data [1] of low binding in growing and high binding in stationary cells, since the former contain a high percentage of cells in the S, G2 and M phases, while the latter are mostly in the G1 phase. In addition, the high level of hormone receptors in the G1 phase was in agreement with the work of Emanuel & Gelehrter [11] who reported that tyrosine transaminase could only be induced by insulin in synchronized hepatoma cells during the G1 phase of the cell cycle.

The results obtained with the second procedure of synchronization, however, did not confirm those obtained by the first method (fig. 3*b*). In cells synchronized by suspension in methyl cellulose the initial binding (3%) was somewhat lower than in cells arrested in plasma. Further, no clear trend in insulin binding was observed as the cells progressed through the cell cycle. The quality of the cycles obtained with these two methods was not similar, since differences existed in the total length and the position of the peak of the S phase.

In conclusion, serum factors and cell density do not have a direct regulatory role on insulin binding in cultured fibroblasts. The number of insulin receptors depends mainly on the growth status of the cells, and is perhaps influenced by the cell cycle [12–18].

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Visualization of rDNA spacer transcription in *Xenopus* oocytes treated with fluorouridine

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Summary. Under the influence of 5-fluoro-uridine, the ultrastructure of the rDNA transcription units in *Xenopus* oocytes is altered. Whereas part of the matrix units maintains a normal aspect or shows various degrees of inhibition, in a strong proportion of the transcription units the alternating pattern of matrix units and fibril-free spacer regions is no longer rec-

ognized. Transcriptional complexes are found along the entire DNP axis, including the regions of the spacers. These observations support biochemical data on transcription in rDNA spacer regions.

Spread preparations of nucleolar cores from amphibian oocytes show the tandemly arranged ribosomal genes consisting of matrix units alternating with fibril-free regions which are widely interpreted as non-transcribed spacer intercepts [1–3]. A detailed study [3] of the rDNA transcription units in *Xenopus* has shown that the majority of the matrix units has an axial length (2.2–2.5 μm) corresponding approximately to the size of the first stable pre-rRNA (40 S or 2.6 million molecular weight on SDS-polyacrylamide gels [3]). However, similar to the situation described for *Triturus* [2], there are matrix units observed in *Xenopus* that are longer than expected from the size of the predominant pre-rRNA (up to 3.2 μm). Moreover, specific arrays of transcriptional complexes have been found in regions corresponding to spacer intercepts. These arrays consist of single lateral fibrils or groups of lateral fibrils, including the so-called "preclude complexes" [2, 3]. Such complexes, indicative of transcriptional activity in the "non-transcribed spacer" of rDNA have meanwhile been observed in a number of animal and plant species (for reviews see [4, 5]).

In a recent biochemical study [6] we have described the effect of the nucleotide analogue 5-fluoro-uridine on the transcription of rDNA. This drug is incorporated into RNA [7] and completely inhibits the maturation cleavages of the 40 S pre-rRNA. At the same time large RNA molecules of heterogeneous size are synthesized which contain sequences of the 40 S pre-rRNA as well as sequences complementary to the "non-transcribed spacer" [6]. In the present communication we describe morphological changes induced by treatment with fluorouridine in the nucleoli of *Xenopus* oocytes.

Material and Methods

Pieces of ovaries were excised from adult females of *Xenopus laevis* anaesthetized in 0.1% MS222 (Sandoz, Basel). Four different females were used in this study. Portions of the ovaries were kept in a modified Barth's solution [8] at 18°C. The nucleoside analogue 5-fluorouridine (Calbiochem, San Diego) was added to the culture medium at concentrations of 10 or 20 mM. After incubation periods ranging from 0.5 to 3.5 h, nuclei were manually isolated from lampbrush stage oocytes and spread according to a modified Miller technique ([3, 4, 9]; see also [1]). In order to avoid clumping inherent to the *Xenopus* material, 0.02% Sarkosyl NL30 (Ciba-Geigy, Basel) was added to the pH 9 borate water ([3]; cf [10, 11]). After positive staining the preparations were metal-shadowed with platinum–palladium (80 : 20) at an angle of 7°. Micrographs were taken with a Zeiss electron microscope EM10 or an AEI EM6G at 60 kV.

Results

In order to test the influence of the incubation conditions, control preparations were made using oocytes from the same ovaries and incubated under the same conditions as in the experimental series, but in the absence of fluorouridine. The nucleolar transcription complexes of these controls retained their normal appearance, i.e. matrix units of normal length alternating with mostly fibril-free spacer intercepts. Only occasionally spacer regions containing single transcriptional complexes or prelude complexes (cf [3]) were seen.

Transcriptional activity was retained over prolonged incubation periods (up to 8 h). Inactive nucleolar chromatin was usually recognized by a reduced density ("gaps" or homogeneously diluted fibril arrays) or by the complete disappearance of lateral fibrils, including the supporting polymerase granules [11–13].

In the oocytes treated with fluoro-uridine, we made the following observations. A variable proportion of the nuclear transcription units still showed normal mor-

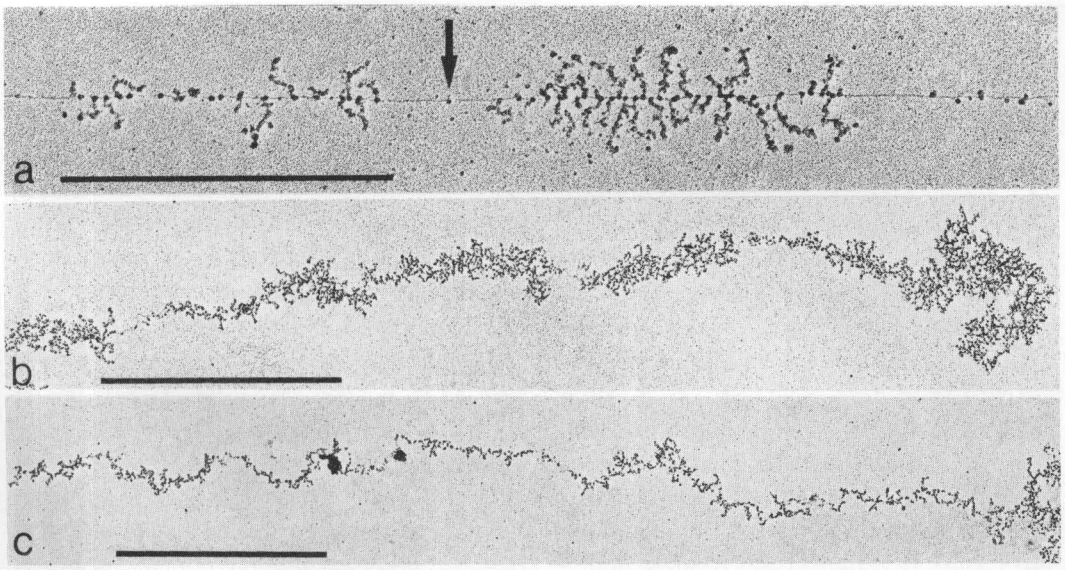


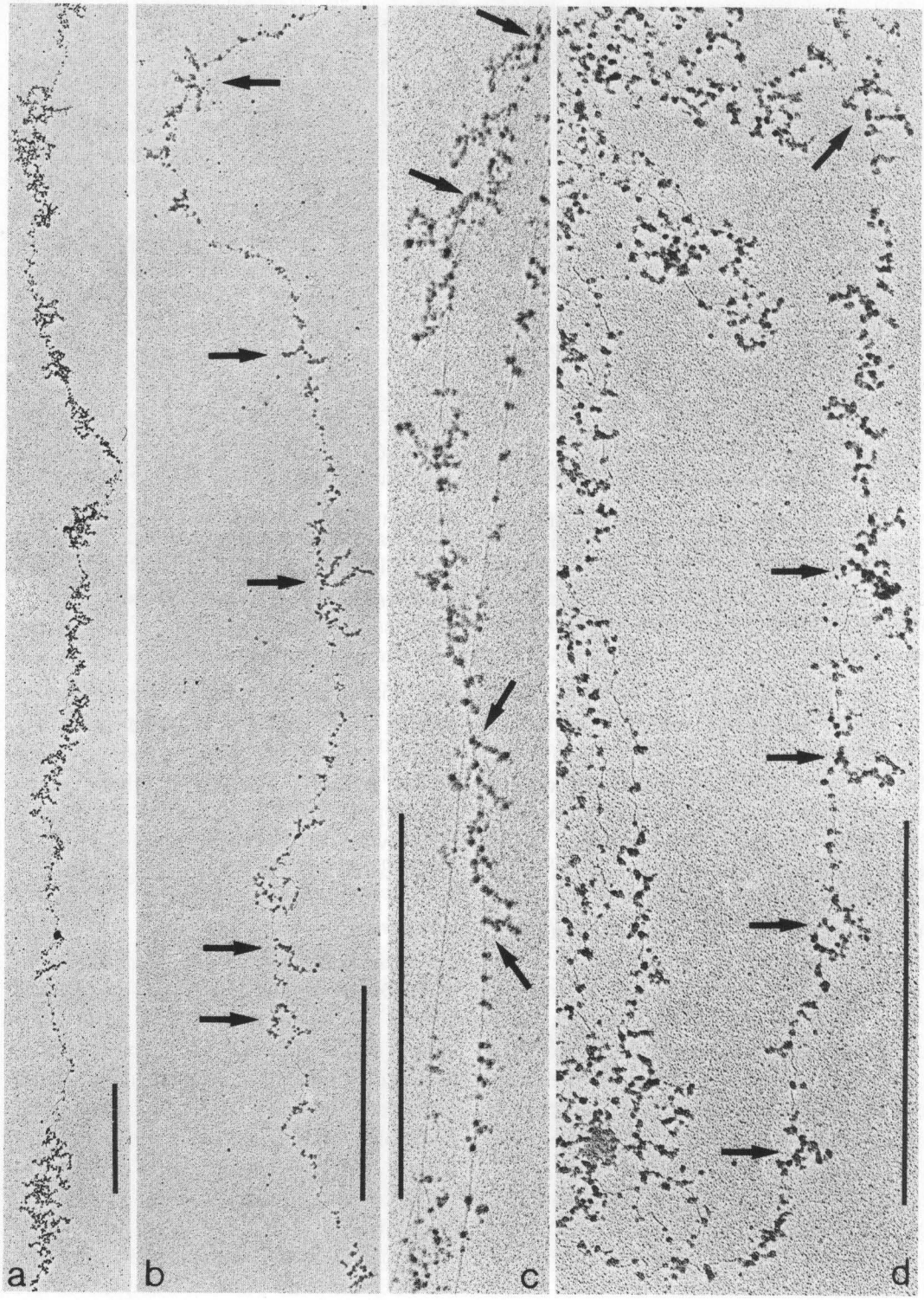
Fig. 1. Various aspects of the morphology of spread nucleolar chromatin after treatment of fully active (vitellogenic) oocytes with fluoro-uridine, (*a*) (20 mM for 3.5 h); (*b, c*) (10 mM for 1 h). Many strands show matrix units with reduced fibril density, indicative of progressive inhibition (*a*) arrow denotes a "gap" as they are typical for inhibited transcription; cf [12, 13]. Other strands show an almost continuous coverage by normally sized (*b*) or anomally short (*c*) lateral fibril material which no longer allows the distinction of spacer regions. Bars, (*a*) 1 μm; (*b, c*) 2 μm.

phology (not shown). In addition, morphological alterations indicative of inhibition of transcription were prominent (e.g. figs 1*a* and 2*b–d*; cf references quoted above). Another relatively large proportion (20–30%) of the transcriptional units showed a different type of alterations so far not observed in nucleoli of untreated *Xenopus* cells [3]. The main characteristic of this alteration is an almost complete disappearance of "naked" spacer intercepts. Association of polymerase granules, with or without attached lateral fibrils, to axial regions corresponding to spacer intercepts can be observed (figs 1, 2). This phenomenon was observed in both, inhibited and fully covered transcriptional units. Frequently we found very long transcriptional units with a reduced density of polymerase granules and attached lateral fibrils that extended over distances considerably longer than normal matrix units or even entire repeating units (fig. 2). Some of these unusually long transcribed regions still exhibited polarity, whereas in other regions polymerases carrying short and long lateral fibrils were distributed more or less randomly along the DNP axis (cf fig. 2*b, d*).

Discussion

Our observations show that, after treatment of *Xenopus* oocytes with fluoro-uridine, the normal arrangement of matrix units alternating with "naked", i.e. seemingly non-transcribed spacers is abolished in a large proportion of the rDNA repeating units. Nucleolar strands are observed which are almost entirely covered with polymerase granules and transcriptional complexes, respectively.

According to the classical view of rDNA transcription, the "spacer" sequences are


a
b
c
d

not transcribed at all and the 40 S pre-rRNA is assumed to represent the full primary rDNA transcript (for reviews see [5, 7, 15]). At least with regard to the initiation site this view has been corroborated by recent experiments [16] in which capping enzymes were used to selectively label 5′di- or triphosphates. Considering the specific capping efficiency, these authors have estimated that at least 85% of the initiation events occur at, or close to, the beginning of the 40 S pre-rRNA sequences. The majority of the 40 S molecules starts with a 5′-terminal (p)ppAAG, but some heterogeneity exists [16].

However, even in untreated *Xenopus* oocytes, "prelude complexes" and isolated transcriptional complexes have been observed in regions corresponding to spacer intercepts [2–4]. In recent biochemical experiments, small RNA molecules (5–23 S) have been detected in untreated cultured *Xenopus* cells complementary to "non-transcribed" spacer sequences [17, 18]. After treatment with fluoro-uridine such small RNAs form a discrete peak at about 23 S in the labelling pattern of total RNA [6], and this material preferentially hybridizes to spacer sequences [17, 18]. In addition, under such conditions large molecules are formed which contain sequences of the 40 S pre-rRNA as well as sequences complementary to the external "non-transcribed" spacer [6, 17, 18]. These findings seem to correspond to the present observations on spacer-associated polymerase granules and transcriptional complexes after fluoro-uridine treatment.

Since fluoro-uridine is known to inhibit processing cleavages in rRNA [6, 7, 19], an attractive possibility to explain the observed changes in rDNA transcription would be that the nucleotide analogue inhibits early cleavages that normally take place on growing RNA chains transcribed from sequences of the external non-transcribed spacer. Early cleavages on growing rRNA chains have been demonstrated in *E. coli,* where the primary transcript normally does not exist as an intact molecule (for review see [5, 20]). Similarly, in *Amphibia,* RNA molecules transcribed from the spacer sequences could be highly unstable, and the effect of fluoro-uridine be due to a protection of these RNA chains from immediate cleavage. It is, however, not clear in what proportion of repeating units spacer transcription occurs. It can also not be decided at the moment whether spacer transcription is due to independent initiation events, or whether it is part of the transcriptional unit coding for the 40 S pre-rRNA.

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Fig. 2. Details of the morphology of nucleolar pre-rRNA genes observed after moderate (*a*) (10 mM for 1 h); and extensive (*b–d*) (20 mM for 3.5 h) treatment with fluoro-uridine. Whereas early stages often show dense and continuous coverage with relatively short lateral fibrils (*a*), prolonged drug action results in the appearance of reduced density (*b–d*) of RNA polymerase granules and associated lateral fibrils (some groups of transcriptional complexes with still relatively long RNP fibrils are indicated by arrows). Note that matrix unit-spacer patterns are only poorly recognized under such conditions, suggesting that polymerase molecules and attached RNP fibrils now also occur in regions corresponding to normal "spacer intercepts". Bars, 1 μm.

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