Brief Report

Reverse Transcriptase Activity in Mature Spermatozoa of Mouse

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Abstract. We show here that a reverse transcriptase (RT) activity is present in murine epididymal spermatozoa. Sperm cells incubated with human poliovirus RNA can take up exogenous RNA molecules and internalize them in nuclei. Direct PCR amplification of DNA extracted from RNA-incubated spermatozoa indicate that poliovirus RNA is reverse-transcribed in cDNA fragments. PCR analysis of two-cell embryos shows that poliovirus RNA-challenged spermatozoa transfer retrotranscribed cDNA molecules into eggs during in vitro fertilization. Finally, RT molecules can be visualized on sperm nuclear scaffolds by immunogold electron microscopy. These results, therefore, reveal a novel metabolic function in spermatozoa, which may play a role during early embryonic development.

Key words: spermatozoa • reverse transcriptase • retroposon • nuclear scaffold • fertilization

Introduction

A growing body of evidence supports the notion that spermatozoa of virtually all animal species can take up foreign DNA molecules and internalize them into nuclei (reviewed by Spadafora, 1998). In the last few years, work in our laboratory has been aimed at characterizing the mechanism(s) of interaction of sperm cells with exogenous DNA. Several factors have been characterized that are implicated in sperm/DNA interactions (Spadafora, 1998). These studies have also revealed unsuspected metabolic features of mature murine spermatozoa, which are traditionally regarded as metabolically inert cells; we have found that foreign DNA molecules are internalized into nuclei, undergo a process of rearrangement, and are eventually integrated into the sperm genome (Zoraqi and Spadafora, 1997), suggesting that at least some site(s) in sperm nuclear chromatin are accessible to the integration and recombination of foreign molecules. Mammalian spermatozoa activate an endogenous nuclease response when incubated with foreign DNA molecules, a response that can similarly be triggered simply after prolonged incubation in medium. Sperm endogenous nucleases degrade the foreign DNA sequences and, when effectively triggered, also cause the localized degradation of sperm chromosomal DNA, with the subsequent release of cleaved hypersensitive chromatin from the spermatozoa into the medium (Maione et al., 1997). That observation indicates that the sperm chromatin is not solely organized in a uniformly compact nucleoprotamine structure, but contains sites at which the chromosomal DNA is sensitive to nuclease activity.

We have recently cloned the released hypersensitive DNA from murine spermatozoa and sequenced randomly selected clones. A high proportion of sequences was found to be of retroposon origin from a variety of families, particularly of the LINE/L1 group (Pittoggi et al., 1999). The findings that these sequences are organized in nucleosomes, are hypersensitive to nuclease cleavage and that many of them contain uninterrupted LINE-derived ORFs suggest that they may derive from LINE elements potentially coding for reverse transcriptase (RT)[1] in male germ cells. These results prompted us to investigate the possibility that an endogenous RT activity exists in mature murine spermatozoa. We reasoned that a direct way to address this question might exploit the spontaneous ability of spermatozoa to internalize foreign molecules. Thus, we took

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[1]*Abbreviations used in this paper:* CTAB, cetyltrimethylammonium bromide; FISH, fluorescent in situ hybridization; FM, fertilization medium; IVF, in vitro fertilization; LCMV, lymphocytic choriomeningitis virus; ORF, open reading frame; RT, reverse transcriptase.

the approach of incubating spermatozoa with purified RNA, and then search for corresponding cDNA copies. We show here that mouse epididymal spermatozoa can indeed retrotranscribe the input RNA. In addition, cDNA products are actually transferred to two-cell embryos obtained by in vitro fertilization assays. Finally, RT molecules can be identified by immunoelectron microscopy on isolated sperm nuclear scaffolds.

Materials and Methods

Preparation of Spermatozoa, In Vitro Fertilization, and Uptake of Foreign RNA

Germ-free mice were from Charles River Italia. Epididymal spermatozoa were obtained from CD1 and C57BL murine strains, while oocytes were collected from superovulated B6D2F1 females. Epididymal spermatozoa collection, in vitro fertilization (IVF) experiments, and embryo cultures were as described (Zaccagnini et al., 1998). Sperm cells were suspended in fertilization medium (FM, Whittingham, 1971) supplemented with 4 mg/ml BSA. Particular care was taken to prepare pure sperm cell samples and avoid contamination by somatic cells. To that aim, we avoided squeezing of epididymis and collected spermatozoa that were spontaneously released through holes made by simply puncturing the epididymis with a needle. After a 30–60-min "swim-up" selection step of mobile cells, the purity of sperm cell preparations was checked by phase contrast microscopy. Preparations containing somatic cells were discarded.

Poliovirus RNA Purification

RNA was phenol-extracted from highly purified poliovirus particles isolated as described (Baron and Baltimore, 1982). After ethanol precipitation, the RNA was resuspended in DEPC-treated distilled water at a concentration of 1 mg/ml. In RNA uptake experiments (see Fig. 1 A), 5 μg of poliovirus RNA were end-labeled using the T4 polynucleotide kinase (Amersham) and γ-[^{32}P]ATP for 1 h at 37°C to a specific activity of 7×10^6 cpm/μg. For PCR and IVF experiments, epididymal spermatozoa were routinely incubated with 50 ng RNA/10^6 spermatozoa in FM for 1 h at 37°C.

Fluorescent In Situ Hybridization (FISH) Analysis

Sperm cells were incubated with poliovirus RNA (200 ng per 10^6 spermatozoa) for 30 min in Ca^{2+}- and Mg^{2+}-free FM containing 1 mM EDTA. Sperm nuclei were prepared using cetyltrimethylammonium bromide (CTAB) detergent (Aldrich) essentially as described (Balhorn et al., 1977) with minor modifications (Maione et al., 1997). Under these conditions, both plasma and nuclear membranes are removed and yet the hooked shape of sperm heads is almost unaltered due to the overall compact structure of sperm chromatin. FISH was performed on sperm nuclei as described (Boyle et al., 1990) with minor modifications (Pittoggi et al., 1999). The probe was a full-length poliovirus cDNA clone (kindly given by M. Chow) that was uniformly labeled using biotin-16-dUTP and a nick-translation kit (Boehringer). Nuclei were counterstained with DAPI. Cells were visualized using ultraviolet excitation and hybridization signals were analyzed under blue-violet illumination using a Zeiss Axiophot microscope. Images were recorded with a cooled CCD camera (Photometrics).

DNA Extraction and PCR Analysis

DNA was purified from sperm nuclei after incubation with proteinase K (500 μg/ml) at 37°C overnight, followed by sequential extractions with phenol/chloroform and ethanol precipitation. Two-cell stage embryos were collected in groups of 20–30, lysed in 20 μl of 200 mM KOH, 50 mM DTT for 15 min at 65°C, then neutralized with the same volume of 0.9 M Tris, 200 mM HCl, and 300 mM KCl. Aliquots corresponding to 10 lysed embryos were subjected to direct PCR amplification using a GeneAmp system 9700 (Perkin-Elmer Corp.) and oligonucleotide primers listed below, corresponding to the indicated regions of the viral genome (accession number V01148) (numbers correspond to positions in the viral map):

V1 (4n-28n) 5′-AAA CAG CTC TGG GGT TGT ACC CAC C-3′
V1REV1 (227n-205n) 5′-ACG GAT CCG TCG CTT TCA ACC AC-3′
V21F (371n-395n) 5′-TAC CTA TGG CTA ACG CAT GGG ACG C-3′
V21R (684n-661n) 5′-CTC AAT GGA GCG GAT CCA GCA AAC-3′
V9 (740n-764n) 5′-AAT GGG TGC TCA GGT TTC ATC ACA G-3′
V10 (1032n-1008n) 5′-CCG CCT CCT GTG TGG TTA TAG TGG A-3′
V19 (1355n-1379n) 5′-CAA CAC CAC TAC CAT GCA CAC CAG C-3′
V20 (1826n-1799n) 5′-CGG TGA CTG GAA GTT GTC TGC AGT AAG A-3′
V15 (1807n-1831n) 5′-CAG ACA ACT TCC AGT CAC CGT GTG C-3′
V2 (2263n-2285n) 5′-CGT GCT GTT GCT AAT CCA TGG CA-3′
B1F (2750n-2774n) 5′-GGA TAA CCC AGC TTC CAC CAC GAA TCT-3′
B1R (3159n-3133n) 5′-AAA GGG AGT CAC CTA GTG CTG CCG A-3′
A1 (3381n-3406n) 5′-GGA TTC GGA CAC CAA AAC AAA GCG G-3′
A2 (3830n-3806n) 5′-GCC TTG TTC CAT GGC TTC TTC TTC G-3′
V5 (4143n-4167n) 5′-ACT GAA GCA TGC CAA CGA GCT AAG G-3′
V4 (4340n-4316n) 5′-TTC CTG GTG TTC CTG ACT AGG GCA T-3′
B4F (4510n-4536n) 5′-CCG GAA CAG GTA AAT CTG TAG CAA CCA-3′
B3R (5604n-5630n) 5′-TGC TTG ATC TTC AAA CAC TTT GGC ATC-3′
B5F (5520n-5546n) 5′-GTC CAC GAC AAC GTG GCT ATT TTA CCA-3′
B5R (6076n-6050n) 5′-ACA TAG TGG AAA GCA CTG GGT TCA AGC-3′
V14 (6309n-6333n) 5′-GAT TTG TCC ACC AGT GCT GGC TAC C-3′
V8 (7223n-7199n) 5′-GCG AAC GTG ATC CTG AGT GTT CCT A-3′.

PCR reactions were set up in 50 μl containing 100 ng of DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 1.25 U of AmpliTaq Gold (Perkin-Elmer Corp.), and 0.5 mM of each primer. Samples were preincubated for 9 min at 95°C, then subjected to 35 cycles of amplification as follows: 15 s at 94°C, 30 s at 60°C, and 1 min at 72°C. A final elongation step was carried out at 72°C for 10 min. Southern blot experiments were carried out as described (Pittoggi et al., 1999), using internal oligonucleotides as probes after end-labeled: V11 (843n-867n) (region between V9 and V10): 5′-GAT TCA GCT AGT AAC GCG GCT TCG A-3′; A3 (3554n-3532n) (region between A1 and A2): 5′-GTT GCA ATT GCA CCT TGC GAT TG-3′; V13 (4312n-4282n) (region between V5 and V4): 5′-GGT GTA TAG TTG AGA TTT GGT TTT CCA GCA-3′.

Preparation of Spermatozoa Nuclear Scaffolds and Immunoelectron Microscopy

The protocol for scaffold isolation from epididymal spermatozoa (Zoraqi and Spadafora, 1997) was adapted from protocols designed for somatic cells (Mirkovitch et al., 1988). In brief, nuclei from at least 10^7 spermatozoa were suspended in 500 μl of buffer (10 mM Tris, pH 8.0, 10 mM NaCl, and 5 mM $MgCl_2$) and incubated with 3,000 units of DNase I (Amersham) at 37°C for 3–4 h; another 3,000 DNase I units were then added and the incubation continued overnight. The mixture was then diluted by adding 14.5 ml of 2 M NaCl, 50 mM Tris-HCl, pH 8.0, 5 mM EDTA, and incubated at room temperature for 30 min under gentle shaking. Scaffolds were pelleted by centrifuging at 10,000 rpm (17,600 *g*) in a swing-out rotor for 15 min at 4°C. For electron microscopy, scaffolds were fixed in 3% *p*-formaldehyde/0.1% glutaraldehyde 1 h at 4°C, rinsed overnight in PBS/5% sucrose, and incubated for 30 min in PBS containing 50 mM NH_4Cl. After centrifugation, the pellets were dehydrated and embedded in Lowicryl K4M (Balzers) at −35°C (Carlemalm et al., 1985). Ultrathin sections cut from embedded blocks were mounted on gold grids and incubated overnight at 4°C with anti-HIV RT monoclonal antibody (Intracel Corporation) diluted in PBS containing 0.1% BSA and 1% goat serum (10 μg/ml final concentration), washed three times in PBS containing 0.1% BSA, and finally incubated with a 1:50 dilution (vol/vol) of anti–mouse IgG conjugated with 10-nm colloidal gold particles (Amersham) for 1 h at room temperature. After incubation, sections were rinsed, stained, and observed using transmission electron microscope CM10 (Philips Electronic Instruments Co.).

Results

Exogenous RNA Molecules are Internalized in Murine Spermatozoa

In previous work, we noticed that a fraction of sperm chromatin has a molecular organization typical of actively ex-

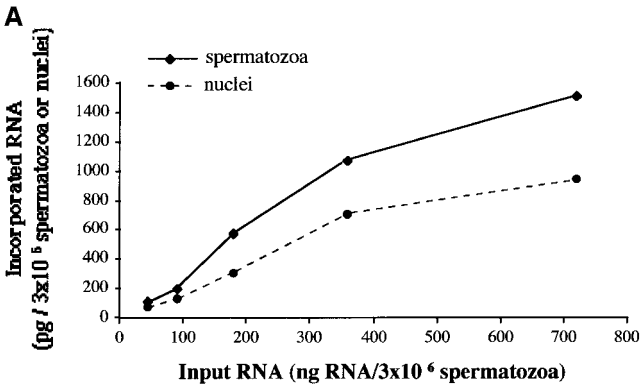
pressed nuclear domains and is enriched in LINE elements carrying uninterrupted ORFs (Pittoggi et al., 1999). To further develop those observations, we sought to establish whether a RT activity is present in murine epididymal sperm cells. We reasoned that if such an activity exists, then spermatozoa incubated with RNA should be able to retrotranscribe input RNA into cDNA copies.

We preliminarily characterized the association of poliovirus RNA with sperm cells and its nuclear internalization. Since it was essential to work with homogeneously pure RNA and avoid any possible DNA contamination, we used the 7,433-nucleotide-long poliovirus chromosomal RNA. This virus replicates through an RNA(−) strand, such that no DNA intermediate is present at any time during the replication process (reviewed by Richards and Ehrenfeld, 1990). We further verified the absence of contaminating DNA in poliovirus RNA preparations in preliminary PCR experiments with and without RT, and pretreating the RNA preparations with DNase I and RNase A (not shown). In the experiment shown in Fig. 1 A, increasing amounts of RNA were incubated with a constant number of sperm cells. After extensive washes, spermatozoa were either directly counted, or subjected to nuclei extraction before counting. As can be seen, RNA molecules bind to spermatozoa and are internalized into nuclei.

Nuclear internalization of exogenous RNA molecules was further confirmed by fluorescence microscopy. Representative results in Fig. 1 B, d, show specific signals within nuclei isolated from spermatozoa that were incubated with unlabeled poliovirus RNA and subsequently processed for FISH analysis using a poliovirus-specific probe. RNA molecules are not evenly distributed throughout sperm nuclei, but appear to preferentially concentrate on the subacrosomal segment of the head, a region of mature sperm cells that was previously characterized as the preferred site of binding by exogenous molecules (Spadafora, 1998).

A RT Activity in Mature Murine Spermatozoa

To follow up the fate of the exogenous RNA in spermatozoa the following experiments were designed. Sperm cells were incubated with poliovirus RNA in FM for 1 h and then split into two aliquots. The first one was used to purify nuclei, from which genomic DNA was extracted and extensively treated with RNase. The second one was used to fertilize oocytes in vitro, which were subsequently allowed to grow to the two-cell stage. Direct PCR amplification experiments were performed with both DNA extracted from spermatozoa and with two-cell lysed embryos. Various combinations of oligonucleotides were designed to cover almost the entire viral chromosome (Fig. 2 A). All oligonucleotide combinations shown in Fig. 2 A (see Materials and Methods) yielded amplified fragments of the expected length in control RT-PCR reactions



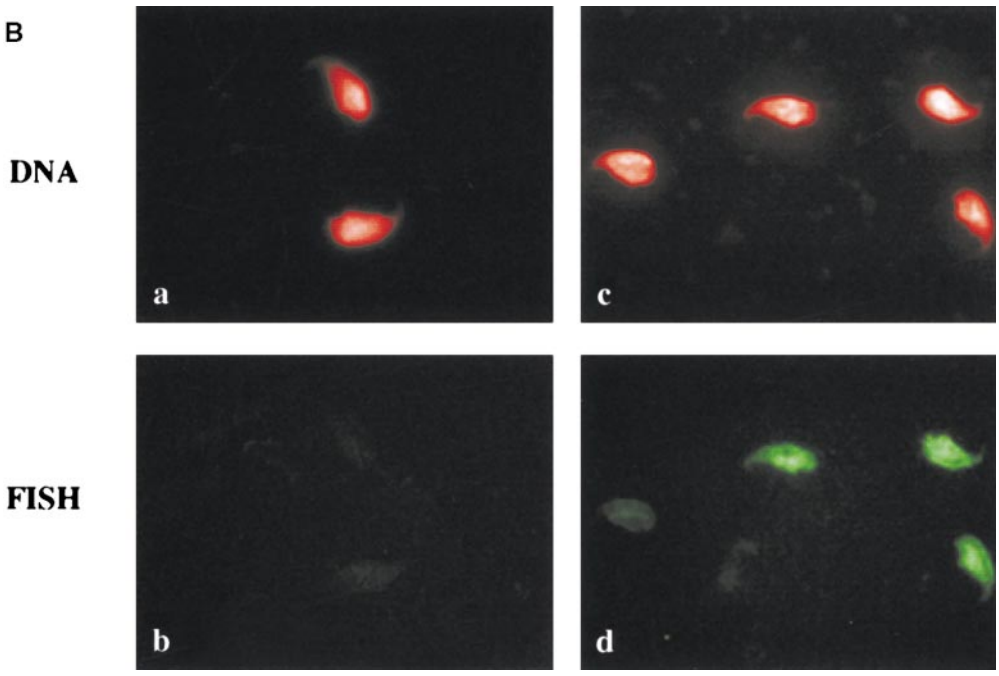


Figure 1. (A) Association of end-labeled poliovirus RNA with mouse epididymal sperm cells. Spermatozoa aliquots were incubated with 45, 90, 180, 360, and 720 ng of poliovirus RNA. After 30 min, spermatozoa from each incubation mixture were washed and divided in two aliquots. The first one was dissolved in soluene/toluene scintillation cocktail and counted to measure the RNA uptake; the second aliquot was used for nuclei purification, then treated and counted as for whole cells to measure nuclear internalization. (B) FISH localization of poliovirus RNA in isolated nuclei from RNA-challenged spermatozoa. (a and c) Sperm nuclei stained with DAPI and pseudocolored in red; (b) FISH experiment with nuclei from spermatozoa incubated with buffer only; (d) FISH experiment with nuclei from spermatozoa incubated with poliovirus RNA. The signal associated with the biotinylated probe is pseudocolored in green.

with poliovirus RNA (data not shown). The results of DNA amplification experiments from sperm cells and two-cell embryos are shown in Fig. 2, B and C, respectively. Three segments from the poliovirus RNA genome (solid boxes) were consistently and faithfully retrotranscribed in cDNA molecules in spermatozoa and subsequently transferred to the embryos. The oligonucleotide pairs V9/V10, A1/A2, and V5/V4 yielded amplified cDNA products of the expected length using DNA extracted from both spermatozoa that had been incubated with poliovirus RNA and from embryos. No amplification was detected using genomic DNA from sperm cells that had been incubated with buffer alone, nor from embryos obtained in IVF experiments using buffer-incubated spermatozoa. No retrotranscribed products were significantly amplified from the remaining portions of the viral chromosome (empty boxes), except for products occasionally detected using the V1/V10 and V14/V8 pairs. The faithfulness of retrotranscription for the three poliovirus regions that were consistently amplified was confirmed by hybridizing the amplified cDNA products with end-labeled internal oligonucleotides (Fig. 2, B and C). In addition, gel-purified amplified products were sequenced; this confirmed a 99% identity with the original poliovirus RNA segments (data not shown). Attempts were made to establish whether cDNAs were or were not integrated into the sperm genome, following two major experimental approaches. On the one hand, we constructed and screened a partial library using DNA extracted from spermatozoa incubated with poliovirus RNA, and, on the other hand, we employed a ligation-mediated PCR approach (Pfeifer et al., 1999). Both approaches failed to demonstrate a junction between viral and murine sequences (not shown), suggesting that the cDNA fragments remain in a nonintegrated state in sperm nuclei.

RT Is Associated with Sperm Nuclear Scaffolds

Results thus far are highly suggestive of the existence of a functionally active RT in mature spermatozoa. Hence, we sought to ascertain whether the enzyme could be directly visualized in mature sperm cells. Immunogold electron microscopy methods were employed to analyze sections of intact spermatozoa, purified nuclei, and high salt-extracted nuclear scaffolds. No positive gold staining was detected in intact sperm heads or purified nuclei, while clustered gold particles were reproducibly localized on sections of purified sperm scaffolds, as shown in Fig. 3. Clusters of gold particles were of different size (a–d), with some clusters being remarkably large (d) by comparison with an HIV particle from infected T-lymphocytes (e). No gold particles were revealed on scaffolds incubated only with secondary antibody (f). Most sections contained only one cluster of gold particles, though some samples contained two independent signals, such as shown (d). RT appears to be specifically and stably associated with the sperm nuclear scaffold, since it is neither removed by CTAB, the ionic detergent used for nuclei purification, nor in 2 M NaCl extraction step during the preparation of scaffolds. Control experiments ruled out the possibility that sperm nuclear scaffolds acted as sticky structures and caused antibodies to bind in a nonspecific manner. Anti-

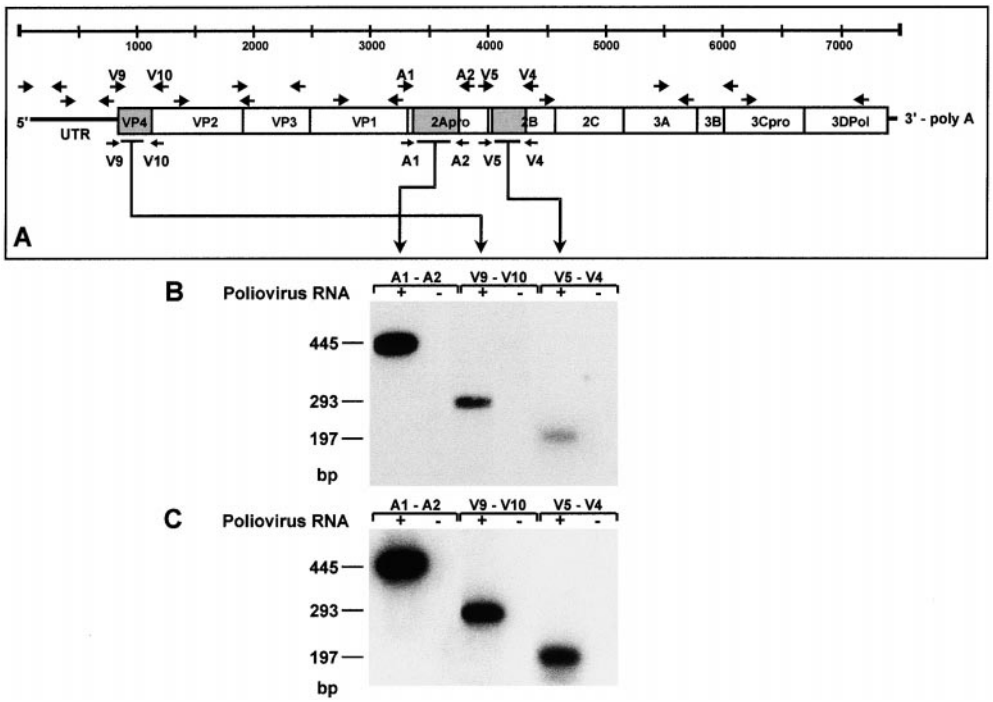


Figure 2. PCR amplification of poliovirus cDNA copies in sperm cells and two-cell embryos after incubation with poliovirus RNA. (A) Map of the 7,433-nt poliovirus RNA chromosome. Solid boxes indicate regions of the viral genome that were found to be amplified in the DNA extracted from both poliovirus RNA-incubated spermatozoa and from two-cell embryos. Arrows indicate the oligonucleotide combinations used to amplify the viral chromosome. Viral cDNAs fragments were PCR-amplified in DNA samples extracted from: (B) poliovirus RNA-challenged (+), but not from buffer-incubated (−) spermatozoa, and (C) two-cell embryos after egg fertilization with poliovirus RNA-incubated (+), but not with buffer-incubated (−), spermatozoa. Amplified cDNAs were visualized by hybridization with specific internal oligonucleotide probes.

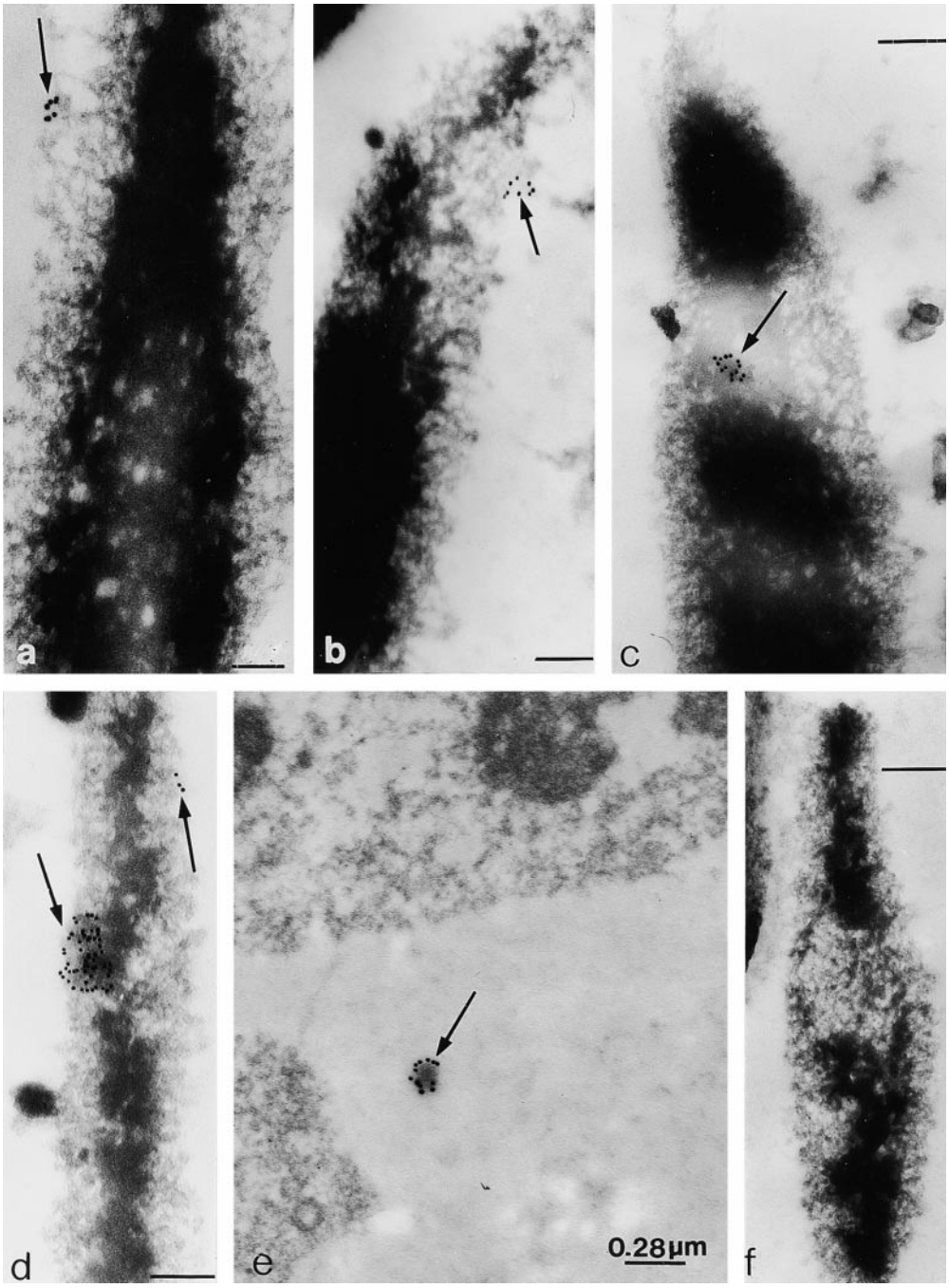


Figure 3. Localization of RT molecules (arrowed) on sperm nuclear scaffolds by immunogold electron microscopy. (a–d) Sperm nuclear scaffolds; (e) HIV-infected T-lymphocyte; (f) scaffolds incubated with secondary antibody conjugated with colloidal gold particles. Bars: (a, c, d, and f) 32 μm; (b) 25 μm.

bodies against proteins unrelated to RT, i.e., a monoclonal α-tubulin antibody and a polyclonal anti-actin antiserum, were tested with intact sperm cells and with purified scaffolds. We found that neither antibody binds to sperm scaffolds, nor did the secondary anti-mouse and anti-rabbit IgGs (not shown). Both primary antibodies were highly specific, because anti-tubulin binds the microtubules of the axoneme and anti-actin binds the fibrous sheath, as expected (not shown). Therefore, these experiments indicate that purified sperm scaffolds are not intrinsically sticky structures. Hence, the binding of anti-RT antibody to scaffolds can be regarded as a specific scaffold-bound antigen recognition.

Discussion

The present data represent the first evidence that an endogenous RT is active in mouse spermatozoa. The enzyme is localized on the sperm nuclear scaffold and its activity is revealed when spermatozoa are incubated with foreign RNA, eventually yielding retrotranscribed cDNA copies. In our experiments, retrotranscription did not cover the entire poliovirus RNA genome, but was confined to three genomic regions. The reasons for this specificity are unclear and several possibilities may be envisaged. First, it is possible that specific rearrangements occur in the viral chromosome upon exposure to sperm endoge-

nous nucleases. Nuclease activation was previously depicted upon spermatozoa incubation with exogenous DNA (Maione et al., 1997), and may also be triggered by RNA binding. It is worth noting that in some experiments occasional amplifications were obtained using oligonucleotide combinations V1/V10 and V14/V8, which amplify the 5′ and the 3′ end of the viral RNA, respectively. Products from such occasional amplifications were of smaller size than expected: this observation would support the view that the RNA is rearranged within sperm nuclei. Another, nonexclusive, possibility is that sperm RT preferentially binds to, and selectively retrotranscribes, specific RNA sequences. A third possibility is that regions of the viral RNA genome, after nuclear internalization, interact with proteins in ribonucleoprotein complexes that make the RNA locally inaccessible to the retrotranscription machinery. On the other hand, the specificity of amplification does not apparently reflect intrinsic structural hallmarks in the poliovirus genome, since no significant sequence or structural similarity was found among the amplified regions, nor did any of them share homology with known secondary structure-forming sequences. Control RT-PCR amplifications covering the entire viral chromosome using various oligonucleotide combinations produced DNA fragments of the expected size, and hence rule out the possibility that the amplification products detected here reflect experimental artifacts. Retrotranscribed cDNA fragments were detected in two-cell embryos, indicating that they were transmitted from sperm cells to zygotes through fertilization. These experiments together depict a novel unsuspected function in mature spermatozoa. We have recently found that a nucleohistone fraction of sperm chromatin is hypersensitive to cleavage by endogenous nuclease(s) and is enriched in retrotransposon DNA, particularly of the LINE/L1 family (Pittoggi et al., 1999), which is the major source of RT activity in eukaryotic cells. The present results formally identify the existence of functional RT in mature spermatozoa. It is possible that sperm RT molecules such as those implicated in the present experiments are the products of retroposon, and/or endogenous retroviral gene, expression which may occur during spermatogenesis. RT molecules appear to be retained as components of nuclear scaffolds in mature spermatozoa. The possibility of an exogenous retroviral infection can be excluded, since appropriate tests established that animals were germ free.

A close relation between RT, endogenous retroviruses and the male genital tract in the mouse was observed previously (Kiessling et al., 1987, 1989). Those reports showed that epididymal spermatozoa from healthy mouse males are absorbed with retroviral particles, and that mouse epididymis is a preferential site of retroviral expression. Experiments presented here were carried out with the CD1 strain. Since retroviral expression varies among strains, we also performed experiments using spermatozoa from the C57BL strain, in which the level of retrovirus expression is low (Kiessling et al., 1987). The results substantially confirmed those obtained with CD1 mice, yielding retrotranscription of the same A1-A2, V5-V4, and V9-V10 fragments (data not shown). Thus, the sperm RT activity identified here is not correlated with the extent of expression of retroviruses in the epididymis. It is also unlikely that sperm RT reflects a telomerase activity, based on recent evidence that telomerase is present in spermatogonia/primary spermatocytes, yet is not retained in mature spermatozoa (Wright et al., 1996; Eisenhauer et al., 1997; Ravindranath et al., 1997). On the other hand, a direct association between germ cells and mRNA retrotranscription was recently reported (Zhong and Kleene, 1999). The authors showed that reverse transcribed cDNA copies of lactate dehydrogenase-C mRNA, a testis-specific isoform, are present in meiotic and haploid spermatogenic cells; in addition, cDNA copies are not integrated in the genome.

The present findings suggest that RT is stored in spermatozoa in a potentially inducible condition; activity may be triggered upon interaction with RNA molecules. In this respect, RT appears to behave like other enzymatic activities identified in spermatozoa, including endogenous nucleases implicated in degradation events resembling those occurring in the apoptotic cascade (Maione et al., 1997; Zaccagnini et al., 1998), as well as enzymes catalyzing DNA recombination and integration events (Zoraqi and Spadafora, 1997).

In striking analogy with our results, though in a different cellular context, are the findings by Klenerman et al. (1997), who reported the synthesis of unintegrated cDNA fragments from the RNA-replicating lymphocytic choriomeningitis virus (LCMV) both in mice and in murine and hamster cultured cells expressing RT, but not in cell types lacking this activity. These data support the conclusion that foreign RNA can be retrotranscribed in cDNA when transfected in cells expressing RT of retrotransposon/endogenous retrovirus origin.

At this stage, it is not yet possible to draw conclusions on the physiological role, if any, of sperm RT. This enzyme was previously hypothesized to play a role in development and evolution (Temin, 1971, 1982). Sperm RT activity can reasonably be expected to be associated with the conformationally active nucleohistone subfraction of sperm chromatin, which is enriched in retrotransposon DNA (Pittoggi et al., 1999). The sperm nucleohistone component has also been implicated in early events after fertilization (Gatewood et al., 1987). Those observations, together with earlier findings that DNA-dependent DNA and RNA polymerases are also present in mammalian spermatozoa (Philippe and Chevaillier, 1976; Fuster et al., 1977), support the view that potentially active domains exist in the sperm genome. In this framework, RT may mediate the mobilization of retrotransposable elements from, or within, accessible histone chromatin domain(s). The potential ability of LINE-L1 to reshuffle the genome has been recently demonstrated by the finding that L1 elements integrate into transcribed genes by retrotransposing their 3′ sequences into new genomic locations (Moran et al., 1999). The results reported here raise the possibility that RT is involved in the reshuffling of genetic material in a subfraction of sperm chromatin: whether this reshuffling is random or specifically directed is a question which will require further work.

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