ISOLATION OF CENTROMERIC DNA SEQUENCES FROM CATTLE AND SHEEP

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SUMMARY

The goal has been to isolate and characterise centromeric and repetitive DNA sequences from cattle and sheep in order to undertake a comparative analysis. Using selective endonuclease digestion and PCR, it has been possible to rapidly isolate a series of repetitive sequences for analyses. These sequences have been initially characterised by sequencing, cross-hybridisation, and physical mapping.

INTRODUCTION

The relative role of DNA in the structure and function of centromeres has not been well delineated. One approach to the study of centromeric DNA is a comparative analysis of sequences from different species. For such analysis, we have attempted to isolate sequences associated with the centromeres of cattle and sheep in order to compare their structure and organisation with known centromeric sequences in humans, primates, and mouse. The objective is to isolate both conserved as well as species-specific centromeric sequences.

Satellite sequences associated with centromeres have been isolated in the past by density gradient centrifugation of bulk genomic DNA and recovery of the density fractions. We have examined two simpler methods to isolate DNA sequences of unusual higher-order repeat structure. These methods will also allow the isolation of other non-satellite DNA repetitive regions (eg., short interspersed elements).

CLONING OF SATELLITE DNA FROM SHEEP AND CATTLE RESTRICTION DIGESTS

Method

In the first method, 4 bp restriction enzymes have been used to digest total genomic DNA. If these restriction enzymes have a bias of either AT or GC in their restriction recognition sites, then large fragments (> 3 kb) and distinct bands are more frequently observed than is normally observed when using 4 bp recognition endonucleases. These bands and smears of large fragments are likely to be enriched for repetitive DNA with higher order repeat structure (Callen, et al. 1990).

Results
We have used a range of such enzymes to digest cattle and sheep DNA and have successfully cloned a series of restriction DNA fragments in both species. High quality genomic DNA from several cattle and sheep has been digested with a series of four base cutting enzymes including *Mse*I, *Sau*3A, *Nla*III, *Hin*PI, *Msp*I, and *Taq*I.

High molecular weight fragments were produced by the *Msp*I, *Hin*PI and *Taq*I digestion in cattle and these are being cloned. Of particular interest are the patterns of bands also observed with *Mse*I, *Nla*III, *Hin*PI, and *Taq*I digestion of cattle genomic DNA. These bands are being isolated as well for cloning and characterisation.

In sheep, digestion with *Hin*PI, *Msp*I, and *Taq*I gave high molecular weight fragments. Bands were observed with *Mse*I, *Nla*III, *Hin*PI, *Msp*I, and *Taq*I digestion of sheep genomic DNA. These fragments and bands are being cloned and characterised.

Among the sequences cloned by this method and characterised thus far are satellite I, gamma globulin gene, and an Alu-like repetitive region from cattle and satellite I from sheep.

CLONING OF SATELLITE DNA FROM SHEEP AND CATTLE BY PCR AMPLIFICATION

Method
The second method to obtain satellite DNA has been to use PCR oligonucleotides designed to eukaryotic putative centromeric consensus sequences to amplify cattle and sheep genomic DNA. Examples of such sequences would include the conserved repeat DNA sequence $(GGAAT)_n$ with unusual hydrogen bonding properties (Grady, et al., 1992) and the CENP-B box with the centromeric protein CENP-B binding motif (Willard, 1990). Oligonucleotides for PCR amplification of the livestock satellite probes have also been derived from the limited GENBANK and EMBL data (eg., cattle satellite IV).

Results
This method has proved successful in the isolation of DNA sequences from both cattle and sheep. For example, amplification using the oligonucleotide $(AAGGT)_n$ (Grady, et al. 1992) results in a 1 kb fragment in cattle and three fragments (500 bp, 700 bp, and 900 bp) in sheep. Among the sequences cloned by this method and characterised thus far are satellite I and an immunoglobulin gene from sheep.

CHARACTERISATION OF CENTROMERIC DNA SEQUENCES

Methods
Once the satellite DNA has been isolated and cloned using these methods, the DNA is sequenced for comparison to known centromeric DNA sequences, analysed by partial restriction digestion for higher repeat order, examined for cross-species hybridisation by zoo blots, and physically mapped by fluorescence *in situ* hybridisation.

Results

Preliminary characterisation reveals that at least some cattle and sheep satellite sequences are related and can cross-hybridise to each other. For example, using *in situ* hybridisation we have demonstrated that a bovine satellite I probe will cross-hybridise to sheep chromosomes at low stringency. This bovine satellite I probe hybridises to all of the cattle acrocentric autosomes, but only a few of the sheep autosomes.

The ovine satellite I probe labelled the sheep acrocentric chromosomes heavily, while the natural fusion metacentric chromosomes showed little hybridisation. Interestingly, Robertsonian translocations resulting in new fusions of sheep acrocentric chromosomes retained the heavy labelling.

Like the bovine satellite I probe, the ovine satellite I probe does not cross-hybridise except under very low stringency conditions. In contrast to the limited cross-hybridisation of these satellite I probes, a bovine Alu-like repetitive region probe hybridises equally well to cattle, sheep and deer. Similar to the results observed using Alu repeats in human chromosomes, this probe labels the euchromatin of metaphase chromosomes and not the centromeres.

FUTURE STUDIES WITH CENTROMERIC DNA SEQUENCES

It is clear that these methods will allow the rapid isolation of a series of satellite DNA clones for future studies. Those centromeric clones with consensus sequences, putative centromere protein binding sequences, or unique DNA sequences can be used in DNA mobility shift assays and hyperchromicity studies. The DNA mobility shift assays using extracted nuclear proteins can determine if the sequence may have a functional role in centromere protein binding. Hyperchromicity studies can determine if the sequences have the unusual hydrogen bonding properties associated with consensus repeats such as $(GGAAT)_n$.

The organisation of centromere DNA can be analysed by whole mount electron microscopy using those clones that map to the same centromere, to single centromeres or to chromosomal rearrangements and those clones that represent novel satellite families, unique DNA sequences, or boundary satellite sequences. The centromere structure can also be examined by transmission electron microscopy to delineate the position of the satellite sequences relative to the kinetochore.

REFERENCES

1. Callen DF, Ringenbergs ML, Fowler JCS, Freemantle J, and Haan EA (1990) *J. Med. Genet.* 27:155-159.
2. Grady DH, Ratliff, Robinson DL, McCanilies EC, Meyne J, and Moyzis RK (1992) *Proc. Natl. Acad. Sci.* 89:1695-1699.
3. Willard HF (1990) *Trends in Genetics* 6:410-415.

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