SUPPLEMENTARY INFORMATION

Purification of probes and Oligonucleotides sequence

Oligonucleotides were purchased from Eurogentec, purified by denaturing gel electrophoresis and recovered by electroelution. Labelling was performed using $\gamma^{32}$P ATP and T4 PNK (Promega). All probes were prepared by incubation at 85°C in 20 mM Tris-Cl pH8, 50 mM NaCl, followed by slow cooling overnight followed by purification by native gel electrophoresis. The sequences are as follows:

**nspHJ**

Strand 1

5’ GGAATCATCGTCCTAGCAAGGTATTGGTGGAGGGGCTGCTACCGGCACTGCGTT

Strand 2

5’ AACGCAGTGCCGGTAGCAGCCCCCTCCACCAATATGAGCGGTGGTTATCCAGGTT

Strand 3

5’ AACCTGGATAACCACCGCTCATATTGGTGAGGACTCAACTGCAGTCGCTTGCG

Strand 4

5’ CGCAAGCGACTGCAGTTGAGTCCTCCACCAATACCTTGCTAGGACGATGATTCC

**dsT2**: strand 1 from tHJ and the following sequence

5’ AACGCAGTGCCGGTAGCAGCCCCCTCCACCAATACCTTGCTAGGACGATGATTCC

**dsT4**

Strand 1

5’ ATCGTCTAGCAAGGTTAGGGTTAGGGTTAGGGTTAGGGGCTGCTACCGGCACTG

Strand 2

5’ GTGCCGGTAGCAGCCCCCTCAACCCTAACCCTAACCCTAACCCTTTGCTAGGACGAT

**dsBG2**: strand 1 from tHJ and the following sequence

5’ AACGCAGTGCCGGTAGCAGCCCCCTCCACCAACCTTGCTAGGACGATGATTCC

**dsBL2**: strand 1 from tHJ and the following sequence
5' AACGCAGTGCCCGGTAGCAGCCCCCTTTCTCCAACCTTGCTAGGACGATGATTCC

18dsT2:
5' CTAGGTTAGGGTTAGCTC
5' GAGCTAAACCCTAACCTAG

S1:
S1 top strand
5' AACCTGGGATACACCAGCTCAGGATCCATCGTCCTAGCAAGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGCTGCTACGGCACTGCGTTACTCAAGCTTACTGCTCGTTTCG
S1 bottom strand
5' GACGCTGGCTCTCTAGAGCTTAGAGCTTAGAGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGCTGCTACGGCACTGCGTTACTCAAGCTTACTGCTCGTTTCG

S2:
S2 top strand
5' CGCAAGCGACTAAGCTTGAGTAAACGCAGTGCCCGGTAGCAGCCCCCTTTCTCCAACCTTGCTAGGACGATGATTCC
S2 bottom strand
5' AACCTGGGATACACCAGCTCAGGATCCATCGTCCTAGCAAGGTTAGGGTTAGGGTTAGGGCTGCTACGGCACTGCGTTACTCAAGCTTACTGCTCGTTTCG

hS2:
hS2 top strand
5' CGCAAGCGACTAAGCTTGAGTAAACGCAGTGCCCGGTAGCAGCCCCCTTTCTCCAACCTTGCTAGGACGATGATTCC
hS2 bottom strand
5' AACCTGGGATACACCAGCTCAGGATCCATCGTCCTAGCAAGGTTAGGGTTAGGGTTAGGGCTGCTACGGCACTGCGTTACTCAAGCTTACTGCTCGTTTCG
Peptides sequence

B peptide        MAGGGGSSDG SGRAAGRRAS RSSGRARRGR HE PGLGGPAE RGAGE
B 11-32         SGRAAGRRAS RSSGRARRGR HE

Histidine 31 is underlined
**Supplementary Figure 1**
The telomeric HJ is constructed by hybridization of four 54 nt oligonucleotides. (A) Sequence of the four 54 nt oligonucleotides used for the construction of tHJ. (B) Comparative migration on acrylamide gel: strand 1 (lane 1), species obtained after hybridization of strands 1 and 2 (lane 2) or strands 1, 2 and 3 (lane 3), the four purified tHJs labelled on strand 1, 2, 3 or 4 (lanes 4 to 7 respectively) and a 108 bp double stranded probe as size marker (lane 8).
Supplementary Figure 2

The B domain is essential for efficient binding of TRF2 on a telomeric HJ. (A) Schematic drawing of the tHJ center showing the telomeric homologous region (boxed sequence) and 3 of the 13 possible positions of the junction branch point (numbered from -6 to +6). (B) Schematic drawings of the different mutant and wild type forms of TRF2, letters above indicate the different domains of TRF2 and numbers the starting and ending positions of each domain. B stands for N-terminal basic domain, D for dimerization domain, L for linker domain and M for Myb-like domain. (C) EMSAs performed on labelled tHJ (5 nM) using 10, 20, 30, 50, 75 and 100 nM of either TRF2, TRF2^ΔB or TRF2^ΔM. Lanes 1 show the junction in the absence of protein.
Supplementary Figure 3

The B domain of TRF2 exhibits a marked preference for the Holliday junction structure. (A) Competition experiments: 5 nM of labelled tHJ was incubated with 50 nM of TRF2 (top gel), 200 nM of TRF2ΔB (middle gel) or 50 nM TRF2ΔM (bottom gel) in the presence of increasing amounts of cold competitor, tHJ, nspHJ (a semi-mobile junction containing a scrambled sequence of the telomeric repeat) and dsT2 (a double stranded probe containing two telomeric repeats). The concentrations of competitors were 0, 5, 6.25, 7.5, 10, 15 and 25 nM (lanes 1 to 7 respectively). (B) Graph representing the variations of the ratio of the DNA bound fractions (fraction bound in the sample with competitor, fb, over fraction bound in the sample without competitor, f0) as a function of the ratio between the concentrations of labelled tHJ and the cold tHJ (closed squares), cold nspHJ (open squares) and cold dsT2 (open triangles) competitors.
**Supplementary Figure 4**

GEN1\(^{1-527}\) cleaves a telomeric HJ in a sequence-dependent manner. (A) GEN1\(^{1-527}\) cleavage products obtained after incubation of tHJ labelled on either of the four strands, the cleavage pattern is compared to that obtained following a Maxam and Gilbert sequencing (A+G) reaction on the corresponding strands. One can observe the preferential cleavages in the guanine tracts of strands 1 and 3. (B) Cleavage sites positions on tHJ, arrows and asterisks represent the major and minor cleavage sites respectively.
Supplementary Figure 5

The B domain of TRF2 controls protection while the Myb-like domain is responsible for the 5' side activation. (A) 5 nM of thJ labelled on strand 2 was incubated with 200 nM of TRF2, TRF2ΔB or TRF2ΔM prior to cleavage with GEN1-527. The first lane shows the uncleaved junction and in the three last lanes only telomeric proteins were added. Numbers below the gel represent the variations of intensities of cleavage in % (ΔI %) in the samples containing the telomeric proteins compared to the sample only containing the enzyme. Negative numbers show protection, positive numbers activation of cleavage. Note the apparent resistance of the 5' located cleavage site (*) to TRF2-mediated inhibition. (B) Same experiment as in (A) with strand 3.
Supplementary Figure 6

A. T7 Endo I cleavage profile

B. 

\[
\begin{align*}
\Delta I \% &= \frac{I_{\text{Endo I} + \text{TRF2}} - I_{\text{Endo I}}} {I_{\text{Endo I}}} \\
&\times 100
\end{align*}
\]

\[
\begin{align*}
\text{[TRF2]} \; \text{nM} &\quad 0 &\quad 50 &\quad 100 &\quad 150 &\quad 200 \\
\Delta I \% &\quad -100 &\quad -50 &\quad 0 &\quad 50 &\quad 100
\end{align*}
\]

C. 

\[
\begin{align*}
\Delta I \% &= \frac{I_{\text{Endo I} + \text{TRF2}^{AB}} - I_{\text{Endo I}}} {I_{\text{Endo I}}} \\
&\times 100
\end{align*}
\]

\[
\begin{align*}
\text{[TRF2}^{AB}] \; \text{nM} &\quad 0 &\quad 50 &\quad 100 &\quad 150 &\quad 200 \\
\Delta I \% &\quad -100 &\quad -50 &\quad 0 &\quad 50 &\quad 100
\end{align*}
\]

D. 

\[
\begin{align*}
\Delta I \% &= \frac{I_{\text{Endo I} + \text{TRF2}^{AM}} - I_{\text{Endo I}}} {I_{\text{Endo I}}} \\
&\times 100
\end{align*}
\]

\[
\begin{align*}
\text{[TRF2}^{AM}] \; \text{nM} &\quad 0 &\quad 50 &\quad 100 &\quad 150 &\quad 200 \\
\Delta I \% &\quad -100 &\quad -50 &\quad 0 &\quad 50 &\quad 100
\end{align*}
\]
TRF2 protects tHJ from cleavage by T7 Endo I in a B domain-dependent manner. (A) Cleavage experiment performed on 5 nM of tHJ labelled on strand 1 in the presence of increasing amounts of TRF2, TRF2^ΔB or TRF2^ΔM. Concentrations used were 10 nM, 20 nM, 50 nM, 100 nM and 200 nM. Lane 1 shows undigested strand 1 and lane 2, background T7 Endo I cleavage. In lanes 18-20 only 200 nM of telomeric proteins were added. Positions of cleavage sites were assigned by comparison to a Maxam and Gilbert sequencing reaction of the strand (not shown). On the left side is presented the cleavage profile obtained in the absence of telomeric protein and the position of each cleavage. (B) Left panel: graph representing the variations in % of the intensities of each cleavage band as a function of TRF2 concentration. The lines correspond to average values calculated for bands exhibiting similar behaviour. Three behaviours could be observed: some band intensities were not altered (open symbols), some were increased (grey symbols) or decreased (black symbols). Middle panel: comparison of cleavage profiles for T7 Endo I alone (grey line) or in the presence of 200 nM of TRF2 (black line). The symbols above the profiles are the ones used in the left panel and each correspond to the peak below them. Right panel: data obtained for all strands are summarized, empty boxes corresponding to unaltered positions, grey boxes to sites of increased cleavage and black boxes to sites of decreased cleavage. (C) Same as (B) for TRF2^ΔB. (D) Same as (B) for TRF2^ΔM.
Supplementary Figure 7
Supplementary Figure 7

TRF2 protects tHJ from T7 Endo I cleavage in a B domain-dependent manner. (A) top: cleavage experiment performed with 5 nM of tHJ labelled on strand 2 in the presence of increasing amounts of TRF2, TRF2\[^{AB}\] and TRF2\[^{AM}\]. Concentrations of telomeric proteins used were 10, 20, 50, 100 and 200 nM. The first lane shows undigested strand 2 and the second T7 Endo I cleavage. In the last three lanes 200 nM of telomeric proteins were added alone. Top graph: graph representing the variations of the intensities of each cleavage band (ΔI %) as a function of TRF2 concentration. The lines were drawn using average values for bands exhibiting the same behaviour. Open symbols correspond to invariant intensities, grey symbols to increasing intensities and black symbols to decreasing intensities. Middle graphe: same graph as above for TRF2\[^{AB}\]. Bottom graph: same graph for TRF2\[^{AM}\]. (B) and (C) correspond to the same experiment as in (A) performed on strands 3 and 4 respectively.
Supplementary Figure 8

A. CCE1 cleavage profile

B. CCE1 cleavage profile

C. RusA cleavage profile

D. RusA cleavage profile

E. RusA cleavage profile

F. CCE1 cleavage profile

G. CCE1 cleavage profile

H. 

I. 

Supplementary Figure 8
Supplementary Figure 8
TRF2 protects tHJ from cleavage by CCE1 and RusA in a B-dependent manner. (A) MBP-CCE1 cleavage experiment performed with 5 nM of tHJ labelled on strand 4 in the presence of increasing amounts of TRF2 and TRF2^AB. Concentrations of telomeric proteins used were 10, 20, 50, 100 and 200 nM. The first lane shows undigested strand 4 and the second lane MBP-CCE1 cleavage. In lanes 13 and 14 only 200 nM of telomeric proteins were added. (B) Comparison of cleavage profiles for MBP-CCE1 alone (grey line) or in the presence of 200 nM of TRF2 (black line) or TRF2^AB (dotted line). (C) and (D) Same experiments as in (A) and (B) for MBP-RusA. (E) MBP-CCE1 and MBP-RusA cleavage experiments performed with 5 nM of tHJ labelled on strand 4 in the absence or presence of 200 nM of TRF2 or TRF2^AB. The first lane shows undigested strand 4 and lanes 2 and 5 MBP-CCE1 and MBP-RusA cleavage respectively. In lanes 8 and 9 only 200 nM of telomeric proteins were added. (F) Comparison of cleavage profiles for MBP-CCE1 alone (grey line) or in the presence of 200 nM of TRF2 (black line) or of TRF2^AB (dotted line). (G) Same comparison as in (F) for MBP-RusA cleavage. (H) Graph representing the variations in % of MBP-CCE1 cleavage intensity (ΔI %) as a function of the concentration of TRF2 (closed squares) or TRF2^AB (open squares). Values were obtained by averaging those obtained for the two major cleavage bands since they exhibited the same behaviour. Error bars correspond to the maxima and minima obtained for these values. (I) Same as (H) for MBP-RusA.
Supplementary Figure 9

TRF1 does not protect against Endo I, CceI and RusA cleavage (A) Endo I cleavage experiment performed with 5 nM of tHJ labelled on strand 4 in the presence of increasing amounts of TRF1 (lanes 3 to 7). Concentrations of TRF1 used were 10, 20, 50, 100 and 200 nM. The first lane shows undigested strand 4, the second Endo I cleavage and in lane 8 only 200 nM of TRF1 was added. Note the activation of cleavage obtained for the 5’ located cleavage sites (*). (B) Comparison of cleavage profiles for Endo I alone (light grey line) or in the presence of 200 nM of TRF1 (dark grey line). (C) MBP-CceI and MBP-RusA cleavage experiments performed with 5 nM of tHJ labelled on strand 4 in the presence of 200 nM of TRF1. The first lane shows undigested strand 4 and in lane 6 only 200 nM of TRF1 was added. (D) and (E) Comparison of cleavage profiles for MBP-CceI or MBP-RusA alone (light grey lines) and in the presence of 200 nM of TRF1 (dotted lines).
Supplementary Figure 10
Supplementary Figure 10
The migration assay. (A) Reaction scheme of the migration assay. (B) Electrophoresis migration of the S1 substrate (lane 1) and the sample obtained after 6 hours incubation (lane 2). (C) Electrophoresic migration of the S1 substrate (lane 1), *BamH*I digested (lane 2) or *Hind*III digested (lane 3) S1 substrate, P1 product (lane 4), *BamH*I digested (lane 5) or *Hind*III digested (lane 6) P1 product. B and H marks show the positions of the *BamH*I and *Hind*III restriction sites respectively. (D) Reaction scheme of the migration assay performed in the presence of the hS2 substrate which stops migration. (E) Electrophoretic migration of the S1 substrate (lane 1), outcome of the assay after 6 hours incubation using the S2 substrate (lane 2) or the hS2 substrate (lane 3).
Supplementary Figure 11

TRF2 greatly increases the junction formation but slows down its migration. (A) Migration assay performed in the presence of 100 nM of TRF2, aliquots were taken at long time points. (B) Variations of the percentage of the S1 substrate, junction and P1 product through time corresponding to the experiment shown in (A). The lines represent the fitting obtained with the rates noted inside the figure which were calculated using the experimental data shown in Figure 3G. One can note the good fitting of the experimental data with the parameters obtained with an independent shorter experiment.
Supplementary Figure 12

TRF2 gives complexes similar to those of the dimeric TRF1 with a double stranded telomeric probe. Acrylamide gel electrophoresis of samples obtained by incubation of 5 nM of labelled dsT4 probe with increasing amounts of TRF2 (lanes 2 to 4), TRF2AΔB (lanes 5 to 7) or TRF1 (lanes 8 to 10). Concentrations used are indicated above the gel.
Supplementary Figure 13
Supplementary Figure 13
Permanganate probing of tHJ on strands 2 to 4. (A) EMSAs on tHJ labelled on strand 1 (top panel), strand 2 (middle-top panel), strand 3 (middle-bottom panel), strand 4 (bottom panel) in the presence of 500 nM of either TRF1, TRF2, TRF2ΔB or TRF2ΔM and 5 µM of the B peptide. (B) permanganate probing experiment performed with 5 nM of tHJ labelled on strand 2 in the presence of 500 nM of either TRF1, TRF2, TRF2ΔB or TRF2ΔM and 5 µM of the B peptide. Lane 1 shows undigested strand 2. Lane 2 and 3 show the background cleavage obtained through piperidine cleavage alone and with permanganate respectively. Sequence of the telomeric tract is given on the left side. On the right side are presented the relative intensity profiles obtained after quantification of the sample lanes. (C) and (D) same experiments as in (B) with strands 3 and 4 respectively.
Supplementary Figure 14

Quantitative analysis of permanganate hypersensitivity as a function of proteins concentration on strand 4. (A) Schematic representation of tHJ, the boxed sequence corresponds to the homologous telomeric core in strand 4. (B) Graph showing the stimulation of permanganate reactivity (ratio between the intensity of the corresponding bands with, I, and without added protein, I0) on thymines T40, T41 and T42 of strand 4 in the presence of increasing concentration of wild type and various mutants of TRF2. For TRF2\(^{\Delta B}\), TRF2\(^{\Delta M}\) and the B peptide the values obtained for the three thymines were averaged since they exhibited the same behaviour. For TRF2 wild type, T40 reactivity was different from that of T41 and T42 and was therefore considered separately. Errors bars correspond to standard deviations between values for the corresponding thymines in different sets of experiments. One can note the weak response obtained for TRF2\(^{\Delta B}\) and the reactivity of thymines in the presence of TRF2 wild type, TRF2\(^{\Delta M}\) and the B peptide. The same experiment performed on the other three strands gave similar results (data not shown).
Supplementary Figure 15

TRF2^{ΔM} preferentially binds to a T2 bulge. (A) Left panel: agarose gel of the competition experiment performed using 5 nM of the labelled dsNSP probe, 200 nM of TRF2^{ΔM} and increasing concentrations of cold dsNSP probe. Middle panel same experiment as in the left panel with the T2 bubble competitor (dsBL2). Right panel same experiment as in the left panel with the T2 bulge competitor (dsBG2). (B) Graph representing the variations of the ratio of the DNA bound fractions (fraction bound in the sample with competitor, fb, over the fraction bound in the sample without competitor, f0) as a function of the ratio between the concentrations of labelled dsNSP and cold dsNSP (open diamonds), cold dsBL2 (grey triangles) and cold dsBG2 (closed triangles) competitors.
Supplementary Figure 16
Supplementary Figure 16
The TRF2B H-A mutant does not protect tHJ from cleavage by GEN1\textsuperscript{1-527}, T7 Endo I and CceI. (A) GEN1\textsuperscript{1-527} cleavage experiment performed on 5 nM of tHJ labelled on strand 1 in the absence (lane 2) or the presence of 200 nM of TRF2B H-A (lane 3). Lane 1 shows undigested strand 1 and in lane 4 only 200 nM of TRF2B H-A was added. (B) T7 Endo I cleavage experiment performed on 5 nM of tHJ labelled on strand 1 in the presence of 200 nM of TRF2 or increasing amounts of TRF2B H-A. Concentrations of TRF2B H-A used were 50, 100, 200, 500 and 1000 nM. The first lane shows undigested strand 2. In the last two lanes the highest concentration of each telomeric protein was added alone. (C) Same experiments as in (A) on strand 2 using T7 Endo I and MBP-CceI (D) Same experiment as in (B) on strand 3. (E) Same experiment as in (C) on strand 4.