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Different Chromatin Structures in *Physarum polycephalum*

A Special Form of Transcriptionally Active Chromatin Devoid of Nucleosomal Particles

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Abstract. Nonnucleolar chromatin from interphase nuclei of *Physarum polycephalum* plasmodia occurs in two different structural configurations as seen in electron microscopic spread preparations. While the majority of the chromatin is devoid of nascent ribonucleoprotein (RNP) fibrils and compacted into nucleosomal particles, a minor proportion (10–20%) is organized differently and reveals a smooth contour. It is this form of smooth chromatin which is rich in transcription units (mean length: 1.36 ± 0.21 μm). Only occasionally are solitary nascent RNP fibrils observed which are associated with beaded strands of chromatin. In transcribed smooth chromatin nucleosomal particles are not only absent from the transcription units but also from their nontranscribed flanking regions, indicating that this special structural aspect is not merely a direct consequence of the transcriptional process. The existence of ca. 10–20% of *Physarum* chromatin in the smoothly contoured form is discussed in relation to reports of a preferential digestibility of a similar proportion of *Physarum* chromatin by DNAse I (Jalouzot et al., 1980) and to the altered configuration of "peak A" chromatin subunits after micrococcal nuclease digestion (Johnson et al., 1978a, b).

Introduction

It is established that transcribed regions of extranucleolar chromatin occur in a structural configuration different from that of nontranscribed chromatin as demonstrated by their preferential digestibility by DNAse I (Garel and Axel, 1976; Weintraub and Groudine, 1976) and micrococcal nuclease (Bellard et al., 1978; Tata and Baker, 1978; Bloom and Anderson, 1979). While micrococcal nuclease appears to recognize a structural aspect of chromatin that is dependent on the continuous presence of transcriptional complexes (Bloom and Anderson, 1979), the selective susceptibility of various genes to digestion by DNAse I apparently is not directly correlated with the transcriptional process but rather seems to reflect a configuration ready for transcription (Weintraub and Grou-

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dine, 1976; Garel et al., 1977; Garel and Axel, 1978; Miller et al., 1978; Weintraub, 1979; Stalder et al., 1980).

At the ultrastructural level a different chromatin arrangement devoid of nucleosomal particles have so far been detected in transcribed nonribosomal chromatin regions only in very actively expressed genes (Franke et al., 1976, 1978; McKnight et al., 1978; Scheer, 1978; Lamb and Daneholt, 1979; Trendelenburg et al., 1980). By contrast, genes with a relatively low density of transcriptional complexes usually show, in regions between the complexes, a beaded chromatin aspect indistinguishable from that of completely inactive chromatin (Foe et al., 1976; Laird et al., 1976; McKnight et al., 1978; Scheer, 1978; Busby and Bakken, 1979). From these observations it has been concluded that transcriptional events are accompanied by an "unfolding" of the nucleosomal particles, but that the nucleosomal organization can be almost immediately restored after the passage of an RNA polymerase (Scheer, 1978; McKnight et al., 1978).

In this report we present electron microscopic evidence for a long-sustained structural alteration of chromatin structure that occurs not only in transcribed regions but extends far into the adjacent, nontranscribed regions.

Materials and Methods

Microplasmodia of *Physarum polycephalum* strain M_3C VII were maintained as submerged shaken cultures as described by Daniel and Baldwin (1964). Mitotically synchronous macroplasmodia were set up as detailed by Mittermayer et al. (1965). Samples were taken during S-phase and G_2-phase after the second post-fusion mitosis (M_{II}). Generation time between M_{II} and M_{III} was 9 h ± 30 min at 26° C.

A small piece of a macroplasmodium was scraped off the supporting filter paper and immediately transferred into 5 ml of an ice-cold solution containing 80 µg/ml tRNA and 0.1% Sarkosyl NL-30 buffered with 0.1 mM sodium borate to pH 8.5–9.0. The material was gently homogenized using a hand-operated glass-teflon Potter-Elvejhem homogenizer and kept on ice. Within the next 10 min a few drops were loaded on top of a cushion consisting of 1% formaldehyde (prepared from paraformaldehyde), 0.1 M sucrose, 0.1 mM borate buffer, pH 8.5, in a centrifugation chamber as described by Miller and Bakken (1972). The carbon-coated electron microscopic grids (300 mesh) were freshly glow discharged to render them hydrophilic. Centrifugation was for 20 min at 3,500 × g at 10° C or room temperature. After drying of 0.4% Kodak-Photoflo, the grids were positively stained with 1% ethanolic phosphotungstic acid (Miller and Bakken, 1972), and dehydrated with 100% ethanol. The dried grids were finally rotary shadowed with platinum/palladium at an angle of ca. 8° C.

Results

The presence of relatively high levels of nucleases in *Physarum* (Melera and Rusch, 1973) made it necessary to develop a rapid method to prepare chromatin in the presence of a RNAse inhibitor. Addition of tRNA (Foe et al., 1976) turned out to be not only an efficient RNAse inhibitor with this material but also seemed to promote swelling of chromatin (probably by the transfer of very lysine-rich histones to the tRNA; see Ilyin et al., 1971). Inclusion of 0.1% Sarkosyl NL-30 yielded a rapid and sufficient dispersion of chromatin within a few minutes after lysis of the plasmodium without affecting the basic nucleosomal organization of chromatin (for effective dehistonizing concentrations of Sarkosyl see Scheer, 1978). Preliminary experiments using chromatin

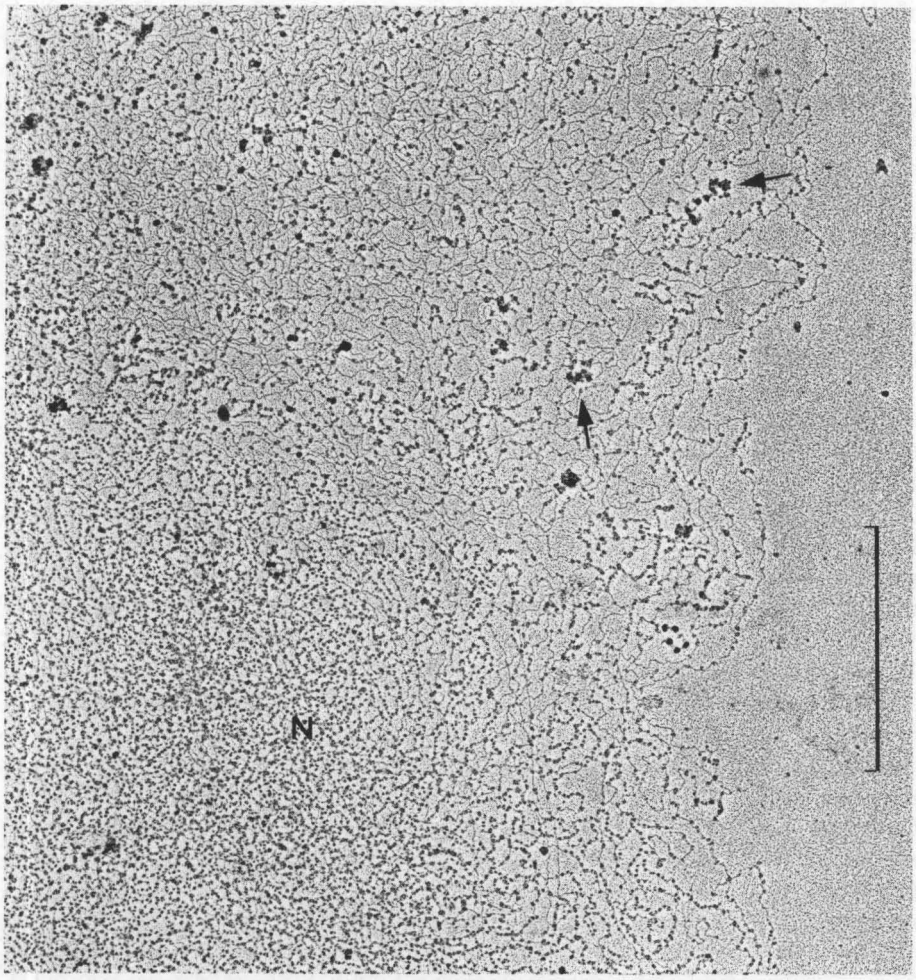

Fig. 1. Survey micrograph of chromatin prepared for electron microscopy 6 h after mitosis. Two different structural aspects can be distinguished: a beaded, nucleosomal (bottom part, *N*) and a smooth organization (upper part). The *arrows* denote nascent RNP fibrils associated with the smooth chromatin. Bar indicates 1 μm

from various sources had shown that the structure of nucleosomal particles was fully maintained in buffers containing such low concentrations of Sarkosyl together with tRNA.

When chromatin taken from various postmitotic stages (S and G_2) was prepared by this procedure for electron microscopy, we observed that roughly 80–90% of the chromatin recovered on the grids was arranged in the "beads-on-a-string" configuration characteristic of transcriptionally inactive chromatin (cf. Johnson et al., 1976). The remaining 10–20% was organized completely different and appeared as relatively thin, smoothly contoured fibrils of 3–5 nm diameter to which very sparsely and irregularly distributed particles and frequently nascent RNP fibrils were attached. Figure 1 shows the transition between both types

of chromatin (in a preparation made during G_2 phase, 6 h after mitosis) which can be clearly distinguished even at relatively low magnifications. Virtually the same structures were seen in early S-phase 10 min after mitosis (Fig. 2a): Chromatin with a beaded organization occurred next to, and intermingled with, long nonbeaded nucleofilaments which were frequently associated with laterally projecting RNP fibrils. Direct transitions between both types of chromatin organization were often observed on the same strand (Fig. 2b). The smooth aspect usually involved long stretches of individual chromatin strands, which often occurred over larger areas (Fig. 1). Because of this tendency of the smoothly contoured chromatin to form clusters, this type of organization could be easily identified in spread preparations, even in the presence of excess amounts of beaded chromatin.

Multiple arrays of transcripts arranged in continuous length gradients (transcriptional units, TUs) were found exclusively associated with the smooth chromatin (Fig. 3b–e). These TUs were clearly different from TUs coding for ribosomal RNAs (ribosomal TUs) based on the following criteria (compare Fig. 3a with 3b–e): (i) Ribosomal TUs with a length of 3.8–4.3 μm were located towards the ends of extrachromosomal, linear chromatin fragments (Fig. 3a; c.f. Grainger and Ogle, 1978). (ii) The "nontranscribed terminal spacer" of rDNA, i.e. the short nontranscribed chromatin tail distal to the transcript release site, was another useful diagnostic feature for the identification of ribosomal TUs. In contrast to the transcribed regions, these free ends with a length of ca. 0.5 μm invariably showed a beaded organization and contained about 16 nucleosomes (Fig. 3a). This indicates that the nontranscribed terminal spacer of *Physarum* rDNA has a length of about 3 kb and therefore is considerably shorter than the rDNA segment distal to the 26 S rRNA gene (Molgaard et al. 1976; Campbell et al., 1979; Johnson et al., 1979; Gubler et al., 1980). (iii) The "central nontranscribed spacer" of rDNA, i.e. the palindromic axial region between two ribosomal TUs, also showed a beaded conformation and length measurements indicated a corresponding foreshortening of the DNA (see also Grainger and Ogle, 1978; Johnson et al. 1979)[1]. (iv) The growing RNP fibrils of ribosomal TUs were characterized by conspicuous terminal knob-like thickenings (Fig. 3a). While, occasionally, particles of higher order folding of nascent nonribosomal RNP were also observed (Fig. 3b, insert), they were never confined to the RNP fibril termini.

The TUs shown in Fig. 3b–e represent a selection of transcribed nonribosomal genes most commonly found in our preparations. They were generally separated from each other by long distances. The frequency of transcripts usually ranged from 9 to 12 per micrometer chromatin length, i.e., considerably less than in TUs of ribosomal RNAs (compare Fig. 3a). Occasionally, however, solitary transcriptional complexes were also observed (Fig. 3e). The appearance of both the transcribed chromatin regions, i.e. the intercepts between adjacent

[1] The term "spacer" as used here in the context of a palindromic sequence organization is not equivalent to the spacer of tandemly arranged rRNA genes (Franke et al., 1979). Nontranscribed spacer chromatin of tandemly arranged rRNA genes appears, under the same conditions, not to be compacted into nucleosomes (Franke et al., 1976; Scheer et al., 1977; Scheer, 1980; Reeder et al., 1978)

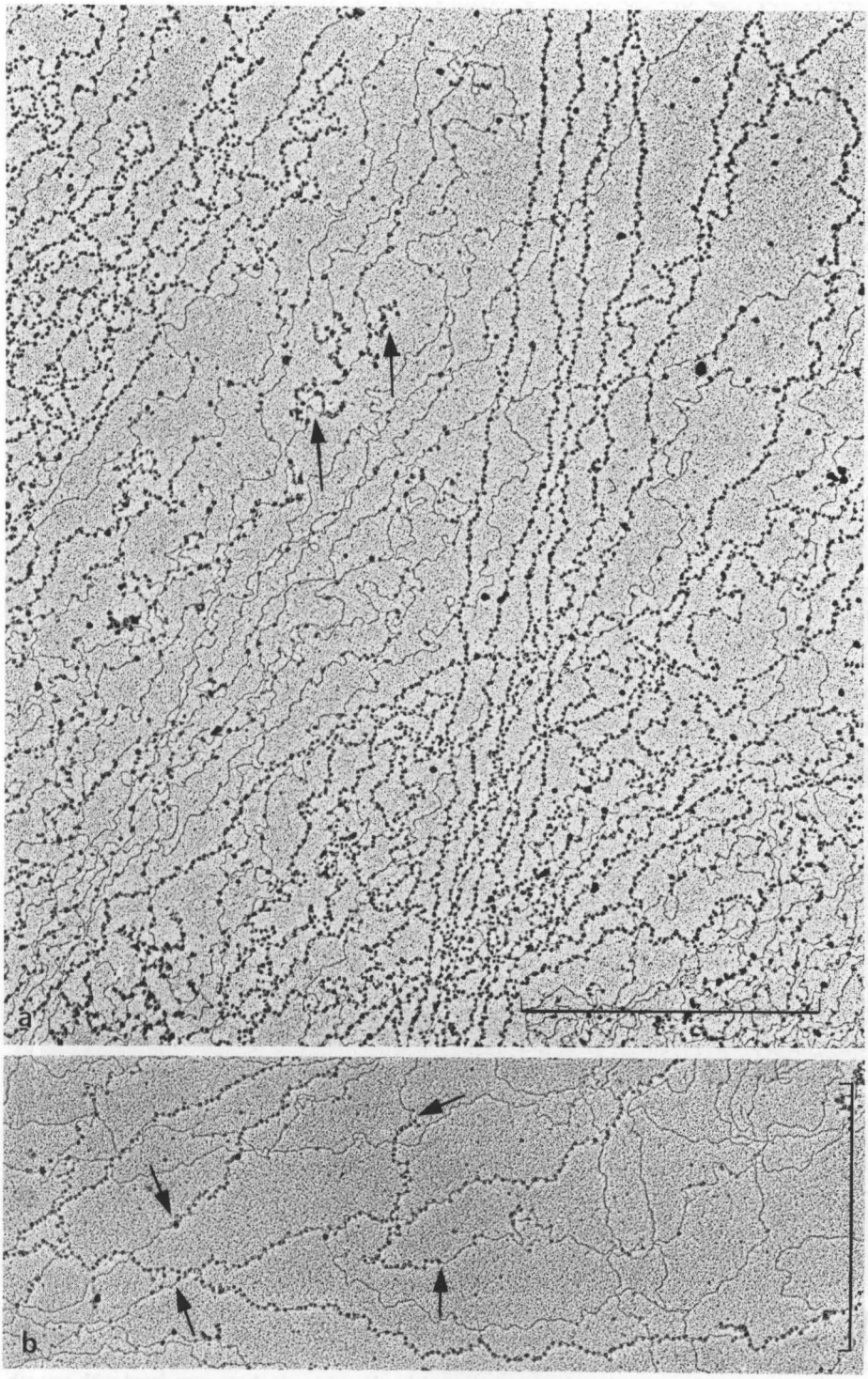

Fig. 2a and b. Closely adjacent chromatin strands of the beaded and smooth type in a preparation made 10 min after mitosis. The *arrows* in **a** indicated lateral RNP fibrils arranged in a length gradient. Structural transitions between both types of chromatin organization are often recognized (*arrows* in **b**). Bars indicate 1 μm

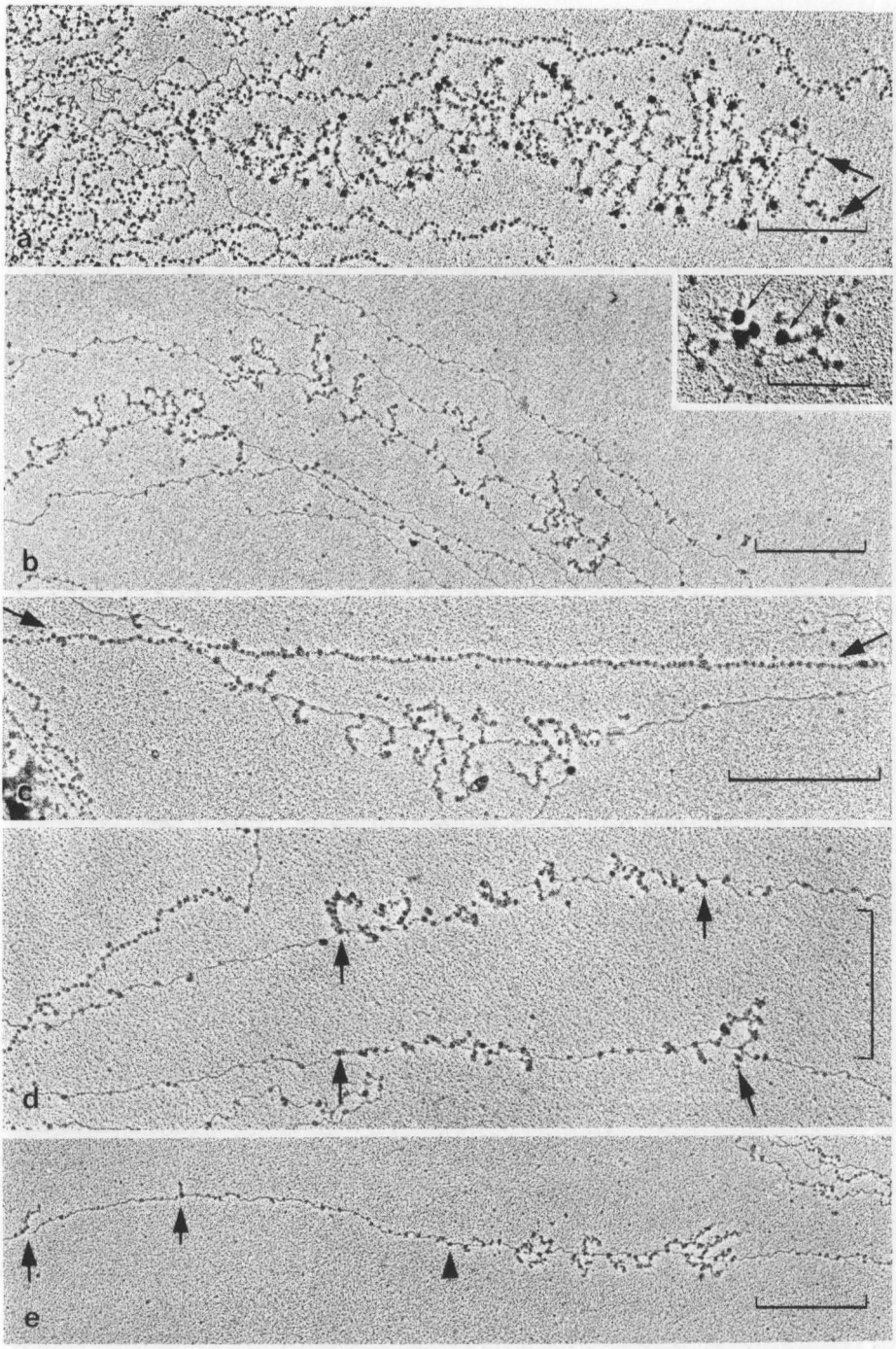

Fig. 3a–e. Ribosomal (**a**) and nonribosomal (**b–e**) transcription units (*TUs*) in preparations made 10 min (**a**), 40 min (**b**) and 6 h (**c–e**) after mitosis. Ribosomal TUs are identified by a short "terminal nontranscribed spacer" of beaded morphology (pair of *arrows* in **a**). Nonribosomal TUs (transcriptional initiation and termination sites are indicated by the *arrows* in **d** are associated with smooth chromatin which is clearly different from adjacent nontranscribed beaded chromatin strands (denoted, e.g., by the *arrows* in **c**). Single transcripts are occasionally recognized (*arrows* in **e**; the *arrowhead* denotes the start region of a TU). The insert in (**b**) shows a globular form of higher order compaction of nascent RNP (*arrows*). Scale bars indicate 0.5 μm (**a–e**) and 0.2 μm (insert in **b**)

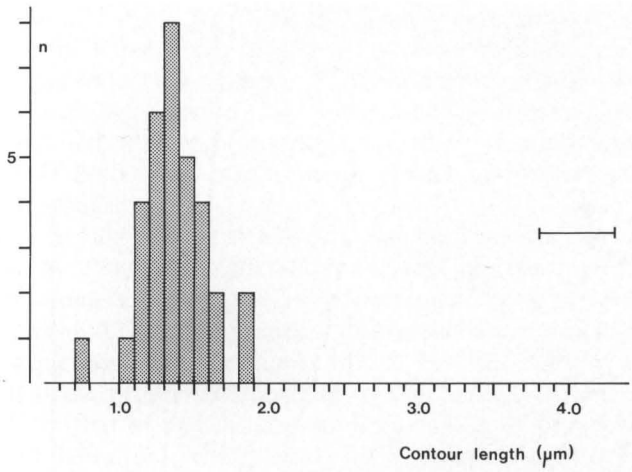

Fig. 4. Length distribution of nonribosomal TUs from various interphasic stages of *Physarum* plasmodial nuclei. The bar indicates the length range of ribosomal TUs found in the same preparations

RNP fibrils, and the nontranscribed regions flanking the TUs was identical: both were uniformly smooth and could be clearly distinguished from adjacent nucleosomal chromatin strands (Fig. 3c). It should be emphasized that the smooth character of the chromatin within and around these nonribosomal TUs resembled that of the ribosomal TUs, with the notable difference that the latter were immediately flanked by beaded "spacer" chromatin[1].

The lengths of the nonribosomal TUs (estimated as the distances between sites for initiation and transcript release; for definition see Laird et al., 1976) were surprisingly homogeneous (Fig. 4). The mean length was 1.36 ± 0.21 μm ($n = 33$). Assuming full extension (B-conformation) of the DNA axis, as is indicated by the absence of nucleosomes (see also Discussion), one can estimate that these TUs have at the average a size of about 4 kb and that their full-sized primary RNA transcripts have a mean molecular weight of about 1.4×10^6. A similar size distribution of total nucleoplasmic RNA from *Physarum* has been determined by gel electrophoresis (Davies and Walker, 1978). Our results indicate that the 26–30 S peak observed by these authors most likely contains primary transcripts and not intermediate processing products derived from larger TUs.

Occasionally we have observed individual transcripts attached to the beaded form of (nonribosomal) chromatin (Fig. 5). Such transcripts, however, were never arranged in multiple arrays forming fibril length gradients but were widely scattered and apparently reflected a rather small proportion of the total transcriptional activity present in these nuclei.

Discussion

The results of this study show that nonnucleolar interphase chromatin of *Physarum*, when dispersed by low salt treatment and prepared for electron microscopy, occurs in two morphologically distinct states. The majority of chromatin was

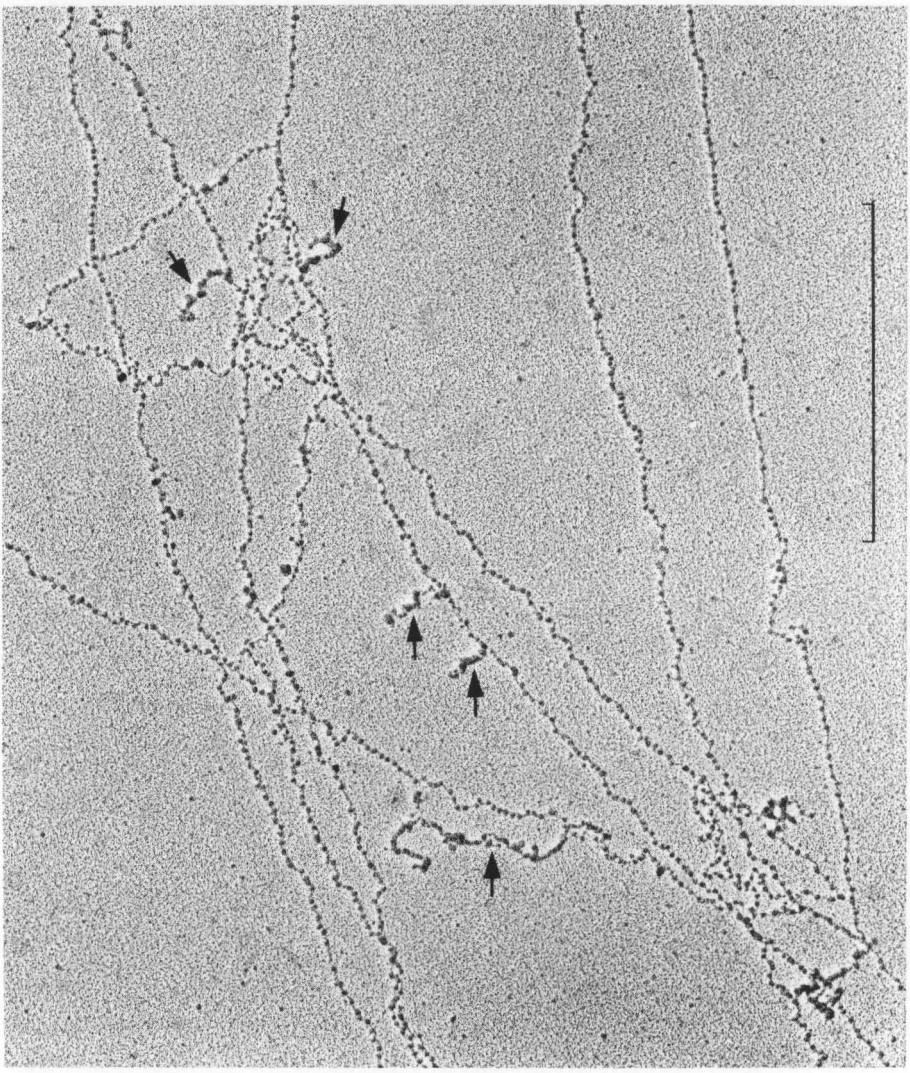

Fig. 5. Association of lateral RNP fibrils (*arrows*) with beaded chromatin strands. These transcripts occur usually singly and never form continuous length gradients. Bar indicates 1 μm

found to be organized in nucleosomes as indicated by the characteristic "beads-on-a-string" appearance (for nucleosome DNA repeat length determination in *Physarum* see Johnson et al., 1976). The other type of chromatin strand was smooth and thin (3–5 nm diameter) and was frequently associated with arrays of nascent RNP fibrils arranged in length gradients. These chromatin strands were interpreted to be transcriptional units (TUs) of a rather homogeneous size class (1.36 ± 0.21 μm). Although the nonbeaded chromatin represented only a minor proportion of the total chromatin (10–20%), it could be readily and reproducibly observed in all preparations because of its tendency to form foci of smooth fibers surrounded by chromatin of the beaded type. The observa-

tion that many structural continuities and transitions from smooth to beaded chromatin occurred, clearly excludes the possibility that the two conformational states reflect the existence of two different subpopulations of plasmodial nuclei containing exclusively one type of the two forms of chromatin. It rather indicates that each nucleus of a synchronously growing plasmodium simultaneously contains both types of chromatin during interphase, even right after the onset of S-phase.

In this context it is worth mentioning that digestion of plasmodial nuclei from *Physarum* with staphylococcal nuclease yielded, besides nucleosome monomers and oligomers, a prominent peak of slowly sedimenting particles ("peak A") that contained rDNA sequences as well as a variety of nonribosomal sequences (Johnson et al., 1978a, b, 1979). These authors have also shown that the protein composition of the peak A chromatin subunits was different from that of nucleosomes and that its DNA occurred in a relatively extended configuration (Allfrey et al., 1978; Johnson et al., 1978b). From these results and our present observation of the structural similarity between the extended chromatin of ribosomal TUs and the smoothly contoured chromatin of nonribosomal TUs, including their flanking regions, we suggest that the peak A subunits were derived from all chromatin regions that appeared nonbeaded in our electron microscopic preparations. The smooth contour would then indicate an almost full extension of the B-form DNA (Johnson et al., 1978b), as opposed to the about twofold compaction in nucleosomes.

Our finding that nucleosomes are not only absent from chromatin regions active in RNA synthesis but also from the nontranscribed regions flanking the putative protein-coding genes shows that this special aspect is not a direct consequence of transcriptional events per se. Whether it reflects a structural rearrangement preceding transcription (for comparison in nucleolar chromatin see Foe, 1978) or a metastable alteration related to transcription but extending into adjacent nontranscribed regions, remains to be examined.

From our present findings we suggest that the structural rearrangements responsible for the smooth contour of chromatin are related to the altered structure recognized by DNAse I. This notion is supported by the finding that about 15% of interphase chromatin of *Physarum* is different from the bulk chromatin and is preferentially digested by DNAse I (Jalouzot et al., 1980). In addition, from studies on vertebrate cells it is known that the structural rearrangements conferring DNAse I-sensitivity to certain chromatin regions can precede the onset of gene expression (Garel et al., 1977; Miller et al., 1978; Weintraub, 1979; Stalder et al., 1980) and that these structural alterations extend into apparently nontranscribed regions surrounding the transcribed genes (Bellard et al., 1980; Lawson et al., 1980).

Although the vast majority of transcripts were seen in association with nonbeaded chromatin strands we have also found several examples of lateral RNP fibrils attached to chromatin organized in nucleosomal particles. However, RNP fibrils present on beaded chromatin were never arranged in multiple arrays and fibril length gradients were not observed. Furthermore, these single nascent RNP fibrils occurred at relatively great distances. This sporadic occurrence of transcriptional complexes indicates a very low level of transcription.

In conclusion, our observations suggest that, in nuclei of *Physarum* plasmo-

dia, competence for transcription manifests itself in a structural change, probably an unfolding of the nucleosomal particles, which may reflect a drastically altered protein composition (Allfrey et al., 1978; Johnson et al., 1978a). In addition, our results indicate that this type of pre-transcriptional rearrangement is not restricted to the specific individual gene region but rather involves larger chromatin domains. Transcription of specific genes within such a potentially active domain would then depend upon other factors like, e.g., promotor-recognizing molecules. A similar successive regulatory principle acting at two different levels has also been proposed in the control of ribosomal RNA gene expression (Foe, 1978; Scheer, 1978). However, it cannot be excluded that most of the smooth chromatin is transcribed at some time during the mitotic cycle of *Physarum* as could be inferred from saturation hybridization experiments (Fouquet and Braun, 1974) and an estimate of the sequence complexity of the RNA which amounts to about 20–30% of single copy DNA (Baeckmann, 1980).

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