Developmental Biology
A COMPREHENSIVE SYNTHESIS

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Developmental Biology

A COMPREHENSIVE SYNTHESIS

Volume 1
Oogenesis

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the polytrophic-meroistic ovaries of *Calliphora erythrocephala*, *Drosophila hydei*, and *Sarcophaga barbata*, *Chromosoma* **53**:131–140.
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Chapter 9

Functional Organization of the Amphibian Oocyte Nucleus

ULRICH SCHEER and MARIE-CHRISTINE DABAUVALLE

1. Introduction

Nuclei of amphibian oocytes ("germinal vesicles") can grow to considerable size. For example, the nucleus of a mature *Xenopus laevis* oocyte has a diameter of about 0.5 mm and, after isolation, can be seen with the naked eye. This exceptional size (for comparison, the diameter of somatic cell nuclei usually ranges from 5 to 30 μm), the ease and rapidity with which they can be manually isolated, and the occurrence of lampbrush chromosomes and amplified nucleoli makes amphibian nuclei choice material not only for studies of chromosome structure and genetic activity at different levels of resolution, but also for analysis of the biochemical composition of individual nuclei and nuclear components as well. A single manually isolated nucleus from an amphibian oocyte is sufficient to analyze its major protein constituents by gel electrophoresis, and a few nuclei provide sufficient RNA for analysis by gel electrophoresis or electron microscopic spreading methods. In addition, the size of amphibian oocyte nuclei greatly facilitates the introduction of certain substances by microinjection. In fact, *Xenopus* oocytes are presently used in numerous laboratories as "living test tubes" to study the expression of cloned DNA sequences after microinjection into their nuclei with the aim of identifying those DNA sequences necessary for transcriptional initiation and termination events, as well as the factors involved in gene regulation. Another important approach is the microinjection of proteins into amphibian oocytes in order to analyze their resulting nucleocytoplasmic distribution and to classify them as "karyophilic," "karyophobic," or "amphiphilic" proteins. This approach requires a very rapid isolation of nuclei in order to prevent redistribution

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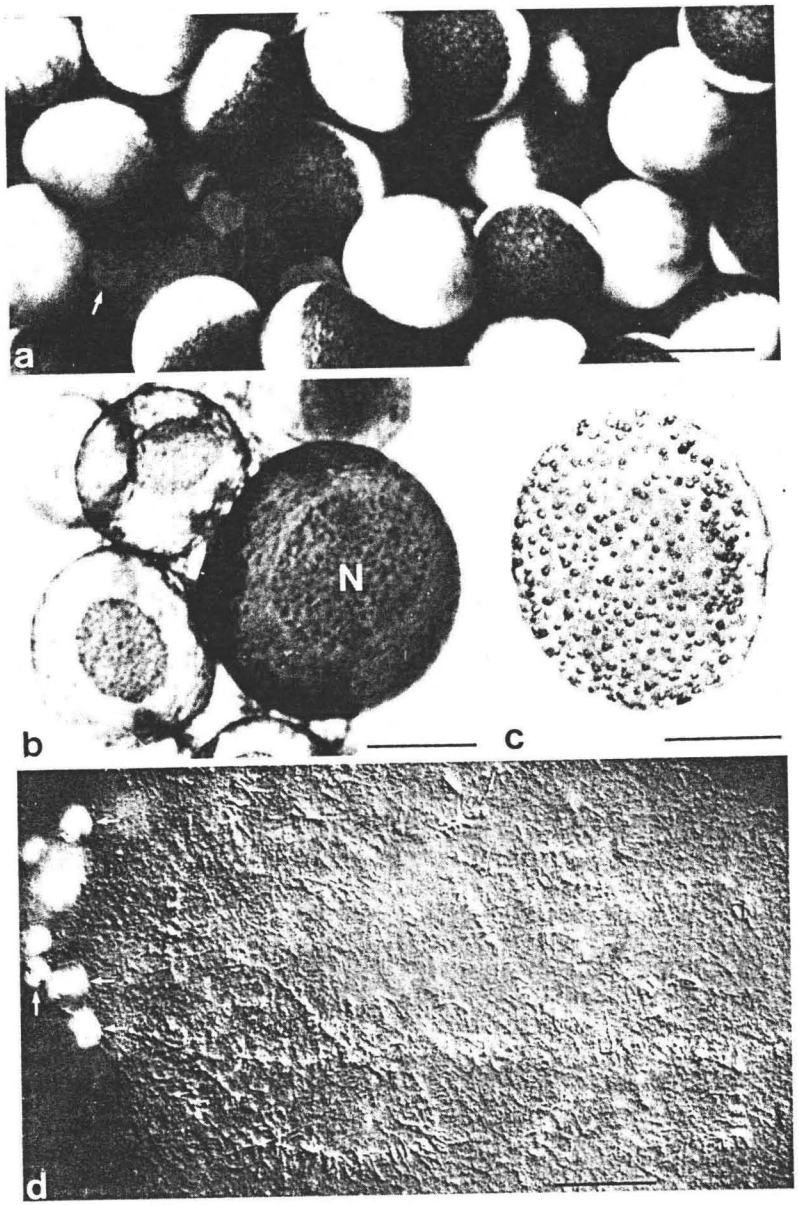

Figure 1. Amphibian oocytes. (a) Portion of a *Xenopus laevis* ovary with small previtellogenic (arrow) and full-grown oocytes characterized by a white equatorial band. (b)Nuclei (N) containing numerous amplified nucleoli are visible in living previtellogenic *Triturus alpestris* oocytes. (c) Nucleus isolated from a vitellogenic oocyte of *T. alpestris*. Note the abundance of amplified nucleoli. (d) In optical cross sections of the *T. alpestris* nucleus (the nuclear envelope has been removed for clarity), the tight packaging of the lampbrush chromosomes is evident (arrows denote some amplified nucleoli). Scale bars: 1 mm (a), 0.2 mm (b,c), and 50 μm (d).

or leakage of proteins and therefore excludes in most cases the use of conventionally isolated nuclei from somatic cells.

Thus, nuclei of amphibian oocytes are not only of general interest for studies that attempt to correlate the structure and function of nuclear components, but also provide a very useful experimental system for micromanipulation.

For the purpose of this chapter it is sufficient to divide amphibian oogenesis into three main stages: (1) previtellogenesis, (2) vitellogenesis, and (3) postvitellogenesis or maturity. A portion of an ovary from *Xenopus laevis* is shown in Fig. 1a to illustrate the difference in size between translucent previtellogenic and full-grown oocytes (the commonly used staging system is that of Dumont, 1972.)

In the first part of this chapter we discuss the main components of the amphibian oocyte nucleus, i.e., **lampbrush chromosomes, amplified nucleoli,** and the **nuclear envelope,** which can be recognized in the living oocyte (Fig. 1b) or in freshly isolated nuclei (Fig. 1c,d). It is worth recalling that these structures were identified and described in detail almost a century ago using stained paraffin sections of ovaries (Born, 1894; Carnoy and Lebrun, 1897, 1898). Next, we consider the soluble phase of oocyte nuclei—the **nucleoplasm** or nuclear sap—which contains the majority of nuclear proteins. Of special interest are histone and nonhistone proteins, which are stored in a soluble form and are most likely used to package DNA into chromatin during the extremely rapid cell divisions of early embryonic development. Finally, we discuss the possibility of interfering with gene expression in the living oocyte by microinjection of specific antibodies into its nucleus.

2. Lampbrush Chromosomes

2.1. General Morphology

Lampbrush chromosomes are the most conspicuous and are certainly the most attractive constituents of amphibian oocyte nuclei. As chromosomes of the diplotene stage of meiotic prophase, they occur in the form of homologous pairs, being joined by one or more chiasmata (Fig. 2a). Because of their size, lampbrush chromosomes can be readily observed under the light microscope (Fig. 2a,b) (for methods of preparation, see Callan and Lloyd, 1960; Gall, 1966). The axis of each chromosome appears as a linear

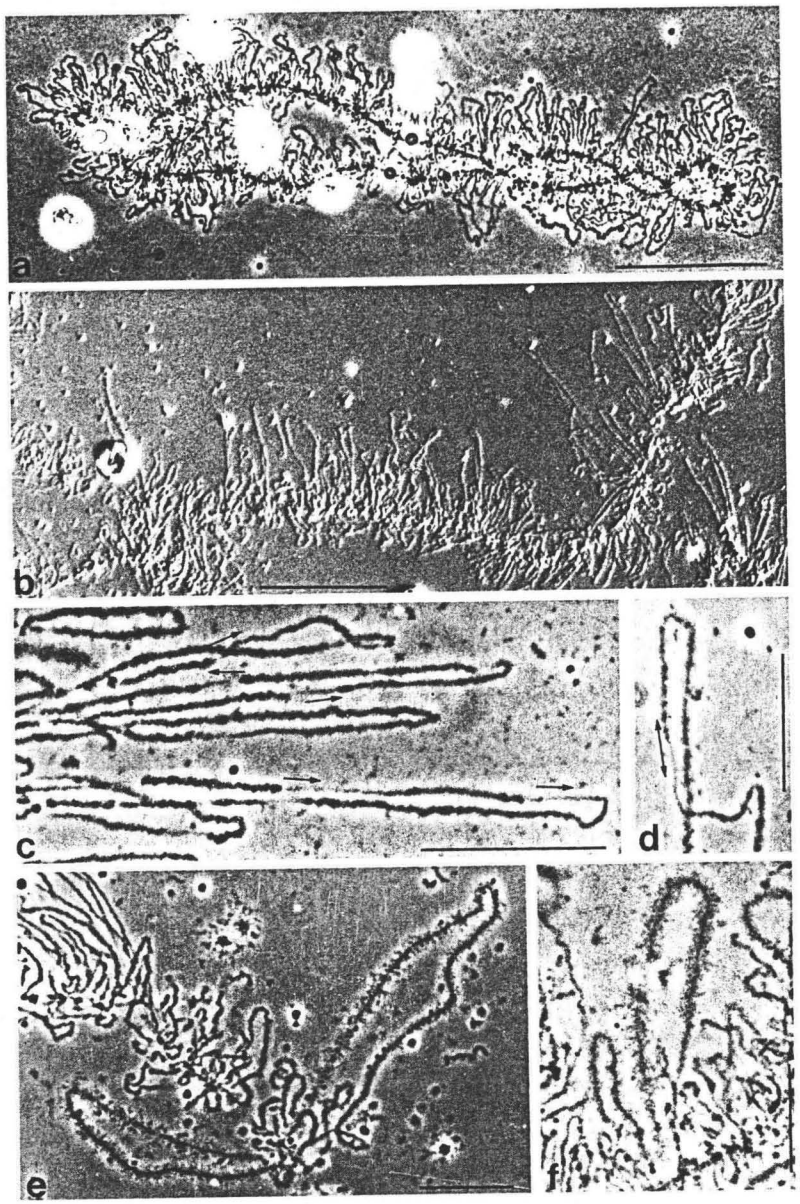

Figure 2. Isolated lampbrush chromosomes from *Pleurodeles waltlii* (a), *Triturus alpestris* (b–e) and *T. cristatus* (f), photographed with phase contrast (a, c–f) and Nomarski interference contrast optics (b). The matrix material of the giant loops (e,f) contains numerous fine fibrils of increasing lengths, so that a thin and thick loop end can be distinguished. Other loops contain two or more thin–thick gradients of identical or opposite polarities (arrows in c and d). Scale bars: 50 μm (a,b) and 20 μm (c–f).

aggregate of dark globules, the **chromomeres,** from which numerous **loops** extend laterally. In *Triturus* species, most of the loops are 30–50 μm long, but some can well exceed 100 μm in axial length (Fig. 2e). Although the chromosomal axis normally appears single, each chromosome consists of two identical chromatids, so that the loops extend as symmetrical sister pairs (Fig. 2e). Specific loops, which are recognizable by size and/or unique morphology, occur at genetically fixed positions and can serve as reference structures to identify the chromosomes. "Working maps" of lampbrush chromosomes have been constructed for numerous amphibian species (Callan and Lloyd, 1975).

The precise stage at which lampbrush chromosomes form is difficult to determine, because the unfolding of the lateral loops is a gradual process. It is clear, however, that the lampbrush form develops very early in diplotene shortly after the pachytene stage, lasting until the oocyte approaches maturity.

The lateral loops are the manifestation of ongoing transcription (see also Section 8). When lampbrush chromosomes are spread for electron microscopy according to the procedure introduced by O. Miller (Miller and Beatty, 1969; Miller and Hamkalo, 1972), numerous individual lateral fibrils are seen attached to the loop axes (Fig. 3a,b). These lateral fibrils have been identified as nascent ribonucleoprotein (RNP) transcripts, each containing a growing RNA chain complexed with proteins. They are anchored to the chromatin axis by virtue of a 12–15-nm-thick particle containing an RNA polymerase molecule (for details, see Miller *et al.*, 1972; Scheer *et al.*, 1979a; Sommerville, 1981). When viewed under the light microscope, numerous loops appear asymmetrical in the sense that they reveal a continuously increasing thickness of the RNP matrix between their two insertion sites on the chromomere (Fig. 2e,f). This polarized RNP distribution reflects the increasing transcript length as the polymerases move along the loop axis from the thin end containing the initiation site to the thick end containing the termination site for transcription. These loops therefore contain one transcriptional unit. On the other hand, loops with multiple thin–thick gradients of identical, opposite, or varying polarities can also be found reflecting the occurrence of several transcription units within a single loop (Fig. 2c,d) (Scheer *et al.*, 1979a). This interpretation is confirmed by electron microscopic spread preparations (Fig. 3c). A situation such as that shown in Fig. 3c, in which the origins of two transcription units of opposite polarity are separated by an extremely short spacer region, clearly excludes the possibility that in the living cell the spacer has formed a part of the chromomere and was pulled out artificially due to mechanical stress during the spreading procedure.

Whereas the progressive size increase of the nascent RNP fibrils can be clearly seen in electron microscopic spreads near the origin of transcription, i.e., in the proximal portions of transcriptional units (Fig. 3a–c), the lateral fibrils of more distal regions are difficult to trace because of their

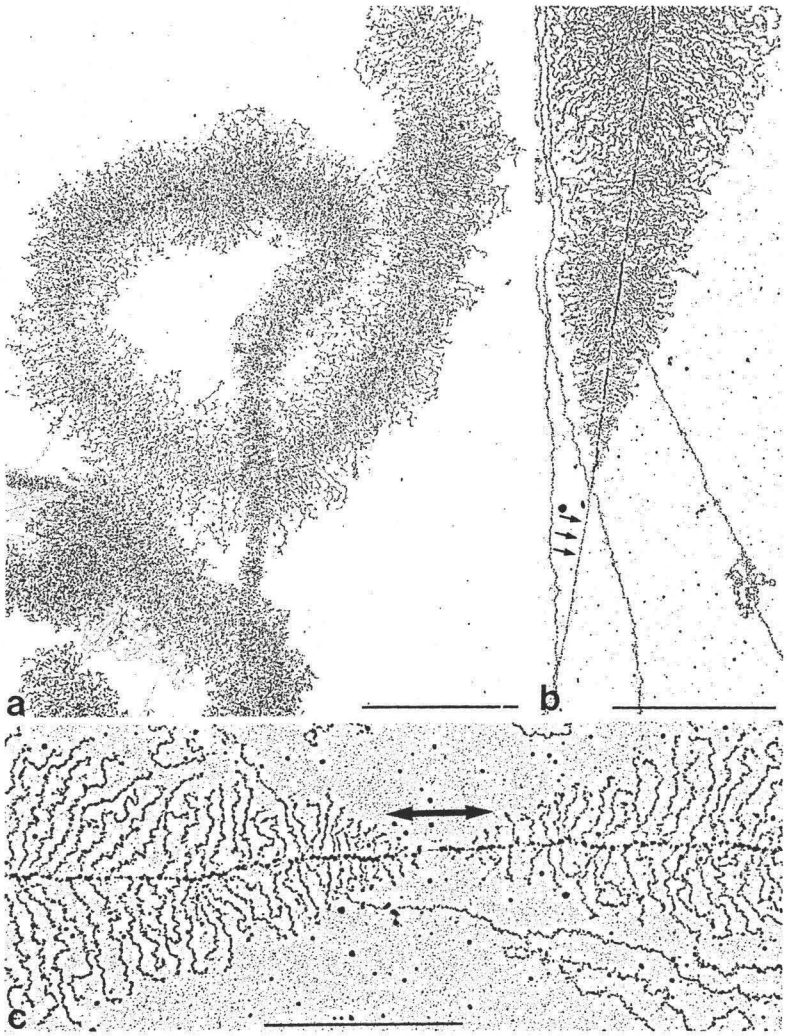

Figure 3. Electron microscopic spread preparation of lampbrush chromosomes from *Pleurodeles waltlii*. Note the high packing density of transcribing RNA polymerase particles. The nontranscribed chromatin colinear with the origin of the transcription unit shown in (b) has a beaded appearance (arrows). Two closely adjacent transcription units of opposite polarities (denoted by the double-headed arrow) are shown in c. Scale bars: 5 μm (a), 2 μm (b) and 1 μm (c).

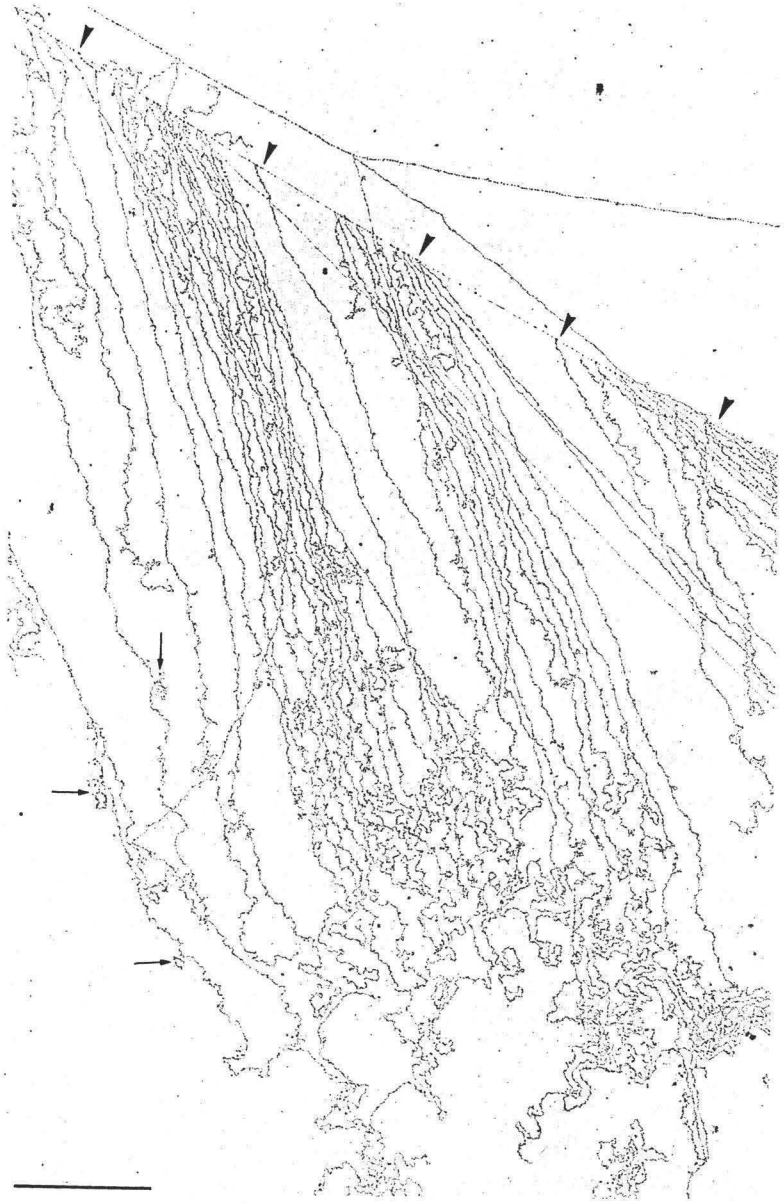

Figure 4. Terminal region of a transcription unit from a lampbrush chromosome of *Triturus helveticus*. The chromatin axis is denoted by arrowheads. The extremely long nascent ribonucleoprotein (RNP) fibrils are folded at numerous sites into complex ring- and bushlike structures (e.g., at the arrows). Scale bar: 2μm.

tendency to form extensive aggregates. In favorable spreads, however, nascent RNP fibrils can be seen to reach lengths of ≥ 20 μm (Fig. 4).

2.2. Structural Organization of Nascent RNP Transcripts

A comparison of the highly compact state of the RNP matrix as seen at the light microscopic level (Fig. 2a–d) with the extended configuration of individual lateral RNP fibrils in electron microscopic spread preparations (Fig. 4) demonstrates that *in vivo* the RNP transcripts are tightly packed but can be transformed experimentally into extended fibrils. This transformation is brought about by exposure of the lampbrush chromosomes to mildly alkaline buffers of very low ionic strength (0.1–1 mM borate buffer, pH 8–9; for details, see Miller *et al.*, 1972; Miller and Hamkalo, 1972; Scheer *et al.*, 1979a). Electron micrographs of sections through flat embedded lampbrush chromosomes isolated in a solution containing 0.1 M saline probably reflect the natural packaging state of nascent RNP transcripts (Fig. 5a) (Mott and Callan, 1975; Spring and Franke, 1981). Under such conditions, the RNP material consists of periodically arranged globular particles with a size ranging from about 25 to 40 nm. These globular units of compacted RNP are unraveled by lowering the ionic strength, as seen in spread preparations (Fig. 5b–d). An intermediate stage in this unfolding process is presented in Fig. 5b. Maximally extended RNP transcripts appear as thin fibrillar structures 5–12 nm thick (Figs. 4 and 5c,d). Occasionally, however, they display a particulate morphology due to the presence of closely spaced 20-nm particles. Such regions can alternate with regions of smooth morphology along a given fibril (Fig. 5c). Thus, under the specific spreading conditions, nascent RNP material apparently can assume two alternative morphological aspects but is—at the next higher level of organization—compacted into linear arrays of globular structures with diameters of 25–40 nm.

Lateral fibrils commonly display other, unique morphological features such as loop- and ringlike structures distributed along their entire lengths (Fig. 5d) (for further examples, see Sommerville, 1981). The most commonly found structure is an RNP loop of variable circumference connected to the main axis of the lateral fibril via a very thin stem, which most likely represents a double-stranded RNA region with few, if any, associated proteins (Fig. 5d) (Sommerville, 1981). It is important to point out that similar or more complex branching or bushlike RNP structures are also found in somatic cells of amphibian (Fig. 5e) and other species (e.g., Beyer *et al.*, 1981).

Loop-stem structures do not occur at random but rather at corresponding positions in the lateral fibrils of a transcriptional unit, shown schematically in Figure 6. Such a situation indicates that stable RNA duplex structures form at defined positions by a foldback mechanism of the nascent RNA chain shortly after synthesis of inverted repeat sequences.

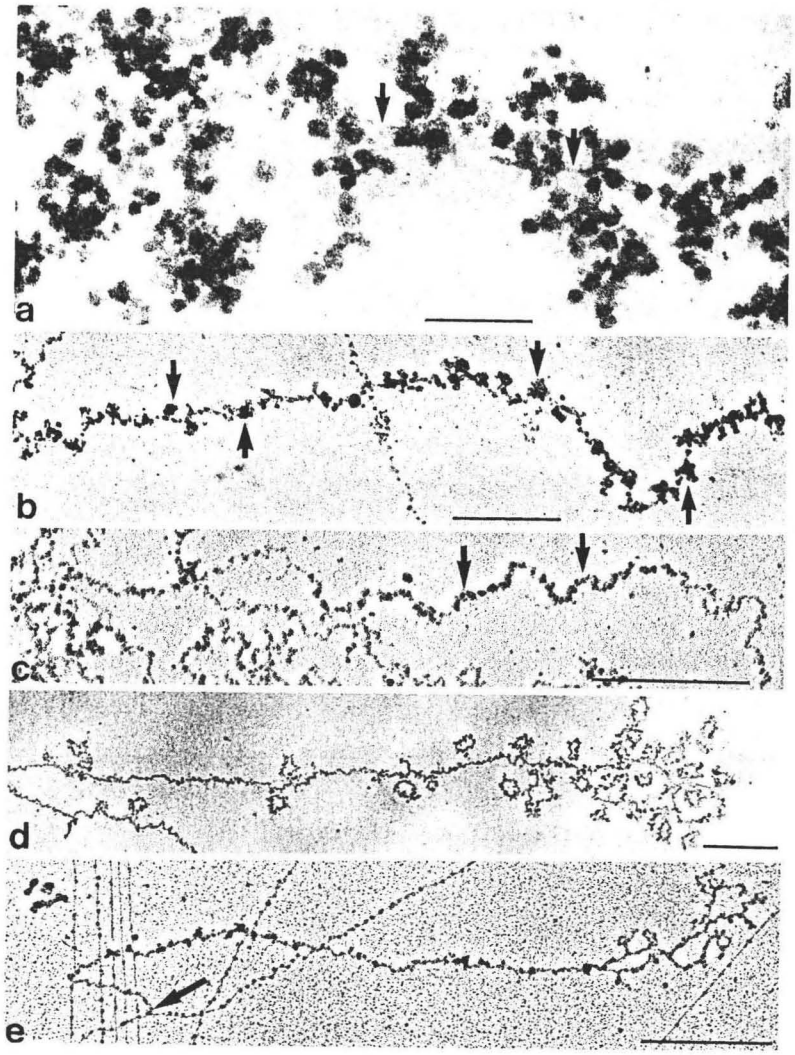

Figure 5. Morphology of nascent ribonucleoprotein (RNP) as seen in an ultrathin section through flat-embedded lampbrush chromosomes of *Pleurodeles waltlii* (a) and in spread preparations of lampbrush chromosomes from *P. waltlii* (b), *Triturus cristatus* (c), and *Necturus maculosus* (d). For comparison, spread chromatin from cultured *Xenopus* kidney cells with an attached RNP fibril is shown (e). (a) The RNP material of lampbrush chromosomes isolated in 0.1 M saline appears as strings of 25–40-nm granules around the loop axis. The loop axis is denoted by arrows. (b–d) Low salt treatment unfolds these linear aggregates of particles into fibrillar strands. (b) An intermediate state of the relaxation process is depicted, where the arrows denote some residual large globular units of condensation. (c) The extended RNP fibrils occasionally show a beaded morphology (arrows). (d) Complex folding of the RNP fibril into numerous circular structures. (e) Corresponding structures are also found in somatic cell nuclei (arrow points to the attachment site of the RNP fibril to the beaded chromatin axis). Scale bars: 0.5 μm (b–e) and 0.2 μm (a).

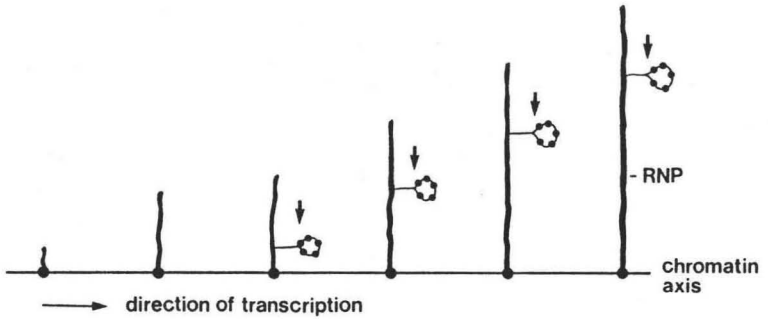

Figure 6. Analysis of a transcription unit from a lampbrush chromosome of *Triturus helveticus*. The scheme illustrates that loop formation is not a random process but occurs at corresponding sites along the nascent ribonucleoprotein (RNP) fibrils (arrows). Compare the length reduction of the third RNP fibril with that of the second RNP fibril, caused by loop formation.

2.3. Proteins Bound to Nascent RNA

Morphological evidence from spread preparations suggests that proteins bind to nascent RNA with the possible exception of double-stranded regions (see Section 2.2). Whereas the protein composition of RNP particles containing heterogeneous nuclear RNA (hnRNP) isolated from *Triturus* oocytes has been studied in detail by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Sommerville, 1981), it is as yet not clear whether the same set of proteins is also associated with nascent RNA. Immunofluorescence microscopy has been used to show that the ubiquitous core proteins of hnRNP particles with molecular weights ranging from 34,000 to 40,000 are constitutive elements of the RNP material of all loops of lampbrush chromosomes (Martin and Okamura, 1981). This observation indicates that proteins immunologically related to mammalian RNP core proteins bind to nascent RNA and are probably involved in folding the growing chain. In addition, there is considerable evidence for the occurrence of loop-specific or RNA sequence-specific proteins in addition to these ubiquitous packaging proteins (Sommerville, 1981).

2.4. Sizes of Chromosome Loops, Transcription Units, and hnRNA

The relative amount of DNA engaged in transcription at any one time can be calculated directly from light microscopic preparations of lampbrush chromosomes by determining the axial lengths of all lateral loops per chromosome set. Irrespective of large variations of genome sizes, especially of tailed amphibia, the percentage of genomic DNA contained in the lateral

loops is relatively constant at 5–10% (Scheer and Sommerville, 1982). A similar figure has been obtained from molecular hybridization experiments (see Davidson, 1976).

In *Triturus* species, most of the lateral loops have axial lengths of 30–50 μm (Fig. 2b–d). The dimensions of transcriptional units as measured in electron microscopic spread preparations are similar (3–30 μm) (Fig. 3a), although this technique tends to underestimate the true lengths of transcriptional units due to the difficulty in identifying their termini. Primary transcripts of such transcriptional units—provided that they represent contiguous RNA chains—are therefore expected to have molecular weights ranging between 3×10^6 and at least 30×10^6.

An electron micrograph showing hnRNA molecules isolated from nuclei of previtellogenic *Triturus* oocytes by a method that minimizes chain breakage is presented in Fig. 7. It is worth mentioning that a substantial proportion of the RNA molecules form stable intramolecular duplex structures under the partially denaturing conditions of surface spreading. These are visible as hairpin or loop-stem structures, which indicate the extensive occurrence of inverted repeat sequences (for details, see Sommerville and Scheer, 1982). It is tempting to correlate such RNA loops with RNP loops seen in chromatin spreads (see Section 2.2). The results of contour-length measurements of RNA molecules are summarized in Fig. 7b. It is apparent that most of the nuclear hnRNA molecules are relatively short, comparable to the sizes of poly(A)$^+$ RNA isolated from total oocyte RNA (Fig. 7c), and they do not exceed 10 μm in length (corresponding to a molecular weight of 10×10^6). Thus, nascent RNA molecules contained in apparently continuous lateral RNP fibrils such as those shown in Fig. 4 must be nicked at several sites, with the integrity of the RNP being maintained through protein–protein interactions or through double-stranded RNA regions (see Sommerville, 1981; Scheer and Sommerville, 1982).

2.5. Model of Lampbrush Transcription Units

On the basis of molecular hybridization of hnRNA and on *in situ* hybridization of various DNA probes to nascent RNA transcripts attached to chromosomal loops (DNA/RNA-transcript hybridization), the following general picture of a lampbrush transcription unit has emerged (Sommerville and Malcolm, 1976; Varley *et al.*, 1980a; Diaz *et al.*, 1981; Callan, 1982; Gall *et al.*, 1983). The origin of a transcription unit (usually the thin end of the loop), reflects initiation of transcription at a promoter sequence adjacent to the protein-coding region. The RNA polymerases continue transcription beyond the coding region without interruption all along the loop axis. According to this model, a primary transcript would contain gene sequences including introns as well as flanking noncoding sequences with various types of repetitive sequences. In fact, repetitive sequences are tran-

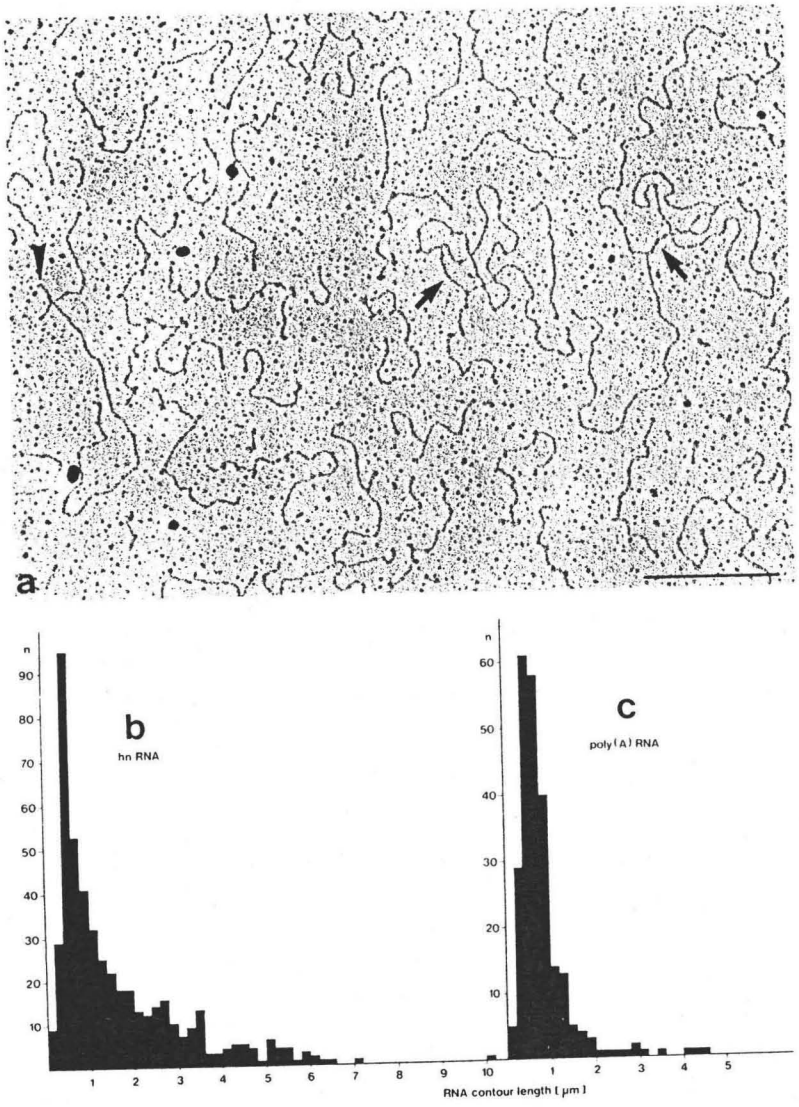

Figure 7. Analysis of hnRNA (a,b) and total poly $(A)^+$ RNA (c) from oocytes of *Triturus cristatus*. (a) Electron micrograph of spread hnRNA molecules isolated from nuclei of previtellogenic oocytes. Intrastrand duplex formation results in loop-stem structures (double-stranded "stem" regions denoted by arrows) or in hairpin structures (arrowhead). Scale bar: 0.5 μm. The histograms illustrate the contour length distributions of hnRNA (b) and polyadenylated RNA from total ovaries (c).

scribed on lateral loops, as shown by DNA/RNA transcript hybridization (Varley *et al.*, 1980a,b; Diaz *et al.*, 1981; Sommerville and Scheer, 1982; Jamrich *et al.*, 1983) and are also present in isolated hnRNA molecules, as shown by their ability to form rapidly intermolecular base-paired structures (Sommerville and Scheer, 1982). In addition, a sizeable amount of poly$(A)^+$ RNA isolated from the cytoplasm of *Xenopus* oocytes also contains interspersed repetitive sequences, in contrast to the poly$(A)^+$ RNA isolated from tadpoles (Anderson *et al.*, 1982) (see Chapter 12).

2.6. Visualization of Tandemly Repeated, Very Short Transcription Units That Do Not Form Typical Lateral Loops

Besides the huge transcription units, which are readily visualized at the light microscopic level as lateral loops, small genes such as those coding for 5 S rRNA, tRNA, small nuclear RNA (especially U1; Forbes *et al.*, 1983) and the recently discovered OAX-RNA (Wakefield *et al.*, 1983; Ackerman, 1983) are also transcribed in lampbrush chromosomes. By incubating isolated oocyte nuclei in the presence of different concentrations of α-amanitin, Schultz *et al.* (1981) were able to demonstrate convincingly that RNA polymerase II is responsible for all RNA synthesis on lateral loops, whereas polymerase III mediates RNA synthesis at a few sites scattered on the chromomeric axes of the chromosomes. This observation clearly excludes the possibility that genes transcribed by RNA polymerase III (such as the highly reiterated 5 S rRNA genes; see Chapter 10) form a typical loop.

The special chromatin organization shown in Fig. 8a,b probably represents tandemly arranged transcription units of 5 S rRNA genes. This type of chromatin organization is characterized by a periodic alternating pattern of thick and thin regions—the thick regions containing two or three closely spaced RNA polymerase particles, which are separated from each other by a beaded chromatin spacer. Nascent RNA-containing fibrils are not visible because they are below the critical size of resolution (for details, see Franke *et al.*, 1976a). The size and the pattern of arrangement of the thickened regions along a chromatin strand are compatible with the known sequence arrangement of DNA containing 5 S rRNA genes (for details, see Scheer, 1982).

Another class of slightly larger transcription units containing approximately 940 base pairs of DNA is shown in Fig. 8c. They are also tandemly repeated, separated by chromatin spacers with a nucleosomal configuration, and occur in clusters of 100 or more copies (Scheer, 1981). At the moment, they cannot be correlated with repetitious genes of defined genetic content known thus far.

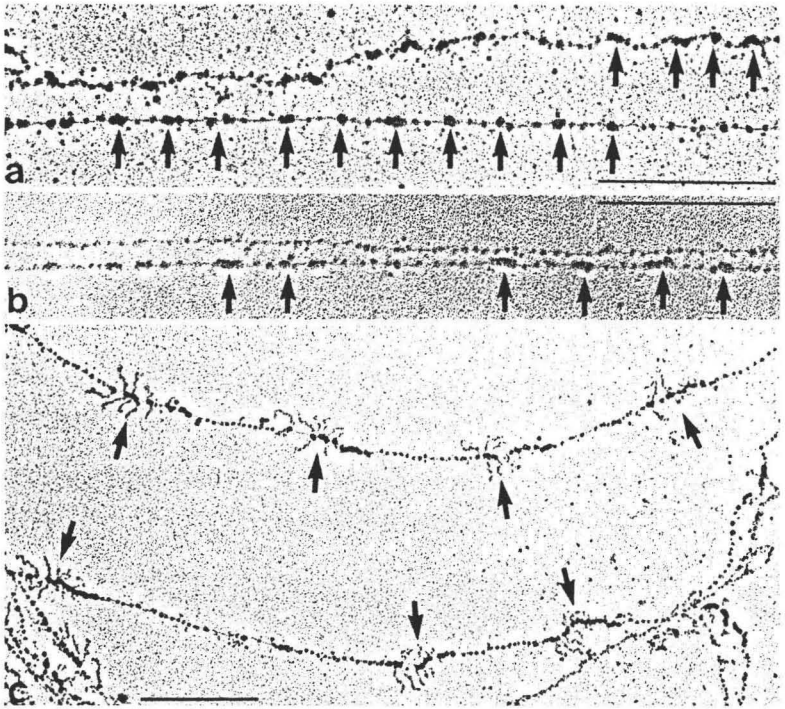

Figure 8. Spread lampbrush chromatin from *Pleurodeles waltlii* (a,c) and *Xenopus laevis* (b) oocytes. The periodic alternating thick–thin pattern seen in (a) and (b) probably reflects tandemly arranged, transcriptionally active 5 S rRNA genes (arrows) separated by beaded spacer chromatin. Another class of repeated genes is shown in c (arrows). Here, the transcripts are long enough to be visualized as lateral fibrils. Scale bars: 0.5 μm.

2.7. Chromatin Organization of Chromomeres and Lateral Loops

The transcriptionally inactive chromatin contained in the chromomeres shows a beaded, nucleosomal organization when spread for electron microscopy (Fig. 9a). This organization, which is characteristic of inactive chromatin (Olins and Olins, 1974; Oudet *et al.*, 1975), extends next to the origin of transcription units (Figs. 3b and 9a). By using immunofluorescence techniques, the presence of histones in the transcriptionally inactive chromomeric regions of lampbrush chromosomes can be demonstrated directly (Fig. 9b,c) (see also Sommerville *et al.*, 1978; Scheer *et al.*, 1979b).

Maximally transcribing loop axes are saturated with RNA polymerases with no detectable interspersed nucleosomal particles and are thought to occur in an extended state equivalent to the B conformation of DNA (Fig.

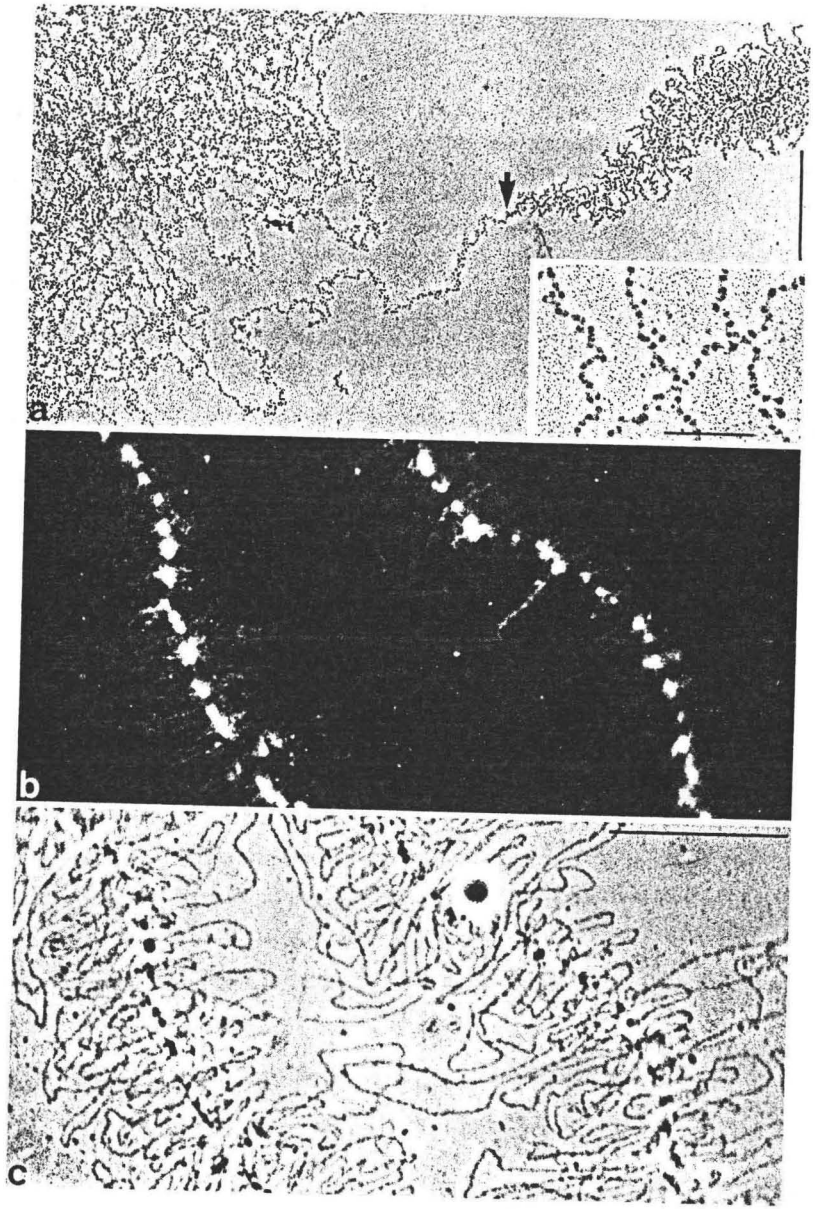

Figure 9. Chromomere organization. (a) Nucleosomal organization of transcriptionally inactive chromatin contained in the chromomere of a lampbrush chromosome of *Triturus helveticus* (arrow indicates the origin of a transcription unit). Inset shows, at higher magnification, the "beads-on-a-string" appearance of chromatin. (b) Histones are located in the chromomeres of lampbrush chromosomes (in this case from *T. cristatus*), as demonstrated by immunofluorescence microscopy using antibodies to histone H2B. (c) Corresponding phase-contrast photograph. Scale bars: 1 μm (a), 0.2 μm (inset in a) and 20 μm (c).

3a) (see Franke *et al.*, 1976b, 1978; Scheer *et al.*, 1979a). Indirect evidence suggests that histones are still associated with the transcriptionally active loop chromatin, although in an apparently nonnucleosomal mode (see Section 8). In states of reduced RNA synthesis (e.g., in maturing oocytes), the RNA polymerases are more distantly spaced, and the chromatin of such "gaps" assumes a beaded nucleosomal organization (Fig. 10a,b). Situations

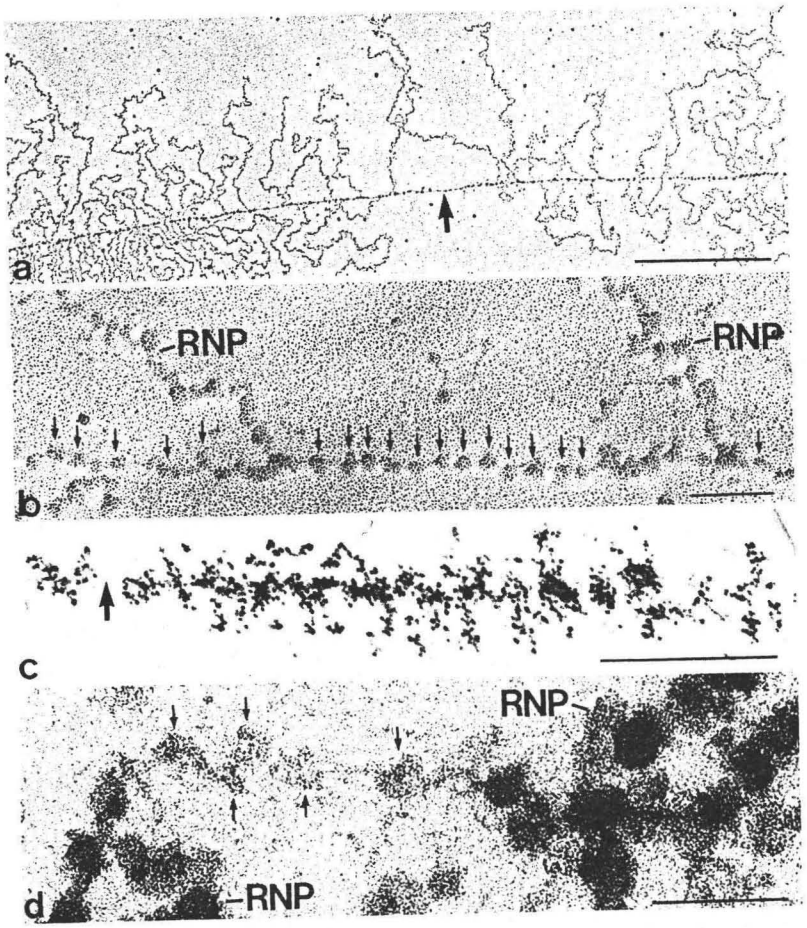

Figure 10. Morphology of transcript-free regions of loop chromatin as seen in electron microscopic spread preparations of lampbrush chromosomes of *Triturus cristatus* (a,b) and in ultrathin section through flat-embedded lampbrush chromosomes of *Pleurodeles waltlii* (c,d). Gaps within transcriptional units are recognized at low magnification (a,c) (arrows). Higher magnification displays the presence of nucleosomal beads between the distantly spaced transcripts (b,d, arrows). Scale bars: 1 μm (a,c) and 0.1 μm (b,d).

such as that shown in Fig. 10b, in which transcribing polymerases are surrounded on both sides by nucleosomes, suggest that nucleosomes unfold directly in front of a polymerase and reform rapidly following transcription (Scheer, 1978; McKnight *et al.*, 1978).

The same structural features are found when chromosomes are prepared under conditions that largely preserve their native state (Fig. 10c,d). Intragenic chromatin stretches free of transcripts invariably reveal the presence of nucleosomal beads (Fig. 10d). Thus, it appears that nucleosomes will form transiently within transcription units whenever transcribing RNA polymerases are sufficiently far apart.

2.8. Function of Loop Transcription

The conventional view assumes that lampbrush chromosomes generate a complex set of maternal as well as of polysomal mRNAs (see Sommerville, 1977; MacGregor, 1980; Davidson, 1976). However, this concept is difficult to reconcile with the finding that not only the total poly(A) RNA but also individual poly(A) RNA sequences reach their final levels early in oogenesis and remain thereafter at an approximately constant level throughout oogenesis (Rosbash and Ford, 1974; Golden *et al.*, 1980; van Dongen *et al.*, 1981, 1983). This apparent paradox was explained recently by Anderson *et al.* (1982), who showed that nuclei of *Xenopus* lampbrush stage oocytes do indeed synthesize poly(A) RNA of a complexity similar to that already stored in the ooplasm but that this RNA production is just sufficient to compensate for the continuous degradation due to turnover of the very large pool of maternal mRNA.

In order to prove that transcription of lampbrush chromosomes leads in fact to functional mRNAs, we have used an experimental approach originally developed by DeRobertis and Gurdon (1977). This approach involves injecting somatic cell nuclei into amphibian oocytes and analyzing changes in the synthetic pattern of proteins by two-dimensional gel electrophoresis. However, instead of injecting somatic cell nuclei, we have transferred single, manually isolated nuclei of early vitellogenic oocytes of *Pleurodeles waltlii* into full-grown oocytes of *Xenopus laevis*. The rationale of this experiment was to identify the synthesis of *Pleurodeles*-specific proteins in the *Xenopus* oocyte. A comparison of the [^{35}S]methionine-labeled total proteins of mature *Xenopus* (Fig. 11a) and early vitellogenic *Pleurodeles* (Fig. 11b) oocytes by two-dimensional gel electrophoresis reveals (besides some common spots such as actin) marked differences in the overall pattern of the polypeptides. This is shown more clearly when only a selected area of the gels is analyzed with actin as a reference spot (Fig. 12a,b). Figure 12d shows the result of a nuclear transfer experiment. *Xenopus* oocytes containing *Pleurodeles* oocyte nuclei were incubated for a few days, then labeled for 24 hr with L-[^{35}S]methionine, and the proteins were analyzed

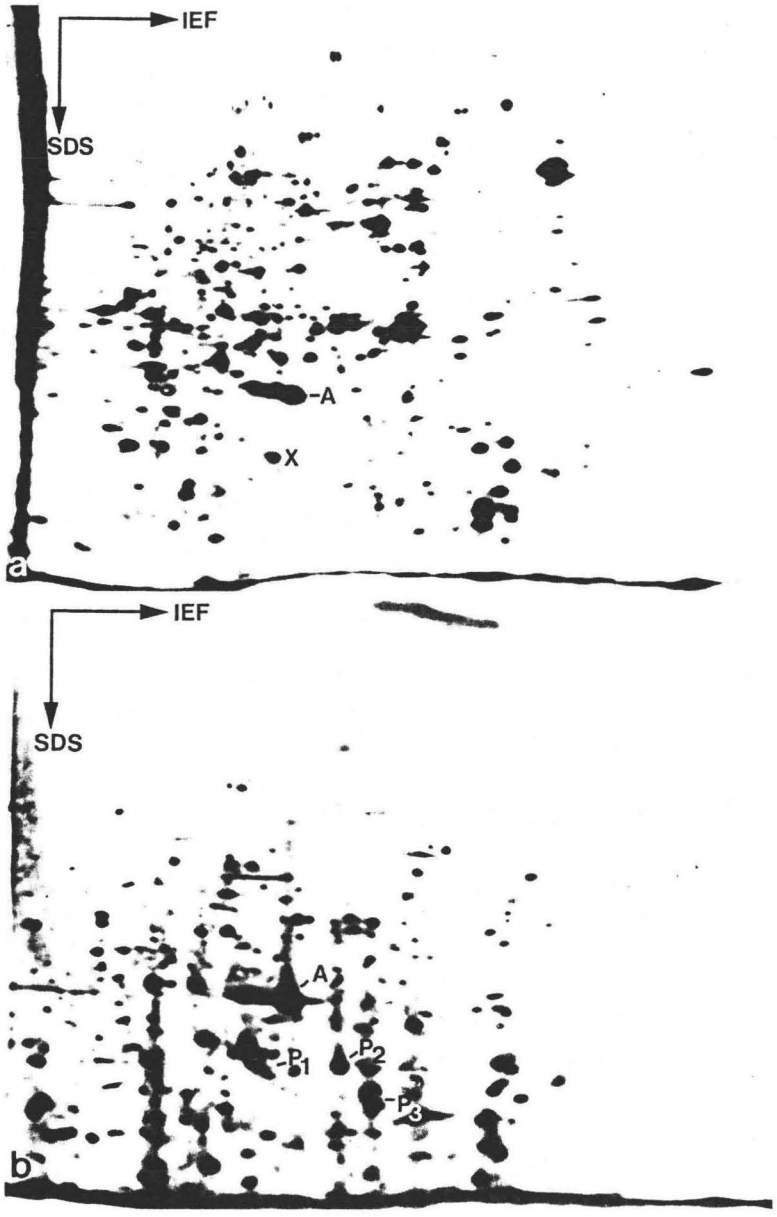

Figure 11. Radioactive proteins from full-grown oocytes of *Xenopus laevis* (a) and from previtellogenic oocytes of *Pleurodeles waltlii* (b) analyzed by two-dimensional gel electrophoresis (first dimension: isoelectric focusing, IEF; second dimension: 12% polyacrylamide in the presence of SDS). Oocytes were incubated for 24 hr in Barth medium containing [^{35}S]methionine and were then homogenized; the proteins of the low-speed supernatant were analyzed. Gels were processed for autoradiography. A, actin; X, *Xenopus*-specific protein; $P_{1\text{-}3}$, *Pleurodeles*-specific proteins.

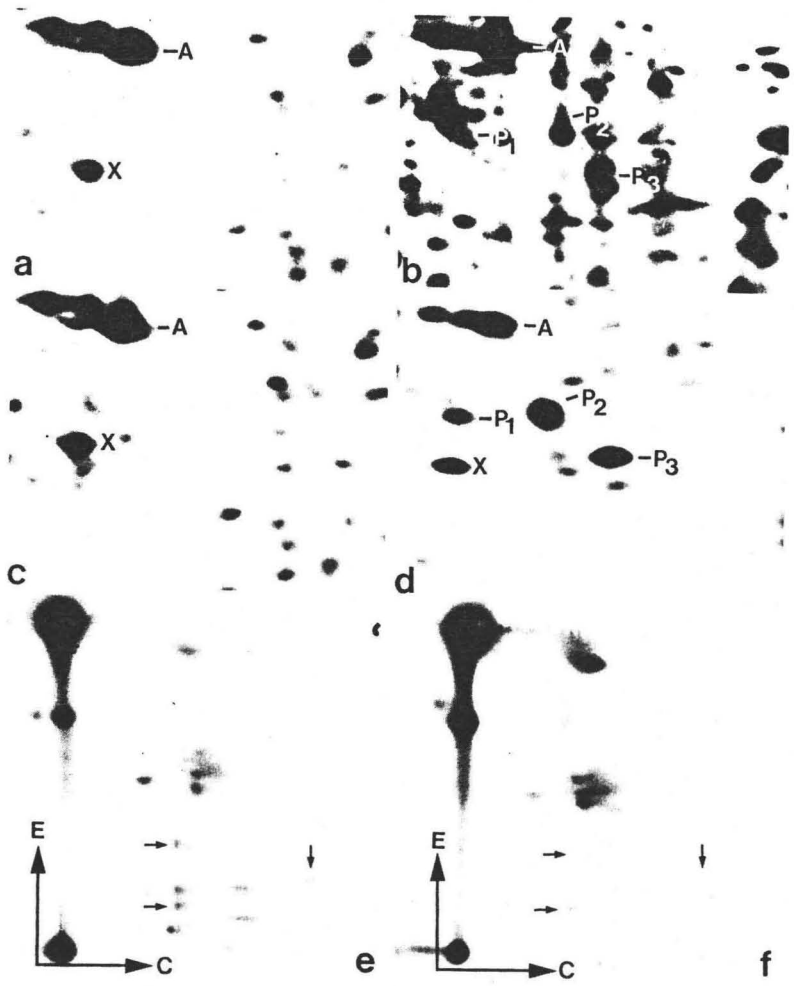

Figure 12. (a,b) The regions below actin were selected from the gels of the previous figure and are shown at higher magnification. Actin (A) serves as reference; X, *Xenopus*-specific protein, $P_{1\text{-}3}$, *Pleurodeles*-specific proteins. After transfer of the nucleus from a previtellogenic *P. waltlii* oocyte into a mature *X. laevis* oocyte, successfully healed oocytes were incubated for 5 days, followed by labeling with [^{35}S]methionine for 24 hr. *Xenopus*- and *Pleurodeles*-specific proteins are synthesized (d). As a control, *Xenopus* oocytes were injected with α-amanitin (final concentration, 1mg/ml shortly after the nuclear transfer, followed by labeling. As seen in (c) *Pleurodeles*-specific proteins are absent. (e,f) Tryptic peptide maps of *in vivo*-labeled P_2 (e, authentic P_2; f, P_2 synthesized in *Xenopus* oocytes after nuclear transfer). The pattern of cleavage products is essentially identical, with some minor spots denoted by arrows (e, f). E, electrophoresis; C, chromatography.

by two-dimensional gel electrophoresis. It is evident that *Pleurodeles*-specific proteins (designated P_1–P_3 in Fig. 12d) are synthesized. The occurrence of P_1–P_3 depends on transcriptional activity, since injection of α-amanitin at concentrations sufficient to inhibit lampbrush chromosome transcription prevents their synthesis (Fig. 12c). That P_1–P_3 are actually proteins encoded by the genome of *Pleurodeles* is suggested by the similarity of the peptide maps between authentic P_2 (Fig. 12e) and P_2 synthesized in *Xenopus* oocytes after transplantation of *Pleurodeles* oocyte nuclei (Fig. 12f).

Taken together, these results provide direct evidence to support the view that lampbrush chromosomes are synthesis sites for functional mRNAs.

3. Amplified Nucleoli

3.1. General Features

Ribosomal RNA genes are amplified in amphibian oocytes (Brown and Dawid, 1968). As a consequence, a huge number of extrachromosomal rRNA genes occur (in addition to the few hundred rRNA genes clustered at the chromosomal nucleolus organizer regions) in numerous nucleoli that are not associated with the lampbrush chromosomes (Fig. 13a,b). Although the amplification process begins in premeiotic oogonia, the main period for the selective replication of rDNA is pachytene of meiotic prophase, followed by completion in early diplotene. In *Xenopus laevis*, the amount of extrachromosomal rDNA per oocyte nucleus is about 30 pg, corresponding to approximately 2 million copies of rRNA genes, which are distributed in about 1000 amplified nucleoli (reviewed in MacGregor, 1972).

It is obvious that amplification of the rDNA enables amphibian oocytes to support unusually high rates of rRNA synthesis. Thus, a single *Xenopus* oocyte synthesizes in stages of maximal growth about 300,000 ribosomes per second (Scheer, 1973), whereas a normal somatic cell produces in the same time span only 10–100 ribosomes (Hadjiolov, 1980). The majority of the oocyte ribosomes are stored in the cytoplasm as monosomal particles for use in future embryonic growth.

In growing oocytes, the amplified nucleoli are distributed toward the periphery of the nucleus and are often firmly attached to the inner nuclear membrane by a network of fibrillar strands (Franke and Scheer, 1970). Amplified nucleoli appear either as compact spheroidal bodies 2–10 μm in diameter (Figs. 1c; 13a, and 17a) or as ringlike structures (Fig. 13b). In several amphibian species, transitions between both nucleolar forms have been described in specific stages of oogenesis (see MacGregor, 1972).

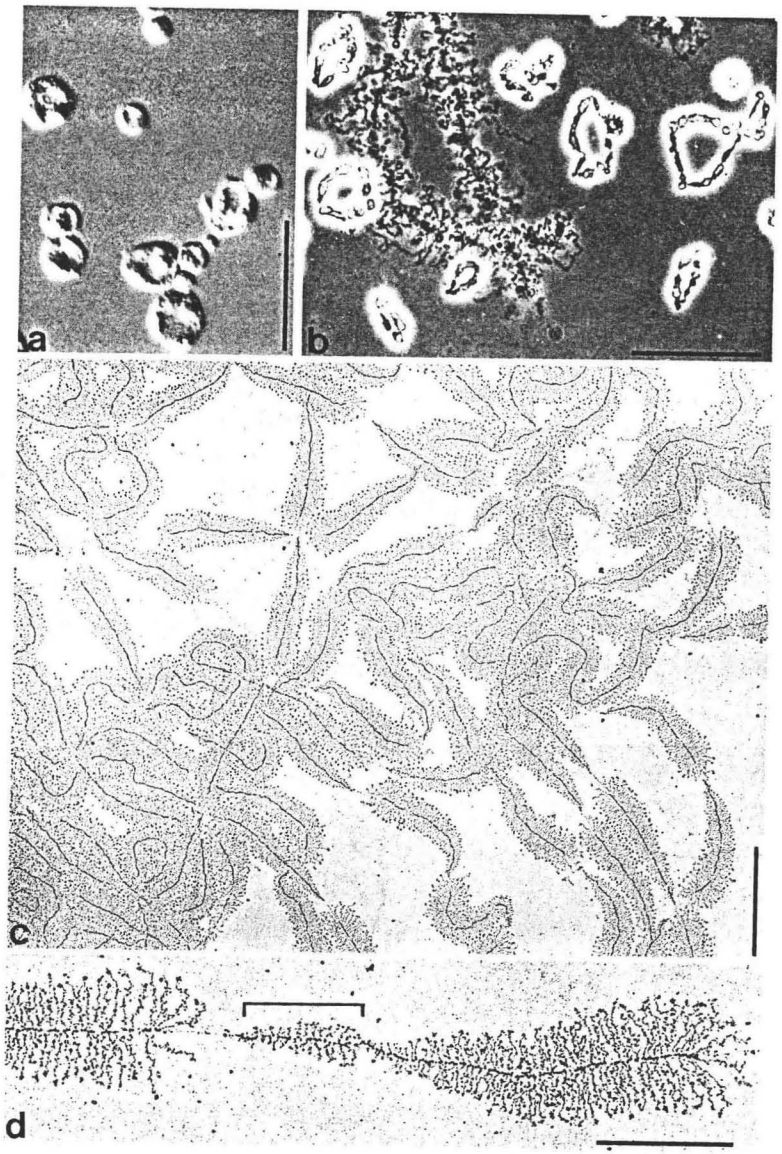

Figure 13. Amplified nucleoli (a,b) and nucleolar chromatin (c,d). In light microscopic preparations, amplified nucleoli appear as compact spheroidal entities (a, from *Triturus alpestris*, Nomarski interference contrast) or as ringlike structures (b, from *Pleurodeles waltlii*, phase contrast). When spread for electron microscopic study, nucleolar chromatin displays the characteristic tandem arrangement of transcriptionally active rRNA genes (c, from *P. waltlii*; d, from *X. laevis*). Note the small transcriptional unit in the spacer region between two adjacent rRNA genes (bracket, d). Scale bars: 50 μm (a,b), 2 μm (c), and 1 μm (d). (Figure 13d courtesy of Dr. M. Trendelenburg.)

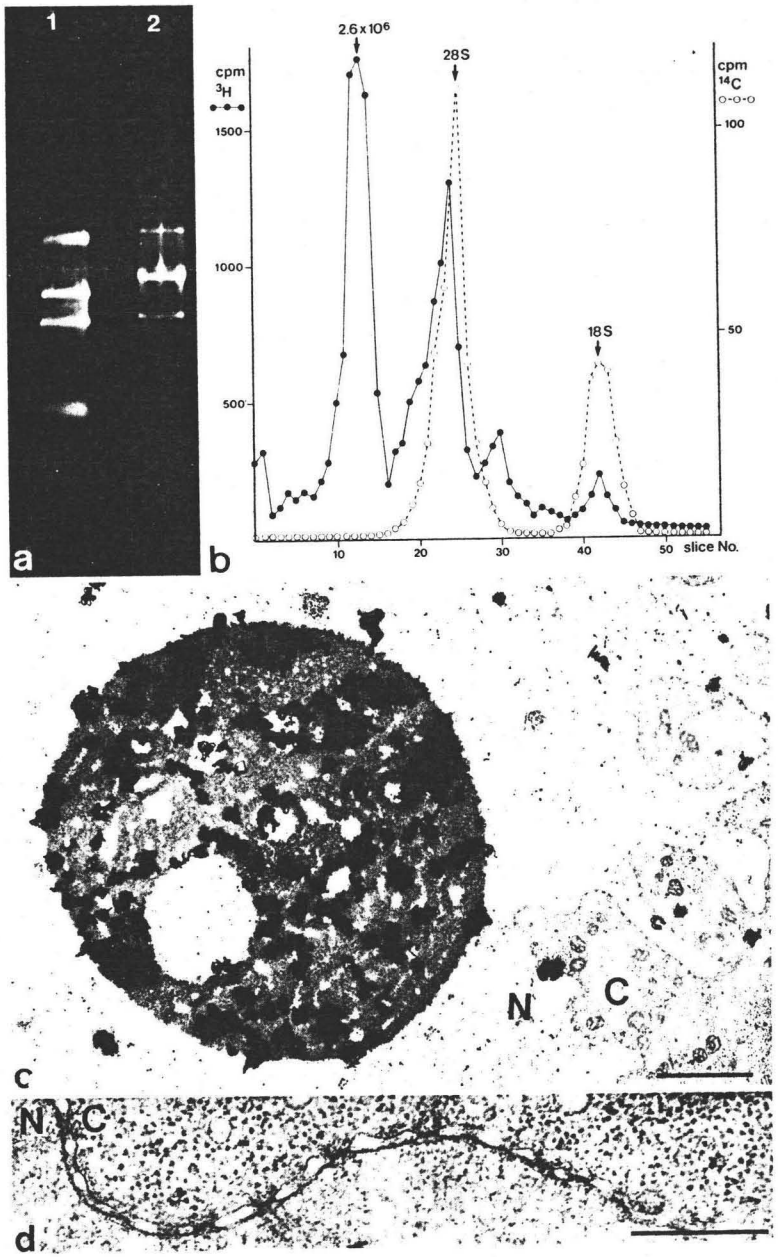

Figure 14. (a,b) Gel electrophoretic analysis of RNA from manually isolated nuclei. Total RNA from 150 *Xenopus laevis* oocyte nuclei was separated on a 1.5% agarose gel and stained with ethidium bromide (a, lane 2). The three bands correspond to 40 S pre-rRNA, 30 S, and 18 S rRNA. Reference RNAs are, from top to bottom, tobacco mosaic virus RNA, *Escherichia coli* rRNAs, and tRNA (a, lane 1). After incubation of *Triturus alpestris* oocytes in a medium containing [^{3}H]uridine, nuclei were isolated and the RNA was analyzed on a composite gel (2.25% acrylamide, 0.5% agarose) together with added ^{14}C-labeled rRNA from *Xenopus* as internal reference (b). Note that 28 S rRNA migrates ahead of the nuclear radioactivity peak. (c) In cytological preparations, silver grains are preferentially localized over the amplified nucleoli (electron microscopic autoradiography after labeling the oocytes of *T. alpestris* with [^{3}H]uridine). (d) Typical ribosomal particles are absent from the nucleoplasm, as demonstrated in the ultrathin section through the nuclear periphery of a *T. alpestris* oocyte. N, nucleus; C, cytoplasm. Scale bars: 2 μm (c) and 0.5 μm (d).

3.2. Visualization of Transcribing rRNA Genes

By introducing a new spreading technique to prepare chromatin for electron microscopic examination, Miller and Beatty (1969) were able to visualize directly the process of transcription of amplified rRNA genes from amphibian oocytes. An example of spread nucleolar chromatin from a *Pleurodeles* oocyte is shown in Fig. 13c. Numerous maximally active rRNA genes are seen in linear arrays separated from each other by so-called nontranscribed spacers. Each gene contains about 100 nascent RNP transcripts arranged in a length gradient, thereby giving rise to the characteristic Christmas tree appearance. Each transcript is anchored to the chromatin axis by a 12–15 nm particle that contains a RNA polymerase I complex (for details, see Franke *et al.*, 1979; Scheer and Zentgraf, 1982). It should be emphasized that the "nontranscribed" spacer regions not infrequently contain small transcription units ("prelude complexes") (Fig. 13d), which (in *Xenopus laevis*) seem to initiate at positions corresponding to reduplicated promoterlike sequences (Trendelenburg, 1981). It has been hypothesized that spacer transcription might serve to concentrate RNA polymerases around the ribosomal gene promoter (Moss, 1983).

3.3. Synthesis of rRNA and Formation of Preribosomes

The amplified rRNA genes are transcribed as 40 S pre-rRNA molecules with an apparent molecular weight of 2.6×10^6 (Fig. 14a,b) (for review, see Sollner-Webb *et al.*, 1982). When total RNA extracted from manually isolated vitellogenic *Xenopus* oocytes is analyzed by gel electrophoresis, it is evident that rRNA species predominate (Fig. 14a). The prevalence of rRNA synthesis can also be demonstrated by autoradiography after labeling the oocytes with [^{3}H]uridine. Most of the silver grains are located over the peripheral, amplified nucleoli (Fig. 14c).

Specific ribosomal proteins bind to pre-rRNA molecules while they are still attached to the template. This has been shown convincingly in *Drosophila* by combining Miller spreads with immunolocalization techniques (Chooi and Leiby, 1981). By contrast, other ribosomal proteins such as S 1 attach to preribosomes only at later stages of their maturation pathway, i.e.,

after translocation of the newly synthesized rRNA into the granular component of the nucleolus (Hügle *et al.*, 1985). The coordinate association with ribosomal and nonribosomal proteins and processing of the pre-rRNAs leads to the formation of different kinds of nucleolar RNP particles (Rogers, 1968), which finally mature into ribosomal subunits (reviewed in Hadjiolov, 1980). The final maturation steps occur in amphibian oocytes immediately before, during, or shortly after the passage through the nuclear pores, since not only morphologically identifiable ribosomal particles are absent from the nucleoplasm (Fig. 14d), but the 28 S rRNA component of the large subunit of cytoplasmic ribosomes is also missing (Fig. 14b). Instead, an RNA precursor sedimenting at about 30 S is detected in isolated nuclei by gel electrophoresis (Fig. 14b).

The protein complement of preribosomal particles of amphibian oocyte nuclei is so far unknown. Recent experiments based on microinjection of ribosomal proteins into oocytes of *Xenopus laevis* suggest that certain ribosomal proteins migrate into the nucleus and take part in the formation of preribosomal particles (Kalthoff and Richter, 1982).

3.4. Regulation of rRNA Synthesis

Although rDNA amplification takes place very early in oogenesis (the pachytene stage), synthesis of rRNA reaches its peak value only after the onset of yolk deposition (Ford, 1972; Sommerville, 1977; Scheer *et al.*, 1976a). On the basis of biochemical data, it has been calculated that previtellogenic and mature oocytes of *Triturus alpestris* synthesize rRNA at only about 0.01% and 13%, respectively, of the rate of vitellogenic oocytes (Scheer *et al.*, 1976a). In states of reduced synthetic activity the rRNA genes reveal a reduced number of RNP transcripts (Fig. 15a–c). This observation indicates that regulation of rRNA synthesis occurs at the level of transcription by modulation of the frequency of initiation events. Each gene represents an independent unit of regulation, since genes fully loaded with RNA polymerases can alternate with sparsely loaded genes or with totally inactive ones (Fig. 15a) (Scheer *et al.*, 1976a).

3.5. Structure of Nucleolar Chromatin at Different States of Activity

It is now generally accepted that transcriptionally active nucleolar chromatin—including gene and spacer regions—is organized differently from inactive chromatin, which is packed into nucleosomes (Franke *et al.*, 1976b; Scheer, 1978; Labhart and Koller, 1982; for reviews, see Franke *et al.*, 1979; Scheer and Zentgraf, 1982). The absence of nucleosomes is especially evident in transcription units with a reduced number of transcripts

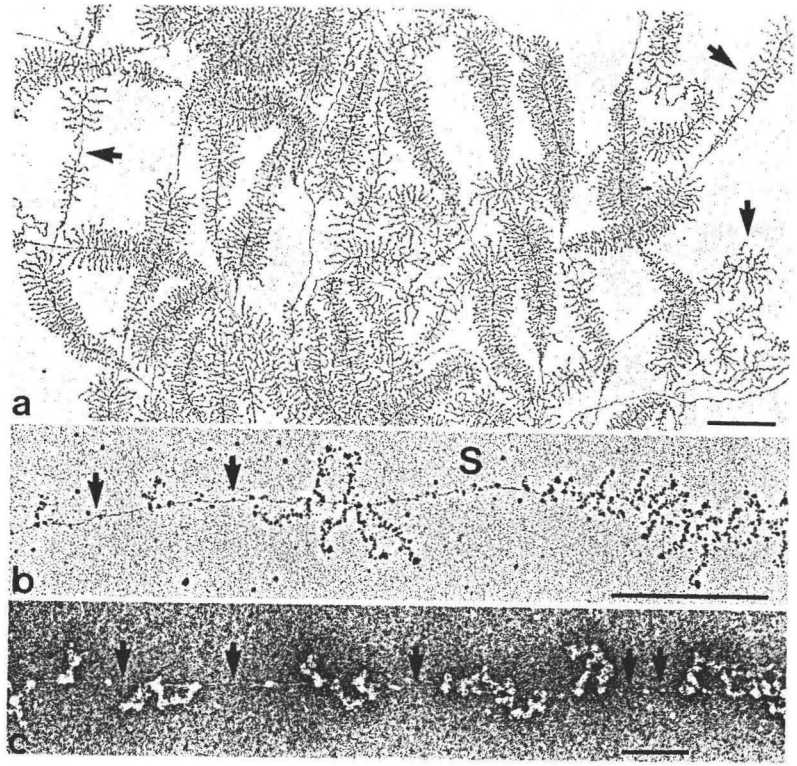

Figure 15. Spread preparations of nucleolar chromatin from full-grown oocytes of *Pleurodeles waltlii* (a,b) and *Triturus cristatus* (c) after positive staining and additional metal shadowing (a,b) or negative staining (c). Several rRNA genes are only sparsely covered by transcripts (arrows, in a). The transcript-free intragenic regions (arrows in b and c) and the spacer (S, b) exhibit a nonbeaded organization. Scale bars: 1 μm (a,b) and 0.1 μm (c).

(Fig. 15b,c): The chromatin intervals between adjacent RNA polymerase particles are thin and nonbeaded. The same holds true for the spacer regions, although—depending on the specific spreading conditions—irregularly sized and distributed particles can be present, which are, however, of a nonnucleosomal nature (Fig. 15b) (Scheer, 1980). When the contour lengths of transcription units, spacer regions, and the total repeat units are compared with the corresponding lengths of deproteinized rDNA, it is obvious that nucleolar chromatin occurs in electron microscopic spread preparations in a fully extended state equivalent to the length of the B-form DNA (Scheer *et al.*, 1977; Reeder *et al.*, 1978). The important question as to whether histones are associated with transcriptionally active rDNA is as yet not settled. Biochemical data indicate that histones with the excep-

tion of H1 are present in the rDNA-containing nucleolar "cores" isolated from amplified nucleoli of *Xenopus* oocytes (Higashinakagawa, 1982). However, unless the histones:rDNA ratio is determined, it cannot be excluded that active nucleolar chromatin is deficient in histones. Thus, at the moment it is not clear whether the specific organization of active nucleolar chromatin reflects a deficiency in histones or a nonnucleosomal arrangement of the full complement of histones.

Completely inactive nucleolar chromatin assumes a nucleosomal organization indistinguishable from that of other portions of inactive chromatin (Scheer, 1978). Thus, it appears that the extended conformation reflects a state of transcribability but that the actual transcriptional intensities of individual rRNA genes are controlled by other factors.

3.6. Protein Filaments

Recent evidence suggests that protein filaments occur within the nucleoli. These filaments might be involved in maintaining nucleolar structural integrity and/or in storage, maturation, and intranucleolar translocation of the preribosomal particles.

3.6.1. Medusoid Fibril Bodies

In spread preparations of *Xenopus laevis* oocyte nuclei, transcribed rRNA genes are often seen in conspicuously close vicinity to roundish aggregates of a distinct filament type (Fig. 16a). These filamentous aggregates, which have been termed **medusoid fibril bodies** (Moreno Diaz de la Espina *et al.*, 1982), contain two structurally different components: the filaments proper with a diameter of 8–12 nm and a distinctly beaded appearance and densely stained 18–30-nm particles, which are attached at numerous sites to these filaments (Fig. 16b). It has been suggested that the medusoid fibrils form part of the nucleolar cortex and provide structural support for the attachment of nucleolar RNP particles (Moreno Diaz de la Espina *et al.*, 1982).

3.6.2. Residual Skeleton Structures

Amplified nucleoli can be isolated in a highly pure state by fluorescence-activated particle sorting using an argon ion laser (Fig. 17a) (Franke *et al.*, 1981a). Extraction of the nucleoli in a buffer of high ionic strength (1 M KCl) containing 1% of the nonionic detergent Triton X-100 leaves a residual fibrillar meshwork of about 3–5-nm-thick filaments (Fig. 17b,c). Digestion of the nucleoli with DNAse and RNAse before the salt extraction does not alter the morphological appearance of these fibrillar aggregates; trypsin, on the other hand, effectively destroys them (Franke *et al.*, 1981a).

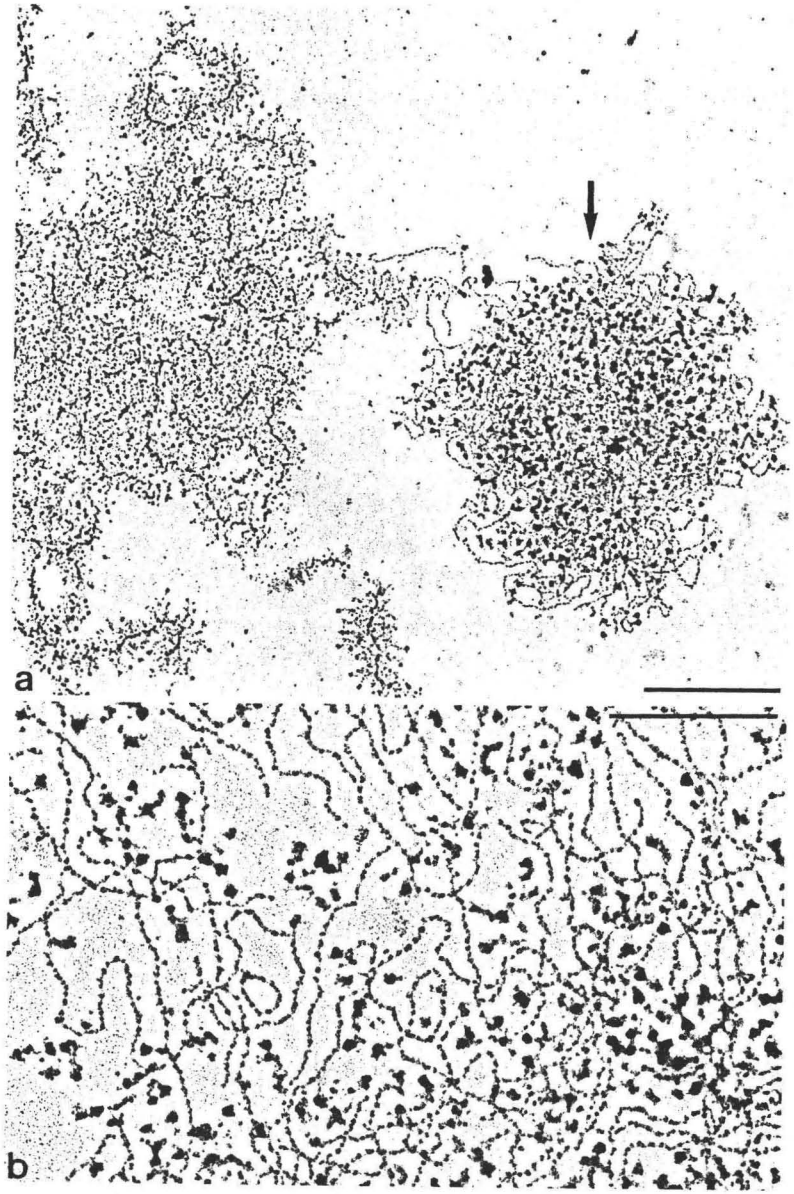

Figure 16. Spread preparation of nucleolus-enriched material from *Xenopus laevis* oocytes. (a) The "medusoid body" composed of a network of fibrils (arrow) lies next to a group of rRNA genes. (b) Numerous large particles are attached to the filaments, which exhibit a periodic substructure. Scale bars: 1 μm (a) and 0.5 μm (b).

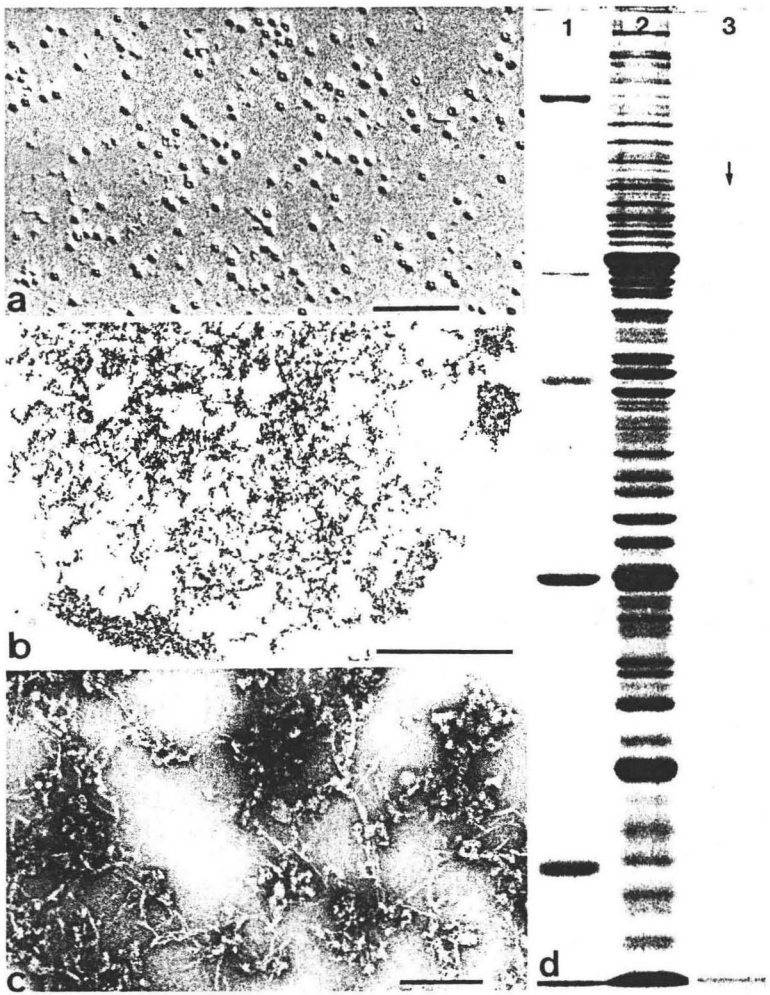

Figure 17. Insoluble protein component of amplified nucleoli from *Xenopus laevis* oocytes. Nucleoli isolated by fluorescence-activated particle sorting are shown in a (Nomarski interference contrast). After extraction with 1 M KCl and 1% Triton X-100, residual fibrillar structures remain as seen in ultrathin sections (b) and in negatively stained preparations (c). Upon one-dimensional gel electrophoresis, only one predominant polypeptide with a molecular weight of 145,000 is recognized in such residual fractions (d, lane 3, arrow). For comparison, the complex protein pattern of total nuclei is also shown (d, lane 2). Reference proteins are, from top to bottom, myosin heavy chain, phosphorylase a, bovine serum albumin (BSA), actin, and chymotrypsinogen (d, lane 1). Scale bars: 50 μm (a), 1 μm (b), and 0.1 μm (c).

Fractions enriched in high-salt-resistant nucleolar fibrils contain only one major protein with a molecular weight of approximately 145,000 (Fig. 17d, lane 3) and an isoelectric pH value of about 6.15 (Franke *et al.*, 1981a). Antibodies against this protein were used to demonstrate that the nucleolar skeletal fibrils are not uniformly distributed throughout the amplified nucleoli but are more concentrated toward their peripheries (Krohne *et al.*, 1982; Benavente *et al.*, 1984a,b). Furthermore, these workers showed that immunologically related proteins occur also in nucleoli of somatic *Xenopus* cells.

4. The Nuclear Envelope

4.1. Morphology and Distribution of Pore Complexes

The nuclear envelope of amphibian oocyte nuclei is perforated by numerous pore complexes, which—in freeze-fractured (Fig. 18a) or negatively stained (Fig. 18b) preparations—appear as ringlike structures with diameters of 70–80 nm. In transverse section, it is obvious that the pores are sites of local fusion of the inner and outer nuclear membrane (Fig. 19d). Pore complexes are ubiquitous structures found in all eukaryotic cells and have a very characteristic and highly regular architecture, the most prominent nonmembranous components being eight symmetrically arranged annulus subunits on either pore margin (Figs. 18b and 19d) (for reviews, see Franke and Scheer, 1974; Franke *et al.*, 1981b). The number of pore complexes per unit area is remarkably high in amphibian oocytes (50–60 pores/μm^2) (Fig. 18a,b), so that about 25% of the surface area of the nucleus is occupied by pores. This high density—together with the enormous size of the germinal vesicle—results in a total number of about 38 million pore complexes per nucleus of a mature *Xenopus* oocyte. They are formed during oogenesis at a rate of about 8 pores/sec (Scheer, 1973).

4.2. Architectural Components of the Pore Complexes: High-Salt-Resistant Proteins

Isolated oocyte nuclei can be manually subfractionated into nuclear envelopes and gelled nuclear contents (Fig. 19a–c). The nuclear envelopes thus obtained are extremely pure and represent a fraction that is highly enriched in pore complexes (Fig. 19d) (Scheer, 1972). When exposed to mechanical stress (such as during surface spreading on a drop of water), the pore complexes remain intact. With progressive disintegration of the interporous membrane, a network of resistant fibrils appear, which seems to interconnect all pore complexes (Fig. 19e; Scheer *et al.*, 1976b). After extraction with 1.5 M KCl and 1% Triton X-100, the basic architecture of

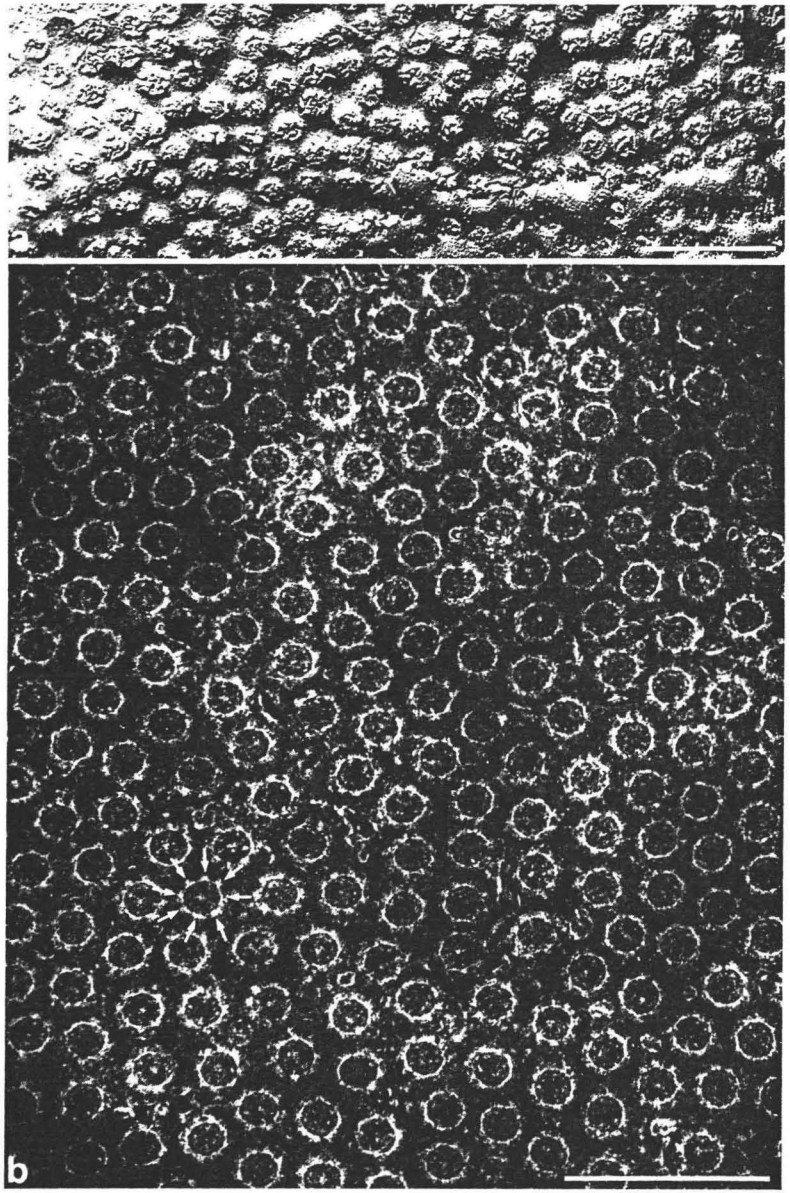

Figure 18. Nuclear envelope from *Xenopus laevis* oocytes as seen in freeze-etched (a) and negatively stained (b) preparations. Note the eight symmetrically arranged annulus subunits lying on the pore rims (e.g., at the arrows in b) and the central granules. Scale bars: 0.5 μm.

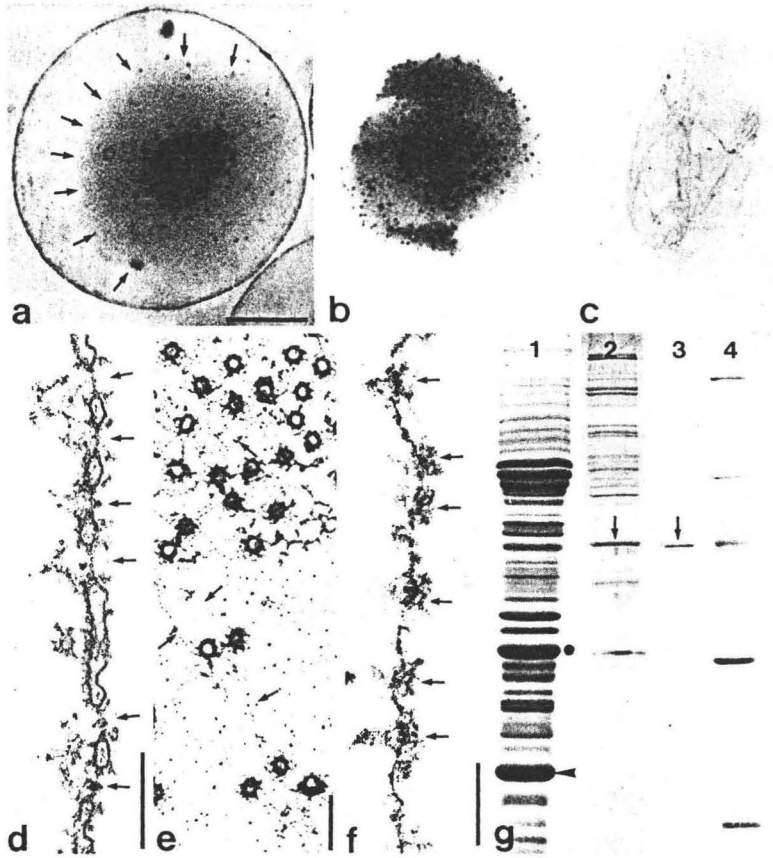

Figure 19. Isolation and high salt extraction of the nuclear envelope. (a–c) Isolation procedure. An isolated nucleus (in this case from *Triturus alpestris*) is transferred into a medium containing 5–10 mM $MgCl_2$, in which the nucleoplasm forms a gel (denoted by arrows, a). The nuclear envelope is then punctured and separated from the gelled nucleoplasm (b,c). Note the purity of the nuclear envelope fraction as demonstrated by electron microscopy of ultrathin sections (d, port complexes denoted by arrows). Mechanical stress (e) or extraction with 1 M KCl plus 1% Triton X-100 (f) destroys the interporous membrane and exhibits a network of fibrils interconnecting the very stable pore complexes as shown by surface spreading (e) or ultrathin sectioning (f) (interporous fibrils denoted by arrows, e; high salt-stable pore complexes denoted by arrows, f). (g) One-dimensional gel electrophoresis of proteins from total nuclei (lane 1), nuclear envelopes (lane 2), and extracted nuclear envelopes (lane 3) shows the enrichment of one polypeptide band with a molecular weight of 68,000 (arrows). The dot denotes actin and the arrowhead indicates nucleoplasmin (lane 1). Reference proteins are, from top to bottom, myosin heavy chain, phosphorylase a, bovine serum albumin (BSA), actin, and chymotrypsinogen (lane 4). (Figures 19 f,g courtesy of Dr. G. Krohne.) Scale bars: 0.2 mm (a; a–c are magnified to the same scale) and 0.2 μm (d–f).

the pore complexes is still retained; in ultrathin sections, it is evident that they are interconnected by a thin layer ("lamina") at the level of the inner nuclear membrane (for further details, see Franke *et al.*, 1981b; Benavente *et al.*, 1984b). The protein composition of the extracted nuclear envelopes is very simple with only one major component having an apparent molecular weight of 68,000 (Fig. 19g, lane 3) (Krohne *et al.*, 1978, 1981). Immunological data indicate that the high-salt-resistant protein with a molecular weight of 68,000 is not only a structural component of the lamina proper, but contributes to the architecture of the pore complexes as well (Stick and Krohne, 1982; Benavente *et al.*, 1984b).

4.3. Permeability of the Nuclear Envelope and Nucleocytoplasmic Flow Rate of Ribosomal RNP through the Pore Complexes

Various substances—including proteins and colloidal gold particles of known sizes—have been microinjected into living oocytes in order to study the permeability characteristics of the nuclear envelope (reviewed in Bonner, 1978; Paine and Horowitz; 1980; Feldherr and Ogburn, 1982). The results are consistent with the view that macromolecules enter the nucleus by passing through the pore complexes and that the passage is restricted to a central channel with a patent opening much smaller than the actual pore diameter. The upper size limit for passage through the pore complexes is about 10–15 nm; this is also the size to which large RNP particles constrict during their nucleocytoplasmic translocation (Franke and Scheer, 1970). Experiments involving mechanical disruption of the nuclear envelope in intact oocytes have suggested that the nuclear envelope does not play a major role for the intracellular compartmentation of endogenous proteins (Feldherr and Ogburn, 1982). However, the model view of free diffusion through the pores and selective retention in the nucleoplasm to explain the unequal nucleocytoplasmic distribution of numerous proteins (Bonner, 1978) is not consistent with recent experiments favoring instead a selective entry mechanism for nucleoplasmin (Dingwall *et al.*, 1982) (see also Section 5.1). Furthermore, it has been shown that the cytoplasm of *Xenopus laevis* oocytes contains an abundant class of relatively small, soluble proteins, which are excluded from the nucleus ("karyophobic" proteins) (Dabauvalle and Franke, 1984). Thus, at the moment it is not clear to what extent the pore complexes are involved in the regulation of nucleocytoplasmic exchange and the maintenance of the differential distribution of proteins in both compartments (see also DeRobertis, 1983).

In growing oocytes of *Xenopus laevis*, two to three rRNA molecules are transferred every minute through a pore complex to the cytoplasm (Scheer, 1973). It is interesting to note that this rate does not represent the maximal transport capacity of a pore complex for ribosomal RNP material. In macronuclei of exponentially growing *Tetrahymena pyriformis*, for instance, the flow rate is higher by a factor of at least ten (Franke and Scheer, 1974). Thus, it is unlikely that the main function of the millions of pore complexes on the surface of oocyte nuclei is simply to provide enough export sites for ribosomal RNP material.

5. The Nucleoplasm

5.1. Identification of Some Major Stored Proteins: Nucleoplasmin, N1/N2, Histones, HMG Proteins, and Actin

The term nucleoplasm was originally used to describe the nonstructured ground substance of cell nuclei. In the present context the **nucleoplasmic fraction** is defined preparatively as the supernatant fraction obtained after centrifugation of a nuclear homogenate at 100,000 × g for 1 hr.

Total proteins extracted from manually isolated oocyte nuclei reveal a complex pattern upon gel electrophoresis (Fig. 20a). A major protein is **nucleoplasmin** (Laskey and Earnshaw, 1980), with a molecular weight of approximately 30,000. Nucleoplasmin is quantitatively recovered in the high speed supernatant fraction of nuclei and can be readily identified by two-dimensional gel electrophoresis (Fig. 20b). The amount of nucleoplasmin in oocyte nuclei can be determined by quantitative scanning densitometry of Coomassie blue-stained gels and determination of the total protein content of individual nuclei. Nuclei from mature *Xenopus laevis* oocytes contain about 0.25 μg of nucleoplasmin, which is equivalent to a concentration of 4.2 mg/ml (Krohne and Franke, 1980; Mills *et al.*, 1980), whereas nuclei from *Pleurodeles* oocytes contain 0.4 μg, which is equivalent to 6.1 mg/ml; i.e., about 7.5×10^{12} molecules per nucleus. Nucleoplasmin is located exclusively in the nucleus and is not detectable in the cytoplasmic fraction (Fig. 20c–e).

Other major nucleoplasmic proteins are the very acidic, high-molecular-weight proteins N1 and N2, which—depending on the species—occur as a pair of closely related proteins (Fig. 20a, lane 2) or as a single protein termed N1/N2 (Fig. 20a, lane 3) (cf. Bonner, 1978; Dabauvalle and Franke, 1982). In *Xenopus* oocytes, N1 and N2 (100,000 and 110,000 M_r, respectively) represent approximately 12% of the total nuclear protein; i.e., 0.34 μg per nucleus, which is equivalent to a concentration of 5.2 mg/ml.

N1/N2 and nucleoplasmin are typical "karyophilic" proteins, since they are located with a remarkable specificity in the oocyte nucleus (Fig. 20c–e) and, upon microinjection into the cytoplasm, are rapidly sequestered into the nucleus (Bonner, 1978; DeRobertis *et al.*, 1978; Dabauvalle and Franke, 1982; Dingwall *et al.*, 1982; DeRobertis, 1983) (see also Section 4.3).

Analysis of the nucleoplasmic proteins from *Xenopus laevis* oocytes by

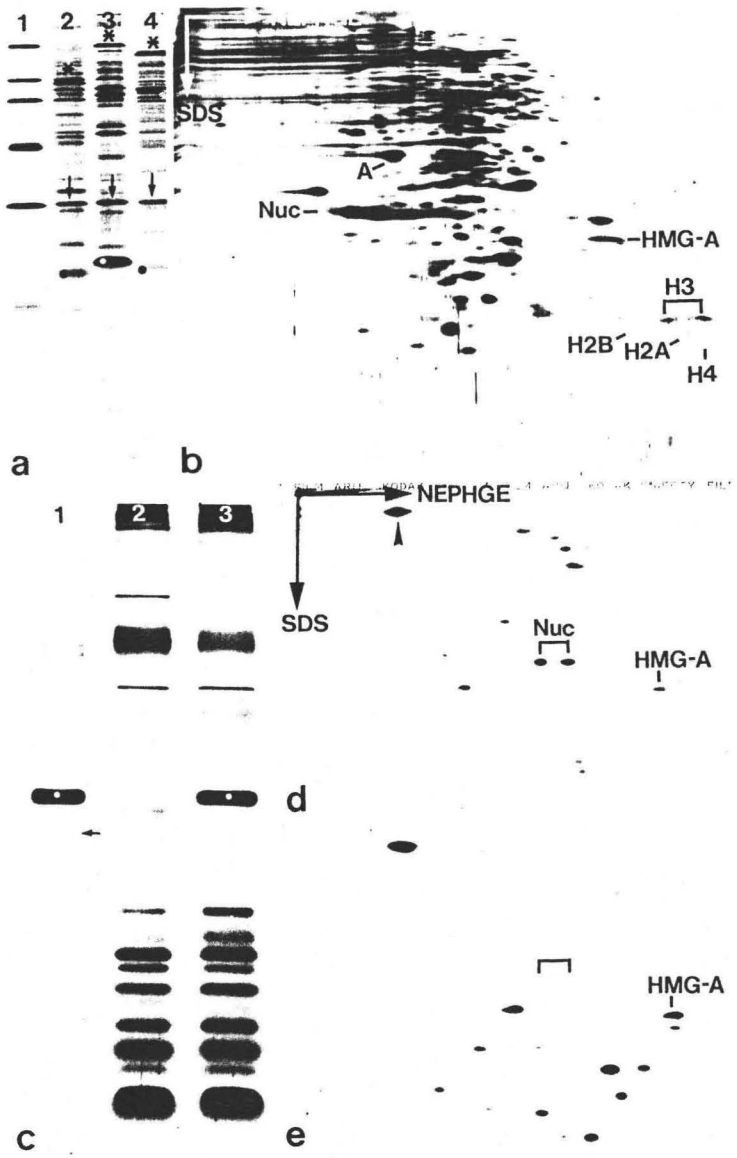

Figure 20. Analysis of nuclear proteins from amphibian oocytes by one- and two-dimensional gel electrophoresis. (a) Total proteins from oocyte nuclei of *Xenopus laevis* (lane 2), *Pleurodeles waltlii* (lane 3), and *Rana temporaria* (lane 4). Coomassie blue staining. Dots denote nucleoplasmin, arrows actin, and asterisks N1/N2. Reference proteins are (from top to bottom) myosin heavy chain, β-galactosidase, phosphorylase a, bovine serum albumin (BSA), actin, and chymotrypsinogen (lane 1). (b) Two-dimensional gel electrophoresis (first dimension: nonequilibrium pH gradient electrophoresis, NEPHGE; second dimension: 18% polyacrylamide containing SDS) of the high-speed supernatant of 10 *X. laevis* nuclei. Silver staining. Note the presence of the core histones and HMG-A. (c) Proteins extracted with 5% PCA from total *X. laevis* oocytes (lane 3) and manually separated nuclei (lane 1) and cytoplasm (lane 2). Nucleoplasmin (denoted by white dots) is not detectable in the cytoplasm. Arrow denotes HMG-A. Silver staining. (d,e) Two-dimensional gel electrophoresis of radioactively labeled proteins extracted with 5% PCA from nuclei (d) and cytoplasmic portions (e) of *P. waltlii* oocytes. Nucleoplasmin is recovered from the nuclear fraction (d) and is not detectable in the cytoplasm (e; the position of nucleoplasmin is indicated by bracket). N1/N2 is also confined to the nucleus (arrowhead, d), whereas HMG-A is present in both compartments. The high-molecular-weight acidic polypeptide of the cytoplasm (e) is not related to N1/N2. Nuc, nucleoplasmin; A; actin.

two-dimensional gel electrophoresis with nonequilibrium pH gradient electrophoresis as the first dimension (Fig. 20b) indicates the presence of a large pool of stored histones (Woodland, 1980; Kleinschmidt and Franke, 1982) and of the HMG-1 like protein HMG-A (A for amphibia) (Kleinschmidt *et al.*, 1983). It should be pointed out that these proteins, which are normally bound to DNA, occur in a free form in large excess over the DNA present in oocyte nuclei. The stored histones H3 and H4 are associated with the highly acidic proteins N1/N2, thus forming defined, negatively charged complexes (Kleinschmidt and Franke, 1982). Recently, a second type of a soluble acidic histone complex was described in the nuclei of *Xenopus laevis* oocytes, which contains all four core histones in association with nucleoplasmin (Kleinschmidt *et al.*, 1985). By contrast, HMG-A occurs in free monomeric form and is apparently not bound to other proteins (Kleinschmidt *et al.*, 1983). *Xenopus* oocyte nuclei contain about 14 ng HMG-A, which is equivalent to a concentration of 0.2 mg/ml. Approximately equal amounts of HMG-A are also stored in the cytoplasm (Fig. 20d,e) (Kleinschmidt *et al.*, 1983).

Actin is present in relatively large amounts in isolated nuclei (Fig. 20a) and in the high-speed supernatant (Fig. 20b). *Xenopus* oocyte nuclei contain about 0.15 μg of actin, which is equivalent to a concentration of $\sim$4.5 mg/ml (Clark and Rosenbaum, 1979). Similar values have been reported for *Pleurodeles* oocyte nuclei (Gounon and Karsenti, 1981). In both species, the amount of globular (G) actin exceeds that of filamentous (F) actin. It has been reported that 37% of the total actin of freshly isolated *Xenopus* oocyte nuclei occurs in form of F actin (Clark and Rosenbaum, 1979), whereas the corresponding value is less than 10% in *Pleurodeles* nuclei (Gounon and Karsenti, 1981). These differences in the relative amounts of F actin are apparently reflected by differences of the consistency of the nuclear sap, which is very stiff in *Xenopus* and almost fluid in *Pleurodeles*. The soluble G actin behaves differently from muscle actin, since it does not assemble spontaneously into filaments *in vitro*.

Although the role of nuclear actin is not yet clear, recent evidence suggests that this protein may be involved in transcriptional events. Injection of antibodies to actin into amphibian oocyte nuclei interferes with RNA polymerase II-mediated transcription and causes retraction of the lateral

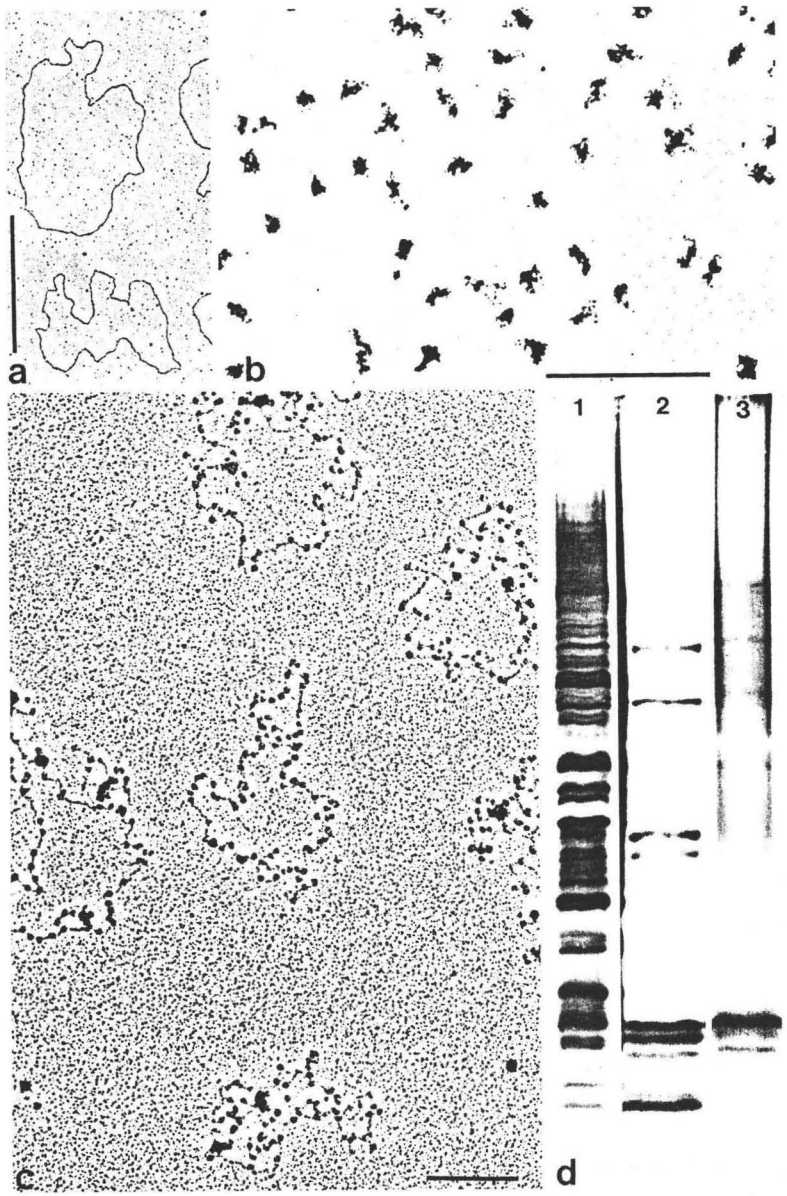

Figure 21. *In vitro* chromatin assembly. (a) The recombinant plasmid shown is converted into nucleoprotein complexes by incubation with the 100,000 × g supernatant fraction from isolated *X. laevis* oocyte nuclei. The newly formed nucleoprotein complexes appear—depending on the spreading conditions—as compact particles (b, 0.1 M salt) or as relaxed beaded chromatin circles (c, low salt). (d) Major proteins bound to the plasmid DNA are the four core histones as shown by one-dimensional gel electrophoresis and silver staining (d, lane 3). Several minor nonhistone proteins are also detected. Note the absence of H1. The complex protein pattern of the incubation mixture is shown in lane 1. Reference proteins are (from top to bottom) glutamate dehydrogenase, actin, calf thymus H1, and core histones (lane 2). Scale bars: 1 μm (a,b) and 0.2 μm (c).

loops of the lampbrush chromosomes. The same inhibitory effect is also observed upon injection of actin binding proteins from different sources, such as fragmin from the slime mould *Physarum polycephalum* and the actin modulator from pig stomach smooth muscle (Scheer *et al.*, 1984). Although this experimental approach is unable to distinguish whether the actin involved in transcriptional events includes actin polymers or monomers, we favor the concept that monomeric or oligomeric nuclear actin interacts directly with the transcriptional machinery. This possibility is supported by the recent finding that a soluble protein factor required *in vitro* for accurate transcription by RNA polymerase II contains actin (Egly *et al.*, 1984). In this connection, it is worth mentioning that, when transcription of the lampbrush chromosomes from *Triturus* or *Pleurodeles* species is inhibited by drugs such as actinomycin D or α-amanitin or by injection of antibodies into the cell nucleus (see Section 8), the formation of an extensive fibrillar network containing actin is induced after dispersing the nuclear contents in 0.1 M saline containing 0.1 mM $CaCl_2$ (Fig. 23d). The same phenomenon, though to a lesser degree, is also seen in stages of natural inactivation: i.e., in fully mature oocytes. This observation indicates that the equilibrium between G and F actin in the oocyte nucleus is somehow dependent on transcriptional activity of the lampbrush chromosomes and thus might imply that actin is involved, directly or indirectly, in transcriptional processes or post-transcriptional maturation events of the hnRNP.

5.2. Chromatin Assembly

It has been shown recently that double-stranded DNA is converted into chromatinlike structures by incubation in a supernatant fraction obtained from homogenized *Xenopus laevis* eggs (reviewed in Laskey and Earnshaw, 1980). The components involved in this assembly process include the large pool of stored core histones and nucleoplasmin, which is believed to catalyze the ordered interaction between histones and DNA. Chromatin assembly is also achieved by using the nucleoplasmic fraction of *Xenopus* oocyte nuclei. The newly formed nucleoprotein complexes can be purified by sucrose gradient centrifugation and either visualized in the electron microscope (Fig. 21b,c) or analyzed by SDS-gel electrophoresis (Fig. 21d) (for details, see Scheer *et al.*, 1980).

The morphology of these nucleoprotein complexes is dependent on the ionic strength of the spreading medium. In the presence of 0.1 M salt con-

centrations, they appear as very compact particles (Fig. 21b), whereas low salt treatment relaxes them into chromatin rings with a regularly beaded aspect (Fig. 21c). That the beads represent nucleosomes is indicated by an approximate twofold compaction of the original DNA in the chromatin (the contour length of the chromatin circle is about one-half that of the naked DNA; compare Fig. 21a,c). The major protein components of the newly formed nucleoprotein complexes are the four core histones, demonstrating that they are indeed chromatinous structures, which, however, lack histone H1 (Fig. 21d, lane 3). How closely their nonhistone protein composition resembles that of the *in vivo*-formed embryonic chromatin is an interesting question that awaits further analysis.

6. Storage at Two Different Levels: Proteins and Maternal mRNA

As shown in Section 5 of this chapter, *Xenopus* oocyte nuclei contain sufficient amounts of protein components for future assembly of chromatin during early embryogenesis, when extremely rapid nuclear divisions take place (Woodland, 1980). It is interesting to note that amphibian oocytes store not only the proteins needed for the assembly of embryonic chromatin but the corresponding mRNAs as well. When total *Xenopus* ovary RNA is translated in a rabbit reticulocyte lysate and the proteins are analyzed by two-dimensional gel electrophoresis, the four core histones and HMG-A are among the major translation products (Fig. 22a,b) (see also Rud-

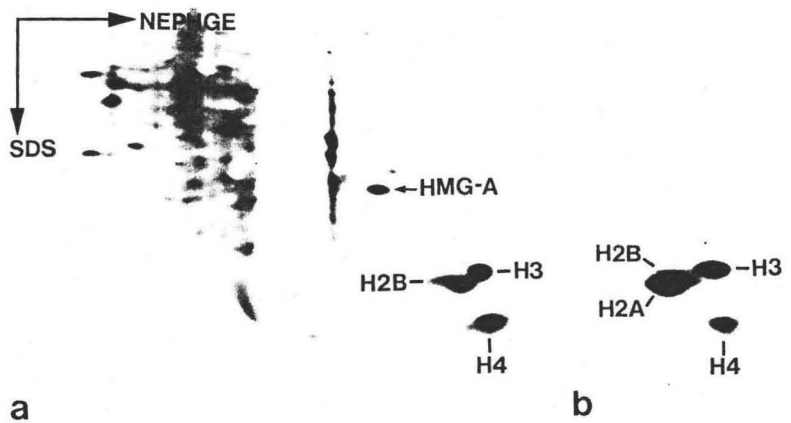

Figure 22. Two-dimensional gels of the translation products obtained by incubating total *Xenopus laevis* ovary mRNA in a rabbit reticulocyte lysate containing [^{35}S]methionine (a) or ^{14}C-labeled amino acids (b). Major translation products are the core histones and HMG-A.

erman *et al.*, 1979). The large pool of histone mRNAs stored in *Xenopus* oocytes (for quantitative determinations see Van Dongen *et al.*, 1981) is mobilized during maturation, resulting in an about 50-fold rise in histone synthesis (Woodland, 1980). As a consequence, the histones (and probably also HMG-A) contained in newly assembled embryonic chromatin are a mosaic of molecules made months before and stored in the oocyte and of molecules newly synthesized by translation of the preformed maternal mRNAs (see also Woodland, 1980, 1982).

7. Microinjection of DNA into Oocyte Nuclei

The purpose of injecting purified gene sequences into oocyte nuclei is generally to examine the function of specific nucleotide sequences of the introduced DNA for regulation of transcription. This aspect is beyond the scope of this chapter. However, some information about the functional status of the germinal vesicle can be obtained from such studies.

Most of the injected DNA molecules are converted into chromatin structures similar in appearance to the *in vitro*-assembled chromatin (see Section 5.2), whereas only a relatively small proportion is transcribed. Trendelenburg and Gurdon (1978) injected a recombinant plasmid containing a full repeat unit of *Xenopus* rDNA into *Xenopus* oocyte nuclei and examined its fate by electron microscopic spread preparations. They observed that—in contrast to nontranscribed chromatin circles, which showed a regularly beaded morphology—the transcribed circles were free of nucleosomes except for a region containing the plasmid vector. Thus, the conformation of the transcribed chromatin containing the exogenous rDNA is indistinguishable from that of the endogeneous nucleolar chromatin. The occurrence of a few maximally active injected genes next to a majority of transcriptionally inactive ones packaged into nucleosomes implies that some component present in limiting amounts in the oocyte nucleus is necessary to induce and maintain their extended nonnucleosomal state.

The *Xenopus* oocyte nucleus is not only capable of transcribing the injected genes but also of processing the transcripts and producing functional mRNAs (Gurdon and Melton, 1981; Etkin, 1982; Green *et al.*, 1983). Electron microscopic analysis indicates that proteins bind in an apparent specific mode to the nascent RNA chains, since the lateral fibrils of transcription units formed on injected genes show a characteristic ultrastructural organization and foreshortening of the RNA (Trendelenburg, 1983). It is remarkable that in the *Xenopus* oocyte nucleus heterologous RNA molecules synthesized on injected genes are converted into RNP structures by association with oocyte-specific RNA-binding proteins and are correctly processed and spliced. This indicates that the maturation steps for the production of functional mRNAs follow a general principle.

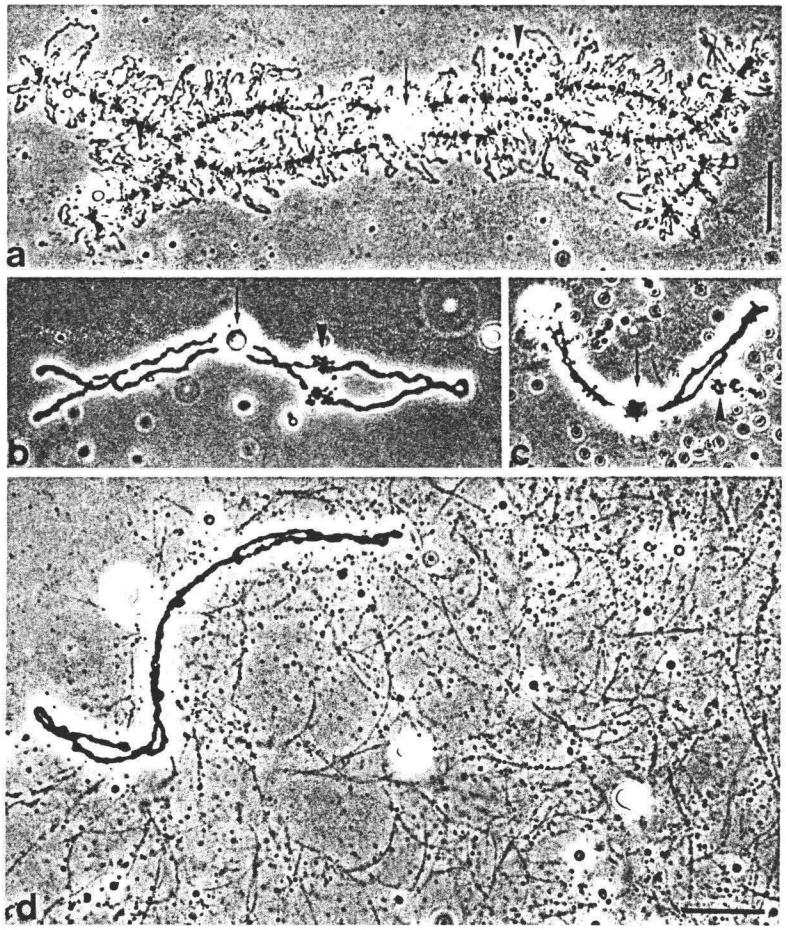

Figure 23. (a–c) Retraction of the lateral loops of lampbrush chromosomes after injection of antibodies to histone H2B into nuclei of *Pleurodeles waltlii* oocytes. Shown here is chromosome bivalent No. XI, identified by the sphere (arrows) and the granular loop (arrowheads). (a) Control preparation after injection of nonimmune IgG. (b,c) Chromosome preparations 1 hr and 2 hr, respectively, after injection of the antibodies to H2B. Note that the granular loop does not retract into the chromosome axis (arrowheads, b,c). The condensed chromosomes are often embedded in a finely filamentous network. This is especially clearly seen after complete inhibition of transcription by incubation of the oocytes for several hours in the presence of 50 μg/ml actinomycin D (d). Scale bars: 20 μm (a–c are magnified to the same scale).

8. Injection of Antibodies into the Oocyte Nucleus: A Means of Interfering with Gene Expression in the Living Cell

When antibodies directed against RNA polymerase II are injected into the nucleus of *Pleurodeles* oocytes, transcription of the lateral loops is immediately and effectively inhibited (Bona *et al.*, 1981). Inhibition of transcription of the lampbrush chromosomes results in dramatic structural rearrangements, which are visible in the light microscope as retraction of the lateral loops. It is expected that this test system will provide a valuable means to decipher the highly complex process of gene expression, since it offers the possibility of interfering selectively with the whole cascade of transcription and post-transcription events involved in the formation of functional mRNAs. In fact, this experimental approach permitted the proposal of a novel function of nuclear actin in transcription of protein-coding genes (Scheer *et al.*, 1984) (see Section 5.1). Antibodies to chromosomal components such as histone H2B and HMG-1 also induce loop retraction (Fig. 23b,c) (Scheer *et al.*, 1979b; Kleinschmidt *et al.*, 1983). A possible explanation for this finding is that, by binding of the antibodies to their DNA-associated antigens, a steric hindrance of the further progression of the RNA polymerase II is imposed, which in turn results in the premature release of the transcripts followed by the collapse of the loop axis. The observed loop retraction is therefore indicative of the presence of histones and HMG-1 on the heavily transcribed loop chromatin.

Antibodies directed against oocyte nuclear RNP and hnRNP core proteins do not inhibit transcription. However, it is an intriguing idea that injection of antibodies that bind to the nascent RNA itself or to defined RNA associated proteins might be used in the future to alter normal processing pathways.

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Chapter 10

5 S Ribosomal Gene Transcription During *Xenopus* Oogenesis

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1. Introduction

In contrast to somatic cells, *Xenopus* oocytes accumulate large numbers of ribosomes for their subsequent use in early embryogenesis (Davidson, 1976). To accommodate the need for ribosomal RNAs, the genes encoding the large ribosomal RNAs (i.e., 18 S, 28 S, and 5.8 S rRNA) are amplified in the oocyte to yield about 2×10^6 extrachromosomal copies per nucleus (Brown and Weber, 1968b; Gall, 1968). Since the genes coding for 5 S rRNA are not part of the repeating unit that encodes the large rRNAs (Brown and Weber, 1968a; Brown and Dawid, 1968), initial investigations on 5 S rRNA focused on elucidating the organization and structure of these genes.

The presence of two *Xenopus* 5 S rRNA gene families, which are developmentally regulated, has generated further interest in this system. The somatic gene family is expressed in both oocytes and somatic cells, whereas the oocyte-type genes are only expressed during oogenesis and early embryogenesis (Wegnez *et al.*, 1972; Ford and Southern, 1973; Brown *et al.*, 1981). Thus, the introduction of a second set of 5 S rRNA genes, which are only transcribed when large amounts of the RNA are needed, provides extra templates comparable to the amplified 18 S and 28 S rRNA genes.

The question of how the expression of the related 5 S gene families is regulated has led to an investigation of the basic mechanisms of 5 S rRNA synthesis. The use of *Xenopus* oocyte microinjection and the development of soluble cell-free systems, both of which direct the accurate transcription of cloned genes by RNA polymerase III, has facilitated the search for signals encoded in the DNA that are essential to transcription initiation and

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