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ELECTRON MICROSCOPIC ANALYSIS OF CHROMATIN AND GENE EXPRESSION

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One factor involved in the regulation of gene expression is the specific nucleoprotein organization of chromatin. Differences in chromatin structure between transcribed and nontranscribed regions can be identified both by biochemical and electron microscopic (EM) methods. As originally shown by Weintraub and Groudine (47) and subsequently confirmed in a wide variety of organisms and cell types (reviewed in 17) transcribed DNA sequences are much more sensitive to DNAse I digestion within intact nuclei than are transcriptionally inactive regions. EM analysis of spread chromatin is another approach to study changes in chromatin architecture that accompany gene activation and transcription. This chapter focuses on the specific form of arrangement of various types of transcriptionally active chromatin as compared to the appearance of transcriptionally inactive chromatin.

ULTRASTRUCTURE OF INACTIVE CHROMATIN

The spreading technique originally developed by Miller and coworkers (22,23) essentially consists of extensive dispersion of chromatin in a buffer of very low ionic strength and centrifugation of the solubilized chromatin through a sucrose containing formaldehyde solution onto a freshly glow-discharged (i.e. hydrophilic) carbon-coated EM grid. The preparation is then positively stained with ethanolic phosphotungstic acid and, in addition, may be rotary-shadowed to enhance contrast.

When chromatin is exposed to media of very low ionic strength all higher order packing structures such as the 25-35 nm supranucleosomal globules of condensed chromatin progressively unravel (reviewed in 48). The unfolded state of chromatin is finally stabilized at the level of nucleosomal arrays, i.e. the "primary nucleofilament" displaying the typical beaded organization (Fig. 1a; cf also 24). This characteristic "beads-on-a-string" pattern reflects the ubiquitous nucleosomal subunit organization of chromatin at the first level of DNA-histone interaction. A nucleosome is a roughly wedge-shaped 10-13 nm particle which contains a defined octamer of the four histones H2A, H2B, H3 and H4 and a total of about 200 base pairs of DNA which is wound around the surface of the protein core interacting primarily with the amino terminal hydrophilic regions of the histones (for details see 18). Since the length of a DNA stretch of 200 base pairs is about 64 nm (200 x 3.4 Å) as opposed to the diameter of a nucleosome of only 10-13 nm, it follows that the nucleosomal organization of chromatin involves an apparent foreshortening of the DNA by a factor of 5 to 6.4. However, since in spread preparations nucleosomal particles are usually separated by a short stretch of internucleosomal linker DNA, the average frequency of nucleosomes is only

about 28-33 per micrometer chromatin length (Fig. 1a). Therefore, the apparent contraction ratio of the DNA under such preparative conditions is about 2 (1,4,8,15,21). Thus it is possible to estimate the length of DNA (in micrometer, molecular weight or number of base pairs) contained in a given length of nucleosomal chromatin.

MORPHOLOGY OF NUCLEOLAR CHROMATIN

In chromatin spreads of most eukaryotic cells active rRNA genes are recognized by (i) their tandem arrangement, adjacent genes being separated by usually nontranscribed spacer regions, (ii) a rather homogeneous distribution of their contour lengths with mean values characteristic for a given species, (iii) a high packing density of the transcriptional complexes, thus causing the characteristic "Christmas tree" appearance, and (iv) terminal knobs at the free ends of the lateral ribonucleoprotein (RNP) fibrils (Fig. 1b,c; for reviews see 7,35). In several lower eukaryotes amplified rRNA genes are not arranged in tandem but with opposite polarities in linear chromatin units containing two genes each, i.e. in palindromic arrangements (see below).

A. Transcribed rRNA genes and adjacent nontranscribed spacers exist in a non-nucleosomal form

1. In fully active rRNA genes with maximal packing density of the RNA polymerases additional nucleosome sized particles are absent (Fig. 1b; ref. 5). The polymerases are so closely spaced that they often form a continuous thickening of the gene axis (Fig. 1b).
2. The contour length of the rRNA genes correspond to the molecular weight of their primary transcription products, the pre-rRNAs, only under the assumption that the rDNA exists in an almost extended form, i.e. close to the length of DNA in B-conformation (5,36,38,42).
3. The spacer regions between tandemly arranged rRNA genes are different from the bulk of transcriptionally inactive chromatin (Fig. 1b,c; 5,14, 30,31,44). By variations of the spreading procedure (e.g. inclusion of monovalent salt or lowering the pH of the dispersal medium to neutrality) it has been demonstrated that particles of nucleosomal size frequently occurring in spacer regions (Fig. 1d) behave differently from nucleosomes (14,31,35; see, however, also 25). This non-nucleosomal form of organization has also been seen in the spacer regions of cloned rRNA genes injected into the nuclei of Xenopus oocytes (43).
4. There is a good correspondence of the contour lengths of repeating units in chromatin and in isolated rDNA (28,29,38,45). Especially conclusive examples are the amplified nucleolar chromatin circles in oocytes of the water beetle Dytiscus marginalis and the house cricket, Acheta domesticus (45).

Taken together, these data exclude the possibility that nucleolar chromatin, both the transcribed genes and the spacer regions, is arranged in nucleosomal particles since then a two-fold contour length difference between deproteinized rDNA and chromatin should be found. These observations do not exclude the possibility that transcribed nucleolar chromatin is organized in a mode resembling nucleosomes but is much more susceptible to unravelling during exposure to low salt concentrations, compared to inactive chromatin.

B. Transcribed palindromically arranged rRNA genes exist in non-nucleosomal form but not their intergene regions

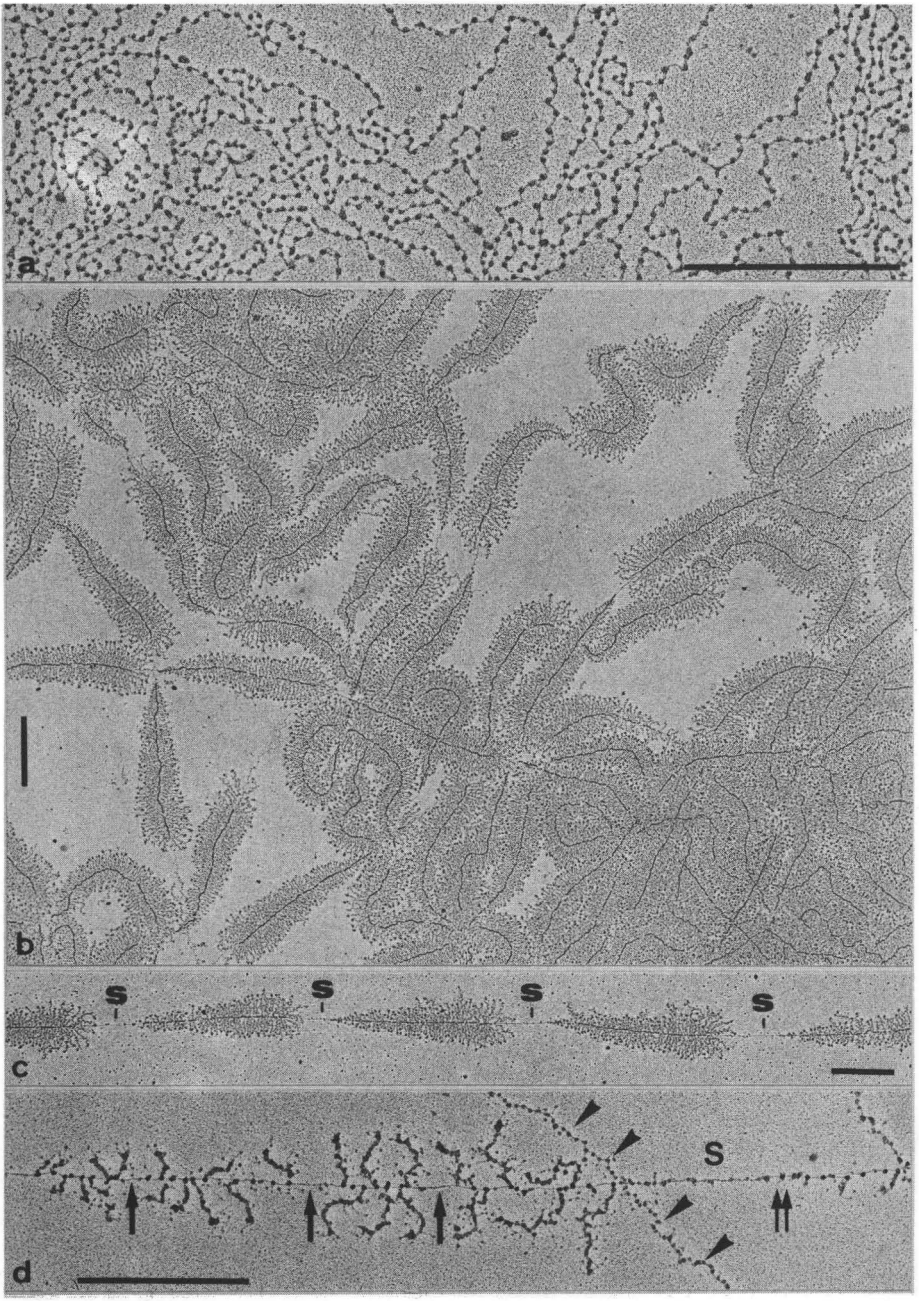
Linear nucleolar chromatin units with two rRNA genes at either end in palindromic arrangement have so far been reported in Physarum, Dictyostelium and Tetrahymena (for reviews see 7,35). The transcribed chromatin regions exhibit a nonbeaded structure (9,35,40). By comparing the contour lengths of the genes with the molecular weights of the corresponding pre-rRNAs it is clear that the rDNA is largely extended (9). However, the nontranscribed intergenic regions exhibit a beaded pattern and the DNA is foreshortened by a factor of about 2 (9,10,35). Thus, the intergenic regions of palindromically arranged rRNA genes are compacted into nucleosomes, as opposed to the spacers of tandemly arranged rRNA genes (one has to bear in mind that both situations are principally different), and morphologically appear as other regions of transcriptionally inactive chromatin.

C. Morphological changes accompanying activation and inactivation of rRNA genes

In amphibian oocytes the regulation of rRNA synthesis occurs at the level of transcription as shown by a combined biochemical and ultrastructural analysis (37). Ribosomal RNA genes of reduced transcriptional activity are characterized by a reduced packing density of transcriptional complexes (37). The chromatin between the distantly spaced RNA polymerases is uniformly thin (Fig. 1d; 5,14,30,31; the same aspect is found in a variety of other organisms such as sea urchins, insects and mammals, see 2,13,26). However, when larger domains of nucleolar chromatin are completely inactivated such as during late stages of amphibian oocyte development or after application of the drug actinomycin D which inhibits transcription, rearrangement into the nucleosomal form takes place (30,44). This structural reorganization is accompanied by an apparent contraction of the rDNA as this has been shown in the chromatin circles of Dytiscus oocytes (34).

The activation of rRNA genes can first be recognized by an altered chromatin structure. During early embryogenesis of the milkweed bug Oncopeltus fasciatus the actual transcription of the rRNA genes is preceded by an interconversion of the chromatin from a beaded to a non-

Figure 1. a. Typical appearance of transcriptionally inactive chromatin in spread preparation. The beaded fibers represent linear arrays of nucleosomes. Chromatin was prepared from polytene chromosomes of salivary glands of the dipteran insect, Sciara coprophila. b. Clusters of transcriptionally active rRNA genes from oocytes of the salamander Pleurodeles waltlii. c. The tandem arrangement of rRNA genes ("matrix units" or "Christmas trees") separated by nontranscribed spacer regions (S) is clearly seen. Same preparation as in Fig. b. d. When few transcripts are attached to rRNA genes such as in late stages of amphibian oocyte growth, fibril-free gene regions reveal a thin and smooth contour (arrows) and are clearly distinguished from nucleosomal, beaded chromatin (arrowheads). Note the occurrence of some irregularly spaced particles in the spacer region (S). The start region of the adjacent rRNA gene is indicated by a double arrow. Bars indicate 0.5 (a, d) and 1 (b, c) µm.


a
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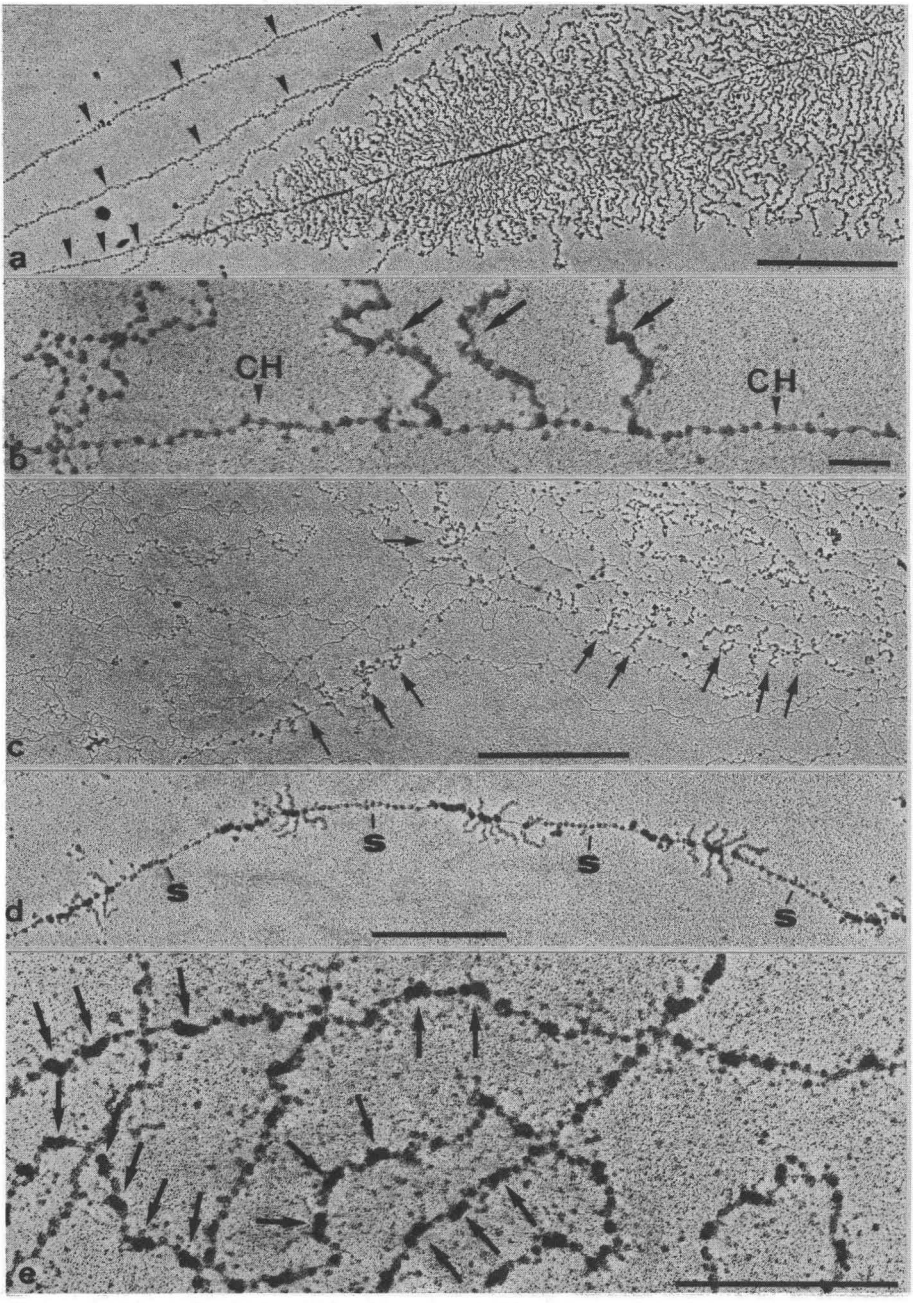
beaded state (3). Thus, the non-nucleosomal configuration of rRNA genes seems to be a prerequisite for their transcribability.

MORPHOLOGY OF NON-NUCLEOLAR TRANSCRIPTION UNITS

Unlike rRNA genes, non-nucleolar genes are characterized by a highly variable packing density of RNA polymerases, reflecting differing degrees of transcriptional activities, a usually solitary occurrence and very heterogeneous contour length distribution. When RNA polymerases are spaced at maximal density, additional nucleosome-sized particles are absent and the underlying DNA seems to be largely extended (Fig. 2a; 5,19, 46). However, when the polymerases are more distantly spaced, nucleosome-like particles can often be recognized in the chromatin regions in between them (Fig. 2b; see also 1,2,4,11,12,15,16,20,21,30). The nucleosomal nature of these particles has been demonstrated by their sensitivity towards low concentrations of the detergent Sarkosyl which remove histones but leave the transcriptional complexes intact (30) and their specific reaction with antibodies against histones H3 and H2B (20). Furthermore, antibodies against H2B cause a retraction of the lampbrush chromosome loops upon injection into nuclei of amphibian oocytes suggesting that H2B is actually associated even with heavily transcribed chromatin (39). Regions of thin chromatin axes obviously devoid of nucleosomal particles have also been recognized in the transcriptionally active chromatin of loops of lampbrush chromosomes after fixation in solutions of physiological salt concentrations and processing for EM observation of sections (41).

A completely different picture is found in some lower eukaryotes such as the slime mold Physarum polycephalum and yeast. Here most of the transcriptional units (TUs) are characterized by a smooth chromatin configuration (27,40). Moreover, this nonbeaded aspect also extends into the flanking regions of the TUs (Fig. 2c). These chromatin domains devoid of nucleosomal particles may represent potentially transcribable regions.

Figure 2. a. Start region of a TU of a lampbrush chromosome loop from a Pleurodeles oocyte. Note the increase in length of the lateral RNP fibrils. The RNA polymerase particles are closely spaced except in several gaps. The chromatin region preceding this TU as well as adjacent nontranscribed chromatin strands (arrowheads) exhibit the characteristic beaded pattern of nucleosomal organization. b. High magnification of a part of a lampbrush TU showing three distantly spaced transcriptional complexes (arrows). The chromatin axis (CH) is beaded indicative of a nucleosomal organization. c. Chromatin of the slime mold Physarum polycephalum prepared for EM 10 min after mitosis. A minor proportion (about 10-20 %) of the chromatin occurs in a smooth configuration. Transcripts (some are denoted by arrows) are preferentially associated with this smooth chromatin. d. Tandemly repeated relatively short TUs (mean length 0.32 µm or 940 base pairs of DNA) from lampbrush chromosomes of Pleurodeles. The nontranscribed spacers (S) are beaded. e. Tandemly repeated very short TUs from lampbrush chromosomes of Pleurodeles. Each thickened region (some are denoted by arrows) represents a TU containing only 2 RNA polymerases. Note the beaded appearance of the spacer regions. Bars indicate 0.1 (b), 0.5 (d,e) and 1 (a,c) µm.


a
CH
CH
b
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e

TANDEMLY REPEATED NON-RIBOSOMAL RNA TRANSCRIPTION UNITS

In lampbrush chromosomes of the salamander Pleurodeles waltlii a homogeneously sized family of tandemly repeated TUs occurs (Fig. 2d; 32). These TUs containing approximately 940 base pairs of DNA are densely covered by RNA polymerases and are separated by nontranscribed spacers with the beaded aspect of nucleosomal chromatin.

Very short TUs cannot be identified by their transcriptional products because a nascent RNP fibril has to reach a certain length before it is recognizable in spread preparations (6). However, even very short TUs accomodating no more than two RNA polymerases can be clearly visualized provided that they occur in a highly repeated and uniform pattern of arrangement. This is shown in Fig. 2e. The regular thickenings separated by beaded spacer chromatin represent very short TUs with an average DNA content of 130 base pairs (33). Considering their very high reiteration number (10,000 or more) these TUs may represent 5S rRNA or tRNA genes.

In sea urchin embryos another class of tandemly repeated TUs has been described which were tentatively interpreted as histone genes (2). It is interesting to note that in all three cases the intervening nontranscribed spacer regions are beaded indicative of a nucleosomal compaction of the spacer DNA, contrary to the situation found in tandemly repeated rRNA genes.

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