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Transcription patterns of amplified *Dytiscus* genes coding for ribosomal RNA after injection into *Xenopus* oocyte nuclei

(electron microscopy/chromatin morphology/nucleoli/RNA synthesis/living oocytes)

M. F. TRENDELENBURG*, H. ZENTGRAF†, W. W. FRANKE†, AND J. B. GURDON*

* MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England; and † Institute of Experimental Pathology and Institute of Virology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany

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ABSTRACT **Oocytes of the frog *Xenopus* were injected with purified circular DNA containing amplified rRNA genes of the water beetle *Dytiscus*. Nuclear contents of injected oocytes were spread and examined by electron microscopy. Most of the *Dytiscus* DNA seen in injected nuclei contained regions densely packed with polymerases and nascent transcripts. Apparently normal, as well as abnormal, patterns of transcription were observed. By this type of experiment, it may become possible to recognize the transcribed regions and immediate transcripts of cloned DNA molecules whose activity cannot be seen by electron microscopy of normal nuclei.**

Purified DNA is extensively transcribed after injection into oocyte nuclei (1). Transcripts from injected 5S DNA are believed to be predominantly correct (ref. 2 and unpublished data). However, it is generally hard to distinguish accurate initial transcription from inaccurate transcription followed by a rapid processing of incorrect primary transcripts. The initial transcripts of genes as well as the transcriptionally active regions of DNA can be recognized most reliably by electron microscopy of nuclear spread preparations, a technique that has been applied with special success to oocyte nuclei (3–6). Therefore, the possibility exists of recognizing the initial transcripts and other transcriptional properties of any kind of DNA that has been purified by electron microscopic examination of oocyte nuclei injected with DNA. Injected oocytes should be technically favorable for this procedure because their nuclei may contain up to 100 times more DNA of the injected type than their total content of amplified DNA that codes for rRNA (rDNA) (7).

In the experiments described here, we have injected amplified rDNA of *Dytiscus marginalis* oocytes into oocytes of *Xenopus laevis*. This heterologous combination was chosen because the amplified rDNAs of *Dytiscus* and *Xenopus* differ in many respects. On the basis of these differences, it should be possible to say whether the transcription complexes that we see have originated from injected or endogenous DNA.

MATERIALS AND METHODS

Preparation of DNA. DNA was extracted from the ovaries of *D. marginalis* females by a procedure (8, 9) that involves lysis with sodium dodecyl sulfate and prolonged incubation in proteinase K and Pronase. This was followed by banding in CsCl isopycnic gradients. Material with a density of 1.710–1.724 g/cm^3 was collected and precipitated with ethanol, thereby excluding most of the chromosomal DNA (density 1.696 g/cm^3). In addition, some samples were analyzed in CsCl/ethidium bromide gradients (0.65 mg of ethidium bromide per ml of DNA solution; final refractive index = 1.3380). Electron microscopy of DNA spread preparations showed that 5–20% of the DNA used for injection was in circular molecules of the kind expected in amplified rDNA (8, 9).

Injection of DNA into *Xenopus* Oocyte Nuclei. The injection procedure by which DNA is aimed for the oocyte nucleus has been described (10); DNA is deposited in the nucleus in about half of all injections (1, 2). To facilitate the identification of successfully injected nuclei, we supplemented the sample of *Dytiscus* DNA with bovine serum albumin that had been labeled *in vitro* with [^{125}I]iodine (11). After incubation for 1–2 days, the nuclei of injected oocytes were dissected out and radioactivity was measured individually in a γ counter. Only nuclei that contained ^{125}I radioactivity above background were processed further.

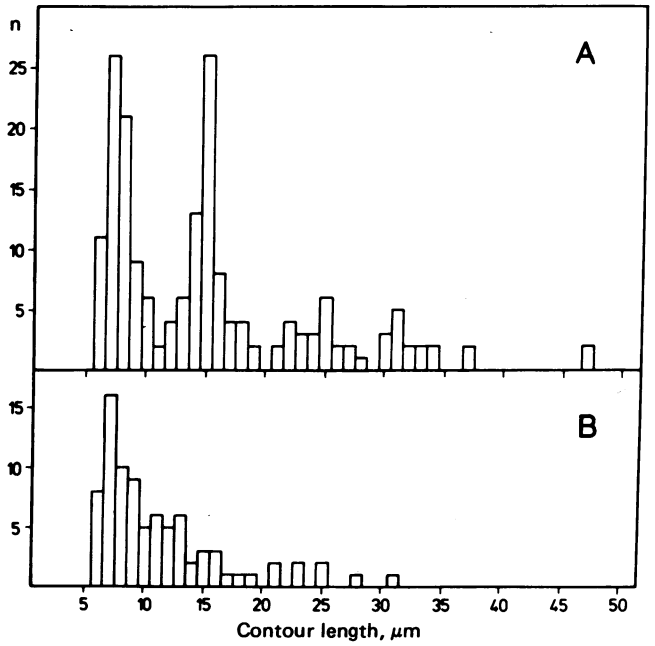

FIG. 1. Contour lengths of circular DNA molecules. (*A*) Purified rDNA molecules of *Dytiscus*, as used for injection. (*B*) DNA in transcriptionally active chromatin circles seen in spread preparations of five *Xenopus* oocyte nuclei 24 hr after injection of *Dytiscus* rDNA. Only circles longer than 5.7 μm have been included. The procedure used selects against the inclusion of large circles, which are susceptible to fragmentation and whose contours are difficult to trace.

Abbreviations: rDNA, DNA that is enriched in, and may exclusively consist of, the genes for the common precursors to 18S and 28S rRNA sequences. Pre-rRNA, the largest size class of stable molecules that contain 28S and 18S rRNA sequences. Matrix unit, a region of DNA containing a pre-rRNA gene that is covered with a series of growing lateral fibrils. Spacer, a region of DNA that is not covered with lateral fibrils and is located between two matrix units.

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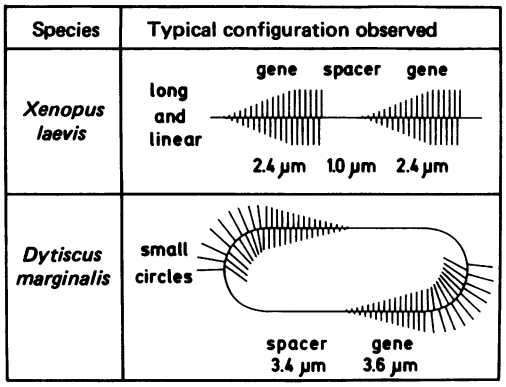

FIG. 2. Patterns of transcriptional units (matrix units) of amplified pre-rRNA genes in large vitellogenic oocytes. The diagrams are based on electron microscopy of nuclear spread preparations (long and linear rDNA molecules seen in such preparations might well represent fragmented large circles of rDNA). For details of frequency of different configurations and for heterogeneity of spacer lengths see refs. 5, 9, and 16 for *Dytiscus*, and refs. 3, 4, and 6 for *Xenopus*. Transcribed small rDNA circles are extremely rare in vitellogenic oocytes of *X. laevis* (unpublished observation and O. L. Miller, personal communication). For a ring containing 10 pre-rRNA genes, see ref. 3.

Spread Preparations of Chromatin. Nuclei of injected *Xenopus* oocytes were manually isolated in "5:1" medium (4, 5) and transferred into small drops of 0.1–0.5 mM borate buffer (pH 9) containing either 0.01–0.05% of the detergent Joy (12) or 0.02–0.1% Sarkosyl NL-30 (6, 13, 14). The dispersed content of one oocyte nucleus (for procedure, see refs. 4–6) was then centrifuged onto a carbon-coated electron microscope grid (MSE high speed 18 centrifuge, 6000 × *g*, 30 min, 10°), positively stained, and metal shadowed (6). Electron micrographs were taken with a Philips 300 or a Zeiss EM-10 at 60 kV. Length measurements were made exactly as described (9). Previous experience has shown that the length of rDNA seen as a transcription unit in a spread of nucleolar chromatin is similar to the length of the same kind of rDNA seen in a spread with cytochrome *c* (6, 9).

Table 1. Patterns of transcription of *Dytiscus* rDNA in *Xenopus* oocytes

Oocyte	No. of molecules Examined	Tran-scribed	Dense Polar	Non-polar	Irregular	None
A	30	30	26	26	16	0
B	7	7	5	9	3	2
C	6	5	3	5	9	1
D	10	6	2	5	4	4
E	16	8	5	9	8	3

Five oocytes in which the endogenous pre-RNA genes showed normal patterns of transcription were examined, and all DNA circles 6–17 μm long were micrographed. A molecule was classified as transcribed if more than one-third of its length was covered with transcripts (compare Figs. 4, 5, and 6*A* with 6*B*). Within each molecule, regions approximately the length of a pre-RNA gene were classified untranscribed (e.g., Fig. 6*B*), irregularly transcribed (parts of Fig. 4), or densely transcribed (Fig. 4). Densely transcribed regions were further classified as polar (a gradient of transcript lengths, e.g., Fig. 5, top) or nonpolar (dense long transcripts, not in an obvious length gradient, e.g., Fig. 6*A*).

RESULTS

Properties of Injected DNA. The sample of *Dytiscus* DNA used for injection in these experiments was enriched for its content of rDNA. At least 5% of the molecules in the prepara-

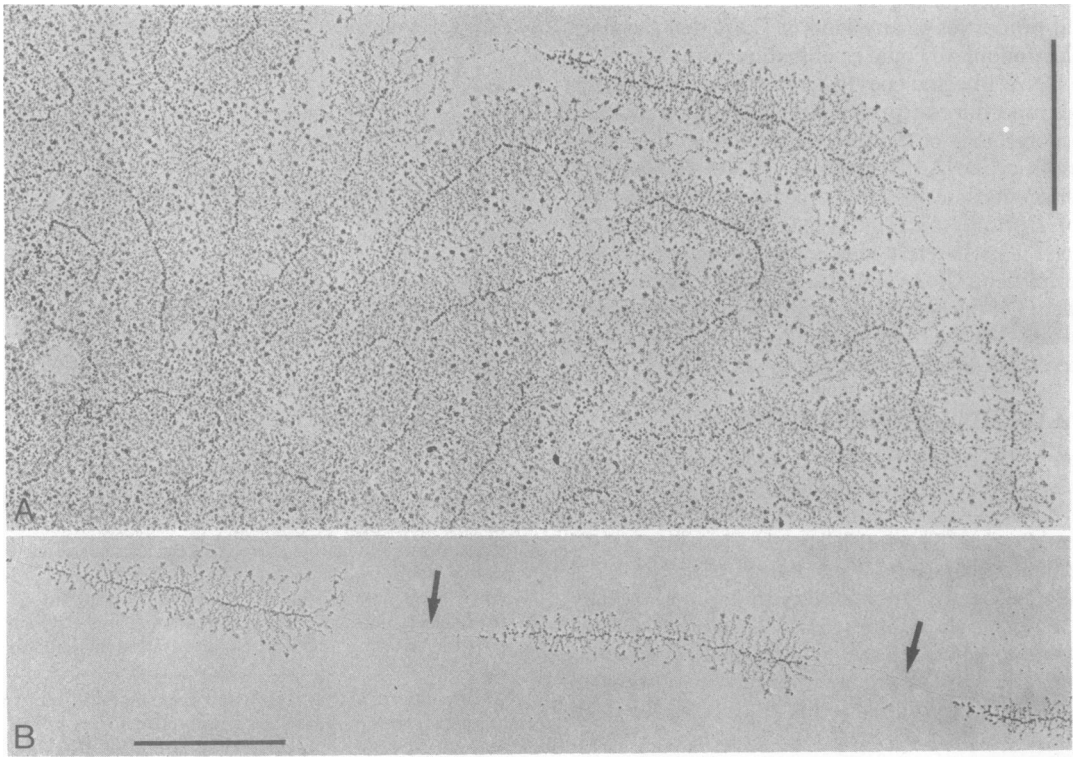

FIG. 3. Electron microscopy of spread nucleolar chromatin from *Xenopus* oocyte nuclei injected with *Dytiscus* rDNA and incubated for 24 hr. The endogenous nucleolar chromatin is fully transcribed (*A*) and shows an arrangement of matrix units and apparent spacers (arrows) that is typical for *X. laevis* (*B*). Length measurements of matrix and spacer regions do not differ from values of untreated oocytes. Scales in these and all subsequent figures represent 1 μm.

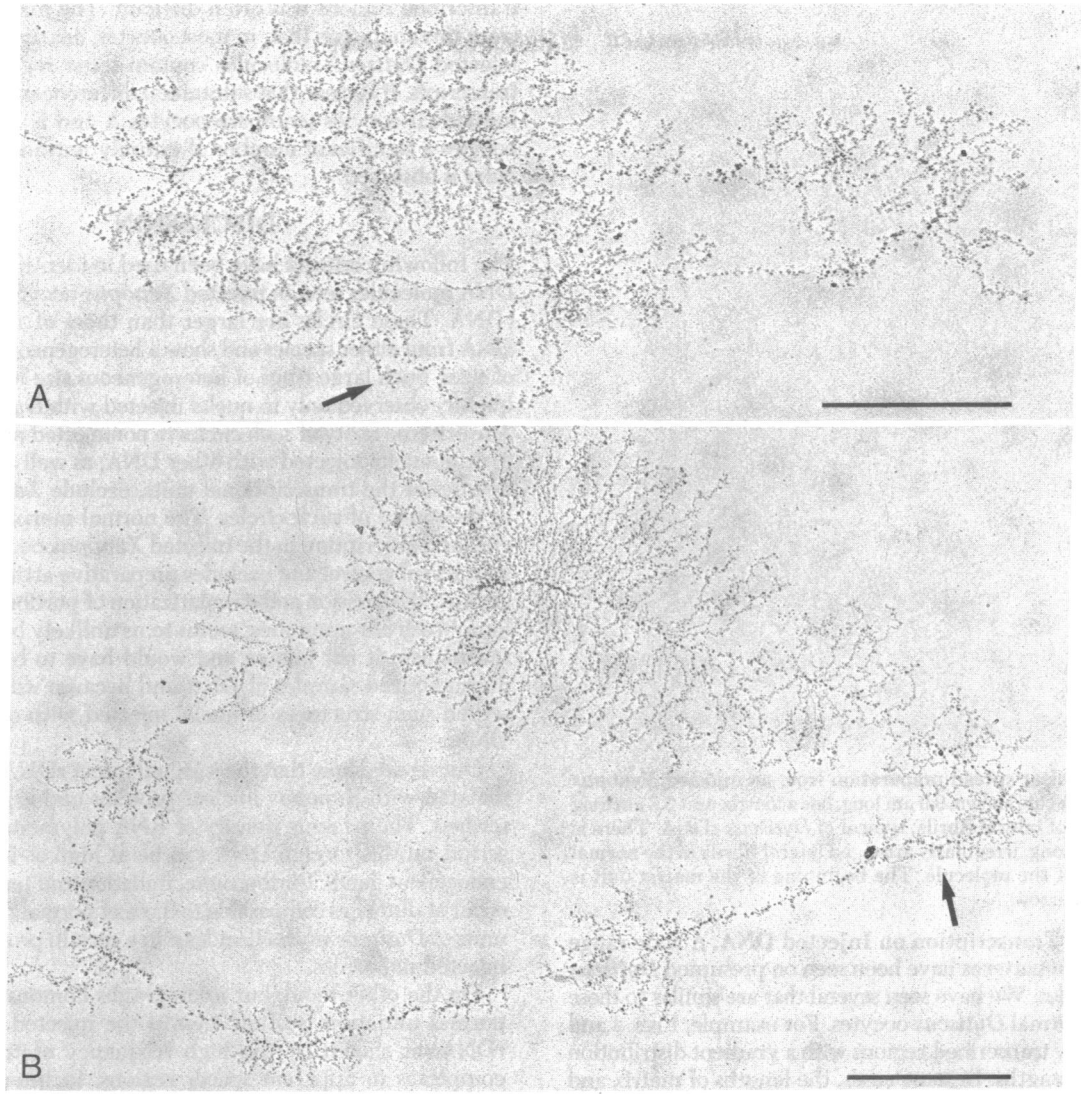

FIG. 4. Spread preparations of *Xenopus* oocyte nuclei injected with *Dytiscus* rDNA. (*A* and *B*) Circular molecules 9.9 and 12.9 μm long, respectively. Each molecule has transcription complexes arranged in a gradient of lateral fibrils (beginnings indicated with arrows). In addition, each molecule has irregular clusters of lateral fibrils in positions corresponding to normal spacer regions.

tion were circular. Of the circular molecules, a few were below 5.6 μm long and probably consisted of mitochondrial DNA (average length 5.26 μm, ref. 9) and very small circles (0.1–2.2 μm) found in the nuclei of various cells, including amphibian oocytes (15). The sample of DNA used for injection contained circles of various lengths, but most were 7.53 μm long or multiples thereof (Fig. 1*A*), a value similar to that reported previously (9). As has been shown (9, 16), such circles contain one to three or more pre-rRNA genes, each separated by long regions of spacer. Thus, the amplified rDNA of *Dytiscus* differs substantially from that of *Xenopus* oocytes in the lengths of the matrix unit, spacer, and repeat unit (Fig. 2).

Nucleolar Genes of *Xenopus* Oocytes Are Unaffected by Injection. Adverse conditions rapidly result in the disappearance of lateral fibrils from transcription complexes. This is seen as "gaps" within matrix units and as an increasing number of pre-rRNA gene regions without any associated lateral fibrils (14, 17, 18). Most *Xenopus* oocytes that had been injected with DNA, and from which nuclear spreads were successfully prepared, had entirely normal nucleolar transcription units, as shown in Fig. 3 and as described (6). We conclude that normal rDNA transcription continues for at least 24 hr in injected oocytes, and therefore that the presence of large amounts of *Dytiscus* rDNA, the injection procedure, and the culture conditions used are not harmful.

Identification of *Dytiscus* rDNA in *Xenopus* Oocytes. Fig. 1*B* shows the contour lengths of circular molecules found in injected oocytes. Only molecules that were well separated from any adjacent material were scored. A substantial number of DNA circles of the length distribution expected for *Dytiscus* rDNA were seen after a detailed examination of 10 grids, each grid carrying the nuclear contents of one successfully injected oocyte. An equally careful survey of 20 grids with nuclei from uninjected or saline-injected oocytes revealed no DNA circles between 6 and 25 μm long. Small circles of amplified rDNA are seen only rarely in large yolky oocytes of *Xenopus* (3, 4, 6).

As described below, the arrays of transcriptional complexes on small DNA circles in injected oocytes are totally dissimilar to those of *Xenopus* rDNA. We emphasize the point that free DNA circles were examined in only those oocytes whose endogenous rDNA and chromosomal DNA showed normal transcriptional patterns. Therefore, the frequency, length, and transcriptional pattern of free DNA molecules in apparently normal oocytes indicate to us that most, if not all, of these molecules are injected *Dytiscus* rDNA.

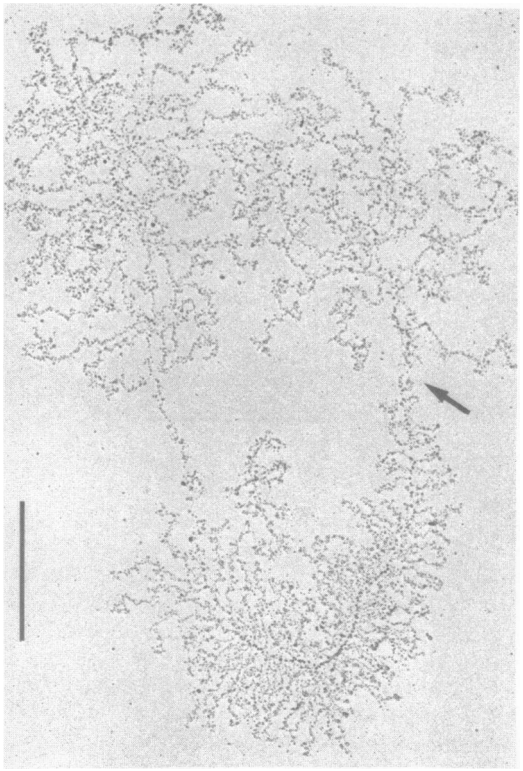

FIG. 5. Nuclear spread preparation from an injected *Xenopus* oocyte. The molecule shown, 9.9 μm long, has a matrix unit 3.3 μm long and a gradient of lateral fibrils, typical of *Dytiscus* rDNA. There is also a group of long, irregularly arranged lateral fibrils in the normal spacer region of the molecule. The beginning of the matrix unit is denoted by an arrow.

Patterns of Transcription on Injected DNA. A wide range of transcription patterns have been seen on presumed *Dytiscus* rDNA molecules. We have seen several that are similar to those observed in normal *Dytiscus* oocytes. For example, Figs. 4 and 5 show densely transcribed regions with a gradient distribution of transcript lengths. In some cases, the lengths of matrix and spacer regions and of lateral fibrils, are typical for *Dytiscus* rDNA (Figs. 4 and 5). In other cases, various degrees of abnormality have been seen. Matrix units slightly larger than normal (4.3 μm in Fig. 4*B* compared to normal 3.5 μm) may have excessively long lateral fibrils (1.2 μm in Fig. 4*B* compared to normal 0.4 μm; refs. 5, 9, and 16). More obviously abnormal matrix units have been seen as much as 8 μm long. In these cases, irregular groups of lateral fibrils were attached to other parts of the molecule including spacer regions; the length and density of lateral fibrils were variable (Figs. 5 and 6). Several examples have been seen of circular molecules almost entirely covered with very long lateral fibrils up to 3.5 μm long but not arranged in a gradient (Fig. 6*A*). Finally, a small portion of DNA circles had no identifiable lateral fibrils and appeared as chains densely covered with evenly sized granules (Fig. 6*B*).

In an attempt to determine the abundance of these various patterns of transcription, we have classified DNA circles as transcribed or not, according to whether more or less than one-third of the length of each molecule was associated with lateral fibrils. The five oocytes listed in Table 1 are representative of those we have examined. Most molecules contain densely transcribed regions. Molecules classed as transcribed were then scored for their content of different patterns of transcribed region (see legend to Table 1). Not all transcribed regions classed as polar were normal in length of matrix and lateral fibril, and the distinction between polar and nonpolar transcribed regions was often difficult. The main conclusion from this analysis is that, in most oocytes, nearly all presumed injected *Dytiscus* molecules contain some regions of dense transcripts. It is clear that substantial differences exist between individual oocytes (compare oocytes A and E), and in some oocytes a significant number of entirely untranscribed molecules is observed.

DISCUSSION

The following criteria have been used to identify the circular DNA molecules seen in injected *Xenopus* oocytes as *Dytiscus* rDNA. These circles are larger than those of mitochondrial DNA from either species and show a heterogeneous distribution of sizes. Such large rings of heterogeneous size have been frequently observed only in nuclei injected with *Dytiscus* rDNA. The extreme rarity of such circles in noninjected nuclei (see Fig. 2) or in nuclei injected with other DNA, as well as the characteristics of the transcriptional units, exclude *Xenopus* rDNA as the source of such circles. The normal morphology of nucleolar transcription in the injected *Xenopus* oocytes serves as an internal control and excludes preparative artifacts. Another artifact, the excision and circularization of portions of *Xenopus* lampbrush chromosomes, seems to us unlikely because such a mechanism is not known and would have to be induced by deproteinized samples of DNA and because we have not observed such structures in nuclei injected with other kinds of DNA.

Our results show that the injected insect rDNA becomes associated with *Xenopus* nuclear proteins and is actively transcribed. The packing density of RNA polymerases and transcripts on this foreign DNA can be as high as in transcribed endogenous genes. Furthermore, initiation and termination can occur at distances comparable to those of normal transcriptional units of *Dytiscus* nucleoli, at least in a certain proportion of the injected molecules.

On the other hand, our micrographs demonstrate that abnormal initiation is often seen in the injected heterologous rDNA, as shown by the high frequency of transcriptional complexes in apparent spacer regions. In interpreting such "odd" transcriptional events, however, transcription in apparent spacers has been observed, though much less often, in endogenous nucleoli of various cells, including *Xenopus* oocytes (4, 6, 19, 20). Another frequent abnormality observed in the transcription of injected DNA is the high frequency of unusually long lateral fibrils; these are also seen, though rarely, in preparations of insect oocytes (16, 20). Such structures could reflect the transcription of rDNA regions not normally transcribed or they could result from failures of proper termination, of processing of nascent RNA, or of packaging of nascent RNA with protein.

The excess RNA synthesized on the injected heterologous insect rDNA appears to be efficiently complexed with proteins, as shown by the staining procedure used. This indicates that the nucleus of *Xenopus* oocytes contains large stores of proteins that may be used for the complexing of nascent rRNA. *Xenopus* oocytes contain a great excess of RNA polymerase (21), histones (22), and chromatin-forming materials (23, 24).

Although some abnormal transcription is seen from the DNA injected in these experiments, we believe our results encourage the hope that it may eventually be possible to obtain useful information about the structure and the transcription of cloned pieces of chromosomal DNA. This would be particularly valuable for recognizing transcribed regions and the initial transcripts of single-copy genes, which at present cannot be identified in normal nuclei.

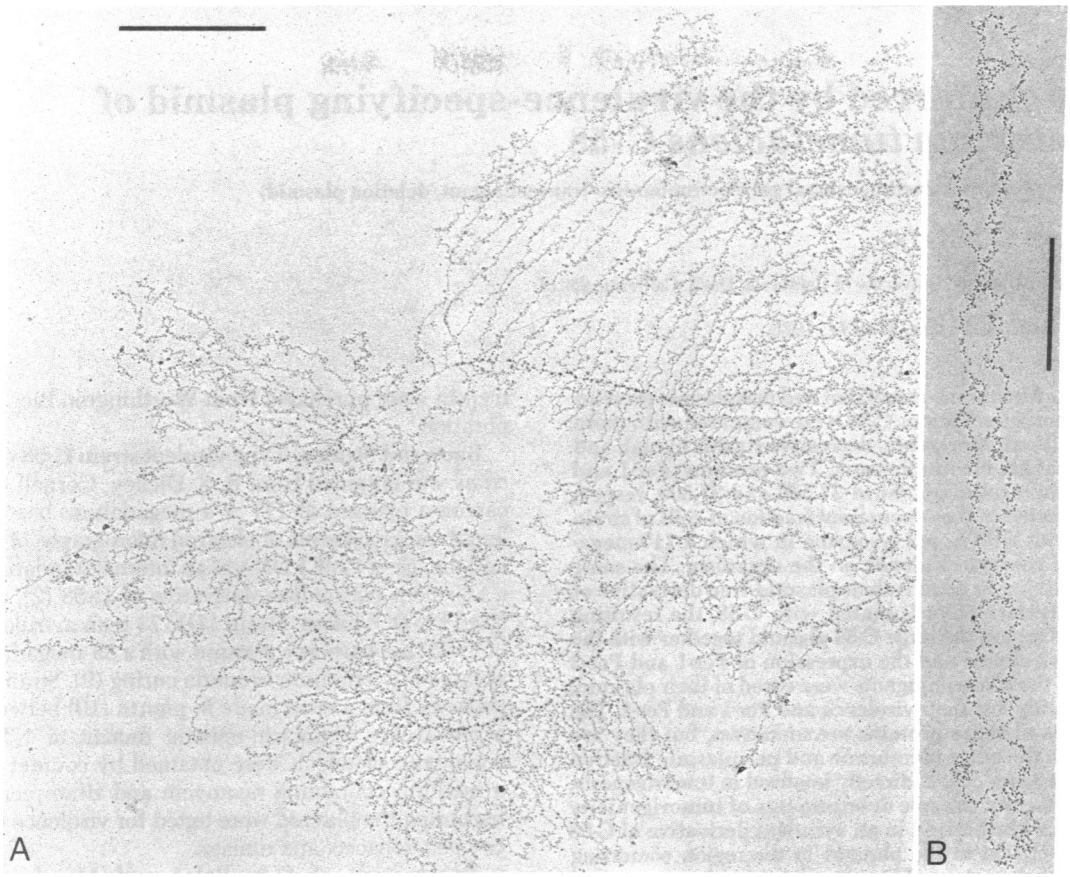

FIG. 6. Two extreme morphological forms of circular molecules seen in nuclear spread preparations of *Xenopus* oocytes injected with *Dytiscus* rDNA. Both circles are densely covered with small, intensely staining particles. (*A*) Nearly the whole circumference (8.4 μm) of the circle has enormously long lateral fibrils which do not show the typical length gradient. (*B*) The circle, 15.2 μm long, has no lateral fibrils.

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1. Mertz, J. E. & Gurdon, J. B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1502–1506.
2. Brown, D. D. & Gurdon, J. B. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2064–2068.
3. Miller, O. L. & Beatty, B. R. (1969) *Genetics,* suppl. **61,** 134–143.
4. Scheer, U., Trendelenburg, M. F. & Franke, W. W. (1973) *Exp. Cell Res.* **80,** 175–190.
5. Trendelenburg, M. F. (1974) *Chromosoma* **48,** 119–135.
6. Scheer, U., Trendelenburg, M. F., Krohne, G. & Franke, W. W. (1977) *Chromosoma* **60,** 147–167.
7. Gurdon, J. B. (1977) *Proc. Roy. Soc. London Ser. B.* **198,** 211–247.
8. Gall, J. G. & Rochaix, J.-D. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1819–1823.
9. Trendelenburg, M. F., Scheer, U., Zentgraf, H. & Franke, W. W. (1976) *J. Mol. Biol.* **108,** 453–470.
10. Gurdon, J. B. (1976) *J. Embryol. Exp. Morphol.* **36,** 513–540.
11. Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89,** 114–123.
12. Miller, O. L. & Bakken, A. H. (1972) *Acta Endocrinol.,* suppl. **168,** 155–177.
13. Franke, W. W., Scheer, U., Trendelenburg, M. F., Spring, H. & Zentgraf, H. (1976) *Cytobiologie* **13,** 401–434.
14. Scheer, U. (1978) *Cell,* **13,** 535–549.
15. Stanfield, S. & Helinski, D. R. (1976) *Cell* **9,** 333–345.
16. Trendelenburg, M. F., Franke, W. W. & Scheer, U. (1977) *Differentiation* **7,** 133–158.
17. Scheer, U., Trendelenburg, M. F. & Franke, W. W. (1975) *J. Cell Biol.* **65,** 163–179.
18. Scheer, U., Trendelenburg, M. F. & Franke, W. W. (1976) *J. Cell Biol.* **69,** 465–489.
19. Trendelenburg, M. F., Scheer, U. & Franke, W. W. (1973) *Nature (London) New Biol.* **245,** 167–170.
20. Franke, W. W., Scheer, U., Spring, H., Trendelenburg, M. F. & Krohne, G. (1976) *Exp. Cell Res.* **100,** 233–244.
21. Roeder, R. G. (1974) *J. Biol. Chem.* **249,** 249–256.
22. Adamson, E. D. & Woodland, H. R. (1974) *J. Mol. Biol.* **88,** 263–285.
23. Laskey, R. A., Mills, A. D. & Morris, N. R. (1977) *Cell* **10,** 237–243.
24. Wyllie, A. H., Laskey, R. A., Finch, J. T. & Gurdon, J. B. (1978) *Dev. Biol.,* in press.