BOVINE ORNITHINE DECARBOXYLASE (ODC): 
cDNA CLONING AND DNA POLYMORPHISMS

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SUMMARY
A cDNA coding for ornithine decarboxylase was isolated from a bovine liver cDNA library. The clone (1760 bp) consisted of 5'- and 3'-untranslated regions of 187 nucleotides each and an open reading frame of 1383 nucleotides encoding an ODC protein (Mr. 51,342 daltons) of 461 amino acids. Comparison of the nucleotide and the predicted amino acid of the cDNA to other mammalian ODCs showed a very high degree of homology both at the DNA and protein levels. The bovine ODC mRNA was identified by Northern blot to be a single species with a molecular size of 2.35 kb. Primer extension analysis indicated that the 5'-untranslated region of the bovine ODC mRNA was 314 nucleotides long. Southern blot analysis of bovine genomic DNA revealed restriction fragment length polymorphisms when cleaved with restriction enzymes PstI, MspI, TaqI and BglII.

INTRODUCTION
The polyamines, putrescine, spermidine, and spermine, are low molecular weight polycations that have been shown to exert an extraordinary degree of control over the growth, development and division of all cells (Pegg, 1986). Ornithine decarboxylase (ODC; EC 4.1.1.17) catalyses the conversion of ornithine to putrescine, the first and rate limiting step in polyamine biosynthesis. The level of ODC activity is close to zero in quiescent, nonproliferating cells, but is readily induced by a wide variety of trophic stimuli (Pegg and McCann, 1982; Pegg, 1986). The cDNA and the structural gene for ODC have been cloned from several mammalian species including human (Hickok et al., 1987; 1990) rat (van Kranen et al., 1987; Wen et al., 1989) and mouse (Kahana and Nathans 1985; Katz and Kahana, 1988). As revealed by sequence analysis, the coding sequence is highly conserved, as is the general arrangement of introns and exons in the gene.

Studies on mammalian ODCs so far have been mainly confined to experimental animals and humans and very little is known about the characteristics and regulation of the enzyme in domestic animals such as cattle. In some species, ODC has been implicated as a candidate gene which is potentially involved in increased growth rates of animals (Bulfield et al., 1988; Gray and Tait, 1993). Similarly, in dairy cattle, genetic variants of ODC might be associated with increased mammary gland activity or interact with trophic hormones during lactation. As a first step in characterizing ODC at the molecular level, we have cloned a bovine ODC cDNA and shown it to be highly polymorphic in Holsteins.

MATERIALS AND METHODS
A cDNA library was constructed from mRNA isolated from the liver of a Holstein cow using the lambda ZAP II cloning kit according to the manufacturer’s protocol (Stratagene). Approximately, 500,000 plaques were screened using radiolabelled chicken ODC cDNA (Zhang et al., 1992). Four positive plaques were isolated. Following phagemid recovery, strands were partially sequenced using a T7 sequencing kit (Pharmacia). Preliminary sequence analysis of the 5' and 3' ends of these clones revealed that three of them were truncated cDNA products but otherwise identical to the largest clone (pY1). To facilitate sequence analysis, the cDNA insert (1760 bp) from pY1 was subcloned into pUC18 following digestion with HindIII, HincII, Aval and MboII to generate overlapping fragments and the sequence for both + and - strands was completely determined.
For Northern blot analysis, 20 µg of total RNA isolated from cultured bovine mammary epithelial cells (Huynh et al., 1991) were separated on a denaturing gel, transferred to Zeta-probe membrane (BIO-RAD) and hybridized with the radiolabelled bovine ODC cDNA (pY11).

The transcription start site was estimated by primer extension using a synthetic oligonucleotide primer (21-mers; 5'-AAACACACAATCTGCTGTCTC-3') complementary to nucleotide position -180 to -160 (the A of the AUG translational initial codon is designated number 1) of the ODC mRNA. The primer was end-labelled, hybridized to 12 µg of total RNA and reverse transcribed as described (Sambrook et al., 1989). The extended products were fractionated on a 7 M urea/8 % polyacrylamide sequencing gel and visualized by autoradiography.

For Southern blot analysis, high molecular weight genomic DNA was isolated from bovine leucocytes and digested with various restriction enzymes (Pharmacia). The digested DNA (5 µg) was separated by electrophoresis in a 1 % agarose gel and transferred overnight to Zeta-probe membrane (BIO-RAD). The cDNA insert from the bovine ODC cDNA clone (pY11) was 32P-labelled by random primer extension. Hybridization and autoradiography were performed as described (Kuhnlein et al., 1989).

RESULTS

The sequence of bovine ODC cDNA (GenBank and EMBL Accession number M92441) covered 1760 nucleotides consisting of 5'- and 3'-untranslated regions (UTR) of 187 nucleotides each and a coding region of 1383 nucleotides. The protein predicted from the open reading frame contains 461 amino acids (51,342 daltons) which is identical to that reported for other mammalian ODC cDNAs. The amino acid sequence shared a high degree of homology to human (93 %; Hickok et al., 1987), rat (91 %; van Kranen et al., 1987) and mouse (90 %; Kahana, and Nathans, 1985) ODC cDNAs. About 40 % of the differences in amino acid residues occurred in the 50 residues at the carboxyl terminus but these differences did not substantially change the PEST regions (stretches enriched in proline, glutamic acid, serine, and threonine) which have been shown to modulate protein turnover (Loetscher et al., 1991).

Comparison of bovine with other mammalian ODC cDNAs showed that nucleotide sequences within the open reading frames are also highly conserved and share overall homology of 89 %, 86 % and 86.5 % with the human (Hickok et al., 1987), rat (van Kranen et al., 1987) and mouse (Kahana and Nathans, 1985), respectively.

To determine the size of the bovine ODC transcript, the bovine cDNA was used to probe RNA isolated from bovine mammary epithelial cells by Northern blotting. Hybridization of the blot to the probe revealed a single mRNA species of about 2.35 kb (data not shown). This result is in agreement with that reported in human (Hickok et al., 1987) in which only a single transcript of about 2.25 kb was detected, but is different from that in the mouse (Kahana and Nathans, 1985) and rat (Wen et al., 1989) where two mRNA species of 2.2 and 2.6 kb are always present due to the alternative use of the polyadenylation signals (Kahana and Nathans, 1985; Hickok et al., 1986).

To determine the transcription start site of the bovine ODC mRNA, we examined the bovine RNA by primer extension. The extension product was shown by electrophoresis to be a single band with the size of 155 bp (data not shown) as determined by comparison to a known sequence fragment. This indicates that the 5'-UTR of the bovine ODC mRNA is 314 nucleotides long which is 127 bp longer than that present in pY11. This size is very close to the reported length of 5'-UTR of human (335 nt; Hickok et al., 1987) and murine mRNA (330 nt; Gupta and Coffino, 1985).

Southern blot analysis of bovine genomic DNA isolated from leucocytes of 15 individual Holstein cows revealed restriction fragment length polymorphisms (RFLPs) when cleaved with restriction enzymes PstI, MspI, TaqI and BglI, but not with EcoRI, SacI, HindIII, BamHI, Rsal, and KpnI. Cleavage with PstI revealed 2 hybridization patterns (Fig. 1A). The individual shown in lane 1 is homozygous for the 5.0 kb PstI fragment, while the individual in lane 2 is heterozygous for the
5.5 and 5.0 kb bands. DNA samples digested with MspI also revealed 2 patterns (Fig. 1B). The majority of animals appeared homozygous for the 3.5 kb fragment (lane 1). Animals showing the other pattern demonstrated two bands of 3.5 and 4.8 kb (lane 2). Digestion with the restriction enzyme TaqI exhibited 3 distinct patterns (Fig. 1C), with one heterozygous for the 6.9 kb and 6.3 kb fragments (lane 1) and two homozygous for 6.9 kb and 6.3 kb fragments, respectively (Lane 2 and 3). Two patterns were observed when DNA was digested with BgII. The individual shown in lane 1 (Fig. 1D) showed a weak 2.7 kb band but a strong 2.1 kb band, while the individual in lane 2 is just the opposite, i.e. the 2.7 kb band is stronger than the 2.1 kb band. This result indicates that the bovine ODC gene is highly variable.

Fig. 1. Southern blot analysis of bovine genomic DNA showing restriction fragment length polymorphisms when cleaved with PstI (A), MspI (B), TaqI (C) and BgII (D). The sizes (kb) of the restriction fragments are shown on the right of the respective blots.

DISCUSSION
The present work describes the isolation and sequence characterization of a bovine ODC cDNA clone. The authenticity of the clone was established by the observation that the nucleotide sequence of the cDNA clone showed a very high degree of homology to those of other mammalian ODC cDNA clones. Using this clone as a probe, DNA polymorphisms were identified with PstI, MspI, TaqI and BgII at the ODC locus in Holsteins.

There is a large difference in homology among species between the 5'- and the 3'-UTRs, respectively. Homology at the 5'-UTR amongst the bovine, human, and mouse was low (less than 60% in 187 nucleotides upstream of the initiation codon), whereas homology at the 3'-UTR was high (greater than 80% in 187 nucleotides downstream of the stop codon). The 5'-UTR is considered to have a major role in control of translation of ODC yet is relatively poorly conserved amongst mammalian species. The extraordinary degree of nucleotide conservation which occurs in the coding region (more than 86%) is indicative of a very slow rate of evolutionary divergence and the importance of this enzyme in animal growth and differentiation. That the 3-UTR should also be conserved to the same extent may imply a key role in regulation. What this role may be is at this time not clear. Deletion of this region has been shown to decrease the rate of translation (Greens and Scheffler, 1990; Manzella and Blaksear, 1990) and an interaction between the 5'- and 3'-UTRs has been proposed as a mechanism for reducing the inhibition of transcription imposed by the 5-UTR secondary structure in vitro (Greens and Scheffler, 1990). Computer analysis of complementarity between the 3-UTR sequence and sequence in both the 5-UTR and the coding region did not reveal
any obvious interacting domains.

ODC is the first and key regulatory enzyme in the biosynthesis of polyamines, which are essential for protein biosynthesis and DNA replication (Pegg, 1986). In general, ODC protein concentration and the catalytic enzyme activity correlate with the growth state of the cell and change rapidly upon exposure to trophic stimuli such as hormones, drugs and growth factors, and the increase in ODC activity is tightly regulated at the transcriptional, translational and post-translational levels (Pegg, 1986). In chickens, it has been shown that this enzyme has over 20-fold higher bioactivity in breast muscle of broiler strains when compared with layer strains at a week of age (Bulfield et al., 1988). Recently, Gray and Tait (1993), using replicate lines of mice, have demonstrated that the peak of ODC activity is consistently higher in all the high lean mass lines than the low lean mass lines, and the ODC gene has a 1.9 kb HaeIII restriction fragment present in all the high lines but not in the low lines, indicating that ODC is a growth trait-gene. The association between levels of ODC activity and growth provides evidence that ODC might also be associated with mammary gland activity in dairy cattle. Variations in the ODC gene could cause altered bioactivity of the enzyme in mammary gland, leading to different milk production. Thus, the identification of DNA polymorphisms at the ODC gene in the present study serves as the first attempt to correlate ODC genetic variants with milk production traits. We are currently investigating the association of different allelic variants with superior level of milk production in Holsteins.

REFERENCES
