

Differential Expression and Subcellular Localization for Subunits of cAMP-Dependent Protein Kinase During Ram Spermatogenesis

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Abstract. The expression of mRNAs for the RI_α, RII_α, and C_α subunits of cAMP-dependent protein kinase has been studied in different ram germ cells. The sizes of the specific RI_α, RII_α, and C_α mRNAs, observed in germ cells were 1.6, 2.0, and 2.6 kb, respectively. RI_α and C_α mRNAs were mainly expressed in primary spermatocytes. A postmeiotic expression predominating in early spermatids was unique to RII_α mRNA. The location of RI, RII_α, and C subunits in well-defined organelles of ram spermatids and epididymal sperm was assessed by immunogold electron microscopy. In spermatids, RI, RII_α, and C were es-

entially present in the forming acrosome and, to a lesser extent, in the nucleus. During sperm epididymal maturation, the protein kinases disappeared from the acrosome and were detected in a variety of sperm functional areas, such as the tip of the acrosome, the motility apparatus, and the membrane network. The present study on subunits of cAMP-dependent protein kinase supports the concept that specific functions are attached to the different subunits in that it shows differential expression and differential subcellular localization in germ cells.

TYPE I and type II cAMP-dependent protein kinases are distinguished by their regulatory subunits RI and RII, respectively. Four different regulatory subunits (R) and two different catalytic subunits (C) for cAMP-dependent protein kinases have now been identified at the gene/mRNA level. These should be designated RI_α (formerly called RI), RI_β, RII_α (RII₅₄), RII_β (RII₅₁), C_α, C_β, (Øyen et al., 1988). In the early 1970's, the presence of type I and type II of cAMP-dependent protein kinases was demonstrated in bovine epididymal sperm (Hoskins et al., 1972; Garbers et al., 1973). Over the last few years, several different attempts have been made to detect the regulatory subunits RI and RII of cAMP-dependent protein kinases at the subcellular level in sperm (Pariset et al., 1984; Horowitz et al., 1984; Atherton et al., 1985; Noland et al., 1986; Horowitz et al., 1988; Paupard et al., 1988; Liebermann et al., 1988). Changes in cAMP-dependent protein kinase activity during spermatogenesis have been reported (Conti et al., 1983; Feinberg et al., 1983; Pariset et al., 1985). The expression of mRNAs for the cAMP-dependent protein kinase subunits in several testicular cell types has been determined (Øyen et al., 1987; Øyen et al., 1988). However, subcellular distribution at the electron microscope level of cAMP-dependent protein kinase subunits in germ cells during spermatogenic differentiation and epididymal maturation has not been described and interpreted along with the mRNA data. In the present work,

mRNA levels were measured by probing Northern blots of ram germ cell fractions with specific RI_α, RII_α cDNAs, or C_α oligonucleotide. The cAMP-dependent protein kinase subunits were characterized and localized at the subcellular level with the use of specific antibodies in immunoblotting and immunoelectron microscopy.

Materials and Methods

Ram Germ Cell Preparation

Ram spermatids were separated by elutriation following the method of Loir and Lanneau (1982). Ram caput, corpus, and cauda epididymal sperm were collected as described by Dacheux (1980). Flagella and heads were obtained by sonication followed by centrifugal elutriation as described by Pariset et al. (1984).

Preparation of Total RNA and Northern Analysis

RNA extraction from the elutriated ram germ cell fractions was performed by homogenization in guanidinium isothiocyanate as previously described (Øyen et al., 1987). The samples were electrophoresed on a 1.5% agarose gel containing formaldehyde as denaturing agent and with recirculating 20 mM sodium phosphate as running buffer, according to Øyen et al. (1987). 20 μg total RNA was used in each lane, separated on the gel and transferred to a nylon filter (ICN Radiochemicals, Irvine, CA) by capillary blotting technique. The resulting filter was prehybridized and hybridized using either nick-translated (α³²P-dCTP) cDNA probes (50% formamide, 5× SSC, 250 μg/ml denatured salmon sperm DNA, 42°C) or end-labeled

(γ - 32 P-ATP) oligonucleotide probes (40% formamide, $5\times$ SSC, 250 μ g/ml denatured salmon sperm DNA, 50 μ g/ml *Escherichia coli* tRNA, 42°C). Washing was performed with $0.5\times$ SSC at 50°C. The filter was autoradiographed.

Specific DNA Probes

The RI $_{\alpha}$ probe was a 0.8-kb Eco RI fragment containing 0.1 kb 5' noncoding sequence and 0.7 kb of the open reading frame from a human cDNA clone (Sandberg et al., 1987). The probe used for RII $_{\alpha}$ was a 1.3-kb Eco RI fragment representing 1.1 kb of coding sequence and 0.2 kb of 3' noncoding sequence from a human cDNA clone (Øyen et al., 1989). An oligonucleotide (40 mer) corresponding to the far 3' nontranslated region of human C $_{\alpha}$ cDNA (nucleotides 2,462–2,501; Maldonado and Hanks, 1988) was purchased from Genetic Design Inc. (Houston, TX).

Affinity-purified Antibodies to the cAMP-dependent Protein Kinase Subunits

The RI subunit from bovine skeletal muscle and the RII $_{\alpha}$ subunit from bovine heart muscle were purified to homogeneity by affinity chromatography according to Rannels et al., (1983) and Dills et al., (1979), respectively. Antibodies against these subunits were raised in rabbit as described by Lohmann et al. (1980). The C subunit was purified to homogeneity from bovine heart muscle, as reported by Demaille et al. (1977). After conjugation to keyhole limpet haemocyanin, antibodies were elicited in rabbit according to Schwach et al. (1980) and affinity purified, as previously described (Weinman et al., 1986a).

Immunoblotting

10^{10} cells were sonicated for 2 min in 5 vol of 4 mM EDTA, 100 mM

2-mercaptoethanol, 20 mM potassium phosphate buffer, pH 7.0 (buffer A), containing 2 μ g/ml antipain, 1 μ g/ml pepstatin, 1 μ g/ml leupeptin, and 100 μ g/ml aprotinin as antiproteolytic agents. The suspension was centrifuged at 100,000 g for 30 min. The supernatant referred to as the soluble fraction, was processed for SDS-PAGE. The pellet was extracted for 15 min in buffer A containing 10% SDS. It was sonicated and sedimented. The supernatant was referred to as the particulate fraction. In separate experiments, sperm membranes were solubilized in buffer A containing 1% NP-40. Soluble and particulate germ cell proteins (100 μ g) were electrophoresed on a 7% polyacrylamide gel according to Laemmli (1970) and transferred to nitrocellulose as described by Towbin et al. (1979). The antigens were detected with specific antibodies according to Weinman et al. (1986a).

Immunocytochemical Procedures

Cells were fixed in 0.5% glutaraldehyde, 5 mM CaCl $_2$, 0.1 M cacodylate buffer, pH 7.2, embedded in Lowicryl K4M, and processed for immunogold labeling, as previously described (Weinman et al., 1986a).

Results

Messenger RNAs for cAMP-dependent Protein Kinases in Ram Testicular Germ Cells

As shown in Fig. 1 A, the mRNAs for RI $_{\alpha}$, RII $_{\alpha}$, and C $_{\alpha}$ subunits of the cAMP-dependent protein kinases were detected in all of the elutriated ram germ cell fractions. Using the human RI $_{\alpha}$ cDNA probe, two mRNAs were detected at 1.6 and 3.2 kb. The high leveled message (1.6 kb) was de-

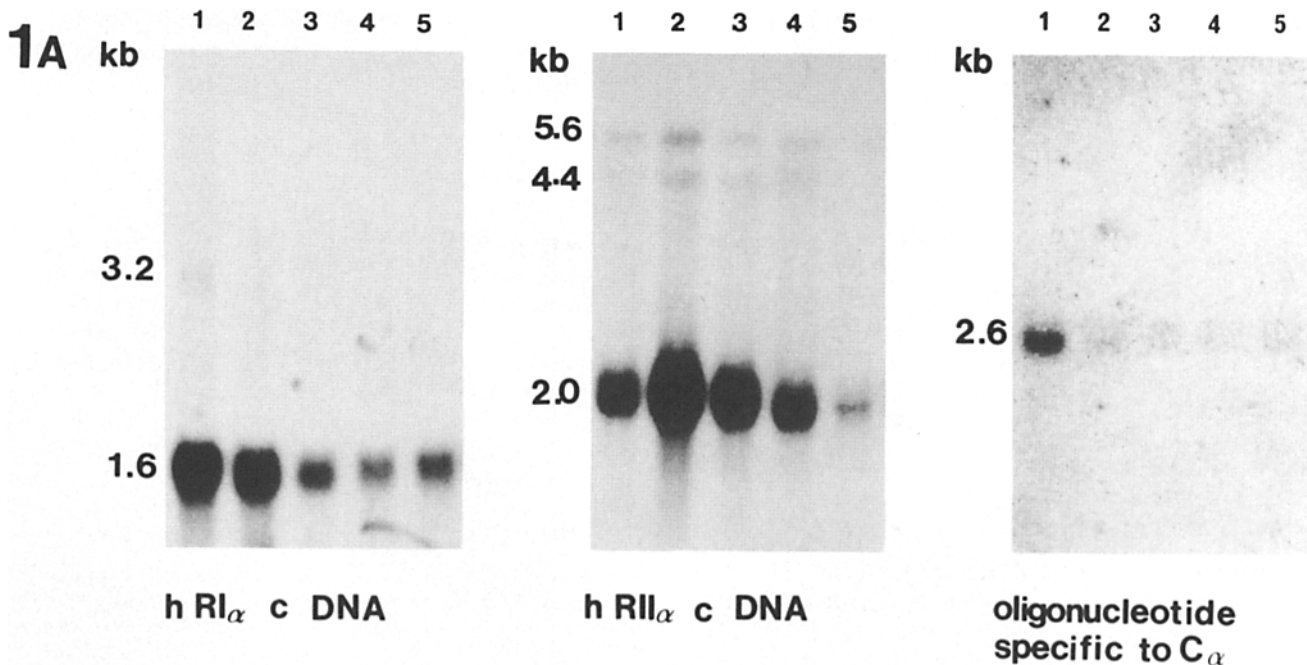


Figure 1. (A) Northern blot. Total RNA (20 μ g) from different ram germ cell fractions was probed with human RI $_{\alpha}$, RII $_{\alpha}$ cDNAs, and a C $_{\alpha}$ specific oligonucleotide, as described in Materials and Methods. Lane 1, primary spermatocytes (95%); lane 2, early round spermatids (95%); lane 3, late round spermatids (95%); lane 4, late round spermatids (60%) and elongating spermatids (40%); lane 5, elongated spermatids (98%). The percentage in parentheses in this figure legend indicates the purity of the cell preparations used. The size of the different mRNAs is expressed in kilobases. (B) Characterization of the antibody to RII $_{\alpha}$ and identification of the RI, RII $_{\alpha}$, and C subunits of the cAMP-dependent protein kinase in ram germ cell and epididymal sperm. (Part a) Purified rat brain RII $_{\alpha} + \beta$ (lane 1) and bovine heart RII $_{\alpha}$ (lane 2) were coelectrophoresed with ram testis (lane 3) and epididymal sperm (lane 4) extracts on 7% acrylamide gels. The proteins were detected with Coomassie brilliant blue G or antibody to RII $_{\alpha}$ after transfer to a nitrocellulose membrane. RI (part b), RII $_{\alpha}$ (part c), and C (part d) subunits were immunodetected on Western blots in soluble (lanes 2, 4, 6, 8, 10, and 11) and particulate (lanes 3, 5, 7, and 9) fractions, as described in Materials and Methods. Extracts from primary spermatocytes (lanes 2 and 3), round spermatids (lanes 4 and 5), elongated spermatids (lanes 6 and 7), cauda epididymal sperm (lanes 8 and 9), flagella (lane 10), heads (lane 11), and 1% NP-40 sperm extract (lane 12) were compared with the corresponding purified antigens (lane 1).

tected mainly in the primary spermatocyte fraction. Its intensity decreased from primary spermatocytes to elongated spermatids. The amount of 3.2-kb mRNA present was much less. With the use of the same filter and probing with human RII α cDNA three mRNAs at 5.6, 4.4, and 2.0 kb were detected. High level expression of the 2.0-kb mRNA was demonstrated. It reached its peak intensity in early round spermatids and gradually decreased to low levels in elongated spermatids. The larger mRNAs 5.6 and 4.4 kb were present at very low levels. When the filter was probed with the C α specific oligonucleotide, only one band was seen at 2.6 kb. C α mRNA appeared in the primary spermatocyte fraction and decreased more markedly than RII α mRNA in spermatids.

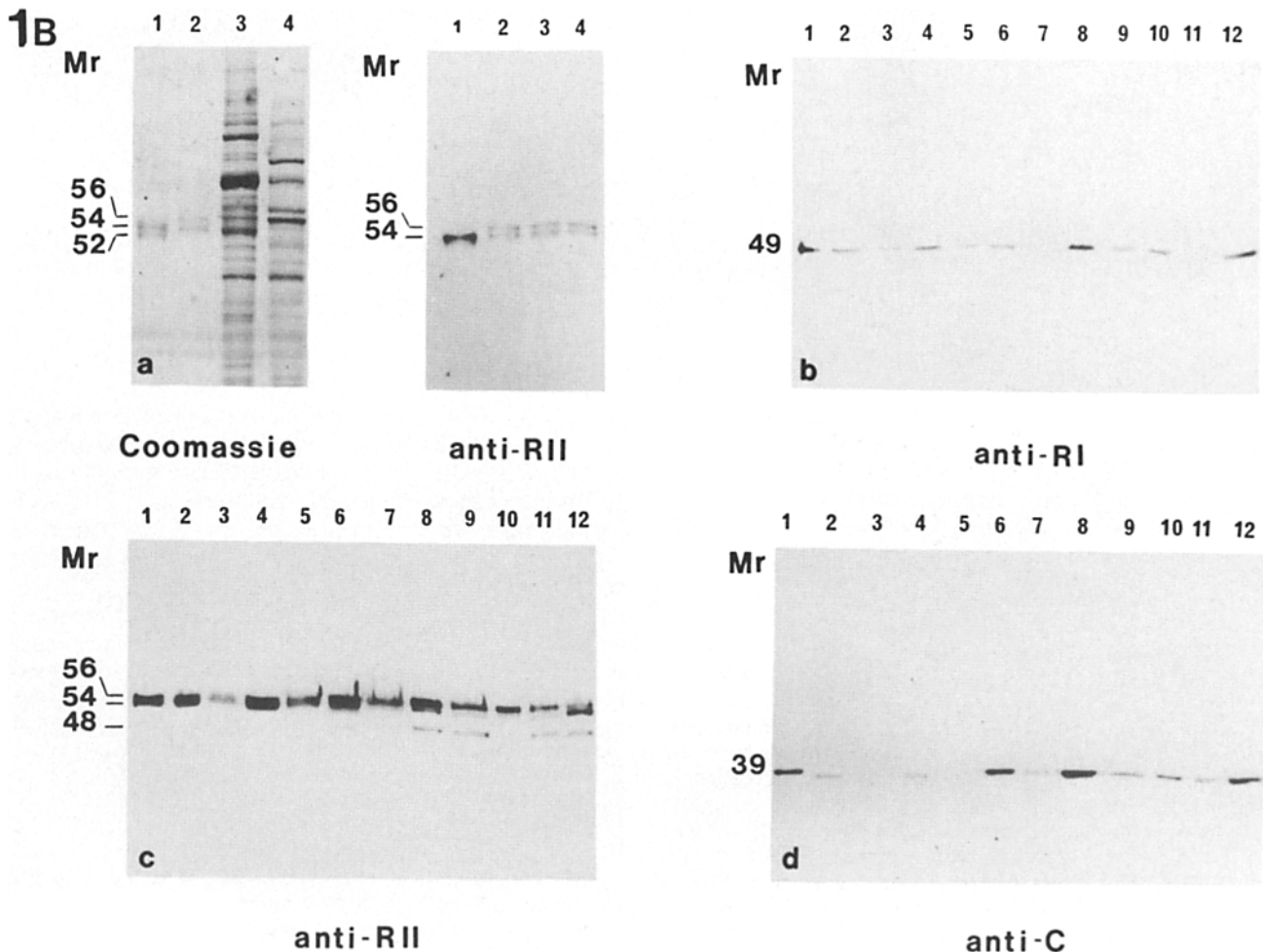
Characterization of the Antibody to RII Subunit

To characterize the antibody to RII α , purified rat brain RII α (54 kD) and RII β (52 kD) and purified bovine heart RII α (56–54 kD) were run on a 7% polyacrylamide gel. As shown in Fig. 1 B part a, the antibody detected only one band at 54 kD in the purified rat brain RII, whereas it recognized a 56–54-kD doublet both in purified bovine heart RII and ram extracts. Thus, the antibody is specific to the RII α subunit. The 56–54 kD doublet do most probably represent phosphorylated and dephosphorylated forms of RII α .

Identification of cAMP-dependent Protein Kinase Subunits in Ram Germ Cells and Sperm

The monospecificity of the affinity-purified IgGs to RI, RII α , and C subunits and the identification of cAMP-dependent protein kinase subunits in soluble and particulate extracts of ram spermatids and sperm are shown in Fig. 1 B, parts a, b, c, and d.

As determined by immunoblot analysis, the IgGs against RI, RII α , or C subunits recognized only proteins of 49, 56–54, or 39 kD, respectively. Purified bovine skeletal muscle RI, heart RII α , and C subunits comigrated with these respective bands. As demonstrated in Fig. 1 B part b, generally more RI was detected in soluble than in particulate fractions of testis germ cells (lanes 2–7). The same was seen for cauda epididymal sperm (lanes 8 and 9). This antigen was also detected in the soluble fraction from sperm flagella but not in the heads. Moreover, RI was present in the 1% NP-40 extract of sperm. The doublet at 56–54-kD specifically detected by the RII α antibody was observed in all the extracts (Fig. 1 B; part c, lanes 2–12). It predominated in the soluble fractions. It increased however from spermatocyte to sperm in the particulate fractions. The C subunit (Fig. 1 B, part d) was mostly present in a soluble form in the testis germ cells and sperm (lanes 2, 4, 6, and 8), as well as in flagella and heads (lanes 10 and 11). The NP-40 extract was also immunoreactive for this antigen (lane 12).



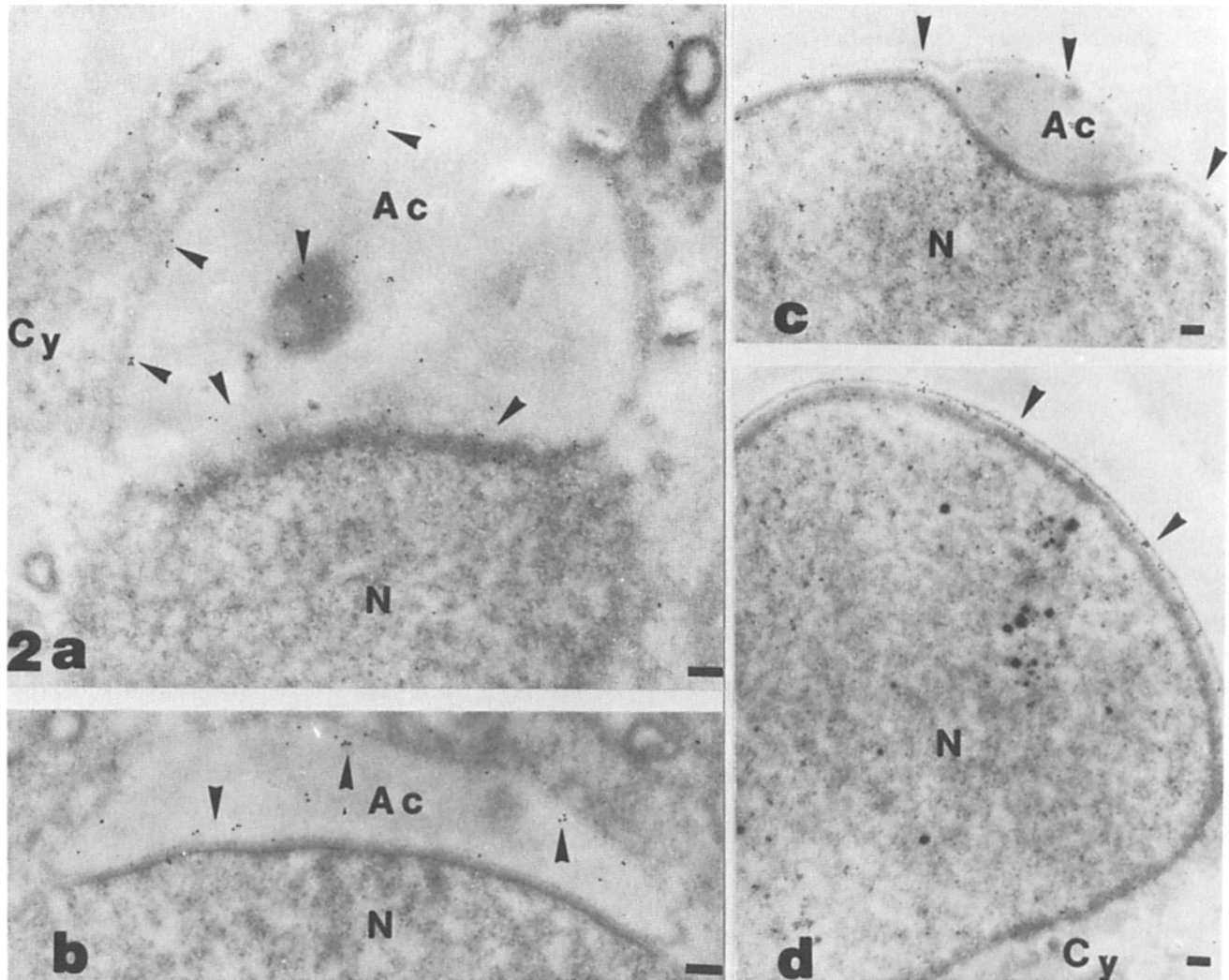


Figure 2. Immunogold electron micrographs of ram spermatids using antibody against the RI subunit of cAMP-dependent protein kinase. Gold particles are sparsely present throughout the nucleus (*N*) and the cytoplasm (*Cy*). Most of the gold particles are confined to the forming acrosome (*Ac*, arrowheads) in early (*a* and *b*), round (*c*), and elongating (*d*) spermatids. The proacrosome of early spermatids (*a*) is also labeled. Bars, 200 nm.

Subcellular Distribution of RI, RII_α, and C Subunits in Ram Testis and Epididymal Germ Cells

Ram spermatids and epididymal sperm were examined by immunogold labeling. Ultrastructural location of the subunits was investigated through the use of affinity-purified antibodies against RI, RII_α, and C subunits. In all cases, specificity of immunolabeling has been carefully assessed by comparison to labeling patterns obtained with preimmune IgGs or with antibodies preabsorbed with an excess of the complementary antigen. Under these conditions, very few background signals could be observed (micrographs not shown).

In early spermatids (Figs. 2, 3, and 4), immunogold staining was essentially associated with the nucleus and the forming acrosome, where the three subunits were broadly codistributed. A diffuse labeling was also present throughout the cytoplasm. Fig. 2 shows the location of the RI subunit in spermatids. These cells contained relatively low levels of type I cAMP-dependent protein kinase. A few gold particles

were randomly present over nuclear and cytoplasmic areas and the RI subunit was most usually located in the forming acrosome. The distribution of the regulatory subunit RII_α in spermatids is shown in Fig. 3. In all these cells, RII_α labeling was more abundant than RI labeling. A few patches of gold particles could be seen all over the nucleus and at the boundary between the acrosome and the nucleus. RII_α subunit was localized all over the forming acrosome, throughout the acrosomal matrix, and close to the acrosomal membrane far from the nucleus. As shown in Fig. 4, the C subunit exhibited a similar distribution. A significant gold label was seen over the nucleus. The forming acrosome was stained. Moreover, a noticeable level of immunoreactivity was detectable in the cytoplasm. Labeling could also be seen for the RI, RII_α, and C subunits in the cytoplasmic droplets and residual bodies (data not shown).

In late spermatids and epididymal sperm (Figs. 5, 6, and 7), obvious changes in protein kinase subunit location occurred. Fig. 5 shows the low concentration of RI subunit. Immunoreactive sites were only observed on the head mem-

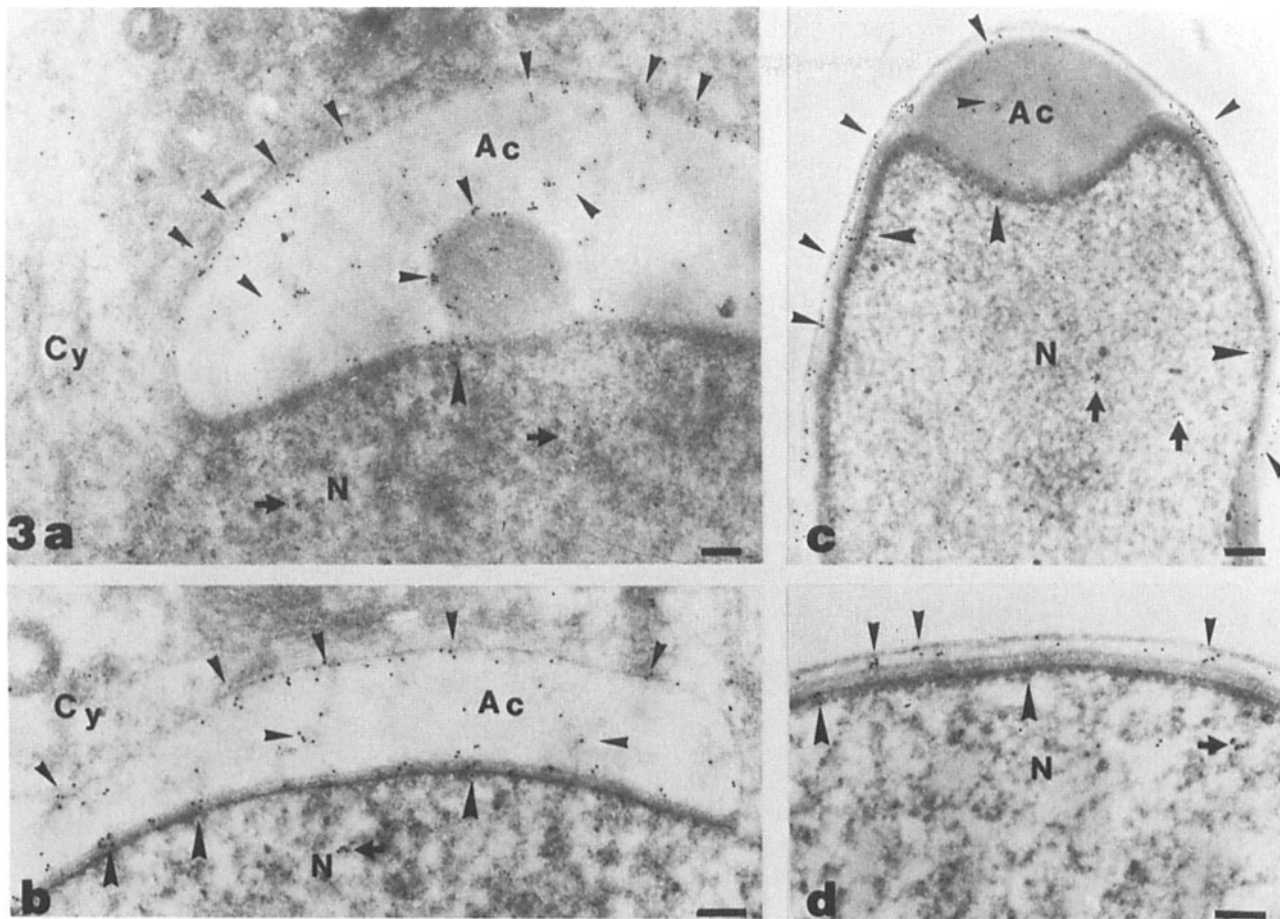


Figure 3. Immunogold electron micrographs of ram spermatids using antibody against the RII α subunit of cAMP-dependent protein kinase. Gold particles are detected in the nucleus (N, arrows). The cytoplasm (Cy) is randomly labeled. Gold particles are highly concentrated all over the forming acrosome on the acrosomal granule, near to the acrosomal membrane away from the nucleus (Ac, small arrowheads) and at the acrosome/nucleus boundary (large arrowheads) in early (a and b) and elongating (c and d) spermatids. Bars, 200 nm.

branes of elongating spermatids (Fig. 5, a and b) and epididymal sperm (Fig. 5 c). Fig. 6 highlights the specific localization of the RII α subunit. In the heads of late spermatids and testicular sperm, RII α was preferentially associated with the nucleus and the acrosome (Fig. 6 a). In caput epididymal sperm, the same pattern could be seen, but the intensity of RII α immunolabeling on the acrosome was lower (Fig. 6 b). In corpus and cauda epididymal sperm, the nucleus still exhibited patches of gold particles, whereas there was no longer any immunoreactivity over the acrosome except at its tip (Fig. 6 c). In the flagellum of sperm from caput as well as from corpus and cauda epididymidis, a noticeable labeling was associated with the axoneme and the fibrous sheath (Fig. 6, d-g, i, and j). Moreover, in corpus and cauda epididymal sperm, a unique localization of RII α (Fig. 6, g and h) appeared between coarse fibers and mitochondria. Immunogold staining of late spermatids and epididymal sperm by C subunit antibodies is shown in Fig. 7. The distribution of the C subunit coincided with that of the RI and RII α subunits. Gold particles could also be seen in midpieces over the mitochondria (Fig. 7, e and i).

Morphometric Analysis of cAMP-dependent Protein Kinase Staining Density

Quantitation of the cAMP-dependent protein kinase staining

density in spermatids and sperm is shown in Table I. These data allow for a comparison of the relative labeling densities for each of the cAMP-dependent protein kinase subunits in the nucleus and acrosome during spermatogenesis and sperm epididymal maturation. The most striking result of this analysis is a significant decrease in the labeling intensity for the three subunits in the acrosome of early to elongated spermatids and epididymal sperm. In the nucleus, the labeling density remained at a constant level for RI and C subunits, whereas an apparent increase was observed for the RII α subunit.

Discussion

Cell Specific Expression of mRNA for cAMP-dependent Protein Kinase Subunits

Our results show specific expression of the RI α , RII α , and C α mRNAs in ram germ cells similar to the data obtained in rat testis germ cells (Øyen et al., 1987). Unique smaller sized mRNAs are preferentially expressed during spermatogenesis. Larger sized ("somatic") mRNAs are only faintly detected in ram germinal cells. A premeiotic expression pattern is common to rat and ram RI α , and C α mRNAs. The occurrence of a postmeiotic pattern of expression has been

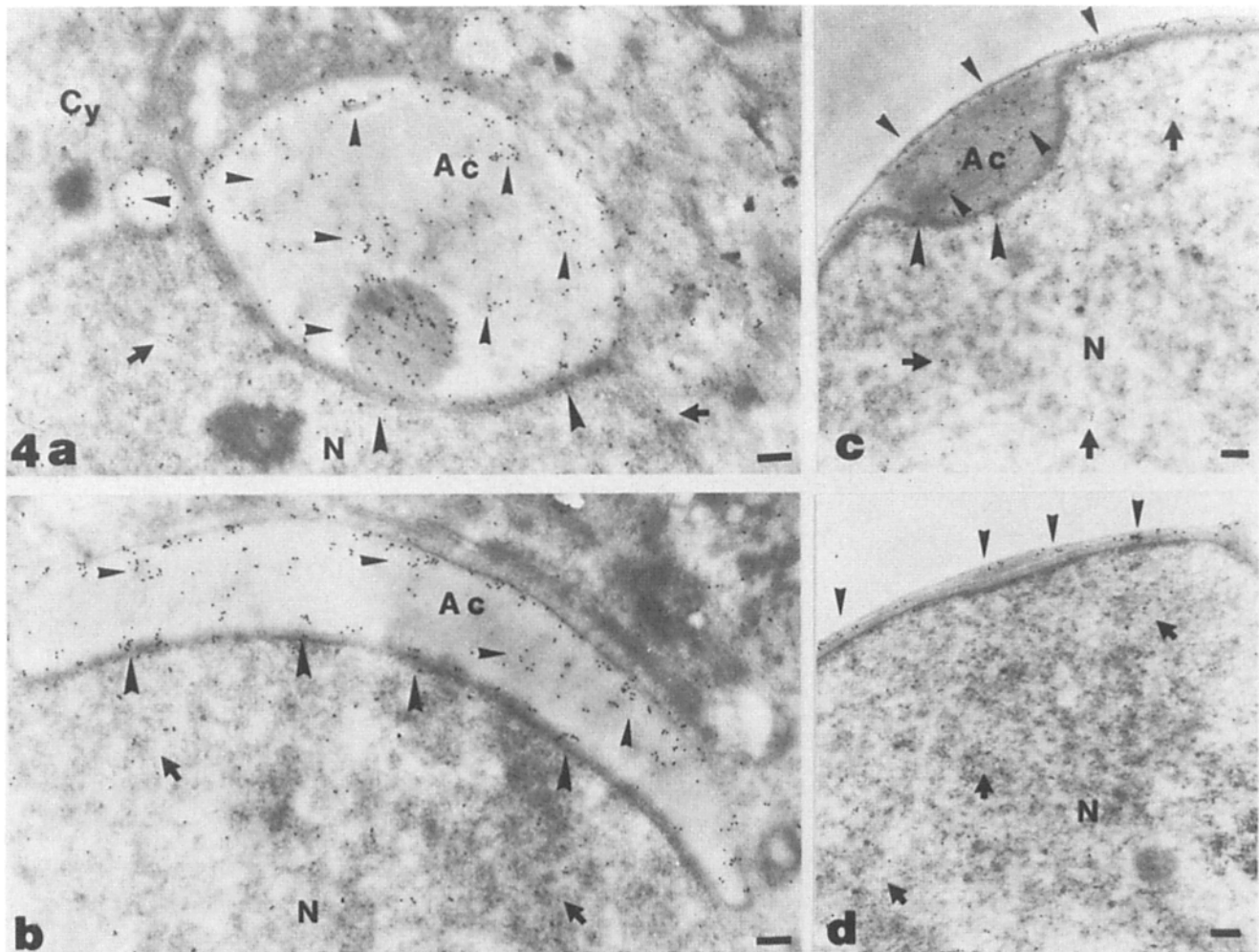


Figure 4. Immunogold electron micrographs of ram spermatids using antibody against the C subunit of the cAMP-dependent protein kinase. The micrographs show a preferential location of gold particles all over the areas labeled by the RI and RII_α subunits: the nucleus (*N*, *arrows*), the forming acrosome (*Ac*, *small arrowheads*), the boundary between acrosome and nucleus (*large arrowheads*). The cytoplasm (*Cy*) is also labeled. (*a* and *b*) Early spermatid; (*c*) round spermatid; (*d*) elongating spermatid. Bars, 200 nm.

observed only for RII_α mRNA. The low degree of contamination of the ram early spermatid fraction by primary spermatocytes (<3% of the total cell number) strongly supports this conclusion. All these observations are in agreement with Oyen et al. (1987) results on isolated rat germ cells.

Subcellular Distribution and Possible Role of cAMP-dependent Protein Kinases

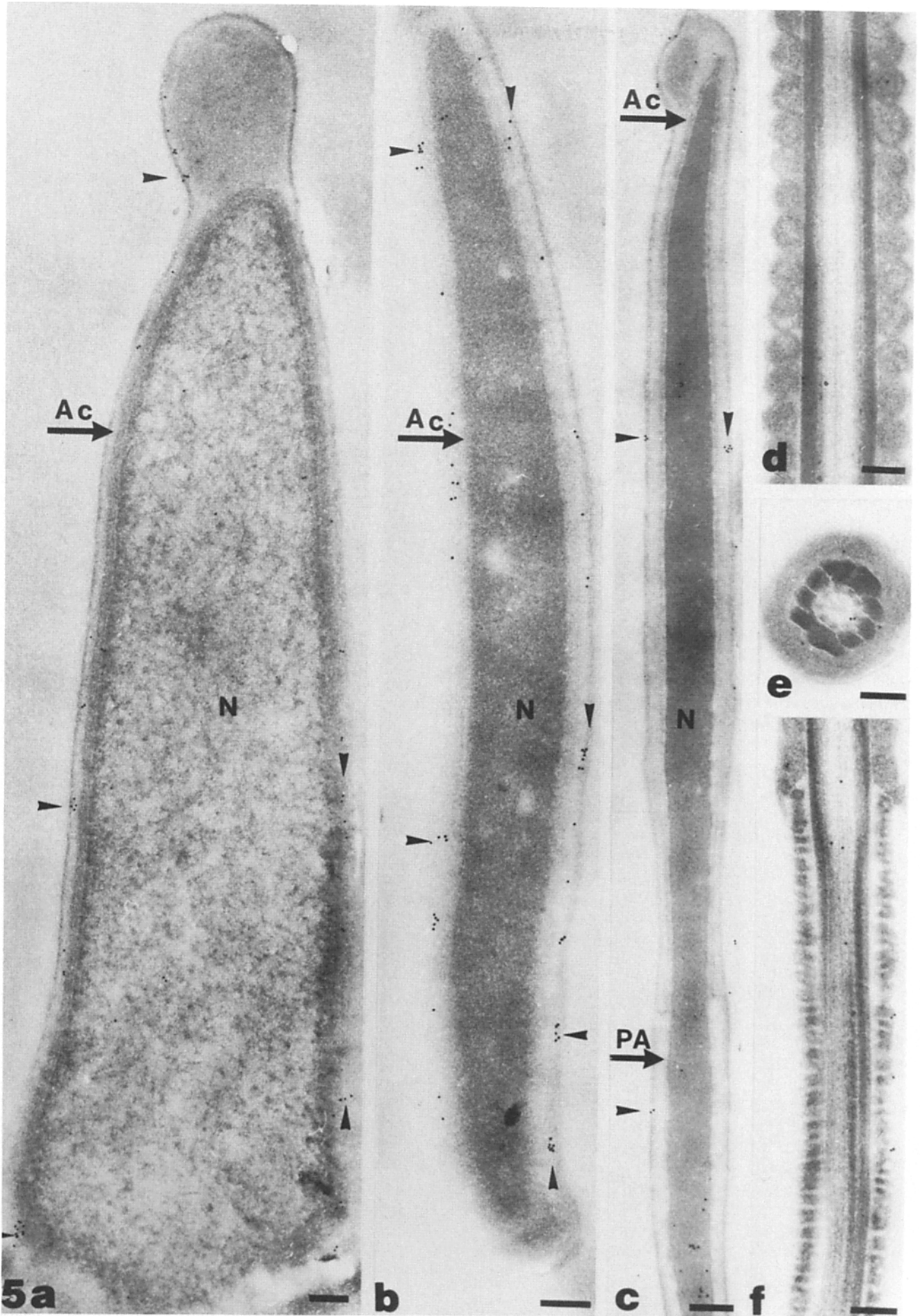
The three antibodies used in the present study proved to cross react specifically with the corresponding proteins in ram germ cell extracts (Fig. 1 *B* parts *a-d*). As shown in Fig. 1 *B* part *a*, our antibody to RII_α subunit from bovine heart was shown to be specific to its reported phosphorylated

and dephosphorylated forms, 56–54 kD, respectively (Robinson-Steiner et al., 1984). Immunoblotting detected the subunits of cAMP-dependent protein kinases in soluble and particulate fractions of ram testis germ cells and epididymal spermatozoa (Fig. 1 *B*). Interestingly, the amount of membrane-bound RII_α protein increased during germ cell differentiation and followed the increase in mRNA of RII_α.

The present study provides the first description of the ultrastructural distribution of the cAMP-dependent protein kinase subunits during ram germ cell differentiation and epididymal sperm maturation. The physiological relevance of the redistribution and the role of cAMP-dependent protein kinases in ram germ cell differentiation will be discussed.

The ultrastructural study provides evidence for the pres-

Figure 5. Immunogold electron micrographs of ram late spermatids and epididymal sperm using antibody against the RI subunit of cAMP-dependent protein kinase. Patches of gold particles (*arrowheads*) are located on the plasma membrane of late spermatids (*a* and *b*) and caput epididymal sperm (*c*). The labeling decreases with the maturation of the cells but remains at the tip of the acrosome. Gold particles are present throughout the flagellum of caput epididymal sperm. More precisely, they are detected in midpieces between coarse fibers and mitochondria (*d* and *e*) and in principal pieces near to the axoneme and on the fibrous sheath. Bars, 200 nm.



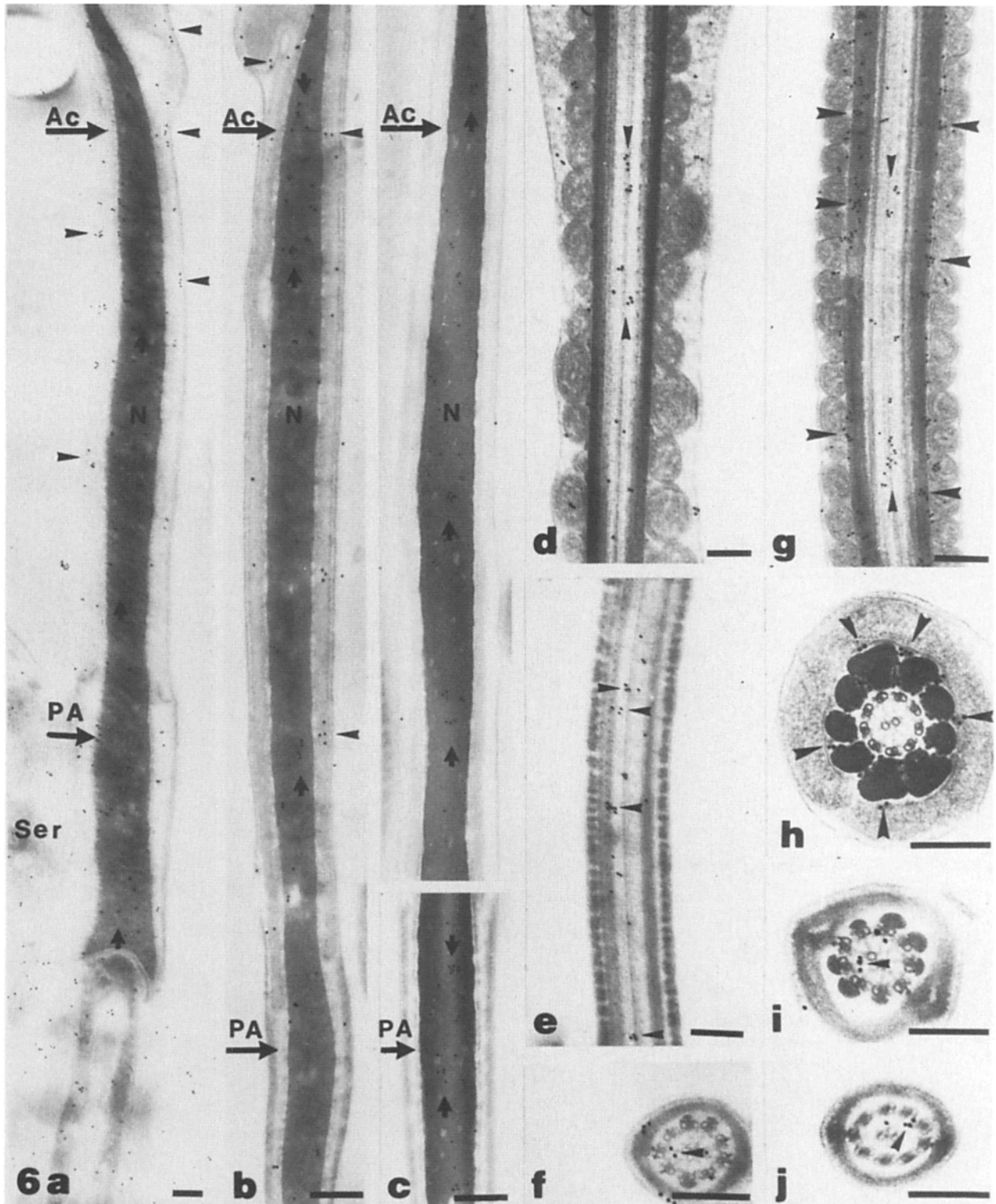


Figure 6. Immunogold electron micrographs of ram late spermatids and epididymal sperm using antibody against the RII α subunit of cAMP-dependent protein kinase. The acrosome (*Ac*, *arrowheads*) is intensely labeled in testicular sperm (*a*) and, to a lesser extent, in caput epididymal sperm (*b*). In cauda epididymal sperm (*c*), few gold particles are found at the tip of the acrosome. Note the absence of immunolabeling in the postacrosomal area (*PA*) of epididymal sperm (*b* and *c*). Gold particles are associated with the axoneme all along the flagellum (*d-g*, *i*, and *j*, *small arrowheads*) and with the fibrous sheath in principal and end pieces (*e*, *f*, *i*, and *j*, *small arrowheads*). Labeling is present between the coarse fibers and the mitochondria of sperm from corpus and cauda epididymidis (*g* and *h*, *large arrowheads*). *Ser*, Sertoli cell. Bars, 200 nm.

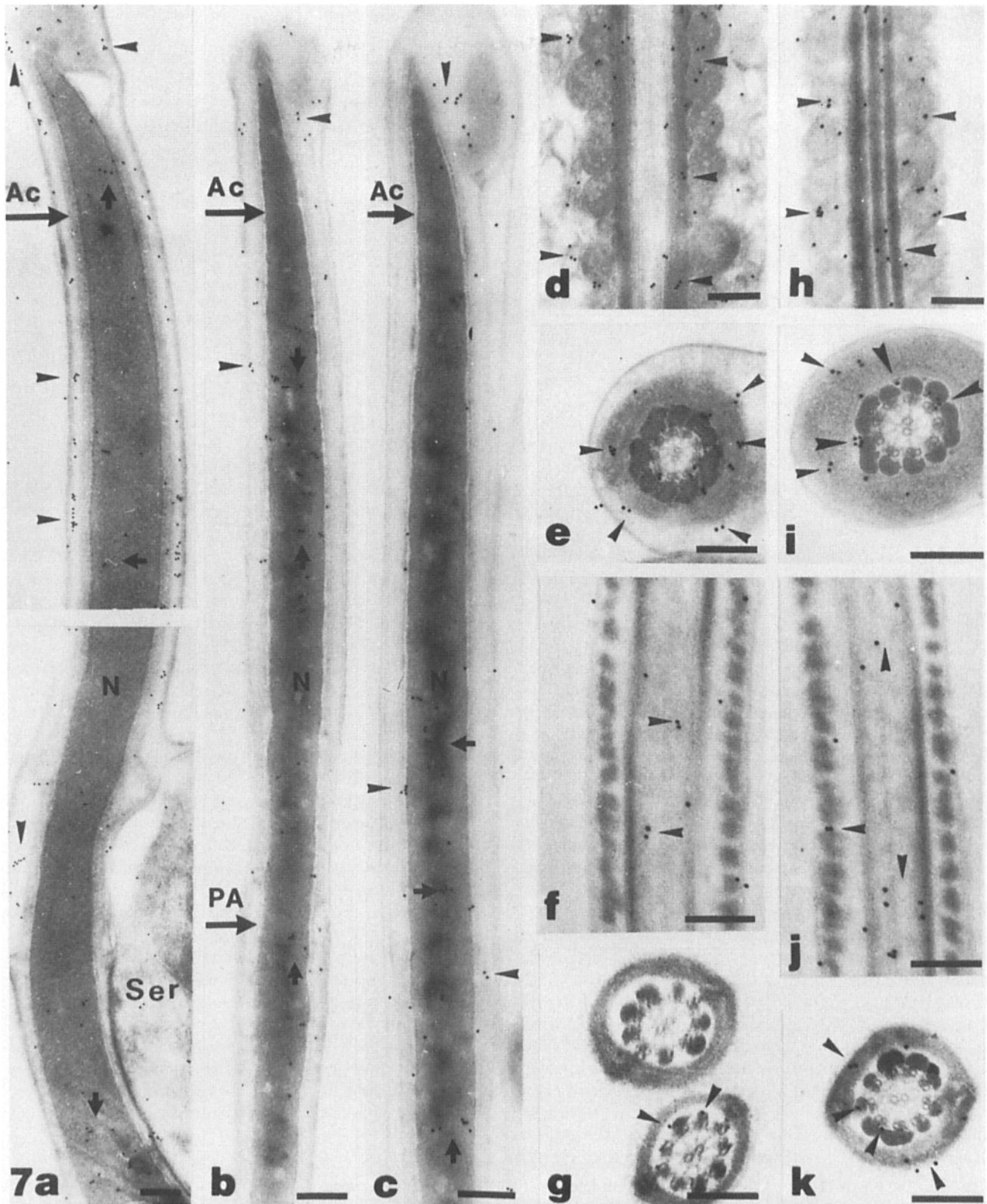


Figure 7. Immunogold electron micrographs of ram late spermatids and epididymal sperm using antibody against the C subunit of cAMP-dependent protein kinase. The micrographs show localization of gold particles all over the areas labeled by RI and RII subunits, i.e., the nucleus (*N*, arrows) in late spermatids (*a*), caput epididymal sperm (*b*), and cauda epididymal sperm (*c*); the acrosome in late spermatids (*a*) and caput epididymal sperm (*b*); the fibrous sheath (*k*); and the space located between coarse fibers and mitochondria (*h* and *i*) (small arrowheads). Moreover, mitochondria are also labeled in caput and cauda epididymal sperm (*e*, *h*, and *i*; small arrowheads). *Ser*, Sertoli cell. Bars, 200 nm.

Table 1. Morphometric Quantitation of cAMP-dependent Protein Kinase Subunit Localization in Ram Germ Cells

Cell type	Staining intensity					
	Nucleus			Acrosome		
	RI	RII _α	C	RI	RII _α	C
	(gold particles/μm ² ± SEM)					
Round spermatids	3.8 ± 0.4	4.3 ± 0.7	12.8 ± 0.5	36.1 ± 1.3	43.0 ± 2.5	89.2 ± 2.6
Elongating spermatids	2.7 ± 0.3	6.3 ± 0.8	16.8 ± 0.4	29.8 ± 3.1	49.2 ± 5.4	61.0 ± 4.5
Elongated spermatids	4.7 ± 0.2	41.3 ± 3.0	15.1 ± 1.1	9.0 ± 0.6	53.1 ± 5.1	30.3 ± 4.3
Caput epididymal sperm	4.1 ± 1.3	41.9 ± 2.6	11.8 ± 2.1	6.8 ± 1.3	20.2 ± 1.8	10.2 ± 2.6
Corpus epididymal sperm	8.7 ± 1.9	35.0 ± 2.2	4.4 ± 1.0	8.2 ± 2.9	3.4 ± 0.7	8.1 ± 2.4
Cauda epididymal sperm	3.7 ± 0.7	47.1 ± 2.1	7.5 ± 2.2	2.2 ± 0.7	7.4 ± 1.6	10 ± 1.3

Ram spermatids and epididymal sperm were fixed in 0.5% glutaraldehyde and sections were processed using the immunogold procedure, as described in Materials and Methods. The data concerning the acrosome include both membrane- and matrix-associated immunolabeling. For each measure, eight cells were analyzed.

ence of cAMP-dependent protein kinases, with a marked predominance of type II, in spermatid and sperm nuclei, in accordance with the immunoblot analysis. Morphometric quantitation of the relative labeling density of the nucleus indicated a modulation in the concentration of each protein during spermiogenesis. Taking into account the fact that, in ram, the nuclear volume decreases from 235 μm³ in round and elongating spermatids to 30–15 μm³ in elongated spermatids and epididymal sperm, it can be concluded that RI and C subunits disappeared progressively from the nucleus whereas the RII_α subunit remained at an approximately constant level during spermatogenesis.

We have demonstrated the presence of cAMP-dependent protein kinases in the acrosome of spermatids during spermiogenesis and the progressive disappearance of such a location in sperm during epididymal maturation. In early spermatids, RII_α and C subunits and, to a lesser extent, the RI subunit, were concentrated in the acrosome. The RI, RII_α, and C-specific labeling predominated on the external side (i.e., opposite the nucleus) of this organelle. The *trans* acrosomal localization of the cAMP-dependent protein kinase subunits in round and elongating spermatids reflects the Golgi origin of this organelle (Nigg et al., 1985; De Camilli et al., 1986). During epididymal maturation, a decrease in acrosome cAMP-dependent protein kinase level occurred. No cAMP-dependent protein kinase could be visualized in cauda epididymal sperm acrosome, except at its tip. The disappearance of cAMP-dependent protein kinase in the maturing sperm acrosome contrasts strongly with the presence of high levels of these enzymes in the spermatid acrosome. Considering the progressive decrease in the acrosome volume from 12–10 μm³ to 6–4 μm³ during ram spermiogenesis, the morphometric analysis indicates a marked decrease in the concentration of the three subunits in the acrosome from early to late spermatids and epididymal sperm. The appearance of cAMP-dependent protein kinase subunits in spermatid flagella occurred simultaneously with this decrease. Similar changes in acrosomal calmodulin contents have been described (Weinman et al., 1986b). The simultaneous presence of these two major regulatory proteins in the spermatid acrosome, and their absence in the cauda epididymal sperm acrosome suggest that a concerted modulation by cAMP and Ca²⁺ takes place in the acrosome during spermiogenesis.

The spermatid acrosome might constitute an internal storage of these two types of regulatory proteins. In the known absence of any protein synthesis in elongated spermatids (Dadoune et al., 1981), the appearance of the three cAMP-dependent protein kinase subunits in mature sperm functional areas could be explained by the exportation of the proteins previously stored in the acrosome.

Furthermore, we have detected that RII_α and C subunits were associated with the sperm motility apparatus. Our study has demonstrated a close association between RII_α and C subunits and the sperm axoneme. In addition, a specific localization of the RII_α subunit between the coarse fibers and the mitochondria occurs during epididymal transit as sperm acquire flagellar motility. These findings concur with the recently reported stimulatory effects of cAMP and ATP on reactivated sperm motility (Tash et al., 1984, 1986). According to these authors, dynein and RII phosphorylations are necessary for triggering and maintaining sperm flagellar motility. We have previously reported a dramatic rise in the cAMP-dependent protein kinase activity correlated to the acquisition of flagellar beat during epididymal maturation of sperm (Pariset et al., 1985). The distribution of RII_α and calmodulin in sperm flagellum (Weinman et al., 1986a,b) supports the concept of a concerted regulation of sperm motility by cAMP and Ca²⁺.

In conclusion, we have demonstrated differential expression and subcellular localization of cAMP-dependent protein kinase subunits in ram germ cells during germ cell differentiation. Our data indicate that different subunits of cAMP-dependent protein kinase have specific functions in these cells. Much more work is needed to understand, at the molecular level, how sperm functions are regulated, most probably in a concerted manner with other second messenger systems.

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