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Tau could protect DNA double helix structure

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Abstract

The hyperchromic effect has been used to detect the effect of tau on the transition of double-stranded DNA to single-stranded DNA. It was shown that tau increased the melting temperature of calf thymus DNA from 67 to 81 °C and that of plasmid from 75 to 85 °C. Kinetically, rates of increase in absorbance at 260 nm of DNA incubated with tau were markedly slower than those of DNA and DNA/bovine serum albumin used as controls during thermal denaturation. In contrast, rates of decrease in the DNA absorbance with tau were faster than those of controls when samples were immediately transferred from thermal conditions to room temperature. It revealed that tau prevented DNA from thermal denaturation, and improved renaturation of DNA. Circular dichroic spectra results indicated that there were little detectable conformational changes in DNA double helix when tau was added. Furthermore, tau showed its ability to protect DNA from hydroxyl radical (·OH) attacking in vitro, implying that tau functions as a DNA-protecting molecule to the radical.

Keywords: Tau; DNA; DNA-protecting protein; DNA double-stranded structure

1. Introduction

Tau is a major microtubule-associated protein of the neuron [1]. A major known function of tau is its role in the promotion of assembly and the maintenance of microtubules that are required for axonal transport and morphogenesis [2]. In addition to its association with microtubules, a major location, tau has been found to be associated with ribosomes [3,4] and has been shown to be localized in nuclei of human neuroblastoma cells, human cervical carcinoma, human macrophages, monkey kidney and PC12 cells [5–7]. Its localization has been observed at the nucleolar regions of the acrocentric chromosomes of human neuroblastoma cells, associated with both fibrillar regions of interphase nucleoli and the nucleolar organizer regions [5,8]. Microtubule-associated proteins have been shown to have a higher affinity to DNA than to microtubules and their removal from the microtubules by DNA causes microtubule breakdown [9]. Separation of tau from LA-N-5 nuclei in the chromatin fraction has indicated the association of nuclear tau with DNA either directly or indirectly [10]. Previously, we showed that phosphorylated tau catalyzed by neuronal cdc2-like kinase could associate with DNA detected by electrophoretic mobility shift assay [11]. Similar to native tau, phosphorylated tau could still increase the melting temperature (T_m) of calf thymus DNA (CTDNA). However, when tau was aggregated, neither native tau nor phosphorylated tau kept the ability to interact with DNA, suggesting that binding of tau to DNA was in an aggregation-dependent, and a phosphorylation-independent, manner. Nevertheless, the physical meaning of tau binding to DNA is still unclear. The potential function of tau interacting with DNA needs further investigation.

DNA is made up of two polynucleotide chains wound about each other to form a double helix. The chains are held together at intervals by hydrogen bonds [12]. When a DNA solution is heated enough, the noncovalent forces that hold the two strands together weaken and finally break. When this happens, the two strands come apart in a process known as DNA denaturation, and the temperature at which the DNA strands are half-denatured is called the melting temperature, or T_m [13]. The thermal denaturation and renatu-

Abbreviations: T_m, melting temperature; phen, phenanthroline; ROS, reactive oxygen species; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; BSA, bovine serum albumin; CD, circular dichroism; CTDNA, calf thymus DNA

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ration, so far, have been widely used to study the interaction of protein with DNA, for example, investigating winding and unwinding proteins [14,15]. Furthermore, circular dichroism (CD) was employed to detect whether the interaction of DNA with a protein could perturb the secondary structure of DNA.

In addition, there is a considerable amount of evidence revealing an association between DNA structure damage and some human problems, such as cancer and aging [16,17]. Now that tau has been found to bind to DNA, we are interested in whether or not this neuronal protein can protect DNA structure. Fortunately, the luminescence is widely used in antioxidation evaluation [18]. The oxidative DNA damage induced by phenanthroline (phen)-Cu/ascorbate/H_2O_2 leads to light emission, which can be used as an indicator for evaluating the extent of DNA damage. In the present study, we show that human neuronal tau can prevent DNA from thermal denaturation, improve renaturation and protect DNA from damage induced by free radicals.

2. Materials and methods

2.1. Materials

Sephadex G50, Q-Sepharose and SP-Sepharose were purchased from Pharmacia. CTDNA (readily soluble, suitable as substrate in DNase assays) absorbance was measured with a Perkin-Elmer Lambda-12 VIS/UV spectrophotometer. CD spectra were carried out on JAS-CO J-720 spectropolarimeter.

2.2. Expression and purification of recombinant human neuronal tau40

The prokaryotic expression vector Prk172, bearing tau_{441}, was kindly provided by Dr. Goedert (Medical Research Council, Molecular Biology Unit, Cambridge, UK). Tau_{441} was purified in queues with Q-Sepharose, SP-Sepharose and Sephadex-G75 columns and the concentrations of the purified protein were determined spectrophotometrically by using $E^{280}_{mg/ml} = 0.27$ [19,20].

2.3. CD absorption spectra

CD spectra were obtained in 0.1-mm-path length cells at room temperature (RT), and recorded from 300 to 230 nm, at a scanning rate of 50 nm/min. CD was expressed as θ, the measured ellipticity in degrees. Each spectrum was the average of four collections.

2.4. Thermal denaturation of DNA

DNA samples were incubated with the protein at desired temperatures (25, 40, 50, 60, 70, 80 and 90 °C) for 10 min, followed by measurements of the absorbance (260 nm) of the mixture. The data were analyzed by the Origin 5.0 application software and differential method.

2.5. Kinetic measurements of DNA denaturation

A cuvette was incubated in an ultraviolet spectrophotometer and the temperature was kept by a thermal bath at 80 °C. When a sample was added into the cuvette, the absorbance at 260 nm was immediately measured for 20 min. All the data were analyzed as described previously [21].

2.6. Kinetic measurements of DNA renaturation

Samples were incubated at 100 °C for 10 min. The absorbance at 260 nm was recorded for 20 min, following the addition of the sample into the cuvette at room temperature. All the data were analyzed as described previously [21].

2.7. Determination of the effect of tau on the antioxidation

According to Ma et al. [18], copper, ascorbate and 1,10-phenanthroline were premixed in 0.1 M NaOAc/HOAc (pH 5.2) buffer; tau at different concentrations was premixed with DNA for at least 20 min in NaOAc/HOAc buffer solution. Samples were incubated with phen-Cu/ascorbate at 37 °C for 5 min. Afterwards, H_2O_2 was added to the solution to give a final volume of 1.2 ml. The chemiluminescence produced in the phen-Cu/H_2O_2/ascorbate system was immediately recorded with a computerized high-sensitivity single-photon counter (type BPCL-4, manufactured at the Institute of Biophysics, Academia Sinica, China). The voltage in the photomultiplier was kept at 1000 V.

3. Results

3.1. Effect of tau on T_m of DNA

To detect the hyperchromic effect of tau on the transition of double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA), it was necessary to investigate whether the absorbance of tau would interfere with that of DNA at 260 nm measurements during thermal denaturation. Fortunately, the absorbance (240–300 nm) of tau at the concentrations used in this study was less than 0.05 at room temperature (data not shown). Such a low absorbance was mainly because the primary structure of tau did not contain any Trp residues [22], and thus, the absorbance of tau is in background.

Through measurements of the hyperchromic effect with ultraviolet spectroscopy, we found that adding tau to solutions of CTDNA increased the T_m from 67 to 81 °C (Fig. 1A). The melting transition was shifted upward by 14 °C as the input protein concentration was increased, with little

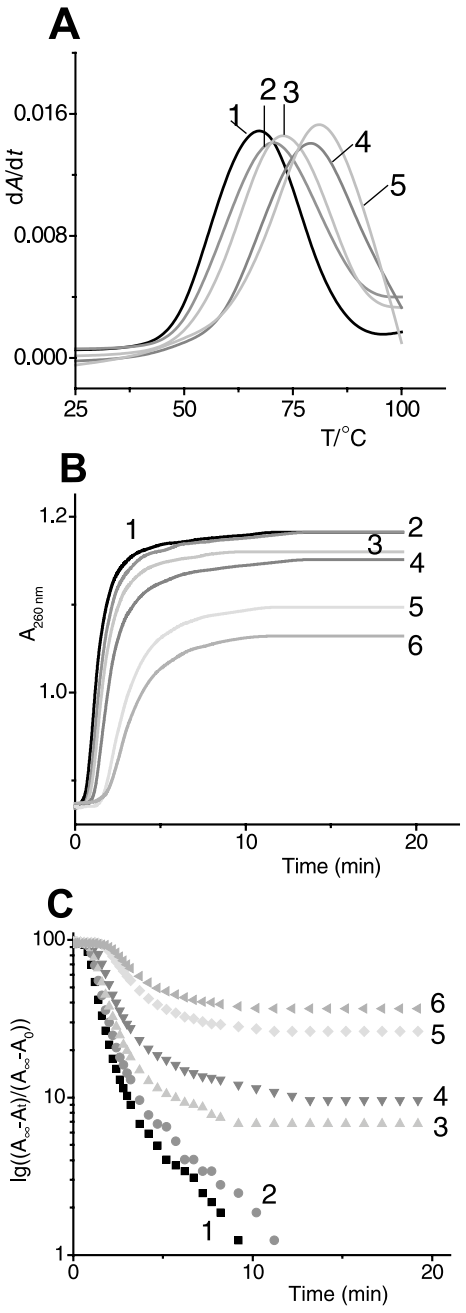

Fig. 1. (A) Heat denaturation of calf thymus DNA at different concentrations of tau. Fifty micrograms per milliliter DNA (final concentration) in 10 mM Tris–EDTA buffer (pH 8.0) was heated and kept for 10 min at desired temperatures, and the absorbance at 260 nm was measured. The ordinate (d*A*/d*t*) represented the differential of the absorbance at 260 nm before and after heat treatment. Curve 1, DNA control; curves 2–5 represented DNA with tau at tau/DNA mass ratios of 0.1, 0.25, 1 and 2, respectively. (B,C) Time kinetics of thermal denaturation of calf thymus DNA in the presence of tau. (B) Forty-two micrograms per milliliter DNA with protein at different concentrations in 10 mM Tris–EDTA buffer (pH 8.0) was added and incubated at 80 °C, and the absorbance at 260 nm was measured. All ratios were by weights. Curves 1 through 6 were represented as follows: DNA with BSA (BSA/DNA = 1), DNA control, DNA with tau at the ratio of tau/DNA = 0.1, 0.25, 1 and 2, respectively. (C) The same data were plotted in semilogarithm according to Tsou [21].

change in melting profile shape. Similar results were obtained when tau was added to pBluescript-II SK whose T_m rose from 75 to 85 °C [23]. These studies suggested that the double strands of both CTDNA and pBluescript-II SK were stabilized by tau. Some eukaryotic DNA-binding proteins, such as HMG_1 and HMG_2, have been shown to display similar properties. In those cases, addition of HMG1 or HMG2 could cause the T_m of T7 DNA to shift to a higher temperature [24,25].

3.2. Effect of tau on denaturation of DNA

Kinetics of thermal denaturation of CTDNA alone or incubated with bovine serum albumin (BSA) as control was a biphasic change, with a fast and a slow phase (Fig. 1B). Such a biphasic denaturation, which occurred over a narrow temperature range [26], might have resulted from the cooperativity among the hydrogen bonds of the complementary bases between the double helix of DNA when it was unwinding during denaturation. As a result, the double helix unwound to form two single strands. Theoretically, the whole unwinding procedure should contain a short relaxation phase, followed by a fast cooperative phase and a slow phase. The relaxation phase was a transient procedure, which was hardly detected by the conventional methods. Thus, just two phases were exhibited, the cooperative unwinding phase and the following slow phase in case of DNA alone or with BSA.

However, kinetics in denaturation of CTDNA incubated with tau was a triphasic procedure: an initial slow phase, followed by a fast and a slow phase. The initial slow phase could correspond to the relaxation phase of DNA unwinding, which was readily detectable by conventional ways in DNA solutions containing tau, suggesting that tau probably extended the relaxation phase when binding to DNA (Fig. 1C). The rate of the fast phase was at least 20 times greater than that of the relaxation phase, and 30 times greater than the following slow phase when the mass ratio of tau/DNA was 2:1 (Table 1). At this ratio, the kinetic rate of the fast

Table 1
The first-order rates of increase in the absorbance at 260 nm of CTDNA incubated with $htau_{441}$ at different concentrations during thermal denaturation

Tau/DNA (mass ratio)	First phase rate	Second phase rate	Third phase rate
0.0	–	133.50 ± 4.50	51.65 ± 5.05
0.1	14.55 ± 2.25	56.70 ± 5.10	4.83 ± 0.38
0.25	14.89 ± 7.21	50.80 ± 2.80	3.65 ± 1.19
1	2.86 ± 0.51	32.75 ± 0.35	1.37 ± 0.05
2	1.14 ± 0.57	22.70 ± 1.50	0.71 ± 0.08
BSA/DNA = 1	–	136.00 ± 1.00	49.45 ± 0.75

Cuvette was incubated in an ultraviolet spectrophotometer and the temperature was kept by a thermal bath at 80 °C. When a sample was added into the cuvette, the absorbance at 260 nm was measured (within 20 s) for 20 min. All the data were analyzed according to Tsou [21]. For sake of convenience, all the kinetic rates (means ± S.D.) were in 10^4 s^{-1}. The concentration of DNA was 42 μg/ml.

phase was at least five times slower than that of DNA alone. Thus, it appeared that tau affected the unwinding of DNA, not only by extending the relaxation phase but also by reducing the cooperative procedure. We speculate that tau might inhibit the heat-induced breakage of the cooperativity among the hydrogen bonds of the paired bases. Similarly, the slow phase following the rapid phase was also distinguishably reduced when tau was added (tau/DNA = 2:1), with the first-order rate being at least 70 times slower than that of DNA alone. Under the same conditions, BSA as control had little effect on unwinding of the double strands. The fact that tau extended the relaxation phase and affected both the cooperative fast and the following slow phases during DNA unwinding, suggested that it could prevent DNA from thermal denaturation.

3.3. Effect of tau on renaturation of DNA

According to Lehninger et al. [27], renaturation of DNA was a rapid one-step process. We investigated the effect of tau on renaturation of thermally denatured DNA when immediately transferred to room temperature. We found that renaturation of CTDNA was a rapid biphasic procedure with a fast and a slow phase (Fig. 2A, Table 2). The slow phase might suggest a further refolding of the DNA, following the formation of the double helix. The rate of the fast phase of DNA refolding incubated with tau (tau/DNA = 2:1) was at least eight times greater than that of the control (Fig. 2B). Under the same condition, the rate of the slow phase was 15 times greater than that of the control. Thus, tau distinguishably improved DNA refolding on renaturation after thermal denaturation, in the way of accelerating both the fast phase and the slow phase. The fact that tau could discriminate the double strands from single strand and then bind to DNA implied that the native ⇆ denatured DNA equilibrium was progressively shifted from the denatured to the native form, induced by tau.

Table 2
The first-order rates of decrease in the absorbance at 260 nm of CTDNA incubated with $htau_{441}$ at different concentrations during renaturation

Tau/DNA (mass ratio)	Rates of fast phase	Rates of slow phase
0.0	5.82 ± 1.89	0.94 ± 0.09
0.1	28.10 ± 1.10	1.40 ± 0.04
0.25	37.35 ± 0.85	2.15 ± 0.03
1.0	40.90 ± 1.25	3.03 ± 0.06
2.0	50.35 ± 3.85	14.10 ± 0.90

Samples were incubated at 100 °C for 10 min before annealing (renaturation) was performed. The absorbance at 260 nm was detected within 20 s, following the addition of the sample into the cuvette at room temperature. All the data were analyzed according to Tsou [21]. For sake of convenience, all the kinetic rates (means ± S.D.) were in 10^3 s^{-1}. The concentration of DNA was 40 μg/ml.

3.4. CD measurement of DNA incubated with tau

To investigate whether the interaction of DNA with tau could perturb the secondary structure of DNA (backbone conformation and vicinal base–base interactions) [28], we conducted CD of DNA complexed with tau. Optical activity,

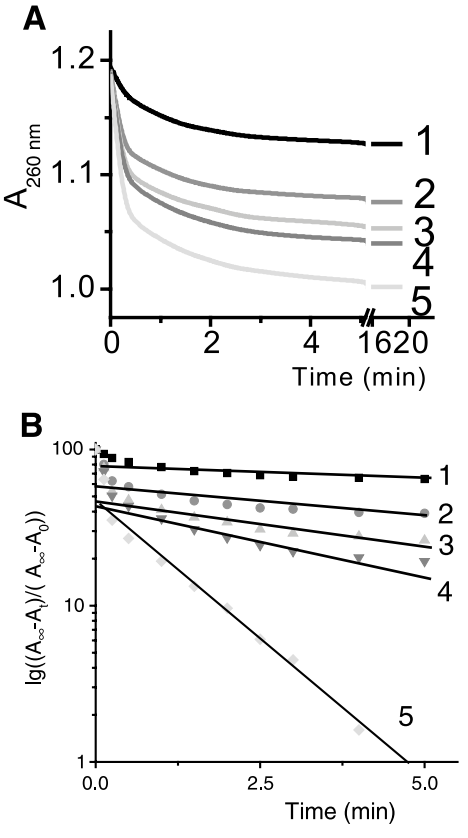

Fig. 2. Kinetics of renaturation of calf thymus DNA with tau when thermal conditions were removed. (A) Renaturation curves of CTDNA (40 μg/ml) with tau at different concentrations. All ratios were by weights. Curves 1 through 5 were as follows: DNA control, DNA with tau at the ratio of tau/DNA = 0.1, 0.25, 1 and 2, respectively. (B) The same data were plotted in semilogarithm according to Tsou [21].

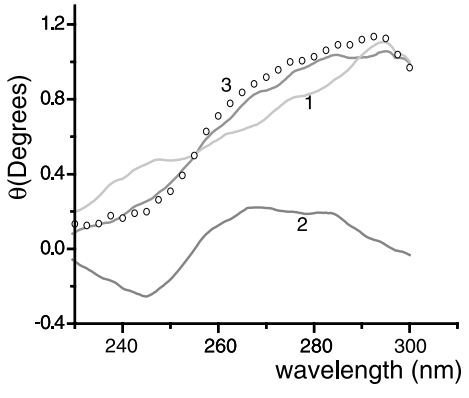

Fig. 3. CD spectra of calf thymus DNA, tau and DNA/tau complex. Concentration of calf thymus DNA and tau were 80 μg/ml. Samples were resuspended in 25 mM Na_2HPO_4, 25 mM NaH_2PO_4, pH 7.2, RT. Curves 1 through 3 represented tau alone, CTDNA alone and DNA–tau complex, respectively. Circles were the sum of the control DNA and protein ellipticities calculated at 2.5-nm intervals across the spectrum.

as measured by CD absorption spectroscopy, was sensitive to details of local conformational changes in the secondary structure. In our experiments, no changes could be detected in the CD spectrum of CTDNA in near ultraviolet. The complex spectrum closely approximated the sum of the ellipticities contributed by protein and DNA alone, suggesting that the binding of tau might not induce any significant conformational deformation of CTDNA double helix under these solvent conditions at room temperature (Fig. 3). Similar results were obtained when plasmid T7TxRX1 was employed (data not shown). That is to say, although neuronal tau facilitated DNA folding, it could not change the double helix structure. Similar property was reported when some DNA-binding proteins, such as HMG1 and HMG2, were studied by Yu et al. [25]. These two proteins had been demonstrated as DNA chaperones [29].

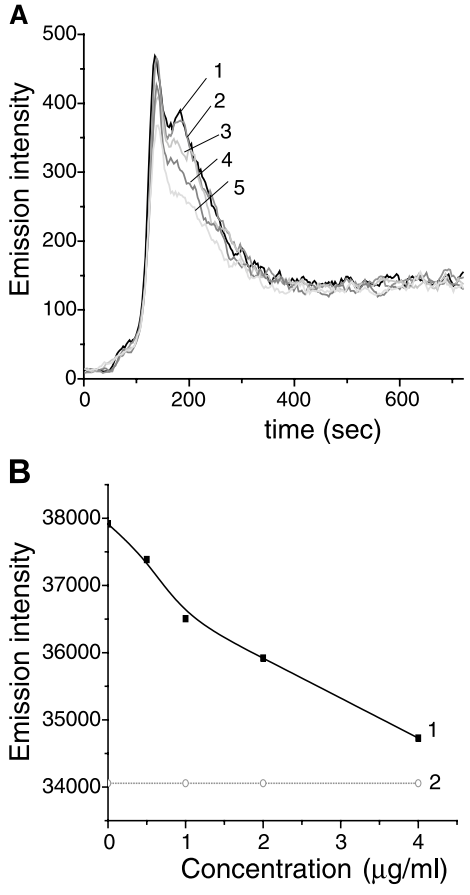

Fig. 4. Effect of tau on the chemiluminescence of DNA. (A) Chemiluminescence profile of DNA (1 μg/ml) incubated with tau protein in the phen-Cu/H_2O_2/ascorbate system. One point was recorded every 3 s, which represents the total intensity of this time interval. The final concentrations of tau were 0 (curve 1), 0.5 μg/ml (curve 2), 1 μg/ml (curve 3), 2 μg/ml (curve 4) and 4 μg/ml (curve 5). The concentrations of other reagents were phen, 3.5×10^{-4} M; $CuSO_4$, 5×10^{-5} M; H_2O_2, 0.5%; ascorbate, 3.5×10^{-4} M. (B) Plot of chemiluminescence lightsum (720 s) vs. tau concentration. The same data from (A) were analyzed by integration. Curves 1 and 2 represent the effect of tau protein and the background luminescence (solution without DNA).

3.5. Antioxidation evaluation

As we know, DNA is an important information-stock macromolecule whose function depends on its exquisite structure. There is a considerable amount of evidence revealing an association between DNA damage and dysfunction of cells [16,17]. Whereupon, we were interested in if tau could protect DNA from damage induced by free radical, for instance, reactive oxygen compounds. The free radical might attack and damage DNA, lipids and proteins [30]. We employed the phen–copper complex as a model, described previously by Ma et al. [18], for studying the effect of tau on the oxidative DNA damage. The system can produce hydroxyl radical (·OH) and induce several kinds of DNA damage in the presence of hydrogen peroxide and ascorbate, including single-strand breaks, modified bases, and nonbasic sites [31]. The emission intensity of chemiluminescence was related to the level of DNA damage [18]. The chemiluminescence concomitant with phen-Cu^{2+}/ ascorbate/ H_2O_2-induced DNA damage and protection effect of tau was shown in Fig. 4. The luminescence was greatly decreased on addition of tau, which bound specifically to DNA in solution (Fig. 4A). That is to say, the decrease was in a tau concentration-dependent manner. At the same time, tau had little influence on the background luminescence (Fig. 4B), suggesting that the effect of tau here was not scavenging reactive oxygen species (ROS) directly but preventing DNA from being attacked in vitro, because the background light emission was observably caused by ROS.

4. Discussion

Tau is found as a major neuronal microtubule-associated protein that promotes assembly and stabilizes the structure of microtubules [1]. Subsequent studies have shown the presence of tau in nuclei of neuronal cell lines [5–10,32], and the binding of tau to both eukaryotic DNA and phage DNA [9]. However, the function of tau in the neuronal nuclei has been unknown. In the present study, we show that tau could stabilize DNA double helix by preventing its denaturation and accelerating its refolding to the native conformation, and could protect DNA from hydroxyl radical (·OH) attack. In short, tau could protect DNA double-helical structure. Here, we suppose that tau might be a DNA-protecting protein, at least in vitro, aside from a microtubule-associated protein.

We have found that neuronal tau has the capability of increasing the T_m of DNA from both prokaryote and eukaryote, and kinetically prevents DNA from denaturation. Furthermore, this protein could accelerate the renaturation procedure of DNA from a thermal-denatured state. The findings presented here indicate that tau binds to DNA with an effect of stabilizing the double strands. Such interaction may not induce a further conformational change in DNA

double stands, similar to the results obtained from HMG family proteins [25]. Furthermore, there is increasing evidence that free radical-induced oxidative damage may play a role in the pathogenesis of Alzheimer's disease. Using phen–copper complex as a model for studying oxidative DNA damage, we have shown that tau acts as a safeguard to protect DNA structure directly.

As described previously [29], tau has some interesting features during interaction with DNA: (1) to bind to dsDNA without any discrimination of its nucleotide sequence (our unpublished work); (2) to stabilize DNA by preventing its denaturation and accelerating its refolding to double helix; and (3) to bind to DNA reversibly and allow itself replacement from the double strands of DNA by histones (our unpublished results). That is to say, tau protects DNA double-helical structure. It appears that nuclear tau might act as a DNA chaperone-like function.

The results presented here and elsewhere establish tau as a multifunctional protein. Although the function of nuclear tau is a matter of speculation, its localization to the NORs and the fibrillar component of the nucleolus is of particular significance because these regions contain the rRNA genes [5]. Hence, it appears that tau may play a role in initiating nucleolar reformation and/or mediating the onset of rRNA synthesis. Nevertheless, effects of reagents or factors on DNA damaging need further investigation. Here, we suppose the novel role of tau as a DNA-protecting protein, besides promoting assembly of microtubule and stabilizing microtubule system.

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