ISOLATION OF DEOXYRIBONUCLEIC ACID FROM THE YOLK PLATELETS OF XENOPUS LAEVIS OÖCYTE*

By E. Baltus, J. Hanocq-Quertier, and J. Brachet

LABORATOIRE DE MORPHOLOGIE ANIMALE,
FACULTÉ DES SCIENCES DE L'UNIVERSITÉ LIBRE DE BRUXELLES

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The problem of the origin of cytoplasmic DNA in amphibian eggs has been extensively studied by Dawid,[1, 2] who showed that high-molecular-weight DNA can be extracted from *Xenopus laevis* eggs, and that it is almost exclusively localized in the mitochondrial fraction. This conclusion was partly based on the fact that the amount of DNA he could extract from the mitochondrial fraction corresponds roughly to the amount found in the whole egg by a modification of the diphenylamine test.[3]

On the other hand, using a fluorometric technique, we[4] obtained much higher values for the egg DNA content than those given by Dawid: 0.02–0.09 μg/egg, instead of 0.003 μg/egg reported by Dawid. Moreover, we found that 63 per cent of this DNA is bound to yolk platelets, in agreement with autoradiographic observations made on centrifuged oöcytes[5] and with our previous biochemical results.[6]

In the present work, it is shown that highly polymerized, double-stranded DNA can be extracted from yolk platelets of *Xenopus* eggs in quantities which amount to about ten times the amount found in the mitochondria.

Haggis[7] had suggested that the egg DNA might not be totally extractable by the conventional methods of preparation, and that only nuclear and mitochondrial DNA were quantitatively recovered with these procedures. This is probably true, since we found[4] that cytoplasmic DNA, in *Xenopus* oöcytes, is strongly bound in a nucleoprotein complex, which is not accessible to deoxyribonuclease without previous digestion with proteolytic enzymes. For this reason, methods which might be expected to break down such a complex have been used in the present investigation.

Materials and Methods.—Preparation of cytoplasmic particles: Yolk platelets and mitochondria: Ovaries were removed from *Xenopus laevis* females anesthetized with 222 Sandoz.

For the isolation of the particles (yolk platelets and mitochondria), either whole ovaries or single cells, from which follicle cells were separated as already described,[4] were used. The yolk platelets and mitochondria were separated according to the procedure of Dawid:[2] ovaries or oöcytes were ground in a loose Teflon homogenizer in 10 vol of 0.03 *M* Tris, 0.25 *M* sucrose, 0.001 *M* EDTA, pH 7.4. The homogenate was filtered on several layers of cheesecloth to eliminate membranes and debris, and then centrifuged for 15 min at 2000 rpm (480 *g*) in a Servall RC_2 centrifuge. The pellet was washed once in the same way; it contained the yolk platelets and some pigment. The supernatant was then centrifuged for 20 min at 10,000 rpm (12,000 *g*) in the same centrifuge; the resulting pellet consisted of mitochondria and it was washed once.

In a few cases, the method described by Wallace and Karasaki[8] for the isolation of yolk platelets has been used.

DNA extraction: The method devised by Kirby and Cook[9] for the isolation of 12–16*S* DNA from mammalian tissues has been used. This method was designed in order to

eliminate polysaccharides. After its extraction, the DNA was precipitated by the addition of 2 vol of cold absolute alcohol.

Analytical ultracentrifugation of DNA: Three to five μg of the DNA to be studied and 2 μg of LP_7 DNA (d = 1.740) were dissolved in 0.65 ml of 0.001 M Tris, 5.10^{-3} M NaCl, pH 8.0. CsCl (882 mg) was added so that the average density was 1,700. The solution was centrifuged at 44,770 rpm for 20 hr in a standard 12-mm Kel —F cell of an AN-D rotor of a Spinco model E. Photographs were taken at 265 mμ and scanned with a Joyce-Loebl microdensitometer. Buoyant density was calculated according to the methods of Schildkraut *et al.*[10]

Purification of DNA on CsCl gradients: The DNA extracted by the Kirby and Cook method[9] was dissolved in 10^{-3} M Tris, 5.10^{-3} M NaCl, pH 8.0, and centrifuged for 45 hr at 35,000 rpm on a CsCl gradient of which the average density was 1,700. The centrifugation was carried out at 15°C in a Beckman L_2 ultracentrifuge. Four-drop fractions were collected and the DNA fractions, localized by their UV absorption, were pooled and precipitated by the addition of 2 vol of cold absolute alcohol.

Sedimentation coefficient of DNA: Fifty μg of DNA were dissolved in 0.5 ml of the following buffer: 0.1 M Na acetate, 0.01 M EDTA, pH 7.0. The dissolved DNA was layered on top of a 5–20% sucrose gradient made up in 0.05 M Na phosphate buffer 0.1 M NaCl, pH 6.7, and run at 47,000 rpm for 195 min at 4°C. As a marker 1 μg of H^3-uridine-labeled *E. coli* RNA was added. Fractions were collected by puncturing the bottom of the tube, and the positions of the DNA and of the marker RNA were determined, respectively, by UV absorption and by radioactivity measurements.

Thermal denaturation: Twenty μg of the DNA purified by centrifugation on a CsCl gradient and precipitated by the addition of 2 vol of absolute alcohol were dissolved in 1 ml of 5.10^{-4} M Tris buffer, 10^{-3} M NaCl, pH 8.0. The optical density was recorded at increasing temperatures. Each temperature was maintained for 20 min, or until the UV absorption no longer changed.

Examination under the electron microscope: Spreading of the DNA was performed following the method of Kleinschmidt and Zahn,[11] and was examined under a Hitachi electron microscope model HS 6 after a circular shading with uranium oxide.

Fluorometric estimation of DNA: The method described by Baltus and Brachet[6] was used.

Estimation of proteins: We used a modification of the Biuret technique described by Zamenhof.[12]

RNA polymerase: The method of Chamberlin and Berg[13] was used.

Results.—DNA extraction: We successively tried the following methods.

(1) The method of Marmur,[14] with chloroform and isoamyl alcohol, that Dawid[1] had used for the extraction of somatic DNA. No substantial amounts of DNA could be extracted in that way from whole oöcytes or yolk platelets.

(2) The method of Yolles and Freeman,[15] which implies a long extraction period with phenol and SDS. After a 40- to 65-hour extraction, we actually obtained some DNA from oöcytes previously freed of their follicle cells, but the preparation was heavily contaminated with polysaccharides. Treatment of the preparation with amylase (50 μg/ml of 0.01 M Tris, 0.005 M EDTA, pH 7.0) for one hour at 37°C was insufficient to remove the contaminating sugars.

(3) The method of Kirby and Cook[9] gave us a good recovery of the DNA. The yield of DNA was 65–75 per cent in the yolk platelets as well as in the mitochondria and in the sperm. A fluorometric method[6] was used in order to estimate the DNA initially contained in the particles and the DNA which had been extracted from them. The mitochondrial fraction contained only 5–10 per cent of the amount of DNA found in the yolk platelets isolated from the same ovary, in confirmation of our previous results.[4] The DNA extracted from yolk was

fibrous, like that of sperm; however, the UV-absorption spectrum was more satisfactory for nuclear than for yolk DNA. The latter was highly contaminated by proteins: for 100 μg DNA, we found 688 μg of protein in the yolk preparation, and 35 μg only in the sperm preparation. The protein contamination disappeared after a run on a CsCl gradient.

DNA analysis by analytical centrifugation: Analytical ultracentrifugation on CsCl density gradients showed the same density, in 15 experiments, for the DNA's prepared from yolk platelets, from mitochondria, and from sperm. The density was 1.699 or, exceptionally, 1.698, corresponding to a GC content of 39.8 per cent.[16] In a few cases, we found the density of the mitochondrial DNA to be 1,700; this small difference cannot be considered significant, since it has not been regularly observed (Fig. 1*a*–*c*). The same results were obtained when the yolk platelets were isolated by the method of Wallace and Karasaki[8] instead of that of Dawid.[2]

Since the preceding results were obtained on particulate fractions isolated from whole ovaries, it was suspected that a contamination due to the nuclei of follicle cells might occur. But exactly the same characteristics were obtained for the DNA isolated from yolk platelets prepared either from single oöcytes freed of their follicle cells or from unfertilized eggs (their jelly had been removed by a 5-min treatment with 0.2% cysteine–0.05% pronase, pH 7.6).

After treatment of the yolk DNA with 0.03 per cent DNase, dissolved in 0.001 *M* Tris, 0.005 *M* NaCl, 0.001 *M* $MgCl_2$, pH 8.0, for 30 minutes at 37°C, no peak remained visible in the analytical ultracentrifuge (Fig. 2).

Following a thermal denaturation at 100°C for ten minutes in 5 mM KF, the density of the yolk DNA amounts to 1,717 (Fig. 3).

Thermal denaturation (Fig. 4): In a 0.001 *M* NaCl solution, sperm DNA had a melting temperature of 58°C. The hyperchromicity observed was 22.5 per cent. Renaturation by slow cooling did not occur.

Under the same conditions, yolk DNA had a melting temperature of 55°C, with a hyperchromicity of 28.5 per cent. Renaturation was slight (2%). It is clear that both nuclear and yolk DNA's must be double-stranded, and probably heterogeneous since they do not easily reanneal.

Electron microscopic examination (Fig. 5): In confirmation of Wolstenholme and Dawid's results,[17] we found circular molecules in the mitochondrial preparations, some of them in the supertwisted form. On the other hand, yolk and sperm DNA molecules were always linear. The molecule length was quite variable, especially in the case of yolk DNA. The following dimensions were found: (a) *Yolk DNA:* from 0.67 to 22.6 μ, values which correspond to molecular weights of 1.3–43 $\times$ 10^6. The average value was 1 μ, which corresponds to a molecular weight of 1.9 $\times$ 10^6. (b) *Sperm DNA:* from 8.1 to 26.8 μ, which corresponds to molecular weights of 15–48 $\times$ 10^6.

Primer activity of yolk DNA in RNA polymerase reaction: Preliminary experiments showed that yolk DNA is capable of supporting the RNA polymerase reaction. However, the reaction was much stronger with nuclear DNA; this might be due to its higher purity. More refined experiments would be necessary to clarify this point.

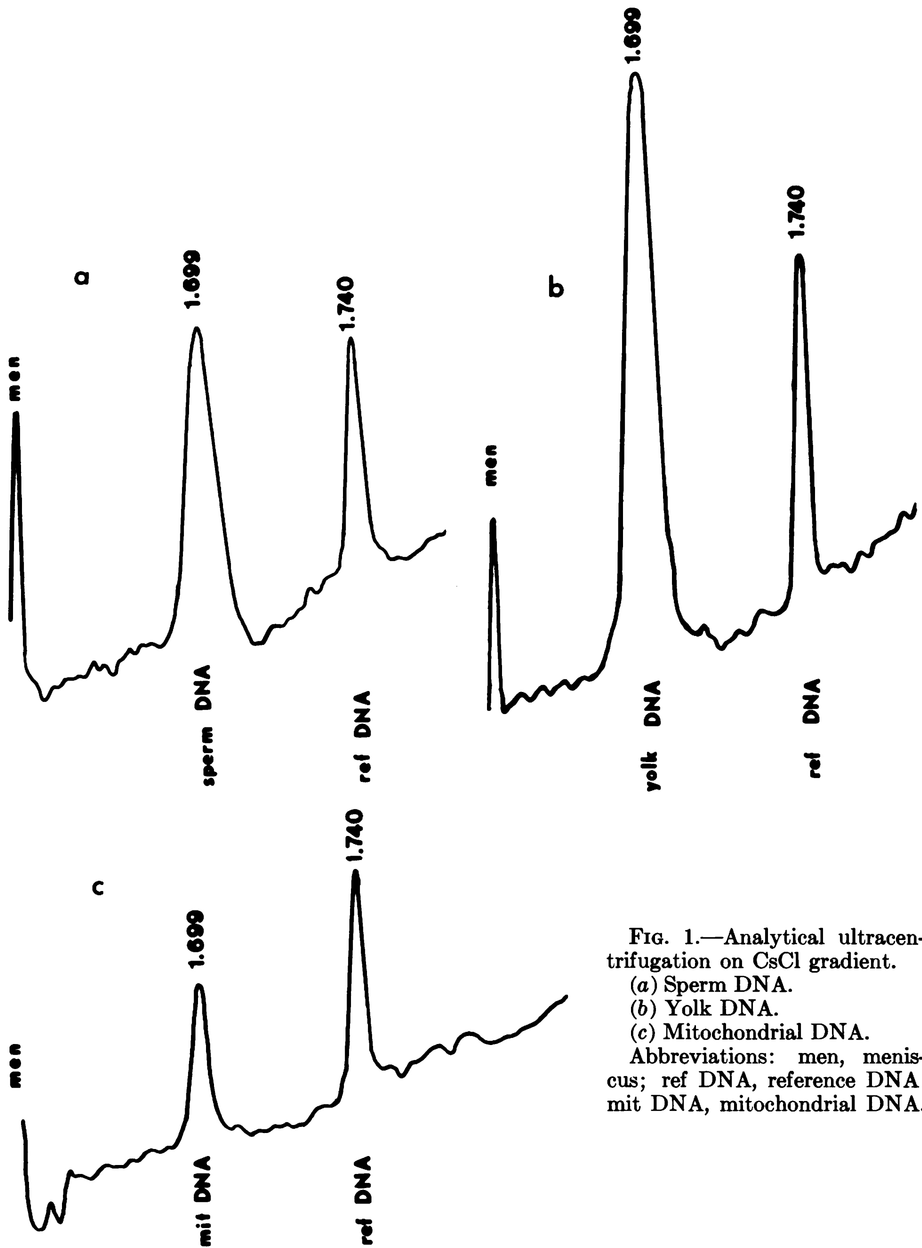

FIG. 1.—Analytical ultracentrifugation on CsCl gradient.
(*a*) Sperm DNA.
(*b*) Yolk DNA.
(*c*) Mitochondrial DNA.
Abbreviations: men, meniscus; ref DNA, reference DNA; mit DNA, mitochondrial DNA.

Discussion.—So far, the method described by Kirby and Cook[9] is the only one that allows the extraction of the DNA from the yolk platelets of amphibian eggs and the removal of the contaminating polysaccharides.

In confirmation of our previous results, based on fluorometric estimations,[4] we found that, in *Xenopus* oöcytes, mitochondrial DNA represents at most 10 per cent of the total DNA.

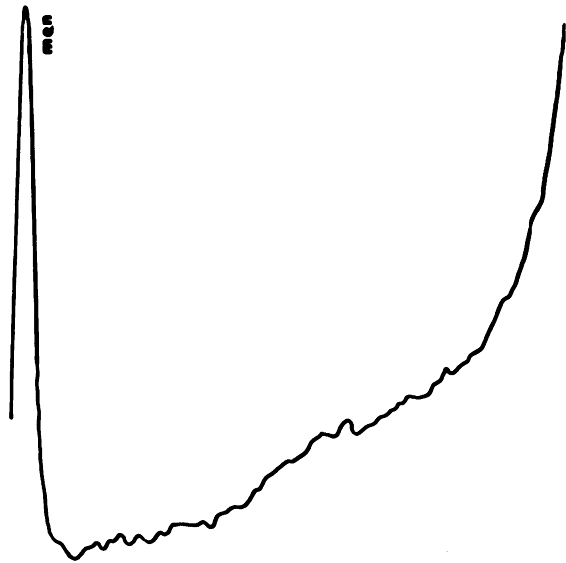

FIG. 2.—DNase-treated yolk DNA. Analytical ultracentrifugation on CsCl gradient.

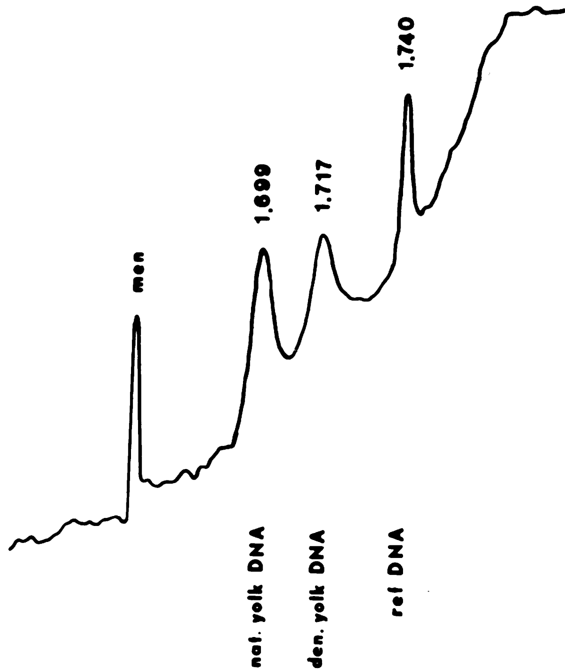

FIG. 3.—Native and denatured yolk DNA. Analytical ultracentrifugation on CsCl gradient.

The density of yolk and mitochondrial DNA's is the same as that of nuclear DNA (from sperm or from swimming tadpoles). Further studies may show more subtle differences between these three types of DNA. Their aspect under the electron microscope is totally different and this, in itself, eliminates the possibility of any considerable contamination of one particulate fraction by the other.

It cannot be decided yet whether the heterogeneity of sizes observed for yolk

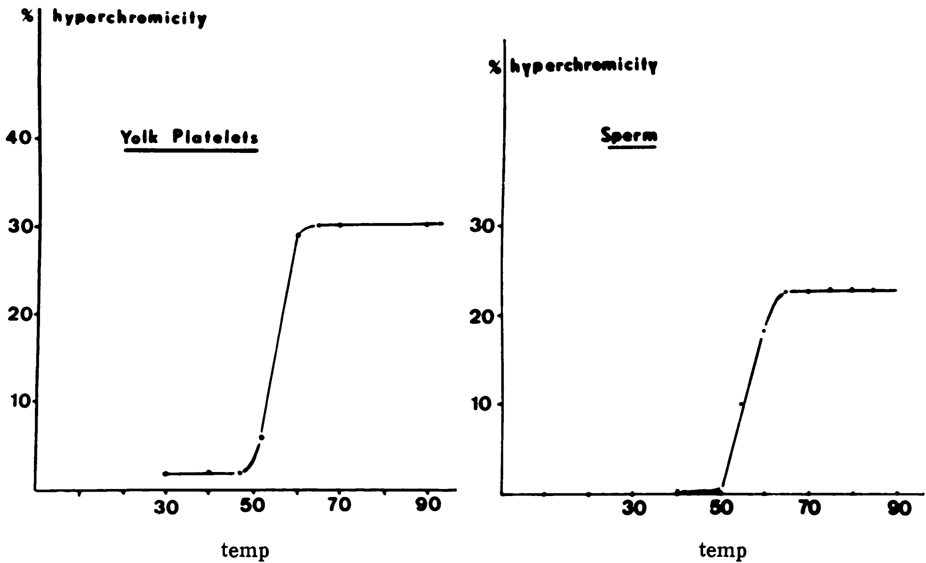

FIG. 4.—Thermal denaturation of DNA. *Left,* yolk DNA; *right,* sperm DNA.

DNA molecules is an artifact due to the isolation procedure; but the presence of molecules as long as 22 μ shows that at least part of yolk DNA is of high molecular weight; the sharpness of the peaks obtained in the analytical ultracentrifuge confirms this conclusion.

The double-stranded nature of these DNA molecules is indicated by the denaturation experiments, which give rise to molecules having a higher chromicity and a density increased by the classical value of 0.017 gm/cm^3.

The role of the cytoplasmic DNA's is far from clear. Yolk DNA has a high molecular weight and is able to serve as a primer in the RNA polymerase reaction; it is therefore likely to play an active role and probably represents more than a prospective pool of deoxyribonucleosides. It might, for instance, play a part in controlling the synthesis of the enzymes which break down the yolk platelets during development. In favor of such a hypothesis is the fact that ethidium bromide, an intercalating agent that reacts with DNA, markedly inhibits the utilization of the yolk platelets in amphibian embryos (J. Brachet, unpublished).

Like phosvitin,[18] yolk DNA may well originate from the liver of the maternal organism, since we have observed (unpublished) a considerable breakdown of liver cells in female frogs at the time of vitellogenesis. Moreover, we had found earlier[4] that a complex between DNA and a protein with a high serine content, resembling phosvitin, can be isolated from the yolk platelets and is remarkably resistant to deoxyribonuclease; this complex might therefore be formed in the liver, transferred to the blood stream, and incorporated in the growing oöcytes.

Summary.—Highly polymerized DNA has been extracted from yolk platelets of *Xenopus laevis.* It is double-stranded and can be used as a primer in the RNA polymerase reaction. Examination under the electron microscope shows that

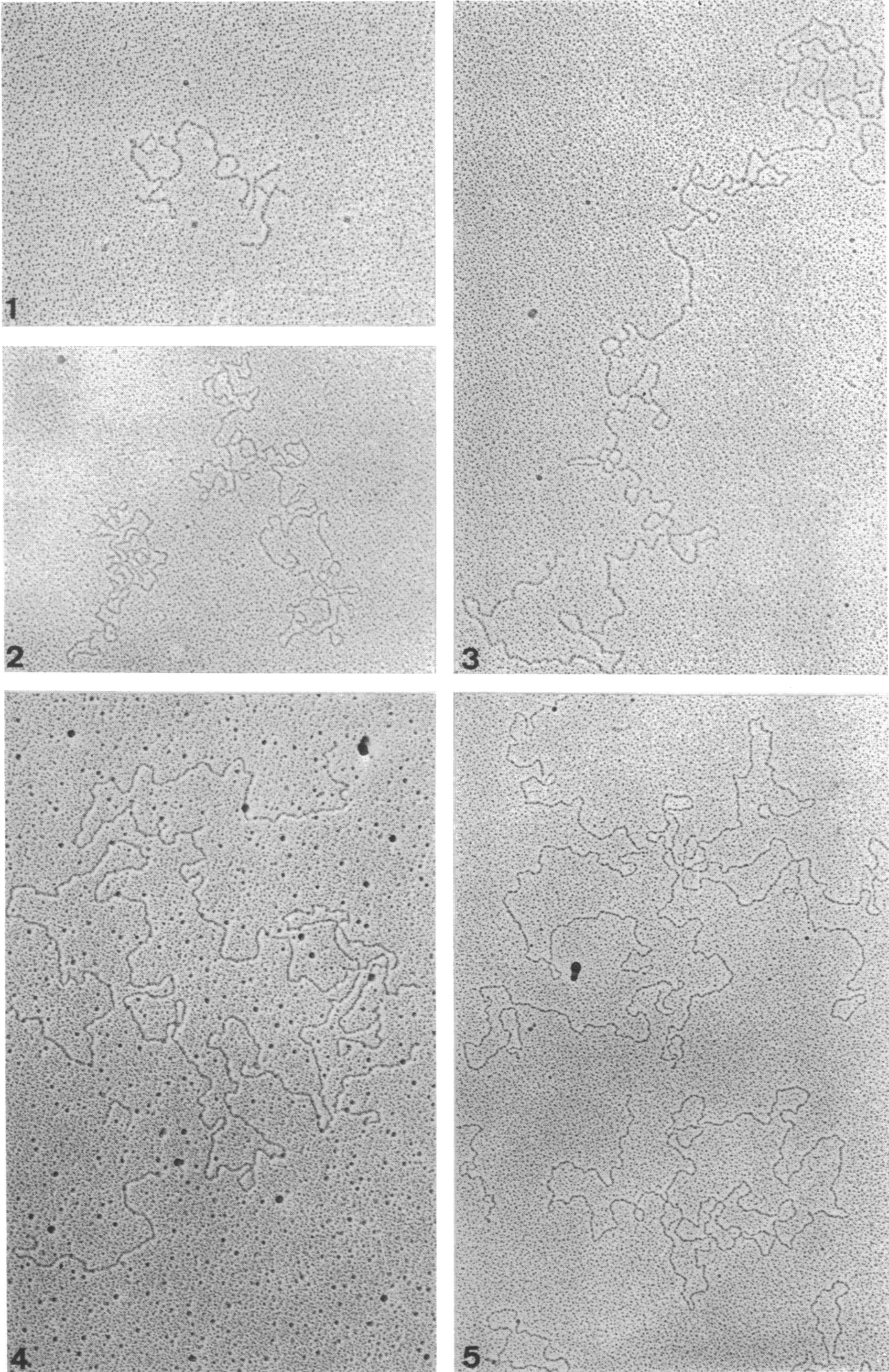

FIG. 5.—(*1*) Mitochondrial DNA, 34,560×. (*2*) Sperm DNA, 23,040×. (*3*) Sperm DNA, 25,540×. (*4*) Yolk DNA, 28,800×. (*5*) Yolk DNA, 23,040×.

this yolk DNA is linear, as opposed to mitochondrial DNA, which is circular. There is ten times more DNA in the yolk platelets than in the mitochondria of an oöcyte.

We are very grateful to Mrs. A. Miller, who kindly performed the electron microscopy on our DNA preparations.

Abbreviations used: DNase, deoxyribonuclease; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; EDTA, ethylenediaminetetraacetate; SDS, sodium dodecylsulfate.

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