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# Accessibility of Ribosomal Genes to Trimethyl Psoralen in Nuclei of *Physarum polycephalum*

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We have probed the accessibility of the genes for rRNA in *Physarum polycephalum* by using the photoreactive DNA cross-linking agent 4,5',8-trimethyl psoralen. Nuclei isolated from actively growing *Physarum* were treated with trimethyl psoralen and irradiated with 360-nm light in order to form cross-links. The palindromic, extrachromosomal rDNA then was isolated, and the positions of cross-links were determined by electron microscopy of the DNA under totally denaturing conditions. The results indicate that the frequency of cross-linking, after correction for base sequence bias of the reaction, is up to sixfold higher in the transcribed regions than in the central or the terminal spacer regions. There is no detectable heterogeneity among the different rDNA molecules or between the halves of a single molecule. Cross-linked molecules invariably occur in a linear as opposed to a cruciform structure. The preferential cross-linking of the transcribed region is nearly eliminated in spherules, a dormant transcriptionally inactive form in the *Physarum* life cycle.

The genes coding for ribosomal RNA (rDNA) in the acellular slime mold *Physarum polycephalum* are located on linear extrachromosomal DNA molecules of a discrete size, 60 kilobases (kb) (30). Each of the several hundred molecules per nucleus has the structure of a palindrome, with a transcription unit near each end separated by a large central spacer region. The facts that rDNA is repetitious and that it is unlinked to chromosomal DNA and differs from it in GC content facilitate the purification and therefore the study of these genes.

The structure of the repetitive unit common to all eucaryotic chromatin, the octamer of four different histones called the nucleosome, has been elucidated over the past several years (8, 16). Much less is known about the role of nonhistone proteins in chromatin, however, or about the features that distinguish those portions of the chromatin that are actively transcribed from those that are not. Both types of regions appear to contain nucleosomes, as defined by staphylococcal nuclease digestions (for example, see reference 32). At the same ionic strength, transcribed chromatin appears by electron microscopy to be more extended than nontranscribed chromatin (22, 23, 29). At least for certain vertebrate and invertebrate genes, an operational characteristic of transcribed chromatin is that it is preferentially attacked by DNase I (9, 10, 18, 21, 31, 35). The nonhistone proteins HMG-14 and HMG-17 appear to play an essential role in this DNase I sensitivity (32, 33). However, it is not certain that DNase I sensitivity is either a necessary or a sufficient condition for active transcription of chromatin. Experiments with yeast (17) as well as our own experiments with *Physarum* (Swofford and Vogt, unpublished results) failed to demonstrate differential DNase I sensitivity. Also, some chromatin has been found to be DNase I sensitive without concurrent transcription (18, 20, 26, 31).

Recently Hearst and collaborators (12, 34) and Cech and collaborators (2, 4, 5) have shown that the psoralen class of DNA cross-linking agents can serve as probes for chromatin structure in vivo. Trimethyl psoralen intercalates into DNA, and upon excitation with near UV radiation it forms convalent cross-links between the DNA strands. When the DNA of intact *Tetrahymena pyriformis* cells was cross-linked in this fashion and then analyzed by electron microscopy (2), links were found to occur predominantly at intervals of 200 base pairs and multiples thereof, consistent with preferential intercalation of the drug between nucleosomes. Furthermore, the rDNA showed cross-linking preferentially in the transcribed portion of the rDNA of this organism.

We have adapted the same methodology to study the differences in chromatin structure between the transcribed and the spacer portions of *Physarum* rDNA. We show that cross-linking occurs preferentially in the transcribed region,

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as in *Tetrahymena*. This preferential cross-linking is largely abolished in *Physarum* spherules, a transcriptionally inert form of this organism.

## MATERIALS AND METHODS

**Cultures and isolation of nuclei.** Liquid cultures of *P. polycephalum* strain CH918 (from C. E. Holt) were grown in semidefined medium (7). This colorless variant of *Physarum* was chosen because the pigment present in normal strains absorbs strongly at the wavelength required for psoralen cross-linking.

Nuclei were prepared by a modification of the procedure of Mohberg and Rusch (19). Harvested microplasmodia (5-ml packed volume) from a 2-day-old, actively growing liquid culture were washed once with distilled water and suspended in 150 ml of ice-cold lysis buffer (0.1% Triton X-100, 0.25 M sucrose, 0.01 M $CaCl_2$, and 0.01 M Tris-hydrochloride [pH 7.0]), and then blended at 0°C at high speed for 30 s in a Sorvall Omnimixer. After centrifugation at 5,000 rpm for 5 min in an SS-34 rotor the nuclei were suspended in about 50 ml of ice-cold lysis buffer.

**Cross-linking of nuclei and DNA.** Trimethyl psoralen (a gift of the Paul Elder Drug Co., Bryant, Ohio) was added to the above suspension of nuclei at a concentration of 2 mg/5 ml of packed plasmodia (equivalent to about 0.5 mg of DNA). The suspension was gently mixed in the dark at 0°C for 3 min to allow for trimethyl psoralen uptake into the nuclei and then irradiated for various amounts of time under a standard mercury vapor lamp (Sylvania H33GL 400/DX) at a distance of 10 cm. Irradiation took place through a 1-cm solution of 40% (wt/vol) $CoNO_3$ in a Pyrex dish to filter out most light of wavelength below 300 nm. Nuclei were constantly mixed during this period and kept on ice until they were lysed. Lysis took place no more than 15 min after homogenization of the microplasmodia. After irradiation the nuclei were collected by centrifugation, washed with cold distilled water, and suspended in 5 ml of 0.1 M EDTA (pH 7.5).

Trimethyl psoralen was added to purified deproteinized rDNA (25 μg/ml, prepared as described below) to a final concentration of 3 μg/ml. The solution was then irradiated for various amounts of time by a low-intensity, long-wave UV light source (two General Electric F20T12 BL bulbs). DNA was kept in the dark after treatment.

**Isolation of DNA.** Nuclei were lysed by the addition of sodium dodecyl sulfate to 2% and pronase to 1 mg/ml and then incubated for 30 min at room temperature. The DNA was spooled on a glass rod after the addition of 2 volumes of ethanol. It was suspended in 0.05 M EDTA (pH 7.5) and then centrifuged in a 5-ml KI density gradient (refractive index, 1.4213) at 33,000 rpm for 48 h in a 50 Ti rotor in the presence of 2 μg of the fluorescent dye Bisbenzimide H (Riedel-De Haen Ag, Seelze-Hannover) per ml. The denser rDNA band, visualized by near-UV light, was removed from the lighter chromosomal DNA band with a syringe and either centrifuged again in a second density gradient for repurification or dialyzed against Tris-EDTA buffer (0.01 M Tris-hydrochloride, 0.001 M EDTA, pH 7.5) and a small amount of Dowex-50 resin overnight at room temperature. After dialysis the purified rDNA was precipitated with ethanol and suspended in Tris-EDTA buffer.

For cross-linked samples, several extra steps were taken. Samples were kept in the dark after irradiation to prevent further cross-linking. Also, after being lysed, trimethyl psoralen-treated nuclei were phenol extracted once and then exhaustively extracted with chloroform-isoamyl alcohol (24:1) at 0.15 M NaCl to remove un-cross-linked TMP.

**Isolation of cross-linked DNA from spherules.** Microplasmodia (7.5-ml packed volume) were harvested from an actively growing liquid culture of strain CH918, washed two times with sterile distilled water, suspended in 50 ml of starvation salts medium (7), and continually shaken at 28°C in the dark. After 2 days microscopic examination indicated that spherule formation had occurred. At this time the spherules were washed with distilled water, placed in a Sorvall Omnimixer at full speed for 15 s to destroy remaining microplasmodia, and suspended in 30 ml of ice-cold lysis buffer without Triton X-100 (0.01 M Tris-hydrochloride [pH 7.0], 0.05 M sucrose, 0.01 M $CaCl_2$). All steps from this stage until lysis of nuclei were carried out on ice. The spherules were incubated with 2 mg of trimethyl psoralen for 30 min with gentle stirring, irradiated for 5 min with a mercury vapor lamp as described above, incubated with an additional 2 mg of trimethyl psoralen for 5 min, and irradiated for 5 min. The spherules were collected by centrifugation and suspended in ice-cold lysis buffer without Triton X-100 (total volume, 10 ml). They were broken by passage through a French press at 8,000 $lb/in^2$, and the nuclei were recovered by centrifugation for 5 min at 5,000 rpm in an SS-34 rotor. DNA was isolated from nuclei as described above.

**Electron microscopy.** Cross-links were visualized by denaturing DNA in the presence of glyoxal and spreading the DNA for electron microscopy essentially as described by Cech and Pardue (3). DNA (10 μl, approximately 25 μg/ml) was added to a solution containing 73 μl of 99% formamide, 10 μl of 0.1 M Na phosphate–0.01 M EDTA buffer (pH 6.9), and 7 μl of glyoxal (40% in water) and heated at 37°C for 60 min. Of this solution 10 μl was added to 43 μl of 99% formamide, 10 μl of 1.0 M Tris–0.01 M EDTA (pH 8.4), 30.5 μl of water, 5 μl of cytochrome *c* (1 mg/ml), and 1.5 μl of φ X174 DNA (3 mg/ml) and spread onto a 0.01 M Tris–0.001 M EDTA (pH 8.5) hypophase containing 17% formamide. After 1 min the DNA was picked up onto parlodion-coated copper grids, stained for 30 s in uranyl acetate ($5 \times 10^{-5}$ M in 90% ethanol), rinsed for 10 s in 90% ethanol, and rotary shadowed with 80 to 20% Pt-Pd. Molecules were traced with the aid of a Numonics electronic graphics calculator from projections of negatives made on a Phillips 301 electron microscope. Phage φ X174 DNA was included as a standard in each spread.

## RESULTS

**Cross-linking of nuclei from plasmodia.** The use of trimethyl psoralen to probe the chromatin structure of genes requires that the DNA be accessible to the drug and that after cross-linking the DNA be purifiable as an intact molecule of defined size. Most previous studies on psoralen cross-linking have employed living cells, since both procaryotes and eucaryotes

readily take up the drug. We found that *Physarum* plasmodia (the syncytial form of this slime mold) appear to be impermeable to trimethyl psoralen. In numerous experiments with DNA extracted from microplasmodia that had been treated with trimethyl psoralen and then irradiated, we saw no highly cross-linked DNA by electron microscopy under denaturing conditions. Neither increasing the times of irradiation nor increasing the external trimethyl psoralen concentration affected this result. Cultures grown in the presence of the drug for 1 h and then irradiated also contained DNA with too few cross-links to be useful for analysis. By contrast, *Physarum* nuclei, which can be easily and rapidly isolated from actively growing plasmodia, do take up trimethyl psoralen and form DNA cross-links. Preliminary experiments showed that at the drug concentration used, the frequency of cross-linking on unfractionated nuclear DNA increased with time over the range of 10 to 150 s of irradiation. Hence, detailed studies on the ribosomal genes were all performed on rDNA purified from intact nuclei that had been incubated briefly with trimethyl psoralen and then irradiated.

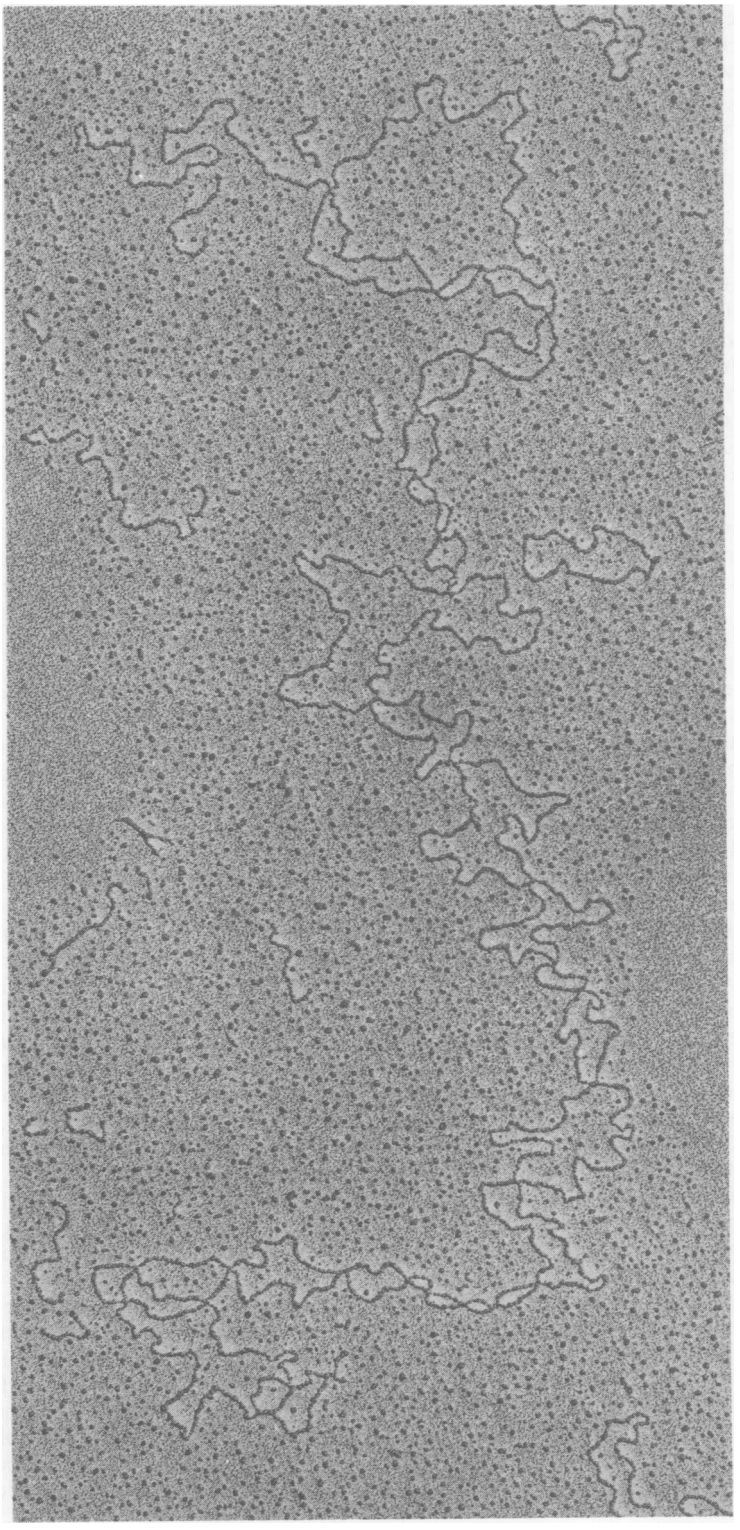
The cross-linking pattern of the rDNA was analyzed by measuring the point of each cross-link from one end of the DNA and then plotting its position in normalized units of 2% of the rDNA, or about 1.2 kb. An electron micrograph of a typical molecule is shown in Fig. 1. The criteria for inclusion of molecules in the statistics were that they be unambiguous to trace and that they be of correct size, about 60 kb. In the samples irradiated for 150 s, the average size was 61.6 kb, and the molecules had an average of 28 cross-links each. We selected this time of irradiation because the frequency of cross-linking was high enough to generate a large statistical sampling, but not so high that closely spaced links would lead to the apparent collapse of intervening single-stranded DNA. At this frequency distortion of the DNA in the nuclei due to the presence of intercalating TMP molecules should also be low.

The distribution of cross-links from 30 rDNA molecules is shown in Fig. 2A. It is apparent both from the micrograph and from this distribution that the central and terminal regions are more lightly cross-linked than are the other sections. Quantitation of this difference requires correction for any base sequence dependence of the reaction. The exact base sequence specificity of the photoreaction is unknown; long stretches of pyrimidines on one strand prevent cross-linking, whereas an alternating pyrimidine-purine sequence such as poly d(AT) · d(AT) favors the reaction (6). To correct for sequence bias, purified and deproteinized rDNA was also cross-linked at a frequency similar to that in isolated nuclei (29 cross-links per molecule). The distribution of cross-links for this sample is presented in Fig. 2B. The fact that this reaction was conducted in a solution not resembling the ionic environment of the nucleus is unlikely to affect the interpretation of this control experiment. It has been demonstrated for *Tetrahymena* rDNA that the sequence dependence of the cross-linking reaction is the same in Tris-EDTA as in a more physiological environment (2).

Two methods have been used to define a parameter, which we call accessibility, that quantifies the effect of chromatin structure on the susceptibility of specific portions of DNA to trimethyl psoralen-induced cross-linking. In one, for each interval on the DNA the normalized cross-linking frequency for naked DNA is subtracted from the normalized frequency for the DNA as a chromatin complex. In the second, the quotient of these two values is taken to represent accessibility of the DNA. If chromatin proteins affect cross-linking by exerting a shielding effect on the DNA, which seems likely, then the chromatin structure and the base sequences should act independently, and thus in a multiplicative way, to determine the final extent of cross-linking. Hence, we have chosen the second method to describe the frequency of cross-linking for *Physarum* rDNA.

Figure 2C displays this accessibility of the rDNA in nuclei to trimethyl psoralen. Comparison of this profile with the transcription map shown in Fig. 2D leads to the conclusion that the DNA sequences corresponding to the pre-rRNA, which extend from about 8 to 29% on the map (28), are about six times more accessible to the intercalating drug than the central spacer region and about four times more accessible than the terminal spacer. This conclusion is qualitatively similar to that drawn for *Tetrahymena* rDNA by Cech and Karrer (2). Unlike in *Physarum*, however, in *Tetrahymena* the central spacer is extremely small. The several-kilobase region proximal to the transcribed pre-rRNA sequences, 29 to 32% on the map in Fig. 2D, also appears more accessible to trimethyl psoralen than the spacer. Whether the drop in accessibility near 26% on the map is a significant feature of the chromatin or represents a statistical fluctuation is unclear to us.

Two other conclusions can be drawn from the appearance of the cross-linked rDNA molecules. The first is that in nuclei freshly prepared from growing plasmodia, the rDNA molecules do not assume any of the alternative secondary structures theoretically possible for inverted repetitious sequences. As naked DNA in solution, simple palindromic molecules can exist either as linear forms or as branched, intrastrand



base-paired "cruciform" structures. Due to extensive blocks of inverted repetitious sequences in the central spacer region, *Physarum* rDNA could also exist as more complex branched forms (30). Intrastrand cross-links formed during irradiation should stabilize any branches in palindromic DNA. However, in over 100 molecules of rDNA carefully examined, none showed any deviation from linearity under native or denaturing conditions. Since in the same sample cross-links occurred approximately every 2 kb, it is unlikely that branches larger than about 1 kb exist at an appreciable frequency in nuclei. This conclusion is consistent with the results of Cech and Karrer (2), who showed at a much higher level of resolution that *Tetrahymena* rDNA does not exist as cruciforms in vivo.

The second inference is that the population of rDNA molecules is homogeneous with respect to accessibility to trimethyl psoralen-induced cross-linking. If differences existed among the molecules in nuclei, then the relative standard deviation of the average number of cross-links within a given molecule should be larger for samples cross-linked in nuclei than for samples cross-linked as naked DNA. In fact, however, these standard deviations are similar—21% for the first class and 23% for the second. An analogous calculation can be made to show that the two halves of each nuclear rDNA molecule are equivalent. The average ratio of the number of cross-links in one half of the rDNA to cross-links in the other was 0.74 (relative standard deviation, 23%) for DNA cross-linked in nuclei. The same ratio for naked DNA was 0.82 (relative standard deviation, 19%).

**Cross-linking of spherules.** We wanted to compare the relative accessibilities of transcribed and spacer sequences under conditions where rDNA transcription is shut off. *Physarum* spherules, or microsclerotia, are the diploid, dormant form of this organism, and are thought to be transcriptionally inactive. Spherules cannot be lysed without extensive shearing, however, which leads in our hands to fragmentation of nuclei, to partial degradation of the rDNA, and thus presumably to disruption of the chromatin structure. Since spherules are permeable to trimethyl psoralen, as is evidenced by the data presented below, cross-linking can be performed in vivo. But the fragmentation of the rDNA (to a mean size of about 15 kb) upon disruption of spherules prevents direct measurements of the cross-link distribution as described for plasmodial rDNA. The fragmentation also results in a broadening of the rDNA and chromosomal DNA bands in isopycnic centrifugation, thus leading to contamination of the rDNA with chromosomal fragments.

To circumvent these problems, we designed a series of selective restriction enzyme digestions to generate easily recognizable DNA fragments of unique size from rDNA isolated by isopycnic centrifugation from cross-linked spherules. The purity of the rDNA was approximately 50%. The enzymes were selected such that portions of the rDNA not of interest, as well as any contaminating chromosomal DNA, would be cut into very small fragments, whereas the unique desired fragment would be the largest fragment and thus easily recognizable in the electron microscope. The relevant features of the rDNA restriction map (1; Ferris and Vogt, submitted for publication) that allowed this selection are shown in Fig. 3. A 7.4-kb fragment derived from the transcribed region of spherule rDNA was generated by digestion with enzymes *Pst*I, *Hpa*I, and *Bgl*I. To demonstrate the fidelity of this procedure, this DNA was further digested either with *Bam*HI, which has no cleavage sites in this fragment, or with *Xho*I, which cleaves it at three sites. The length distribution for the DNA resulting from each of these digests (Fig. 4A, B, and C) suggests that the 7.4-kb fragment was relatively pure. A similar procedure was used to generate a 5.7-kb fragment from the central spacer portion of the rDNA, by digesting a separate sample of partially purified spherule rDNA with *Taq*I and *Mbo*I. The specificity of this cleavage scheme was tested by further digesting the resulting fragments either with *Xho*I, which does not cut the 5.7-kb fragment, or with *Bgl*I, which cuts it at nine sites. Length distributions for these samples (Fig. 4D, E, and F) again were consistent with a sufficient purity of the fragment to allow its analysis.

Table 1 compares the difference in accessibility to trimethyl psoralen-induced cross-linking of transcribed and nontranscribed fragments in spherule rDNA (cross-linked as intact spherules) and in microplasmodial rDNA (cross-linked as isolated nuclei). Since the positions of the 7.4- and 5.7-kb fragments on the rDNA are known, the expected cross-linking values for microplasmodial rDNA fragments were calculated directly from the distribution in Fig. 2A. Table 1 shows that the relative difference in accessibility was decreased in spherules as com-

FIG. 1. Electron micrograph of cross-linked rDNA. Purified rDNA isolated from cross-linked nuclei was denatured in 72% formamide and 2.8% glyoxal, diluted, and then spread for microscopy along with $\phi$ X174 phage DNA as a standard. A circular phage DNA molecule is visible above the rDNA molecule.

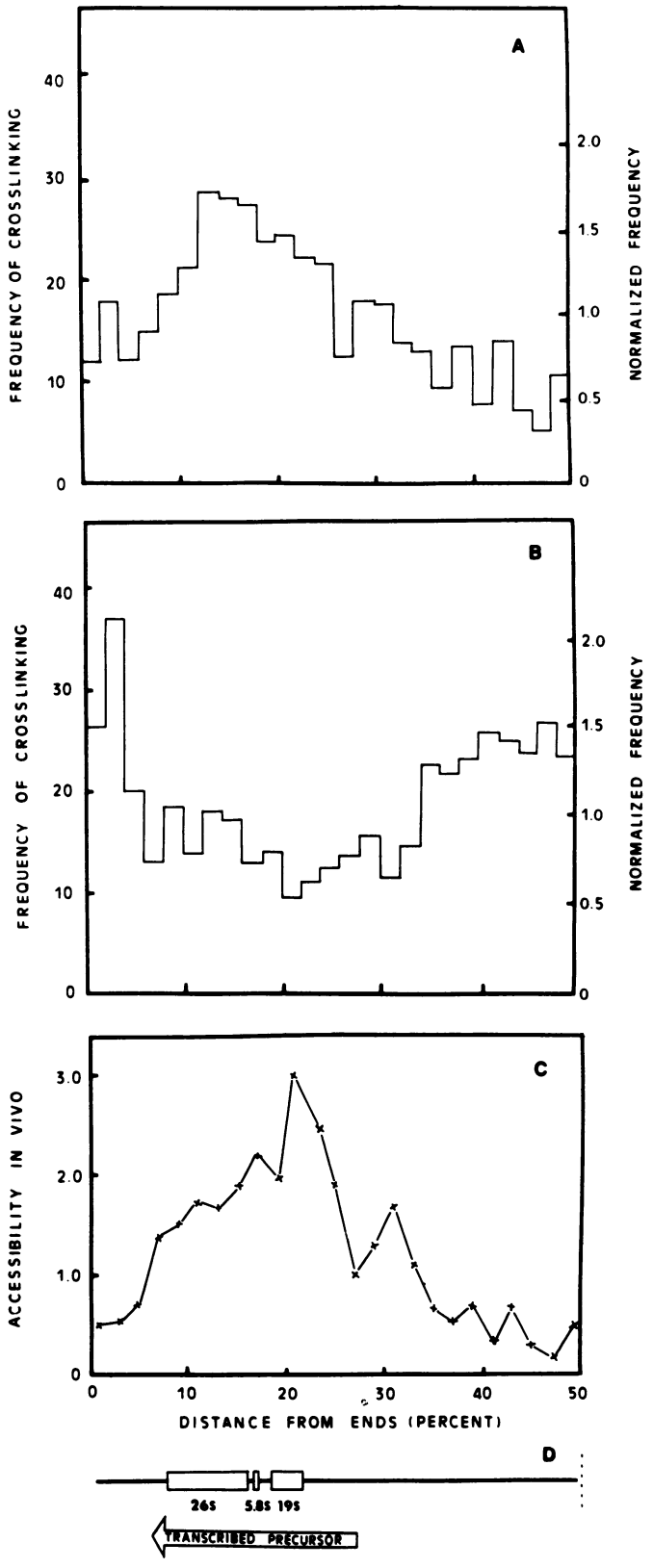

A
FREQUENCY OF CROSSLINKING
NORMALIZED FREQUENCY
0
10
20
30
40
0
0.5
1.0
1.5
2.0
B
FREQUENCY OF CROSSLINKING
NORMALIZED FREQUENCY
0
10
20
30
40
0
0.5
1.0
1.5
2.0
C
ACCESSIBILITY IN VIVO
0
1.0
2.0
3.0
0
10
20
30
40
50
DISTANCE FROM ENDS (PERCENT)
D
26S
5.8S
19S
TRANSCRIBED PRECURSOR

FIG. 2. Distribution of cross-links on rDNA. rDNA was isolated from cross-linked nuclei (A) or was isolated and then cross-linked as purified, naked DNA (B). Thirty molecules, with averages of 28 cross-links (A) or 29 cross-links (B) per molecule, were measured, and the position of each cross-link from one end was recorded. The length of the DNA (61.6 ± 3.2 kb [standard deviation] in A, and 61.7 ± 2.7 kb in B) was normalized to 100%, and then the frequency of cross-links (left-hand ordinates in A and B) was plotted in intervals of 2%. Since the DNA is palindromic, and thus the two halves are equivalent, the frequency of cross-links shown is for both halves. The absolute frequency of cross-links was normalized to make the mean frequency per interval equal to 1.0 (right-hand ordinates in A and B). The accessibility of the rDNA in nuclei, which is defined as the ratio of normalized cross-linking frequency in nuclei to normalized cross-linking frequency in naked DNA, is shown in C. The position of the ribosomal transcription unit is drawn in D.

pared with microplasmida by more than threefold. In nuclei from the transcriptionally active microplasmodia the sequences near the 19S ribosomal RNA were 6.2 times as accessible as the sequences from the nontranscribed central spacer, whereas in the dormant spherules the same sequences were only 1.7 times as accessible. (These ratios have been defined so that if no difference existed between the regions, this value would be 1.0.) These data suggest that the transcriptional state of rDNA sequences is reflected in the accessibility of transcribed sequences to trimethyl psoralen.

## DISCUSSION

We have shown that the transcribed portions of the extrachromosomal rDNA in isolated *Physarum* nuclei are four to six times more sensitive to cross-linking with trimethyl psoralen than are the terminal and central spacer regions. This preferential cross-linking is nearly abolished in dormant, transcriptionally inactive spherules. Statistical comparison of the distributions of cross-links in molecules cross-linked in nuclei and as naked rDNA indicates that the population of rDNA molecules in the nuclei is homogeneous in accessibility to trimethyl psoralen.

Although the boundaries between the regions of high and low cross-linking approximately match the boundaries between transcribed and nontranscribed DNA, this correspondence in fact may not be exact. Recent evidence indicates that transcription is initiated near 17.3 kb, or 28 to 29% from one end of the rDNA (28). The region of preferential cross-linking extends to about 32%, a point that is in a 2.1-kb stretch of DNA consisting of repeats of a 30-base-pair unit (Ferris and Vogt, submitted for publication). The significance of this observation and of the

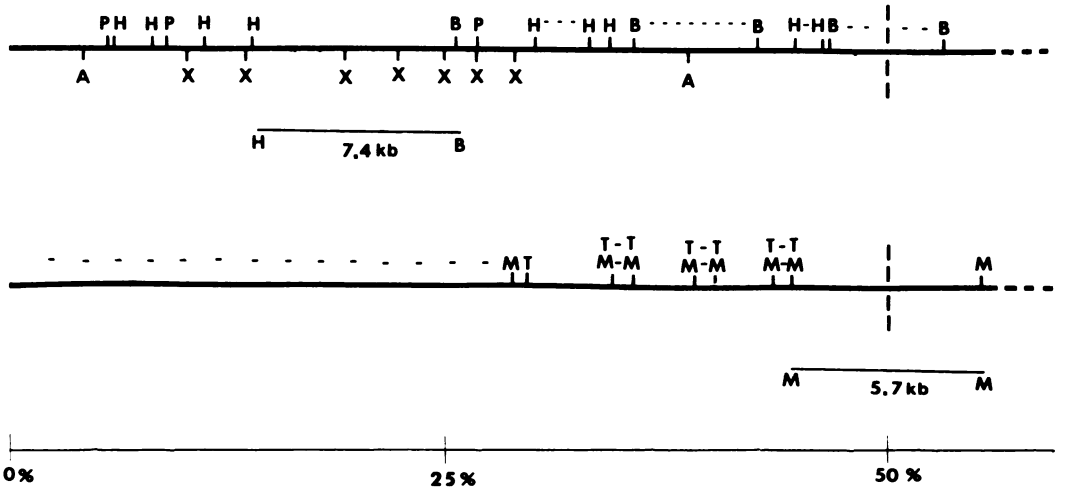



FIG. 3. Restriction map of rDNA. To generate large recognizable restriction fragments from the transcribed and central spacer regions, rDNA from spherules was digested either with *Bgl*I, *Hpa*I, and *Pst*I (upper line) or with *Mbo*I and *Taq*I (lower line). The largest rDNA fragments left after these digestions are the 7.4- and 5.7-kb pieces shown. Additional cleavage by *Xho*I or *Bam*HI (7.4-kb fragment), or by *Bgl*I or *Xho*I (5.7-kb fragment) was used as described in the text and in the legend to Fig. 4 to evaluate the specificity of these cleavages. The horizontal dashed lines indicate many cleavage sites. The vertical dashed line marks the central palindromic symmetry axis of the rDNA. Abbreviations: P, *Pst*I; H, *Hpa*I; B, *Bgl*I; X, *Xho*I; A, *Bam*HI; T, *Taq*I; M, *Mbo*I.

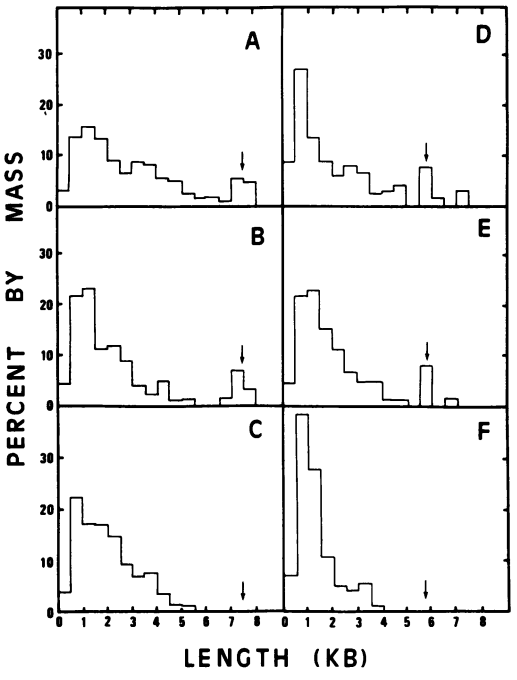



FIG. 4. Length distribution of DNA from restriction fragments. Spherule rDNA was digested with the enzymes indicated, and then the length distribution of the resulting fragments was measured by electron microscopy under nondenaturing conditions. Panels: A, *Pst*I, *Bgl*I, and *Hpa*I; B, as in A but plus *Bam*HI; C, as in A but plus *Xho*I; D, *Taq*I and *Mbo*I; E, as in D but plus *Xho*I; F, as in D but plus *Bgl*I. The arrows in A and D show the sizes expected of the 7.4-kb transcribed and 5.7-kb spacer fragments, respectively.

local decrease in accessibility to trimethyl psoralen near 27% remains to be assessed.

As already discussed by others (12), the most likely interpretation of the observation that trimethyl psoralen intercalates into DNA preferentially in regions of transcribed chromatin is that these regions are less shielded by proteins. Electron microscopic studies suggest that the transcribed portion of rDNA chromatin complexes in *Physarum* (11) and in other organisms (22, 23, 29) is in a more extended conformation than surrounding nontranscribed DNA. The nature of this "open" configuration is not well understood. It could be related to the presence of RNA polymerase molecules, HMG proteins, modified histones, or to altered conformation of nucleosomes. Another possibility that cannot be excluded is that in the chromatin the pitch of the DNA helix is not constant from one region to another. Intercalation of trimethyl psoralen is known to be sensitive to tightening of the helix (25). Whatever the exact origin of this open configuration, for *Physarum* rDNA it appears to be altered in dormant spherules. We interpret the reduction in accessibility of the spherule rDNA to be a result of its transcriptional inactivity, but have not excluded two other possibilities. This reduction simply may reflect the fact that cross-linking in one case occurred in nuclei and in the other occurred in vivo. Or some aspect of spherule physiology other than transcription rate of rDNA might affect the cross-linking pattern.

Numerous studies have led to the conclusion that transcribed chromatin, at least in higher eucaryotes, is preferentially digested by DNase I (9, 10, 18, 21, 31, 35). It is interesting in this regard that in our hands, under the conditions for isolation of nuclei used in this study, transcribed rDNA sequences in *Physarum* are not more rapidly digested than the sequences in the central spacer (Swofford and Vogt, unpublished results). An earlier report by Stalder et al. (27) of selective DNase I digestion of rDNA sequences in *Physarum* nuclei can be explained, at least in part, by their use of too high a stringency ($T_M$ –

TABLE 1. Analysis of cross-linked rDNA fragments from spherules[a]

| Fragment (kb) | Spherule rDNA | | | Plasmodial rDNA | | |
|---|---|---|---|---|---|---|
| | Cross-links/kb | Relative accessibility | | Cross-links/kb | Relative accessibility | |
| | | Uncorrected (A/B) | Corrected[b] | | Uncorrected (A/B) | Corrected[b] |
| A. Transcribed (7.4) | 0.74 | 0.91 | 1.7 | 1.4 | 3.3 | 6.2 |
| B. Nontranscribed (5.7) | 0.84 | | | 0.43 | | |

[a] The 7.4-kb transcribed and 5.7-kb spacer rDNA fragments, generated as described in the text and in the legend to Fig. 4, were measured, and the number of cross-links for each was determined. The average lengths for these fragments, based on internal $\phi$ X174 DNA standards, were 7.42 kb ($n$ = 29, standard deviation = 0.21) and 5.63 kb ($n$ = 33, standard deviation = 0.25). The cross-linking frequencies for the same regions of intact, plasmodial rDNA were calculated directly from the data in Fig. 2A. The base sequence correction multiplier (1.9) was determined from Fig. 2B. This factor is simply the ratio of number of cross-links in the 5.7-kb fragment to the number of cross-links in the 7.4-kb fragment, as determined for naked plasmodial DNA.

[b] Corrected for base sequence dependence.

$T$ = 13°C) in the hybridization of rDNA fragments to rRNA. Under the conditions used by these authors, a large fraction of the digested DNA fragments would have been scored as DNase sensitive simply because they were too small to anneal, yet large enough to be precipitable by perchloric acid. We have also been unable to find preferential digesiton of *Physarum* DNA sequences corresponding to abundantly transcribed mRNA. The chromatin of yeast cells also is reported to lack differential DNase I sensitivity (17). These several results suggest the possibility that trimethyl psoralen and DNase I probe different aspects of chromatin structure.

In contrast to DNase I, staphylococcal nuclease, which introduces breaks into DNA between nucleosomes, generally has not been found to be selective for transcribed chromatin. In *Physarum*, however, staphylococcal nuclease digestion of nuclei appears to yield, in addition to single and oligomeric nucleosomes, a minor class of particles with the same DNA content as nucleosomes, but with a slower sedimentation rate (13–15). These "A particles" appear to be enriched for the transcribed rDNA sequences relative to the central nontranscribed spacer sequences. Thus, it is possible that some aspect of chromatin structure recognized by staphylococcal nuclease is also reflected in preferential trimethyl psoralen intercalation. The observation that intercalation occurs preferentially between nucleosomes (2), i.e., in the same regions cleaved by staphylococcal nuclease, is consistent with this possibility.

The molecular mechanism of the selective cross-linking of transcribed sequences is *Physarum* and other species remains to be clarified. The recently described techniques for purifying *Physarum* rDNA as an intact chromatin complex (24) should help in the unravelling of this mechanism.

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