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Organization of Transcriptionally Active Chomatin in Lampbrush Chromosome Loops

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I. Introduction

A. *Transcriptional Units—hnRNA and mRNA*

Although it is generally accepted that functional mRNA molecules are derived from larger nuclear precursor molecules (see, e.g., Scherrer, 1974) which are collectively termed "heterogeneous nuclear RNA" (hnRNA; for possible exceptions of this principle, see Daneholt, 1976; Timberlake and Shumard, 1977), a definite precursor–product relationship so far has not been established for any specific mRNA species. Moreover, the relationship between hnRNA molecules and the initial, primary transcripts likewise remains obscure. No hnRNA class has yet been identified as an entire, full-length primary transcript (for reviews, see Lewin, 1975a,b; Daneholt, 1976; Perry *et al.*, 1976) although several lines of evidence suggest that the molecular weight distribution of hnRNA reflects directly the size distribution of complete primary products of transcription, corresponding to the sizes of the specific transcriptional unit* (e.g., Georgiev *et al.*, 1972; Bajszár *et al.*, 1976; Derman *et al.*, 1976; Giorno and Sauerbier, 1976; Schmincke *et al.*, 1976). Needless to say that the analysis of transcriptional units and their primary transcripts is of utmost importance for the understanding of gene expression and of the regulatory mechanisms that control gene expression at the transcriptional and at the posttranscriptional level.

What is the size, composition, and structure of primary transcripts in comparison with the corresponding functional mRNA's? Several techniques have been developed to approach this problem (e.g., Stevens and Williamson, 1973; Curtis and Weissmann, 1976) but one has always to bear in mind the possibility that a primary, full-length transcript does not exist. If transcription and cleavage events occur simultaneously, the largest hnRNA class detectable in biochemical analyses might represent the first stable processing product and not the size of the complete product of the specific transcriptional unit. For instance, we have shown that in certain insect species the transcriptional units of rDNA-containing nucleolar chromatin are much longer (up to twofold) than expected from the molecular weight of the corresponding ribosomal RNA precursor (pre-rRNA), an observation which we have

* In the context of this chapter we define a transcriptional unit as a section of a chromatin strand which is limited by an initiation and a termination site for the RNA polymerase and which is transcribed into one polyribonucleotide sequence. Note that this definition does not necessarily imply that the covalent state of the transcript is maintained until the RNA polymerase has reached the termination signal.

explained by cleavage or processing of the nascent rRNA precursor molecules during transcription (Trendelenburg *et al.*, 1973, 1976; Franke *et al.*, 1976a; see also Franke *et al.*, Chapter 2, this volume; for detailed discussion, see Hadjiolov and Nikolaev, 1976; Rungger and Crippa, 1977). Similar mechanisms may well act during transcription of genes coding for mRNA's (Old *et al.*, 1977), and in this chapter we will present some morphological evidence compatible with the concept of superimposed transcription and processing.

A way out of this obvious dilemma is to analyze directly transcriptional units and to relate them to the mRNA derived therefrom. Old, Callan, and Gross have done pioneering experiments in this direction using lampbrush chromosomes of newt oocytes (Old *et al.*, 1977), and it is the purpose of this chapter to demonstrate that lampbrush chromosomes, when prepared for electron microscopy according to the spreading technique (Miller and Beatty, 1969; Miller and Bakken, 1972) offer new insights into the mode of arrangement and size distribution of transcriptional units of the nuclear genome of eukaryotes.

B. Occurrence and Morphology of Lampbrush Chromosomes

Lampbrush chromosomes are found in growing oocytes of a great variety of animals, both invertebrates and vertebrates (see the compilation in Davidson, 1976). They are characteristic for the diplotene stage of the prophase of the first meiotic division and seem to be composed of serially arranged, cytologically defined globular subunits, the chromomeres (for definition, see Lima-de-Faria, 1975). Parts of the chromomeric deoxyribonucleoprotein (DNP) appear to be unraveled into lateral loops which are visible in the light microscope due to the presence of a relatively thick matrix coat consisting of ribonucleoprotein (RNP). In cross sections this structural organization leads to a starlike appearance of the lampbrush chromosomes, i.e., the lateral loops extend radially from the dense chromomeric core. In fact, in the first description of lampbrush chromosomes, Flemming (1882) already compared the appearance of cross-sectioned chromosomes of axolotl oocytes with "a star with dark center" (see also Born, 1894; Carnoy and Lebrun, 1897, 1898; Dodson, 1948). Rückert (1892) was the first who isolated germinal vesicles (he studied oocytes of the dogfish *Pristiurus*) and determined the length of the chromosomes, which he compared with lampbrushes, within the nuclei. Six decades later, Gall (1954) introduced the use of an inverted phase contrast microscope for high resolution studies of isolated lampbrush chromosomes (for methodological details, see Callan and Lloyd, 1960; Gall, 1966), and since

then remarkable progress has been made in identifying the individual chromosomes and in distinguishing certain loops by their unique architecture. These studies have resulted in the construction of "working maps" and have allowed, for instance, the analysis of heterozygosity and chromosomal mutations (e.g., Mancino, 1973; Lacroix and Loones, 1974; Callan and Lloyd, 1975; Hartley and Callan, 1977). Furthermore, such chromosome preparations have been combined with a variety of cytochemical and autoradiographic procedures, and have led to an increased knowledge of composition of lampbrush chromosomes and the processes involved in loop formation and maintenance (for reviews, see Wischnitzer, 1957; Macgregor and Callan, 1962; Callan, 1963; Hess, 1971; Davidson, 1976; Macgregor, 1977; Sommerville, 1977; Sommerville *et al.*, 1978).

We have described similar lampbrush-type chromosomes with defined axes, chromomeres, and lateral loops in the primary nucleus of the green alga, *Acetabularia* (Spring *et al.*, 1974, 1975). Since in this organism we could not find paired chromosomes resembling bivalents it may well be that the chromomere–loop configuration, in general, is not restricted to the paired chromosomes of the meiotic prophase.*

The Y chromosome in primary spermatocytes of *Drosophila* forms at least six morphologically different structures which resemble lampbrush chromosome loops (Meyer, 1963; Hess, 1970, 1971; Hennig *et al.*, 1974). It is not clear, however, whether these structures represent lampbrush chromosome loops of the type described above since they do not laterally project from chromomeres but are serially arranged. On the other hand, they are reminiscent of certain exceptional amphibian chromosome loops with respect to some morphological and transcriptional features (Hennig *et al.*, 1974).

Shortly after the introduction of electron microscopic techniques, whole mount preparations of lampbrush chromosomes were studied (Tomlin and Callan, 1951; Gall, 1952, 1956; Guyénot and Danon, 1953; Lafontaine and Ris, 1958). Because the chromosomes have usually been prepared at moderately high salt concentrations or at low pH in order to preserve the "matrix material" associated with the loops in a relatively compact state, the information obtained has not added essentially new aspects to what has already been known from light microscopy. Examination of thick sections through isolated and then

* It is not clear whether and how the observation of Benyajati and Worcel (1976) showing that the interphase genome of *Drosophila* is folded in a defined pattern into numerous DNA loops is related to the typical chromomere–loop organization of the lampbrush chromosomes (for loop arrangements in mammalian metaphase chromosomes, see Paulson and Laemmli, 1977).

"end-embedded" spread lampbrush chromosomes by high voltage electron microscopy has revealed that under isotonic conditions the matrix is composed of tightly packed arrays of about 20–30 nm thick RNP particles (Mott and Callan, 1975). Furthermore, this study has clearly demonstrated the frequent occurrence of more than one pair of loops per chromomere (cf. Sommerville and Malcolm, 1976), an observation which is at variance with the earlier simplistic view that the number of chromomeres is equivalent to the number of "genes" expressed (for detailed discussion, see Vlad and Macgregor, 1975; Sommerville, 1977). As will be shown below, the numerical correlation between chromomeres and transcriptional units is rather complex and by no means a constant one, which is at least partly due to the occurrence of multigenic loops. Direct structural analyses of transcriptional units became possible when Miller introduced his electron microscopic spreading technique (Miller and Beatty, 1969; Miller and Bakken, 1972; Miller *et al.*, 1972). An important step in his procedure is the swelling of the lampbrush chromosomes by exposing them to very low salt conditions at slightly alkaline pH. Under such ionic conditions the matrix material is rapidly dispersed and no longer visible in the light microscope (Tomlin and Callan, 1951; Guyénot and Danon, 1953; Gall, 1954, 1956; Wischnitzer, 1957; Callan, 1963). At the electron microscopic level, however, one sees that the matrix material has unraveled into numerous lateral RNP fibrils which are attached to the loop axis via RNA polymerase-containing granules (Miller and Beatty, 1969).

C. *Models of the Organization of Transcription in Lampbrush Chromosome Loops*

Most previous models of lampbrush chromosome loop architecture and function were based on the assumptions (1) that initiation and termination sites for transcription are located close to the loop bases, i.e., at the insertion sites of the loops at the axial chromomere knob, (2) that one transcriptional unit extends over the entire loop length, and (3) that the lateral RNP fibrils containing the nascent RNA are densely packed and arranged in a continuous length gradient from one end of a loop to the other thus causing the asymmetric form of most of the loops seen in light microscopic preparations (Miller, 1965; Miller *et al.*, 1972; Mott and Callan, 1975; Macgregor, 1977; Sommerville, 1977). In addition, it has been postulated that each primary transcript consists of a mRNA sequence ("informative" part) linked to middle repetitive sequences ("noninformative" or "regulatory" part; Sommerville and

Malcolm, 1976; Sommerville, 1977; cf. Georgiev, 1974; Scherrer, 1974; Davidson *et al.*, 1977).

This generalized "one loop represents one transcriptional unit" concept is not compatible with some recent biochemical and morphological findings. Certain transcriptionally active loops of amphibian lampbrush chromosomes contain rDNA, i.e., tandemly arranged repeating units consisting each of a pre-rRNA gene and interspersed spacer regions (Macgregor, 1977), or contain the similarly arranged multiple genes coding for 5 S rRNA (5 S DNA; Pukkila, 1975; Barsacchi-Pilone *et al.*, 1974, 1977), or repeated sequences of unknown function (Gould *et al.*, 1976; Macgregor and Andrews, 1977). In addition, electron microscopic spread preparations of primary nuclei of *Acetabularia* (Spring *et al.*, 1974, 1975; Scheer *et al.*, 1976a) and oocyte nuclei of various urodelan and anuran amphibia (Angelier and Lacroix, 1975; Scheer *et al.*, 1976a) have indicated that some loops can contain more than one transcriptional unit (for details see below).

D. Dynamic Aspects of Loop Formation and Changes of Loop Morphology

Lampbrush chromosome loops as such are dynamic structures in the sense that they develop in early stages of oogenesis by unfolding from the tightly packed chromatin of the chromomeres, their lateral extension reaches a maximum at the so-called "lampbrush chromosome stage" during early vitellogenesis, and in maturing oocytes they retract again to form finally the highly condensed chromosomes of meiotic metaphase I. The important point which is still a matter of debate is whether only a fixed portion of the chromomeric DNP is spun out into a loop and transcribed during the total lampbrush chromosome phase, or whether the unraveling is a continuous process which successively exposes all gene-containing chromomeric DNP to transcription. The "continuous spinning out and retraction" hypothesis (Callan and Lloyd, 1960; Gall and Callan, 1962; Snow and Callan, 1969) has recently been supported by the finding that *in situ* hybridization of labeled middle repetitive DNA-to-RNA transcripts associated with loops (DNA/RNA–transcript hybridization) can result in different patterns of labeled loops, depending on the specific oogenic stage (Macgregor and Andrews, 1977). On the other hand, these authors have also shown with the same technique that certain other loops were labeled in an invariable pattern independent of oocyte size. A similar constant labeling pattern has been reported for the histone–gene-containing loops of *Triturus* (Old *et al.*, 1977). Thus, it seems reasonable to conclude that some chromomeres are permanently "looped" in a fixed

position, at least throughout mid-oogenesis, whereas other chromomeres extend different portions of their DNP into loops in different stages of oogenesis.

E. Meiotic versus Somatic Transcription

It has been questioned whether transcription of loops of meiotic lampbrush chromosomes bears any significance for transcription in somatic interphase cell nuclei (Edström and Lambert, 1975; Edström, 1976). In fact, it is unknown whether the chromomeric organization is also present in interphase chromatin (see above). On the other hand, it seems rather unlikely that, as hypothesized by Edström, meiotic transcripts have an almost exclusively "meiotic" function and are not destined to become cytoplasmic mRNA's. During oogenesis of *Xenopus laevis*, one of the most thoroughly studied animals, about 20×10^{10} poly(A)-containing RNA molecules are synthesized and stored in a mature oocyte (Cabada *et al.*, 1977). This RNA fraction consists of approximately 20,000 different sequences (Perlman and Rosbash, 1978), is transcribed preferentially, if not exclusively, from unique DNA sequences (Rosbash *et al.*, 1974) and is indistinguishable in its sedimentation profile from poly(A)-containing RNA of cultured *Xenopus* kidney cells (Rosbash *et al.*, 1974) and consists largely of functional or potentially functional mRNA molecules as has been demonstrated in a cell-free wheat germ translation assay (Darnbrough and Ford, 1976). According to Müller (1974) the mean total length of the lampbrush chromosomes of one chromosome set in *Xenopus* oocytes is about 1.5 mm. The axial chromomere frequency in urodeles is roughly constant at 0.5 chromomere per micron chromosome length (Wischnitzer, 1957; Vlad and Macgregor, 1975). When we correct for the smaller size of *Xenopus* lampbrush chromosomes (by a factor of 2), we end up with an estimate of 1500 chromomeres per chromosome set, i.e., 3000 for the total chromosome bivalents. Assuming a similar loop/chromomere ratio as determined in *Triturus cristatus* (Sommerville and Malcolm, 1976) the estimated total number of loops per nucleus is about 12,000. At maximal packing density of the RNA polymerases (center-to-center distance ca. 200 Å; see below) and a RNA chain elongation rate of about 30 nucleotides per second (Scheer, 1973), it can be calculated that every two seconds a newly synthesized mRNA molecule is released from the loop axis. During the total lampbrush chromosome stage, which in *Xenopus* lasts about 6 months (Davidson, 1976), this would allow a synthesis of 9.3×10^{10} mRNA molecules (assuming conservative processing, one structural gene per

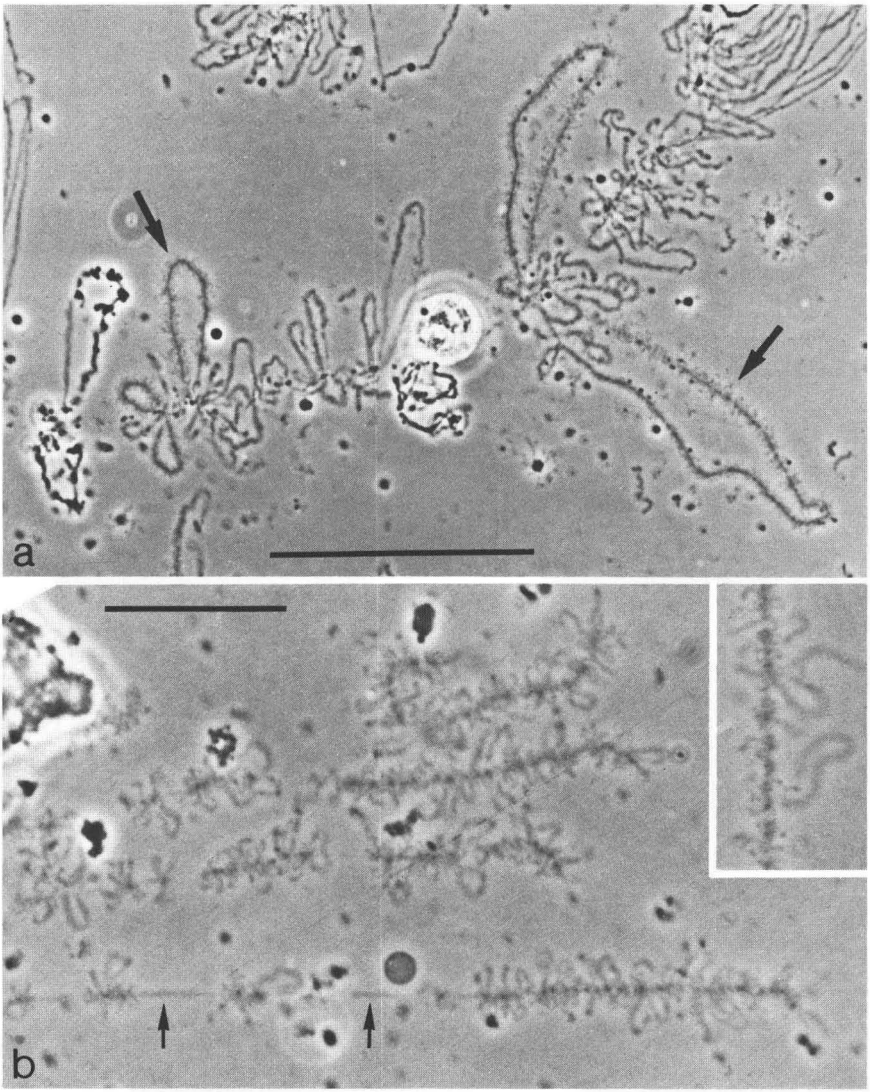


Fig. 1 Phase contrast photographs of isolated lampbrush chromosomes from oocytes of *Triturus alpestris* (a) and primary nuclei of *Acetabularia mediterranea* (b). Note the size difference of the lateral loops of the two species. The loop denoted by the right arrow in (a) has a total contour length of 150 μm, whereas the longest loop observed in *Acetabularia* is only 25 μm [insert in (b)]. The matrix material of "normal" loops of *Triturus* [two are denoted by arrows in (a)] is composed of numerous fine fibrils of increasing lengths from the "thin" to the "thick" loop insertion site at the chromosome axis. Mechanical stress may have induced local stretching of the chromosome axis,

loop and negligible turnover). This crude estimation demonstrates that in amphibian oogenesis the transcription occurring in lampbrush chromosome loops correlates with the amount of mRNA's accumulated in mature oocytes which seems to exclude a solely "meiotic" function of this transcription. At the moment the evidence available favors the concept that transcription of lampbrush chromosome loops reflects the activity of "normal" genes and that the transcriptional units seen in loops represent functional stages of unusually high activity of genes that can be active in somatic cells as well, though perhaps at reduced levels of activity.

The only exception so far known are the genes coding for the ribosomal 5 S RNA. These highly reiterated genes represent a complex population. The 5 S rRNA synthesized in *Xenopus* oocytes differs from 5 S rRNA synthesized in somatic *Xenopus* cells by 6 nucleotides out of 121 (e.g., Ford and Southern, 1973; Brown *et al.*, 1977), indicative of differential transcription of subsets of 5 S RNA genes. According to Denis and Wegnez (1977), however, both sets of genes are transcribed in oocytes but only the 5 S rRNA of the "oocyte type" is stored in the form of the typical 42 S RNP particle.

II. General Light and Electron Microscopic Appearance of Lampbrush Chromosomes

A. *Appearance of Isolated Lampbrush Chromosomes at Moderately High Salt Concentrations*

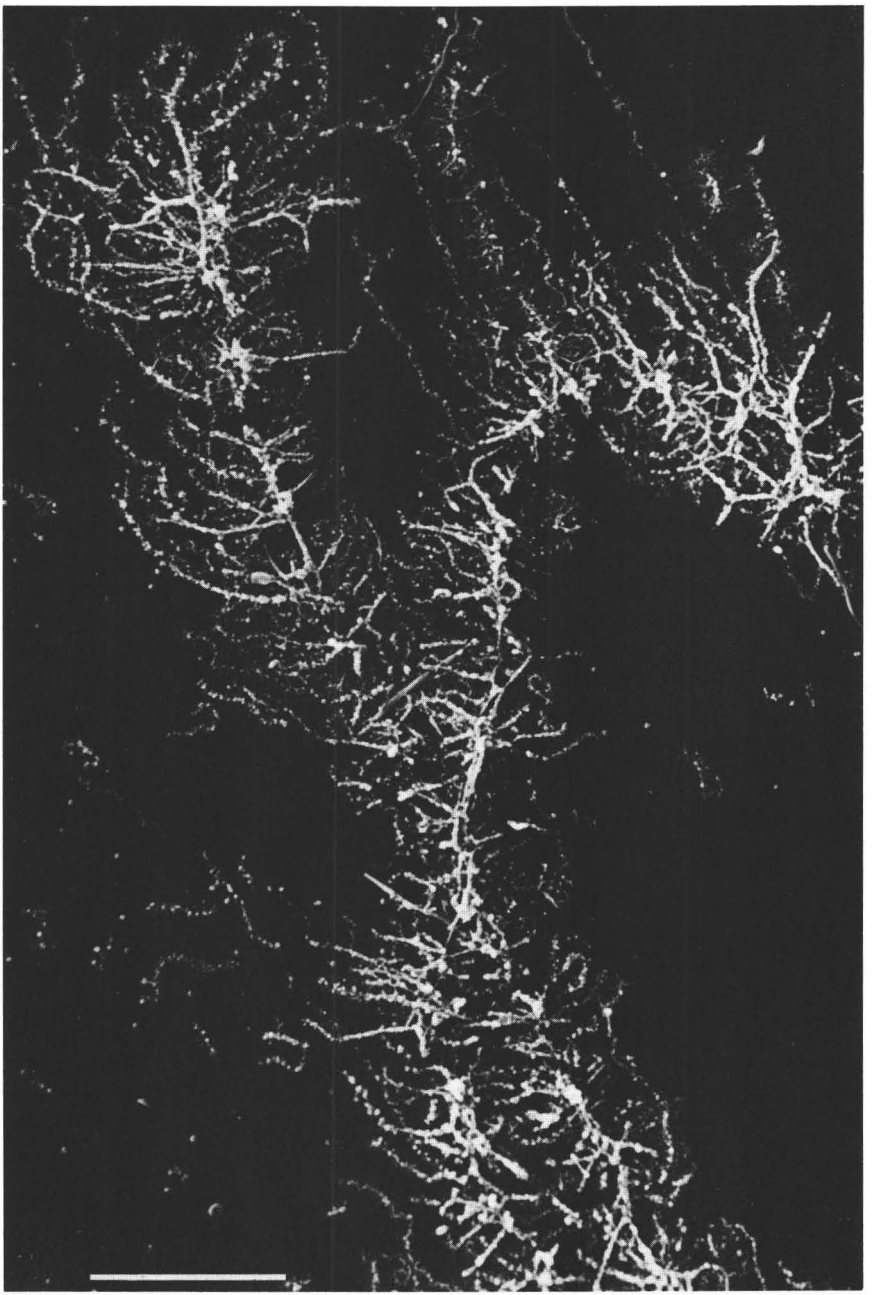
Lampbrush chromosomes from newt oocytes reveal the familiar aspect in light microscopic spread preparations when isolated in alkaline salt solutions in the range from 50 to 100 m*M* ("medium B" of Callan and Lloyd, 1960; Müller, 1974; see also Gall, 1966). The quality of the spread preparations is usually greatly improved when the nuclear content is first dispersed in the microscope slide chamber (for details, see Callan and Lloyd, 1960; Gall, 1966) and then centrifuged at about 2000 *g* for 10 min (Sommerville, 1973). Under such conditions the chromosomes and their loops are evenly spread out and firmly attached to the coverslip but still show Brownian motion of the loop matrix material (Fig. 1a). The characteristic details of the loop mor-

resulting in the formation of "double loop bridges" [arrows in (b)]. The chromosomes were isolated in the medium specified by Müller (1974) and centrifuged at 2000 *g* for 10 min. Photographs were taken with the inverted Zeiss microscope IM 35 (Zeiss, Oberkochen, FRG) and the oil immersion objectives Zeiss Planapo 40/1.0 and 63/1.4. Bars indicate 50 μm (a) and 20 μm [(b) and insert of (b)].

phology are preserved and this allows the recognition of special chromosomal loci. The matrix material of large "normal" loops *sensu* Callan (1963) appears to consist of numerous fine fibrils which laterally project from the loop axis and are frequently arranged in a continuous length gradient between the two loop bases, i.e., the insertion sites at the chromomere (Fig. 1a). The average length of the lateral fibrils near the thick end of such loops ranges from 2 to 4 μm; the diameter is near the resolving limit of the light microscope (Fig. 1a; see also Fig. 6a, c). The general structural aspect of the lampbrush chromosome loops presented in Fig. 1a (from a growing *Triturus alpestris* oocyte) is entirely comparable and identical to that known for isolated lampbrush chromosomes of oocytes of a great variety of other animal organisms (for references see Section I,B). Chromosomes isolated and prepared in the same way from primary nuclei of the green algae of the genus *Acetabularia* exhibit basically identical features, though at much smaller sizes (Fig. 1b; cf. Spring *et al.*, 1975). The majority of the loops measure 5–10 μm but some can reach lengths of 15–20 μm. These "giant" loops of *Acetabularia* often show an asymmetric distribution of the associated matrix material (insert of Fig. 1b), comparable to the situation described in amphibian oocytes (Callan, 1963).

When the lampbrush chromosomes are prepared for electron microscopy under similar ionic conditions, no essentially new information is obtained. This holds for both scanning electron microscopy of critical point-dried (Fig. 2, see also Fig. 6f) and transmission electron microscopy of air-dried (see Fig. 7) lampbrush chromosomes. The matrix material of the lateral loops of the air-dried and positively stained chromosomes usually appears collapsed into a very compact electron-dense mass that cannot be resolved into the constituent fibrils (e.g., see Fig. 7). However, in some loops thin and thick ends can still be distinguished at the chromomeric insertion sites although an increase in the thickness of the loop matrix coat often is significant only in proximal portions of the loops (see Fig. 7a). When the chromosomes are dried according to the critical point method and viewed in the scanning electron microscope, many loops are covered by a gradient of thin lateral fibrils whereas other loops show a compact and granular matrix (Fig. 2; see also Fig. 6f).

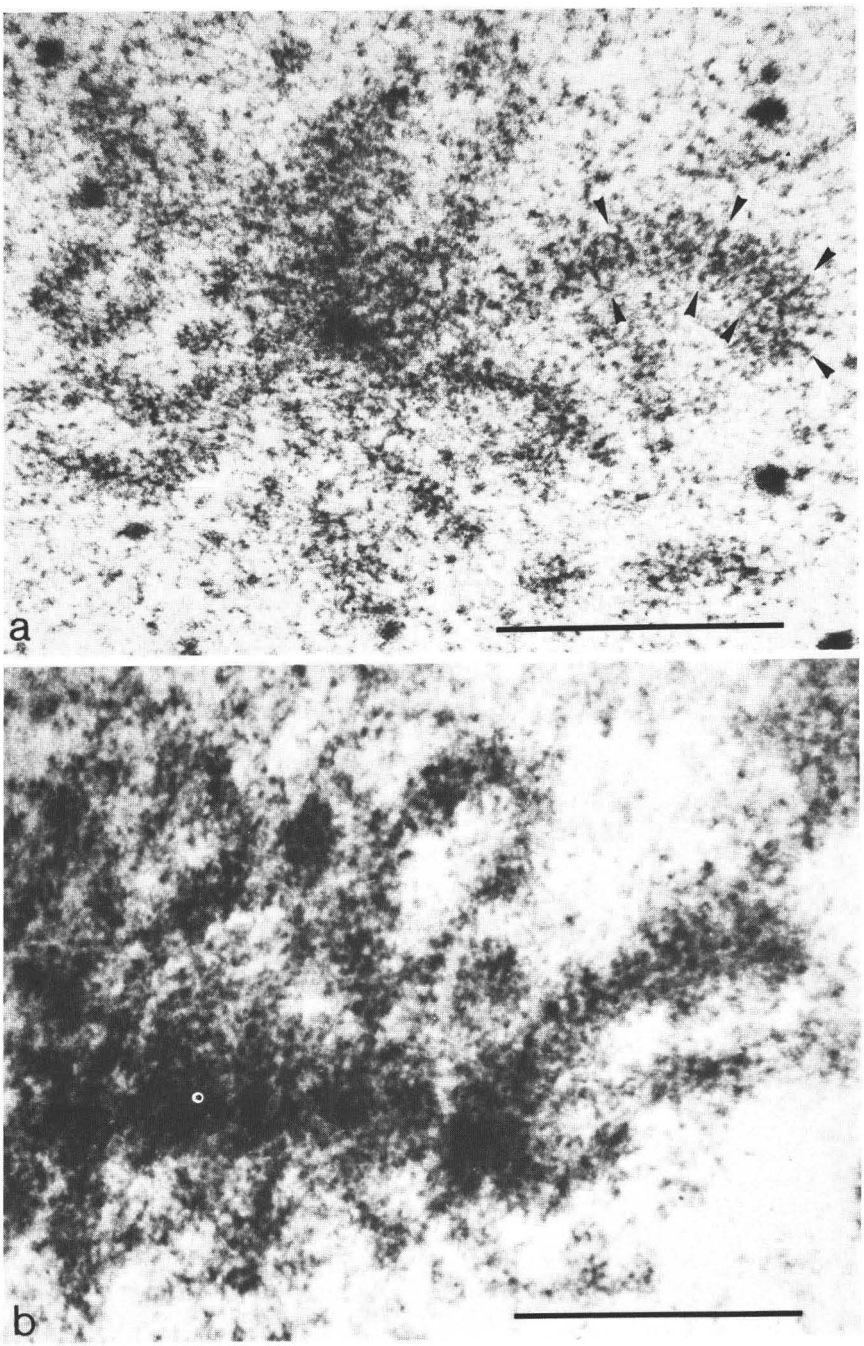
Fig. 2 Scanning electron (Zeiss Novascan 30) micrograph of a lampbrush chromosome bivalent of *Pleurodeles waltli* prepared as described in the legend of Fig. 1, then fixed in 4% formaldehyde, dehydrated in graded acetone solutions, and dried according to the critical point method. Numerous loops have a fibrillar matrix coat (especially those in the upper left part of the figure). Bar indicates 10 μm.



B. Appearance and Spatial Organization of Lampbrush Chromosomes in Situ

The argument that the isolation and the spreading may have led to drastic artificial alterations of lampbrush chromosome structure can be examined by a comparison with the chromosome structure revealed in sections after fixation in intact cells. The small lampbrush chromosomes of *Acetabularia* are especially well suited for such comparative studies of the structural relations between the chromosomal axis and lateral loops since here the probability that relatively sizable chromosome portions are included in a given section is much higher than with large amphibian chromosomes. Conventional transmission as well as scanning transmission electron micrographs of thick sections (0.5–2.5 μm) of epoxy resin-embedded primary nuclei of *Acetabularia* show the characteristic linear arrangement of dense chromomere knobs from which the loops extend, apparently under a nearly right angle (Fig. 3b). Similar structures have been described in high voltage electron micrographs of thick sections of isolated and then "end-embedded" lampbrush chromosomes of *Triturus* (Mott and Callan, 1975). Cross sections of lampbrush chromosomes usually reveal a starlike appearance: several lateral loops project from the electron-dense chromomeric center (Fig. 3a). Similar configurations have been described from light microscopic studies of paraffin sections through nuclei of growing amphibian oocytes fixed *in toto* (Flemming, 1882; Carnoy and Lebrun, 1897, 1898; Born, 1894; Dodson, 1948). This indicates that the subdivision of lampbrush chromosomes into linearly arranged chromomere–loop complexes exists *in situ*, and most probably also *in vivo*. Moreover, these observations show that the loops project radially from the central axis into the nucleoplasm and that, at least in some chromomeres, more than two loops can originate from one chromomeric knob (cf. also Mott and Callan, 1975; Sommerville and Malcolm, 1976). In addition, such sections show the dense cover-

Fig. 3 Electron micrographs of sections through primary nuclei of *Acetabularia* allow the analysis of the spatial chromosome organization. In cross sections the chromosomes have a starlike appearance with a dark core (chromomere) and radially projecting lateral loops [(a) transmission electron microscopy at 100 kV of a 0.5-μm-thick section through an isolated primary nucleus of *Acetabularia cliftonii*]. The loop axes are coated with asymmetrically distributed fibrillar material [arrowheads in (a)]. When longitudinally sectioned, the chromosome axis is seen to consist of numerous closely spaced chromomeric masses from which lateral loops extend [(b) scanning transmission electron micrograph of a 2.5-μm-thick section through the rhiziod of *Acetabularia mediterranea*; Siemens Elmiskop 102, equipped with the scanning transmission device, Siemens AG, West Berlin]. Bars indicate 1 μm.


a
b

age of the loops by matrix material, frequently in a typical asymmetric distribution, and its composition by numerous, densely spaced lateral fibrils (Figs. 3a, b). In *Acetabularia* these lateral fibrils, which usually are oriented perpendicularly with respect to the loop axis, have a diameter of approximately 20 nm (Fig. 3a) and sometimes show associated larger globular structures (Fig. 3b).

C. *Appearance of Isolated Lampbrush Chromosomes at Low Salt Concentrations*

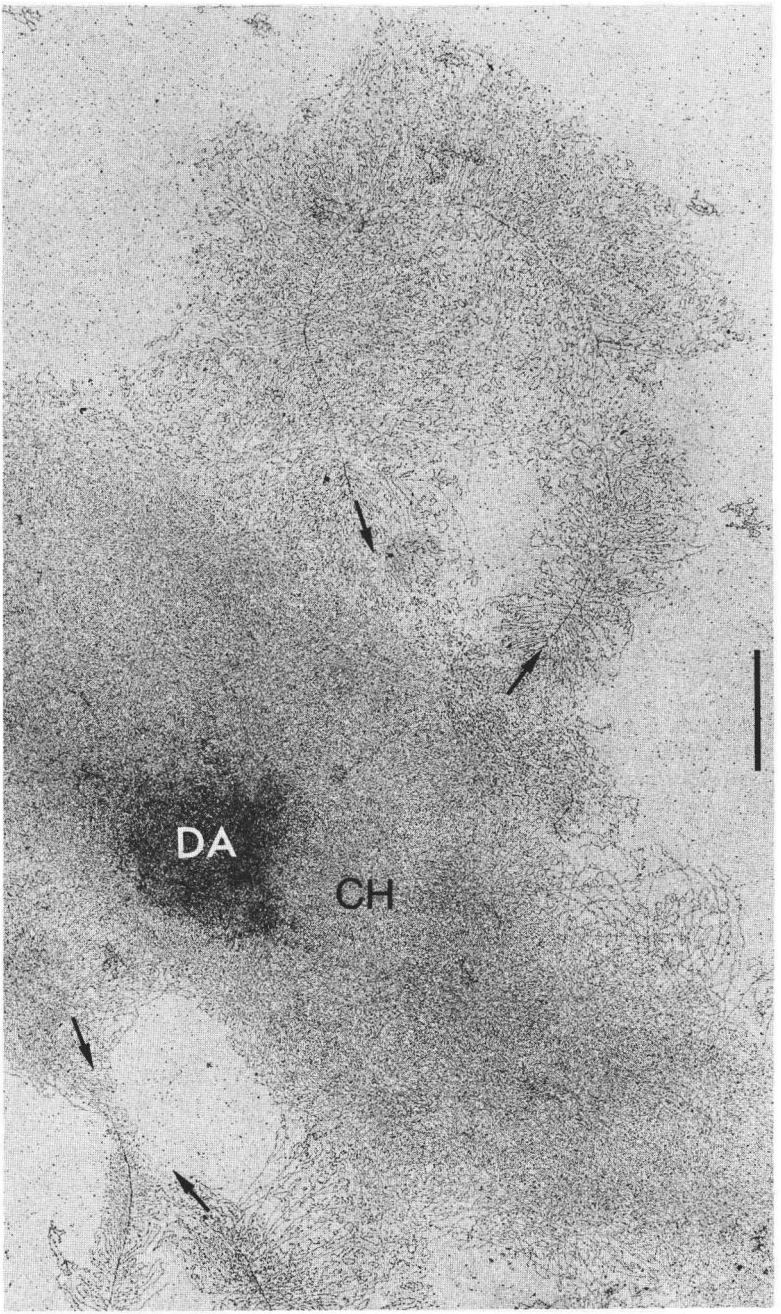
The procedure developed by Miller and co-workers for the visualization of "genes in action" requires extensive swelling of the chromatin in "pH 9 water" (0.1 m*M* borate buffer, pH 9). Under these low salt concentrations the matrix material undergoes a rapid transition from the compact state, visible in the light microscope, to a highly dispersed state (as seen under the light microscope, the matrix "dissolves"; see references quoted in Section I,B; see also Malcolm and Sommerville, 1977). After centrifugation onto an electron microscopic grid and positive staining it is seen that the matrix is composed of numerous individual lateral fibrils attached to the loop axis (Figs. 4 and 5; cf. Miller and Beatty, 1969; Miller and Bakken, 1972; Miller *et al.*, 1972). A detailed electron microscopic analysis of lampbrush chromosome loops prepared with this technique is presented below.

III. Electron Microscopic Visualization of Lateral Loops in Spread Preparations

A. *Loops Covered Entirely by One Transcriptional Unit*

The characteristic structural features of lampbrush chromosomes observed in light microscopic preparations and described above are still recognizable in electron microscopic spread preparations (Figs. 4 and 5). Although some unraveling apparently occurs in the

Fig. 4 Electron microscopic appearance of the chromomere–loop complex of *Pleurodeles waltli* after dispersal in "pH 9 water," centrifugation and positive staining. The majority of the chromatin axes are condensed into the chromomeric masses (CH) which show a central dense aggregate (DA). Two loops with a length of about 20 μm each extend on either side (only one loop is shown here completely, insertion sites are indicated by arrows). The loop axis is entirely covered by numerous lateral RNP fibrils arranged in a length gradient starting from the "thin" insertion region. Bar indicates 2 μm.


DA
CH

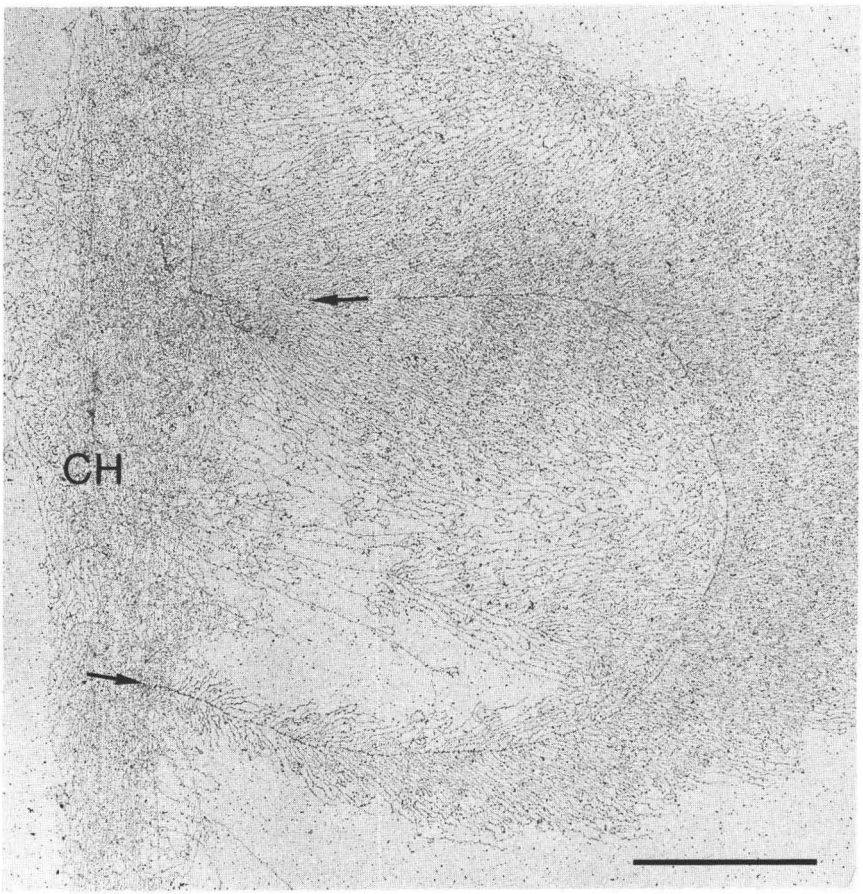


Fig. 5 Same preparation as in Fig. 4. The chromomere (CH) is composed of numerous extended parallel chromatin fibrils. A loop (about 15 μm long) projects from the chromomere (insertion sites are denoted by the arrows). The loop axis is accentuated by the attached, densely stained, and closely spaced basal granules of the lateral fibrils. Bar indicates 2 μm. (From Scheer *et al.*, 1976a.)

chromomeric region it can be seen that the pairs of lateral loops extend from, and are anchored to, the chromomere (Fig. 4). It is emphasized that the vast majority of DNP fibrils are entangled in the chromomeric mass whereas only a relatively small proportion of chromosomal DNP is extended into the lateral loops. The chromomeres appear either very compact, with centrally located foci of much denser aggregates (Fig. 4), or can be resolved into numerous, often parallel DNP axes

(Fig. 5). The chromatin axes contained in the chromomeric masses are usually free of lateral RNP fibrils whereas the loop axes are densely covered by numerous lateral fibrils of increasing lengths from the thin to the thick insertion site (Figs. 4 and 5). Each lateral RNP fibril is anchored with a basal granule at the loop axis and represents a transcriptional complex containing an RNA polymerase molecule and the attached nascent RNA complexed with specific proteins (e.g., Scott and Sommerville, 1974; Sommerville and Malcolm, 1976). In the examples shown in Figs. 4 and 5 the loop axes are transcribed entirely, with the initiation and termination sites being close to the insertion point of the loop ends in the chromomere. This demonstrates that the dispersal of chromatin, together with the mechanical forces exerted during the centrifugation, does not necessarily pull out the lateral loops from their anchoring sites, thus unraveling chromomeric DNP. The axial lengths of the transcriptional units shown is about 20 μm (Fig. 4) and 15 μm (Fig. 5). Frequently, we have observed much longer transcriptional units which, however, were extremely difficult to trace. Loops covered by shorter transcriptional units are also often recognized, especially in anuran amphibia and *Acetabularia* (see below).

B. Loops Containing More Than One Transcriptional Unit

In the light microscope, the organization of the loop matrix often shows discontinuities which are clearly distinguishable from mere "gaps" (e.g., Fig. 6a). Several different situations can be distinguished. The matrix is arranged either in two successive (Fig. 6c) or several, sometimes tandemly repeated, thin-to-thick gradients, with either identical (Fig. 6b) or opposite polarities (Fig. 6d). The polarity of adjacent matrix units can even change within one loop as illustrated in Fig. 6e (for similar observations, see Markarev and Safronov, 1976). Scanning and transmission electron microscopy of chromosomes isolated and prepared under similar conditions, which maintain the compact state of the matrix material, reveal similar situations (Figs. 6f and 7).

Figures 8 to 10 and 13 illustrate that these discontinuities in the distribution of matrix coat along an individual loop can be explained by the occurrence of several transcriptional units which lie close together and are oriented either in identical (Fig. 8a), opposite (Figs. 8c, 9a, c, and d, 10; see also Fig. 13b and c), or changing polarities (Figs. 8b and 9b). In arrangements of matrix units of opposite polarity, both "head-to-head" and "tail-to-tail" configurations are noted (Figs. 8 and

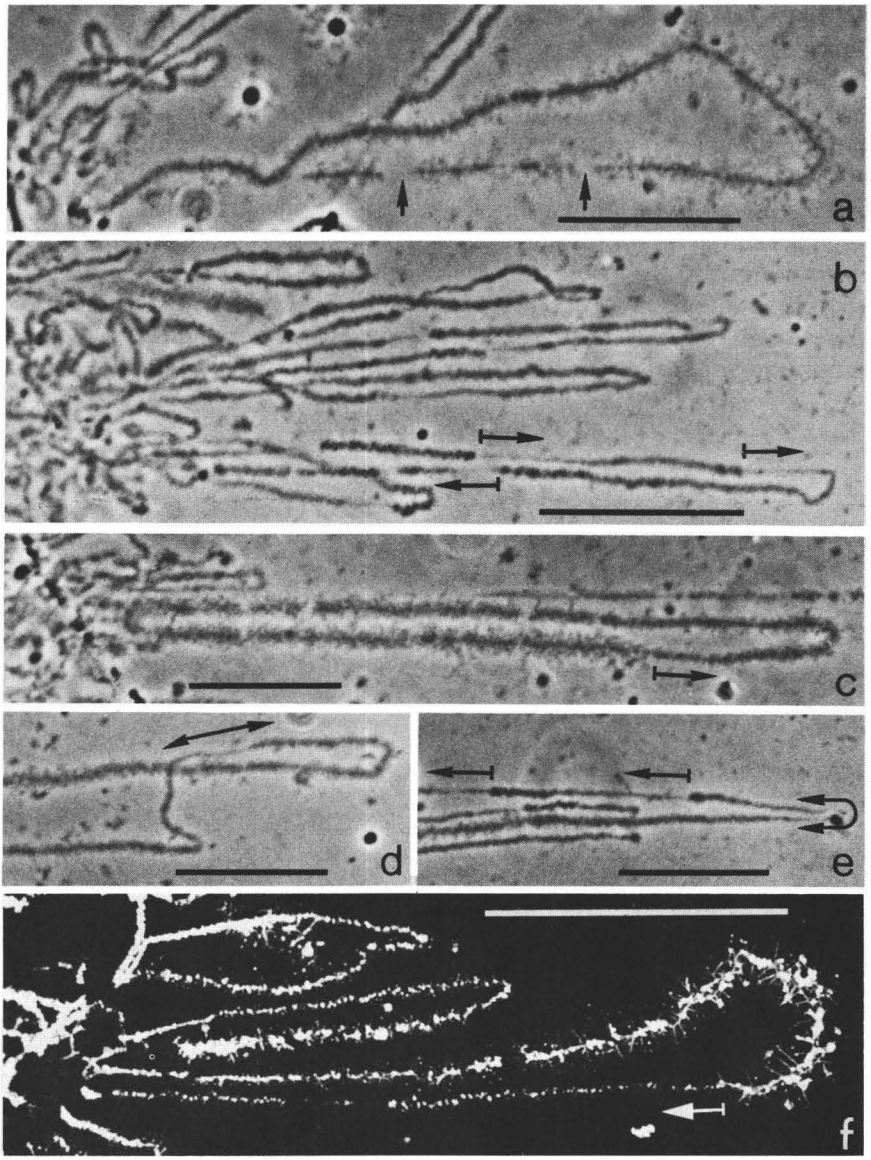


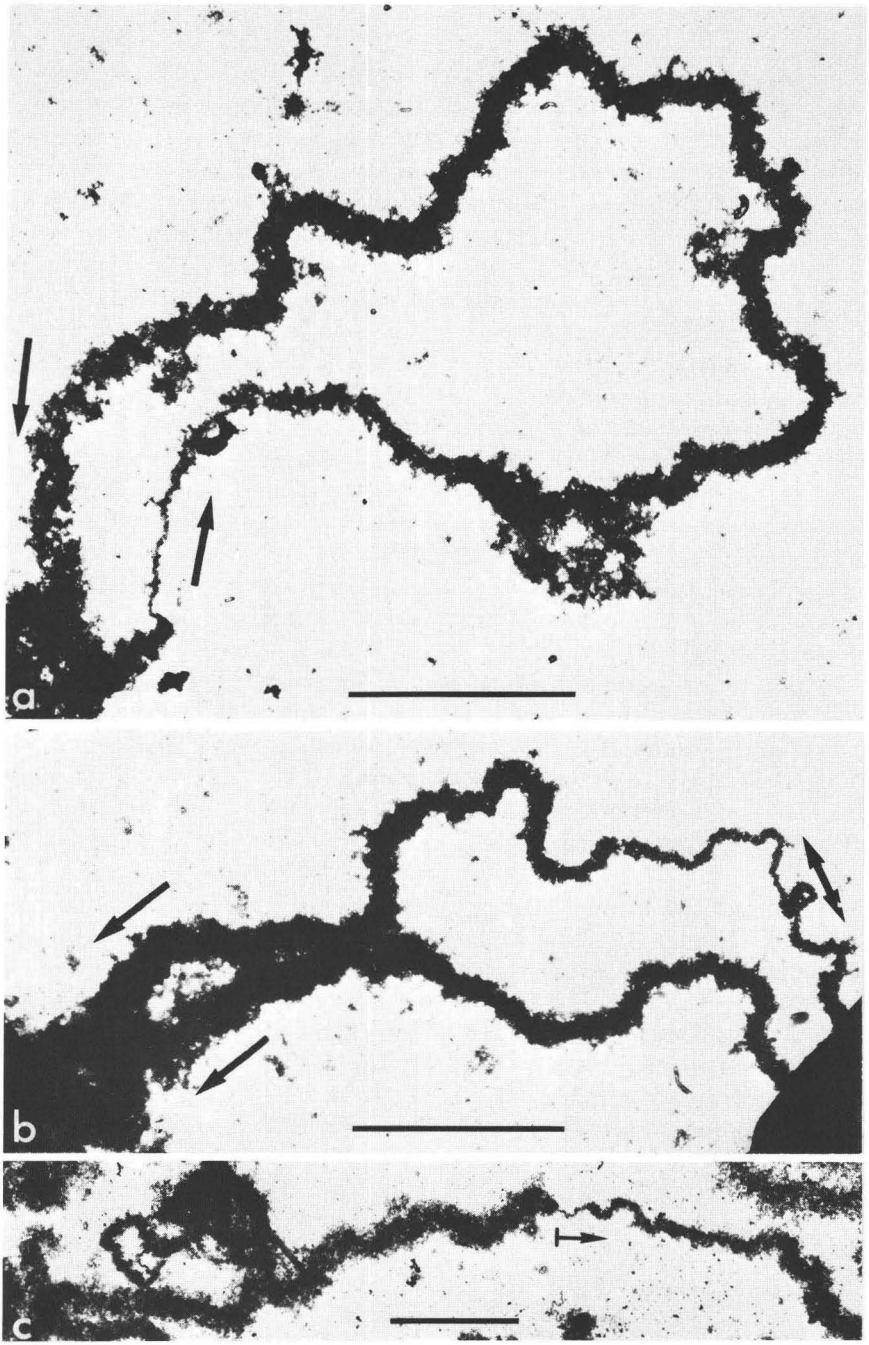
Fig. 6 Discontinuities in the matrix gradients of lampbrush chromosome loops of *Triturus alpestris* (a–e) and *Pleurodeles waltli* (f) oocytes as revealed in the light microscope (a–e; for technical details see the legend to Fig. 1) or scanning electron microscope (f; see the legend to Fig. 2). Contrary to the situation shown in (a) where "gaps" (arrows) interrupt the otherwise continuous thickness gradient of matrix material, the loops presented in (b)–(f) exhibit a clear repeated "thin-to-thick" pattern, with either identical (b,c,f,), opposite (d), or changing polarities (e). Bars indicate 20 μm (a–e) and 10 μm (f).

9; see also Fig. 13b and c; for further examples, see Angelier and Lacroix, 1975; Scheer *et al.*, 1976a). In the case of "head-to-head" arrangements the apparent initiation sites of two adjacent transcriptional units are separated by a certain distance (e.g., see Fig. 13b) or lie close together (e.g., Figs. 8b, c, 9a–d). We have also found situations suggesting that the extrapolated apparent initiation sites (e.g., Franke *et al.*, 1976a; Laird and Chooi, 1976; Laird *et al.*, 1976) of two transcriptional units arranged in opposite polarities overlap (Fig. 10).

The number of transcriptional units per loop varies widely. In some loops we have identified up to five units, a figure which corresponds to the maximal number of repeats seen in light microscopic preparations (Figs. 6b–e). Loops can contain transcriptional units of similar or different lengths (Figs. 8, 9, 13b, c; for further examples, see Scheer *et al.*, 1976a). In our opinion, the immediate succession, or even overlapping, of transcriptional units along the loop axis rules out the possibility that such "multigenic" situations are a result of the artificial extension of chromomeric DNP by mechanical stresses during the preparation. If this were so, clear "spacer"* sections should separate adjacent transcriptional units.

When transcription is inhibited by actinomycin D, a drug which is known to generally strip off the matrix material from the loop axis (Snow and Callan, 1969; Sommerville, 1973) and to remove transcriptional complexes from the template (Scheer *et al.*, 1975; Scheer, 1978), the loops collapse within a short time (Izawa *et al.*, 1963). This behavior has been taken as support for the concept that the extension of a loop is brought about by its transcription and concomitant accumulation of large amounts of RNP material. Figures 11 and 12, however, demonstrate that occasionally loops contain both transcribed and extended, apparently nontranscribed "spacer" regions. These spacer regions are located before and/or subsequent to the transcriptional unit and, in some loops, may be much longer than the transcribed part (Fig. 12). In addition, we have frequently observed that short or relatively long spacer intercepts separate adjacent transcriptional units and are inserted between either two initiation (Fig. 13b) or two termination (Fig. 13c) sites. Such situations are clearly different from "gaps" occurring within otherwise normal transcriptional units which may have been caused by an artificial removal of lateral fibrils during the prep-

* The term "spacer" is used here in a strictly morphological sense to characterize axial intercepts free of lateral RNP fibrils which are apparently not transcribed at the point of time of the preparation. It does not imply that this region is never transcribed or is not potentially transcribable.


a
b
c

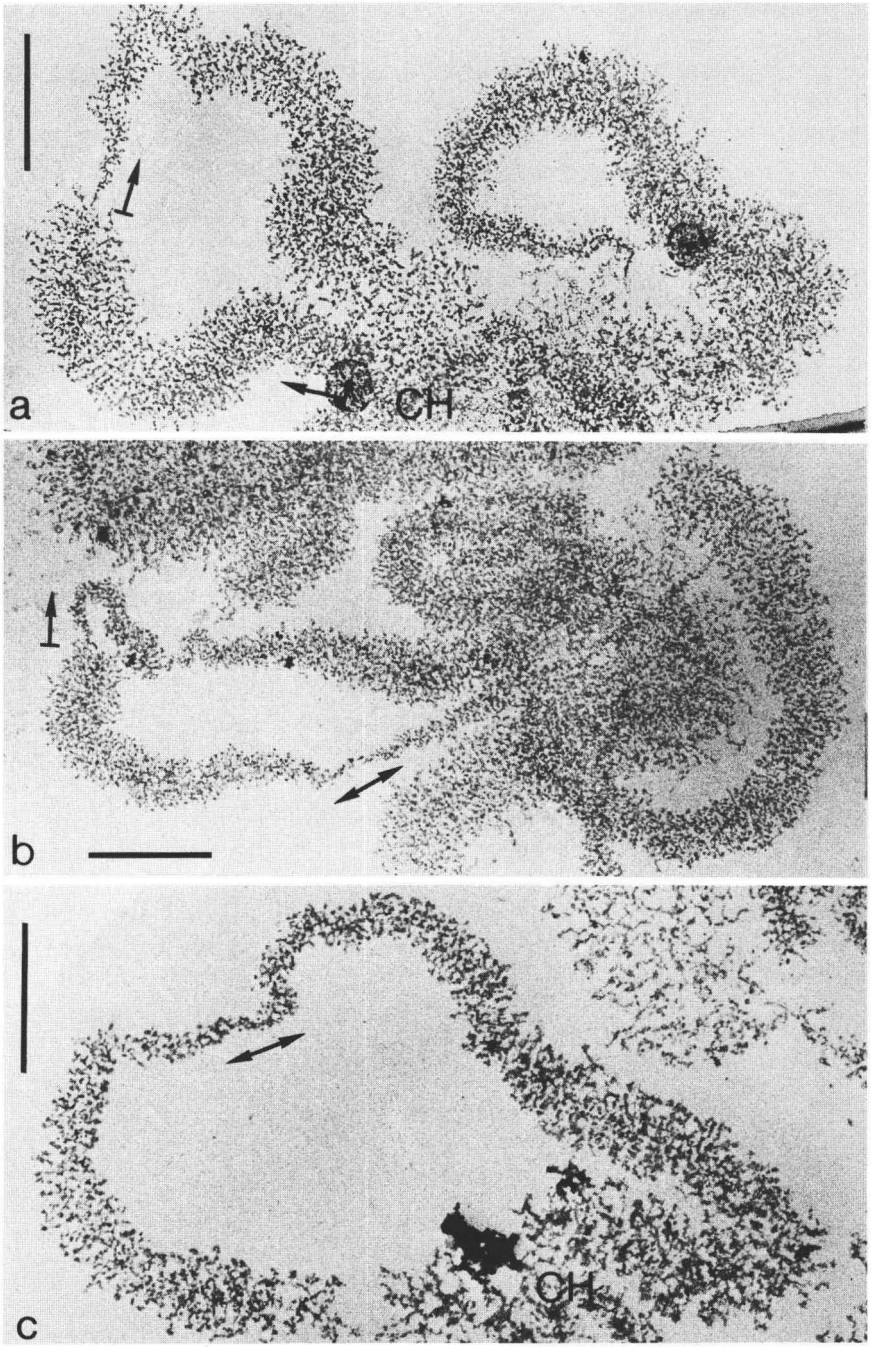
aration or might reflect true discontinuities in transcriptional events (Fig. 13a; for further examples, see Franke *et al.*, 1976b; Scheer *et al.*, 1976a).

IV. Cleavage of RNP Fibrils during Transcription

In most transcriptional units analyzed, the lengths of the lateral fibrils increase gradually, though not always steadily and/or linearly, as a function of the distance of the specific transcriptional complex from its initiation site. The length of the lateral fibrils, however, is much shorter than the corresponding axial intercept of the transcribed chromatin strand, indicative of a high degree of condensation and packing of the nascent RNP fibril. Consequently, differences of lengths among adjacent lateral fibrils are not per se indicating losses of portions of the RNA in the specific lateral fibril but may as well reflect local differences in RNP packing and dispersal during the preparation, respectively. In some loops, however, distinct and abrupt discontinuities in the length gradient of the lateral fibrils are evident. Relatively large proportions of the lateral fibrils seem to be cleaved off at a specific site (Figs. 14a, b). It should be emphasized that after this "cleavage site" the fibril length gradient does not start at zero but that the first lateral fibril already has a sizable length.

Evidence that functional processing occurs long before the RNA polymerase has reached the terminal signal comes from the study of Old *et al.* (1977). They have incubated denatured, tritiated plasmid DNA with inserted sequences coding for sea urchin histones with lampbrush chromosome preparations of *Triturus cristatus* under conditions favouring a DNA/RNA–transcript hybridization. Such an *in situ* hybridization allows the localization of transcripts containing histone mRNA sequences. Figure 15a shows the remarkable result. One of the few labeled lateral loop pairs, among several thousand unlabeled ones, is seen with a characteristic silver grain distribution

Fig. 7 Electron micrographs of loop–chromomere complexes of *Triturus cristatus* oocytes after very short exposure time to low salt conditions or spreading in the presence of 50 m*M* salt. The matrix material appears very condensed. A normal asymmetric loop with a thin and thick insertion site (arrows) is shown in (a). A symmetrically arranged loop is presented in (b). The double-headed arrow indicates the start regions of two matrix gradients that run in opposite directions so that this loop is inserted with two thick ends (arrows). A small portion of the loop is obscured by a bar of the grid [right corner in (b)]. A repeated "thin-to-thick" pattern is shown in (c) (arrow). Bars indicate 5 μm.


a
CH
b
c
CH

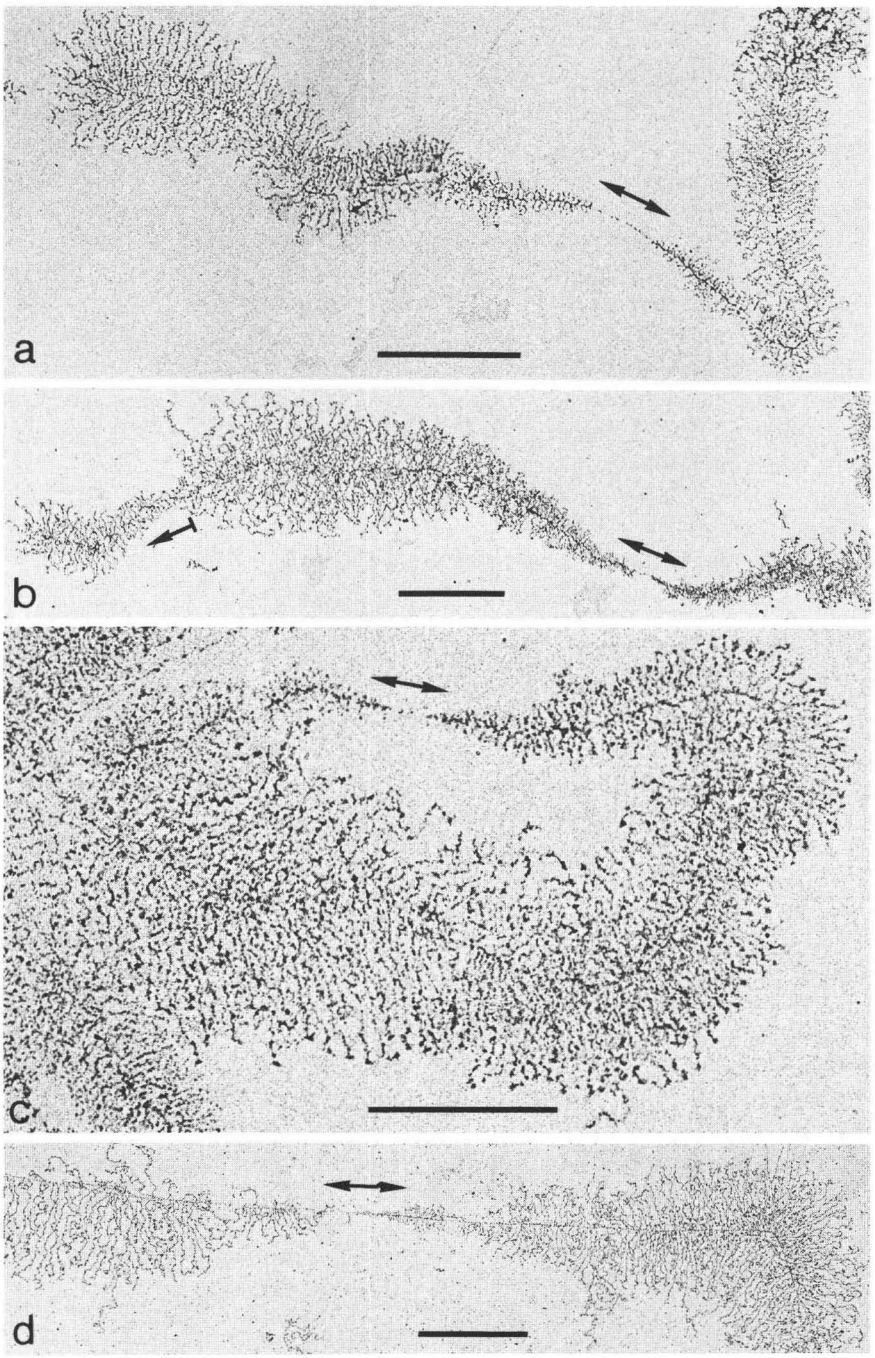
indicating that transcripts containing histone mRNA sequences are confined only to a certain section of the loop. However, these histone gene-containing loops are entirely and uniformly labeled after a short pulse with [^{3}H]uridine which means that transcription proceeds throughout the whole loop. This conclusion is also in agreement with the presence of asymmetrically distributed matrix material along the loop (Fig. 15a). The diagram in Fig. 15b illustrates the rather complex process by which histone mRNA-containing molecules are generated (for further details, see Old *et al.*, 1977). At the site "P," defined by the ending of the labeled region in the autoradiogram, distal portions of the lateral RNP fibrils including the histone mRNA sequences are clipped off whereas transcription proceeds further until the "thick" terminal insertion site of the loop axis.

V. Structure of Loop Chromatin in Different States of Activity

During mid-oogenesis the lateral loops of lampbrush chromosomes of amphibia are densely covered with transcriptional complexes in an almost maximal packing density (center-to-center distances of the basal RNA polymerase particles range from 17 to 25 nm; Figs. 16 and 18a). This close arrangement leaves little if any space for interspersed nucleosomes (for further discussion, see Franke *et al.*, 1976b, 1978a; Franke and Scheer, 1978). In fact, no nucleosomal beads can be recognized in between the basal particles of the lateral fibrils (Figs. 16 and 18a), even in regions of a somewhat reduced lateral fibril density. This appearance contrasts to that of the nontranscribed chromomeric DNP which is arranged in the characteristic beaded configuration indicative of nucleosomal packing of the chromatin (Fig. 16).

In maturing oocytes the lateral loops progressively retract into the chromosome axis (Fig. 17), concomitant with a drastic reduction of the frequency of the lateral RNP fibrils in the retracting loops (Figs. 18b, c, 19a–c). This observation suggests a progressive transcriptional inactivation during late oogenesis, similar to the situation found in the genes coding for ribosomal RNA's (Scheer *et al.*, 1976b) and is difficult to reconcile with the proposition that full-grown *Xenopus* oocytes syn-

Fig. 8 Electron micrographs of moderately spread segments of lampbrush chromosomes isolated from *Acetabularia mediterranea* with tandemly repeated transcriptional units of identical [arrows in (a)], opposite [double-headed arrow in (c)], or alternating [arrows in (b)] polarities. Note that the loop axes are covered with lateral fibrils over their entire lengths, CH, chromomere. Bars indicate 1 μm.


a
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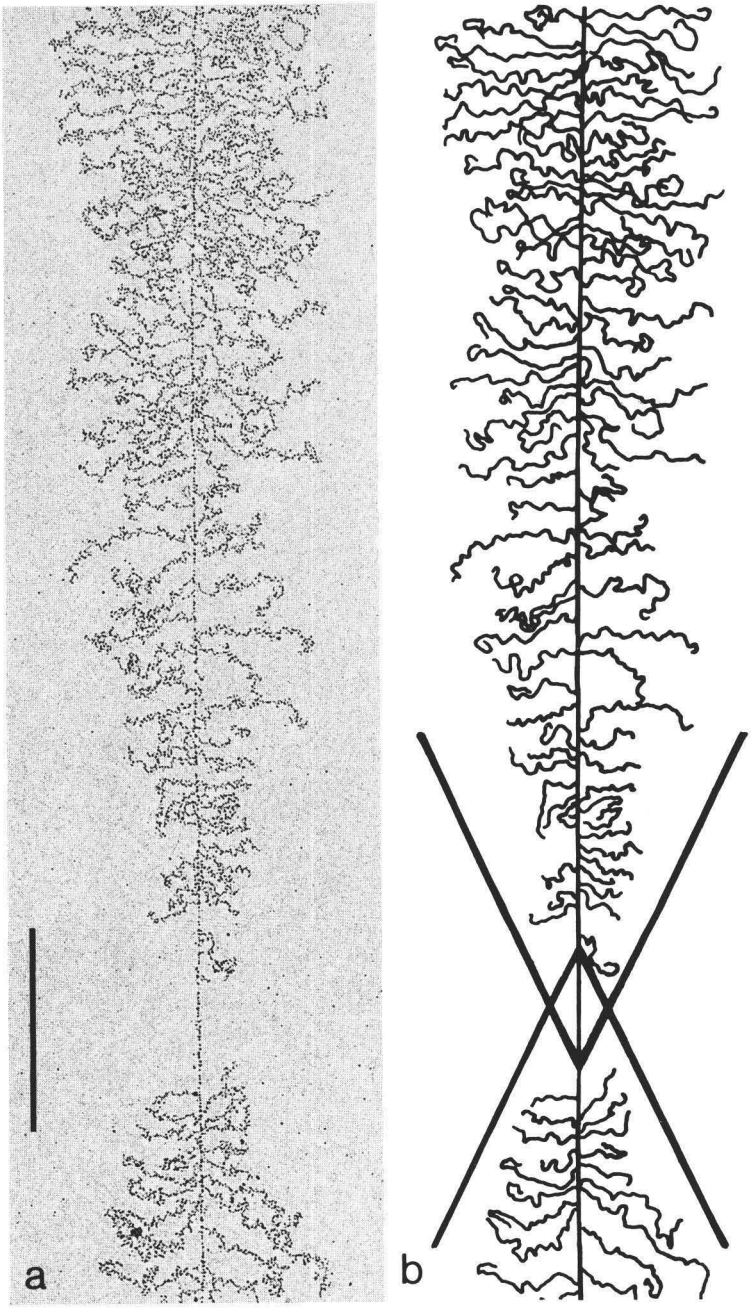
d

thesize identical amounts of hnRNA as compared to the stage of maximal loop extension of lampbrush chromosomes during mid-oogenesis (Anderson and Smith, 1977).

In loops of greatly reduced lateral fibril density the loop chromatin axis in between the widely distant transcriptional complexes sometimes appears as a very thin and smooth strand, especially after positive staining alone (Fig. 18b). In other loops, however, and especially after additional metal shadowing, such axial intercepts free of transcriptional complexes show a conspicuous beaded organization (Figs. 18c and 19a, b; cf. also Foe *et al.*, 1976; Laird *et al.*, 1976; McKnight *et al.*, 1978; Scheer, 1978). This pattern is indistinguishable from that of completely inactive somatic chromatin prepared under identical conditions (Franke *et al.*, 1976b; Scheer, 1978). However, the image of a linear array of "beads" with diameters in the range from 80 to 130 Å per se does not allow the conclusion that this represents a nucleosome chain. For example, when RNA polymerases are closely spaced along a DNA axis and most of the growing RNA chains is released by enzymatic cleavage so that they are not visible as lateral fibrils, a "beads-on-a-string" pattern is also obtained. Therefore, we developed a method to discriminate in electron microscopic spread preparations between nucleosomes and RNA polymerase particles (Scheer, 1978). At sufficiently high concentrations the anionic detergent Sarkosyl removes most of the DNA-associated proteins, histones included, but leaves initiated RNA polymerases attached. Therefore, under defined conditions, this detergent can be used as a probe to characterize the nature of chromatin-associated "beads." The result is presented in Fig. 19. When the spreading is performed in the presence of Sarkosyl the beaded structure of the chromatin axis disappears but the few transcriptional complexes remain stably bound to the DNA (compare Figs. 19b and 19c). This clearly demonstrates that in the course of the inactivation of the genes present in the chromosome loops a transition occurs from smooth to beaded morphology of the chromatin axis. The data also indicate that spacious, transcriptionally inactive, regions within an initiated transcriptional unit can assume the nucleosomal configuration, i.e., nucleosomal packing is reformed between two transcriptional events that are separated by extended periods of time and/or space.

Fig. 9 Selected loops of *Acetabularia mediterranea* (a–c) and *Pleurodeles waltli* (d) with tandemly arranged transcriptional units in opposite [double-headed arrows in (a,c,d)] or alternating polarities [arrow in (b)]. Bars indicate 1 μm (a–c) or 2 μm (d).


a
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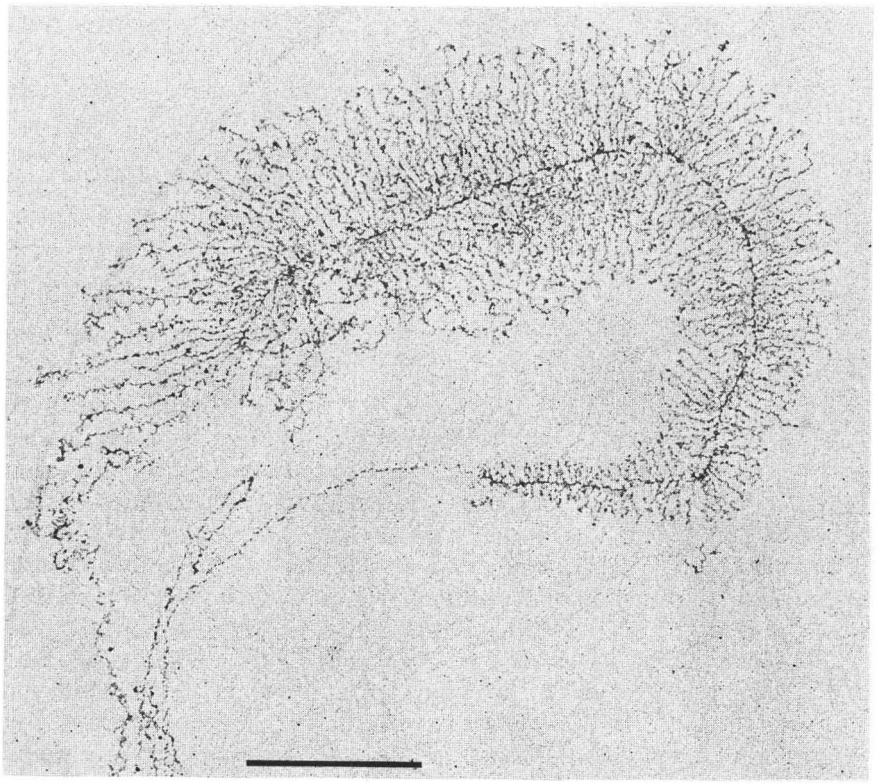
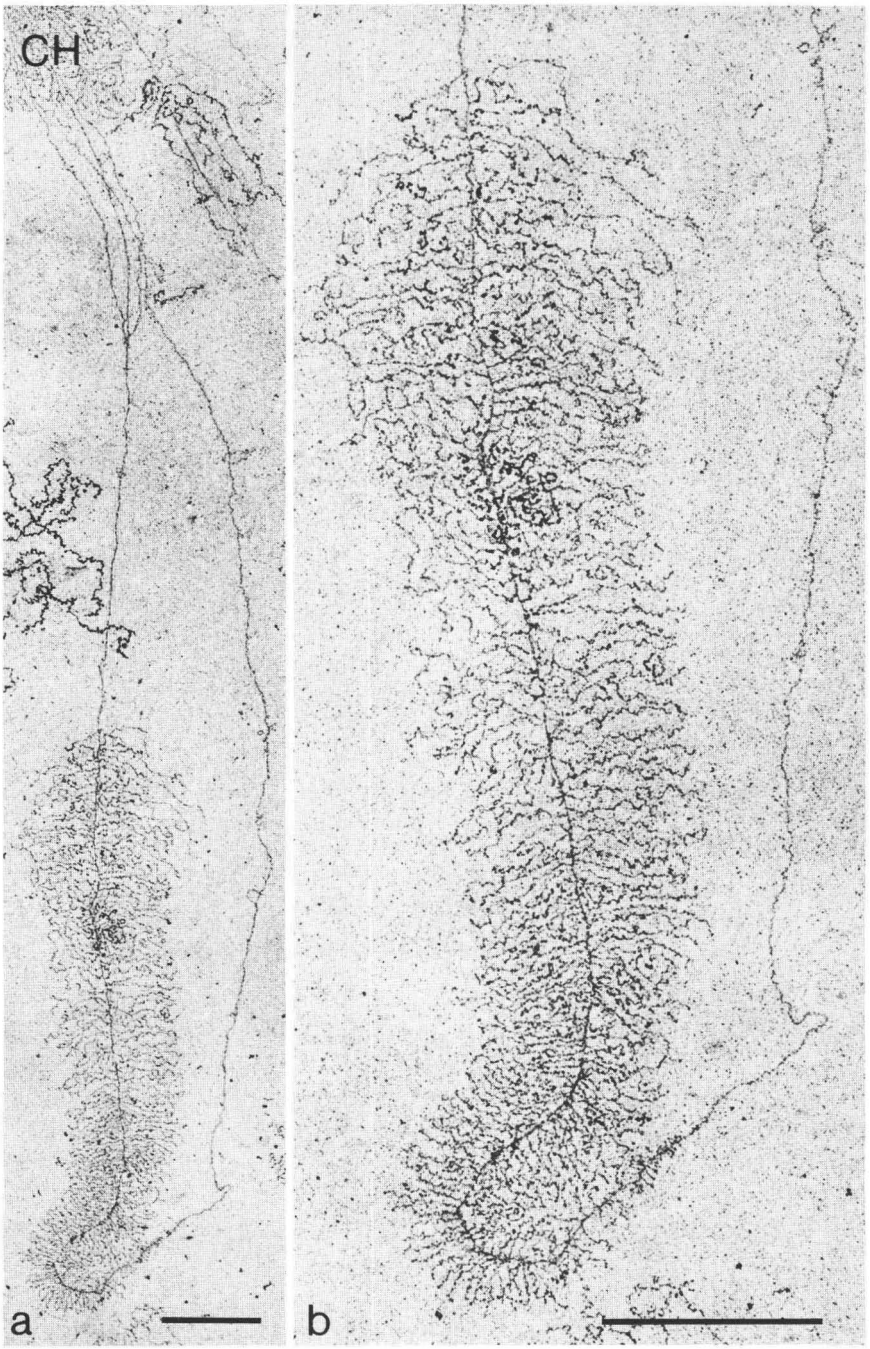


Fig. 11 Electron micrograph of a loop of *Acetabularia cliftonii*. The loop axis contains one transcriptional unit but also long apparently nontranscribed spacer regions on either side of the transcribed portion. Bar indicates 1 μm.

VI. Structural Organization of the Lateral RNP Fibrils

When the lateral fibrils have reached a certain length they tend to coil up at certain intervals, preferentially in the more distal portions, and often form complex bushlike or even ringlike structures (Figs.

Fig. 10 Part of a lampbrush chromosome loop isolated from a *Xenopus laevis* oocyte showing the start regions of two transcriptional units of opposite polarity (a). As illustrated schematically in the diagram (b), the initiation sites as extrapolated from the increase of lengths of lateral fibrils overlap considerably. No sizable transcriptional complexes are attached to the start regions of both transcriptional units. Bar indicates 2 μm.


CH
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b

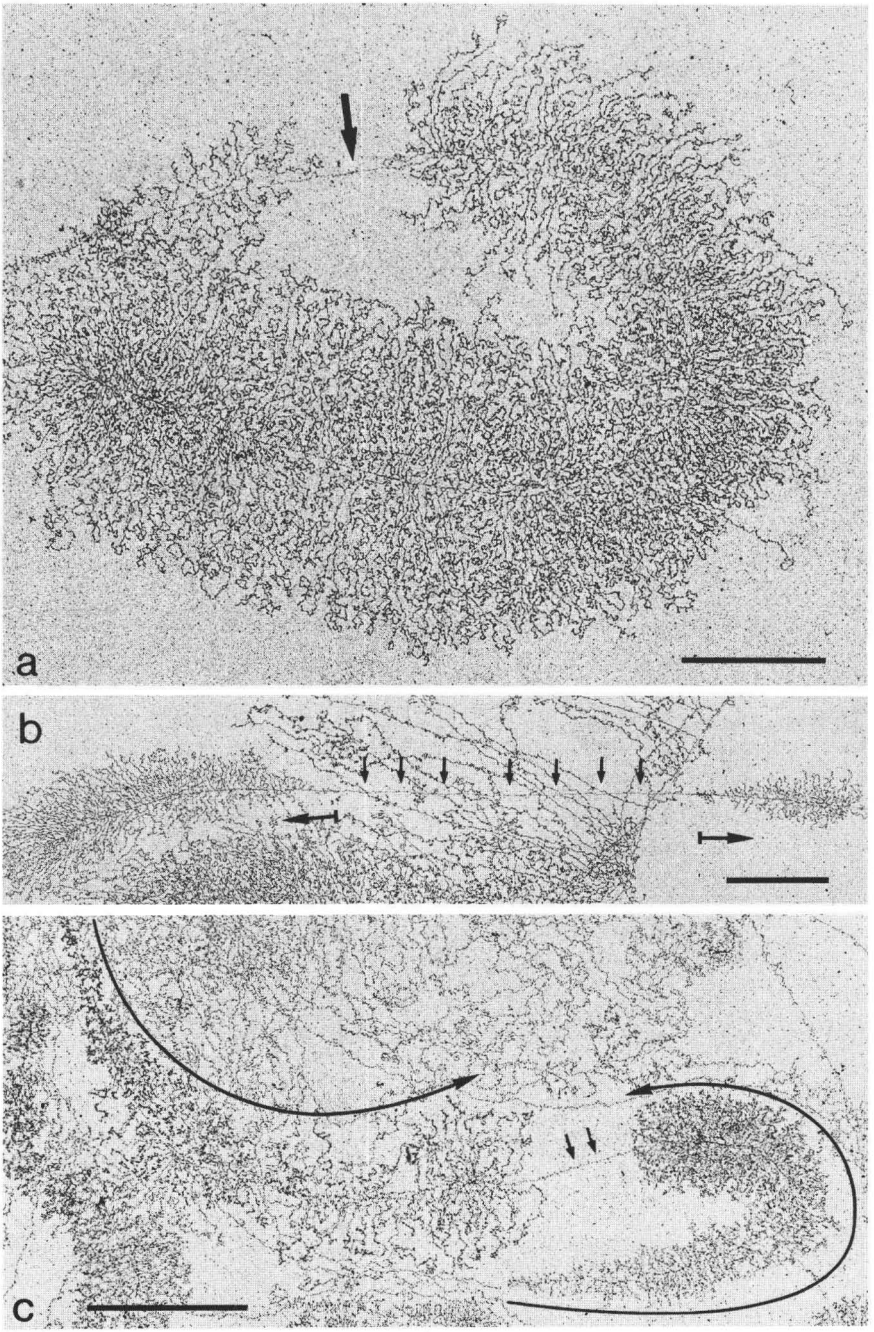
20a, b, e). Similar morphological features which also may be used as diagnostic criteria for the identification of nonribosomal RNP fibrils have been described in germ line and somatic cells of several species (e.g., Angelier and Lacroix, 1975; Glätzer, 1975; Laird and Choii, 1976; Laird *et al.*, 1976; Foe *et al.*, 1976; McKnight and Miller, 1976; Puvion-Dutilleul *et al.*, 1977; McKnight *et al.*, 1978). Occasionally, small annular structures with diameters of 50–150 nm are seen to be aligned along a lateral fibril without any visible connection to it (Figs. 18c and 20e). Depending of the specific spreading condition, the lateral fibrils have a nearly uniform thickness (range 5–12 nm) or show a more granular appearance. The granular formations within the lateral fibrils are either sparsely and irregularly distributed (Fig. 20c) or are arranged regularly (Fig. 20d). In the latter case one gains the impression that the lateral fibrils are composed of linear aggregates of periodic globular granules with diameters at about 20 nm (Figs. 14b and 20d; cf. Malcolm and Sommerville, 1974, 1977; Mott and Callan, 1975; Franke *et al.*, 1976b).

The observation that the lateral RNP fibrils apparently can occur in several structural states, in a beaded or totally extended configuration as well as in numerous intermediate formations, is in agreement with the observation of Malcolm and Sommerville (1977) that both high and low salt concentrations destroy the "beads-on-a-string" pattern of the lateral fibrils and unfold the RNP coils. It seems to be a general principle in eukaryotic nuclei that specific proteins associate with the nascent RNA and form periodically arranged units of condensation, i.e., aggregates of globular 20 nm particles which are interconnected by RNAse-sensitive strands (e.g., Malcolm and Sommerville, 1977; Karn *et al.*, 1977).

VII. Concluding Remarks

Lampbrush chromosomes contain several thousands of simultaneously active genes and hence offer a unique possibility of studying the modes of arrangement and the sizes of transcriptional units within the eukaryotic genome. Such analyses are facilitated by some remark-

Fig. 12 This loop with a total length of 53 μm of an oocyte of *Triturus cristatus* contains only one transcriptional unit with a length of 15 μm and a preceding and a subsequent fibril-free, i.e., apparently nontranscribed intercept (25 and 13 μm in length). The transcriptional unit is shown at higher magnification in (b). CH, chromomere. Bars indicate 2 μm. (From Scheer *et al.*, 1976a.)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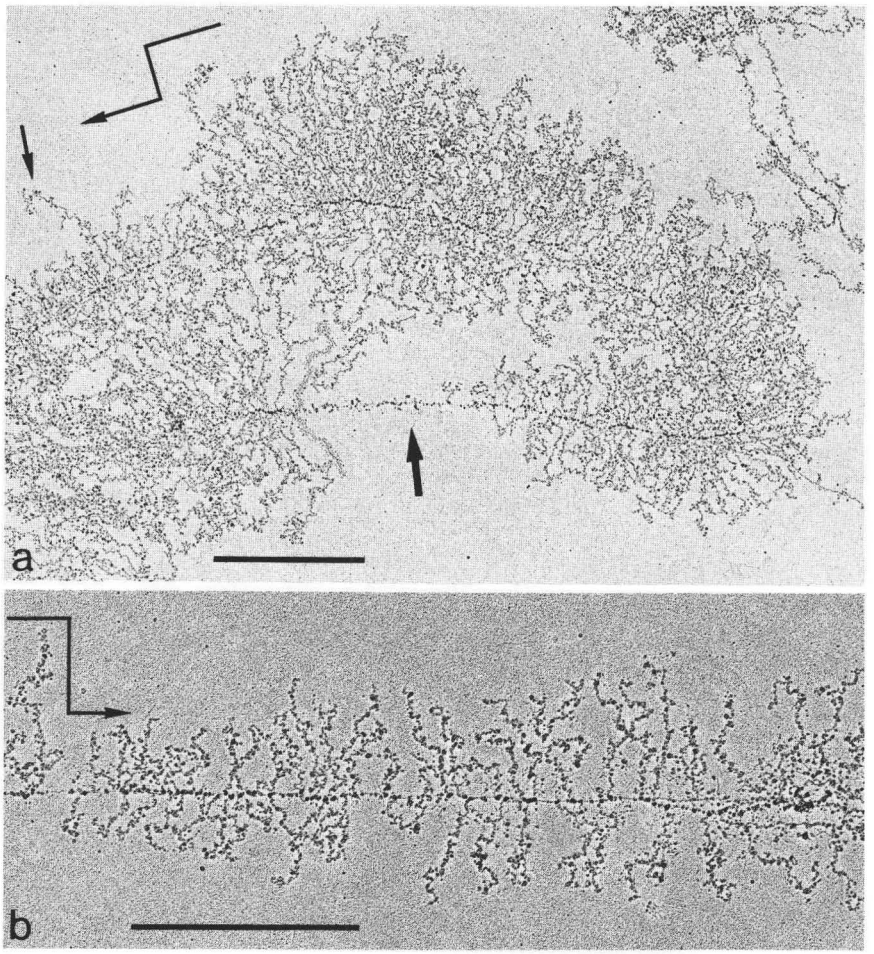
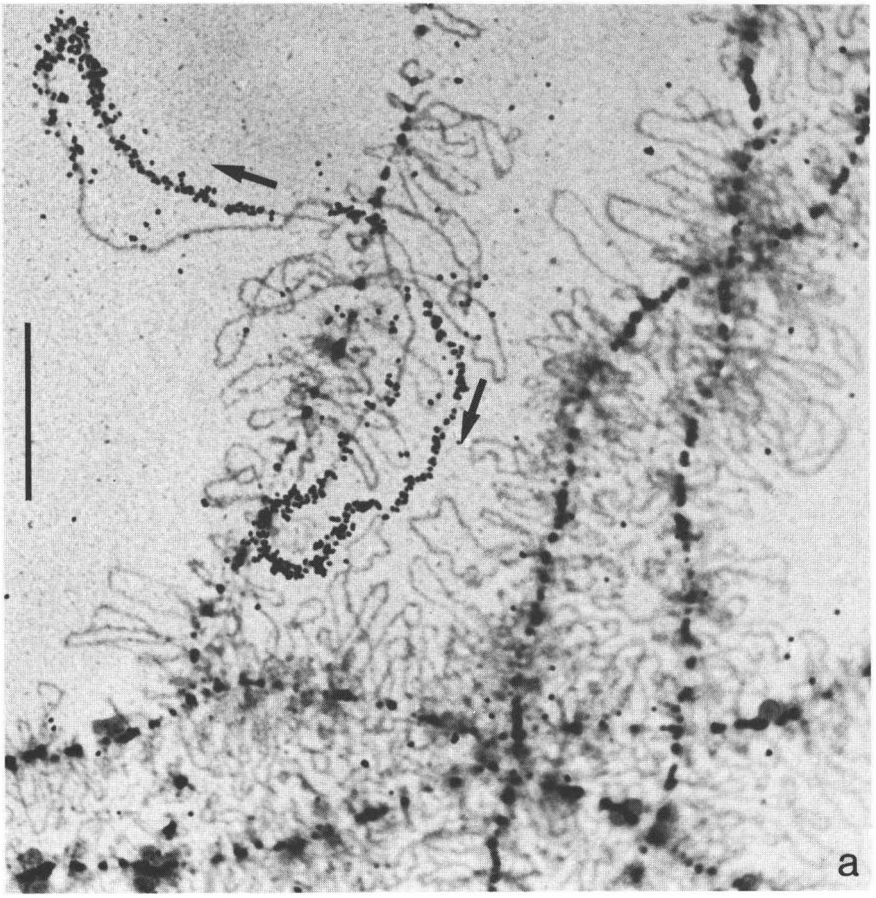


Fig. 14 Morphological indications for the occurrence of distinct cleavage processes in the nascent RNP fibrils. The steplike arrows denote regions in which abrupt reduction of the lengths of lateral fibrils is seen. Such a situation is clearly distinguishable from two tandemly arranged transcriptional units the start region of one transcriptional unit is denoted by the thick arrow in (a). Some of the fibrils seem to have escaped the cleavage process [e.g., small arrow in the upper left of (a)]. Note the granular substitutes in the lateral fibrils. The lampbrush chromosomes were isolated from oocytes of *Xenopus laevis*. Bars indicate 1 μm.

Fig. 13 Adjacent transcriptional units are often separated by nontranscribed "spacer" intercepts [small arrows in (b) and (c)], a situation which is clearly distinguishable from "gaps," i.e., short fibril-free sections within a given transcriptional unit [arrow in (a)]. The lampbrush chromosomes were obtained from oocytes of *Pleurodeles waltli* (a,b) and *Xenopus laevis* (c). The long arrows in (b) and (c) indicate the polarity of the specific transcriptional units. Note the complex folding and/or coiling of the terminal portions of the nascent RNP fibrils [(a), total loop length 21 μm]. Bars indicate 2 μm.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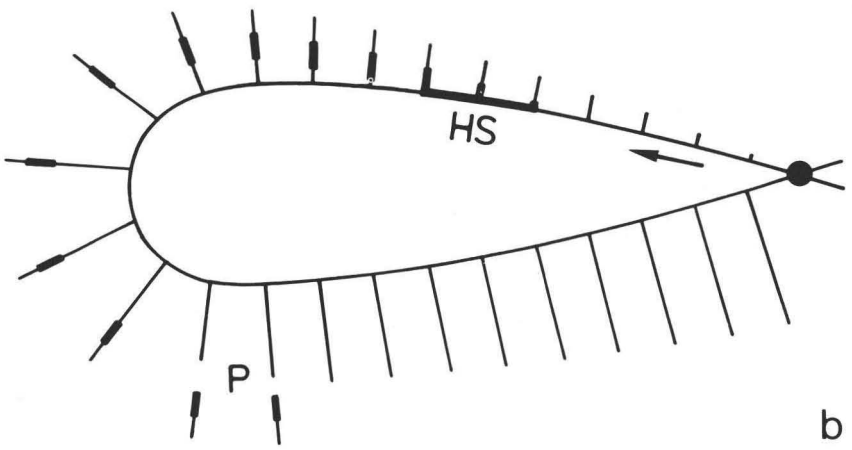
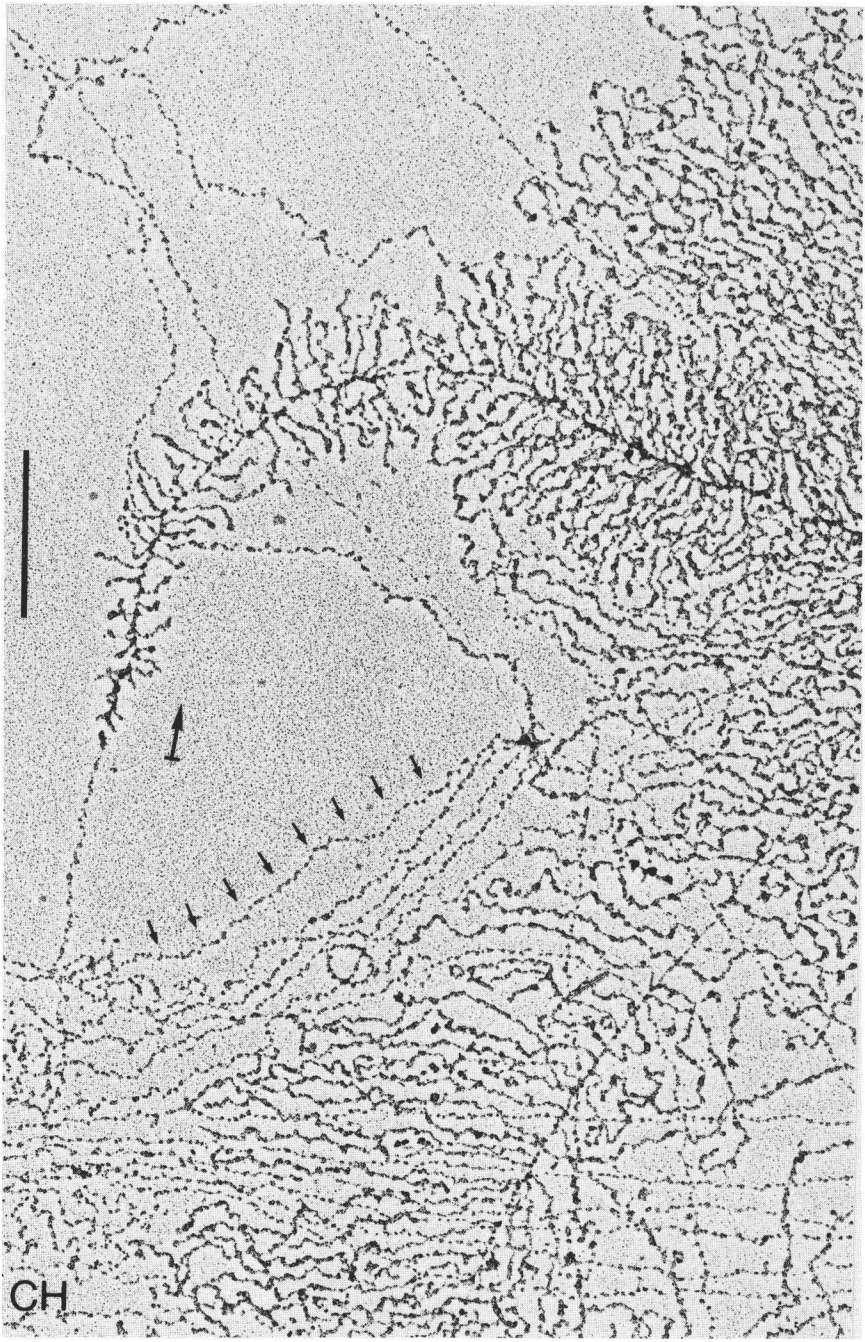

HS
P
b

able structural and functional features of lampbrush chromosomes such as the subdivision into periodic chromomere/loop complexes and the very high transcriptional activity which is observed during phases of cell and nuclear growth as in amphibian oocytes and the green alga, *Acetabularia*. The packing density of the RNA polymerases along the transcriptional units of the lateral loops is generally very high (15–25 nm center-to-center distance) and close to its maximal possible value (about 13 nm, corresponding to the average diameter of the RNA polymerase-containing globule; see Franke *et al.*, 1976b). A rough estimation of the maximal rate of hnRNA synthesis in *Xenopus laevis* oocytes calculated from the data presented in Section I,E (22×10^6 transcripts/hr/nucleus) and below (average molecular weight of the primary transcripts 7×10^6) agrees reasonably well with the corresponding biochemical measurements (Anderson and Smith, 1977) and supports the concept of an extremely high transcriptional activity in the chromosome loops.* We would like to emphasize, however, that certain genes of somatic cells have similarly high "transcriptional efficiencies," i.e., rates of release of RNA transcripts (Kafatos, 1972; Hunt, 1974; Palmiter, 1975). Recently, one of these highly transcribed genes, the silk fibroin gene of *Bombyx mori*, has been putatively iden-

* One germinal vesicle of *Xenopus laevis* contains about 12,000 chromosome loops (see Section I,E) with axial lengths of the majority of the loops ranging from 5 to 10 μm (Müller, 1974). As discussed below, the loop length roughly corresponds to the same length of DNA in B conformation. This means that during mid-oogenesis 2.3% of the total chromosomal DNA is extended into lateral loops (taking a value of 3.2 pg of DNA per haploid chromosome set; e.g., Thiébaut and Fischberg, 1977) and transcribed by a total of about 4.2×10^6 RNA polymerases of the type II (or B). This figure is by two orders of magnitude higher than that determined to be present in nuclei of metabolically active somatic cells (e.g., Cochet-Meilhac *et al.*, 1974) but is considerably lower than the number ($80–200 \times 10^6$) of the nuclear RNA polymerases involved in the transcription of the amplified rDNA in the same nuclei ($1–2 \times 10^6$ pre-rRNA genes, see Birnstiel *et al.*, 1971; 80–100 transcriptional complexes per gene, see Scheer *et al.*, 1977).

Fig. 15 Light microscopic autoradiogram of lampbrush chromosomes of *Triturus cristatus* after *in situ* hybridization of tritiated plasmid DNA containing sea urchin histone gene sequences to nascent RNA transcripts (a). Note the high specificity of the labeling. The arrows in (a) indicate the polarities of the loops. The diagram presents an illustration and interpretation of the result of this hybridization study (b). The region of the loop which contains the histone sequences is drawn thicker and denoted HS. Thickened portions of the lateral fibrils represent histone mRNA sequences which are cut off at the region indicated P. Bar indicates 20 μm. (From Old *et al.*, 1977, see there for further details.)


CH

tified using the spreading technique and is characterized by a very close packing of transcriptional complexes (McKnight *et al.*, 1976).

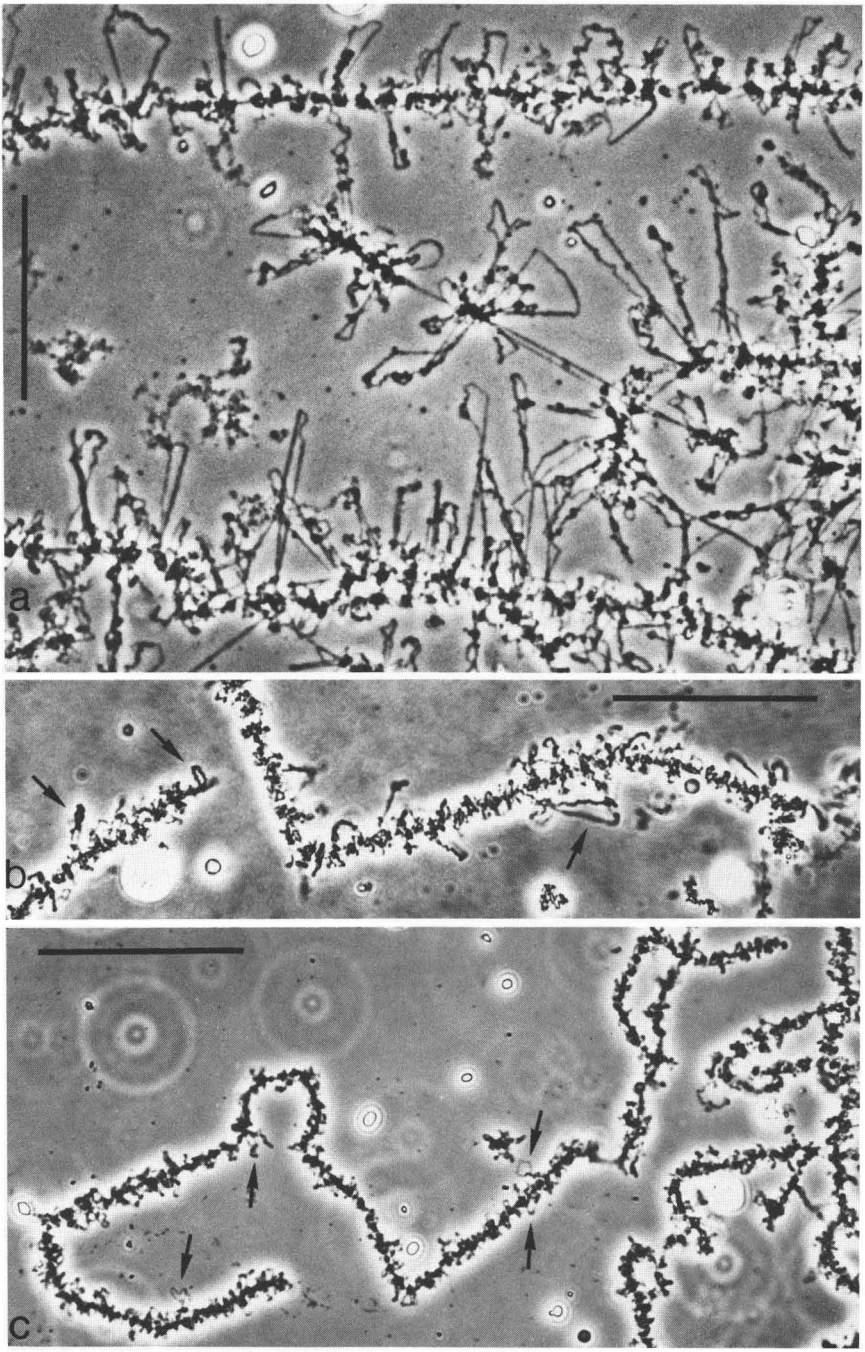
Toward the end of oogenesis the loops retract and the number of transcriptional complexes per unit length of chromatin decreases drastically, indicative of regulative processes acting at the level of transcription, i.e., through the frequency of initiation events of the RNA polymerases. It is obvious that lampbrush chromosomes are of great potential of studying structural and regulatory aspects of the transcription of defined genes and transcriptional units, respectively.

Loops with different patterns of arrangement of transcriptional units can be distinguished (diagrammed in Fig. 21). The "classic" scheme is depicted in Fig. 21a. Here one loop represents one transcriptional unit. The largest loop of this type and, consequently, the largest transcriptional unit which we have been able to clearly trace in an electron microscopic spread preparation of an urodelan oocyte has been measured to be 28 μm long and thus falls in the average size class of large loops seen in the light microscope (10–40 μm). Whether the exceptionally large loops observed in some organisms (over 100 μm in axial length) can be equated with transcriptional units of about the same size is unclear at present; it would be extremely difficult to trace such "giant" transcriptional units in electron microscopic preparations. Other loops also contain only one transcriptional unit but additional "spacer" regions (Fig. 21b). We do not know whether these fibril-free spacer intercepts contain genes which are not expressed at the time of the preparation or represent true nontranscribed spacer sequences. Other types of loops (Figs. 21c–e) illustrate multigenic situations.*

The transcriptional units present in a single loop are either largely identical as this is the case, for instance, for the tandemly arranged 5 S rRNA and pre-rRNA genes (Fig. 21d) or are of different length and/or sequence with or without interspersed "spacer" intercepts (Figs. 21c–e). The orientation of the transcriptional units can vary within a

* The number of transcriptional units per loop is not necessarily identical with the number of genes but represents the minimal number of genes per loop. A transcriptional unit may contain several structural genes, and certain genes of a given loop may be inactive.

Fig. 16 "Thin" insertion site of a loop axis in the chromomeric mass (CH) of a lampbrush chromosome of *Triturus alpestris*. The start region of the transcriptional unit is denoted by the arrow. Note the beaded appearance of the chromatin axes of the chromomere (e.g., at the small arrows) and of the axial intercept preceding the transcriptional unit. Bar indicates 0.5 μm.


a
b
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single loop (Fig. 21e). This indicates either intra-axial switches in DNA polarity (Wolff *et al.*, 1976) or, what seems more likely to us, transcription from both DNA strands in a manner similar to situations observed in prokaryotic systems (e.g., Szybalski *et al.*, 1970; Guha *et al.*, 1971; Acheson, 1976; Ptashne *et al.*, 1976; Evans *et al.*, 1977). An interesting type of arrangement of two bidirectional transcriptional units is diagrammed in Fig. 10b and indicates the possibility of overlap of adjacent units in the start regions. Such an arrangement reminds one of the overlap of transcriptional units, be it in divergent or convergent manner, described in prokaryotic systems (e.g., Szybalski *et al.*, 1970; Ptashne *et al.*, 1976; Evans *et al.*, 1977). At present it remains a matter of speculation to regard such multigenic loops as whole sets of transcriptionally coordinated units.

The majority of the loops of *Acetabularia* lampbrush chromosomes have lengths from 5 to 10 μm as determined in light microscopic preparations. When measured in electron microscopic spread preparations the transcribed loop axes fall within the same size range. This is an indication that the DNA in both types of preparations has the same structural arrangement and extension. Several observations strongly suggest that DNA in heavily transcribed chromatin is in a fully extended state, both in nucleolar (see Franke *et al.*, Chapter 2, this volume) and nonnucleolar chromatin (cf. Franke *et al.*, 1976b, 1978a; Franke and Scheer, 1978). Nucleosomes are obviously absent in regions of very high packing densities of RNA polymerase particles. Moreover, the length of the silk fibroin transcription unit of *Bombyx mori* corresponds to the molecular weight of the silk fibroin mRNA (or a slightly larger precursor; Lizardi, 1976), suggesting that the transcribed DNA is largely extended in its B conformation and not compacted into nucleosomes (McKnight *et al.*, 1976).

Transcriptional units of *Acetabularia* (genome size about 1 pg; Spring *et al.*, 1978) range from 0.3 to 10 μm with a modal peak of about 4 μm, corresponding to sizes of the primary transcripts between 900 and 30,000 nucleotides. The transcriptional units of the amphibian species studied fall within a total size range from 3 to 28 μm. Our preliminary analyses indicate that the sizes of the transcriptional units

Fig. 17 Light microscopic preparation of typical lampbrush chromosomes isolated from different size classes of *Triturus alpestris* oocytes to illustrate the retraction of lateral loops towards the end of oogenesis. Chromosomes were isolated from oocytes with diameters of 0.8 mm [(a) late lampbrush stage], 1.3 mm (b), and 1.5 mm [(c) almost mature]. Note the striking size reduction of the lateral loops [some remaining small loops are denoted by the arrows in (b) and (c)]. Same magnification in all three micrographs, bars indicate 50 μm.

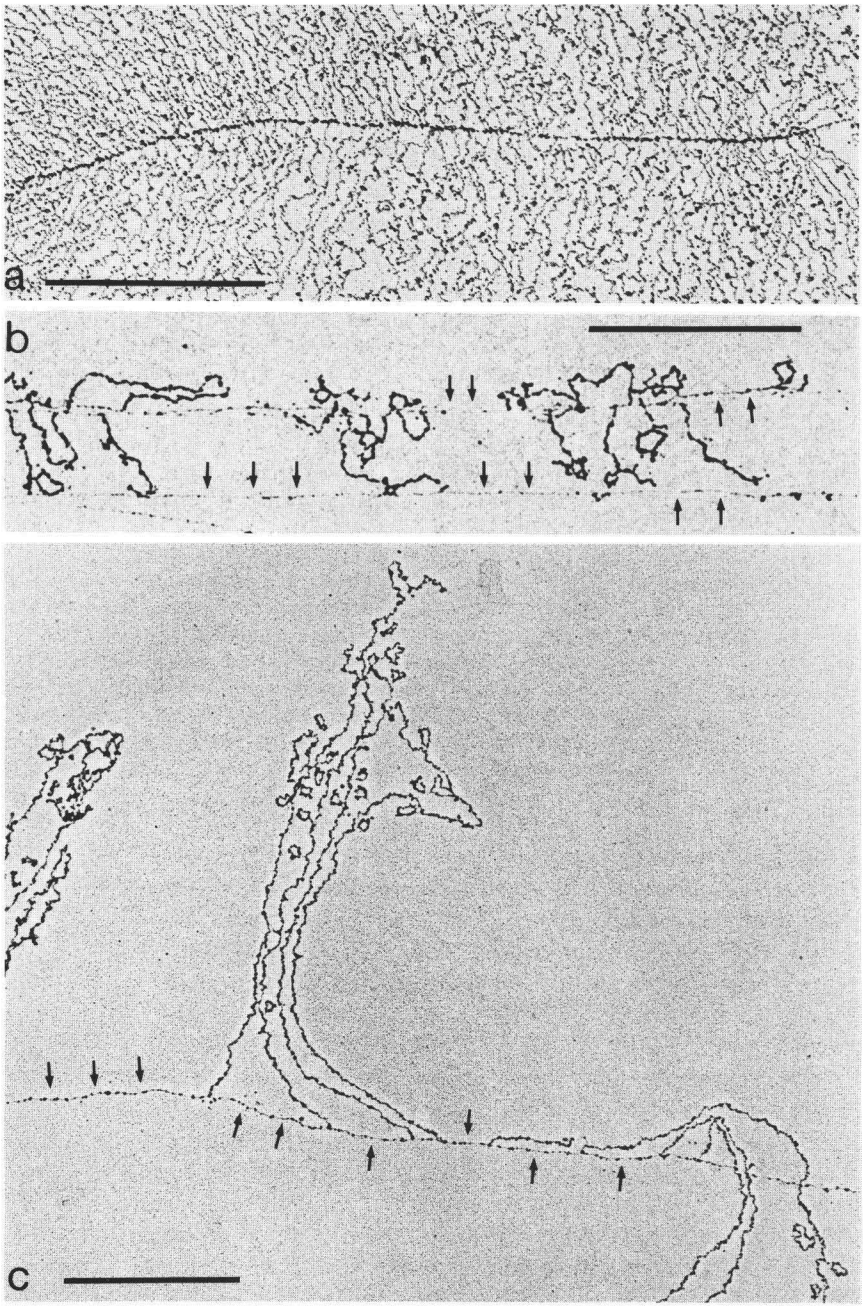

Fig. 18 (See page 43 for legend.)

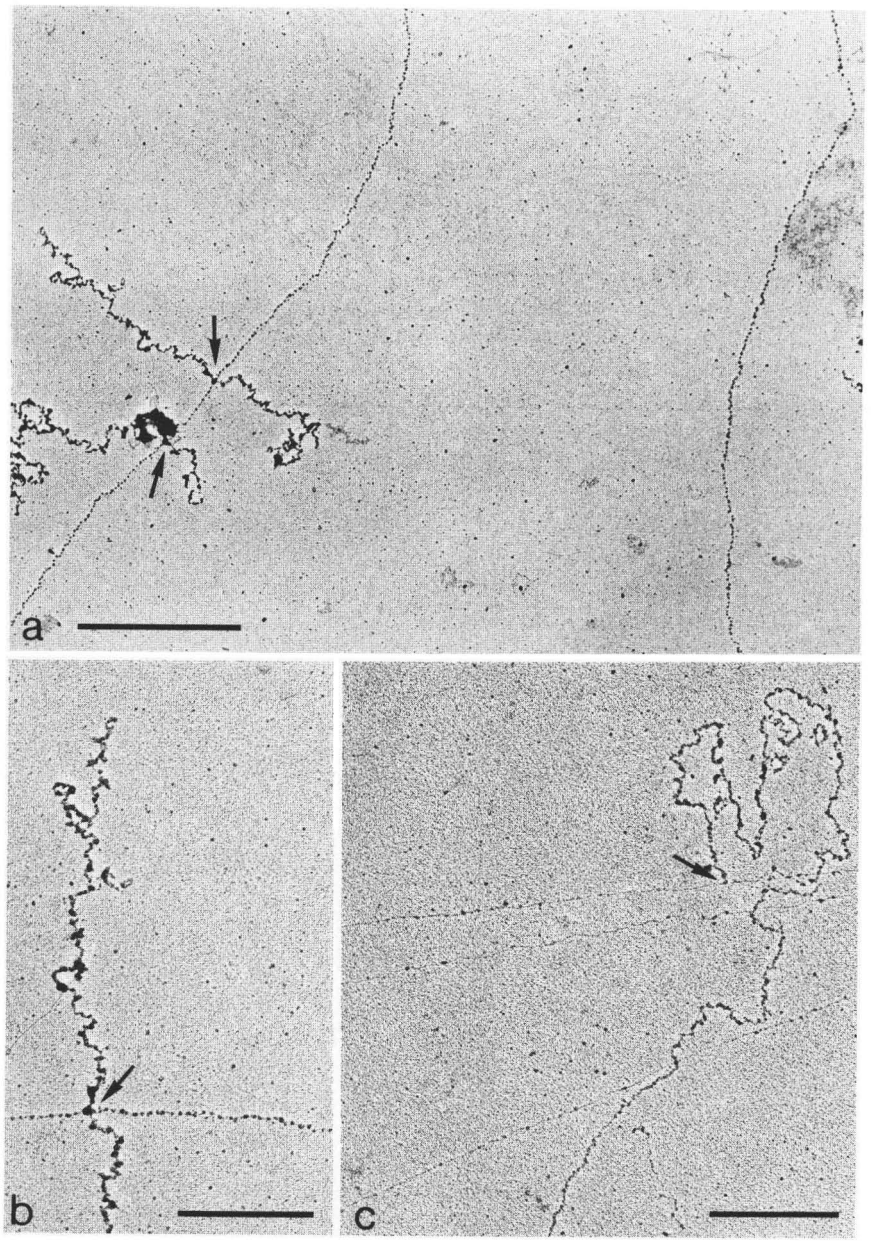

Fig. 19 (See page 43 for legend.)

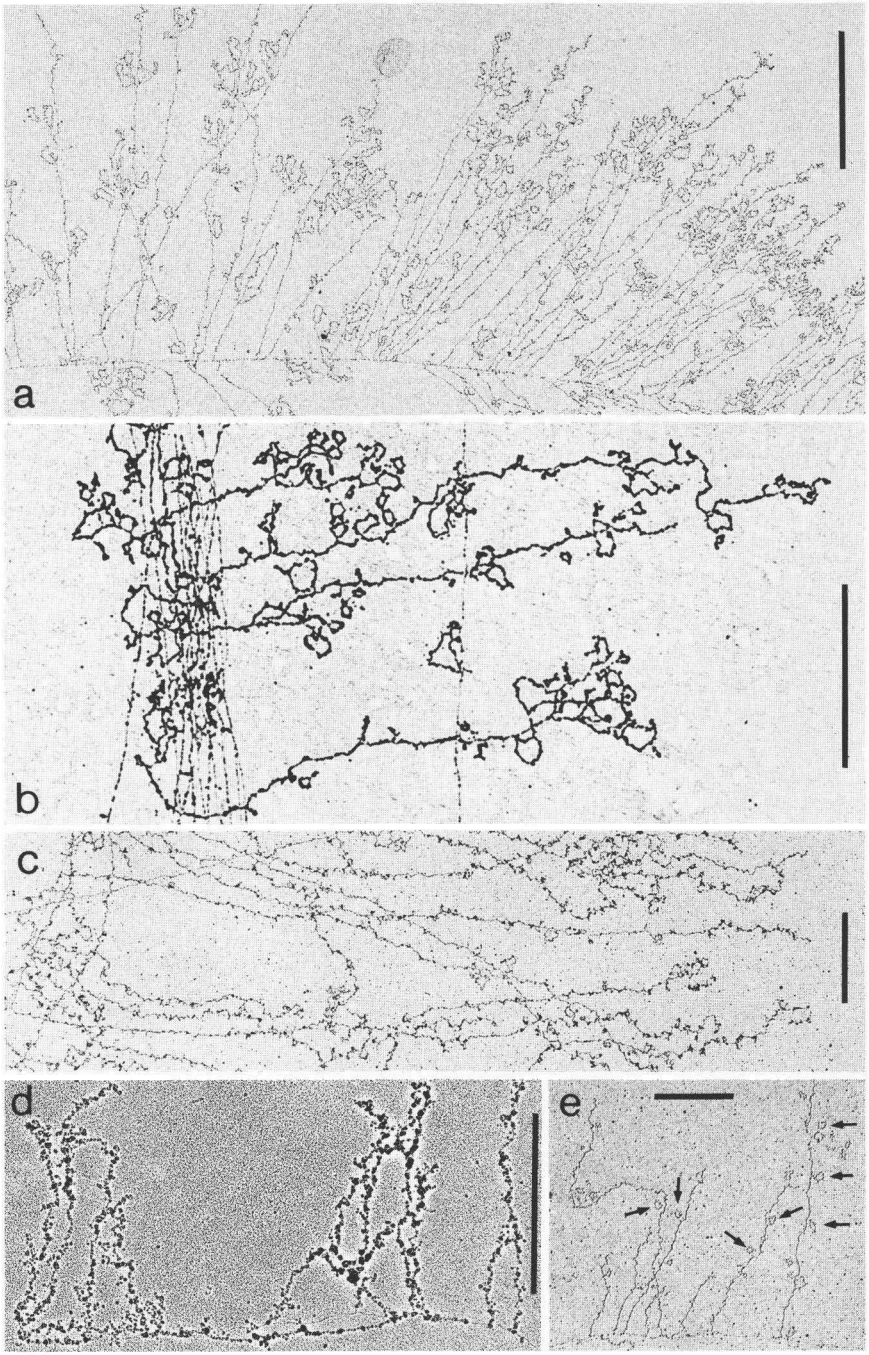

Fig. 20

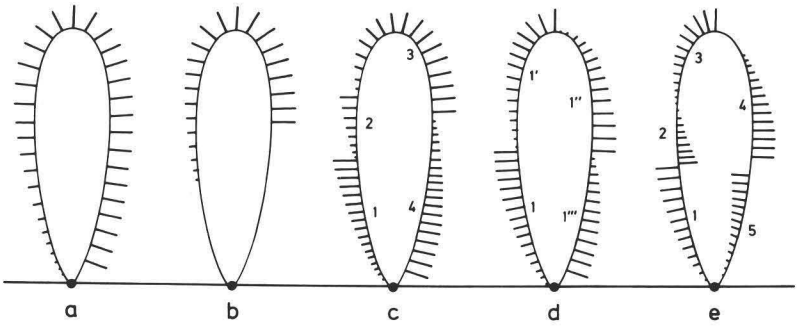

Fig. 21 Schematic presentation of various arrangements of transcriptional units within individual loops of lampbrush chromosomes as revealed in spread preparations. The numbers 1-1''' denote units of equal lengths, 1-5 denotes units of different lengths. For further details see text. (From Scheer *et al.*, 1976a.)

Fig. 18 Comparison of the packing densities of lateral RNP fibrils attached to loop axes of lampbrush chromosomes in growing (a) and nearly mature (b,c) oocytes of *Triturus cristatus*. The packing density of the RNA polymerase-containing granules in early and mid-oogenic stages is so high that the particles seem to touch each other (a). In maturing oocytes the lateral fibrils are much more distant (b,c). The fibril-free loop axis appears sometimes smooth and thin [arrows in (b)], but usually, especially after additional metal shadowing, a beaded configuration is recognized [arrows in (c)]. Bars indicate 1 μm.

Fig. 19 Lampbrush chromosome loops of almost mature *Triturus alpestris* oocytes, spread without (a,b) and in the presence of 0.5% Sarkosyl NL-30 (c). The beaded organization of the nontranscribed portions of the chromatin axis is also visible between adjacent distant lateral fibrils [arrows in (a); the same situation is shown at higher magnification in (b)], is destroyed under the action of the detergent, and the loop axis appears as a very thin, nonbeaded fibril [(c), the arrow denotes the attachment site of the lateral fibril]. This sensitivity of the "beads" allows their tentative identification as nucleosomes. Bars represent 1 μm (a) and 0.5 μm (b,c).

Fig. 20 Morphology of nascent lateral RNP fibrils attached to lampbrush chromosome loops isolated from oocytes of *Pleurodeles waltli* (a,c), *Triturus cristatus* (b,e), and the insect *Acheta domesticus* (d). The preparations shown in (d) and (e) were made in the presence of 0.1% Sarkosyl NL-30. Note the brush-like structures in the terminal portions of the fibrils shown in (a) and (b). A particular structure of the RNP fibrils is apparent in (c) and (d). Only few particles can be recognized along the lateral fibrils of the preparation shown in (c), whereas the RNP fibrils of (d) appear to be composed of linear aggregates of about 20 nm-particles. Note also the occurrence of small ring structures associated with the lateral RNP fibril [arrows in (e)]. Bars indicate 1 μm (b–e) and 2 μm (a).

of *Xenopus laevis* are distributed preferentially around 7 μm (corresponding to mean primary transcripts of about 21,000 nucleotides), whereas those from *Pleurodeles* and *Triturus* are evenly scattered within the limits of 9,000 and 82,000 nucleotides. Similar sizes of hnRNA molecules from *Triturus* and *Pleurodeles* oocyte nuclei have been determined by sedimentation or gel electrophoretic analyses (Scheer *et al.*, 1976b; Denoulet *et al.*, 1977; Sommerville and Malcolm, 1976). At present we are unable to find a correlation between the size distribution of transcriptional units and the genome size as this has been implied from the inverse correlation between light microscopic loop length and genome size among anuran and urodelan amphibia (e.g., Sommerville, 1977).

The wide size distribution of the lengths of transcriptional units (for related data derived from somatic chromatin of the insects *Drosophila* and *Oncopeltus*, see McKnight and Miller, 1976; Laird and Choii, 1976; Foe *et al.*, 1976) indicates that functional mRNA molecules can be derived from very large (molecular weights up to 28×10^6 or 82,000 nucleotides) or moderately large (about 7×10^6 or 21,000 nucleotides) primary transcripts or are synthesized and transported in the cytoplasm without any reduction in size. This view is compatible with biochemical analyses which have suggested that primary transcripts reveal the same broad size spectrum as the corresponding hnRNA (e.g., Derman *et al.*, 1976; Giorno and Sauerbier, 1976).

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