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MORPHOLOGY OF TRANSCRIPTIONAL UNITS OF rDNA

Evidence for Transcription in Apparent Spacer Intercepts and Cleavages in the Elongating Nascent RNA

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SUMMARY

Several types of "irregular" structures in the arrangement of lateral fibrils were noted in electron microscopic preparations of transcriptionally active nucleolar chromatin from various plant and animal cells. Such forms include:

1. Disproportionately long lateral fibrils which occur either as individual fibrils or in groups;
2. "Prelude complexes" and other arrangements of lateral fibrils in apparent spacer intercepts;
3. Thickening of the rDNA chromatin axis at the starting end of pre-rRNA matrix units;
4. Extremely long matrix units, the length of which exceeds that of the rDNA (double-strand) sequence complementary to the specific pre-rRNA (for abbreviations see text).

In addition, the stability of high molecular weight RNAs contained in the nucleolar ribonucleoproteins during the preparation for electron microscopy was demonstrated by gel electrophoresis. The observations indicate that the morphological starting point of a pre-rRNA matrix unit is not necessarily identical with the initiation site for synthesis of pre-rRNA, but they rather suggest that the start of the transcriptional unit is located at least 0.2–0.8 μm before the matrix unit and that parts of the "apparent spacer" are transcribed. It is proposed that the pre-rRNA molecules do not represent the primary product of rDNA transcription but rather relatively stable intermediate products that have already been processed during transcription.

The pathways and mechanisms of formation of ribonucleic acids necessary for protein synthesis in eukaryotic cells have been the subject of a considerable number of investigations. However, although some advances have been made in understanding the processing and regulatory principles, the primary events of transcription remain rather obscure. Recently it became clear that the demonstration of a complete primary transcriptional product of high molecular weight as such is very difficult. This holds in particular for the best studied type of high molecular weight RNA, the precursor molecules common to the two large ribosomal RNAs, the 28S and the 18S rRNA (for reviews see [3, 6, 7, 22, 34]). Although in most nuclei a very prominent and distinct pre-rRNA molecule can be identified as a long and covalently connected unit, there are several reasons to doubt that these molecules represent the

complete transcripts of the rDNA (for references see [21, 38, 39, 44, 52] and Discussion). Evidence has been accumulated from studies on some special *Escherichia coli* mutants that in prokaryotes the processing of the pre-rRNA usually begins in the nascent molecule, i.e. already during transcription (see also the concept of "primary processing" [4, 8, 11, 13, 17, 32, 33]). In the present article we describe a series of morphological details of transcriptional units in nucleoli from various animal and plant cells which suggest that similar processes, i.e. superpositions of processing and transcription, might also occur in eukaryotic cells (see also references [37, 38]).

ABBREVIATIONS

S values of ribosomal RNAs (rRNAs) are nominal; 28S and 18S are assigned to the largest and second largest species of cytoplasmic rRNA of eukaryotic organisms. The term "rDNA" is used to designate DNA that is enriched in, and consists of, the genes for the common precursor to 18 and 28S rRNAs, the interspersed spacer regions (cf [45, 58, 59]), if present, and possibly some other genes. The term "apparent spacer" is defined here strictly morphologically as intercepts in rDNA chromatin strands which lie between the matrix units (the fibril covered transcriptional units containing the pre-rRNA). The term "spacer" is used to define all regions in repeating units of rDNA that do not contain sequences complementary to pre-rRNA (cf [35]) or to RNA which is transcribed from the same promoter as pre-rRNA (cf [39]). This is not necessarily identical with the "non-transcribed spacer" as defined by others ([5, 58]; for detailed discussion see text). The term "pre-rRNA" designates the largest molecules that can be isolated, contain rRNA sequences and appear as a distinct size class.

MATERIAL AND METHODS

Electron microscopy

Nucleolar material from various cell types (vegetative stages of the green algae *Acetabularia mediterranea* and *A. major*, diplotene oocytes from the insects, *Acheta domesticus* and *Dytiscus marginalis*, and from the various amphibia listed in ref. [39]) was prepared and evaluated as previously described [39, 41, 47, 49, 51–53, 55]. In the present study only well extended fibrils were considered for evaluation.

Examination of the stability of RNA during the preparation for electron microscopy

Freshly collected newts, *Triturus alpestris*, were injected intraperitoneally with 200 μCi each of the four tritiated ribonucleosides ([^{3}H]cytidine, 25 Ci/mM; [^{3}H]guanosine, 10 Ci/mM; [^{3}H]adenosine, 22 Ci/mM; [^{3}H]uridine, 45 Ci/mM; The Radiochemical Centre, Amersham, UK). After 48 h nuclei were isolated from oocytes (lampbrush-chromosome stage), and their contents were dispersed and spread according to the above mentioned technique (for details see [39]). The spread material was centrifuged directly onto thin epoxy-resin discs (Epon 812; Serva Feinbiochemica, Heidelberg) that had been coated with a carbon film and exposed to glow-discharge. The diameter of the discs was the same as that of the normal electron microscope grids. The material obtained from five nuclei was used per Epon-disc. After centrifugation, the supernatant liquid was collected with pipettes, the discs were removed from the centrifugation chamber, washed in borate buffer and collected in ice-cold 70% ethanol. To the combined supernatants 2 vol of ice-cold absolute ethanol were added. After storage at −20°C for at least 24 h and centrifugation at 3000 g for 30 min the ethanol was removed, and RNA was extracted from 10 to 20 combined discs as well as from precipitates of the supernatant fraction using 0.5 ml of a pronase-SDS solution for 30 min at 37°C (for details see [39, 40]). After adding 20 μg of *Escherichia coli* rRNA the total RNA was precipitated with 2.5 vol of cold absolute ethanol. The RNA samples were analysed in cylindrical 2.4% acrylamide gels following the procedure of Loening [20]. After the run (11 V/cm, 20°C, ca 2.5 h) the gels were scanned at 260 nm in a Gilford Spectrophotometer 2400 S equipped with a linear gel transport device. The gels were then frozen on solid CO_2, cut into 1.1 mm thick slices, incubated at 40°C overnight in a toluene-based scintillation fluid containing 4% NCS (Nuclear Chicago Solubiliser, Amersham/Searle, Ill., USA) and counted in a scintillation spectrometer (Betaszint BF 5000; Berthold Frieseke, Wildbad, BRD).

RESULTS AND DISCUSSION

The spreading technique introduced by Miller and associates [24–28] allows the direct visualization of spread and positively stained, transcribed and untranscribed chromatin strands in the electron microscope. With this technique fully transcribed transcriptional units are recognized as "matrix units" (for nomenclature see also [24–28, 39, 47, 49]), that is, intercepts on the chromatin axis covered by densely packed, regularly spaced, about 70–150 Å thick la-

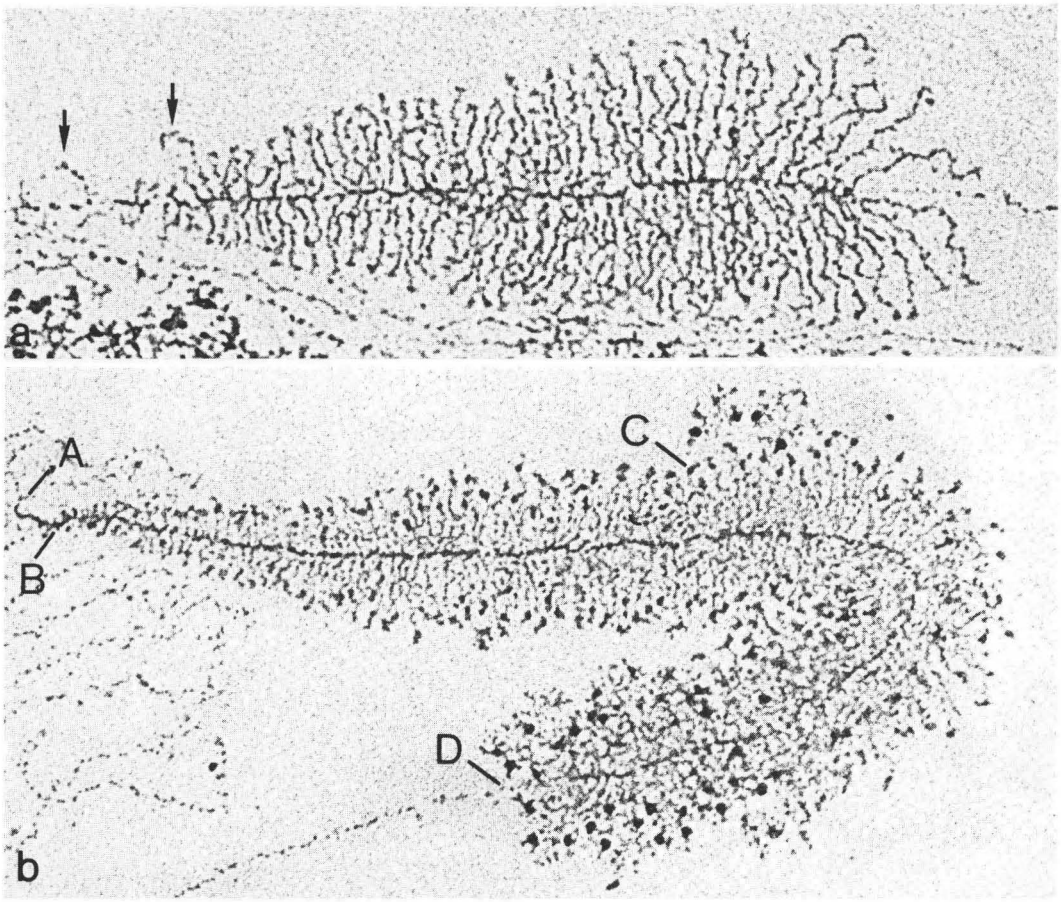


Fig. 1. Representative examples of short (*a*) and long (*b*) nucleolar transcription units ("matrix units") that are maximally active in terms of packing density of associated RNA polymerase molecules and lateral ribonucleoprotein (RNP) fibrils containing the nascent pre-rRNA molecules. The matrix unit presented in fig. (*a*), from a primary nucleus of the green alga, *Acetabularia major* (for details see [2, 47, 49, 53]), is about 1.9 μm long and displays a rather constant length increment of its lateral RNP fibrils over the entire axial intercept included in the matrix unit. Note also the small sizes (15–20 nm) of the terminal granules at the free ("leading") ends of the lateral fibrils. The transcription unit shown in (*b*), from an oocyte (diplotene stage) of the water beetle, *Dytiscus marginalis* (for details see [51, 55]), is 3.7 μm long, and an increment in lateral fibril length is seen only in the first half of the unit. Note that the terminal granules on these lateral fibrils are small in the first half of the unit but are much larger (up to 32 nm) in the second half. In both size classes of matrix units the dense packing of the basal granules that contain the polymerases and connect the lateral fibrils with the axial fibril frequently results in the appearance of a nearly uniform thickening of the axis (12–22 nm width). Within the matrix units, especially in the long ones, one can sometimes clearly distinguish three intramatrical regions (*b*): (1) a start region which is usually very short (limited here by *A* and *B*) and shows a thickened axis but no associated lateral fibrils (see also fig. 4*a*); (2) a proximal ("initial") intercept (between *B* and *C*) which is ca 1.7–2.5 μm long (see also [52]) and is characterized by a gradual increase in lateral fibril length and the presence of small terminal granules, and (3) a distal portion (between *C* and *D*) which is prominent only in the long matrix unit classes and is characterized by the nearly equal length of its lateral fibrils and relatively large terminal granules. Occasionally, near the beginning of a matrix unit, individual lateral fibrils are detected which are longer than expected from their position in the fibril sequence of the length gradient ("disproportionately long fibrils", arrows in fig. 1 (*a*); for details see text). (*a*) ×50 000; (*b*) ×49 000.

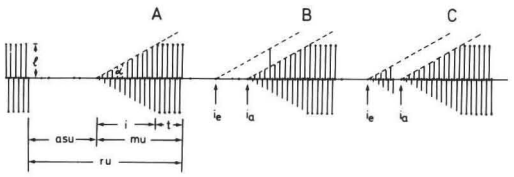
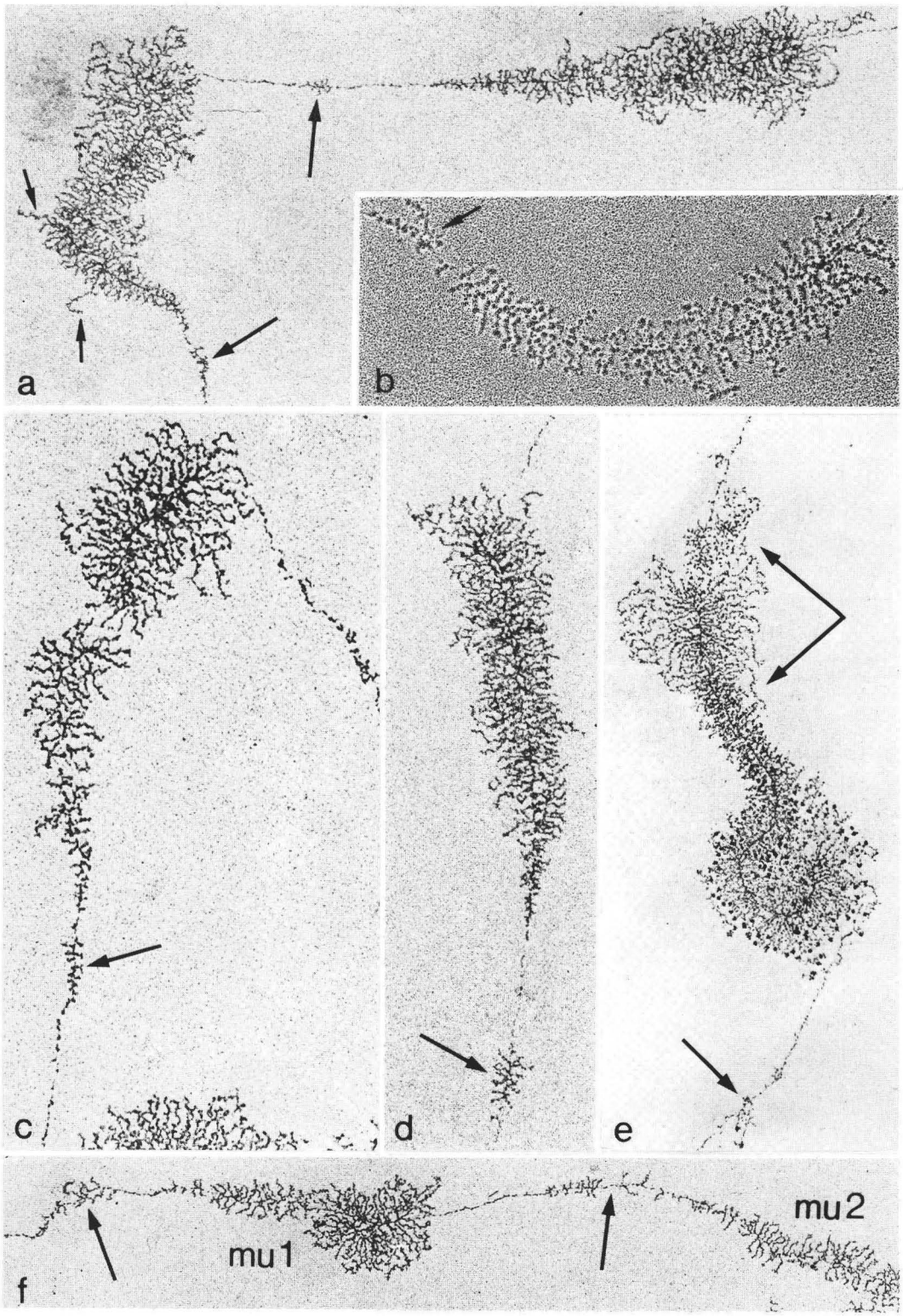


Fig. 2. Schematic drawing which shows a series (*A, B, C*) of nucleolar matrix units (*mu*), the transcribed genes of pre-rRNA, separated from each other by axial intercepts that are usually free from associated lateral fibrils ("apparent spacer units", *asu*). In such gene clusters each matrix unit plus an adjacent spacer unit constitutes one repeating unit (*ru*). The length (l) increment of the lateral fibrils within the first part of a matrix unit is defined by tanα, i.e. the ratio of the length of the specific lateral fibril to the length of the axial intercept measured from the apparent beginning of the matrix unit (i_a) to the basal attachment point of this fibril. Within each matrix unit one may distinguish, at least in some organisms, the initial (proximal) region (*i*) from the terminal (distal) region (*t*) in that dense and large terminal granules are present on the lateral fibrils in the latter (see fig. 1*b*). The existence of both extraordinarily long individual lateral fibrils in some matrix units (*B*) and "prelude regions" (*C*; for definition see [39]) in some apparent spacer units might indicate that transcription of rDNA may sometimes or generally begin in the apparent spacer unit (i_e, extrapolated initiation site; for explanation see text). The dots on the rDNA axis represent distinct, usually irregularly distributed granules which may contain polymerase molecules (cf [23, 25, 27, 40, 41]).

teral fibrils which are arranged in a characteristic length gradient (figs 1, 2). Adjacent matrix units of identical (such as in the nucleolar pre-rRNA gene clusters, see references quoted above) or different (such as in lampbrush chromosomes loops; cf [1, 48]) nature and size are separated by the "apparent spacers" which are usually fibril-free intercepts of lengths that are variable among different organisms and even different nucleolar strands of the same organism but often are highly regular in the specific nucleolar strand (cf [49]). According to the prevailing interpretation of such electron micrographs, the lateral fibrils are ribonucleoproteins (RNP) containing the specific nascent RNA [24–28]. Moreover, it has been assumed that the RNA contained in a given lateral fibril represents the complete transcript of the DNA sequence contained in the axial intercept between the start of the matrix unit, as defined by the smallest recognizable lateral fibril of the gradient, and the basis of this specific fibril. This interpretation has been supported by demonstrations that the mean molecular weights of pre-rRNAs as determined by biochemical methods correspond to the average lengths of the rDNA intercepts contained in the nucleolar matrix units as determined by electron microscopy, assuming the B-conformation for most of the DNA [23–25, 27, 28, 39, 40, 47, 49]. This correlation appears to hold for some organisms and cell systems (see references mentioned) but not for others such as the oocytes of some insects [51, 52, 55] and the amoeba [31] in which the nucleolar transcriptional units are much longer than expected from the mean sizes of the pre-rRNA molecules. In the course of our studies of nucleolar transcription in various cell systems we repeatedly observed distinct "irregularities" in the arrangements of lateral

Fig. 3. Some examples which demonstrate the occurrence of small, extraordinary regions associated with lateral fibrils in spread and positively stained nucleolar chromatin isolated from oocytes of newts (*a, c, d, Triturus alpestris*; *b, f, Triturus helveticus*) and the house cricket (*e, Acheta domesticus*). Transcriptional complexes in apparent spacer intercepts are denoted by long arrows and disproportionately long lateral fibrils within matrix units are denoted by small arrows. Note that transcriptional complexes in the apparent spacer units can be located either immediately preceding the (large) pre-rRNA matrix units ("prelude pieces", (*a, c, f*); see also [39]) or in more central regions of the spacer (e.g. *a, d, e*). Prelude pieces are demonstrated in both adjacent matrix units (mu_1 and mu_2) of (*f*). The pair of arrows in 3(*e*) denotes a situation in which the proximal (initial) region of a matrix unit is covered by a whole group of disproportionately long lateral fibrils (for interpretation see text). Note also the large sizes of the terminal granules at the free ends of the lateral fibrils of the distal part of this matrix unit. The unit shown in (*b*) is from a preparation that has been shadowed with metal as described elsewhere [41]. (*a*) ×22 000; (*b, c*) ×31 000; (*d*) ×25 000; (*e*) ×17 000; (*f*) ×19 000.


a
b
c
d
e
mu1
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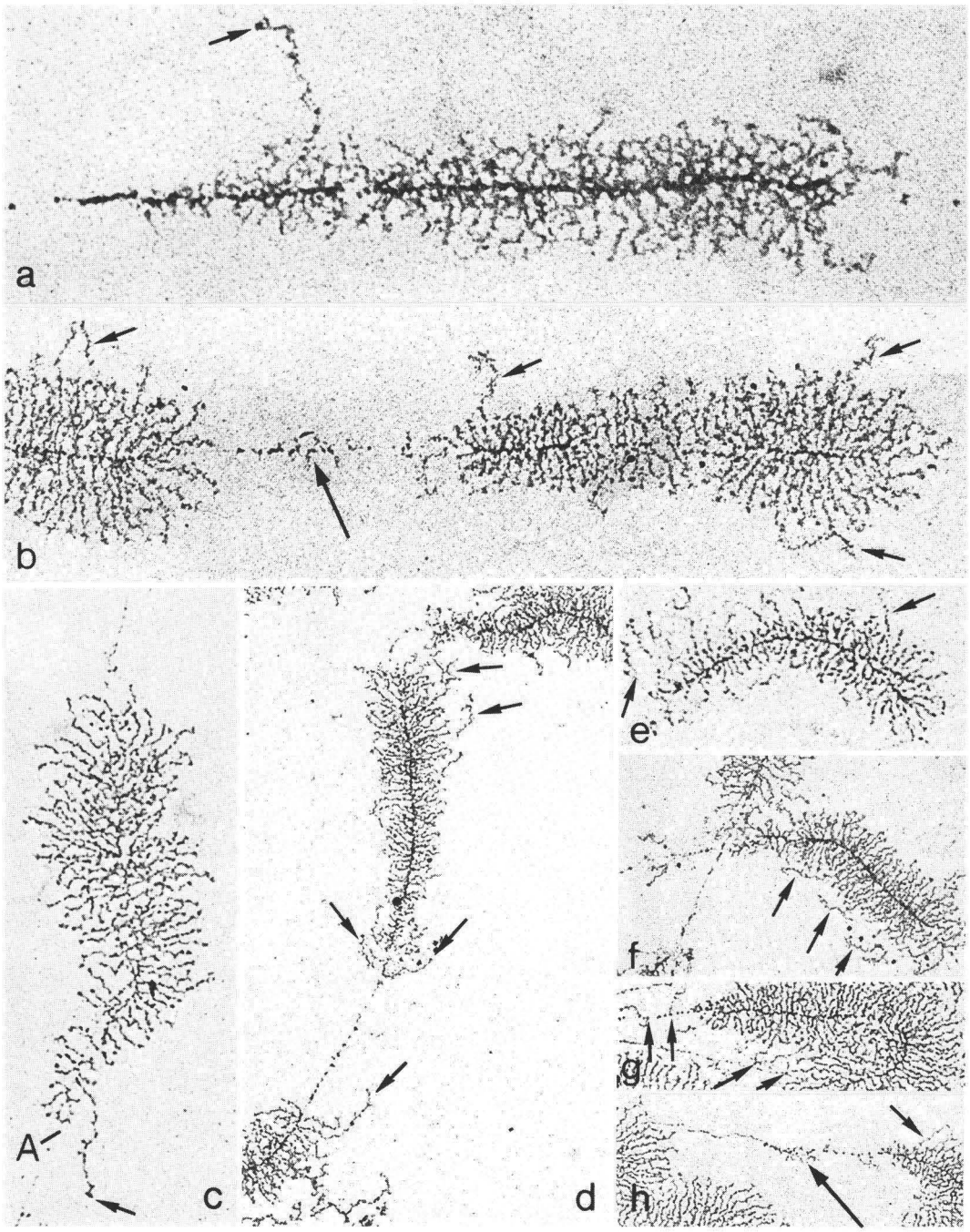

Fig. 4. Various examples of the occurrence of individual, extraordinarily long matrix unit fibrils (small arrows) and of individual lateral fibrils and small transcriptional complexes within apparent spacer regions (long arrows in (*b, h*) in spread and positively stained nucleolar chromatin from the primary nucleus of the plants *Acetabularia mediterranea* (*a–c, e*) and *A. major* (*d, f–h*). Note that disproportionately long lateral fibrils can occur in initial (proximal) as well as terminal (distal) regions of the matrix units. Some situations suggest that distinct fibril fragments may remain in loose association with the matrix unit (e.g. as denoted by the lower arrow in (*b*), by the uppermost two arrows in (*d*), and by the arrows in (*e*) and (*f*)). (*a*) ×56 000; (*b*) ×36 000; (*c*) ×32 000; (*d*) ×21 000; (*e*) ×27 000; (*f*) ×19 000; (*g*) ×29 000; (*h*) ×25 000.

fibrils which would be compatible with an alternative interpretation, namely that the matrix unit fibrils usually do not contain the complete transcription product but rather portions of relatively high stability. Some of these observations and some related considerations are specified in the following.

(*a*) *Disproportionately long lateral fibrils*

The typical morphology of nucleolar matrix units is presented by the examples shown in fig. 1 and in the scheme of fig. 2. Major differences between relatively short (fig. 1*a*) and long (fig. 1*b*; see also fig. 3*e*) matrix units exist in the distal (terminal) part which in the latter size class does not show any significant gradual increase of lateral fibril length but conspicuous, dense and relatively large granules on the free ends of these fibrils (cf [51, 52]). The gradual increment in the length of the lateral fibrils can be expressed by the tan α (the enclosed angle, for definition see fig. 2). This angle is characteristic and rather constant for each specific type of matrix unit, but only in its first (proximal, initial) part (α_i, fig. 2). (The increment in the length of the lateral fibrils seems to gradually decrease in the more distal parts so that, for example, in one and the same matrix of *Acetabularia major* a mean value for tan α of 0.35 was determined in the initial third (tan α_i), 0.32 for the first two thirds and 0.25 for the entire matrix unit. The differential increment (increase of lateral fibril length per axial unit interval) appears to approach zero in the terminal regions of most matrix unit types. This decrease of fibril length increment may also reflect terminal degradation of the nascent RNA molecules but alternative explanations such as changes in the packing ratio and morphology can also be discussed. In making such comparisons it is important that the fibrils are maximally unravelled and spread (for detailed discussions of possible morphological differences due to the specific preparation conditions see also [39]). Consequently, the angle α_i, which in all examples so far studied is much smaller than 45° (in pre-rRNA genes, for example, values determined for tan α_i were about 0.33 in *Acetabularia major*, 0.2 in *Triturus alpestris*, 0.17 in *Dytiscus marginalis*), defines the position of a lateral fibril of a given length in the sequence of fibrils of the initial region of a matrix unit.

In our preparations of spread nucleolar material from various animal and plant nuclei we observed individual lateral fibrils which were significantly longer than the adjacent ones, i.e. they were longer than expected from their position in the sequence of fibrils (e.g. figs 1*a*; 3*a, b, e*; 4*a–h*). Such extraordinarily long lateral fibrils could occur at all sites within matrix units but were particularly conspicuous in the very initial portions. Occasionally, such unusually long fibrils occurred in groups as illustrated in fig. 3*e*. The occurrence of these disproportionately long, odd fibrils could be explained by one of the following alternatives which are, of course, not at all mutually exclusive: (*i*) Certain fragmentation processes which normally take place in the growing fibrils and perhaps reflect special steps of very early processing (see p. 234) are sometimes omitted. (*ii*) Transcription is initiated much before the apparent beginning of the matrix unit, and the products of this very first transcribed region are normally either split off and/or not covered with proteins; occasional omissions would then be indicated by the irregularly long fibrils. (*iii*) The RNA contained in some fibrils is better covered with proteins and thus more readily extended during the preparation. The exceptionally

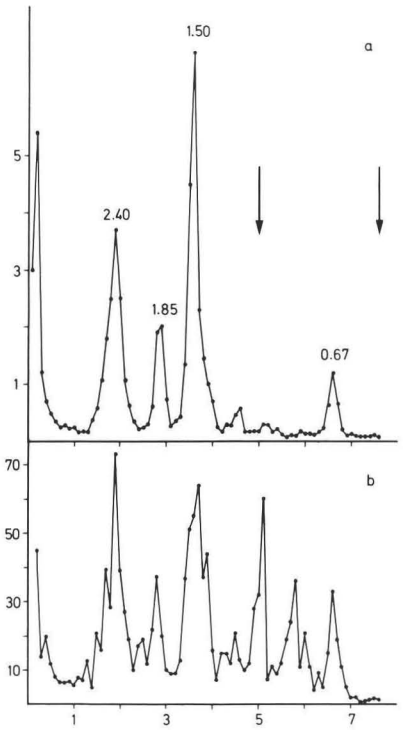

Fig. 5. Abscissa: distance migrated in cm; *ordinate:* (*a*) cpm$\times 10^{-3}$; (*b*) cpm.

Gel electrophoretic analysis of the radioactive RNA contained in spread and sedimented ribonucleoprotein preparations from 65 nuclei of *Triturus alpestris* oocytes (lower panel (*b*)), in comparison with the material that did not sediment under the conditions of the technique by Miller & Beatty [25] used for visualization of nucleolar pre-rRNA genes (upper panel, (*a*)). Only about 4% of the total radioactive RNA could be recovered from the preparation discs (for details see Material and Methods), whereas most of the radioactivity remained in the supernatant. The electrophoretic pattern of the RNA sedimented onto the discs (*b*) is nearly identical to that of the non-sedimentable material (*a*) and that of freshly isolated nuclei and nucleoli (cf [39–41]). The apparent molecular weights are given in million daltons and are slightly lower than those determined in composite polyacrylamide-agarose gels (cf [39]); the two arrows denote the positions of the co-electrophoresed *E. coli* 23S and 16S rRNAs). The pattern shows that the major high molecular weight RNA species are not degraded during the preparation and that RNP structures containing processed RNA are sedimenting as well. In the sedimented material two RNA classes with molecular weights of about 1 and 0.82×10^6 D are somewhat enriched (*b*); their nature, however, is unknown.

long lateral fibrils had the same thickness as the "regular" ones and did not exhibit any other significant morphological difference. This renders another possible explanation rather unlikely, namely (*iv*) that differences in the RNA packing ratio and pattern are responsible for the appearance of such unusual fibrils. Alternatives (*i*) and (*ii*), which seem more likely to us, both require the occurrence of processing of the nascent molecule. Assuming that the RNA : protein packing ratio is about the same in all fibrils, one might conclude that a specific, exceptionally long fibril results from a transcriptional process initiated at a site which is located before the beginning of the specific matrix unit, i.e. within the apparent spacer region (i_e, extrapolated initiation site, fig. 2). The lengths of the longest of these unusual lateral fibrils suggest that up to about 0.7×10^6 D of RNA might be produced in this initial region which is normally degraded immediately after synthesis, resulting also in the loss of the nucleoside triphosphate at the 5′-end of the molecule (for related biochemical data see also [44]). In this context it is perhaps worth stating that the preparation technique used for the morphological studies is not detrimental to the pre-rRNA molecules, as is demonstrated in fig. 5. Therefore, it is rather unlikely that the observed differences in lateral fibril lengths are due to nonspecific RNA degradation during the preparation.

(*b*) *"Prelude complexes" and other arrangements of lateral fibrils in apparent spacer intercepts*

Individual or grouped lateral fibrils which are discontinuous with the pre-rRNA containing matrix units and are located in axial regions corresponding to apparent spacer intercepts (see above) were sometimes noted in the spread preparations of nucleolar chromatin [39, 47, 52]. Especially conspicuous and frequent arrangements are

the "prelude complexes" which appear as small groups of fibrils of gradually increasing lengths which are located immediately before the beginning of a "normal" pre-rRNA matrix unit (e.g. figs 3*a, c, d, f*; 4*b, h*; for further examples see [38, 39]). These prelude complex formations might again indicate that (*i*) the transcription of rDNA has been initiated much before the apparent beginning of the pre-rRNA matrix units (a corresponding, extrapolated initiation site, i_e, is designated in the scheme of fig. 2) and represent occasionally preserved transcriptional products of this region which are usually split off somewhere near the growing point (see also [39]). However, alternative explanations may also be possible such as (*ii*) that these prelude complex fibrils contain early transcriptional products of rDNA units which are normally not visualized due to insufficient or unstable coverage with proteins. Another alternative could be (*iii*) that these arrangements represent transcriptional units of their own, qualitatively different from those containing the pre-rRNA, and merely occur in the close vicinity of rDNA units and are transcribed at the same time. Again it is interesting to note that the largest prelude complexes encountered correspond to transcriptional products of $0.7–0.8\times10^6$ D molecular weight (compare the previous paragraph).

In nucleoli from various cells, groups of lateral fibrils, frequently also with a pronounced length gradient, were noted in more central portions of the apparent spacer intercepts (fig 3*a, e*; see also [39, 52]). In addition, individual isolated lateral fibrils occurred sometimes in spacer regions at various distances from the adjacent matrix units (see references mentioned above). It is unclear whether these structures that strongly suggest transcriptional events in apparent spacer intercepts are in any way related to the matrix units that contain the pre-rRNA [39].

(*c*) *Axial thickening at the beginning of matrix units*

The apparent beginning of a matrix unit is commonly defined by the first and smallest identifiable lateral fibril (see above). The detection of a significant lateral fibril is limited by the specific resolution of the spreading and staining technique used. The routinely obtained point resolution of confidence that was significantly above the noise level of the rather coarse background granularity and the pattern of the granules associated with the axes was about 12 nm (these authors, unpublished data). This, for example, means that the production of as much as about 0.35×10^6 D of RNA (assuming high RNA packing densities of about 0.4 D/Å^3 as in ribosomes; for references see [46]), but in any case of 0.15×10^6 D of RNA (assuming lower packing ratios; for preribosomal particles see, e.g. [18, 29]), would not be detected with this method at all (cf also [2]).

The conclusion that the growing RNA has to reach a certain length before it attains the critical size of detection as an RNP fibril is also compatible with the observation of short (up to approx. 0.2 μm) intercepts of a rather uniformly thickened axis (mean width 14 nm) which in some of our preparations seemed to precede the start of the matrix unit proper (e.g. figs 1*b*; 4*a*; see also [49]). We think that such thickened axial regions result from the attached transcription products which may not have been unravelled or may be still too small to be recognized as lateral fibrils. A general conclusion from the existence of the above given methodical limits of detection is that chromatin strands even with fully active transcriptional units coding for less than

0.15–0.35×10⁶ D molecular weight of RNA (depending on the specific RNA packing ratio; see above) would not be recognized as transcriptionally active at all.

(d) Matrix unit lengths that exceed the length of the rDNA sequences complementary to pre-rRNA

The length of the DNA double strand contained in the repeating unit of transcribed nucleolar chromatin (for definition see fig. 2) seems to be about the same as the length of the repeat unit as determined in isolated rDNA (for example, compare the data in references [5, 9, 16, 27, 30, 36, 39, 59, 60]). In some organisms the mean molecular weight of the transcribed strand of the rDNA matrix units is in close agreement with the mean molecular weight of the stable pre-rRNA (for references see above). However, in most nucleoli a certain (small) proportion of the matrix units is significantly larger. In some of these cases, though not in all (cf [39, 49]), this greater length seems to be the result of the association of lateral fibrils with regions that in other repeating units correspond to spacer intercepts (for examples see, e.g., references [38, 39, 47, 49]). Moreover, there are some organisms and/or cell systems in which all matrix unit lengths clearly exceed the length of an axial DNA intercept complementary to the pre-rRNA isolated (references given above; cf also figs 1*b*, 3*e*). Since we could recently demonstrate that axial intercepts engaged in transcription of rDNA are neither strongly extended nor contracted, compared to their fibril-free, i.e. apparently untranscribed state [10], the occurrence of such long transcriptional units suggests that here the isolated pre-rRNA does not contain the complete primary transcript. The relative amount of sequences of the transcription product that are conserved in the stable pre-rRNA then might vary between individual matrix units, but especially between various organisms [52, 54].

CONCLUSIONS

The observations described (see also [39]) suggest that the morphological beginning of a matrix unit as revealed by the technique of Miller & Beatty [25–27] does not necessarily and not always represent the initiation site of the transcription of pre-rRNA genes. Apart from the fact that the very first part of the transcript, up to RNA chain lengths of about 0.1–0.3×10⁶ D molecular weight, might not at all be detected with this method, the various morphological "irregularities" summarized in this article would all be compatible with the concept of occasional or regular initiation processes at sites which are located 0.2–0.8 μm before the actual starting point of the lateral fibril gradient of the matrix unit as defined by Miller and colleagues [24–28]. This interpretation would, of course, also allow the existence of several initiation points in this start region and some variability in the position of the initiation site(s) relative to both regions, the genes for pre-rRNA and the matrix units (cf also [39]). The reason is not known why some of the elongating RNA chains might escape the early cleavage events that may occur in many (as indicated by the disproportionately long fibrils) or in a few preferential (as in the case of the prelude complexes) positions, while the majority of fibrils normally does not (for drug effects interfering with the frequency of such cleavages see [37, 38]). It is conceivable that differences in the degree of protein coverage and/or in the activity of specific endonucleases (for analogous findings in bacteria see references quoted on

p. 234) are responsible for such differences in stability.

The concept of an immediate processing at the 5′-end of the nascent pre-rRNA molecule would also explain the reported failures to detect the native triphosphate end in mature pre-rRNA molecules (e.g. [44]). It also leads to the conclusion that the DNA sequence complementary to the 5′-end of the pre-rRNA molecule is up to several hundred nucleotides distant from the initiation site. Furthermore, it indicates that several types of rDNA patterns which are characterized by very short apparent spacer intercepts might have extremely little, if any, "non-transcribed spacer" sensu Brown et al. [5] but that the whole nucleolar chromatin consists of fully functioning, closely packed transcriptional units of pre-rRNA (for examples see [2, 47, 49, 53]). In fact, the present study as well as others [37–39] suggests that parts of the "non-transcribed spacer" sensu Brown et al. [5] actually are transcribed.

Recently, evidence has accumulated that the sequences coding for the 18S rRNA are in the proximal part of the pre-rRNA gene (for comprehensive reference lists see [7, 14, 19]; for contrasting conclusions see [43, 56, 57]), similar to the situation described in *E. coli* (for references see [13, 33, 50]), and that this 5′-region of the pre-rRNA contains an extended and in evolutionary terms rather variable portion which is lost during processing (the "external transcribed spacer" as defined by other authors; e.g., [43, 45, 57]). Therefore, the cleavage processes which seem to occur in the 5′-part of the nascent transcripts from the transcriptional unit for pre-rRNA would take place in a non-conserved region.

Although this cleavage of initial regions of the nascent pre-rRNA gene transcript resembles the "primary processing" described in *E. coli* in that it "occurs during or very soon after transcription" and may also have "all its requisite signal in the structure of the RNA itself" [33], it is basically different in that in all eukaryotes studied the linkage between the two functional rRNA units, 28S and 18S, remains intact during these cleavages.

The possible functions of such early cleavages in the nascent growing RNA are obscure. Their primary role, however, could be a regulatory one and would then provide a theoretically interesting example of a concerted interplay between post-transcriptional mechanisms (for references see [12, 15, 42]) such as processing and coverage with specific proteins and the ongoing transcriptional process.

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