Ber. Deutsch. Bot Ges. Bd. 97 (1984), S. 315—326

Organization of Transcriptionally Active and Inactive Chromatin

By

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(Read at the Botanical Meeting, Freiburg, September 13th, 1982)

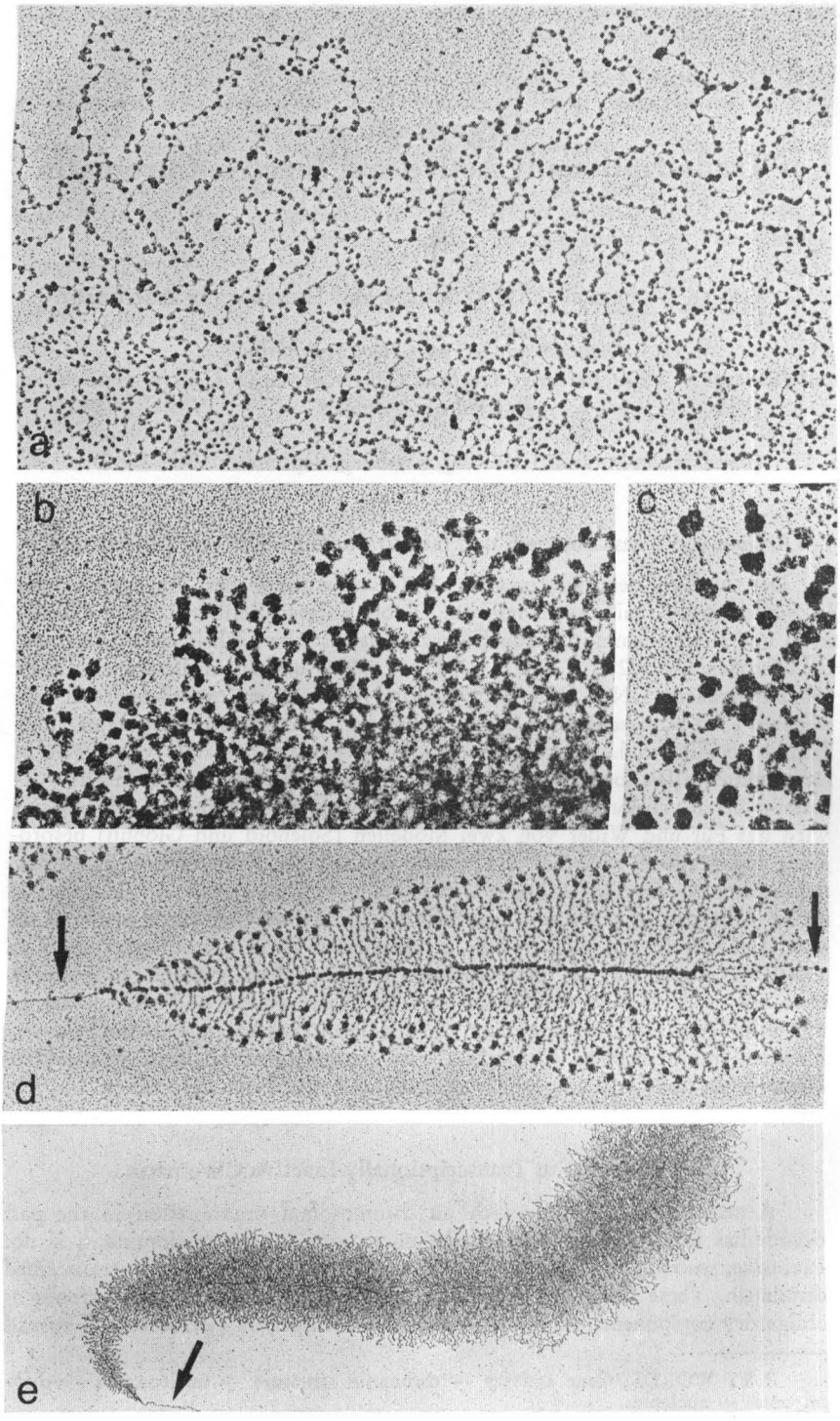
Aufbau von transkribiertem und transkriptionell inaktivem Chromatin

Der Aufbau des Nukleosoms als Grundbaustein des Chromatins der Eukaryontenzelle, mit Ausnahme des Dinoflagellaten-Zellkerns, wird biochemisch und elektronenmikroskopisch vorgestellt als eine Einheit eines Oktamers aus Histonen (2×H2A, 2×H2B, 2×H3, 2×H4) und einem Betrag von etwa 150 Basenpaaren DNA. Diese Partikel sind durch einen Zwischenbereich von weiteren etwa 50 Basenpaaren DNA verbunden, an dem auch Histone der H1-Familie beteiligt sind. Verschiedene nichtnukleosomale Zustände von DNA in Eukaryontenzellen, besonders nach Virus-Infektionen, werden ebenfalls vorgestellt. Bei der Diskussion der supranukleosomalen Anordnung des Chromatins wird das Für und Wider von zwei Modellen (Solenoid und Globuli) besprochen; insbesondere wird auf die Verschiedenheit der Dimensionen der supranukleosomalen Fibrillen (Dicken-Unterschiede von 20 bis 50 nm) in verschiedenen Zelltypen eingegangen. Transkriptionell aktives Chromatin erscheint sowohl strukturell wie biochemisch in geänderter Form vorzuliegen: im Vergleich zur Anordnung in nukleosomalen Partikelketten erscheint es gestreckt, etwa wie es der Länge der B-Form der DNA entspricht, und seine DNA ist wesentlich empfindlicher gegenüber verschiedenen Nukleasen. Die Struktur von maximal transkribiertem Chromatin wird besonders am Beispiel von nukleolären Genen und von Lampenbürstenchromosomenschleifen besprochen.

Nucleosomes in Transcriptionally Inactive Chromatin

A major advance in research on chromosomal organization in the past decade has been the discovery of the nucleosome as the predominant, if not exclusive, subunit particle governing the organization of non-transcribed chromatin. These chromatin subunits, containing only DNA and histones as obligatory components, can best be visualized by electron microscopy of spread

KEY WORDS: Gene activity — chromatin structure — nucleosomes — chromosomes — nucleolus.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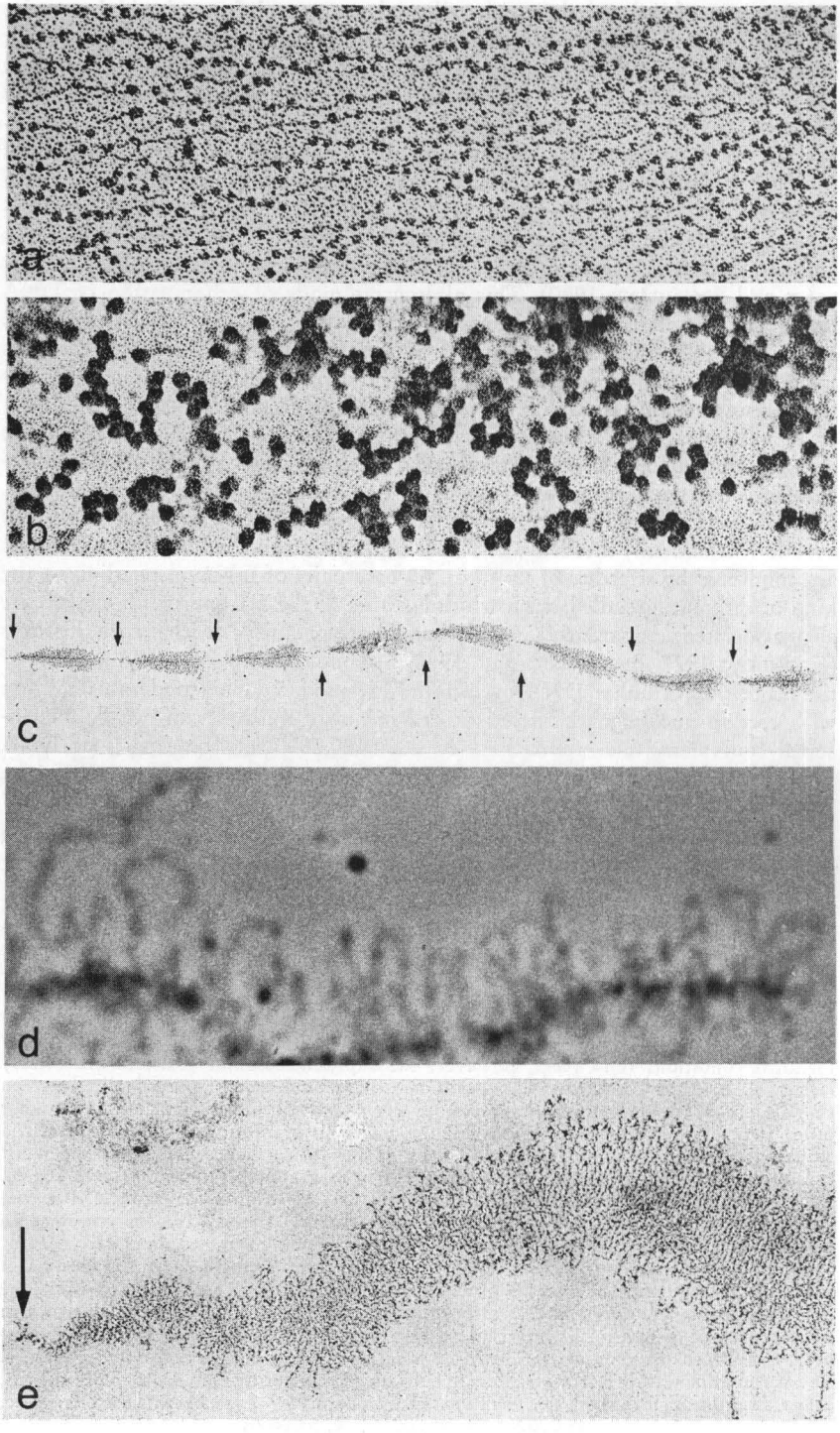
preparations of isolated chromatin allowed to unravel from the higher order packing into extended "beaded chains" (nucleofilaments) by exposure to low salt concentration devoid of divalent cations (Figs. 1 a, 2 a and 3 d; WOODCOCK 1973, OLINS and OLINS 1974; for recent reviews of literature see MCGHEE and FELSENFELD 1980, IGO-KEMENES et al. 1982). However, with adequate preparation nucleosomal particles can also be visualized in ultrathin sections (OLINS et al. 1980). Such nucleosomal particles have been observed in chromatin of both animal (Fig. 1 a) and plant (Fig. 2 a) cells, with the remarkable exception of the dinoflagellate nucleus which does not contain typical histones and also does not reveal the typical beaded nucleofilament organization (LIVOLANT and BOULIGAND 1980). This alternative mode of organization of DNA in the dinoflagellate nucleus reflects the absence of the characteristic histones and is not the result of peculiarities of dinoflagellate DNA, which contains some unusual bases, as demonstrable by the in vitro reconstitution of nucleosomes from dinoflagellate DNA with purified histones (RAE and STEELE 1978).

Biochemically the nucleosome can now be defined as a disk-shaped particle of ca. 10 nm diameter in its longer axis which contains a nuclease digestion-resistant core made up of an octamer of two molecules of each histone H2a, H2b, H3 and H4 and a total of 146 base pairs of DNA wrapped around it in a mode schematically sketched (Fig. 3 a). Two adjacent nucleosomal core particles are connected by stretches of up to ca. 60 base pairs of DNA, depending on the kind of chromatin studied, with which histones of the H1 and/or H5 classes are also associated, apparently in the immediate vicinity of the core histones (KORNBERG 1977, BELYAVSKY et al. 1980, BRADBURY et al. 1981). As a result of the protection of linker DNA by histones H1 and H5 during moderate digestion with certain nucleases, characteristic fragments of multiples of whole nucleosomal equivalents, i. e. approximately 200, 400, 600, etc. base pairs of DNA

Fig. 1. Electron microscopy of transcriptionally inactive (a—c) and active (d, e) chromatin from various animal species as seen in electron microscopic spread preparations. Transcriptionally inactive chromatin from chicken erythrocyte nuclei shows, after exposure to buffers (pH 7—9) containing low salt concentrations and no divalent cations, the typical "beads-on-a-string" appearance of highly dispersed chromatin (a). Practically all the chromatin appears in the form of the nucleosomal particles of a ca. 10 nm diameter. By contrast, very brief exposure to low salt buffers reveals an organization of the chicken erythrocyte chromatin reminiscent to that observed after fixation in situ, i. e. chains of tightly packed moruloid supranucleosomal particles of ca. 30 nm diameter (b). This aspect of chromatin organization can also be observed in spread preparations of various other forms of transcriptionally inactive chromatin such as in sea urchin sperm chromatin prepared under identical conditions (c). Here, however, the supranucleosomal granules are clearly larger (40 to 50 nm diameter; c). Note also some nucleosomal particles in between these supranucleosomal granules, resulting from partial unravelling of the higher order structure. Chromatin regions highly active in transcription are characterized as units containing numerous lateral fibrils of increasing lengths, the nascent RNP transcripts, which thus form a characteristic length gradient ("christmas tree", d, e). Each lateral fibril is anchored to the chromatin axis by a globular particle, the specific RNA polymerase (d, e). The high packing density of the RNA polymerases on a pre-rRNA gene is shown in a nucleolus of an oocyte of the salamander, *Pleurodeles waltlii* (d) where it results in an almost uniform thickening of the chromatin axis. Spacer regions, i. e. intercepts not transcribed at the same time into the same RNA molecule, surround the transcription initiation and termination sites (arrows in d). A part of a non-nucleolar transcription unit from a lateral loop of a *Pleurodeles* lampbrush chromosome is shown at a relatively low magnification in Figure e. The chromatin axis is hardly visible due to the presence of numerous RNP fibrils. The transcription initiation site is indicated by the arrow (e). Magnifications: a—c, 48 000×; d, 35 000×; e, 5 000×.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are found (Fig. 3 b). Histone H1, which occurs in several members of a multigene family, shows species- and cell type-specific patterns of polypeptides and is replaced, to a high degree, by histone H5 during erythropoesis (Fig. 3 c).

When additional DNA molecules are introduced into the cell nucleus, as, for example, by viral infections or by experimental microinjection, they are assembled into nucleosomal chromatin, provided that a sufficient histone pool is present (e. g., GRIFFITH 1975, MÜLLER et al. 1978, TRENDELENBURG et al. 1978, WYLLIE et al. 1978, ZENTGRAF et al. 1979). However, other forms of intranuclear DNA have also been described for certain viral DNAs, such as single-stranded DNA of replicating adenovirus (MATSUGUCHI et al. 1979, BEYER et al. 1981) and of herpes simplex virus (MÜLLER et al. 1980), which appear as uniformly protein-coated chromatin strands of 15—20 nm diameter. This may reflect depletion of the histone pool or association with specific single-strand DNA binding proteins. Moreover, non-nucleosomal, seemingly "naked" DNA has also been found in nuclei infected with viruses that result in an inhibition of protein synthesis (for refs. see MÜLLER et al. 1980) and after drug-induced inhibition of protein synthesis in uninfected cells (RILEY and WEINTRAUB 1979).

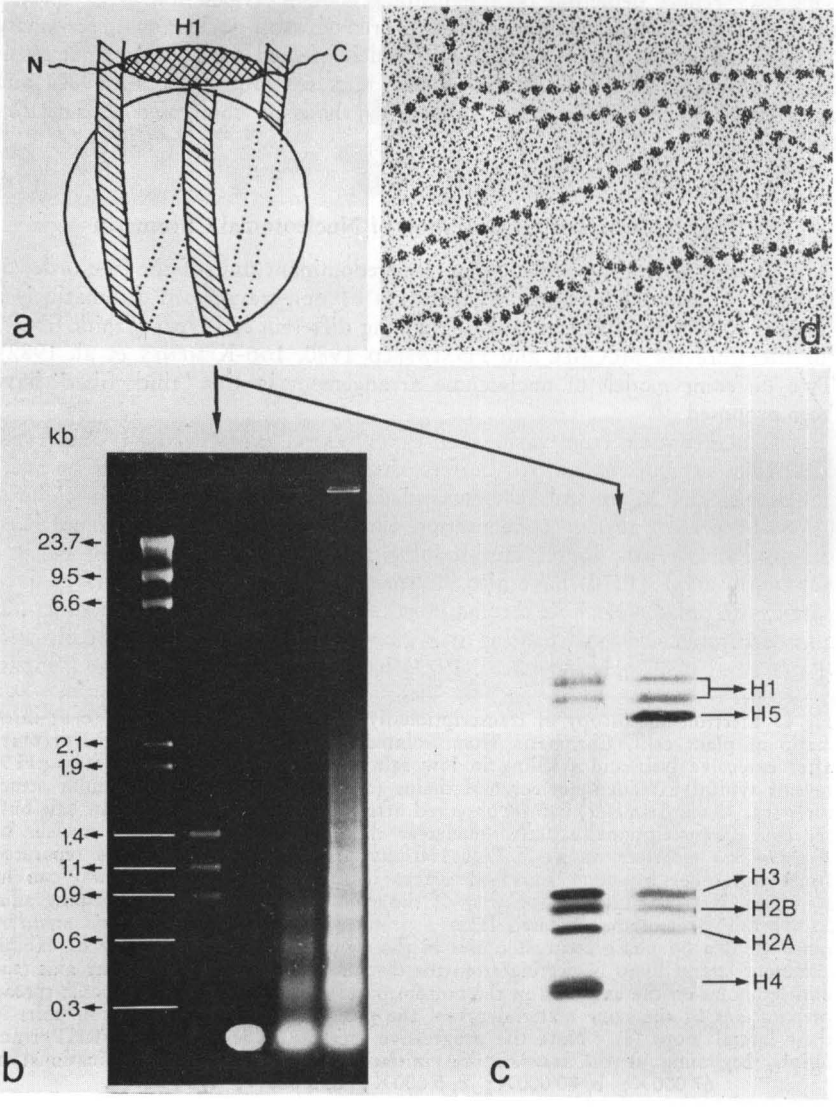
Higher Orders of Organization of Nucleosomal Chromatin

There is general agreement that the predominant unit of the first order of higher, i. e. supranucleosomal organization of non-transcribed chromatin is a fibril of 20—50 nm diameter, varying among different cell types ("thick fiber"; for literature see MCGHEE and FELSENFELD 1980, IGO-KEMENES et al. 1982). Two different models of nucleosome arrangement in this "thick fiber" have been proposed

1. In chromatin from various cell types FRANKE et al. (1976) have observed, after brief exposure to low salt buffers, chains of globular particles of an average diameter of 26 nm and have concluded that "these large chromatin globules (26 nm) represent further condensation and packing of the nucleosomal bead chains, characteristic for certain topological classes of inactive chromatin". KIRYANOV et al. (1976) have also interpreted the existence of similarly-sized particles in isolated rat liver chromatin as an indication that the basic structural unit of supranucleosomal packing is a globular particle. RENZ and colleagues (RENZ et al. 1977, HOZIER et al. 1977) have isolated such particles ("super-

Fig. 2. Electron microscopy of transcriptionally inactive (a, b) and active (c, d) chromatin of plant cells. Chromatin from isolated nuclei from root tips of *Zea mays* after extensive lysis and swelling in low salt buffer (0.5 mM borate buffer, pH 9) reveals typical extended nucleosomal chains (a). Supranucleosomal granular structures (ca. 30 nm diameter) can be observed after very brief exposure to law salt buffer (b). Transcriptionally active nucleolar chromatin from a primary nucleus of *Acetabularia mediterranea* (c). Eight serially arranged pre-RNA genes separated from each other by non-transcribed spacer incepts (indicated by arrows) can be identified. Note the identical polarity of the genes along the rDNA containing axis. Lampbrush chromosomes isolated from a primary nucleus of *Acetabularia mediterranea* (shown by phase contrast optics in the light micrograph of Figure d) exhibit numerous lateral loops projecting from the chromomeres of the chromosome axis (the dark granules on the axis seen in the bottom part of d). Electron microscopic spread preparations of the same material reveal the presence of long transcription units in these lateral loops (e). Note the progressive increase in length of the RNP transcripts, beginning at the transcription initiation site (arrow). Magnifications: a, 67 000×; b, 80 000×; c, 5 000×; d, 3 000×; e, 11 000×.

beads") and have presented evidence that a certain number of nucleosomes is packed together by the action of histone H1 (see also STRÄTLING et al. 1978, BUTT et al. 1979, ZENTGRAF et al. 1981, KIRYANOV et al. 1982). Such supranucleosomal granules have not only been described in animal chromatin (Fig. 1 b, c) but also in chromatin from plant cells (Fig. 2 b; cf. GREIMERS and DELTOUR 1981, ZENTGRAF et al. 1981). Comparison of the sizes of such supranucleosomal granules in different cell types (Fig. 1 b and c, 2 b) shows that they can greatly differ from 25—30 nm in chicken erythrocytes (ZENTGRAF et al. 1980a; cf. PRUITT and GRAINGER 1980) to 40—50 nm large granules in sea urchin spermatozoa (ZENTGRAF et al. 1980b; see also SUBIRANA et al. 1981). Such size differences can also be recognized in nuclei fixed in situ. It is unlikely that these differences



are exclusively explained by the known differences of DNA contents of the specific nucleosomes (cf. KORNBERG 1977) since the total number of nucleosomes per higher order granule seems to be much higher in the large particles of, e. g., sea urchin than in the smaller particles of chicken erythrocytes and rat liver (ZENTGRAF et al. 1980a, b). The observation of dimers and trimers of such granular supranucleosomal particles in rat liver (STRÄTLING et al. 1978, KIRYANOV et al. 1982) as well as in chicken erythrocytes (these authors, unpublished data) also supports models of "discontinuous" supranucleosomal packing of chromatin (Fig. 4 b). Large supranucleosomal granular particles have also been described for inactive states of extrachromosomal DNA of some insects (SCHEER and ZENTGRAF 1978), for the "minichromosomes" of simian virus SV40 (GRIFFITH 1975, MÜLLER et al. 1978) and for chromatin formed on DNA after microinjection into amphibian oocyte nuclei or incubation with soluble nuclear proteins (SCHEER et al. 1980).

2. Several electron microscopic studies, using isolated chromatin processed through various steps of preparation, have emphasized a uniform appearance of 25—30 nm thick chromatin fibrils (values given are for the dehydrated state; the diameter of the corresponding hydrated fibril is approximately 30—35 nm) and have suggested that the nucleofilament is regularly coiled into a solenoid structure with 6—7 nucleosomes per turn (FINCH and KLUG 1976, SUAU et al. 1979, THOMA et al. 1979, MILLER et al. 1978). Measurements of hydrodynamic properties (BUTLER and THOMAS 1980) and X-ray data (BRUST and HARBERS 1981) have also been interpreted in support of this continuous solenoid model (Fig. 4 a). However, it is difficult to envisage how a uniform solenoid of 6—7 nucleosomes per turn could produce 40—50 nm thick chromatin fibrils as present, for example, in sea urchin sperm. An obvious implication of the solenoid model is the existence of a uniform hollow core which, however, has so far not been demonstrated, including studies using heavy metal tracer molecules such as lanthanum. Clearly, future experimental work is required before a definitive decision on the higher order organization of the nucleofilament can be made.

Transcribed Chromatin

Electron microscopy of spread preparations of chromatin has shown that regions of chromatin actively engaged in transcription are recognized by the attachment of lateral fibrils containing nascent RNA associated with proteins

Fig. 3. Organization of the nucleosome as shown in scheme (a; modified after BRADBURY et al. 1981) by gel electrophoretic analysis of DNA after digestion of chromatin by micrococcal into protected DNA fragments (b) by gel electrophoretic analysis of its histones (c), and by electron microscopy of spread chromatin (d; from plasmodial nuclei of the slime mold, *Physarum polycephalum*; 83 000×; for details see SCHEER and ZENTGRAF 1981). In a, the large histone octamer is surrounded by two turns of DNA which are "sealed off" by histone H1 (N, amino-terminus; C, carboxy-terminus). In b, sizes of DNA of chicken erythrocyte chromatin obtained after various degrees of digestion (slot in the right margin: brief digestion, next slot, intermediate digestion; third slot from the right: maximal digestion resulting in core particles) are compared with those obtained by digestion of the DNA of the bacteriophage λ with restriction endonuclease HindIII (kilobases are indicated on the left margin). In c, histones of whole nucleosomes from calf thymus cells (left lane) are compared with those from chicken erythrocytes (right lane). Histones are labelled H1, H3, H2B, H2A and H4 (the latter is somewhat underrepresented in the material shown in the right line). Note that histone H5 is only seen in the erythrocyte chromatin.

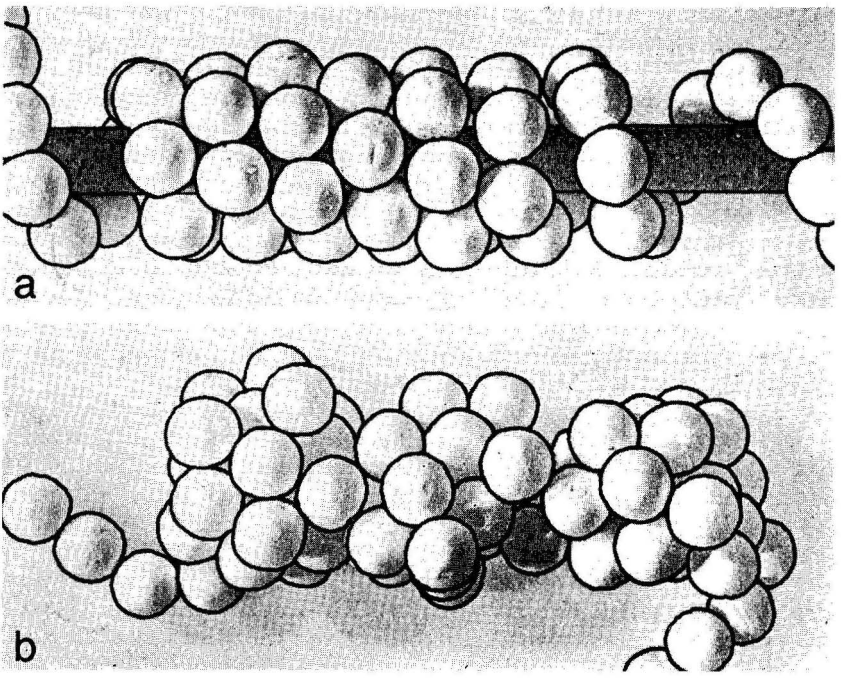


Fig. 4. Schematic diagrams showing alternative model views of packing of nucleosomes into a solenoid (a; partly unravelled) and into a series of supranucleosomal granules (b; also partly unravelled). For the sake of simplicity nucleosomes are depicted here as spheres rather than disks. Note the existence of a hollow core in the solenoidal model (position indicated by the horizontale bar).

(MILLER and BAKKEN 1972). In chromatin regions of maximal transcriptional activity these laterally projecting ribonucleoprotein fibrils and the polymerase molecules from which they emerge are closely spaced (Fig. 1 d, e and 2 c, e). Measurements of lengths of transcriptional units of the ultrastructurally best studied gene class, i. e. the nucleolar genes coding for pre-rRNA (e. g. Figs. 1 d and 2 c), have shown that the transcribed DNA is not condensed into nucleosomes but is extended to the normal length of B-conformation DNA (FRANKE et al. 1976, 1978, FOE et al. 1976, FOE 1978, REEDER et al. 1978, LABHARDT and KOLLER 1982; for further refs. see MATHIS et al. 1980, IGO-KEMENES et al. 1982). This concept of an absence of nucleosomal particles in transcribed rDNA chromatin has also been supported by biochemical studies (e. g., REEVES 1978, CZECH and KARRER 1980, GIRI and GOROVSKY 1980, BORKHARDT and NIELSEN 1981, BORCHSENIUS et al. 1981). A similarly extended state of DNA in nucleolar chromatin has also been described for non-transcribed spacer regions located between pre-rRNA genes (FRANKE et al. 1976, REEDER et al. 1978, SCHEER 1980, LABHARDT and KOLLER 1982).

Among the non-nucleolar genes, transcriptional events are relatively sparse in most of the transcribed chromatin and the individual transcriptional complexes are separated by regions of variable sizes containing nucleosome-like particles. These particles have been identified as histone-rich nucleosomal structures by their reaction with histone antibodies (MCKNIGHT et al. 1978) and by their sensitivity to treatment with a detergent, Sarkosyl, which removes

histones from DNA but does not remove transcriptional complexes (SCHEER 1978, SCHEER et al. 1981). In special chromosomal regions known to be intensely transcribed, i. e. showing a high density of transcriptional complexes, such as lampbrush chromosome loops of amphibian oocytes (Fig. 1 e) and of primary nuclei of the green alga, *Acetabularia* (Fig. 2 d, e), as well as Balbiani rings of polytene chromosomes of salivary glands of *Chironomus* a rather close packing of lateral fibrils containing nascent pre-mRNA associated with proteins has been found on a relatively extended chromatin axis depleted of nucleosomal particles (FRANKE et al. 1976, 1978, SCHEER et al. 1977, 1979, LAMB and DANEHOLT 1979). At least short non-beaded axial intercepts have also been described in transcriptional units of lateral loops of *Acetabularia* chromosomes that have been fixed at physiological ionic strength, flat-embedded and visualized in thin sections (SPRING and FRANKE 1981), thus again supporting the notion that such nucleosome-depleted regions have not been artificially introduced as a result of lowering the ionic strength.

In summary, the evidence available at present suggests that the transcribed chromatin is organized in a mode different from that of transcriptionally inactive chromatin. This has also been demonstrated by various biochemical methods, in particular taking advantage of the observation of WEINTRAUB and GROUDINE (1976) that the nucleohistone material of transcribed or potentially transcribed genes is more sensitive to digestion with certain nucleases, notably DNAse I, than inactive chromatin (for reviews on the voluminous literature see MATHIS et al. 1980, and IGO-KEMENES et al. 1982). Two extreme alternatives of explanation have been offered to account for the structural changes during transcription. One hypothesis, specifically formulated for rDNA chromatin (LABHARDT and KOLLER 1982), proposes that histones are absent from the specific DNA intercept occupied by the polymerase. Other authors emphasize observations indicative of the presence of histones at or near the transcriptional complexes (e. g., MCKNIGHT et al. 1978, SCHEER 1978, SCHEER et al. 1979) but in a loosened form of organization different from that of the nucleosomal particles, resulting in a relatively thin and uniform diameter of the chromatin axis and a higher susceptibility of its DNA to nuclease digestion. In this model a transcriptional complex with its polymerase molecule is translocated to a structurally altered locus of "unfolded" chromatin and then proceeds to the subsequent DNA intercept, whereupon the first locus either rearranges into the nucleosomal organization or is occupied by the next transcriptional complex. The molecular basis for this structural change, however, is not understood.

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