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The Enhancer Domain of the Human Cytomegalovirus Major Immediate-Early Promoter Determines Cell Type-Specific Expression in Transgenic Mice

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The human cytomegalovirus (HCMV) major immediate-early promoter (MIEP) is one of the first promoters to activate upon infection. To examine HCMV MIEP tissue-specific expression, transgenic mice were established containing the *lacZ* gene regulated by the MIEP (nucleotides −670 to +54). In the transgenic mice, *lacZ* expression was demonstrated in 19 of 29 tissues tested by histochemical and immunochemical analyses. These tissues included brain, eye, spinal cord, esophagus, stomach, pancreas, kidney, bladder, testis, ovary, spleen, salivary gland, thymus, bone marrow, skin, cartilage, and cardiac, striated and smooth muscles. Although expression was observed in multiple organs, promoter activity was restricted to specific cell types. The cell types which demonstrated HCMV MIEP expression included retinal cells of the eye, ductile cells of the salivary gland, exocrine cells of the pancreas, mucosal cells of the stomach and intestine, neuronal cells of the brain, muscle fibers, thecal cells of the corpus luteum, and Leydig and sperm cells of the testis. These observations indicate that the HCMV MIEP is not a pan-specific promoter and that the majority of expressing tissues correlate with tissues naturally infected by the virus in the human host.

Human cytomegalovirus (HCMV) is a betaherpesvirus with a viral genome capable of encoding more than 200 proteins (6). The genes encoding these proteins have been subdivided into three kinetic classes which include immediate early (IE), early (E), and late (L) (7, 57). The expression of the IE genes is dependent on host cell factors, while both the early and late genes are interdependent on viral and cellular factors. The region of predominant IE expression occurs in two contiguous areas designated IE-1 (UL123) and IE-2 (UL122) (6, 49–51). Together, these two regions constitute the major IE (MIE) gene which is regulated by the major IE promoter (MIEP). The MIEP controls the expression of three predominant IE protein isoforms (49, 51). The MIE proteins are transcriptional regulatory factors which, together with host cell proteins, temporally regulate subsequent viral gene expression (49). Since these proteins play a pivotal role in determining the permissive state of the virus, activation of the MIEP may be the critical step in determining HCMV tissue-specific expression.

The MIEP is a complex regulatory element which is composed of distinct domains, including a basal promoter with a canonical TATA box (14) and a strong enhancer between nucleotides (nt) −50 and −530 (3, 55). Sequences between nt −750 and −1145 are known to repress the activity of the MIEP in undifferentiated cells (25, 33, 34, 36, 44). In vitro studies have shown that the enhancer appears to be universally functional in multiple cell types (3, 19, 36, 41, 44, 48, 55).

Because HCMV is a species-specific virus, the mechanisms involved in viral pathogenicity have been difficult to approach in animal models. However, transgenic technology has provided a means to examine specific effects of viral proteins or promoter regulatory elements in vivo. To date, two independent HCMV enhancer transgenic mouse studies have reported on tissue-specific expression of genes under the control of the HCMV MIEP in adult mice (10, 43). Observations from these groups suggest that the HCMV MIEP is a pan-specific promoter. However, neither of these studies identified cell types within tissues targeted for expression by the HCMV MIEP. Recently, Koedood et al. (23) examined expression of the HCMV MIEP during embryogenesis in transgenic mice. In their studies, specific expression which was dependent on the tissue and stage of fetal gestation was observed in embryos. These observations suggest that HCMV MIEP expression during embryogenesis is highly regulated by the developing tissues.

In the present study, we examined tissue-specific expression of the HCMV MIEP in adult transgenic mice. The specific cell types within organs were also assessed histochemically and immunochemically for expression. Our results indicate that the enhancer domain of the MIEP targets expression in 19 of the 29 organs examined. Although expression was detected in multiple organ systems, promoter activity within these tissues was restricted to specific cell types. The majority of these tissues correlated with tissues naturally infected by HCMV in the human host. These observations indicate that the HCMV MIEP is not a pan-specific promoter and that expression in transgenic mice is restricted to specific cells.

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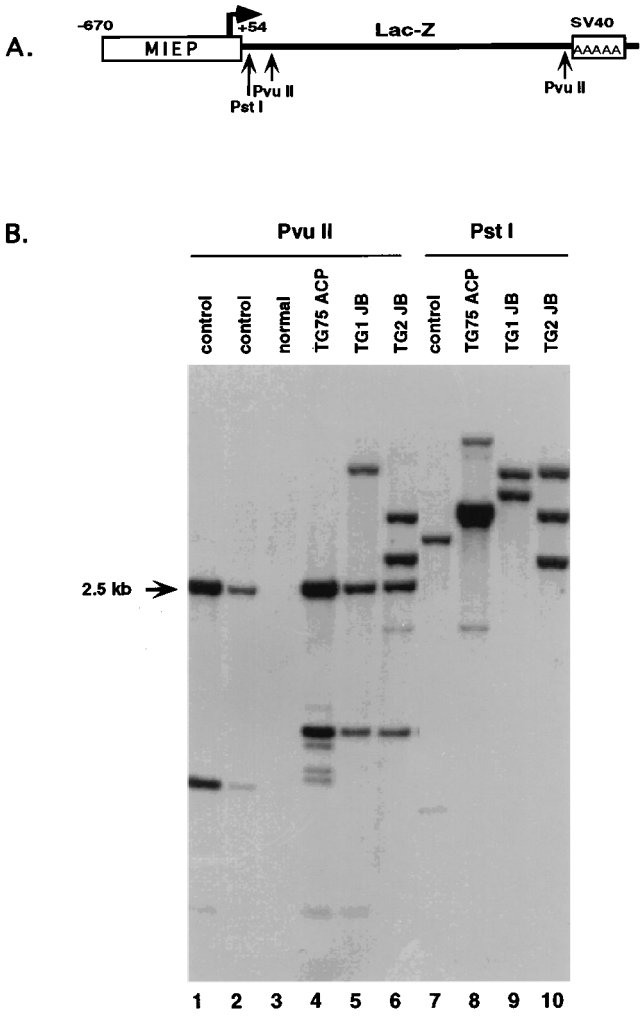

FIG. 1. (A) Construct used to generate MIEP-*lacZ* transgenic mouse lines. MIEP-*lacZ* contains a truncated version of the MIEP (nt −670 to +54, lacking the modulator and NF1 regions) cloned upstream of the *lacZ* coding sequence plus 241 nt of simian virus 40 DNA containing a polyadenylation sequence. Cleavage sites for restriction enzymes *Pvu*II and *Pst*I are indicated. (B) Southern blot of three homozygous transgenic mouse lines, demonstrating the presence of the MIEP-*lacZ* transgene. Ten micrograms of tail DNA was digested with *Pvu*II (lanes 1 to 6) or *Pst*I (lanes 7 to 10), run on a 1% agarose gel, transferred to nitrocellulose, and probed with ^{32}P-labeled MIEP-*lacZ* transgene DNA. Control lanes contained 10 μg of normal mouse tail DNA plus 10 (lane 1) or 1 (lanes 2 and 7) genomic equivalent of MIEP-*lacZ* transgene fragment. Lane 3 contained 10 μg of nontransgenic mouse tail DNA.

MATERIALS AND METHODS

Construction and preparation of the MIEP-*lacZ* transgene. The MIEP-*lacZ* transgene was generated by inserting the HCMV MIEP (nt −670 to +54) as a *Hin*dIII-*Eco*RI fragment from pRR49 (a generous gift from R. Ruger) into p315 DNA (derived from pβactin P E1 IVS SDK *lacZ*pA, a generous gift of R. Scott), which contains a promoterless *Escherichia coli lacZ* gene (GenBank accession no. V00296), starting downstream of a Shine-Delgarno sequence and a Kozak consensus sequence and upstream of a simian virus 40 polyadenylation signal sequence (*Bcl*I to *Bam*HI, nt 2533 to 2770) (Fig. 1A). The resulting plasmid, designated p324, was digested with restriction enzymes *Hin*dIII and *Sac*II to separate the MIEP-*lacZ* transgene from vector sequences prior to microinjection.

Generation of MIEP-*lacZ* transgenic mice. MIEP-*lacZ* transgenic mice were generated by injection of linear DNA, purified after the removal of vector sequences, into the male pronuclei of fertilized ova derived from FVB mice (TG75ACP) or from CB6F2/J (TG1JB and TG2JB) mice as previously described (17). After injection, ova were transferred to surrogate pseudopregnant FBV or CB6F1/J mice for development and delivery. Transgenic mice generated by this procedure were identified by Southern blot analysis (46) of high-molecular-weight DNA extracted from tail biopsies of homozygous transgenic mice by using the proteinase K-sodium dodecyl sulfate extraction procedure (17). Briefly, 10 μg of genomic mouse DNA was digested with restriction enzyme *Pst*I, which cuts once in the transgene, or with *Pvu*II, which cuts twice, to release a 2.5-kb fragment, run on a 1% agarose gel, and transferred to GeneScreen Plus (E. I. Dupont-NEN) by the method of Southern (46). The filters were then probed with ^{32}P-random primer-labeled MIEP-*lacZ* transgene, washed, and exposed to X-ray film. Transgene copy number was estimated by comparing the digestion pattern and band intensities of transgenic mouse DNAs with 1 or 10 genomic equivalents of MIEP-*lacZ* transgene mixed with nontransgenic mouse genomic DNA digested with the same enzyme. Founder lines were established from transgenic mice that transmitted the MIEP-*lacZ* gene to progeny in Mendelian fashion and demonstrated reproducible patterns of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining in tissues over multiple generations. Each transgenic line was inbred to homozygosity.

Histochemical and immunocytochemical analysis of *lacZ* transgene expression. To prepare transgenic mouse tissue for histochemical analysis, adult mice were anesthetized with avertin and perfused with saline–4% paraformaldehyde. After perfusion, tissues were removed and placed in freshly prepared 4% paraformaldehyde for 4 h at 4°C. Tissues were subsequently rinsed in phosphate-buffered saline (PBS) containing 10% sucrose and frozen in OCT (Miles Scientific, Elkhart, Ind.). Twenty-micrometer-thick frozen sections were cut on a Reichert-Jung cryostat and stained with X-Gal (Sigma Chemical Corp., St. Louis, Mo.) overnight at 4°C (42). After staining, slides were rinsed in 0.1 M PBS, counterstained with eosin, and dehydrated through increasing concentrations of isopropyl alcohol. After brief immersion in xylene, slides were coverslipped with permount and examined under a light microscope.

For immunohistochemical analysis, tissues were fixed as above and embedded in paraffin. Ten-micrometer-thick sections were placed on gelatin-coated slides and stained by the method of Nilaver and Kozlowski (37). Briefly, slides were deparaffinized in Americlear (Baxter Sci., McGaw Park, Ill.), hydrated through graded ethanol, rinsed in 0.05 M Tris containing 0.9% NaCl, pH 7.6 (hereafter referred to as Tris-saline), and then treated with 1% hydrogen peroxide for 30 min. Slides were subsequently rinsed in Tris-saline to remove hydrogen peroxide and incubated in Tris-saline plus 0.1% Triton X-100 (hereafter referred to as Tris A) for 15 min to delipidate sections and permeabilize the cell membranes. Slides were then incubated in Tris-saline with 0.1% Triton X-100–0.02% bovine serum albumin (Sigma) (hereafter referred to as Tris B) for 15 min and exposed to rabbit anti-β-galactosidase antibody (Organon Teknika, West Chester, Pa.) that had been preabsorbed with mouse and rat liver powder (Sigma) at a 1:100 dilution in Tris B, at 4°C for 12 h. After incubation, the slides were rinsed in Tris A for 20 min and Tris B for 20 min and exposed to biotinylated protein A (Vector Labs, Burlingame, Calif.) for 45 min at room temperature. Slides were rinsed and exposed to ABC (Vector) for 1 h, and positive cells were visualized using 3-amino-9-ethyl carbazole (AEC) (Dako Corp., Carpintera, Calif.). Slides were counterstained with Mayer's hematoxylin, rinsed, dehydrated through isopropyl alcohol, rinsed briefly in xylene, and coverslipped with permount.

An alternative immunohistochemical staining procedure was also performed for detection of *lacZ* expression (38). In this method, transgenic mice were perfused with 10% formalin, and tissues were fixed in 10% formalin for at least 3 days. Tissues were then embedded in gelatin-egg yolk, sectioned 100-μm thick on a vibratome, and stored in Tris-saline. Sections were then treated with 1% hydrogen peroxide for 30 min, rinsed in Tris-saline, and incubated in Tris A for 15 min, Tris B for 15 min, and anti-β-galactosidase antibody for 12 to 16 h at 4°C. Tissues were rinsed with Tris A and Tris B and incubated in biotinylated protein A for 45 min at 25°C, incubated in Tris A for 15 min, Tris B for 15 min, and ABC for 1 h at 25°C, and then rinsed in Tris-saline. Tissues were then exposed to 3,3-diaminobenzidine (DAB) (Sigma) for 15 to 45 min at 25°C, rinsed with Tris-saline, mounted on gelatin-coated slides, air dried, dehydrated through ethanols, counterstained with thionin (40), and mounted as described above.

RESULTS

Establishment of MIEP-*lacZ* transgenic mice. The HCMV MIEP is one of the first viral promoters to be activated upon viral infection of the cell (7, 57). Since this promoter controls expression of viral proteins which are integral to subsequent HCMV promoter activation, activity of the MIEP may ultimately determine viral tissue specificity. To address this issue, four transgenic mouse lines containing a transgene composed of the *lacZ* gene under the control of the MIEP (nt −670 to +54) (Fig. 1A) were established by the procedures described in Materials and Methods. All founders gave rise to offspring in crosses with wild parent nontransgenic mice and transmitted the introduced gene in Mendelian fashion (approximately 50% hemizygotes), with copy numbers and restriction patterns as expected for a single locus. Three of the four founder lines (TG75ACP, TG1JB, and TG2JB) were analyzed in this study.

TABLE 1. HCMV MIEP-directed expression of the *lacZ* gene in transgenic mouse lines

System and organ	Expression in transgenic mouse lines:		
	TG75ACP	TG1JB	TG2JB
Neural			
Brain	+	+	+
Eye	+	+	−
Spinal cord	+	+	+
Respiratory			
Lung	−	−	−
GI tract			
Esophagus	+	+	+
Stomach	+	−	+
Duodenum	−	−	−
Ileum	−	−	−
Jejunum	−	−	−
Colon	−	−	−
Liver	−	−	−
Pancreas	+	+	+
Genitourinary			
Kidney	+	+	+
Bladder	+	+	+
Testes	+	+	+
Ovary	+	+	−
Lymphoid and blood			
Lymph node	+	−	−
Spleen	+	+	+
Salivary gland	+	+	+
Thymus	−	+	+
Blood	−	−	−
Bone marrow	+	+	+
Muscle, cartilage, and skin			
Cartilage	+	+	+
Striated muscle	+	+	+
Cardiac muscle	+	+	+
Smooth muscle	+	+	+
Skin	+	+	+
Endocrine			
Thyroid	+	−	−
Adrenal	−	+	−

Southern analysis performed on genomic DNA from these mice, digested with the restriction enzyme *Pst*I or *Pvu*II, showed different patterns of DNA restriction fragments, indicating that each of the lines varied in transgene copy number (1 copy for TG1JB, 2 copies for TG2JB, and >10 copies for TG75ACP) as well as integration site (Fig. 1B).

Expression of MIEP-*lacZ* in transgenic mouse tissues. To understand how the activation of MIEP determines cell tropism of HCMV, the expression of *lacZ* in our adult transgenic mouse tissues was assessed by utilizing both X-Gal and antibody directed against the β-galactosidase protein in histochemical and immunocytochemical analyses, respectively. X-Gal staining was confirmed by immunocytochemical staining using antibodies to β-galactosidase. Organs were considered positive for *lacZ* expression if staining was observed in two or more lines. Positive organs from different lines displayed identical patterns of transgene expression; however, when organs with various degrees of expression were compared between the lines, the strongest expression was in TG75ACP followed by TG2JB and TG1JB.

Transgenic mice containing the MIEP-*lacZ* transgene demonstrated expression in 19 of 29 organs (Table 1). These organs included the brain, spinal cord, esophagus, stomach, pancreas, kidney, bladder, testes, ovary, spleen, salivary gland, thymus, bone marrow, cartilage, skin, and striated, cardiac, and smooth muscles. Infrequently, sporadic cells positive for *lacZ* expression were also observed in the thyroid and adrenal glands. However, these tissues were considered negative for expression because the positive sporadic cells probably represented the occasional blood cells trapped in these tissues. In addition, *lacZ* staining was not consistently observed in the small intestines of transgenic mice. Only the TG75ACP line demonstrated staining in the small bowel, which may represent differential expression because of the site of integration. Therefore, this organ was considered negative for transgene expression. Tissues which were consistently negative for *lacZ* staining included the lung, liver, colon, peripheral blood, and peritoneal macrophages. Similarly, all tissues tested from nontransgenic control animals were negative for *lacZ* staining.

MIEP-*lacZ* expression in the CNS. The central nervous system (CNS) is a major target for CMV, especially in patients with AIDS (16, 31, 35). In these patients, virus infection frequently causes encephalitis and retinitis (21, 58). Histologic examination of brain tissues from these individuals indicates that HCMV is capable of infecting most CNS cells, including neurons, astrocytes, and endothelial cells (59). Examination of CNS tissue from MIEP-*lacZ* transgenic mice revealed expression of *lacZ* in the forebrain, midbrain, and hindbrain and in the choriod plexus of the lateral and fourth ventricles as well as limbic brain structures (Fig. 2B through O). Additionally, staining was detected in the retina (Fig. 2A) and neuronal perikarya of the spinal cord (data not shown).

The most intense staining was observed in cells which by morphology and location resemble neurons of the hindbrain corresponding to the brain stem and cerebellum (Fig. 2C and G). Brain stem regions expressing *lacZ* included the locus coeruleus, the dorsal raphae nucleus, the reticulotegmented nucleus of the pons, and the inferior olive (Fig. 2C, D, E, and F). X-Gal staining in the cerebellum was primarily confined to cells resembling Purkinje cells (Fig. 2G).

lacZ expression in the forebrain regions was scattered within the neuronal perikarya of the frontal cortex as well as in a dense concentration of cells in the olfactory nucleus and the islands of Calleja (Fig. 2H and I). Cells resembling neurons in the inferior colliculus and the central gray and ventral tegmental areas of the midbrain also demonstrated *lacZ* expression (Fig. 2J, K, L, and M). β-Galactosidase activity was also observed in the limbic brain regions, which included the dentate gyrus and hippocampus (Fig. 2N and O). In the dentate gyrus, transgene expression was confined to granule cell somata in both the suprapyramidal and infrapyramidal blades (Fig. 2N), whereas in the hippocampus, staining was observed predominantly in the pyramidal cell layer (Fig. 2O).

The above results indicate that although the CNS is a major target for MIEP activity, expression is restricted to defined cell types in these tissues.

MIEP-*lacZ* expression in the genitourinary system. In individuals with acute disease, HCMV viruria is a common feature resulting from infection of the renal tract (4, 45). Examination of the kidneys from these patients commonly reveals the presence of virus in the collecting tubules and proximal tubules near cortical areas (15). Similarly, we consistently observed positive *lacZ* expression in the ductile epithelium of the kidneys (Fig. 3F and G) and in the arterioles of the glomeruli (Fig. 3G) of our transgenic mice. HCMV is also frequently associated with infections in the reproductive organs (28, 32, 53). The virus is reported to infect mature sperm in humans (18). Examination of transgenic mouse testes revealed the presence of β-galactosidase activity in interstitial Leydig's cells and mature sperm cells in seminiferous tubules (Fig. 3H). Expression

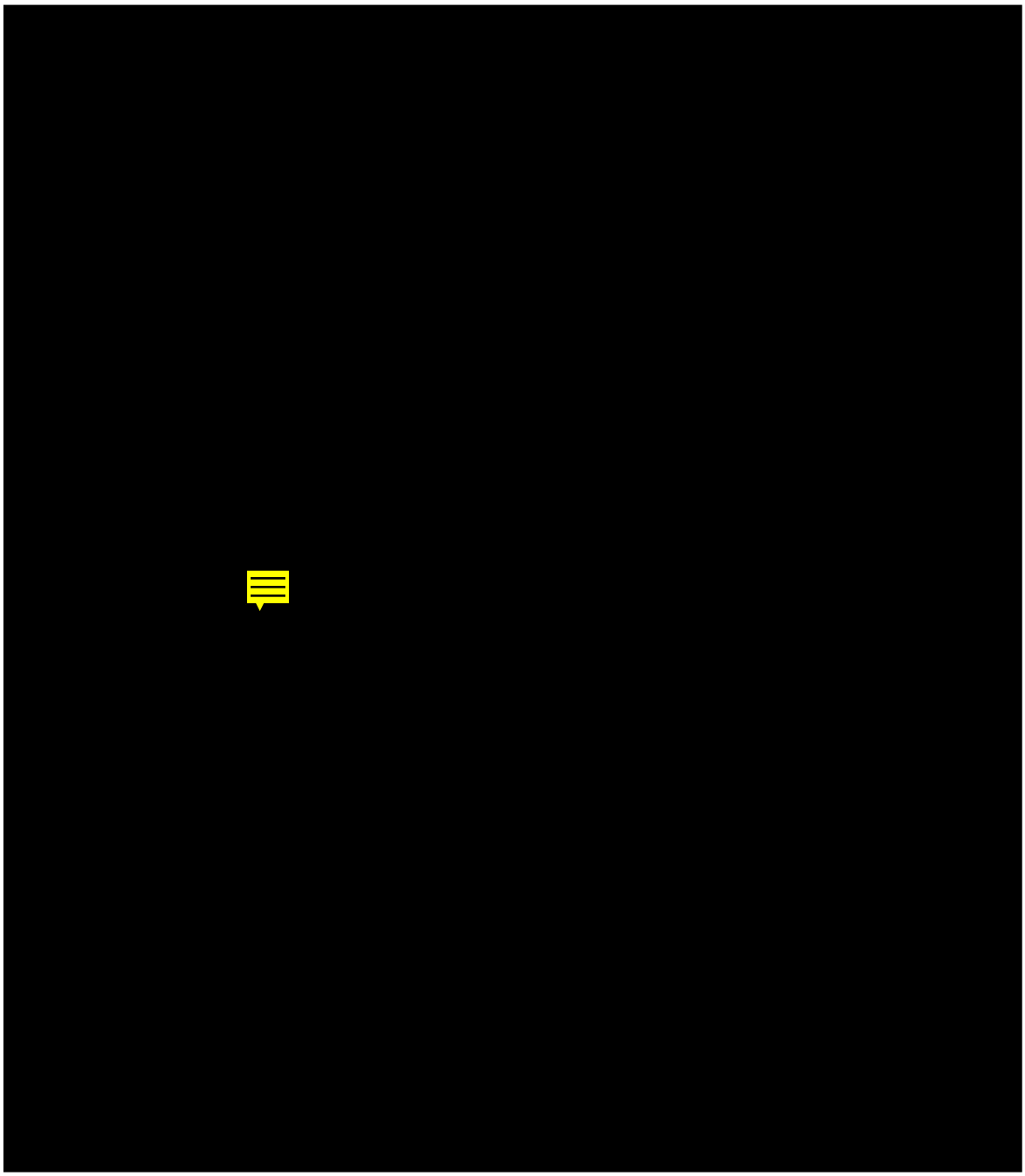

FIG. 2. *lacZ* expression in MIEP transgenic mouse CNS tissues. (A) Paraffin-embedded tissue section of retina stained indirectly for β-galactosidase activity, using biotinylated anti-β-galactosidase antibody detected with AEC. Rods and cones of the retina show positive (red) staining. Magnification, ×220. (B through O) Sagittal brain cryosections from MIEP transgenic mice stained directly for β-galactosidase activity, with positive cells giving a blue stain. (B) Region of the choroid plexus, with staining specific only to ependymal cells. Magnification, ×220. (C) Expression in neuronal cells of the locus coeruleus. Magnification, ×140. (D) Dorsal raphae nucleus. Magnification, ×25. (E) Reticulotegmented nucleus of the pons (magnification, ×25) and (F) inferior olive (magnification, ×43). (G) Positive Purkinje cells in the cerebellum. Magnification, ×220. (H) Expression in the neuronal perikarya of the frontal cortex (magnification, ×46) and (I) islands of Calleja (magnification, ×46). (J and K) Low- and high-power photomicrographs showing positive staining in the inferior colliculus. Magnification, ×20 and 46, respectively. (L) The central gray matter (magnification, ×44) and (M) ventral tegmented midbrain (magnification, ×20). (N) Expression in neurons of the dentate gyrus (magnification, ×240) and (O) hippocampus (magnification, ×160).

of *lacZ* in ovaries was restricted to theca cells of the ovarian corpus luteum (Fig. 3I).

MIEP-*lacZ* expression in the digestive tract. The epithelial cells of the gastrointestinal (GI) tract are a common site for HCMV infection (8, 54). The virus is most commonly found in the colon, followed by the esophagus, rectum, and small bowel. Examination of the GI tracts of our transgenic mice revealed consistent staining for MIEP-*lacZ* in epithelial cells in the esophagus (Fig. 3A) and stomach (Fig. 3B). All other portions of the GI tract were consistently negative for *lacZ* expression.

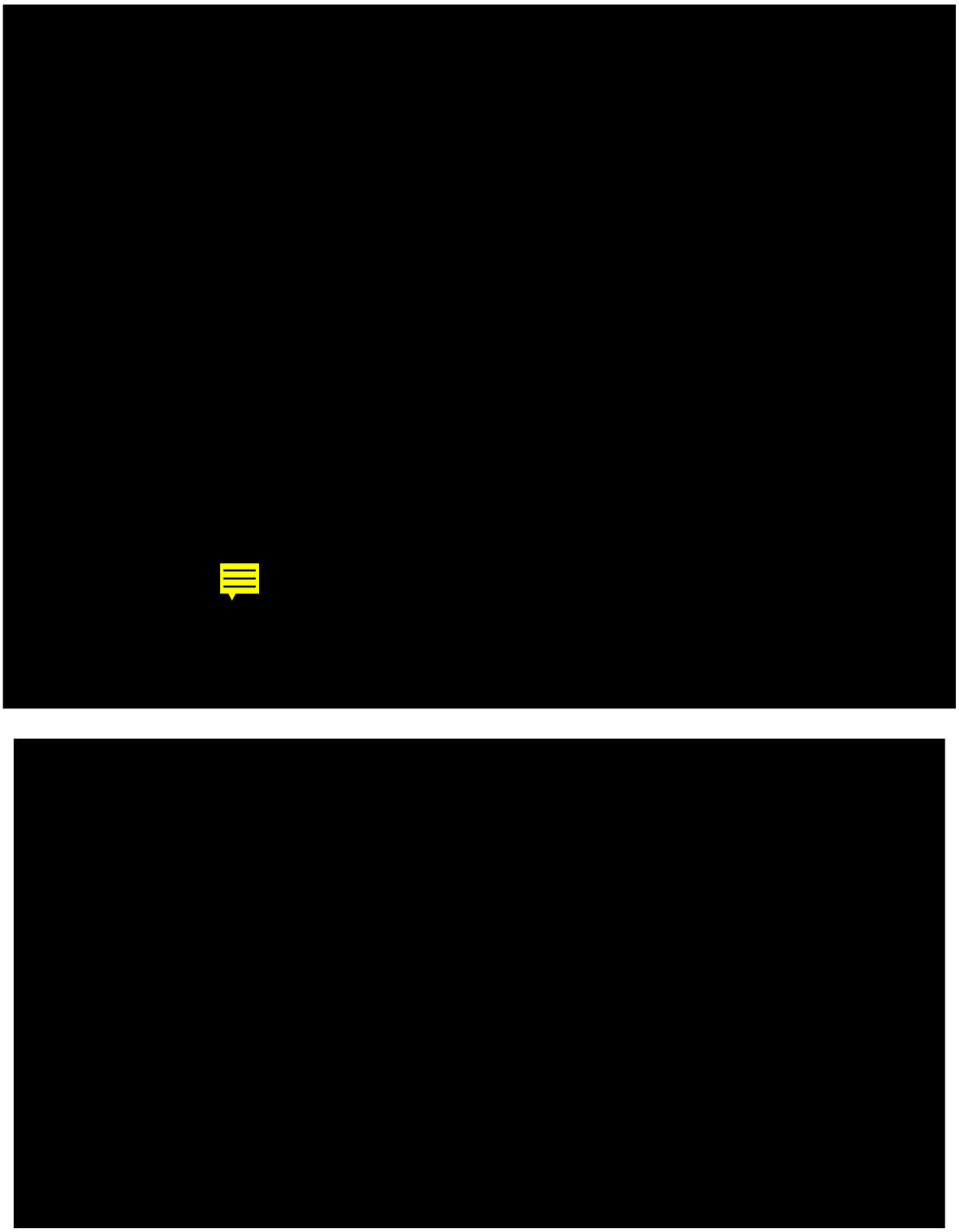

FIG. 3. *lacZ* expression in MIEP transgenic mouse tissues of the digestive system (A to E), genitourinary system (F to I), and muscle (J to L), skin (M), cartilage (N), and bone (O). Cryosections of MIEP-*lacZ* transgenic tissues were stained directly for β-galactosidase activity, with positive cells appearing blue. (A) Expression in epithelial cells lining the esophagus (magnification, ×50) and (B) stomach (magnification, ×100). (C) Expression in ductile epithelial cells in the salivary gland (magnification, ×50) and (D) exocrine cells in the pancreas (magnification, ×100). (E) Gelatin-embedded thick section of pancreas indirectly stained with anti-β-galactosidase antibody detected with DAB. Positive (brown) staining is seen in the exocrine cells as well as cells in the islets of Langerhans (arrow). Magnification, ×200. (F) Expression in epithelial cells of the renal tubules (arrow) and arterioles (arrowhead) of the kidney glomeruli. Magnification, ×100. (G) Kidney section stained as described for panel E, showing expression in the glomeruli (arrow) and renal tubules. Magnification, ×30. (H) Expression in Leydig's cells (arrowhead) and spermatids (arrow) in the testis (magnification, ×140) and (I) theca cells (arrow) in the ovary (magnification, ×100). Positive expression in (J) striated (magnification, ×50), (K) cardiac (magnification, ×120), and (L) smooth (magnification, ×50) muscle fibers. (M) Expression in skin epidermis (magnification, ×160), (N) condrocytes in the cartilage of the sternum (magnification, ×160), and (O) bone marrow (magnification, ×100).

Since HCMV is associated with the salivary glands (9) and pancreas (20), as well as the GI tract, we also examined these organs of the transgenic mice for MIEP-*lacZ* expression. The results revealed consistent expression in the ductile epithelium of the salivary gland (Fig. 3C) and in a subset of exocrine cells in the pancreas (Fig. 3D) as well as in defined cells in the islets of Langerhans (Fig. 3E).

MIEP-*lacZ* expression in muscle, bone, cartilage, and skin. HCMV less frequently infects human muscles, bone, and skin (26, 52, 56). In view of this, we examined these tissues in our transgenic mice for *lacZ* expression. Interestingly, we detected strong expression in skeletal and cardiac muscles. However, only a subset of muscle fibers were positive for *lacZ* expression (Fig. 3J and K). *lacZ* expression was also detected in aortic smooth muscle (Fig. 3L). Transgene expression was also detected in the epidermis as well as in cells lining the hair follicles (Fig. 3M). Examination of cartilaginous and bone tissues revealed *lacZ* expression in condrocytes in the sternum cartilage (Fig. 3N) and sporadic cells in the bone marrow (Fig. 3O).

DISCUSSION

In this study, we demonstrated that the HCMV MIEP was transcriptionally active in multiple organs of transgenic mice. Expression within these organs was restricted to specific cell types in highly differentiated tissues. These results contrast with the results of previous transgenic studies (10, 43) which suggest that the MIEP is a pan-specific promoter in all tissues and cells. Importantly, the majority of tissues and cell types which display MIEP activity parallel tissues naturally infected by HCMV in the human host.

Previous studies with HCMV-MIEP transgenic animals utilized various portions of the MIEP to examine tissue-specific expression. Schmidt et al. (43), using a construct containing MIEP sequences from nt −585 to −14, observed expression in 24 of 28 tissues in transgenic mice. The greatest amount of expression was detected in heart, brain, kidney, and testis tissues. These observations are similar to expression patterns in our transgenic mice, with the exception of those for the pancreas and striated muscle. Expression in the latter tissue was also observed in transgenic mice containing a construct with MIEP sequences from nt −524 to +55 (10). Together, these observations suggest that sequences between nt −14 and +55 alter expression in striated muscle and may determine expression in the pancreas, although the latter tissue was not examined by Furth et al. (10).

Although our observations together with those of the studies above indicate that the MIEP is active in a variety of organs, histologic examinations of these tissues indicate that the promoter exhibits exquisite cellular specificity. For example, in striated and cardiac muscle only a subset of fibers were positive for expression. Tissues such as striated muscle are composed of multiple elements, including red, intermediate, and white fibers. MIEP activity in this tissue may reflect the specificity of this promoter for particular fiber types. Interestingly, only a minor subset of muscle fibers was observed with positive expression in the tongue of developing embryos (2). While these findings contrast with the ability of the MIEP to be active in the majority of cell types in vitro, the restricted expression in transgenic mice may relate to differences in both the differentiation and replicative states of the cells in culture.

The HCMV MIEP contains multiple transcription factor binding sites, the majority of which constitute signal-regulated elements. A number of cellular transcription factors known to interact with the MIEP include SP-1, NF-κB, YY-1, ATF/CREB, AP-1, and RAR-RXR family members (11–14, 39, 41, 48). The variety of transcription factors which bind the MIEP may reflect the diversity of tissues in which the promoter is active and ultimately productively infected by the virus. A number of these transcription factors which interact with the MIEP are regulated through signal transduction factors, such as NF-κB, ATF/CREB, AP-1, and RAR-RXR family members (1, 11–13, 16, 19, 39, 41, 48). Both direct and indirect cell signal-mediated regulatory interactions are likely to be involved in regulating the restricted expression of the MIEP in different tissues. Therefore, signaling pathways may be a fundamental mechanism responsible for cell type-restricted patterns of MIEP expression observed in vivo. Clearly, these pathways operate during embryogenesis, and MIEP expression is highly specific and regulated in embryonic tissues (2, 23).

The tissues targeted for expression by the MIEP relate very well to the various organ diseases associated with HCMV infection. For example, transgene expression detected in brain, eye, spinal cord, esophagus, stomach, pancreas, and adrenal gland tissues correlates highly with HCMV-induced encephalitis, retinitis, esophagitis, pancreatitis, and adrenalitis. Similarly, MIEP activity in the salivary gland, thymus, skin, cartilage, muscle, gonads, and spleen corresponds to sites of natural infection by HCMV. MIEP expression was also detected in kidney ductile epithelium and epithelial cells lining the bladder. The expression may parallel viral infection of these tissues and explain the high amounts of HCMV in urine samples. In addition, specific cells in the bone marrow were also found to express the MIEP-*lacZ* transgene. Although the cells in this hematopoietic organ could not be identified, this tissue is considered to be a potential reservoir of HCMV (24, 29).

Recently, Koedood et al. (23) reported expression of a similar transgene construct in the CNS of developing mouse embryos. In these studies MIEP expression was predominantly detected in cells of the developing CNS vasculature, hindbrain, choroid plexus, and subependymal brain regions. We have also observed similar patterns of MIEP expression in the developing embryos of our transgenic mice (2). By morphology and location in the developing fetal brain, the expressing cell types include endothelial, epithelial, and neuronal cells. MIEP expression was also observed in similar regions and cell types in adult transgenic mouse brains, with the exception of the endothelial cells. Interestingly, adult transgenic mice demonstrate MIEP expression in increased numbers of cells morphologically resembling neurons. Although some differences are observed in embryonic and adult MIEP expression, all of the brain regions and cell types are potential targets for HCMV infection (58).

Interestingly, we observed a lack of transgene expression in the intestine, liver, and lung, organs commonly associated with HCMV infection (22). The most obvious explanation for the absence of MIEP activity in these tissues is the requirement of this promoter for other viral or virally modified host factors. The viral factors may include structural components of the virion or low-level production of IE proteins which have been shown to activate the MIEP (reviewed in reference 14 and 27). Alternatively, HCMV disease may involve sites of tissue regeneration in which the cells revert to a fetal-like phenotype that is susceptible to HCMV infection. This hypothesis is supported by the fact that we and others (2, 23) have observed MIEP expression in a subset of cells in the lungs and livers of developing embryos.

Smooth muscle was another significant tissue demonstrating expression of the transgene. HCMV infection of this tissue is associated with the development of atherosclerosis (30) and restenosis (47). Both diseases are characterized by the excessive proliferation of smooth muscle cells in the vessels. Asso-

ciation of the HCMV IE86 protein with p53 is implicated in the development of restenosis lesions (47). Recently, we have developed transgenic mice which direct the expression of IE86 in smooth muscle tissues (unpublished observation). These mice will provide an ideal model to examine the role of HCMV in these disease processes.

In conclusion, we have completed a full characterization of the organs and cell types targeted for expression by the HCMV MIEP. Our results indicate that although the promoter was active in multiple organs, expression was restricted to specific cell types. The restricted expression of the MIEP in specific cell types and tissues may make this promoter useful for targeting the expression of genes in specific genetic diseases.

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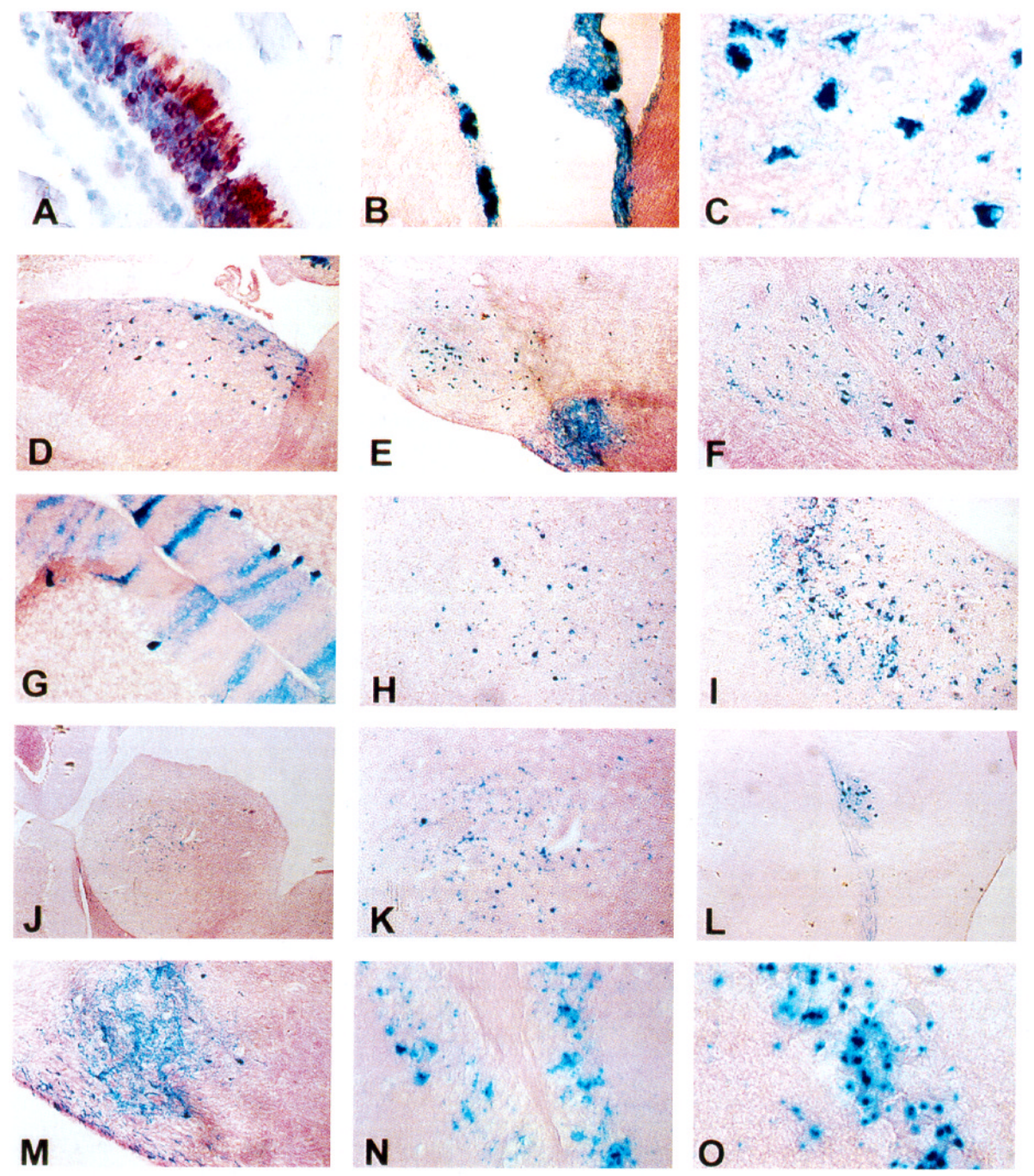
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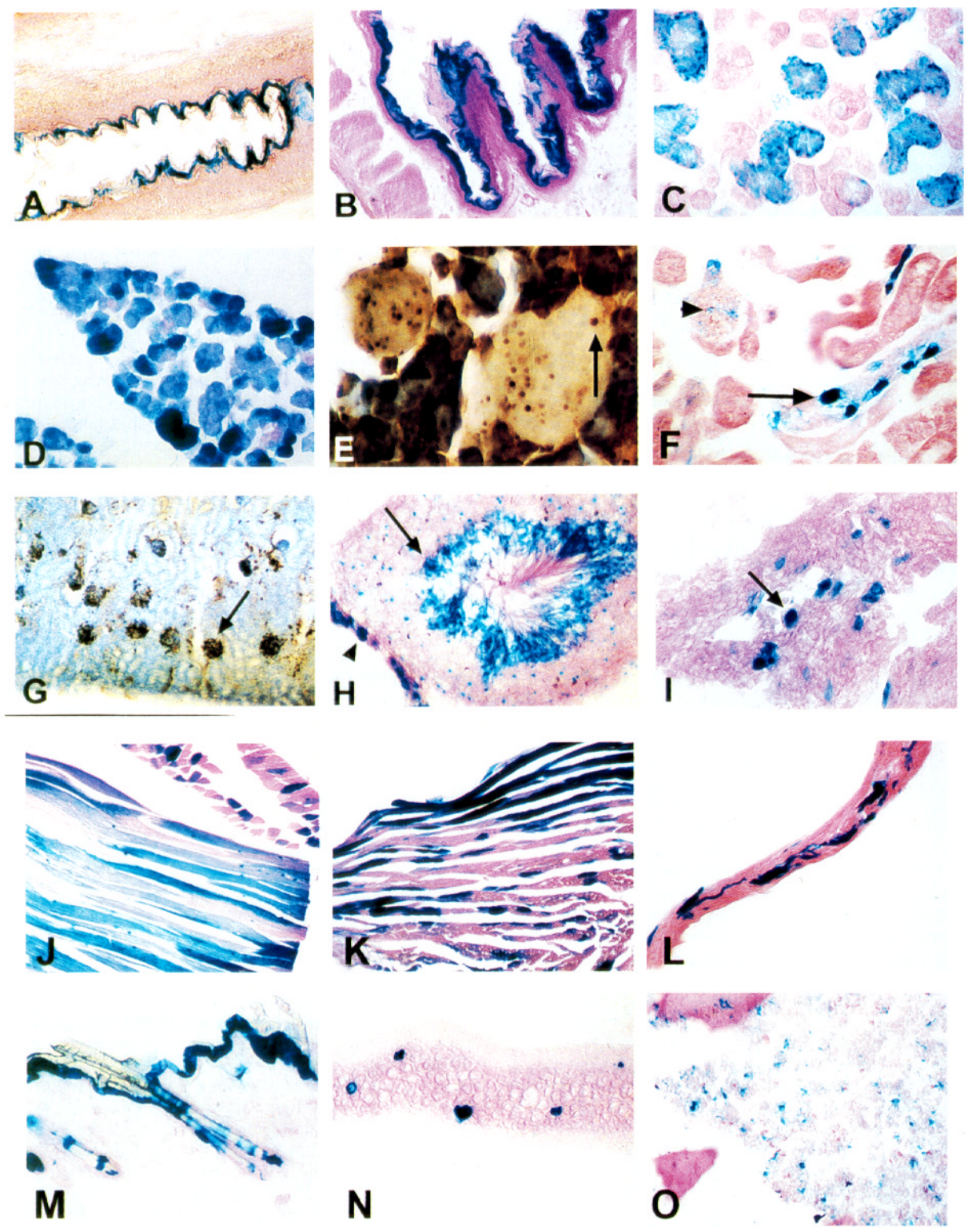
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