Introduction

Alkaline phosphatases (APs, E.C.3.1.3.1., orthophosphoric monoester phosphohydrolases) are ubiquitous enzymes capable of hydrolyzing a wide variety of monophosphate esters at alkaline pH optima. These enzymes exist in nature in species as different as *E. coli* and man, suggesting that APs have fundamental biological roles (for review see McComb et al., 1979). The in vivo function(s) of APs, however, have not been resolved and the physiological substrates have not been identified. APs are membrane-bound glycoproteins encoded by a multigene family composed of four loci in humans and three active loci in the mouse. The isozymes can be distinguished by their thermostability, inhibition kinetics, electrophoretic mobilities and immunoreactivity (for review see Harris, 1989). The tissue non-specific AP (TNAP) is expressed in a wide variety of tissues, including bone, liver, kidney, early placenta, etc. The enzyme is heat-labile and its activity inhibited uncompetitively by L-homoarginine (Fishman and Sie, 1971). Three human and two mouse APs are designated tissue-specific (TSAPs) and show a highly restricted pattern of expression. The human intestinal AP (IAP) and placental AP (PLAP) are primarily expressed in the tissues that confer their name (McComb et al., 1979) and the germ cell AP (GCAP) isozyme is expressed in the testis (Chang et al., 1980), primordial germ cells (Hustin et al., 1987) and also in trace amounts in the thymus (Goldstein et al., 1982). The TSAP isozymes are more heat-stable, resisting temperatures of up to 70°C as in the case of PLAP and GCAP, and are inhibited uncompetitively by L-phenylalanine (Fishman and Sie, 1971). PLAP and GCAP are often overexpressed in cancer (Fishman et al., 1976), particularly ovarian serous cystadeno-carcinoma (Vergote et al., 1987; Nozawa et al., 1989) and testicular seminoma (Lange et al., 1982; Jeppsson et al., 1983), where they serve as useful follow-up markers.

We have recently cloned three TSAP genes from the mouse genome, i.e., embryonic AP (EAP), intestinal AP (IAP) and a putative pseudogene, using the human PLAP cDNA as a probe (Manes et al., 1990). Reverse-transcriptase polymerase chain reaction amplification of RNA revealed that the EAP gene is expressed in the two-cell to blastocyst stage of preimplantation development in the mouse. The isozyme is re-expressed in trace amounts in the thymus, intestine and testis during adult life. In the present report, we find that EAP transcripts can be detected, by RT-PCR analysis, in very low amounts in the testes of newborn mice, but at 24 days of age EAP mRNA levels reach the highest concentrations, remaining high at 40 and even 117 days of age. We produced a synthetic peptide and a corresponding rabbit anti-peptide antiserum (Rb-1434), which was characterized by enzyme antigen immunoassays and reactivity with Chinese hamster ovary cell transfectants, as reacting specifically with EAP. The Rb-1434 antibody enabled us to examine immunohistochemically what cell types in the testis are responsible for the expression of EAP during different developmental stages. No positive cells were recognized in the testis of newborns (day 0) and 8-day-old mice. Positive cells were first observed at day 15 and, at 24 days of age, many positive M-phase cells, morphologically corresponding to spermatocytes in mid to late prophase of meiotic division I, were strongly positive for EAP expression. Positive M-phase cells were also observed at 40 days and 151 days of age. Transgenic mice expressing the human GCAP isozyme in a tissue-specific manner in the testis, showed equivalent stages of M-phase figures when stained immunohistochemically with a specific rabbit polyclonal antiserum. Our results demonstrate that EAP is expressed in M-phase spermatogenic cells during postnatal development, suggesting a role for this AP isozyme during meiosis.

Key words: alkaline phosphatase, mouse embryo, M-phase, spermatogenic cells.
A 24-mer peptide was synthesized on an Applied Biosystems 430A peptide Synthesizer using t-Boc protection. The peptide was cleaved using the TFMSA cleavage protocol according to the Applied Biosystems User Bulletin, Issue No.16. The released peptide was purified by reverse-phase high-potential liquid chromatography. Custom antibody production was performed at East Acres Biologicals (Southbridge, MA). The peptide was conjugated to Keyhole limpet hemocyanin and used to immunize two rabbits. Serum was obtained from the rabbits prior to the initial immunization. The rabbits were boosted on days 21, 42 and 63 and weekly production bleeds commenced on day 70. Animals were boosted monthly during the course of the production bleeds. The rabbit antibodies were purified by ion-exchange chromatography and one part of the immunoglobulin fraction was biotinylated with N-hydroxysuccinimide biotin ester in 1 M NaHCO$_3$ buffer (pH 8.5).

ELISA designs

Microtiter plates were coated with 10 µg/ml of rabbit anti-PLAP antibody (DAKO) in 0.1 M NaHCO$_3$, pH 8.0. The wells were then saturated with a blocking solution composed of 10% casein in Dulbecco’s phosphate-buffered saline (PBS) to reduce non-specific binding. Subsequently, a solution containing 10 µg/ml of biotinylated Rb-1434 antibody and increasing amounts of EAP (0 to 2.8 µg/ml) were added to the microtiter plate and incubated for 3 hours at 37°C. The bound biotinylated antibody was measured using the avidin-biotin-peroxidase procedure (Vecto Lab.) at 492 nm.

For antibody competition experiments, a mixture of 10 µg/ml of biotinylated Rb-1434 and 0.25 µg/ml EAP extract were preincubated in the presence of increasing amounts (0 to 1 mg/ml) of unlabeled Rb-1434 and subsequently added to the microtiter wells coated with anti-PLAP antibody. After 3 hours the bound biotinylated Rb-1434 antibody was measured with the avidin-biotin-peroxidase procedure.

**Materials and methods**

**Stable transfections**

A 4.5 kb EAP and a 5.0 kb IAP genomic fragment (Manes et al., 1990) were subcloned into the pSVT7 mammalian expression vector using standard procedures. An EcoRI site was introduced upstream of the ATG in exon I by PCR to simplify subcloning of the pSV2-dhfr plasmid (Schimke, 1982) was co-transfected to allow selection of the stable transfectants in nucleoside-free culture medium. The stably transfected CHO cells were extracted with n-butanol as described (Narisawa et al., 1989) and the extracts were concentrated with Cen-upstream of the ATG in exon I by PCR to simplify subcloning of the antigenic marker of carcinoma in situ of the testis (Paiva et al., 1983; Koide et al., 1987). However, the cell type(s) expressing this isozyme in the normal testis have not been identified due to difficulties in obtaining and preserving tissue samples for study.

Recently, results obtained in our laboratory with transgenic mice harboring the entire GCAP gene indicated high expression of GCAP in the male germ cells (unpublished data). These results suggested that the germ cell lineage might be responsible for the endogenous expression of EAP in the mouse testis. In this paper we present evidence that EAP is expressed in M-phase spermatogenic cells during postnatal development, suggesting a role of this AP isozyme during meiosis.

**Peptide synthesis and antibody production**

A 24-mer peptide was synthesized on an Applied Biosystems 430A peptide Synthesizer using t-Boc protection. The peptide was cleaved using the TFMSA cleavage protocol according to the Applied Biosystems User Bulletin, Issue No.16. The released peptide was purified by reverse-phase high-potential liquid chromatography. Custom antibody production was performed at East Acres Biologicals (Southbridge, MA). The peptide was conjugated to Keyhole limpet hemocyanin and used to immunize two rabbits. Serum was obtained from the rabbits prior to the initial immunization. The rabbits were boosted on days 21, 42 and 63 and weekly production bleeds commenced on day 70. Animals were boosted monthly during the course of the production bleeds. The rabbit antibodies were purified by ion-exchange chromatography and one part of the immunoglobulin fraction was biotinylated with N-hydroxysuccinimide biotin ester in 1 M NaHCO$_3$ buffer (pH 8.5).

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**Histochemistry and immunohistochemistry**

Testes from FVB mice of different ages were fixed with 10% formalin in PBS for 24 hours, washed with increasing concentrations of sucrose in PBS(10%, 15%, 20%), embedded in OCT compound (Miles Lab), frozen in a dry ice-ethanol bath and maintained at −80°C. 5 µm sections were cut using a Reichert cryostat and mounted on ovoalbumin-coated glass slides. For histochemical stainings, fresh sections were washed in PBS containing 1 mM MgCl$_2$ and incubated in freshly mixed substrate solution made of one volume of 0.2 mg of Naphtol AS-MX phosphate (sodium salt, Sigma) per ml of water and one volume of 1.2 mg FAST Violet B salt (4-bensoylamino-2-methoxy-S-methyl benzenediazonium chloride hemi [zinc chloride] salt, Sigma) per ml of 0.2 M Tris-HCl (pH 8.9), at room temperature for 60 minutes. Slides were washed in water and mounted in glycerol. In the case of the fluorescent simultaneous azo-dye technique, 1.2 mg/ml FAST Red TR (4 chloro-2-methyl benzene-diazonium salt, hemi [zinc chloride] salt, Sigma) was used instead of FAST Violet Salt and the slides were observed with a fluorescence microscope under G excitation.

In order to test the heat-stability of AP in tissue sections, slides were immersed in prewarmed PBS containing 1 mM MgCl$_2$ at 65°C for exactly 5 minutes prior to incubation in the staining solution. For inhibition tests with L-amino acids, sections were preincubated with 10 mM L-phenylalanine or L-homoarginine in 0.2 M Tris-HCl (pH 8.9) for 45 minutes and incubated with the staining solution containing 5 mM L-phenylalanine or L-homoarginine.

**Northern blot and RT-PCR analysis**

Total cellular RNA was extracted by the acid-guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987). Poly(A)$^+$ RNA was isolated from total cellular RNA by standard oligo(dT)-cellulose column chromatography. 10 µg of...
poly(A)⁺ RNA or total cellular RNA were electrophoresed on 1% agarose gels containing 2% formaldehyde. The separated RNAs were transferred to DBM paper (TRANSA-BIND, Schleicher & Schuell) and the filter was probed with a 438 bp fragment of the mouse EAP cDNA generated by RT-PCR from mouse testis RNA (see below). The blot was hybridized at 45°C in 50% formamide and washed with three changes of 0.1× SSPE-0.1% SDS at 45°C during 2 hours.

For RT-PCR analysis, total RNA was dissolved in water, heat-denatured at 94°C for 2 minutes and reverse transcribed (RT) in a reaction mixture containing 40 mM KCl, 50 mM Tris-HCl (pH, 8.3, at 42°C), 10 mM MgCl₂, 0.1 µg/ml BSA, 1 mM dATP, 1 mM dTTP, 1 mM dGTP, 1 mM dCTP, 1.25 i.u./µl RNasin (Promega, WI), 0.1 i.u./µl oligo(dT)₁₅ (Promega, WI), 1.1 i.u./µl reverse transcriptase (Avian Myeloblastosis Virus, Seikagaku America Inc. MD). After incubation at 42°C for 60 minutes, 10 µg E. coli tRNA was added as carrier and the mixture was extracted with phenol-chloroform and precipitated with ethanol. The RT reaction was dissolved in water, heat denatured at 94°C for 8 minutes, chilled on ice and subjected to polymerase chain reaction (PCR) amplification with primers specific for the EAP gene. The primers were derived from the EAP genomic sequence (Manes et al., 1990) as described in Hahnel et al. (1990): the 5′ EAP primer is identical to nucleotides 2464 through 2484 and anneals within exon IX, but was extended in this report to include an EcoRI site upstream from the homology sequence: its 3′ partner is the reverse complement of nucleotides 3082 through 3101 annealing within exon XI and was extended by a BamHI site for easy subcloning into KS+ bluestrip (Stratagene, CA). These primers should amplify a 438 bp fragment from EAP cDNA and a 638 bp fragment from genomic DNA. The PCR reaction mixture contained 10 mM Tris-HCl (pH, 9.3), 50 mM KCl, 1.5 mM MgCl₂, 0.1 mg/ml gelatin (Sigma, MO), 0.2 mM dATP, 0.2 mM dTTP, 0.2 mM dGTP, 0.2 mM dCTP, 1.0 µM of both the forward and reverse primers and 0.03 i.u./µl Taq polymerase (Perkin Elmer Cetus, CT) and was incubated in a thermal controller (MJ Research Inc., MA). Each cycle consisted of annealing at 70°C for 2 minutes, extension at 72°C for 3 minutes and denaturing at 94°C for 1 minute. After 40 cycles, 20 µl of each reaction was loaded on a 1.8% agarose gel and electrophoresed.

Results

The mouse testis expresses considerable amounts of AP activity (approx. 100 i.u./mg), characterized biochemically as TNAP. Additionally, 1% to 3% of the total AP activity is contributed by a heat-stable, L-phenylalanine-sensitive TSAP isozyme. This situation is identical to the human testis where the expressed TSAP isozyme has been found to be GCAP. Histochemical stainings of mouse testis sections reveal that peritubular cells and endothelial cells are strongly AP positive, while dividing germ cells are weakly positive (Fig. 1A, D). Using a fluorescent azo-dye technique (Ziomek et al., 1990), positive M-phase cells are observed, as shown in Fig. 1E. The activity in peritubular cells and endothelial cells corresponds to the expression of TNSAP, as shown by the absence of a GCAP signal in two of the mouse testis samples derived from transgenic mice expressing GCAP transcripts in the testis. Reprobing of the filters using a TNAP-specific probe indicated that the predominant AP mRNA species in the mouse testis is TNAP (Fig. 2B), in agreement with the total AP activity and histochemical results.

EAP transcripts were detected, by RT-PCR analysis, in very low amounts in the testes of newborn mice, but at 24 days of age EAP mRNA levels reached the highest concentrations, remaining high at 40 and even 117 days of age (Fig. 3A). EAP expression was confirmed in the two-cell through the blastocyst stage of embryonic development as well as in the intestine, and even in the D3 embryo-derived stem cell line (Fig. 3B). The latter was established from cells in the inner cell mass of blastocysts (Doetschman et al., 1985). An immortalized germ cell line (GC-1spg), corresponding to a stage between spermatagonia type B and pre-leptotene spermatocyte (Hofmann et al., 1992), was also found to express the EAP transcript (Fig. 3B).

Production of EAP-specific antisera

In order to identify the cell type expressing the EAP isozone in testis, EAP-specific anti-peptide antisera were produced in rabbits. A region of EAP, which resides in a surface loop on top of the molecule, was selected to synthesize a peptide for production of antibody (Table 1). This region is likely to be exposed and accessible for antibody recognition due to estimates of its high hydrophilicity and solvent accessibility. The sequence (Table 1) and secondary structure predictions indicated that this region of EAP is considerably different from the corresponding regions of the other mouse AP isozymes. The peptide and corresponding antisera were produced as described in the Materials and methods section. While antisera were produced in two rabbits, Rb-1434 reached a higher titer and was used for all the experiments reported in this paper. As antigen for characterization, we used recombinant EAP expressed in vitro in a Chinese Hamster Ovary cell system because of the advantage of obtaining sufficient quantities of EAP from the stable transfectants, without the need to sacrifice large numbers of animals for isolation of early stage embryos, for enzyme extraction.
In order to characterize the reactivity of the Rb-1434 rabbit anti-EAP peptide antibody, we analyzed the capture of EAP bound to biotinylated Rb-1434 by immobilized anti-human PLAP antibody. In this simultaneous incubation experiment, the ascending part of the curve represents formation of a ternary complex between the anti-PLAP antibody, EAP and the biotinylated Rb-1434. As expected (Fig. 4A), increasing concentrations of EAP promote the progressive formation of the ternary complex, while at higher concentrations, the decreasing part of the curve represents competition between high concentrations of free EAP and anti-PLAP bound EAP for the limited amount of biotinylated Rb-1434. The capture of EAP-biotinylated Rb-1434 complex by anti-PLAP also decreased in competition experiments using increasing concentrations of non-labeled Rb-1434 antibody (Fig. 4B). The experiment in Fig. 4A shows that the binding of the anti-peptide antiserum to the plate is mediated by the antigen and therefore reflects binding to EAP, while the experiment in Fig. 4B confirms that the binding is not an artefact due to biotinylation. Therefore, these results demonstrate that the reaction between EAP and antibody Rb-1434 is specific.

The possible cross-reactivity of Rb-1434 anti-EAP antibody with IAP could not be analyzed with competition experiments due to the much lower cross-reactivity of the anti-PLAP antibody to mouse IAP. However, using native gel electrophoresis on a Phast System (Pharmacia LKB Biotechnology, NJ), concentrations of Rb-1434 that effectively retarded EAP had no effect on IAP (not shown). Furthermore, we used CHO cells stably transfected with the EAP and IAP genes expressing large amounts of the respective isozymes (Fig. 5) to demonstrate specific immunostaining with Rb-1434. Immunohistochemical analysis of these cells with the Rb-1434 antipeptide antibody produced positive staining results with the EAP-CHO stable transfecants but were negative with the IAP-CHO transfecants (Fig. 5). The antibody Rb-1434 is, therefore, specific for EAP and does not cross-react with IAP.

**Developmental expression**

The Rb-1434 antibody enabled us to examine by immunohistochemical staining what cell types in the testis are responsible for the expression of EAP during different developmental stages. No positive cells were recognized in the testis of newborns (day 0) and 8-day-old mice (Fig. 6A, B). At 15 days, clearly positive dividing cells are observed (Fig. 6C). At 24 days of age, many positive M-phase cells, morphologically corresponding to spermatocytes that are in mid to late prophase of meiotic division I, are strongly positive for EAP expression (Fig. 6D). Positive M-phase cells are also observed at 40 days and 151 days of age (Fig. 6E-G). Transgenic mice expressing the human GCAP isozyme in a tissue-specific manner in the testis, show equivalent
Alkaline phosphatase expression in mouse embryo stages of M-phase figures when stained immunohistochemically with a specific rabbit polyclonal antiserum (Fig. 6H).

**Discussion**

The existence of a distinct EAP gene was not recognized until Lepire and Ziomek (1989) reported the expression of a molecule related to human PLAP in the preimplantation stages of mouse development. After cloning the EAP and related genes from mouse genomic libraries (Manes et al., 1990), we were able to demonstrate the expression of the EAP mRNA in the two-cell to blastocyst stage preimplantation development, as well as in the thymus, intestine and testis.

In the present report we have been able to develop a specific antipeptide antiserum that reacts specifically with EAP. This antibody has allowed us to characterize the cell types expressing EAP in the mouse testis at different developmental stages. We find that only M-phase spermatogenic cells express EAP in the testis. The appearance of cells clearly positive for EAP expression coincides with the onset of meiosis. According to previous studies (Bellvé et al., 1977), at day 15 in the mouse, the most advanced spermatogenic cells are pachytene spermatocytes. The morphology and location of the cells that stain positively for EAP indicate that these cells correspond to spermatocytes in mid or late prophase of meiotic division I. The positively stained cells are large (typical of pachytene cells and not of spermatids or spermatogonia), are located at least 1-2 cell layers away from the basement membrane and show large clumps of chromatin, typical of meiosis I. All of the stained cells in metaphase are located towards the lumen,

### Table 1. Sequence of the synthetic EAP peptide used for production of EAP-specific antisera and sequence comparison with the equivalent regions of mouse IAP and TNAP isozymes

<table>
<thead>
<tr>
<th>AP isozyme</th>
<th>Corresponding peptide sequence in the AP isozymes</th>
<th>No. 405</th>
<th>No. 428</th>
</tr>
</thead>
<tbody>
<tr>
<td>m. EAP</td>
<td>K L H N G A K A D V T E E E S S N P T Y Q Q Q A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m. IAP</td>
<td>V G - T G E R P N V T A A E S S G S S Y R R Q A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m. TNAP</td>
<td>K V V D G E R E N V S M V D Y A H N N Y Q A Q S</td>
<td></td>
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</tbody>
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The numbers indicate the first and last amino acids in the entire primary sequence of the isozymes.

Fig. 5. Immunoreactivity of Rb-1434 anti-EAP antiserum against Chinese hamster ovary (CHO) cells stably transfected with the EAP gene (panels A and D), IAP gene (panels B and E) and non-transfected wild-type CHO cells (panels C and F). Panels A, B and C show the total AP activity staining of the different CHO cells, and panels D, E and F show the immunohistochemical staining using 10 µg/ml of protein A purified Rb-1434 IgG and the Vectostain kit for detection. Bar, 50 µm.
arguing that these are not spermatogonia undergoing mitosis. It is of interest to note that not all cells in meiotic prophase were positive for EAP, indicating that EAP might be only transiently expressed at a particular stage of meiosis I. These results explain the weak signal obtained in northern blots of mouse testis using an EAP probe, since the total number of M-phase cells in the adult testis is small. Since mRNA was also detected by RT-PCR at the newborn stage, EAP expression may not be entirely restricted to meiotic cells in the testis. It is possible that small amounts of EAP are produced during the mitotic cycle of spermatogonia, not sufficient to be detected with our anti-peptide antibody. Alternatively, the EAP mRNA could be transcribed in spermatogonia but stored untranslated until the start of meiotic prophase I.

The similarity between EAP and human GCAP might indicate that in the human testis also GCAP would be expressed at similar stages of meiosis. To support this hypothesis, we found that transgenic mice expressing human GCAP in the testis show M-phase cells staining positively for GCAP at equivalent stages and characteristics to those positive for EAP. Similarly, GCAP in transgenic mice, was found to be expressed at the two- to blastocyst stage of development, in perfect agreement with the expression of EAP during preimplantation development (not shown). These parallels strengthen the notion of equivalent functions of mouse EAP and human GCAP during development. However, differences do exist in some cellular systems with respect to AP isozyme expression; e.g., while mouse primordial germ cells express only the TNAP isozyme, human migrating primordial germ cells express GCAP.

An association between AP expression with cell division has previously been reported. An ultrastructural histochemical study in synchronized HeLa cells indicated that AP activity during the cell cycle was mostly associated with early and middle G1, displaying much lower activity in late G2 and undetectable activity in S phase (Vorbrodt and Borun, 1979). Primordial germ cells have been identified in the mouse as early as 7.0 days p.c. by Ginsburg et al. (1990) and they observed that AP-positive migrating primordial germ cells were frequently found in pairs, suggesting that the cells are mitotically very active. Other cell types known to express high levels of AP such as the early stage embryo, uterine reserve cells (Nozawa et al., 1980), intestinal epithelial cells (Potten et al., 1979) and cancer cells, all have a high mitotic index. It is possible that APs may be involved in cell cycle-controlled dephosphorylation or transphosphorylation reactions. Phosphorylation is a major regulatory mechanism of the cell cycle (Cytet and Thorner, 1989). A number of yeast or fungus genes encoding protein kinases as well as phosphoprotein phosphatases have been shown to control mitosis (Enoch and Nurse, 1991). One serine/threonine phosphatase isozyme was found to be essential for mitosis in Drosophila (Axton et al., 1990). Oncogene products and growth factor receptors have tyrosine kinase activity and tyrosine phosphatases seem to have important roles in cell growth or intercellular communication. There have been suggestions that APs could act as protein tyrosine phosphatases (Swarup et al., 1981; Lau et al., 1989). However, it seems unlikely that APs are protein serine/threonine phosphatases or protein tyrosine phosphatases in vivo since the gene sequences of APs do not have significant homology to the protein phosphatase genes and APs do not have their catalytic domain facing the cytoplasm. In this respect, it is established that human PLAP is anchored to the plasma membrane via a phosphatidylinositol glycan (PGI) anchor attached to Asp484 (Micanovic et al., 1988; Ogata et al., 1988). EAP has a serine residue at position 484 and serine is known to be an acceptor of PGI anchors (Micanovic et al., 1990). However, extraction of AP from mouse blastocysts with n-butanol alone showed much lower yield than Triton X-100 alone or mixture of n-butanol and Triton X-100 (Kim et al., 1989), suggesting that AP in mouse blastocysts does not have a PGI moiety. Since we now know that mouse blastocysts express EAP, the possibility exists that, in some developmental stages, EAP may function as a PGI-anchored and in others as a non-PGI-anchored enzyme, affecting the subcellular localization, and accordingly the target substrates, of this isozyme. Furthermore, TNAP has been reported to bind to collagens type I, II and X (Vittur et al., 1984; Wu et al., 1991) and PLAP and GCAP to have homology to extracellular matrix proteins (Tsonis et al., 1988). Therefore, the possible association of APs with extracellular phosphoproteins must be born in mind as a clue to AP function.

The expression of EAP in critical developmental processes, i.e. the early preimplantation embryo and spermatogenesis, seems to imply a very specialized and possibly a fundamental role of this AP isozyme. This laboratory is now engaged in gene targeting experiments to abolish expression of the EAP molecule in order to analyze the in vivo consequences of inactivating this unique AP isozyme.

This work was supported in part by grants HD 23668 and CA 42595 from the National Institutes of Health. We thank John Leszyk for the synthesis of the 24-mer peptide and Dr Rex A. Hess for his helpful comments regarding the identification of cell types in meiosis, and Dr Marc F. Hoylaerts for his help with the characterization of the anti-peptide antiserum.

References

Alkaline phosphatase expression in mouse embryo


(Received 5 June 1992)
**Fig. 1.** Histochemical staining of alkaline phosphatase activity in adult mouse testis sections. The panels show total AP activity staining (A, D), residual activity after heat treating the specimens at 65°C for 5 minutes (B), residual activity after enzyme inhibition with 5 mM L-homocysteine (C), AP activity of M-phase spermatocytes using fluorescent azo-dye technique (E) and phase-contrast image of E, (F). Bar, 100 μm (A-C) and 25 μm (D-F).
Fig. 6. Immunohistochemical staining of mouse testis of different ages using 10 µg/ml protein A purified Rb-1434 IgG. Testis samples include newborn (A), 8 days (B), 15 days (C), 24 days (D), 40 days (F, G) and 151 days (E, H). Panel H shows immunohistochemical staining of a transgenic mouse testis expressing human GCAP, using a polyclonal anti-PLAP rabbit IgG (10 µg/ml). Bar, 50 µm (A, B) and 25 µm (C-H).