Supplementary Materials and methods

Cloning of Xenopus TRF1 and TRF2

BLAST search for potential *Xenopus* TRF1 and TRF2 sequences in the EST database led to the identification of candidate clones (accession nos. BG552350 and BJ638816). With specific oligonucleotides (for TRF1, 5'-gccttcagaggtcacaatcactgtcaac-3' and for TRF2, 5’-ggaccatgaagaaactggggatcgtgcc-3’) deduced from these clones, the 5’ end of cDNA was determined using a SMART RACE cDNA amplification kit (Clontech) and total *Xenopus* oocyte RNA. Then, the full-length cDNAs were isolated by PCR using an Expand High Fidelity PCR system (Roche Biochemicals) with two oligonucleotides (for TRF1, sense, 5'-GTAACAGAGAGCCAGTTCAAACC-3', and antisense, 5'-cactctcatagaaaagtaattgcc-3'; for TRF2, sense, 5’-GCATAAGGACTTCCAGGAGATCCGGGAC-3’, and antisense 5’-ccttccgttggtagagtacaaggcctc-3’). Three independent clones were isolated and fully sequenced. When nucleotide changes were found, we tentatively assigned the nucleotide that was found in two out of three clones as a correct nucleotide. The thus-obtained full-length *Xenopus* TRF1 cDNA (accession no. AF525882) contains 1,413 bp with an open reading frame encoding 420 amino acids and *Xenopus* TRF2 cDNA (accession no. DQ118429) contains 1,717 bp with an open reading frame encoding 468 amino acids.

In vitro transcription/translation and electrophoretic mobility shift assay

In vitro transcription/translation of xTRF1 were performed using a TnT-T7 coupled reticulocyte lysate system (Promega). Plasmid DNA (0.5 μg per 20 μl reaction) was used in the presence or absence of ^{35}S-methionine. Unlabeled products were diluted 1:1 with buffer D (100 mM KCl, 3 mM $MgCl_2$, 20% glycerol, 0.1% NP-40, 1 mM DTT, 1x Complete (Roche Biochemicals) and 20 mM HEPES-KOH [pH 7.6]), and the resulting aliquot of 2 μl was used for electrophoretic mobility shift assay (EMSA). EMSA was performed as described (Bianchi et al., 1997) using the ^{32}P-labeled insert fragment of pTH5 that contained 27 tandem repeats of TTAGGG (de Lange et al., 1990). Binding reactions (in 5% glycerol, 4% Ficoll, 20 mM HEPES-KOH [pH 7.9], 150 mM KCl, 1

mM $MgCl_2$, 0.1 mM EDTA and 0.5 mM dithiothreitol (DTT)) were carried out at RT for 30 min and run on a 5% polyacrylamide gel (29:1) in 1×TBE at RT at 13 V/cm for 1 hr. Dried gels were analyzed by autoradiography or a phosphorimager.

Chromatin assembly

To prepare the chromatin fractions, sperm chromatin (4000 sperm heads/μl extract) was incubated with the extracts supplemented with ^{35}S-labeled xTRF1 for appropriate times at 22˚C or RT. Aliquots were diluted with 10-fold volume of ice-cold extraction buffer (EB) (100 mM KCl, 5 mM $MgCl_2$ and 20 mM HEPES-KOH [pH 7.5]) containing 0.2% Triton X-100 and placed on ice for 10 min. Then, the chromatin was isolated by centrifugation through a 30% sucrose cushion in EB at 10,000 rpm for 15 min. The isolated chromatin was analyzed by SDS-PAGE and autoradiography or immunoblotting.

Solid-phase chromatin assembly

The pT2AG3 plasmid containing ~800-bp telomeric repeats was digested with *Bsr*F I and *Bbs* I and the *Bsr*F I site was labeled by Klenow enzyme with biotin-14-dCTP. The *Bsr*F I-*Bbs* I fragment was purified by 1% agarose electrophoresis using a Gel Extraction kit (Qiagen), and the labeled DNA fragment was conjugated to streptavidin-coated paramagnetic beads (Streptavidin MagneSphere Paramagnetic Particles, Promega) following the manufacturer’s instructions. As control, pBluescript KS(-) was similarly conjugated to the beads. One μg of DNA conjugated to 50 μg of beads was incubated with 50 μl of egg extracts at 22˚C for 1 hr on a rotating wheel to prepare the reconstituted chromatin. Chromatin-bound proteins were analyzed by SDS-PAGE and phosphorimaging after the beads were separated from the extract and washed with β-GPEB (80 mM β-glycerophosphate, 20 mM EGTA, 5 mM $MgCl_2$ and 20 mM HEPES-KOH [pH 7.5]) containing 0.2% NP-40. For the samples treated with lambda phosphatase, beads were suspended in 50 μl of 1 x lambda phosphatase buffer, 2 mM $MnCl_2$ and 400 U of lambda phosphatase (New England Biolabs) in the presence or absence of 50 mM NaF, 10 mM orthosodium vanadate and 1 μM okadaic acid, and then incubated at 30˚C for 2 hr.

DNA replication assay

DNA replication activity was measured as the incorporation of [α-^{32}P]dATP into sperm DNA according to a previously described method (Fang and Newport, 1991). Briefly, 10 μCi of [α-^{32}P]dATP was added to 50 μl of egg extract containing 500 demembraned sperm nuclei per μl. Aliquots were taken at various time points, and reactions were quenched by adding an equal volume of 2% SDS, 10 mM EDTA and 10 mM Tris (pH 7.0). Samples were then digested with proteinase K (20 μg/ml) at 37˚C for 1 hr and subjected to electrophoresis on a 0.8% agarose gel. The gels were dried and exposed to X-ray film for direct autoradiography.

References

Bianchi, A, Smith, S, Chong, L, Elias, P and de Lange, T (1997) TRF1 is a dimer and bends telomeric DNA. *EMBO J* **16:** 1785-1794

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