Assignment¹ of the telomere-repeat binding factor genes *TERF1* and *TERF2* to Chinese muntjac (2n = 46) chromosome bands 12q3 and 2q33 by in situ hybridization

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¹ To our knowledge this is the first time these genes have been mapped.

Rationale and significance

The *TERF1* and *TERF2* genes encode the telomere-repeat binding factors TRF1 and TRF2 that play a key role in maintenance and protection of chromosome ends (de Lange, 2002). The genus *Muntiacus* is characterized by drastic chromosomal rearrangements such as tandem and centric fusions (Hsu et al., 1975; Shi et al., 1980). While some muntjac species display extremely low chromosome numbers (down to 2n = 6 in the Indian muntjac; Scherthan, 2003), the Chinese muntjac deer (*Muntiacus reevesi*) exhibits a chromosome number of 2n = 46 (Wurster and Benirschke, 1967). In search for a potential role of TRF1 and TRF2 in muntjac karyotypic evolution, we isolated and mapped the Chinese muntjac *TERF1* and *TERF2* genes.

Materials and methods

Full-length cDNA of *TERF1* and *TERF2* was obtained from Chinese muntjac fibroblasts using RT-PCR. Products were cloned into the pGEM-T easy vector (Promega) and sequenced (Acc. nos. AY606022 and AY606028;

Hartmann and Scherthan, 2005). 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 33 kb intron and cDNA sequences was isolated and combined to create a *TERF1* probe. The same strategy was applied to isolate intron and cDNA sequences of muntjac *TERF2*, which comprised approximately 12 kb. Probe DNA pools were labeled with digoxigenin or biotin using nick translation (Roche). FISH on Chinese muntjac metaphase spreads was performed with labeled probe DNAs (50 ng/μl), in the presence of muntjac-specific C₀t-DNA (2 μg/μl), and in case of *TERF1* with the biotinylated MRE chromosome 12 paint probe (Yang et al., 1997). Hybridization was for 72 h at 37°C (Hartmann and Scherthan, 2004). Hybrid molecules were detected by rhodamine-conjugated anti-digoxigenin Fab fragments (Roche) and by Extravidin-FITC (Sigma). 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 names: *TERF1* and *TERF2*

Probe type: Chinese muntjac cDNA and genomic DNA

Insert size: approx. 33 kb and 12 kb

Vector: pGEM-T easy (Promega)

Proof of authenticity: PCR, DNA sequencing

Gene reference: AY606022 and AY606028

Results

Mapping data:

Most precise location: *TERF1* 12q3

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: 33

Number of cells with specific signal: 1 (0), 2 (7), 3 (13), 4 (13) chromatids per cell

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Fig. 1. FISH mapping of the *TERF1* and *TERF2* genes to Chinese muntjac chromosomes. **(A)** *TERF1*-specific signals (green) were detected on chromosome 12q3. **(A')** Mapping to MRE12 was confirmed by colocalization of *TERF1* signals (red) with an MRE12-specific paint probe (green; Yang et al., 1997). **(B)** *TERF2* signals (red) were localized to MRE2q33.

Mapping by FL:

Number of chromosomes examined: *TERF1*: 62

Mean location: 12q3; Bands encompassed: 12q2 → q4

Range: 14% on 12q2, 83% on 12q3, 3% on 12q4

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: 30

Number of cells with specific signal: 1 (0), 2 (15), 3 (6), 4 (9) chromatids per cell

Mapping by FL:

Number of chromosomes examined: *TERF2*: 47

Mean location: 2q33; Bands encompassed: 2q33 → q34

Range: 85% on 2q33, 15% on 2q34

The *TERF1* and *TERF2* genes were mapped to Chinese muntjac chromosomes according to previously established idiograms (Yang et al., 1995). The results obtained are in accordance with Zoo-FISH data (Yang et al., 1997). Colocalization of an MRE chromosome 12 paint (green) with the *TERF1* probe signals (red) confirms the mapping of the *TERF1* gene to MRE12.

References

- de Lange T: Protection of mammalian telomeres. *Oncogene* 21:532–540 (2002).
- Hartmann N, Scherthan H: Characterization of ancestral chromosome fusion points in the Indian muntjac deer. *Chromosoma* 112:213–220 (2004).
- Hartmann N, Scherthan H: Characterization of the telomere complex, *TERF1* and *TERF2* genes in muntjac species with fusion karyotypes. *Exp Cell Res* 306:64–74 (2005).
- Hsu TC, Pathak S, Chen TR: The possibility of latent centromeres and a proposed nomenclature system for total chromosome and whole arm translocations. *Cytogenet Cell Genet* 15:41–49 (1975).
- Scherthan H: Chromosome numbers in mammals, in Cooper D (ed): *Nature Encyclopedia of the Human Genome*, pp 747–754 (Macmillan Press, London 2003).
- Shi LM, Yingying Y, Xingsheng D: Comparative cytogenetic studies on the red muntjac, Chinese muntjac and their F1 hybrids. *Cytogenet Cell Genet* 26:22–27 (1980).
- Wurster DH, Benirschke K: Chromosome studies in some deer, the springbok and the pronghorn with notes on placentation in deer. *Cytologia* 32:273–285 (1967).
- Yang F, Carter NP, Shi L, Ferguson-Smith MA: A comparative study of karyotypes of muntjacs by chromosome painting. *Chromosoma* 103:642–652 (1995).
- Yang F, O'Brien PC, Wienberg J, Neitzel H, Lin CC, Ferguson-Smith MA: Chromosomal evolution of the Chinese muntjac (*Muntiacus reevesi*). *Chromosoma* 106:37–43 (1997).