Formation of sequence-specific telomeric DNA loops via a direct effects of psoralen-photosensitization on telomeres

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ABSTRACT

DNA from HeLa cells was cleaved with RasI and HinfI, and then purified by Bio-Gel P-2 column. Results showed that upon near-UV-irradiation, psoralen could be targeted to the repetitive sequences of telomeres. Large duplex loops with a tail were observed directly by AFM. The loop-tail junction position was assumed to be a tri- or tetra- strand DNA structure according to its apparent height, corresponding structure model was proposed. The psoralen cross-linking products increased with increase of irradiation time. The expression of p53 oncogene was significantly increased.

Keywords telomeres, telomeric DNA, psoralen, HeLa cells, T loop, atomic force microscopy

1. INTRODUCTION

Telomeres are specialized terminal elements in the eukaryotic nucleus, composed of tandem repetitive sequences and specific proteins. Telomeres impart stability on linear eukaryotic chromosomes by acting as caps, protecting chromosome ends from fusion and degradation [1,2]. The telomeric DNA usually consists of a simple repeated sequence with one G-rich strand. This G-strand extends to the 3' end of the chromosome and protrudes to form a single-stranded overhang [3]. In vitro, single-stranded G-rich telomere DNA can form a variety of noncanonical structures including G quartets, triple helices and G:G base pairing [4,5]. Recently, Griffth and Lange showed by electron microscopy (EM) that the single-stranded ends of human chromosomes seem to loop back on themselves in a unique structure termed the t loop [6,7,]. Studies on structure and function of telomeres have received particular attention for its possible significance to aging and cancer.

Psoralen, a three-ringed heterocyclic furocoumarin, has been used for many years as photoactive drugs to treat the dermatological diseases such as psoriasis. The photobiological effects of psoralen are related to the ability to photobind to DNA, to generate either monoadducts or interstrand cross-links at 5'-TpA-3' steps in double helix DNA [8]. Based on its characteristics it was also used as photoactive probes of nucleic acid structure and function to study the mechanisms of mutagenesis and repair processes Telomeres is associated cellular senescence. Less clear, however, is the relevance of progressive telomeric structure change and photobioloical effects of psoralen inside living cells.

AFM has its advantages not only in observing the real morphology with high resolution but also in the rapid sample preparation. Processing with AFM enables the study of transition state to be possible. Here, we used a psoralen as a photoinduced proble, undertaken to examine the response of telomere DNA in HeLa cells upon near-UV-radiation. Thc results of this study provide more new information on the formation of telomere DNA T-loop and its fine structure.

2.MATERIALS AND METHODS

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2.1. Chemicals

Oligonucleotides were synthesized (Applied Biosystem Model 391), purified by HPLC and end-labeled separately using the T4 polynucleotide kinase (purchased from Promega) and γ-[^{32}P] dATP (3000 Ci/mmol, purchased from Dupont Co.). psoralen was obtained from Sigma, proteinase K and RNase were obtained from Sigma. λ DNA/ EcoRI + Hind III marker, Rsal and HinF1 was obtained from Promega. Pancreatic DNase I, and ethidium bromide (EB) were obtained from Fluka Co., RNase is from Pharmacia Co. Other chemicals were homemade and A.R. grade. All fluorescence probes were purchased from Molecular probes Inc. p53 antibiotics were obtained from Santa Cruz Biotechnology CO.

2.2. Cell culture

HeLa cell lines were obtained from Beijing Normal University. Cells were cultured in DMEM medium, supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 IU/ml streptomycin, at 37℃ in a CO_2 incubator. The isolation of cell nuclei was performed according to the method described by Griffith et al.[6].

2.3. Psoralen photocress-linking and Telomere DNA Purification

1 ml of psoralen solution (10 mg/ml stock dissolved in DMSO) was added to 3 ml of nuclei in cross-linking buffer, final concentration was 250 μg/ml. The mixture was spread on a 100 mm plastic petri dish on ice and stirred for 45 min while exposed to a 365 nm UV light bulb at a distance of 2 cm. Then, nuclear suspensions were treated with proteinase K (20mg/ml) in the presence of SDS (0.5%), and DNA was isolated by phenol/chloroform (1:1) extraction and ethanol precipitation (3 Volume). The deproteinized sample was suspended in 9 ml of 10 mM TE buffer (Tris-Hcl, 1 mM EDTA, pH7.5) and cleaved with Rsal (1250 units) and HinF1 (1250 units) solution containing 10 mM Tris, 10 mM $MgCl_2$, 30 mM NaCl, 1 mM DTT, and 100 μg/ml of bovine serum albumin for 12 hr at 37 °C. During the final hour, RNase was added to 20 μg/ml. The sample was then extracted one time with phenol: chloroform: isoamylalcohol =50:49:1, precipitated with ethanol, and suspended in 3 ml of TE. The sample was purified by a 2.5 x 20 cm Bio-gel P-2 column and eluted by water at a flow rate of 0.2 ml/min. Fractions of 0.6 ml were taken and OD_{260} of each determined. All fractions are identified to by ^{32}P-labeled probes $(TTAGGG)_4$ and EB fluorescent probe [10]. All fluorescence measurements were made on a Hitachi M850 model spectrofluorometer. Fluorescence emission spectra were recorded with excitation at 510 nm or emission at 620 nm, respectively. The UV experiments were carried out on a Hitachi U-3200 spectrophotometer.

2.4. Atomic Force Microscopy Observation

A 2 μl drop of the DNA-EB solutions (the concentration of DNA and EB are 5 μg/ml, 2 μg/ml respectively) in the absence or presence of divalent cation (200 mmol/L Mg or Mn, or 2 μmol/L Cu) was deposited onto a freshly cleaved mica surface. About 1 min later, the residual solution on the mica was carefully removed by a slice of filter paper, followed by drying in air. Samples were examined in a nanoscope III (Digital Instruments), and all images were recorded in a Tapping-mode operating at height-mode. The scan rate was usually set to 1.0-2,5 Hz.

3.RESULTS AND DISCUSSION

3.1. Isolation of telomeric DNA in HeLa cell

Protein-free total genomic DNA was digested with Hinfl and Rsal and fractionated on a Bio-Gel P-2 gel-filtration column. As showed in Fig.1A, Bio-Gel P-2 elution profile appeared two peaks. Fluorescence assay with EB and hybridization of ^{32}P-labeled telomere-specific $(TTAGGG)_4$ probe(Fig,1B) showed nearly all of the telomere DNA specie present in the first peak (fraction 3-9). But second peak include the degraded products of DNA, which only gave a weaken

fluorescence and a negative results of $(TTAGGG)_4$ probe test. Psoralen cross-linking did not affect the isolation or fraction of the telomeric fragments from the bulk genomic DNA. The OD value and fluorescence intensity was very low in the cells without psoralen cross-linking (Fig.1-A). When irradiation time is 0, 15, 30, 45 and 60 min, the fluorescence intensity of first peak is 1.86, 18.8, 34.4, 44.6 and 52.1,respectively. This implies that psoralen cross-linking telomeric DNA was produced during UVA irradiation, and progressively increased by increased of irradiation time.

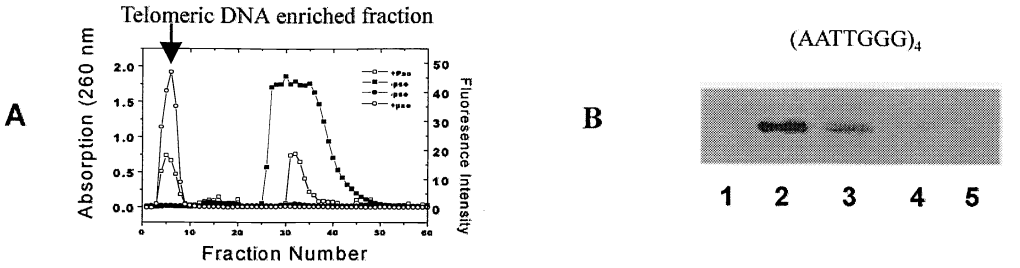

Figure. 1. A, Elution profile of telomeric DNA cleaved with HinfI and RsaI and fluorescence analysis with EB probe; B, Autoradiograph for identification of telomeric TTAGGG repeat sequence on a southern blot. DNA species were pre-incubated in presence of 100 mM magnium ions, and then mixed it with $(TTAGGG)_4$ 1. λ-DNA; 2,4, with Psoralen; 3,5, without psorslen.

3.2. AFM Visualization and analysis of Psoralen cross-linking telomeric DNA

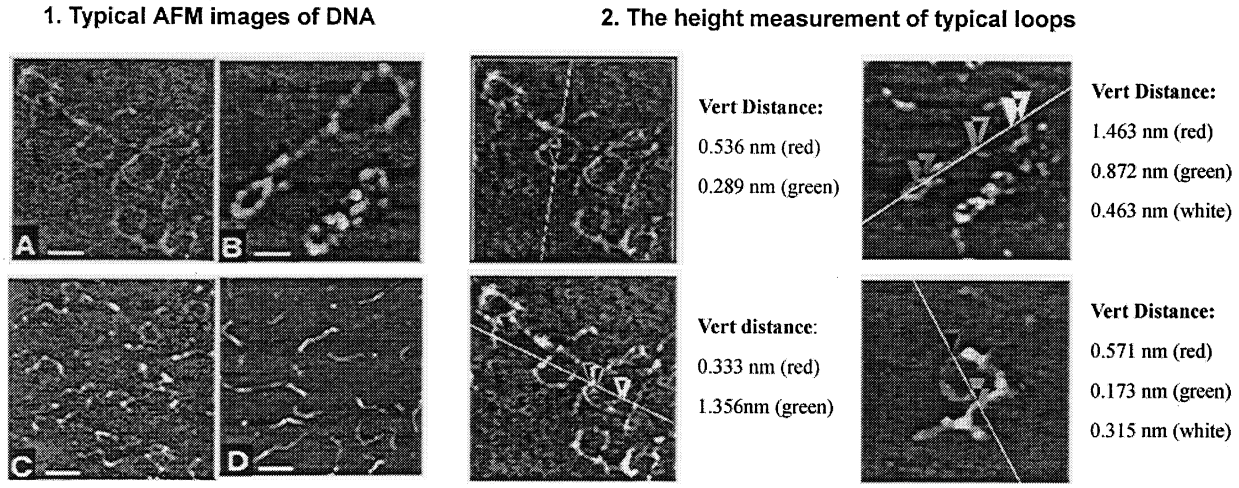

Fig. 2. Typical AFM images of DNA and its corresponding height measurement. DNA was isolated and purified from HeLa cells and diluted to l ng/ml and adjusted to pH 7.0 before aligning. In 1, all samples were treated with psoralen cross-linking expect Fig,1-d. A and: B, typical T-loop structure of telomeric DNA; C, telomeric DNA T-loop and degraded DNA species, Scale bars: 200 nm, 250 nm, 800 nm; D, DNA species of cells without psoralen cross-linking. Scale bars 800; 2. The height measurement of typical DNA T-loops.

The high resolution and cross sectioning measurement function of AFM make it convenient for us to observe telomere morphology and further to analysis its fine structure. Fig2-1 showed that there exactly exists a loop with a tail in the telomeric DNA after the treatment of psoralen plus UVA (F2-1A, B and C), and the length of loop and tail was 2.5 ± 0.5 kb and 2.0 ± 1.5 kb respectively, which is shorter than what have been measured by EM [6]. This may be related to the cell type. For the cells without psoralen cross-linking, the T loop structure can not be observed in the isolated DNA according to same method (Fig.2-1D). However, few similar T loop product can be observed in the crude cell extract without deproteinization It is possible that deproteinization caused the change of telomeric DNA, some experiments proposed that

TRF2 protein bind with the end of telomeric DNA to form T-loop structure, which stabilize the displacement loop and protects human telomeres, So psoralen cross-linking of the DNA strands is predicted to preserve the T-loops after removal of protein. As above mention, psoralen cross-linking play a major role in preserve the T-loop after removal of protein which is mainly TRF2. Maybe the position of psoralen works is the same as TRF2. Form Fig.2, we can see that the height of loop circle portion is uneven, this may be result from psoralen cross-linking T residues of opposite DNA strands randomly, or a variety of noncanonical structures including G quartets, triple helices and G:G base pairing formed by single-stranded G-rich telomere DNA [4].

The T-loop is a large loop-back structure most likely formed through the invasion of the single-stranded telomeric 3' overhang into the duplex telomeric repeat array. The tail-loop junction should be a structure of triplex. Results of AFM showed that the average value of loop circle portion height is about 0.426 + 0.172 nm (n = 41). This value could illustrate that the loop circle portion is duplex DNA. The average height of the tail-loop junction area is about 0.851 + 0.293 nm (n = 26). Due to the height of triplex is two times that of duplex, so we conclude that the structure of junction area is triplex [10,11]. But the height of some tail-loop junction is higher than above average height, it is 1.286 + 0.051 nm (n = 4), suggesting tetra-stranded structure (Fig.2). Fig. 2-2 showed the height of some typical T-loop structure.

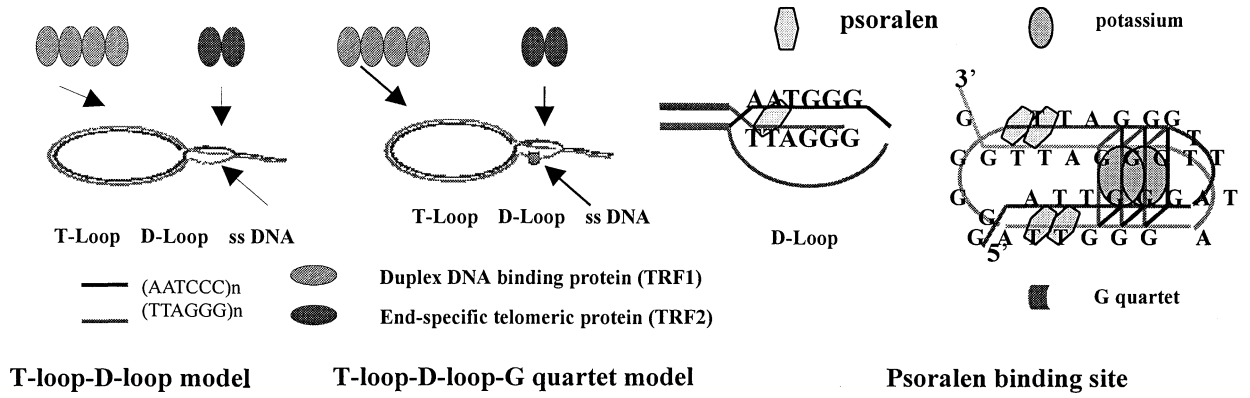

Fig. 3 Telomere structure and pssoralen-binding site

On other hand, the 3'G strand extension invades the duplex telomeric repeats and may form a D-loop (displacement loop). This assume is depend on following experiments, when the t loop were incubated with SSB (single strand binding) protein, 35% of the t loops had one or several SSB protein complexes at the loop-tail junction. The SSB-bearing sites contain a single-stranded segment of possibly 75 to 200 nucleotides [5]. And a specialized telomere-binding protein, TRF2 protein, binds the D loop at the junction of the lasso, which may play this role in stabilizing or allowing formation of the D loop. As showed in Fig.2-2. The exists of D loop were direct observed, the related height is 0.571 (TS), 0.173 (SS) and 0.315 (DS). The height of the tail-loop junction is about 1.9 times that of T-loop circle portion and about 3.4 times that of D-loop circle portion, respectively. A D-loop-T-loop- model was showed in Fig.3 .The telomeric DNA usually consists of a simple repeated sequence with one G-rich strand. Only guanine nucleosides is capable of forming extensive selfstructures in solution, to yield G:G base pairs and G quartets. A D-loop-T-loop-G quartets structure model was proposed (Fig. 3).

Generally speak, the height of DNA duplex is about 0.36~0.76 nm in AFM. So the height of measure in AFM is less than that in theory [11]. There will appear different result of measure under the different condition of measure, such as tip, humidity of environment and so on. The difference of the height of duplex loops in Fig.2 maybe the different humidity of air.

So we should compare the height of different DNA loop in the same images to eliminate these influence.

3.3. Effects of psoralen cross linking on *p53* gene expression

Above results showed psoralen might bind the site of TRF2. Inhibition of TRF2 results in activation of a double strand break checkpoint that includes signaling through ATM and p53 and induces apoptosis in some cells. To identify what would be a possible mechanism underlying the effects of psoplen on cell growth and apoptosis, We have examined the dose-dependent effect of psoralen on *p53* expression, Results showed that psoplen up-regulated the level of *p53* protein. The results from densitometer analysis suggested that up-regulated *p53* protein by psoralen is 1.8 fold higher than that of control. Cells. were treated with *P53* mRNA induction was observed at the 30 min and 60 min time points. Induction of p53 mRNA was in the 1.5-2.0-fold range.

These data indicate that 1). telomeric DNA in human cells do or D-loop-T-loop-G quartet structure. 2) The telomeres can be blocked selectively by psoralen /UVA irradiation, suggesting such psoralen derivative could be used to control gene expression or to induce site-directed damages, and 3) AFMs might be good probes to demonstrate the formation of telomeric DNA in living cells.

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