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TEP-1, a C. *elegans* Telomere Binding Protein, is Required for DNA Bending and Loop Formation at the Telomere

Seung Hyun Kim, Sun Baek Hwang, In Kwon Chung, and Junho Lee

National Research Laboratory, Department of Biology, Yonsei University 134 Shinchon, Seodaemun-ku, Seoul, 120-749 Korea

Abstract

C. elegans is a good model for studying biological functions of key molecules in development using molecular genetic methods. We identified a novel gene, TEP-1, encoding a homeobox-containing protein that specifically binds double-stranded telomeres in C. *elegans* by yeast one-hybrid screening. Structural modeling of TEP-1 revealed that the three-dimensional structure of TEP-1 has a strong similarity to that of hTRF1, a telomere binding protein in humans, although the two proteins do not share any amino acid sequence similarity. TEP-1 bends the DNA molecule by binding to the telomeric sequence as hTRF1 does. Domain studies revealed the distinct functions of the domains of TEP-1: The N-terminal domain and the homeobox domain for telomere binding and the C terminal region for DNA bending activity. Furthermore, by DNA 2D gel electrophoresis and southern hybridization of the genomic DNAs, we found that TEP-1 is required for loop formation at the telomeric region *in vivo,* which is a property of TRF2. Overexpression of TEP-1 enhanced loop formation at the telomeric ends, while a C terminal deletion mutant or a dominant negative mutant failed to form the loop. Either overexpression of the wild-type TEP-1 or the truncated dominant negative form of TEP-1 caused lethality at various stages of development. We propose that TEP-1 is a functional homolog of the mammalian TRF proteins, acting as a balancer protein for chromosome stability.

Purpose of this study

The mechanisms by which to protect the eukaryotic linear chromosomes have been extensively studied. In some species, single stranded telomere binding proteins seem to be major players in protecting telomeres. In other species, telomeres are protected by double stranded telomere binding proteins. Whether using single stranded or double stranded telomere binding proteins, loop formation at the telomeric termini

may be a general mechanism of telomere protection in all species. However, it is not established yet whether loops are the functional units of telomere protection. Furthermore, it is not well established whether loops are formed at the telomeres in other typical experimental species such as S. pombe and C. elegans. We hoped to identify and characterize double stranded telomere binding proteins in the nematode Caenorhabditis elegans in order to elucidate telomere functions in this species. The nematode telomere consists of the TTAGGC repeats, which is different from that in mammals and plants. We utilized a yeast one hybrid screen in which we used the *C. elegans* telomere sequence as a bait. A protein identified in this screen, TEP-1, has a structural domain similar to the homeobox domain, not the Myb-like domain, and specifically binds the nematode telomere to bend the telomere-containing DNA. We show that TEP-1 is structurally similar to TRF proteins despite lack of primary sequence homology. We further show that the telomeres in C. elegans also form loops in vivo and that TEP-1 is required for this process. We further show that TEP-1 is required for chromosome stability in vivo.

Results

TEP-1 specifically binds to the *C. elegans* telomere sequence in vitro and in vivo.

We performed competition assays using the nematode telomere, the human telomere, and the rice telomere as cold competitors. While the nematode cold telomere sequence was able to efficiently compete with the binding ability of TEP-1 to the radioacitve nematode telomere, neither the human telomere nor the rice telomere was able to compete with TEP-1. In order to examine if TEP-1 binds to other sequences than the telomere sequence of the nematode, competition assays with consensus CRX binding motifs. The CRX binding motif sequences did not compete with the TEP-1 binding with the telomere sequence, indicating that TEP-1 probably only binds to the nematode telomere sequence. Gel retardation assays with different numbers of the telomeric repeats showed that the minimal binding site is 1.5 repeats of TTAGGC. We next determined which nucleotides in the TTAGGC sequence are essential for TEP-1 binding. We found that mutations in any single nucleotide from the 4th G through the 9th A abolished TEP-1 binding ability, indicating that these nucleotides, GGCTTA, are the core sequence of the TEP-1 binding site.

In order to examine the subcellular localization of the TEP-1 protein, we examined the localization of GFP reporter protein fused to the TEP-1 protein. We observed that TEP-1 was localized to the tips of the chromosomes in the mitotic cycle. From

these results, we conclude that TEP-1 specifically binds telomeres both in vitro and in vivo

TEP-1 can bend DNA

The human TRF1 is known to bind and bend the telomere sequence. We examined whether TEP-1 was able to bend the DNA by binding to the telomere. Three probes were used in this assay: L, a 200 bp probe with six repeats of the telomere sequences at the left end; M, an identical probe to L except for the telomere sequence located in the middle; and R, a probe with the telomere sequence at the right end. The mobility of the TEP-1- M probe complex was slower than the TEP-1-L or TEP-1-R complexes. This result clearly shows that TEP-1 bends the DNA when binding to the telomere sequence.

3D modeling reveals structural similarity of TEP-1 to TRF proteins, not to Rap1p

Since TEP-1 has sequence similarity to homeobox proteins, which usually act as transcription factors while most identified telomere-binding proteins contain myb domains, we were curious what the three-dimensional structure of TEP-1 would look like. We utilized the SWISS MODEL software (www.expasy.org/swissmod) to perform 3D modeling of the TEP-1 homeodomain. We used the Engrailed homeodomain structure as the template for modeling TEP-1 since Engrailed contains a homeobox domain similar to that of TEP-1. We then fitted the solved 3D structure of TRF1 with the 3D model of TEP-1 in the SPDBV software (www.expasy.org/swissmod). Surprisingly, we found that the two proteins have very similar 3D structures. When fitting the TEP-1 structure with RAP-1, a telomere-associated protein identified in S. cerevisiae and humans, there was little, if any, similarity between the two proteins in terms of three dimensional structure. The structural similarity between TEP-1 and hTRF1 may imply that the nematode telomere-binding protein underwent an evolutionary adaptation in terms of 3D protein structure rather than its primary structure. We propose that the TEP-1 protein, although with no primary structural similarity to TRF1, may be a functional homolog of TRF proteins in terms of 3D structure.

TEP-1 is required for loop formation in the telomeric region

We developed a method to visualize telomere loops by modifying the protocols used for visualizing replication forks and recombination intermediates in the Holiday recombination model. We first examined whether the wild type animals have loops at the telomeric region. We found that most telomeres were linear, but that a small

population of the telomeres were indeed looped. Overexpression of a dominant negative form of TEP-1, which contains only the N terminal domain, also abolished the loop formation. We thus conclude that the C. elegans telomeres indeed form loops at the chromosomal termini, and that TEP-1 is required for the loop formation in vivo.

Discussions

Our results suggest that the nematode telomeres do form loops, but that not all the telomeres seem to form loops. The genomic DNA that we tested was isolated from mixed stage animals, from eggs to adults. It is possible that the chromosomes in different cells at different stages of development or cell cycle may have different state of loop formation. For example, chromosomes at the replication cycle may have linear forms of telomeres, and so on. It was shown in this report that losing telomere binding activity causes more serious defect than the loss of DNA looping at the telomere since overexpression of the wild type or dominant negative form kills a large portion of the affected animals. One possible reason why all the animals without loop formation do not die is that the telomeres may be still under protection without the loop formation. It is possible that telomere binding alone may be able to perform some protection role by protecting the double stranded region from deleterious actions such as exonuclease activities which otherwise would digest longer regions of naked telomeres. Another possible mechanism of telomere protection without loop formation would be mediated by single stranded telomere binding proteins. The capping of the telomeres may be mediated by a single stranded binding protein in C. elegans, a parallel to the situation in other species. It would be interesting to identify and characterize single stranded telomere binding proteins in C. elegans. We had previously reported the existence of a single stranded telomere binding protein in the embryonic nuclear extract..

As shown above, the nematode does not contain any TRF-like proteins, and so far TEP-1 is the only protein that binds the double stranded telomere sequence in C. elegans. Furthermore, TEP-1 was shown to have functions of both TRF1 and TRF2: DNA bending and loop formation. We therefore propose a functional model of TEP-1 biology in which TEP-1 protects the telomeres by binding and bending telomeric DNA, and forming loops at the telomeric ends. In mammals, TRF1 protein may bind double stranded telomeres and bend them to facilitate for the telomeric DNA to fold back, and TRF2 may bind the d loop, stabilizing the loop structure. It was reported that TRF1 did not promote loop formation in vitro. In the nematode, TEP-1 acts in both bending and loop formation. TEP-1 may bind double stranded

telomeres and bend them to facilitate for the telomeric DNA to fold back, and then bind the d loop, stabilizing the loop structure. From more severe phenotypes caused by inhibition of the DNA binding activity of TEP-1, it is also possible to postulate that telomere binding alone can protect the telomeres to some extent in the nematode. To summarize, we propose that TEP-1 protects the telomeres by DNA binding, DNA bending, and loop formation. Consistent with the idea that TEP-1 is involved in telomere protection, we observed a fusion event between a chromosome end and an extrachromosomal array.

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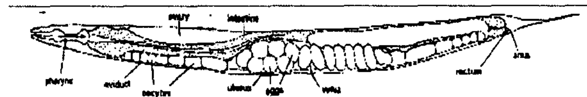
Caenorhabditis elegans, the famous nematode



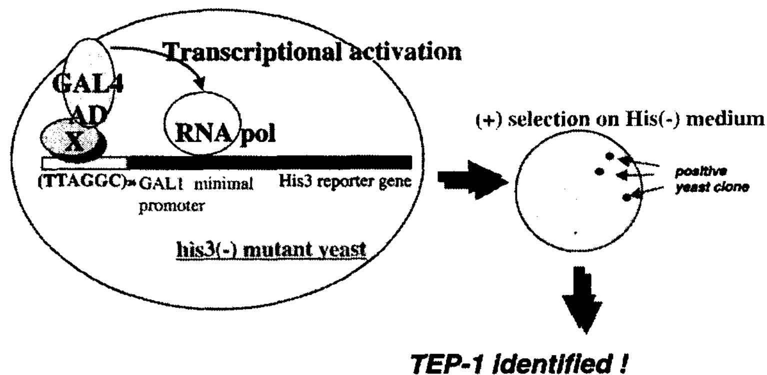
A free-living soil nematode
Genome project completed
Molecular techniques

Life time = 3 weeks
Generation time
= 3.5 days at 20°C

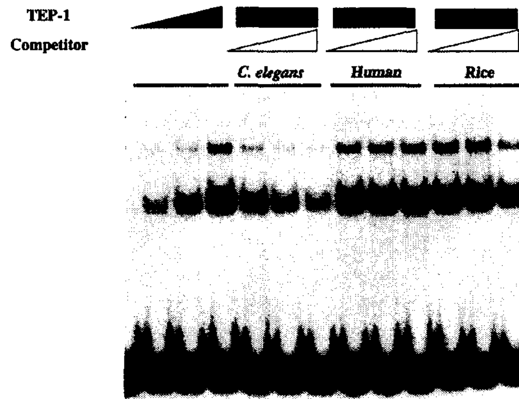
Purpose of this study

- **Identification & Characterization of telomere binding proteins in *C.elegans***
- **Functional studies of the telomere binding proteins**
- **Mechanisms of the telomere binding proteins in telomere protection**

Yeast one-hybrid screening

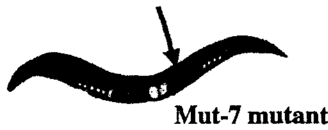


TEP-1 binding is species-specific

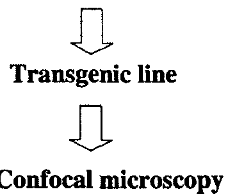


Is TEP-1 localized to the telomere in vivo?

Pie-1 promoter::GFP-TEP-1 cDNA



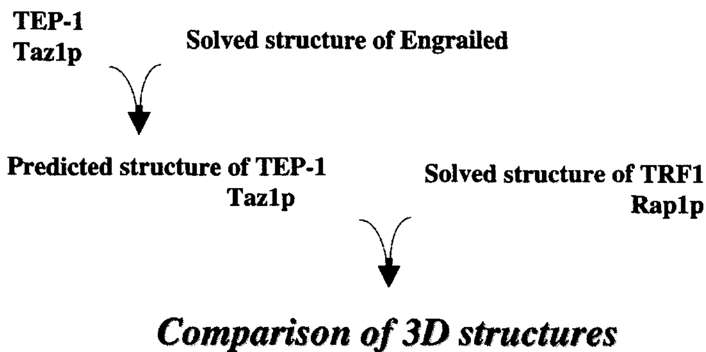
Mut-7 mutant



Transgenic line

Confocal microscopy

Is TEP-1 similar to TRF1 structurally?



TEP-1
Taz1p

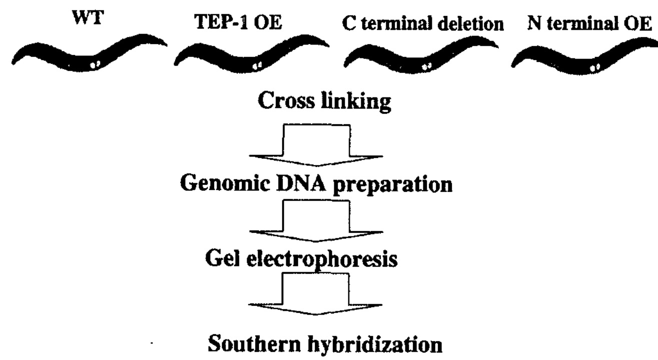
Solved structure of Engrailed

Predicted structure of TEP-1
Taz1p

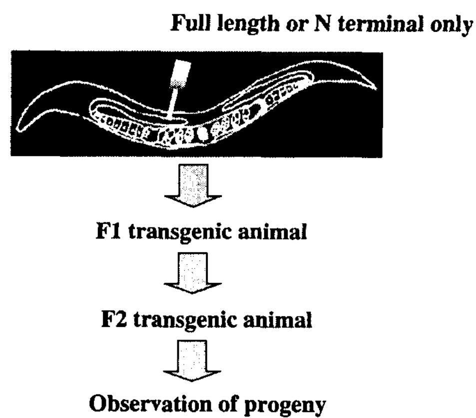
Solved structure of TRF1
Rap1p

Comparison of 3D structures

Is TEP-1 required for loop formation at the telomeric region?



Functional analysis of TEP-1



Acknowledgements

Seung Hyun Kim

Soon Baek Hwang