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PRESENCE OF RNA IN THE SPERM NUCLEUS

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The RNase-colloidal gold procedure for the ultrastructural localization of RNA was used for rat testis. Along with other structures, it was found that the testicular sperm nucleus was well stained. Similar labelling was observed in the nucleus of rat epididymal sperm and human sperm. The RNA was extracted from sperm and analyzed by electrophoresis on 10% polyacrylamide gel and 7 M urea. The electrophoretic profile revealed a complex set of bands ranging in size from tRNA to high molecular weight components. On the average, a content of about 0.1 pg of RNA per rat or human sperm was found.

Spermiogenesis is probably one of the most complex processes of cell differentiation. It culminates in the generation of the sperm that are capable of carrying and introducing the haploid genetic complement into the egg at fertilization. In most species, the genome of the gamete is packed into a complex comprised of DNA and small basic proteins referred to as protamines (1), resulting in a highly compact chromatin, inert with respect to gene expression (2). Interestingly, however, the presence of nuclear DNA polymerase activities has been described (3,4).

We have been studying the RNA present in the chromatoid body, a cytoplasmic organelle especially characteristic of round spermatids (5). Among others, one approach has been the staining of rat testicular sections with RNase-colloidal gold which is quite specific for the ultrastructural localization of RNA (6). During these studies, a consistent staining of the testicular rat sperm nucleus with gold particles was observed. These results are presented here

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along with the electrophoretic characteristics of the RNA extracted from rat and human sperm.

MATERIALS AND METHODS

Sperm and sperm head isolation.

Epididymal rat sperm were prepared as described before (7), with one variation: all the solutions employed contained 10 ug/ml of heparin. For human sperm, the fresh ejaculates were checked under the microscope and those containing a high proportion of abnormal sperm and inmature cells were discarded. The ejaculates were centrifuged at 3,000 x g for 10 min and resuspended in a solution containing 150 mM NaCl and 10 mM sodium phosphate, pH 7.0 (PBS), and 10 ug/ml of heparin. After centrifugation as described, the sperm were washed with the same solution twice. The cells were used inmediately for RNA extraction, or fixed for electron microscopy.

Rat sperm heads were isolated from epididymal sperm treated with cetyltrimethylammonium bromide and 2-mercaptoethanol, as already described (7).

RNA extraction.

Sperm (3×10^8 sperm) were suspended in a final volume of 3 ml of a solution containing 25 mM Tris-HCl (pH 7.5), 100 mM KCl, 300 mM NaCl, 6 mM DTT, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1 % Nonidet P-40 and 100 units of human RNAse inhibitor (Amersham/Searle). After 5 min at room temperature, sodium dodecyl sulfate (SDS) was added to a final concentration of 0.2%, and the mixture was incubated at room temperature for 15 min with occasional shaking. The suspension was centrifuged at 5,000 x g for 15 min, and the supernatant was digested with proteinase K (50 ug/ml) for 20 min at 37 °C. The digest was extracted with phenol-chloroform-isoamyl alcohol and twice with chloroform-isoamyl alcohol (8). The RNA in the aqueous phase was precipitated with ethanol (8), collected by centrifugation at 10,000 x g for 15 min, and dissolved in sterile distilled water. Aliquots were diluted in water and the spectrum from 220 to 330 nm was obtained. Total rat testis polysomal RNA was prepared according to Palmiter (9).

Electrophoresis of the isolated RNA was carried out on 10% polyacrylamide gel containing 7 M urea (10), and stained with silver (11).

Electron microscopy.

The sperm were fixed with 2% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 2 h at room temperature. After washing twice with the phosphate solution, the sperm were postfixed with 0.25% osmium tetroxide. Following dehydration in a series of ethanol, the samples were embedded in Araldite or in Lowycryl K-4M (Balzers Union, Balzers, Liechtenstein) at -30 °C (12). In this latter case, post-fixation with osmium tetroxide was not used.

The RNAse-colloidal gold complex was prepared according to the procedure described by Bendayan (6). The specimens mounted on nickel grids were incubated in a humid chamber with the RNAse-colloidal gold suspension for 1 h at room temperature (6). After washing the grids with PBS and destilled water, they were stained with uranyl acetate and lead citrate (6). Control sections were stained with either RNAse-colloidal gold in the presence of yeast RNA (1 mg/ml) or with bovine serum albumin(BSA)-gold complex (6).

RESULTS AND DISCUSSION

Staining of rat testis section with RNAse-colloidal gold revealed a high density of gold particles in the nucleus of elongated spermatids and fully differentiated sperm, and none in the acrosome or the elements of the tail (Fig.1A). In agreement with the report of Bendayan (6) we have found that this cytochemical technique for the

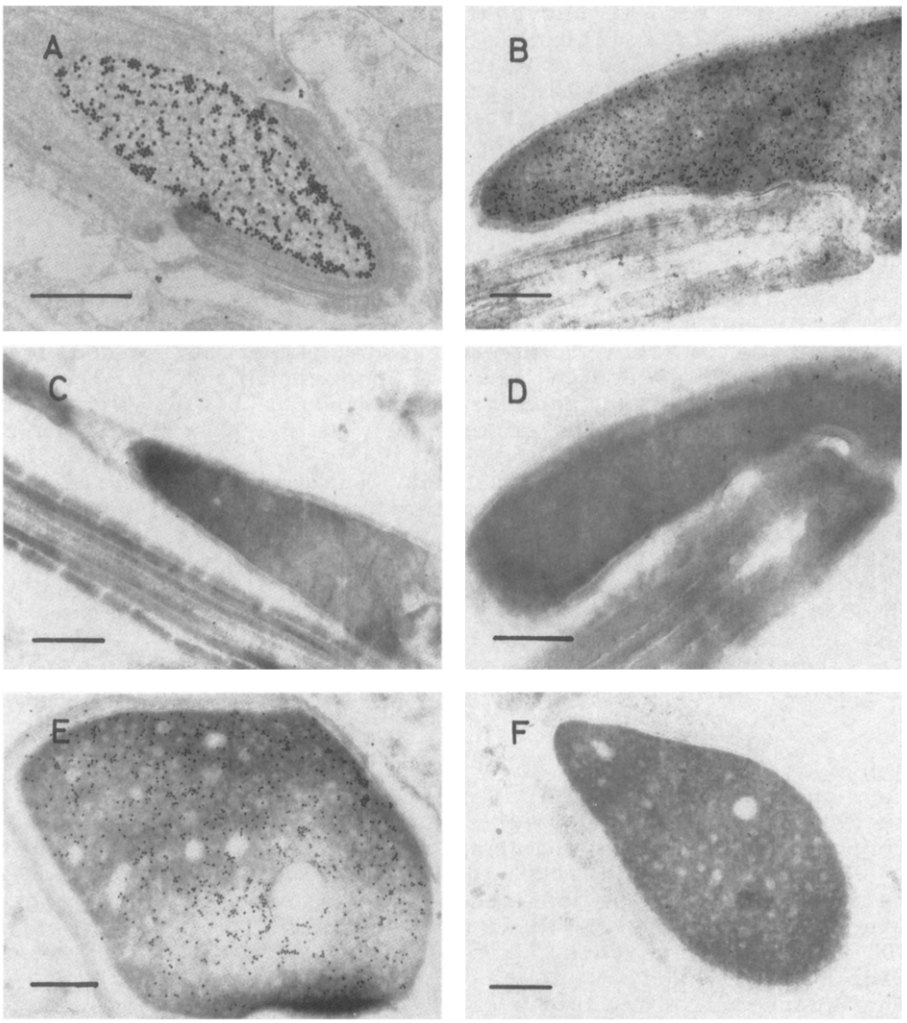


Fig.1.- Ultrastructural localization of RNA in the sperm nucleus. The staining procedure with RNase-colloidal gold complex was carried out as described in Methods.. A, rat testicular section showing an elongated spermatid. B, rat epididymal sperm. C, as B but stained with RNase-gold complex in the presence of yeast RNA (1mg/ml). D, as B but stained with BSA-gold complex. E, human sperm. F, as E but stained with BSA-gold complex. The bar correspond to 0.5 µm.

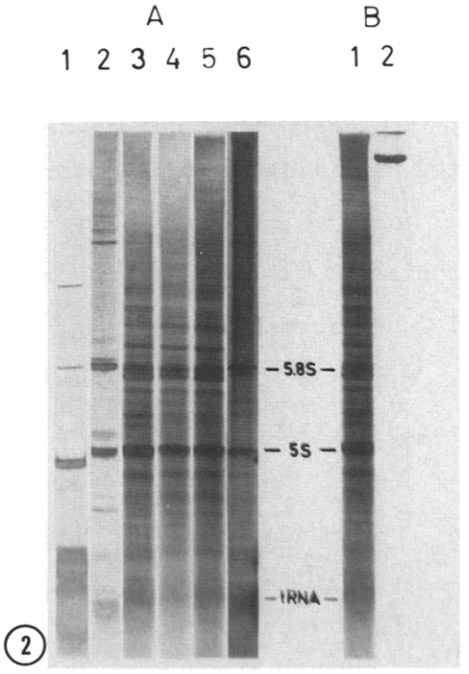
ultrastructural localization of RNA is highly specific (data not shown).

Since it can be argued that the positive labelling of the nucleus of testicular sperm might be related to an incomplete morphogenesis, rat epididymal sperm were subjected to the same staining procedure. The gold particles were highly concentrated in the nucleus of the cell with practically no labelling of the acrosome and the fibrillar elements of the tails (Fig.1B). Moreover, similar results were obtained with human ejaculated sperm (Fig.1E), indicating that the presence of RNA in the nucleus is common to these two species. As expected, control sections stained with RNAse-colloidal gold in the presence of yeast RNA (Fig.1C), or with BSA-colloidal gold (Fig.1D and F), show almost no labelling.

The above results are a clear indication of the presence of RNA in the sperm nucleus. To gain further information on the nature of this RNA, the sperm were subjected to the extraction procedure described in Methods. In several preparations, the yield of RNA from rat sperm was 0.16 ± 0.13 pg per sperm, with an average 260/280 nm ratio of 1.75. In the case of human sperm the yield was 0.07 pg of RNA/sperm.

Electrophoretic analysis of the extracted RNA revealed a complex mixture of components ranging in size from tRNA to large molecules, including 5.8 S and 5 S ribosomal RNA (Fig.2A). It is interesting to note that in three different preparations of RNA, a similar gel pattern was obtained. Moreover, the pattern obtained with RNA extracted from isolated rat sperm heads was quite similar (see track 6, Fig.2A). Finally, treatment of the extracted nucleic acids with RNAse completely removed the set of bands observed previously (Fig.2B).

The electrophoretic profile of the RNA extracted from human sperm is shown in Fig.3. Once again, a complex set of components are



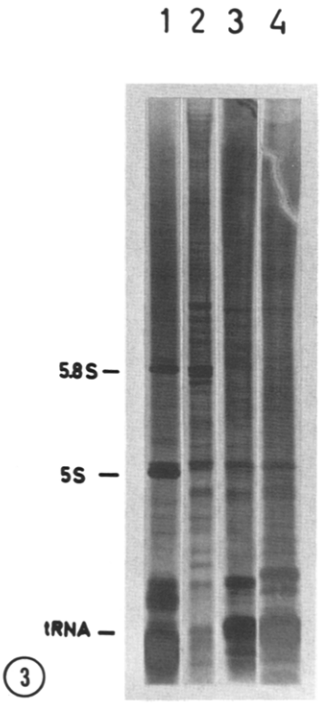


Fig.2.- Electrophoretic analysis of the RNA extracted from rat sperm. The tracks in A correspond to: 1, yeast tRNA; 2, rat testis polysomal RNA; 3 to 5, three different preparations of RNA extracted from rat sperm; 6, RNA extracted from isolated rat sperm heads. In B the tracks correspond to: 1, RNA extracted from rat sperm; 2, the same, but incubated previous to electrophoresis with RNase (1mg/ml) at 37 °C for 30 min. To each track, 2 ug of RNA were applied. The position of tRNA, and 5 S and 5.8 S ribosomal RNA are indicated.

Fig.3.- Electrophoretic analysis of RNA extracted from human sperm. 1) total rat testis RNA; 2) RNA extracted from rat sperm; 3) and 4) two preparations of RNA extracted from human sperm. The amount of RNA analyzed were 2 ug. Reference RNA is on the left.

observed and several bands show similar mobility to those found in RNA extracted from rat sperm (Fig.3).

The staining procedure for electron microscopy clearly shows the presence of RNA in the sperm nucleus. However, with regard to the amount of RNA extracted and the complexity of bands observed, the situation is more difficult to elucidate. A mitochondrial origin for the RNA can be argued, but it seems unlikely. First, no labelling of the mitochondrial sheath (1) was noticed (Fig.1). Moreover, a similar electrophoretic pattern was obtained for the RNA extracted from whole sperm and for that isolated from sperm heads (Fig.2). However, the

possibility that part of this RNA is derived from some other structure, such as the residual body (13) can not be discarded at the present time. With human sperm, the interpretation is more difficult. Although we have discarded those semen with abnormal sperm and inmature cells, a small proportion of the latter can be present, which could account for some of the RNA observed in Fig.3.

It must be emphasized that the present work is the first report in which the combination of ultrastructural staining procedure and biochemical methods, demonstrates the presence of RNA in the sperm nucleus. Early reports claimed the presence of RNA in the sperm (14,15), but the data has not been further discussed.

Altogether the present results open an important question: what is the origin of the sperm nuclear RNA and what might its function be ? Since there is good evidence that the sperm is transcriptionally inactive (2), the origin of this RNA is indeed puzzling and warrants further research. The second question is equally difficult to answer at the present time. Nevertheless, one is tempted to postulate a functional role following fertilization. Recent reports have demonstrated that paternal and maternal contributions are necessary for full development (16,17). Others have proposed that the essential role of the paternal pronucleus may involve nuclear factors other than the DNA (18-20). Whether the RNA found in the sperm nucleus (paternal RNA?) as reported here represents these unknown factors necessary for normal development, remains to be investigated. In this regard, the reference to "paternal RNA" discussed recently by Swain et al.(21), is relevant.

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