A site and strand specific nuclease activity with analogies to topoisomerase I frames the rRNA gene of *Tetrahymena*

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ABSTRACT

Exposure of macronuclear chromatin from *Tetrahymena thermophila* to sodium dodecyl sulfate causes an endogenous nuclease to cleave the extra-chromosomal rDNA at specific sites. All cuts are single-strand cleavages specific to the non-coding strand. Three cleavages map in the central non-transcribed spacer of the palindromic molecule at positions -1000, -600 and -150 bp with respect to the transcription initiation point. A fourth site is located close to the transcription termination point, while no cleavage is observed in the coding region. The position of each cleavage is in the immediate neighbourhood of DNase I hypersensitive sites. Additionally, certain DNA sequence motifs are repeated in the region around the cleavages. Upon cleavage induction a protein becomes attached to the rDNA. Our results indicate covalent binding to the generated 3' end, in analogy to the aborted reaction of topoisomerase I.

INTRODUCTION

Currently much effort is directed towards establishing functional relationships between chromatin structure and transcriptional activity. One approach has been the exposure of chromatin to various nucleases¹⁻¹⁵. Extended regions spanning many actively expressed genes show a generally high susceptibility to DNase I¹⁻⁴ and specific short stretches located at the 5' and 3' ends (where transcriptional control signals are expected to reside) are extremely sensitive⁴⁻¹². The molecular basis for the hypersensitivity has not been elucidated so far, however, irregularities in the nucleosome array, absence of nucleosomes or presence of specific classes of proteins have been suggested^{4,3,12}. Where tested, most of the DNase hypersensitive sites also proved to be sensitive towards the single-strand cutting activity of nuclease S₁^{13,14}. Plasmids carrying

eukaryotic genes with known potential to form hypersensitive sites show S_1 sensitivity at almost the same positions when supercoiled, but not when relaxed¹⁴. Based on these data Weintraub and colleagues concluded that the DNA sequences at these sites contain the potential to form altered secondary structures such as local melting, cruciforms or left handed DNA. Such altered structures, activated or regulated through different degrees of supercoiling, might serve regulatory roles as binding sites for specific proteins of the transcriptional apparatus.

We are studying chromatin structure and transcriptional properties of the genes coding for ribosomal RNA in the macronucleus of the unicellular protozoan Tetrahymena^{10,11,16-18}. The rRNA genes in T.thermophila are situated on extra-chromosomal giant palindromes which contain two transcribed regions framed by a central non-transcribed spacer and two terminal non-transcribed regions (see Fig.1¹⁹⁻²¹). Transcription proceeds in the outward direction¹⁸. We consider the analysis of the chromatin structure of the spacers of particular importance, since regulatory elements for transcription initiation and termination are expected to be localized there²². Recently, we described the existence of defined DNase hypersensitive sites in these regions¹⁰. In addition, we have found that exposure of macronuclei to SDS induces site-specific cleavages in rDNA which map within 100 bp of the hypersensitive sites^{10,11}. In this report we demonstrate that the SDS induced cleavages are single-stranded and specific to the non-coding strand. Furthermore, we demonstrate that protein becomes linked to the rDNA upon cleavage induction. These observations remind of properties of the topoisomerases²³⁻²⁶ or the plasmid relaxing proteins described by Helinski and coworkers^{27,28}.

MATERIALS AND METHODS

Preparation of nuclei. Macronuclei were prepared from early log-phase cells ($\sim 5 \times 10^4$ cells/ml) of Tetrahymena thermophila, strain B1868-7 by cell lysis in non-ionic detergents as described^{16,10}. Phenylmethanesulfonyl fluoride was present in all buffers at a concentration of 0.2 mM.

Cleavage induction, preparation of DNA. SDS (final 1%) and EDTA (final 10 mM) were added to freshly prepared nuclei suspended in nuclei buffer (NB: 10 mM Tris-HCl, pH 7.2, 0.1 M sucrose, 3 mM CaCl₂, 1 mM MgCl₂) at a density corresponding to approx. 0.1 mg DNA/ml. The suspension was mixed carefully for 5 min before NaCl was added to a final concentration of 0.8 M. Inhibition of cleavage in control experiments was achieved by adding NaCl before SDS. The lysate was deproteinized with 200 µg/ml proteinase K (Merck) for 2 hrs at 60 °C followed by sequential extraction with phenol/chloroform/isoamylalcohol (24:24:1) and chloroform/isoamylalcohol (24:1). After ethanol precipitation RNA was digested with 20 u/ml RNase A for 30 min at 37 °C followed by another round of organic extraction and ethanol precipitation. Finally, the DNA was dissolved in TE (10 mM Tris pH 8.0, 0.5 mM EDTA). Restriction of DNA was performed according to suppliers (Biolabs, Amersham, Boehringer) instruction. Digestion with S₁ nuclease (Sigma type III) was done (~0.5 u/µg DNA 20 min at 37 °C) after addition of S₁ buffer (50 mM sodium acetate pH 4.5, 150 mM NaCl, 2 mM ZnSO₄) directly after restriction or after boiling and rapid cooling of unrestricted DNA (snap back formation¹⁰).

Electrophoresis and Southern blotting/hybridization. Electrophoresis was done in horizontal agarose gels submerged in Tris phosphate buffer (36 mM Tris, 30 mM NaH₂PO₄, 1 mM EDTA, pH 7.8) or denaturing gel buffer (30 mM NaOH, 2 mM EDTA). DNA was blotted onto nitrocellulose filters (Schleicher & Schüll, BA 85) according to the method of Southern²⁹. ³²P labelled hybridization probes were prepared by nick translation according to Rigby et al.³⁰ for the plasmid probes. M13 probes were prepared with the hybridization probe primer and Klenow enzyme (PL Biochemicals) according to suppliers instruction. Hybridization and further processing has been described previously¹⁰.

Hybridization probes. Plasmid pTth1 (named pI see Fig.1) originated from E.Blackburn and pJE 298 (pII) from Jan Engberg. Plasmid pEG01 (pIV) was prepared by insertion of the gel purified 4 kb Pst fragment of B7 rDNA into the Pst site of pBR322. Single-strand specific probes (mII-mVI) were prepared by cloning Hind III-EcoRI double digested pJE298 or Hind III-

Pst I double digested pEG01 into the replicative forms of M13 mp7 or mp8³¹ digested with the appropriate enzymes. Verification of the insert was done by Southern blotting/hybridization of restricted rDNA to the labelled plasmid and M13 probes. Strand specificity was verified by dot blotting³² of the M13 DNA and hybridization to 5'- and 3'-end labelled restriction fragments from pJE298 or pEG01.

Preparation of DNA from interphase. Nuclear lysates were prepared as described above. The samples were extracted with phenol after omission of the protease treatment and the water-phase was reextracted once with phenol/chloroform/isoamylalcohol (24:24:1) and once with chloroform/isoamylalcohol (24:1) and precipitated. The organic + interphase of the first phenol extraction was washed twice with 0.6 M NaCl and the interphase was precipitated with 2 vol ethanol. After resuspension in TE + 1% SDS both samples were RNase (20 u/ml; 1 hr), protease K (200 µg/ml; 1 hr) treated and extracted as usual. The waterphases were precipitated and redissolved in TE.

Filter binding assay.³⁵⁻³⁸ The nuclear lysates were extracted three times with chloroform/isoamylalcohol (24:1), precipitated and redissolved in TE. Hind III digestion was performed together with an RNase treatment. The digest was diluted to 3 ml in one of three solutions: a) neutral low salt buffer (10 mM Tris, pH 7.4, 20 mM KCl, 5% DMSO), b) denaturing low salt buffer (100 mM NaOH, 1 mM EDTA) or c) high salt buffer (20x SSC) (the DNA was alkali denatured before addition of 20 x SSC). The solutions were passed through a prewetted nitrocellulose filter (Schleicher & Schüll BA85, ø 2 cm) at a flow rate of approximately 1 ml/min and washed 3 times with the same solution. The filters from sample a) were dipped 20 sec in 0.5 M NaOH and 1 M NaCl and then in 0.5 M Tris, pH 8 and 1.5 M NaCl. The sample b) in the latter solution only. Thereafter, the filters were treated as in the Southern hybridization procedure.

RESULTS

The structure of the extra-chromosomal, highly amplified rDNA molecule from Tetrahymena thermophila is schematically

Figure 1. Schematic map of the extra-chromosomal rDNA molecule from *Tetrahymena thermophila*. Restriction sites (X: XbaI, H: Hind III, P: PstI, Hh: HhaI) and transcriptional map are from 39,47. Only selected HhaI sites are shown. The plasmid probes (pI-pIV) and strand specific M13 probes (mII, mV, mVI) are indicated.

Figure 2. Southern blot analysis of the central region of the rDNA after electrophoresis on a neutral 2% agarose gel. DNA samples from SDS or salt treated nuclei were digested with restriction enzymes (Hind III or PstI) and S_1 nuclease as indicated. The relevant restriction sites and the hybridization probe are shown in the schematic diagram. S_1 sensitive sites ($\blacktriangle$) (single-strand cuts) at positions (a, b, c) and the most prominent double-strand cut ($\blacklozenge$) at position (d) are indicated on the map. The bands derived from these cuts are designated with capital letters.

shown in Fig.1. The plasmid and M13 probes used in the Southern blotting/hybridization experiments are indicated.

SDS treatment causes site-specific cleavages of rDNA. When isolated macronuclei from exponentially growing cells of *T. thermophila* are exposed to a strong detergent in a low ionic strength buffer about half of the molecules receive single-strand cleavages at specific sites. An example of this phenomenon is given in Fig.2, where DNA from macronuclei (lysed in the presence of 1% SDS) is treated with restriction nucleases followed by the single-strand specific nuclease S_1 . After electrophoresis and Southern blotting the central region of the rDNA palindrome was visualized with probe pI. Fig.2 demonstrates that more than 50% of the molecules received site specific cleavages in the central spacer when Hind III (lane 2) or PstI (lane 6) digested rDNA from SDS exposed nuclei were treated with S_1 nuclease. If S_1 treatment was omitted, the majority (>95%) of the molecules appeared intact, when analysed on neutral gels (lanes 1,5). The cleavages were not observed when the nuclei had been incubated in 0.8 M NaCl prior to the SDS treatment (lanes 4,8). Below we will show that the S_1 sensitive sites represent single-strand breaks and are not due to S_1 sensitive altered secondary structure of the DNA helix.

All the S_1 generated bands in Fig.2 shift position when different restriction enzymes were used (lane 2 vs. 6). The Southern blot shown in Fig.2 was probed with probe pI spanning the whole center region (up to the second Hind III site). An identical picture was obtained with probe pII, which covers only a 300 bp stretch upstream of the first Hind III cut (not shown). Bearing in mind the palindromic nature of the molecule, these observations unambiguously locate the SDS induced cleavages and also indicate that very few molecules bear simultaneously two cleavages in the probed region. The two major cuts (designated a and b the schematic map of Fig.2) are located at 850 and 1250 bp distance from the center, corresponding to -1000 and -600 bp with respect to transcription initiation. The bands generated from these cleavages are designated by capital letters in the autoradiogram (e.g. A and A' are the corresponding fragments created from the cleavage at position a).

Figure 3. Southern blot analysis of the transcribed region and the non-transcribed terminal region after electrophoresis on a neutral 2% agarose gel. The DNA samples were restricted with either XbaI, PstI or HhaI and S_1 treated as indicated. Relevant restriction sites and the hybridization probes are shown in the schematic map. The single-strand cut at position (e) is indicated in the map. The positions of the bands derived from this cut are indicated in autoradiogram.

A further minor site (c) is located at 1700 bp from the center (-150 from the transcription initiation point) (lane 6). This site is difficult to see in the Hind III digest (Fig. 2, lane 2) due to less efficient transfer to the nitrocellulose membrane in the low molecular weight range. Better visualization of this cleavage is achieved by snap back/ S_1 analysis (See Fig. 7). A

minor fraction of rDNA molecules received also double-strand cleavages after SDS exposure (Fig.2, lanes 1,5). The most noteworthy bands (at position D) result from a cleavage very close to the center of the palindrome. After S₁ treatment only one slightly displaced band remains. This band is also observed in the salt pretreated sample, thus it is not generated by SDS activated nucleases.

To probe the rDNA molecules for further SDS activated cleavages outside of the non-transcribed central spacer, we restricted the DNA with XbaI, which generates a 9 kb band reaching from -700 to the end of the molecule. The Southern blot was hybridized with probe pII. Fig.3 shows that in the area corresponding to the transcribed region neither double nor single-strand cuts are observed, as the blot shows no bands corresponding to sizes up to 7 kb (Fig.3, lanes 1,2). There is only one further S₁ generated band (E, lane 2). The cleavage designated (e) in the schematic diagram maps close to the 3' end of the pre-35S rRNA. To obtain a more exact position, Pst I or Hha I digestion was used in connection with probe pIV (Fig.3 lanes 5-12). These studies locate the SDS activated cleavage within 100 bp of the end of the coding region. Independent of SDS treatment we observed double-strand cuts very close to the SDS activated cleavage. The fraction of molecules showing these double-strand cuts varied from almost none to ca. 10% between experiments. Presumably the bands are produced by unspecific nuclease digestion during isolation of chromatin, as also a DNase I hypersensitive site is found at the 3' end of the coding region ¹¹.

The SDS induced cleavages are single-strand cuts in the non-coding strand. The S₁ nuclease is known to cleave double-strand DNA at single-stranded nicks but it can also cleave at locally denatured regions ³³. To prove the presence of single-strand cuts in the rDNA from SDS treated nuclei and establish a possible strand specificity we analysed the rDNA from SDS or salt-treated nuclei on denaturing gels and used single-strand specific probes for hybridization.

Fig.4 demonstrates that the M13 probes complementary to either coding or non-coding strand visualize different bands

Figure 4. Southern blot analysis of the central region after electrophoresis on a 1.5% denaturing agarose gels. DNA samples from SDS or salt treated nuclei were Hind III or Pst I digested, divided in two and run in parallel. After blotting the filter was cut in appropriate stripes and hybridized with the M13 probes complementary to either the coding (mIIc) or non-coding (mIInc) strand as indicated. Lanes 9-12 are identical to lanes 5-8 but present a longer exposure. Other features as described in legend to Fig.2.

after digestion with restriction enzymes and Southern blotting. Comparison with Fig.2 indicates that the probe mIInc, which is complementary to the non-coding strand in a 270 bp region upstream of the first Hind III cut, hybridizes to the bands which have been designated A, B and C in Fig.2. The minor band C is visible only in the heavy exposure of the Pst digested sample. The probe mIIc, which is complementary to the coding strand hybridizes to their high molecular weight counter parts, which reach from the restriction cut over the palindromic

Figure 5. Southern blot analysis of the terminal region after electrophoresis on a 1.5% denaturing agarose gel. The DNA samples from SDS or salt treated nuclei were restricted with PstI or not restricted. The M13 probes complementary to the coding strand (mVc, mVic) or the non-coding strand (mVnc, mVinc) are depicted in the schematic diagram. Other features as described in legends to Figs 3 and 4.

centre to the SDS induced cleavage (A',B',C'). Because of the inverted repeat nature of the rDNA it has to be kept in mind that a probe specific to the non-coding strand on one arm of the palindrome visualizes the coding strand on the other arm and vice versa. This establishes the single-strand cleavages at positions -1000, -600 and -150 bp to be specific to the non-coding strand.

The strong overexposure of the Pst digested sample shows several minor cleavages on the coding strand in the region -1000 to -600 bp (Fig.4, lane 11). These sites probably correspond to the double-strand cuts which appeared in the non-denaturing gel (Fig.2, lane 5).

Indirect end labelling of the 4 kb Pst I fragment covering the termination region (Fig.5, lanes 1-4) proves also here that the SDS activated cleavage is specific to the non-coding

Figure 6. Southern blot analysis of the rDNA samples recovered from water and interphase after phenol extraction. Snap-back molecules of rDNA from SDS or salt-treated nuclei were prepared as described in the text (see also ref. 10) and electrophoresed on a neutral 2% agarose gel. The schematic diagram shows the snap-back molecule with the single-strand cleavages and the hybridization probe (pI). Other features as described in the legends to Figs 2 and 3.

strand. Lanes 5-8 of Fig.5 show an experiment, where unrestricted rDNA was probed with M13 probes downstream of the SDS activated cleavage. Together these data confirm that the cut is located 2.0 kb from the end of the molecule.

Protein becomes covalently attached at the cleavage sites. It has been reported ³⁴ that covalently linked protein-nucleic acid complexes accumulate in the interphase upon phenol extraction. In initial experiments we had also observed that a large fraction of the rDNA molecules from SDS treated nuclei but not from salt treated nuclei became trapped in the phenol interphase, if protease treatment was omitted.

We have taken advantage of the palindromic nature of the rDNA and analysed the DNA samples recovered from water or interphase by the snap back/S₁ method ¹⁰. Upon denaturation and

rapid renaturation the rDNA strands fold back on themselves. Downwards from a preexisting single-strand cut these "hairpin" molecules are single-stranded. Removal of this tail by S_1 digestion leaves a double-stranded DNA fragment, measuring the distance of the single-strand cleavage from the centre of the palindrome.

Samples from the water and corresponding interphase are shown in Fig.6. Aliquots derived from equal amounts of nuclei are electrophoresed on a neutral agarose gel. Hybridization to rDNA indicates that roughly equal amounts of cleaved rDNA are found in water and interphase from SDS treated nuclei, while the interphase from salt pretreated nuclei contains no appreciable amount of rDNA. Determination of total DNA content by in vivo ^3H labelling shows that the interphases from SDS and salt treated nuclei contain only very little DNA (3% and 0.3%, respectively, not shown). Thus, the interphase from the SDS sample is highly enriched in SDS induced cleavage products of rDNA. This allows the bands from the cleavages c and e to be more clearly seen in the interphase material (lane 3). In the waterphase the band E is completely lost due to unspecific degradation of the rDNA. In agreement with the report of Philipson and coworkers³⁴ we take these observations as an indication that upon SDS exposure, protein becomes attached to the cleaved rDNA.

To obtain further evidence we performed filter binding studies under low salt conditions, where protein-DNA complexes are known to be retained by nitrocellulose while uncomplexed DNA passes through^{35,36}. For these experiments we extracted the nuclear lysates with chloroform only, since we had observed that chloroform did not trap the cleaved rDNA complexes in the interphase. Before passage through the filters, the DNA was restricted with Hind III and aliquots were protease K treated. After filtration the filters were divided into 3 and the pieces hybridized to different probes. As can be seen in Fig.7 all filters bind equal amounts of rDNA under high salt conditions (20 x SSC). Under low salt conditions (Fig.7A) the central Hind III but very little of a fragment from the transcribed region from SDS treated nuclei was bound to the filter. Neither the

Figure 7. Filter binding of DNA under high salt and of protein-DNA complexes under low salt conditions. The DNA samples were Hind III digested before passage through the filter. Thereafter the filters were cut into sectors and hybridized with different probes. In part A the upper sectors (labelled SDS) represent the DNA samples from SDS treated nuclei, the left sectors (labelled SDS + prot.K) represent the same samples which have been proteinase K treated before passage through the filter, and the right sectors (labelled NaCl) represent the samples from salt treated nuclei. In part B all sectors represent samples from SDS treated nuclei. Filter binding is described in Materials and Methods. The salt conditions and hybridization probes are indicated on the figure. The schematic diagrams show the regions of the rDNA molecule to which the probes hybridize. In part B the strand specificity is indicated by the heavy bars.

sample from salt pretreated nuclei nor the protease digested sample from SDS treated nuclei was retained. Hybridization to the strand specific M13 probes showed that under denaturing low salt conditions only the probe mIIc, complementary to the coding strand, gives a positive hybridization signal (Fig.7B). This indicates that the protein must be bound on the SDS

generated fragments reaching from the Hind III site over the palindrome centre to the SDS activated cleavage (see Fig.4 for comparison). These fragments have the cleavage at the 3' end. Thus, it seems plausible that the protein is located at this end. This can also be inferred from the observation that exonuclease III is not able to degrade the cleaved rDNA starting from the single-strand nick (not shown)²⁴. The resistance of the DNA protein complex against heating to 60 °C in 1% SDS, 0.1 M NaOH (Fig.7, AB) and 6 M guanidinium chloride (not shown) strongly suggests a covalent linkage between protein and DNA.

DISCUSSION

We describe in this report the induction of site specific single-strand cleavages into the rDNA of *Tetrahymena* after exposure of the chromatin to a strong ionic detergent. Altogether about 50% of the rDNA molecules receive cleavages, the separate sites being present at different frequencies. Three cleavages are localized upstream of the transcription initiation point at positions -1000, -600 and -150 bp. The cut close to the initiation site shows considerably lower occurrence than the other two. No cleavage is observed in the coding region, but one further cleavage of intermediate frequency maps very close to the transcription termination site. All the cleavages are single strand cuts specific to the non-coding strand. The central region of the rDNA contains not more than one cleavage per molecule. Fig.8 shows a schematic summary of these observations.

Upon cleavage induction a protein becomes covalently bound to the DNA. The results suggest binding to or very close to the 3' end of the generated fragment. These observations are in line with reports on the abortive reaction of eukaryotic topoisomerase I²⁴⁻²⁶. Topoisomerases are involved in relaxation of superhelical DNA or other topological rearrangements. The reaction of topoisomerase I proceeds via a single strand incision and concomitant bonding of the enzyme to the DNA^{25,26}. Eukaryotic topoisomerase I binds to the 3' end of the cleaved DNA²⁴. The final step - strand closure and release of the protein - can be inhibited by disruption of the protein structure

Figure 8. Location of the SDS induced cleavages on the rDNA of *T. thermophila*. The symbols indicate single (▲) or double-strand cleavages (○). The frequency of cleavages is indicated by the size of the symbol. Hind III restriction sites are shown for orientation.

24-26. Examination of the DNA sequence around topoisomerase I cleavage sites on naked SV40 DNA showed that the enzyme does not recognize a unique sequence³⁷. Cutting occurred on the average approximately 12 bp apart at sites which showed only a slightly biased nucleotide distribution³⁷. Thus, if the cleavages of the rDNA of *Tetrahymena* are caused by a topoisomerase I like enzyme the site specificity must be generated by either additional properties of the enzyme not found in the purified topoisomerase I preparations or by signals lying in the chromatin structure. It has already been noted¹⁰ that the two major cleavages (at -1000 and -600 bp) are located in two 70 bp DNA stretches of ~80% homology. Both regions contain three Dde I recognition sites (R. Pearlman, personal communication). Also around the cleavage at the termination point three Dde I sites are situated, although in a more divergent background³⁹. In the region around the weak cleavage at -150 bp only one Dde I site is located (R. Pearlman, personal communication). Recently, we have mapped the cleavage at -1000 bp on a sequencing gel and found that the site actually consists out of three closely spaced cuts, each located equidistantly to one of the three DdeI sites (Bonven, Gocke and Westergaard, unpublished). These observations suggest a sequence directed location of the enzyme.

What could be the function of a site-specific topoisomerase I like enzyme? The framing of the transcribed region of the rRNA gene suggests a role in the process of transcription, as has been proposed for topoisomerases in other systems^{25,26}. On the rDNA palindrome the polymerase/nascent rRNA complexes

move outward in both directions simultaneously¹⁸. As the DNA cannot rotate both ways at the same time, stress accumulates at the centre of the molecule making an internal swivel mandatory. A relaxation point at the 3' end of the coding region would be needed if the ends of the palindrome are relatively fixed in the nucleolar matrix.

The strict association of the putative enzyme activity with the DNase I hypersensitive sites¹⁰ also could mean that the enzymes play a role in regulation of rRNA synthesis. Topological stress of the DNA template has been implicated in control of RNA synthesis in several instances: 1) In prokaryotes differential effects of changes of DNA supercoiling on gene expression have been reported⁴⁰. 2) Similar effects have been observed with extra-chromosomal plasmids carrying mating type genes in yeast (reported in 41). 3) The fraction of actively transcribed SV40 minichromosomes seems to be under torsional strain⁴². 4) Transcriptional activity in oocyte injection experiments is dramatically affected by the topology of the DNA fiber⁴¹. Weintraub and colleagues argue that the presence of altered secondary structures at DNase hypersensitive sites^{13,14,43} - presumably caused by localized supercoiling - indicates that maintenance of certain levels of topological stress around the hypersensitive sites could influence RNA production.

The topological stress exerted on the DNase I hypersensitive sites of the Tetrahymena rDNA could be regulated by the putative topoisomerase I like enzyme localized at these sites. The enzyme activity itself might in turn be controlled through some sort of feedback mechanism.

In SV40, topoisomerase I⁴⁴ as well as topoisomerase II⁴⁵ has been reported to be associated with the minichromosome. Topoisomerase II action involves a transient double strand break and aborted reactions have been induced by protein denaturation (SDS) in prokaryotes and eukaryotes^{25,46}. Thus, our observation of SDS induction of a low frequency of double strand cleavages in rDNA might be due to topoisomerase II like enzymes.

It will be interesting to see whether SDS induced site-

specific cleavages are restricted to rDNA genes or whether the localization close to hypersensitive sites is a general phenomenon. Furthermore, the high amplification of Tetrahymena rDNA and the possibility of preparing highly intact nucleoli from the macronuclei¹⁶ should facilitate a detailed study of the proteins bound at the cleavage sites.

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