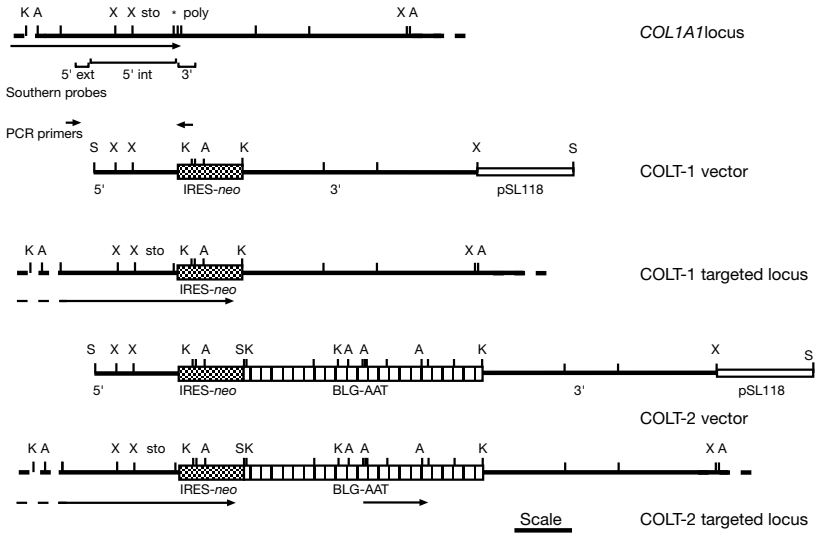

Figure 1 Diagrams of ovine *COL1A1*, COLT-1 and COLT-2 targeting vectors and targeted *COL1A1* locus. The map of ovine *COL1A1* shows the translational stop and polyadenylation sites, the direction of transcription and extent of the *COL1A1* mRNA is indicated by an arrow, an asterisk marks an *Ssp*1 site where the targeted gene insertions were made. Southern hybridization probes and PCR primers are indicated. The maps of the COLT-1 and COLT-2 vectors and the targeted *COL1A1* locus show the IRES–*neo* cassette as a shaded box, the BLG driven AAT transgene by a striped box, and the bacterial vector pSL1180 by an open box. The direction and predicted extent of the *COL1A1* / IRES–*neo* bicistronic mRNA and the AAT transgene mRNA are shown as arrows. The scale bar represents 2 kb. Restriction enzyme sites: A, *Asp*I; B, *Bam*HI; Sc, *Sac*II; S, *Sal*I; K, *Kpn*I; X, *Xho*I.

were derived. About 30 days of culture elapsed between fetal disaggregation and cryopreservation of gene-targeted cell clones. DNA samples of each cell clone were initially screened by polymerase chain reaction (PCR) using primers designed to amplify a 3.4-kilobase (kb) fragment across the 5′ junction of the targeted locus (Fig. 1). In each case, a high proportion of G418 resistant cell clones were found to have undergone gene targeting (5 out of 36 PDFF2 COLT-1, 4 out of 56 PDFF5 COLT-1, and 46 out of 70 PDFF2 COLT-2 cell clones analysed). We called COLT-1 cell clones 'PDCOL' and COLT-2 cell clones 'PDCAAT'. The DNA sequence of PCR products amplified from three PDCOL and two PDCAAT cell clones was determined across the 5′ junction; each was consistent with gene targeting (data not shown).

Figure 2a shows Southern analysis of the 5′ junctions of a series of PDCAAT cell clones. All samples showed the presence of a 7-kb *Bam*HI fragment from the normal *COL1A1* locus. Each clone identified as positive by PCR also showed a diagnostic 4.7-kb *Bam*HI fragment spanning the 5′ junction of the COLT-2 targeted locus. This is consistent with the presence of one targeted and one normal *COL1A1* allele. PDCAAT cell clones 87 and 99 also showed the presence of additional bands, indicating additional integrations of COLT-2.

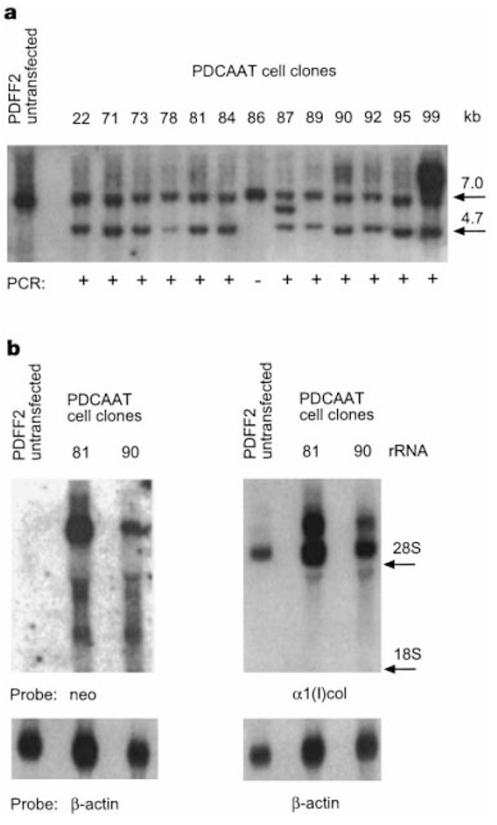

Figure 2 Analysis of COLT-2 transfected (PDCAAT) cell clones. **a**, Southern analysis. Each lane contains *Bam*HI digested genomic DNA from cell samples hybridized with a 3-kb *COL1A1 Sal*I, *Ssp*I fragment corresponding to the 5′ homologous arm of COLT-1 and COLT-2 (see Fig. 1). The cell clone is indicated above each lane and results of the PCR screen are shown below each lane. The positions of the 7-kb *Bam*HI fragment from the non-targeted ovine *COL1A1* locus and the 4.7-kb *Bam*HI fragment from the COLT-2 targeted locus are marked. **b**, Northern analysis. Each lane contains 10 μg total RNA extracted from PDFF2 cells and targeted cell clones PDCAAT81 and PDCAAT90 as indicated. Duplicate blots were hybridized with a *neo* probe (upper left), and full-length human α1(I) procollagen cDNA (upper right). The positions of the 28S and 18S rRNA bands are indicated. The lower two autoradiographs show rehybridization of the same blots with mouse β-actin cDNA as an indication of the amount of total RNA loaded.

PDFF5 cells were also targeted at high frequency with COLT-1. PDFF2 and PDFF5 cells have different parentage from within the PPL outbred flock of Poll Dorset sheep. This indicates that it may not always be essential to isolate DNA from the same individual, or an animal of the same inbred strain, to achieve efficient gene targeting[12]; however, the degree of sequence divergence, if any, between the targeted *COL1A1* alleles in these cells has not yet been determined.

Northern analysis of cell clones PDCAAT 81 and 90 is shown in Fig. 2b. Hybridization with human α1(I) procollagen cDNA detected a 4.8-kb mRNA species in both non-transfected cells and PDCAAT cell clones, consistent with expression of normal *COL1A1*. A larger species of about 6.8 kb was present only in the PDCAAT cell clones, consistent with a bicistronic *COL1A1*–IRES–*neo* fusion mRNA. Hybridization of the same RNA samples with a *neo* probe also detected a 6.8-kb mRNA in the targeted clones, again consistent with a bicistronic mRNA. These results confirmed that gene targeting had occurred. This analysis also showed that, unlike mouse, rat and human, which express two endogenous α1(I) procollagen mRNA species from different polyadenylation sites[13,14], sheep express a single mRNA species.

Although these experiments were designed to avoid disruption of *COL1A1* gene expression, the mRNA from the targeted locus is less abundant than the wild type (Fig. 2b). Whether this reflects different mRNA stability or transcriptional activity has yet to be determined. However, elements which affect transcription have been identified at the 3′ end of *COL1A1* in other species[15,16], and targeted DNA insertion may affect their function.

We carried out northern analysis to determine whether placement of the AATC2 transgene adjacent to the highly expressed *COL1A1* gene resulted in aberrant expression of the BLG promoter in fibroblasts. Hybridization with human AAT cDNA failed to detect AAT mRNA expression in either PDCAAT81 or 90 cells (data not shown), indicating no apparent loss of BLG promoter specificity.

Four targeted cell clones, all derived from PDFF2 female cells (PDCOL6, PDCOL13, PDCAAT81 and PDCAAT90), were selected for nuclear transfer on the basis of their vigour and normal metaphase chromosome number. Nuclear transfer was carried out on twelve occasions and the results are summarized in Table 1. Fourteen lambs were live-born: seven died within 30 hours of birth, and one each after 3 days, 8 days, 7.5 weeks and 12 weeks. Three lambs are currently alive and thriving at almost one year of age. The first two live-born lambs are shown in Fig. 3.

Post mortem examination of lambs that died *in utero* or after birth revealed a range of abnormalities. Although there was no consistent pattern, we observed a high incidence of kidney defects (frequently renal pelvis dilation), liver and brain pathology. These findings are similar to a previous nuclear transfer study using the same cells[3],

Table 1 Summary of nuclear transfer results

Nuclear donor cells	PDCOL 6	PDCOL 13	PDCAAT 81	PDCAAT 90
Reconstructed embryos	109	154	71	83
Embryos recovered from temporary recipient	104	149	62	78
Embryos developed to morula or blastocyst	14	43	4	19
Embryos transferred to final recipients	14	43	4	19
Final recipients	8	22	2	10
Fetuses at day 60	5*	10*	2	3*
Live-born lambs	4	8	0	2
Lambs alive beyond 1 week	2	3	0	1
Lambs alive beyond 6 months (lamb ID)	1 (ID 990502)	1 (ID 990504)	0	1 (ID 990507)

*One twin pregnancy.

and are therefore probably due to some aspect of the cell treatment or nuclear transfer procedure and not a consequence of gene targeting *per se*. Several researchers have reported developmental abnormalities associated with somatic cell nuclear transfer[4,17,18]. Although there is some indication that inappropriate expression of imprinted genes may be involved[19], definitive investigations have yet to be carried out. Understanding and rectifying this problem is a continuing priority.

Figure 4 shows Southern analysis of 5′ and 3′ junctions of the *COL1A1* targeted locus in representative nuclear transfer lambs. Hybridization with the same 5′ and 3′ probes used to analyse PDCAAT cell clones revealed fragments consistent with the presence of one targeted and one normal *COL1A1* allele in the two lambs shown. This was confirmed using a 5′ probe external to the vector.

Sixteen lambs and fetuses were analysed by Southern blot, of which fifteen confirmed the presence of a targeted allele. One lamb (990504), derived from PDCOL13, showed only the normal *COL1A1* gene, which probably indicates that the cell isolate was oligoclonal. Non-transfected cells have been detected within some G418 selected cell isolates in previous experiments (unpublished data).

Lamb 990507 derived from clone PDCAAT90 was hormonally induced to lactate and milk samples analysed by western blotting (data not shown). AAT was detected at a concentration of 650 μg ml^{-1}, which compares favourably with the highest level previously reported for an AAT cDNA transgene in sheep carrying multiple random gene inserts (18 μg ml^{-1})[20]. This indicates that the *COL1A1* locus supports transgene expression even though it is not actively expressed in mammary epithelium[21].

We have shown that gene targeting can be carried out efficiently in somatic cells and that viable animals can be produced by nuclear transfer. We have also obtained preliminary data (that is, PCR fragment size and sequence) indicating similarly efficient targeting at the α-1,3-galactosyl-transferase locus in porcine fibroblasts (unpublished data). Notably, the use of nuclear transfer does not require embryonic stem or embryonic germ cells, and circumvents the generation of chimaeric animals, which would be costly and time consuming in livestock. Fibroblasts are also being used in clinical trials to provide a protein production system after *ex vivo* gene therapy in human patients[22], and it has been suggested that the introduction of therapeutic transgenes by homologous recombination could avoid undesirable effects arising from random integration[23]. Nuclear transfer in animals such as sheep provides a rigorous means of testing the suitability of specific loci for transgene placement. If the *COL1A1*-targeted sheep continue to show no locus-related deleterious effects, this would indicate that this target locus may provide a permissive and benign environment for the insertion of therapeutically useful genes. □

Figure 3 Gene-targeted lambs. Lambs 990502 and 990503, both derived by transfer of nuclei from gene-targeted cell clone PDCOL6.

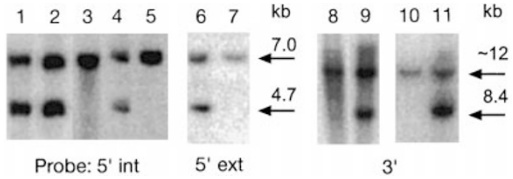

Figure 4 Southern analysis of nuclear transfer lambs. Lanes 1—7 show *Bam*HI-digested genomic DNA samples hybridized with 5′ internal and external probes as indicated. Arrows indicate the 7-kb *Bam*HI fragment from the non-targeted ovine *COL1A1* locus, and the 4.7-kb fragment from the COLT-1 and COLT-2 targeted locus. Lanes 8–11 show *Kpn*I, *Asp*I double-digested genomic DNA samples hybridized with the 3′ probe. Arrows indicate the ~12-kb *Asp*I fragment from the non-targeted locus and the 8.4-kb *Kpn*I, *Asp*I fragment from the COLT-1 and COLT-2 targeted locus. Lane 1, nuclear transfer (n.t.) lamb from cell clone PDCOL6; lane 2, n.t. lamb from cell clone PDCAAT90; lane 3, normal lamb; lane 4, cell clone PDCAAT90; lanes 5, 7 and 8, non-targeted PDFF2 cells; lane 6, n.t. lamb from cell clone PDCOL6; lane 9, cell clone PDCAAT90; lane 10, normal lamb; lane 11, n.t lamb from cell clone PDCOL6.

Methods

Gene-targeting vectors

The promoter trap vector COLT-1 comprised a 3-kb region of the 3′ end of the ovine *COL1A1* gene from a point roughly 2.9-kb 5′ of the translation stop site to an *Ssp*I site 131-bp 3′ of the stop site; a 0.6-kb IRES region[10] corresponding to bases 1,247–1,856 of the pIREShyg vector (Clontech); a 1.7-kb region containing the bacterial *neomycin* gene and a portion of the 3′ end of the human growth hormone gene containing the polyadenylation site, essentially as described[24]; an 8.3-kb region of the 3′ end and flanking region of the ovine *COL1A1* gene from an *Ssp*I site 131-bp 3′ of the translational stop site to a *Xho*I site roughly 8.4-kb 3′ of the stop site; and the bacterial cloning vector pSL1180 (Pharmacia). DNA fragments homologous to the ovine *COL1A1* gene were derived from a single genomic clone isolated from a library of genomic fragments of PDFF2 in bacteriophage λ. The promoter trap transgene placement vector COLT-2 was constructed by inserting an *Mlu*I fragment containing the AATC2 transgene into COLT-1 at a unique *Eco*RV site at the 3′ end of the IRES–*neo* region.

Preparation, culture and transfection of primary fibroblasts

Derivation of ovine PDFF2 and PDFF5 cells has been described[3]. PDFF cells were grown throughout in BHK 21 (Glasgow MEM) medium supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 1X non-essential amino acids and 10% fetal calf serum in standard tissue-culture vessels in a humidified atmosphere composed of 2% CO_2, 5% O_2 and 93% N_2, and were passaged by standard trypsinization. PDFF cells were used at passage three, and had undergone 5–6 days of culture following fetal disaggregation. Cells were plated at 5×10^5 cells in a 25-cm^2 flask and transfected the next day with either 6 μg of *Sal*I linearized COLT-1 or *Sac*II linearized COLT-2, using lipofectAMINE (Gibco, BRL Life Technologies) according to the manufacturer's guidelines. G418 selection (0.8 mg ml^{-1}) was applied 48 h after transfection. On average ~200 G418^r colonies were derived per 5×10^5 cells transfected. Well separated G418^r colonies were isolated, expanded and cryopreserved by standard procedures.

Nuclear transfer

Ovine cell clones were prepared for nuclear transfer by culture in medium containing reduced (0.5%) serum for either 2 or 4 days. Transfer of cell nuclei into Poll Dorset oocytes was carried out essentially as described[3].

Nucleic acid analysis

Putative targeted cell clones were screened by PCR amplification across the 5′ short arm of homology. The position of PCR primers is shown in Fig. 1. Samples of cell clones for screening were lysed in PCR lysis buffer (50 mM KCl, 1.5 mM $MgCl_2$,10 mM Tris-HCl pH 8.5, 0.5% Nonidet P40, 0.5% Tween, 400 μg ml^{-1} Proteinase K) at 65 °C for 30 min. Proteinase K was inactivated at 95 °C for 10 min, and PCR amplification was performed using the 'Expand long template PCR system' (Boehringer) according to the manufacturer's recommended conditions. PCR primer sequences were: COLTPCR4 primer, 5′-GGTTTGTTCCCAGGTGCTCA-3′; COLTPCR8 primer, 5′-GACCTTG-CATTCCTTTG GCGAGAG-3′. Thermal cycling conditions were: 94 °C, 2 min; 10 cycles of 94 °C, 10 s, 55 °C, 30 s, 68 °C, 2 min; 20 cycles of 94 °C, 10 s, 60 °C, 30 s, 68 °C, 2 min + 20 s per cycle; followed by 68 °C, 7 min. PCR products were analysed by agarose gel electrophoresis.

Ovine genomic DNA was prepared from cell pellets, tail samples of live lambs and umbilical cord samples of dead lambs. Southern analysis was carried out by standard procedures. Three hybridization probes were used: a 5′ internal probe, a 3-kb *COL1A1 Sal*I, *Ssp*I fragment corresponding to the 5′ homologous arm of COLT-1 and 2 (Fig. 1); a 5′ external probe, a 520-bp ovine *COL1A1* fragment directly adjacent to but outside the 5′ homologous arm; a 3′ internal probe, a 0.7-kb *COL1A1 Sal*I, *Pst*I fragment immediately 3′ of the integration site (Fig. 1). The diagnostic fragments detected were: a 4.7-kb *Bam*HI fragment extending across the 5′ junction of the targeted locus from a *Bam*HI site within the IRES–*neo* region to a *Bam*HI site in the *COL1A1* gene 5′ of the region contained in the vector; a 8.4-kb *Kpn*I, *Asp*I fragment extending across the 3′ junction of the targeted locus from a *Kpn*I site within the vector to an *Asp*I site in the *COL1A1* gene flank 3′ of the region contained in the vector.

Hormonal induction of lactation

Milk samples were obtained from immature ewes by hormonal induction of lactation, essentially as described[25].

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1. Thompson, S., Clarke, A. R., Pow, A. M., Hooper, M. L. & Melton, D. W. Germ line transmission and expression of a corrected HPRT gene produced by gene targeting in embryonic stem cells. *Cell* **56,** 313–321 (1989).
2. Campbell, K. H. S., McWhir, J., Ritchie, W. A. & Wilmut, I. Sheep cloned by nuclear transfer from a cultured cell line. *Nature* **389,** 64–66 (1996).
3. Schnieke, A. S. *et al.* Human Factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. *Science* **278,** 2130–2133 (1997).
4. Cibelli, J. B. *et al.* Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* **280,** 1256–1258 (1998).
5. Lin, F. L., Sperle, K. & Sternberg, N. Recombination in mouse L cells between DNA introduced into cells and homologous chromosomal sequences. *Proc. Natl Acad. Sci. USA* **82,** 1391–1395 (1985).
6. Smithies, O., Gregg, R. G., Boggs, S. S., Koralewski, M. A. & Kucherlapati, R. S. Insertion of DNA sequences into the human chromosomal β-globin locus by homologous recombination. *Nature* **317,** 230–234 (1985).
7. Yanez, R. J. & Porter, A. C. Therapeutic gene targeting. *Gene Ther.* **5,** 149–159 (1998).
8. Prockop, D. J. & Kivirikko, K. I. Collagens: molecular biology, diseases, and potentials for therapy. *Annu. Rev. Biochem.* **64,** 403–434 (1995).
9. Stacey, A. *et al.* Perinatal lethal osteogenesis imperfecta in transgenic mice bearing an engineered mutant pro-alpha 1(I) collagen gene. *Nature* **332,** 131–136 (1988).
10. Jang, S. K. *et al.* A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* **62,** 2636–2643 (1988).
11. Carver, A. S. *et al.* Transgenic livestock as bioreactors: stable expression of human alpha-1-antitrypsin by a flock of sheep. *Bio/Technology* **11,** 1263–1270 (1993).
12. Sedivy, J. M. & Dutriaux, A. Gene targeting and somatic cell genetics: a rebirth or coming of age? *Trends Genet.* **15,** 88–90 (1999).
13. Mooslehner, K. & Harbers, K. Two mRNAs of mouse pro a1(I) collagen differ in the size of the 3′-untranslated region. *Nucleic Acids Res.* **16,** 773 (1988).
14. Chu, M. L., de Wet, W., Bernard, M. & Ramirez, F. Fine structural analysis of the human pro-alpha 1 (I) collagen gene. Promoter structure, AluI repeats, and polymorphic transcripts. *J. Biol. Chem.* **260,** 2315–2320 (1985).
15. Rippe, R. A., Umezawa, A., Kimball, J. P., Breindl, M. & Brenner, D. A. Binding of upstream stimulatory factor to an E-box in the 3′-flanking region stimulates alpha1(I) collagen gene transcription. *J. Biol. Chem.* **272,** 1753–1760 (1998).
16. Määttä, A., Ekholm, E. & Penttinen, R. P. Effect of the 3′-untranslated region on the expression levels and mRNA stability of alpha 1(I) collagen gene. *Biochim. Biophys. Acta* **1260,** 294–300 (1995).
17. Renard, J. P. *et al.* Lymphoid hypoplasia and somatic cloning. *Lancet* **353,** 1489–1491 (1999).
18. Hill, J. R. *et al.* Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology* **51,** 1451–1465 (1999).
19. Kono, T. Influence of epigenetic changes during oocyte growth on nuclear reprogramming after nuclear transfer. *Reprod. Fertil. Dev.* **10,** 593–598 (1998).
20. McClenaghan, M. *et al.* Production of human α_1-antitrypsin in the milk of transgenic sheep and mice targeting expression of cDNA sequences to the mammary gland. *Anim. Biotechnol.* **2,** 161–176 (1991).
21. Warburton, M. J., Kimbell, R., Rudland, P. S., Ferns, S. A. & Barraclough, R. Control of type IV collagen production in rat mammary epithelial and myoepithelial-like cells. *J. Cell. Physiol.* **128,** 76–84 (1986).
22. Kay, M. A. & High, K. Gene therapy for the hemophilias. *Proc. Natl Acad. Sci. USA* **96,** 9973–9975 (1999).
23. Lai, L-W. & Lien, Y-H. Homologous recombination based gene therapy. *Exp. Nephrol.* **7,** 11–14 (1999).
24. McWhir, J. *et al.* Selective ablation of differentiated cells permits isolation of embryonic stem cell lines from murine embryos with a non-permissive genetic background. *Nat. Genet.* **14,** 223–226 (1996).
25. Ebert, K. D. *et al.* Induction of human tissue plasminogen activator in the mammary gland of transgenic goats. *Bio/Technology* **12,** 699–702 (1994).

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Gigantism in mice lacking suppressor of cytokine signalling-2

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Suppressor of cytokine signalling-2 (SOCS-2) is a member of the suppressor of cytokine signalling family, a group of related proteins implicated in the negative regulation of cytokine action through inhibition of the Janus kinase (JAK) signal transducers and activators of transcription (STAT) signal-transduction pathway[1]. Here we use mice unable to express SOCS-2 to examine its function *in vivo*. SOCS-2$^{-/-}$ mice grew significantly larger than their wild-type littermates. Increased body weight became evident after weaning and was associated with significantly increased long bone lengths and the proportionate enlargement of most organs. Characteristics of deregulated growth hormone and insulin-like growth factor-I (IGF-I) signalling, including decreased production of major urinary protein, increased local IGF-I production, and collagen accumulation in the dermis, were observed in SOCS-2-deficient mice, indicating that SOCS-2 may have an essential negative regulatory role in the growth hormone/IGF-I pathway.

We isolated genomic clones corresponding to three independent loci from two murine libraries using a SOCS-2 coding region complementary DNA as hybridization probe. Comparison of sequence from these clones with that of the SOCS-2 cDNA revealed that one locus, which consisted of three exons and two introns, encoded the predicted SOCS-2 RNA (Fig. 1a). The two other loci

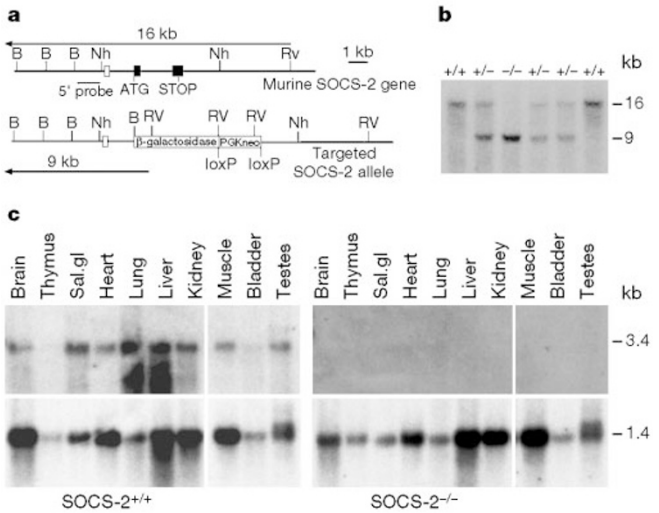

Figure 1 Disruption of the SOCS-2 locus by homologous recombination. **a**, The functional murine SOCS-2 gene (B, *Bam*HI; Nh, *Nhe*I; RV, *Eco*RV) with the exons containing the coding region as shaded boxes. In the targeted allele, the entire SOCS-2 coding region was replaced by a β-gal-PGKneo cassette in which the β-galactosidase coding region was fused to the SOCS-2 initiation codon. **b**, Southern blot of *Eco*RV-digested genomic DNA from the tails of mice derived from a cross between SOCS-2$^{+/-}$ mice. The blot was hybridized with the 5′ genomic SOCS-2 probe, which distinguishes between endogenous (16 kb) and mutant SOCS-2 (9 kb) alleles. **c**, Northern blot showing lack of SOCS-2 expression in organs of SOCS-2$^{-/-}$ mice. Top, the blot was hybridized with a coding region probe, which detects the 3.4-kb SOCS-2 transcript[1]; bottom, the integrity of the RNA was confirmed by hybridization with GAPDH (1.4-kb transcript). Sal gl, salivary gland.

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erratum

Production of gene-targeted sheep by nuclear transfer from cultured somatic cells

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An incomplete version of Figure 1 was printed in this Letter. The correct Figure is reproduced below. □

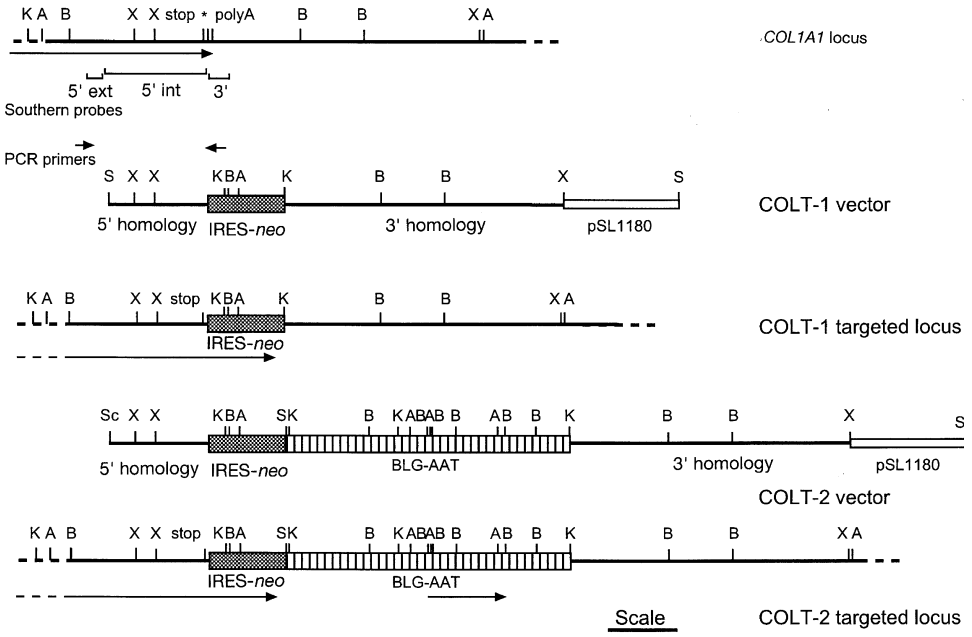

Figure 1 Diagrams of ovine *COL1A1*, COLT-1 and COLT-2 targeting vectors and targeted *COL1A1* locus. The map of ovine *COL1A1* shows the translational stop and polyadenylation sites, the direction of transcription and extent of the *COL1A1* mRNA is indicated by an arrow, an asterisk marks an *Ssp*1 site where the targeted gene insertions were made. Southern hybridization probes and PCR primers are indicated. The maps of the COLT-1 and COLT-2 vectors and the targeted *COL1A1* locus show the IRES–*neo* cassette as a shaded box, the BLG driven AAT transgene by a striped box, and the bacterial vector pSL1180 by an open box. The direction and predicted extent of the *COL1A1* / IRES–*neo* bicistronic mRNA and the AAT transgene mRNA are shown as arrows. The scale bar represents 2 kb. Restriction enzyme sites: A, *Asp*I; B, *Bam*HI; Sc, *Sac*II; S, *Sal*I; K, *Kpn*I; X, *Xho*I.