B94, a Primary Response Gene Inducible by Tumor Necrosis Factor-α, Is Expressed in Developing Hematopoietic Tissues and the Sperm Acrosome*

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B94 was originally described as a novel tumor necrosis factor-α-inducible primary response gene in endothelial cells which was also induced in an in vitro model of angiogenesis. To further characterize its expression, we cloned the mouse homologue and mapped its developmental and tissue specific expression. The predicted amino acid sequence of mouse B94 was found to be 85% similar to its human homologue. The gene was localized to mouse chromosome 12 just centromeric to the immunoglobulin heavy chain locus, in a region that is often rearranged in T-cell neoplasms. To explore the possibility that B94 is expressed during vasculogenesis and angiogenesis, the developmental processes, the expression of its transcript was determined during mouse development by in situ hybridization. In 10-day embryos B94 was expressed prominently in the myocardium and in the aortic arch. By the 15th day of gestation, expression was restricted largely to the liver, the bone forming regions of the jaw, the aortic endothelium, and the nasopharynx; a pattern that was maintained until just prior to birth. Postnatally, expression shifted to the red pulp of the spleen and the thymic medulla. B94 expression was extinguished in most adult tissues but was detectable in lymphopoietic tissues including the spleen, tonsil, and lymphatic aggregates in the gut. Consistent with this was the finding that mononuclear progenitor cells in bone marrow and mature peripheral blood monocytes expressed B94. A truncated testis-specific transcript previously identified by Northern blot analysis was determined to result from the use of an alternate polyadenylation signal which was surprisingly located within the open reading frame. This shorter transcript was expressed at high levels exclusively in late stage spermatids. Immuno-staining with an affinity-purified polyclonal antiserum revealed B94 to be localized to the acrosomal compartment of mature sperm. These studies demonstrate that B94 expression is tightly regulated during development and suggests distinct roles for B94 in myelopoiesis and spermatogenesis.

The influence of cytokines upon their target cells is mediated by biochemical and genetic events which lead to characteristic alterations in cellular behavior. Genes transcriptionally activated by cytokines and growth factors without the requirement of intervening protein synthesis are known as primary response genes and encode key regulatory proteins including transcriptional factors such as c-fos and c-jun, paracrine factors such as interleukin-8, and cell surface molecules that mediate adhesive interactions such as E-selectin (1). Overall, these alterations in biochemical pathways and cell behavior results in the phenotypic change associated with a particular growth factor or cytokine. Tumor necrosis factor-α (TNFα) is a multifunctional cytokine implicated in diverse processes, including acute inflammation (2), angiogenesis (3), blood cell differentiation (4), bone resorption (5), cell proliferation (6), and cell killing (7). It is likely that the primary response genes that TNF induces mediate a subset of these responses. For example, the TNF-inducible protein A20 has been shown to protect cells from TNF-mediated programmed cell death (8).

B94 is a cytokine-driven primary response gene that was originally cloned from TNF stimulated endothelium (9). The B94 gene encodes a novel 73-kDa intracellular protein that exists as a single copy gene on human chromosome 14 near the immunoglobulin heavy chain locus. It is also known that B94 is activated by factors other than TNF; endothelial cells stimulated with interleukin-1β or lipopolysaccharide-induced B94 and Northern blot analysis of murine embryonic tissues revealed it to be expressed in embryonic liver and kidney, organs in which TNF is not present during development (16). Interestingly, a second smaller transcript was highly expressed in the testis.

In the present studies, the expression of B94 was examined in greater detail. The predicted mouse B94 amino acid sequence was compared to its human homologue, and its expression during murine embryonic development was mapped by in situ hybridization. In addition, the expression of B94 in hematopoiesis and male germ cell maturation was examined in detail. The results suggested that B94 may play multiple roles in development, including vasculogenesis, blood cell differentiation, and spermatogenesis.

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1 The abbreviations used are: TNF, tumor necrosis factor-α; dpc, day(s) post-coitum; IGH, immunoglobulin heavy chain locus; Aut-1, α-1-antitrypsin; kb, kilobases; PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor; FG, fibroblast growth factor.
backcross mice were bred and maintained as described previously (10). The testis form of mouse B94 was cloned by reverse transcription-polymerase chain reaction using adult mouse testis RNA as template. The 3’ primer, 5’TGGCTTCGCA-CACCTGCTCC-3’ was specific for mouse B94 sequences just upstream of an EcoRI site in the open reading frame. Full-length cDNA clones for the testis form of B94 were isolated from an adult testis cDNA library in the pCDNA1 vector. Plasmids containing mouse B94 cDNA sequences were purified by CsCl ultracentrifugation and sequenced on both strands with Sequenase (United States Biochemical Corp.) and synthetic deoxyoligonucleotides as primers. Sequence was assembled and analyzed using MacVector release 4.0 (IBI) and the Genetics Computer Group Sequence Analysis Software package (Version 7.0).

Mouse Chromosomal Localization—C3H/HeJ-gld/gld and Mus spretus (Spain) mice and C3H/HeJ-gld/gld X Mus spretus F1, X C3H/HeJ-gld/gld interspecific backcross mice were bred and maintained as described previously (11). Genomic DNA isolated from mouse organs was digested with a panel of restriction endonucleases and 10 µg of each digest was resolved in 0.9% agarose gels, transferred to nylon membranes, hybridized to 32P-radioabeled DNA probes under high stringency conditions, and washed at high stringency as described previously (11). A 2.4-kb XhoI fragment of mouse B94, representing most of the open reading frame and all the 3′-untranslated region, was used as a probe. Gene linkage was determined by segregation analysis with other published markers (11).

Tissue Preparation and in Situ Hybridization—CD1 male mice were mated with virgin females, and embryonic day 1 was established by the presence of a vaginal plug. Embryos and postnatal organs snap-frozen in OCT were cut at 8 µm and collected on acid-washed silane-treated slides. Sections were stored at −80 °C prior to use. Bone marrow cells isolated from an adult murine tibia were washed two times in RPMI media with 1% bovine serum albumin, and 100,000 cells were attached to acid washed slides by cytocentrifugation. The sections were briefly air-dried and then fixed in fresh 2% parafomaldehyde, 0.1% glutaraldehyde (Sigma) in phosphate-buffered saline, pH 7.4 (PBS). Fresh human peripheral blood leukocytes were isolated by centrifugation through Ficoll-Paque (Pharmacia LKB Biotechnology Inc.) for 30 min at 400 x g and were separated by elutriation into fractions enriched in lymphocytes, monocytes, and polymorphonuclear cells. Elutriation was done using a cell elutriation buffer (PBS with 0.05% bovine serum albumin and 1 unit/ml EDTA) with a JE-6B rotor (Beckman) at 2030 rpm and a flow rate varying upwards from 4 to 10 ml/min. Fractions were monitored for specific cell types by morphology and through nonspecific esterase staining for monocytes. The cells were then cytocentrifuged and stained with hematoxylin and eosin. Leukocytes were counterstained with giemsa (Sigma) diluted 1:20 in water for 6 h and washed in 2% acetic acid twice for 1.5 min and 100% ethanol twice for 45 s. The slides were photographed on a Wild M420 darkfield microscope.

Cell Culture and RNA Analysis—Swiss-3T3 cells were cultured and treated as described previously (13). Neonatal Schwann cells were prepared according to Manthorpe and Varon (14) and passaged in DMEM containing 10% fetal bovine serum. The myelomonocytic cell lines HL-60 was obtained from the American Type Culture Collection (Rockville, MD). HL-60 cells were routinely cultured in RPMI-1640 supplemented with 10% horse serum. Before treatment cells were washed twice in PBS. Phorbol 12-myristate 13-acetate (16 ng) and dimethyl sulfoxide (1.25%) were added to the cells, and RNA was extracted at the times indicated. Northern blot analysis was done as described previously (9).

Immunocytochemistry—Testes from a young adult macaque were processed in the same manner as the mouse embryo sections. Adult human sperm from ejaculate was washed by pelleting the cells three times through PBS, freezing in OCT in hexane, and sectioning to 8-µm thickness. The sections were fixed in 1% paraformaldehyde in PBS for 2 min, washed with PBS, and blocked for 20 min in normal goat serum (Vector Labe) diluted 1:20 with PBS. Sections were then incubated for 1 h with affinity-purified rabbit polyclonal antisera raised against the carboxy-terminal 137 amino acids of the human B94 protein (9). Serial sections were incubated with blocked antisera plus either DAPI or antibody specificity. Sections were then washed three times with PBS and incubated for 30 min with fluorescein-conjugated goat anti-rabbit antibody (Sigma) diluted 1:20 in PBS. Sections were rinsed five times with PBS and then examined and photographed on a Leitz Orthoplan fluorescence microscope.

RESULTS

Isolation and Analysis of Mouse B94 cDNAs—Mouse B94 has been shown previously by Northern analysis to be expressed in a variety of tissues including the embryonic kidney (9). Screening of day 17 post-coitum (dpc) embryonic kidney cDNA library with a human cDNA probe resulted in the isolation of eight overlapping clones, the longest two of which were characterized further. The composite cDNA sequence contained one long open reading frame encoding a 650-amino acid polypeptide and a long nonconserved 3′-untranslated sequence terminating in a consensus polyadenylation signal and poly(A) tail (data not shown). Comparison of the predicted amino acid sequence with its human homologue showed that the sequences were 73% identical and 83% similar (Fig. 1). Interestingly, cysteine residues which are usually invariant were relatively conserved.

Chromosomal Localization—Genomic DNA samples prepared from mouse-tail DNA restriction endonucleases and from several interspecific backcrosses was utilized to determine the chromosomal localization of the mouse B94 gene. Initially, DNA from each of the parental mice was digested with various restriction enzymes and hybridized with labeled cDNA to identify a polymorphic restriction site within the B94 locus. A polymorphism detected with the enzyme TaqI (Fig. 2A) was subsequently used to monitor the segregation of B94 with reference to known positional markers in a haplotype analysis of the backcross mice. B94 co-segregated with markers on mouse chromosome 12. Analysis

The sequence is submitted to GenBank with the accession number L24118.
Fig. 1. Comparison of mouse and human predicted amino acid sequence. Numbers refer to the mouse sequence (mB94). Periods indicate identity of residues in the human sequence (hB94) as compared with the mouse. Gaps are indicated by dashes. Regions with high charge content are boxed, and cysteine residues are circled.

Fig. 2. Chromosomal Localization of the mouse B94 gene. A. Southern blot demonstrating a restriction fragment length variation for mouse B94 when DNA from C3H/HeJ-gld/gld x Mus spretus F1 (SC) (right lane, arrows) is compared with DNA from C3H/HeJ-gld/gld (CC) mice that has been digested with TaqI. Molecular size standards are indicated to the left of the blot. B, segregation of the mouse B94 gene (Tnfb94) with markers on chromosome 12. Markers closely linked with Tnfb94 are α-antitrypsin (Aat-1) and the immunoglobulin heavy chain (IGH) locus. Black boxes denote the homoyogous pattern and white boxes the heterozygous pattern. Number of observed crossover events are indicated below the boxes. C, schematic diagrams of syntenic regions of mouse chromosome 12 and human chromosome 14. To the left of the mouse chromosome diagram are the calculated distances in centimorgans (cm) of marker loci from the marker locus and 6.6 centimorgans distal to the α-antitrypsin (Aat-1) locus as evidenced by crossovers between the immunoglobulin heavy chain (IGH) locus and B94 in 4 out of 114 backcross mice and crossovers between Aat-1 locus and B94 in 11 of 114 backcross mice (Fig. 2B). This region of mouse chromosome 12 is syntenic with the most distal region of human chromosome 14, and the result is in agreement with the previous localization of human B94 by in situ hybridization to the q32 band of chromosome 14 (Fig. 2C).

Developmental Expression Patterns—Expression patterns of B94 during murine development were determined by in situ hybridization of 35S-labeled antisense RNA to sagittal sections of embryos aged 10–19 dpc. Serial sections were routinely probed with 35S-labeled sense RNA as a control for nonspecific hybridization.

B94 was expressed in a spatially and temporally restricted pattern that was for the most part consistent throughout the gestational period. At 10 dpc, B94 was present in the forming myocardium and in the aortic endothelium (Fig. 3A). Persistent myocardial expression of B94 was evident throughout embryonic development albeit at reduced levels. The aortic endothelium demonstrated marked expression through 19 dpc.
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**FIG. 3. Expression patterns of B94 mRNA in embryogenesis.** Near midline sagittal sections of embryos aged 10 (A, B), 15 (C, D), 17 (E, F), and 19 (G, H) days hybridized with either antisense (A, C, E, G) or sense (B, D, F, H) mouse B94 32P-labeled riboprobe. Abbreviations are: Ht, heart; Mb, mandible; NC, nasal cavity; Vt, vertebrae; Li, liver. In A, hybridization patterns of B94 to day 10 allantois is shown. In E, a second day 17 section is inset for demonstrating vertebral hybridization.

this developmental period: blood cell production moves from the yolk sac to the liver around gestational day 13 and gradually to the spleen at day 17 (15).

Other tissues positive for B94 expression during embryonic development included the epithelium of the trachea and the oropharynx 17-19 dpc, the hypophysis 15-19 dpc, the submandibular gland 17-19 dpc, and discrete cells in the thymus 19 dpc. Finally, the developing kidney expressed B94 mainly in developing glomeruli, although diffuse signal was present throughout the organ. By adulthood renal expression of B94 was extinguished.

Since the role of TNF in embryonic development is primarily limited to the thymus (16), other factors were likely to be responsible for promoting B94 expression in embryogenesis. To determine if growth factors were capable of inducing B94, quiescent fibroblasts were stimulated with bovine calf serum or purified growth factors and induction of B94 transcript was monitored by Northern analysis. B94 was inducible by serum and to a lesser extent by platelet-derived growth factor and fibroblast growth factor (Fig. 4). Maximal induction occurred 4 h after treatment, was not inhibitable by cycloheximide, and gradually decreased through 48 h.

**B94 Expression in Postnatal Murine Tissues—**Postnatal patterns of B94 expression were also explored by in situ hybridization. Although many tissues expressed little or no B94 postnatally, including the heart, kidney, liver, and lung (data not shown), tissues involved in hematopoiesis and lymphoid development displayed B94-specific hybridization (Fig. 5). In postnatal day 8 spleen, B94 was highly expressed throughout the red pulp and in the white pulp marginal zone but not in the white pulp. By adulthood, expression was limited to cells in the red pulp surrounding the white pulp and to groups of cells just inside the splenic capsule. B94 was also present in the thymic medulla at postnatal day 15 and adulthood and in the luminal region of lymphoid aggregates in the adult small intestine, an area rich in phagocytic antigen presenting cells and T-cells. Additionally, high levels of B94 were detected in human adolescent tonsil epithelium. A small population of cells in the tonsillar germinal centers also produced B94.

**B94 Expression in Hematopoietic Cells—**In order to determine if B94 was expressed by blood cells or their precursors, in situ hybridization was carried out on mouse bone marrow hematopoietic precursor cells and on mature human peripheral blood leukocytes. B94 expression was evident in large mononuclear cells in the bone marrow, likely to belong to the myelomonocytic lineage, but not in smaller mononuclear cells of the lymphoid lineage nor in more differentiated granulocytic or erythroid cells (Fig. 6, A and B). Consistent with this result mature peripheral blood monocytes but neither lymphocytes
nor polymorphonuclear enriched fractions expressed B94 (Fig 6, C–F).

Northern blot analysis of myelomonocytic cell lines demonstrated that B94 was present at low levels in the basal state (data not shown). In the pluripotential cell line HL-60, increased expression of B94 was observed when treated with phorbol 12-myristate 13-acetate, which promotes differentiation down the myelomonocytic pathway (Fig 6G). B94 expression remained at basal levels when HL-60 cells were treated with dimethyl sulfoxide, which causes cells to differentiate down the neutrophil lineage.

**Alternate Polyadenylation of the B94 Transcript in the Testis**—Previous studies have shown that B94 hybridizes on Northern blots with a highly expressed 2.5-kb second transcript (9). To determine how this transcript differed from the originally described and cloned 4.2-kb species, short ³²P-labeled fragments of mouse cDNA were used to probe mouse testis Northern blots at high stringency. Probes including sequence from the 3'-untranslated region and the coding region hybridized with both RNA species, whereas probes from the 5'-untranslated region did not hybridize with the smaller testis-specific transcript (data not shown). Reverse transcriptase polymerase chain reaction of testis RNA with an oligo(dT) adapter and a B94 coding region-specific primer demonstrated that the 2.5-kb transcript was polyadenylated just five nucleotides downstream of the open reading frame termination codon (Fig 7). Surprisingly, the consensus polyadenylation signal sequence was found to be present 13 nucleotides upstream of the stop codon, within the open reading frame. Comparison of human and mouse nucleotide sequences in this region showed that the polyadenylation signal and the sequence surrounding the polyadenylation site were evolutionarily conserved. Full-length cDNA clones of the 2.5-kb species isolated from oligo(dT)-primed testis cDNA library were found by sequence analysis to be identical other than in the placement of the poly(A) tail (data not shown).

The 3'-untranslated region has been implicated in affecting transcript stability as well as translational efficiency (17). Since 3'-untranslated region sequences were not present in the 2.5-kb form of B94, it was possible that one of these two processes was affected. To explore transcript stability it was necessary to find a cell line that expressed both forms of B94. Of many cell lines examined, primary embryonic mouse Schwann cells were the only one found to express both species (Fig 7B). Initial actinomycin D chase experiments did not reveal a significant difference in stability of the two transcripts (data not shown).

**B94 Expression in Reproductive Tissues**—*In situ* hybridization was used to localize B94 expression within the testis. B94 transcript was detectable in a subset of seminiferous tubules over late stage rounded spermatids (Fig 8A). This localization was supported by hybridization of B94 cDNA to a Northern blot of RNA derived from morphologically staged rat seminiferous tubules where B94 was found to be highly expressed in stages 7 and 8, to a lesser extent in stages 9–12, and was undetectable in stages 1–6 and stage 13 (data not shown). Only the 2.5-kb species of B94 transcript was evident in the isolated seminiferous tubule Northern blot, confirming that the smaller transcript was the one present in rounded spermatids.

To determine whether B94 was also present in female reproductive organs, *in situ* hybridization was carried out on sections of an adult mouse ovary. Although no B94 was detectable in the ovary, the fallopian epithelium expressed high levels of transcript (Fig 8, B and C).

An affinity-purified polyclonal antiserum directed against human B94 was used to localize B94 protein in sections of monkey testis. The acrosomal compartment in the head of mature spermatids demonstrated specific reactivity to this antiserum (Fig 9A). Acrosomal staining was not apparent in sections incubated with blocked antiserum (data not shown). To determine whether sperm competent for fertilization also contained B94 protein, frozen sections of sperm from human ejaculate were reacted with the B94 polyclonal antiserum. Staining for B94 was again seen specifically in the acrosomal compartment (Fig 9, B and C). Thus the transcript for B94 was expressed at the sperm developmental stage immediately preceding that in which the protein was found.

**DISCUSSION**

In this paper we have described in detail the expression patterns of B94, a cytokine- and growth factor-inducible primary response gene. In order to carry out these studies, it was first necessary to clone and characterize the mouse B94 homologue. Although the derived amino acid sequence showed a fairly high degree of identity to its human counterpart, some potentially significant structural features of the mouse sequence were not conserved. First, the human B94 protein is predicted to contain 7 cysteine residues, whereas the mouse contains eight. Furthermore, only 5 cysteines are conserved between the two molecules. The nonconserved cysteine residues cluster at the NH₂- and COOH-terminal regions of the proteins. This lack of conservation suggests that either disulfide bridging is not a determinant in B94 secondary structure or that B94 is a rapidly evolving protein. Second, a stretch of charged residues present in the NH₂-terminus of the mouse
**Fig. 6. B94 expression in leukocytes.** Brightfield views of B94 *in situ* hybridization to hematopoetic mouse bone marrow cells (A, B) and to peripheral blood leukocytes (C, D, E, F) with antisense (A, C, E, F) or sense (B, D) 32P-labeled riboprobes. Cells in A and B are mixed populations of bone marrow cells. C and D are purified peripheral blood monocytes. E represents purified lymphocytes, and F represents purified polymorphonuclear cells. G, Northern hybridization of B94 to RNA from HL-60 cells untreated or stimulated with dimethyl sulfoxide for 1 and 4 h or with phorbol 12-myristate 13-acetate for 4 h. Lanes marked CHX were additionally treated with cycloheximide. *Lower panel* shows ethidium bromide staining of 28S RNA to demonstrate equal loading of RNA samples.

**Fig. 7. Differential polyadenylation of the testis B94 isoform.** A, nucleotide sequence of B94 cDNAs near the site of polyadenylation in the testis. Numbering refers to the mouse cDNA sequence, and the carboxyl-terminal 36 amino acid residues are represented below the sequence. The stop codon is indicated with an asterisk. The putative polyadenylation signal is boxed, and the site of polyadenylation in the testis form is indicated. The human cDNA (hB94) is shown above the mouse sequence on the second line, with lowercase *letters* indicating divergent nucleotide residues. B, Northern blot hybridization of B94 to poly(A)+ RNA from mouse embryonic Schwann cells and placenta, demonstrating the existence of a smaller transcript in cells outside the testis.

Predicted protein is reduced in charge density when compared with the human sequence. That this mouse sequence encodes the homologue of human B94 is supported by low stringency Southern blot analysis demonstrating the existence of a single gene in the mouse and human genome (9) and by chromosomal localization of mouse B94 to a region of chromosome 12 syntenic with human chromosome 14 where the human B94 gene is resident.

Expression of TNF during normal development is largely restricted to the thymus (18, 16). When pregnant mice and their progeny are repeatedly injected with anti-TNF antisera, the thymus, spleen, and lymph nodes atrophy, suggesting a role for TNF in immune system development (19). Since TNF is expressed by T-cells in the thymus and B94 expression was apparently widespread during development, TNF may not be the only stimulus necessary for B94 expression. In fact, TNF...
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B94 expression patterns apparently followed the course of hematopoiesis through the developmental stages studied. Hematopoiesis shifts location a number of times during embryogenesis, originating in the yolk sac 8–10 dpc, shifting to the liver 12 dpc where hematopoiesis continues at reduced levels for a few weeks after birth, then to the spleen 16 dpc, which stays active throughout adulthood, and finally to the bone marrow 19 dpc (15). Expression of B94 temporally and spatially mirrored the movement of hematopoiesis during development most prominently in the liver, but also in the allantois and the spleen and to a lesser extent in the bone marrow.

We have shown that B94 is expressed in myelomonocytic cells in the bone marrow, in peripheral blood monocytes, and in a cultured myelomonocytic precursor cell line. Furthermore, this cell line when induced to differentiate toward monocytes but not toward granulocytes exhibit increased expression of B94. This evidence suggests that B94 expression in bone marrow-derived and peripheral blood cells is largely restricted to cells of the myelomonocytic lineage. This finding also suggests that macrophages or other antigen presenting cells are likely the source of B94 expression in the thymic medulla, the adult spleen, the tonsilar germinal centers, and the gut-associated lymphatic aggregate. The hybridization patterns in these tissues correlates with that of antigen presenting cells. For example, in the gut-associated lymphatic tissues, macrophages and specialized epithelial M cells are resident in the luminal region where they sample antigens from the gut and present them to underlying lymphoid cells and in the spleen macrophages populate the red pulp and the white pulp marginal zone (28). Although B34 expression patterns in lymphoid tissues appear to recapitulate the distribution of antigen presenting cells, it was impossible at the resolution of the current analysis to confirm the identity of the B94 expressing cell populations. Thus, other cell types in these organs may express B94.

Many genes have different size transcripts in the testis (29, 30), suggesting that the transcriptional machinery may be specialized for the testicular environment. The near complete absence of 3'-untranslated sequence in spermatic B94 may influence protein expression levels in a number of ways, including transcript stability and translational competence. Given the high level of B94 protein in mature sperm acrosomes, it is likely that the truncated form of B94 in some way increases the ability of the cell to synthesize B94 protein. By in situ hybridization we have determined that B94 transcript in seminiferous tubules is present solely in late stage rounded spermatids. Since this stage follows the second meiotic division, it is likely that B94 is transcribed when the gamete is in the haploid state. Round spermatids are known to be transcriptionally competent as shown in heterozygous transgenic mice in which mutant and wild type alleles are expressed in distinct spermatids (31). Our finding that the 2.5-kb B94 transcript species is co-expressed with the 4-kb species in primary cultures of mouse embryonic Schwann cells provides a model for studying the posttranscriptional regulation of this gene.

The acrosome is derived from the Golgi compartment and is thought to be the sperm equivalent of the lysosome or an exocytic granule (28). Proteolytic enzymes involved in dismantling the egg zona pellucida and penetrating the egg membrane are stored in the acrosome as well as other proteins thought to be involved in fertilization (32, 33). The acrosome contents are released into the zona pellucida by the zona-induced acrosome reaction which is mediated by interaction between the sperm plasma membrane and ZP3, a protein component of the zona pellucida (34). This is followed by fusion of the sperm plasma membrane with the outer acrosomal membrane and punctate

may not control B94 expression during murine development.

Both TNF and its receptors are now recognized as members of expanding gene families. The TNF cytokine family includes lymphotoxin-α and lymphotoxin-β which are expressed almost exclusively on the surface of lymphocytes (20), and the ligands for the lymphocyte receptors CD27 and CD40 (21, 22). Proteins with similarity to the p55 and p75 TNF receptors include the B cell antigen CD40 (23), the lymphocyte antigen APO1/Fas (24), and the widely expressed nerve growth factor receptor (25). Thus, B94 may be regulated by one or more of these related molecules.

That B94 expression can be modulated by factors other than TNF was demonstrated through its induction by mitogenic stimuli, including serum, PDGF, and FGF. Developmentally, PDGF is produced by a majority of cells of epithelial origin, and PDGF receptor is found on most mesenchymal cells (26). Similarly, acidic and basic FGF immunoreactivity is detectable in tissues of mesodermal and neuroectodermal origin, and basic FGF is a mesodermal inducer (27). Mesenchymal expression of B94 (vertebral osteoblasts, nasal conchae, mandible, and the forming vasculature) may thus be driven in part by these growth factors.
formation of pores (35). The pores then enlarge first neutralizing the acidic pH of the acrosome and allowing an influx of extracellular calcium, then later permitting macromolecular diffusion between the acrosomal compartment and the extracellular space. Eventually the pores join and the membrane dissipates as free vesicles, leaving the inner acrosomal membrane as the sperm plasma membrane. It is thus apparent that the suborganellar localization of acrosomal components can assist in predicting function, such as association with the inner acrosomal membrane suggests a role in sperm/egg membrane fusion. It will therefore be important to determine by immunoelectron microscopy the suborganellar localization of B94 in the acrosome. Preliminary results from transiently transfected 293T cells indicate that the B94 protein is associated with the membrane fraction.  

Localization of the B94 gene (TnfB94) to mouse chromosome 12 just proximal to the immunoglobulin heavy chain locus is in agreement with and further refines the previous localization of the human gene to human 14q32 (9). The distal telomeric region of mouse chromosome 12 is syntenic to the most telomeric band of human chromosome 14, and the placement of the B94 gene in this region on both chromosomes further confirms their synteny. Additionally the mouse localization shows the B94 gene to be 3.5 centimorgans proximal to the lgh locus, placing the gene in genomic context near to the akt-1 serine-threonine kinase oncogene (36), the putative ets-like gene Elk-2 (37). and in diversity biological processes, including bone formation, blood differentiation, and spermatogenesis. With the advent of embryonal stem cell techniques, which allow for the production of homozgyous mice deficient in the gene of interest, it should be possible to directly test the role of B94 in development.

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3 V. Sarma and V. M. Dixit, unpublished observations.

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