Brief Gene Mapping Report

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Assignment[1] of telomeric repeat binding factor genes *TERF1* and *TERF2* to Indian muntjac chromosome bands 1p32 and 2q33 by in situ hybridization

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[1] To our knowledge this is the first time these genes have been mapped in the Indian muntjac.

Rationale and significance

The *TERF1* and *TERF2* genes encode the telomeric repeat binding factor proteins TRF1 and TRF2 that are essential components of the nucleoprotein complex at the mammalian chromosome end (Chong et al., 1995; Bilaud et al., 1997; Broccoli et al., 1997). TRF1 and TRF2 play a key role in maintenance of telomeres and confer karyotypic stability by preventing end-to-end fusions (reviewed by de Lange, 2002). The karyotype of the Indian muntjac *(Muntiacus muntjak vaginalis)*, which displays the lowest chromosome number among mammals (2n = 6 ♀, 7 ♂; Wurster and Benirschke, 1970), was generated mostly by numerous tandem fusions (Hsu et al., 1975; Shi et al., 1980) within a relatively short evolutionary time (Wang and Lan, 2000). In an attempt to understand the mechanism of tandem fusion we isolated Indian muntjac *TERF1* and *-2* genes and report their mapping.

Materials and methods

Full-length cDNA of *TERF1* and *-2* was obtained from Indian muntjac fibroblast RNA using RT-PCR. Products were cloned into the pGEM-T easy vector (Promega) and sequenced (Acc. nos. AY606018 and AY606026). Conserved exon boundaries between the human and mouse orthologs were used to design primers that allowed the amplification of gene-specific introns from genomic muntjac DNA. Finally, a total length of approximately 30.5-kb intron and 2.4-kb cDNA sequences were isolated and combined to create a *TERF1* probe. The same strategy was applied to isolate the 9.5-kb intron and 2.5-kb cDNA sequences of muntjac *TERF2* which in total comprised approximately 12 kb. Probe DNA pools were labeled with biotin or digoxigenin using respective nick translation kits (Roche). FISH on Indian muntjac metaphase spreads was performed with the labeled probes (50 ng/μl) and muntjac-specific C_0t-DNA (2 μg/μl) for 72 h at 37 °C (for details see Hartmann and Scherthan, 2004). Hybrid molecules were detected by ExtrAvidin-FITC (Sigma) or by rhodamine-conjugated anti-digoxigenin Fab fragments (Roche). Chromosomes were counterstained with DAPI (0.5 μg/ml) and fluorescent signals were analyzed using a Zeiss Axioskop fluorescence microscope equipped with a cooled CCD camera (Hamamatsu) and an ISIS imaging system (MetaSystems).

Probe name: TERF1
Probe type: Indian muntjac cDNA and genomic DNA
Insert size: approximately 33 kb
Vector: pGEM-T easy (Promega)
Proof of authenticity: cDNA sequencing, size and sequence comparison
Gene reference: AY606018

Probe name: TERF2
Probe type: Indian muntjac cDNA and genomic DNA
Insert size: approximately 12 kb
Vector: pGEM-T easy (Promega)
Proof of authenticity: cDNA sequencing, size and sequence comparison
Gene reference: AY606026

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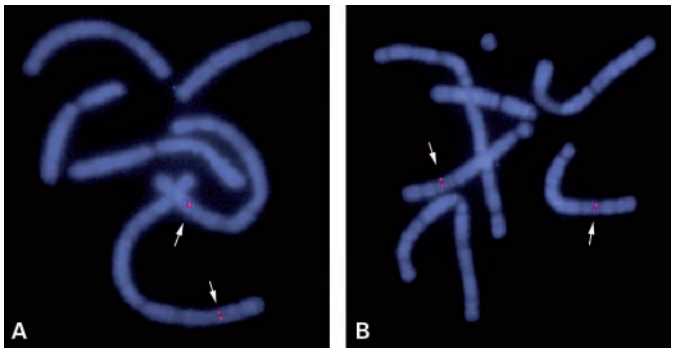

Fig. 1. Chromosomal localization of the *TERF1* and *-2* genes to Indian muntjac (MMV) metaphase chromosomes by fluorescence in situ hybridization. (**A**) *TERF1* signals were detected on MMV1p32 (partial ♂ metaphase, arrows). (**B**) *TERF2* signals were observed on chromosome 2q33 (♂, arrows).

Results

Mapping data:
Most precise location: TERF1: 1p32
Nucleotide position in human chromosome reference sequence: TERF1: chr8: 74,083,662–74,122,281 (according to UCSC Genome Browser, May 2004)
No. of cells examined: 22
Number of cells with specific signal: 1 (0), 2 (6), 3 (9), 4 (7) chromatids per cell

Mapping by FL:
Number of chromosomes examined: TERF1: 34
Mean location: 1p32
Bands encompassed: 1p31→33
Range: 9% on 1p31, 68% on 1p32, 23% on 1p33

Mapping data:
Most precise location: TERF2: 2q33
Nucleotide position in human chromosome reference sequence: TERF2: chr16: 67,947,035–67,977,374 (according to UCSC Genome Browser, May 2004)
No. of cells examined: 34
Number of cells with specific signal: 1 (3), 2 (13), 3 (7), 4 (11) chromatids per cell

Mapping by FL:
Number of chromosomes examined: TERF2: 52
Mean location: 2q33
Bands encompassed: 2q32→34
Range: 15% on 2q32, 70% on 2q33, 15% on 2q34

The *TERF1* and *TERF2* genes were assigned to Indian muntjac chromosomes according to previously established ideograms (Fig. 1; Yang et al., 1995; Frönicke and Scherthan, 1997). The muntjac *TERF1* and *-2* locations are in accordance with Zoo-FISH data (Frönicke and Scherthan, 1997; Yang et al., 1997) and confirm the conservation of large syntenic chromosomal segments during the drastic reduction in chromosome number in muntjac karyotypic evolution.

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