

**Unique *C. elegans* telomeric overhang structures reveal the evolutionarily conserved properties of telomeric DNA**

Petra Školáková<sup>1,2§</sup>, Silvie Foldynová-Trantírková<sup>2,4, §</sup>, Klára Bednařová<sup>1,2</sup>, Radovan Fiala<sup>2</sup>, Michaela Vorlíčková<sup>1,2\*</sup> and Lukáš Trantírek<sup>2,3,5\*</sup>

<sup>1</sup> Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, 602 00 Brno, Czech Republic

<sup>2</sup> Central European Institute of Technology, Masaryk University, Kamenice 735/5, 625 00 Brno, Czech Republic

<sup>3</sup> Department of Condensed Matter Physics, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic

<sup>4</sup> Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovska 31, 375 05 Ceske Budejovice, Czech Republic

<sup>5</sup> Bijvoet Centre for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

§ Equally contributing authors

\*To whom correspondence should be addressed:

[mifi@ibp.cz](mailto:mifi@ibp.cz), [lukas.trantirek@ceitec.muni.cz](mailto:lukas.trantirek@ceitec.muni.cz)

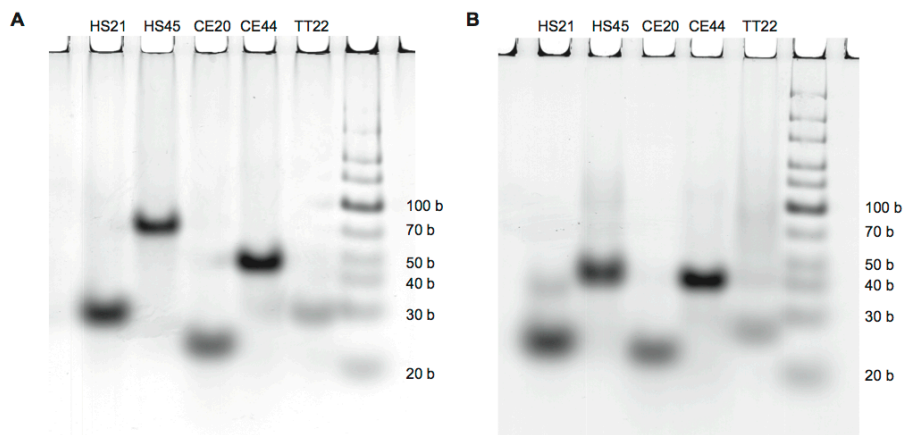


Figure S1: Non-denaturing PAGE of the DNA constructs based on human and *C. elegans* C-rich telomeric DNA repeats at pH 7.3 (A) and 5.5 (B). The individual constructs are labelled according to Table 1.

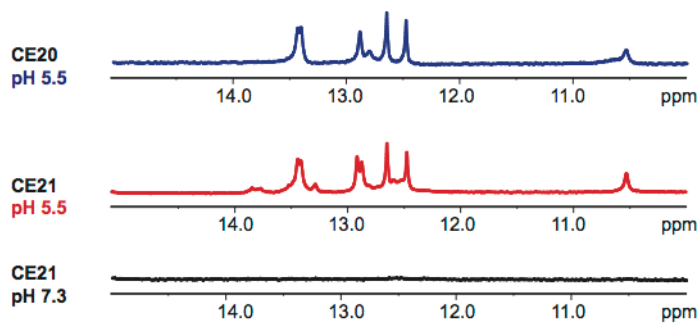


Figure S2. Imino region of the 1D  $^1\text{H}$  NMR spectrum of the CE20 construct at pH 5.5 (blue). Imino regions of the 1D  $^1\text{H}$  NMR spectra of the CE21 construct at pH 5.5 (red) and pH 7.3 (black). The spectra for the CE20 and CE21 were acquired at 700 and 800 MHz, respectively. All the spectra were acquired at 1  $^\circ\text{C}$ .

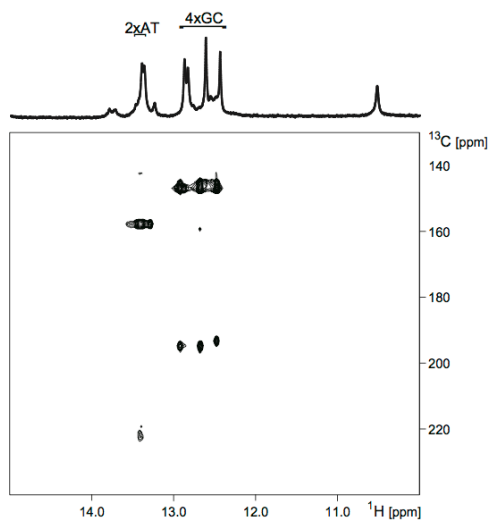


Figure S3: The HNN-COSY spectrum of the CE21 construct recorded at 850 MHz DNA concentration 0.5 mM, pH = 5.5, T = 1 °C, intracellular buffer 90% H<sub>2</sub>O/10% D<sub>2</sub>O).

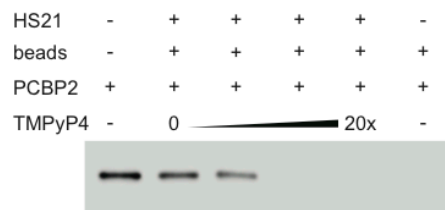


Figure S4. The i-motif stabilizing ligand (TMPyP4) displaces telomere associated human poly-C binding protein (PCBP) from human C-rich telomeric DNA. Pull down of poly-C binding protein (PCBP2) by human C-rich telomeric DNA (HS21) in the absence and presence of the small molecular weight ligand TMPyP4.

Table S1. Nuclear telomeric repeats from species of various eukaryotic phyla.

Organism	Telomeric repeats sequence	
	G-rich	C-rich
<b>ANIMALS</b>		
<i>Homo sapiens</i>	TTAGGG	CCCTAA
<i>Bombyx mori</i>	TTAGG	CCTAA
<i>Caenorhabditis elegans</i>	TTAGGC	GCCTAA
<b>PLANTS</b>		
<i>Othocallis siberica</i>	TTAGGG	CCCTAA
<i>Arabidopsis thaliana</i>	TTTAGGG	CCCTAAA
<i>Chlorella vulgaris</i>	TTTAGGG	CCCTAAA
<i>Chlamydomonas reinhardtii</i>	TTTTAGGG	CCCTAAAA
<b>PROTOZOA</b>		
<i>Trypanosoma brucei</i>	TTAGGG	CCCTAA
<i>Tetrahymena thermophila</i>	TTGGGG	CCCCAA
<i>Oxytricha nova</i>	TTTTGGGG	CCCCAAA
<i>Physarum polycephalum</i>	TTAGGG	CCCTAA
<b>FUNGI</b>		
<i>Saccharomyces cerevisiae</i>	TG <sub>2-3</sub> (TG) <sub>1-6</sub>	(CA) <sub>1-6</sub> C <sub>2-3</sub> A
<i>Neurospora crassa</i>	TTAGGG	CCCTAA
<i>Pneumocystis carinii</i>	TTAGGG	CCCTAA