Molecular characterization of mouse gastric zymogenic cells*

Jason C. Mills\textsuperscript{1,2+}, Niklas Andersson\textsuperscript{1+}, Thaddeus S. Stappenbeck\textsuperscript{1,2}, Christopher C. M. Chen\textsuperscript{1}, and Jeffrey I. Gordon\textsuperscript{1}

Departments of \textsuperscript{1}Molecular Biology and Pharmacology, and \textsuperscript{2}Pathology and Immunology,
Washington University School of Medicine, St. Louis, MO 63110

+Both authors contributed equally

Correspondence to:
Jeffrey I. Gordon
Dept. of Molecular Biology and Pharmacology
Washington University School of Medicine
660 So. Euclid Ave.
St. Louis, MO 63110
Tel: 314:362-7243
Fax: 314:362-7047
jgordon@molecool.wustl.edu

Running title: Molecular characterization of mouse gastric zymogenic cells
Summary

Zymogenic cells (ZCs), acid-producing parietal cells (PCs) and mucus-secreting pit cells are the principal epithelial types in the stomachs of adult mice and humans. Each lineage is derived from the multipotent gastric stem cell and undergoes perpetual renewal within discrete mucosal invaginations (gastric units). In this report we analyze the molecular features of ZCs and their contributions to gastric epithelial homeostasis. GeneChip analysis yielded a dataset of 57 mRNAs encoding known proteins and 14 ESTs enriched in adult mouse ZCs. This dataset, obtained from comparisons of cellular populations purified by counter-flow elutriation and lectin panning, was validated by real time quantitative RT-PCR studies of the in vivo expression of selected genes, using cells harvested from different regions of gastric units by laser capture microdissection. ZC-enriched mRNAs include regulators of angiogenesis (e.g., platelet-derived growth factors A and B). Since PCs are enriched in transcripts encoding other angiogenic factors (e.g., Vegfb, the contributions of these two lineages to vascular development was examined by performing quantitative three-dimensional imaging of the capillary networks that surround gastric units in two types of mice. In normal adult gnotobiotic FVB/N animals network density is, on average, 2-fold higher in ZC and PC containing units located in the proximal (corpus) region of the stomach compared to units positioned in the distal (antral) region that lack these lineages (p<0.01). Gnotobiotic transgenic mice with an engineered ablation of all ZCs and PCs have a 2-fold reduction in capillary network density in their corpus region gastric units compared to the corpus units of normal littermates (p<0.01). These results support an emerging theme that angiogenesis in the adult mouse gut is modulated by cross talk between its epithelial lineages and the underlying mesenchyme.
Introduction

The human gastric epithelium is renewed continuously throughout life with an estimated 500,000 cells shed per minute in adults (1). Dysregulated renewal results in a number of disease states, including gastric adenocarcinoma, the second most common cause of cancer-related deaths worldwide (2). The mouse has been used as a model organism to characterize the cellular and molecular features of this renewal, and to decipher its regulation.

The proximal third of the mouse stomach (forestomach) is lined with a squamous epithelium, and its distal two thirds with a glandular epithelium. The glandular epithelium contains thousands of tubular mucosal invaginations known as gastric units. In the central or corpus region of the stomach, each unit has a steady state population of ~200 epithelial cells, representing three principal lineages (pit, parietal, zymogenic) and two minor lineages (enteroendocrine, caveolated) (3). All lineages are derived from multipotent stem cells located in the mid-portion (isthmus) of each unit (Fig. 1A-C) (4,5).

Karam has used *in vivo* pulse labeling with $[^3]$Hthymidine, followed by electron microscopic autoradiography, to obtain morphologic descriptions of the presumptive multipotent stem cell and its immediate committed oligopotent daughters, as well as the pathways their descendants follow during terminal differentiation (5-10). Mucus-producing pit cells, derived from one of the stem cell’s committed daughters, differentiate during a rapid (3 d) migration from the Stem cell niche located in the middle portion of the unit (isthmus) through the upper portion of the unit (pit region) to the surface epithelium, where they are removed by apoptosis or necrosis(7) (Fig. 1A). In contrast, members of the zymogenic cell lineage differentiate during a *downward* migration from the isthmus through the neck region to base of the unit. During this passage, they undergo a series of morphologic transitions from pre-neck to neck, to pre-zymogenic and finally to mature zymogenic cells (Fig. 1A,B,D) (6). Zymogenic cells (ZCs$^1$), also known as chief cells, are cleared at the base of the unit via necrosis, apoptosis, or are phagocytosed by neighboring cells. The zymogenic lineage turns over every 190d (6). Unlike pit
and zymogenic cells, acid-secreting parietal cells (PCs) differentiate within the isthmus (8,10) (Fig. 1A,B,E). PCs then migrate up through the pit region where they are eliminated by necrosis, exfoliation, or phagocytosis, or down to the base, where they are removed by apoptosis or phagocytosis. The average lifespan of members of this lineage is 54 d (8).

We have embarked on a mouse gastric genome anatomy project (mG-GAP; ref. 11; http://genome.wustl.edu/GSCGAP/; http://www.scgap.org) to delineate the molecular features of these epithelial lineages, with the goal of identifying new mediators/regulators of their renewal, new insights about their functions, and new perspectives about gastric physiology. To date, PCs and isthmal gastric epithelial progenitors (GEPs) have been characterized using a variety of functional genomics methods. Studies of PCs purified by lectin panning from the stomachs of normal adult mice belonging to the FVB/N inbred strain yielded a database of 240 genes preferentially expressed in this compared to all other mucosal lineages (11). Thirty-five percent of these genes encode proteins involved in various aspects of energy metabolism, a finding consistent with the fact that the proton pumps of PCs require high energy generating capacity. PCs are also enriched in mRNAs encoding free radical scavengers that protect against potential damage from high rates of oxidative phosphorylation, proteins involved in various aspects of lipid metabolism (PCs maintain an elaborate tubulovesicular apparatus), and components of calcium signaling pathways (e.g., those involving calcineurin) (11). In another study, we generated a database of 147 transcripts enriched in GEPs using an approach that did not physically disrupt the isthmal stem cell niche (12). A substantial fraction of the transcripts encode products required for processing and cytoplasmic localization of mRNAs, including numerous homologs of Drosophila genes needed for axis formation during oogenesis. These mRNA targeting proteins may help gut epithelial progenitors establish differential communications with their neighbors (12,13).

In this report, we present a molecular profile of ZCs retrieved from adult FVB/N mouse stomachs using counterflow elutriation and lectin panning. A GeneChip-derived dataset of transcripts enriched in ZCs compared to other gastric epithelial lineages was validated by real
time quantitative RT-PCR studies of laser capture microdissected populations harvested from gastric cryosections. One finding from the analysis is that ZCs are a source of platelet derived growth factor (pdgf) A and B. Our previously published molecular profile of PCs (11) revealed that they are a source of another angiogenic factor, vascular endothelial growth factor or B (Vegfb). The results of quantitative three-dimensional imaging studies of the capillary networks that surround gastric units in the proximal and distal glandular epithelium of adult germ-free transgenic mice with an engineered ablation of their PCs and ZCs, and in their normal germ-free littermates, provide direct evidence that these lineages contribute to the regulation of angiogenesis.

Experimental Procedures

Animals – Conventionally raised, specified pathogen-free normal FVB/N mice were maintained under a strict 12 h light cycle and fed a standard chow diet (Picolab rodent diet 20, Purina Mills). Members of a pedigree of germ-free FVB/N transgenic mice that express an attenuated diphtheria toxin A fragment (tox176) under the control of nucleotides -1035 to +24 of the mouse gene encoding the β-subunit of H+/K+ ATPase (14), and their germ-free normal littermates, were maintained in plastic gnotobiotic isolators (15). All experiments involving mice were conducted using protocols approved by the Animal Studies Committee of Washington University.

Isolation of ZCs by elutriation and lectin panning - For each cell preparation, 10 conventionally raised mice were sacrificed at eight weeks of age. Their stomachs were rapidly removed, and put in sterile 150 mM NaCl (pH 7.4). Ligatures were placed at the junction between the esophagus and forestomach, and at the pylorus. A 2 mm incision was made in the forestomach, and the entire stomach everted through the incision. Another ligature was placed at the junction between the forestomach and corpus, and 500 µl of a solution of 1.0 mg/ml Streptomyces griseus Pronase E (7 U/mg; Roche Applied Science) in medium A (70 mM NaCl, 5 mM KCl, 50 mM HEPES, 1 mM Na2HPO4, 0.5 mM NaH2PO4, 20 mM NaHCO3, 10 mM EDTA,
11 mM glucose, 5 mg/ml BSA; pH 7.8) was instilled with a 27 gauge needle. Filled gastric sacs were placed in a flask containing 75 ml of medium A and incubated for 30 min at 37°C (16,17). The everted sac was then put into 75 ml of medium B (medium A with 100 mM CaCl₂, 15 mM MgCl₂ and no EDTA; pH 7.4) and incubated for 30 min at 37°C with constant gentle magnetic stirring. The medium, containing shed epithelial cells, was decanted and passed though a nylon mesh (60 µm pore diameter; Millipore). Cells in the flow-thru were recovered by centrifugation (150 × g, 5 min, 23°C) and re-suspended in 5 ml of medium C (140 mM NaCl, 12 mM MgSO₄, 10 mM CaCl₂, 15 mM HEPES, 11 mM glucose, 0.5 mM DTT, 10 mg/ml BSA; pH 7.4). Cellular viability was defined by staining an aliquot with a solution containing 0.1% neutral Red, 0.1% Brilliant Cresyl Blue, and 0.01% Janus Green in PBS.

Cellular concentration was adjusted to 1.5x10⁷/ml medium C and 5 x 10⁷ cells were subjected to counter-flow elutriation (JE-6 elutriator rotor with standard chamber; Beckman Instruments; injection flow rate 10 ml/min medium C containing 1 mg/ml BSA). Details of the elutriation protocol are provided in Table 1S of the on-line supplemental material. Fractions 1-5 (Table 1S) were each subjected to *Dolichos biflorus* agglutinin (DBA)-streptavidin-conjugated magnetic bead panning (11) to recover PCs (PCs are the only gastric epithelial lineage in FVB/N mice that express GalNAcα(1-3)Gal-containing glycans; ref.18). Lectin panned cells from fractions 2-5 (‘PC+ population’) were pooled, and their viability and purity defined with the vital dye mixture described above (Tables 1S, 2S in the on-line supplemental material), and by transmission EM.

Cells in fractions 4 and 5 that remained after DBA panning (Table 2S) were pooled to generate a “ZC+” fraction. The purified ZC+ population, the purified PC+ population, and the cells in fractions 1-2 remaining after DBA panning (ZC-/PC- population) were each collected by centrifugation (150 × g; 5 min; 23°C), re-suspended in RTL lysis buffer (Qiagen), and RNA was extracted (RNeasy Mini kit with on-column DNAase treatment; Qiagen).
**GeneChip analysis** - Equal amounts of ZC+, PC+, or ZC-/PC- RNA from each preparation (n=6 preparations) were pooled (5 µg/cell population/preparation). Equal-sized aliquots of each RNA pool (9 µg) were used to generate biotinylated cRNA targets (11,19). Each cRNA target, in turn, was used to interrogate a set of Mu11K GeneChips (Affymetrix) according to the manufacturer’s recommendations. Data from each chip were scaled to an average target intensity of 150. A series of pair-wise comparisons were performed (Fig. 2A) using proprietary GeneChip software (version 4.0).

**Real-time quantitative (q)RT-PCR** – Selected findings from the GeneChip study were independently validated by qRT-PCR analysis of the same RNAs employed for generating cRNA targets. Each 25 µl qRT-PCR reaction mixture contained cDNA (20), 12.5 µl of 2X SYBR Green master mix (Applied Biosystems), 900 nM gene-specific primers (Table 3S in the on-line supplemental material), and 0.25 units of UDP-N-glycosidase (InVitrogen). A melting curve (15,20) was used to define a temperature where the amplicon, and not primer-dimers, was the source of SYBR-Green-bound fluorescence. All assays were performed in triplicate with an ABI Prism 7700 Sequence Detector (Applied Biosystems). Data were normalized to glyceraldehyde 3-phosphate dehydrogenase mRNA (C_T method).

**Navigated laser capture microdissection (n-LCM)**—Stomachs from conventionally raised 8 week old mice were (n=5) divided in half along their lesser and greater curvatures, and rinsed quickly in PBS. Each half was imbedded in O.C.T. compound (Sakura Finetek, Torrance, CA), and frozen in Cytocool II (Richard-Allen Scientific, Kalamazoo, MI). Serial, 7µm-thick cryosections were cut, placed on Superfrost/Plus slides (Fisher Scientific) and stained with methyl green/eosin Y (20). Well-oriented gastric units (i.e., having an uninterrupted epithelial column that extended from the base to the apex of the pit), located in the corpus region of the stomach, were subjected to n-LCM (PixCell II apparatus; 7.5 µm spot diameter; CapSure HS LCM caps; Arcturus, Mountain View, CA) as described in Results. Epithelial cells were recovered from three different areas: the lowest ~25% (base of the gastric unit; consisting mainly
of ZCs); the top ~25% (pit; predominantly pit cells); and the mesenchyme immediately below the gastric unit (n=300 gastric units microdissected/cryosection; 4-5 cryosections sections/mouse). RNA was isolated from ~5000 cells/fraction/mouse using the PicoPure RNA Isolation kit (Arcturus) with on-column DNase treatment (Qiagen). Equal amounts of RNA from each area were pooled from three mice, and the material used for qRT-PCR studies.

**Transmission EM** - Cells recovered by elution/lectin panning were subjected to transmission EM analysis using protocols described in a previous publication (11).

**Three-dimensional imaging studies of the gastric microvasculature** - Eight-week-old germ-free normal and tox176 mice were anesthetized, and 200 µl of a 20 mg/ml aqueous solution of high molecular weight (2000 kDa) fluorescein isothiocyanate (FITC)-labeled dextran (Sigma) was injected in their retro-orbital plexus using a 30 gauge needle attached to a 1 ml syringe. Three minutes after the 10-15 sec infusion, animals were sacrificed, their stomachs removed, and perfused with fixation solution (0.5% paraformaldehyde, 15% picric acid, and 0.1 M sodium phosphate buffer; pH 7.0). Each stomach was opened with an incision along its greater curvature, pinned on wax, and shaken at 4°C for 12h in fixation solution. Following three washes in ice-cold PBS (5 min/cycle), the stomach was incubated for 3 h in 10% sucrose/PBS (4°C), and then overnight in 20% sucrose/10% glycerol/PBS (4°C). After freezing the tissue in O.C.T., 60 µm-thick cryosections were cut along the cephalocaudal axis. Sections were air-dried (2 h, room temperature in the dark), re-hydrated in ice-cold PBS (1 min), incubated overnight (4°C) in 3% deoxycholic acid (Sigma), rinsed with deionized water (2 cycles, 5 min each, room temperature), followed by a PBS wash (5 min, room temperature) and stained with either Syto61 (Molecular Probes; 1:1000 dilution in PBS; 1h at room temperature) or DBA. Followed by three more PBS washes (5 min/cycle; room temperature), sections were mounted in 50 % glycerol/PBS, viewed under a LSM 510 confocal microscope (Zeiss), and scanned at 3 µm-thick intervals. Scans were projected in three dimensions by taking 20 serial images, aligning them at 7-10 degree intervals, and compiling/rotating them around the y-axis using LSM 510 software.
Results and Discussion

Purification of zymogenic cells from adult FVB/N mouse stomachs

ZCs were purified from the stomachs of conventionally raised 8 week-old mice belonging to the FVB/N inbred strain. Mucosa from the corpus region of the stomach was dissociated by Pronase E digestion, and the cellular suspension size-fractionated by counter-flow elutriation. Five fractions were recovered, and each fraction was subjected to lectin panning with DBA-magnetic bead conjugates to remove parietal cells. This approach yielded two fractions enriched for ZCs (fractions 4 and 5 in Tables 1S and 2S of the on-line supplemental material). Histo- and immunohistochemical analysis using a previously described panel of lineage-specific lectins and antibodies (11,12,18,21) disclosed that ZCs represented 71-73% of the cells in these fractions, compared to 11% in the starting material (Table 2S; n = 6 independent preparations). Overall cell viability in the two fractions, which together composed the ‘ZC+ population’, was >90%, as defined by vital dye exclusion (Table 1S). Transmission EM studies confirmed that the purified ZCs had retained the characteristic morphologic features of mature members of this lineage (Fig. 2B).

A “PC+” population, obtained by combining the cells from fractions 2-5 that bound DBA, contained 84±1% PCs versus 21±2% in the starting material (Table 2S; Fig. 2C), with an overall viability of 91±3% (n = 6 preparations).

A third population was obtained by pooling DBA non-reactive cells from the two elutriated fractions that contained the smallest average cell size (fractions 1-2 in Tables 1S, and 2S). This ‘ZC-/PC-’ fraction contained an average of 50% pit cells, 20% neck cells, <1% PCs, <2% ZCs and 27% ‘other’ cell types (GEPs, enteroendocrine cells, mesenchymal components) (Table 2S; Fig. 2D).

GeneChip-based dataset of transcripts enriched in zymogenic cells

Total cellular RNA was extracted from the ZC+, PC+ and ZC-/PC- populations obtained from each of the six preparations. Equivalent amounts of RNA from a given
population/preparation were pooled. Two cRNA targets were then independently prepared from each pooled RNA sample (ZC+, PC+ or ZC-/PC-), and each cRNA used to interrogate Affymetrix Mu11K GeneChips containing probesets that recognize ~11,000 mouse genes and ESTs.

Comparisons of ZC+ versus PC+ RNAs yielded a dataset of 187 transcripts (156 encoding characterized proteins; 31 encoding unknown or hypothetical proteins) enriched in the ZC+ population in two separate studies (Table 4S). Duplicate comparisons of ZC+ versus ZC-/PC- RNAs identified 124 ZC+ enriched transcripts (95 encoding characterized proteins; 29 specifying unknown/hypothetical proteins; Table 5S). The union of these two datasets (Fig. 2A) produced a list of 57 known genes and 14 uncharacterized genes from what amounted to quadruplicate comparisons (Table 6S).

A reciprocal analysis of PC+ versus ZC+ and PC+ versus ZC-/PC- datasets identified 83 transcripts enriched in PCs (not shown). This list shared 93% identity (77/83 mRNAs) with our previously published datasets of transcripts enriched in PCs harvested by direct lectin panning (i.e., without an elutriation step; ref. 11). The similarity between the current and previous PC+ datasets indicated that the elutriation protocol used in the present study did not artifactually skew the transcriptional profiles of sorted cells.

**Verification of the dataset**

Twelve genes were culled from the ZC+ dataset for initial real time q RT-PCR confirmation of their enriched expression in ZCs. Assays were performed on the same RNAs used to generate cRNA targets for the GeneChip study. Seven of the 12 genes were known to be expressed in ZCs: *Pepf* (pepsinogen F; ref. 22); *Cckar* (cholecystokinin receptor A; binds cholecystokinin and is involved in regulating pepsinogen secretion; 22); *Amy2* (α-amylase); *Try2/4* (trypsin 2/4); *Gif* (gastric intrinsic factor; intestinal absorption of cobalamin), *Anpep* (alanyl aminopeptidase N, also known as CD13, a metalloproteinase found in ZC vesicles; ref. 23); and *Spp1* (secreted phosphoprotein 1 or osteopontin; an adhesive glycoprotein implicated in
mucosal barrier function; ref. 24). Five of the 12 genes had not been previously reported to be
transcribed in ZCs: \textit{Pdgfb} (platelet derived growth factor-B; see below for discussion of
functions); \textit{Fes} (feline sarcoma oncogene; non-receptor protein tyrosine kinase that co-localizes
with Rab proteins and participates in both endocytic and exocytic pathways; ref. 25,26); \textit{Nucb2}
(nucleobindin 2; a calcium-binding Golgi-associated protein whose expression has been
correlated with differentiation and acquisition of polarity; ref. 27,28); plus \textit{Jun} (c-Jun) and \textit{Jund1}
dimerize with one another or with JunB or Fos family members to form the AP-1 transcription
factor that has pleiotropic effects on intracellular signaling; ref. 29,30).

The qRT-PCR study confirmed that the levels of these 12 mRNAs were 10±1 to 421±97-fold higher in ZC+ versus PC+ RNA (Table 1). The fold-enrichment in the ZC+ population was
less pronounced when the comparison involved the ZC-/PC- fraction (-1.4±0.3 to 35±0.2-fold), a
finding that likely reflects the fact that a substantial proportion (20%) of the ZC-/PC- population
consists of neck cell precursors of mature ZCs.

We combined qRT-PCR with laser capture microdissection (LCM) to quantify \textit{in vivo}
levels of expression of several of these genes. A ‘navigated (\textit{n})’ form of LCM that minimizes
cellular mRNA degradation was used to isolate three populations from gastric units located in the
corpus of the stomachs of 8 week old conventionally raised mice: (i) epithelium from the pit
region (composed predominantly of members of the pit cell lineage); (ii) epithelium from the base
(composed principally of ZCs); and (iii) the mesenchyme surrounding the base of units (Fig. 3A).
Stomachs were serially sectioned, and cells harvested from cryosections that had been briefly
stained with methyl green and eosin Y. Dissection was directed (navigated) using an electronic
image template of the immediate previous section that had been stained with \textit{Griffonia
simplifolica II} lectin to mark neck cells, and DBA to tag PCs (Fig. 3A). The quality of the
microdissection was confirmed by noting that the level of the ZC marker, Gif mRNA, was
760±33-fold higher in n-LCM epithelial cells harvested from the base of gastric units compared
to epithelial cells retrieved from the pit region. The pit cell marker, trefoil factor 1 (Tff1) mRNA,
was 14.3-fold higher in pit compared to base epithelium, while vimentin mRNA was 67 ± 2 and 6 ± 0.8 fold higher in mesenchyme versus the pit and base fractions, respectively (Fig. 3B).

Having established the quality of the dissection, subsequent analysis of Cckar, Fes, Anpep, and Spp1 expression revealed that the concentrations of their mRNAs were 18.6 ± 0.3 to 903 ± 15-fold higher in n-LCM ZC-rich base epithelium compared to the other compartments (Fig. 3C).

ZCs are a highly polarized secretory cell lineage. Further validation of the dataset came from the fact that 47% of its component genes encoded proteins involved in (i) translocation of nascent polypeptides into the ER [e.g., Tram1 (translocating chain-associating membrane), Ssrδ (Signal sequence receptor delta)], (ii) protein modification in the ER and Golgi [e.g., Erε1β (endoplasmic reticulum oxidoreductin1-like beta), P4hb (prolyl 4-hydroxylase)], and (iii) vesicular trafficking (e.g., Gabarap (GABA-receptor associated protein), Copζ2 (coatmot protein complex, zeta 2); and Fes] (Fig. 4).

The impact of ZCs on gastric unit homeostasis

The functions and fates of PCs and ZCs appear to be intertwined. Gastric acid secretion first evolved in Elasmobranches ~350 million years ago (31). In non-mammalian vertebrates, the gastric oxyntopeptic cell secretes acid and pepsinogen, while in mammals, PCs export acid and ZCs produce pepsinogen. Some functions performed by PCs in the human stomach are performed by ZCs in the mouse stomach (e.g., secretion of Gif; ref. 32). In addition, transgenic mice that express an attenuated diphtheria toxin A fragment (tox176) under the control of PC-specific transcriptional regulatory elements not only lose their PCs, but also have a discrete block in terminal differentiation of neck cells to mature ZCs (21). The changes are gastric unit autonomous: in mice with a mosaic pattern of transgene expression, only units with tox176-mediated PC ablation lack mature ZCs. Neighboring units that do not support transgene expression have a normal complement of PCs, and ZCs (21).
Growth factors - Our previous GeneChip profiling of PC gene expression revealed that these cells are enriched for insulin-like growth factor binding protein 2 (Igfbp2) mRNA (11). Igfbp2 decreases the bioavailability of IGFs (33). Isthmal GEPs, in turn, preferentially express transcripts encoding Igf-I and the Igf-I receptor (recognizes both Igf-I and Igf-II) (12). Based on these findings, we proposed that GEPs produce and respond to Igfs through an autocrine loop, while PCs may regulate GEP proliferation by sequestering Igfs (12). In this scheme, tox176-mediated ablation of PCs would increase Igf bioavailability in the isthmal stem cell niche (through loss of Igfbp2), thereby contributing to the stimulation of GEP proliferation that is observed in these mice (12,21,34).

The present study disclosed that furin mRNA is enhanced in ZCs relative to PCs or the ZC-/PC- fraction (see Tables 4S-6S plus the LCM/qRT-PCR results in Fig. 3C). Furin (paired basic amino acid cleaving enzyme; E.C. 3.4.21.75) is a membrane-associated protease that activates a number of secreted or membrane-bound factors, including both Igf-I and Igf-IR (35,36). Murinoglobulin (α 2 macroglobulin) mRNA, another transcript enriched in ZCs (Tables 4S-6S) encodes a secreted protease inhibitor that acts as a carrier for multiple growth factors, including Pdgfs, and can block degradation of Igfbps (37). Together, these findings suggest that ZCs may modulate Igf bioavailability and that ZCs and PCs may be part of a homeostatic regulatory network that modulates Igf-mediated GEP proliferative responses in the isthmal stem cell niche.

PCs are also enriched for transcripts derived from the parathyroid hormone-like peptide gene (Pthlh), a known regulator of proliferation in a number of cell lineages (38,39). Furin expression is prominent in rat stomach (40) and activates Pthlh (41), suggesting another way that ZCs and PCs may interact to control epithelial census in gastric units. Interestingly, Furin is commonly co-expressed with Pthlh in gastric cancers, as well as in gastric carcinoma-derived cell lines (36). Transfection of the MKN28 gastric carcinoma cell line with furin cDNA leads to
increased proliferation, while addition of exogenous Pthlh to the culture medium increases furin expression (36).

Angiogenesis - The GeneChip study revealed that ZCs have enriched levels of Pdgfa mRNA. Follow-up qRT-PCR analysis showed that Pdgfb mRNA was also augmented (Table 1). These findings were confirmed in vivo by qRT-PCR assays of LCM cell populations (Fig. 3D). qRT-PCR assays disclosed that the levels of mRNAs encoding Pdgf receptor subunits, Pdgfrα and Pdgfrβ, were higher in the ZC-/PC- fraction compared to the ZC+ population (17±5 and 2.7±0.3-fold respectively; Table 1). This ZC-/PC- fraction is enriched in mucus-secreting pit and neck cells, GEPs and mesenchymal components. Therefore, to assess Pdgf expression in these populations in vivo, we used germ-free tox176 mice to procure these cells from the pit, isthmal and neck regions by n-LCM. Germ-free rather than conventionally raised tox176 mice were used since the latter develop bacterial overgrowth with gastritis from loss of the acid-barrier to colonization (12,14).

The pit, isthmal and neck domains of germ-free tox176 gastric units were defined in a given eosin Y/methyl green-stained cryosection by marking pit and neck epithelium in the adjacent ‘navigator section’ with AAA and GSII lectins. qRT-PCR assays of RNAs prepared from the three LCM fractions (5000 cells pooled from 3 mice/fraction) revealed that Pdgfrα and Pdgfrβ mRNAs were expressed at similar levels in the GEP-enriched isthmus, as well as in the neck and pit cell zones (data not shown).

The Pdgf family of ligands and their receptors play important roles in regulating vasculogenesis. Pdgfb and Pdgfrb are required for recruitment of vascular smooth muscle cells and pericytes into nascent vessels. Mice homozygous for Pdgfb or Pdgfrb null alleles die prior to birth with vascular abnormalities (42,43). Moreover, administration of dimeric Pdgfb promotes healing of ulcers produced by nonselective cyclooxygenase inhibitors (44). The majority of Pdgfa-/- mice die at E10 (45). While a small fraction survive to postnatal period, those that do typically
succumb prior to the completion of morphogenesis of gastric units, or their assembly of a full complement of mature ZCs (P28) (10, 45). Knocking out Pdgfra produces a more severe phenotype than that observed with a Pdgfa null mutation: death between E8-E16 with various vascular and skeletal abnormalities (43).

These observations, coupled with our earlier functional genomics studies showing that PCs are enriched for vascular endothelial growth factor B (Vegfb) mRNA (11), suggested that PCs, which are long-lived, broadly distributed along the length of gastric units in the corpus region of the stomach and required for terminal differentiation of ZCs, may collaborate with long-lived Pdgf-expressing ZCs to regulate angiogenesis in the gastric mucosa.

To test this hypothesis in vivo, we took advantage of a method we developed for quantitative three-dimensional imaging of mucosal capillary networks in the adult mouse small intestine (46). A solution of high molecular weight FITC-conjugated dextran was injected into the retro-orbital plexus of anesthetized six week-old normal and tox176 littermates. Animals were sacrificed 3 min later, and 60 µm thick cryosections were cut along the cephalocaudal axis of their stomachs. Sections were labeled with Syto61 to mark epithelial nuclei, or with DBA to visualize PCs, and confocal microscopic images collected at 3 µm intervals. Twenty serial images/region/mouse were then assembled into a rotating three-dimensional ‘movie’ and the mucosal capillary network viewed from multiple perspectives. The density of the microvascular network was quantified by scoring the total number of capillaries branches surrounding well-oriented units relative to the length of each gastric unit.

Since angiogenesis in the mouse small intestine is modulated by components of the indigenous microbial community (the ‘microbiota’; ref. 46), all of our comparisons of capillary network complexity in normal and tox176 littermates were performed using mice raised under germ-free conditions to circumvent the potentially confounding variable of bacterial overgrowth with pan-gastritis encountered in conventionally raised tox176 mice.
Gastric units in the distal glandular epithelium (antrum; Fig. 5A) lack ZCs and PCs (they are primarily populated by mucus-producing pit and neck cells; ref. 3). Therefore we first examined the effects of PCs/ZCs on angiogenesis by comparing the density of capillary networks surrounding ZC and PC-containing units in the proximal corpus region of the stomach of normal germ free mice versus those surrounding their antral units. If factors elaborated by PCs and ZCs are important for establishing and/or maintaining the network, then the network should be more complex (dense) in corpus units. Indeed, quantitative three-dimensional image analysis (n = 15 units scored/region/animal; 20 serial optical sections/region/mouse; 3 mice/group) confirmed that the corpus region contains twice the number of capillary branches per micron length of their gastric units compared to antral units (p<0.01; Students t-test) (Fig. 5B,C,F).

To further test the hypothesis that ZCs and PCs affect angiogenesis, we compared capillary network density in the same regions of the stomachs of germ-free normal mice and age- and gender-matched tox176 littermates that lack PCs and ZCs in all regions of their glandular epithelium. ZC/PC ablation from the corpus region of tox176 animals reduced gastric unit microvascular network density 2-fold (p<0.01) without affecting network density in antral units (Fig. 5B-F). Indeed, antral gastric unit capillary density in tox176 mice was not significantly different from that of normal mice, suggesting that the effects of PC/ZC ablation are confined to the region of the stomach where these cells are lost (i.e., the effects on appear to be gastric unit autonomous). In all regions of the tox176 stomach, vessels run parallel to the base-to-pit axis of gastric units, with few encircling branches - a pattern similar to that encountered in the antral units of normal mice (Fig. 5C-E).

Together, these results reveal that ZCs and PCs have a positive role in regulating formation of the microvascular network surrounding gastric units. Our findings support an emerging theme that epithelial-mesenchymal cross talk plays an important role in modulating angiogenesis in the adult mouse gut: e.g. removal of Paneth cells, which differentiate at the base of discrete mucosal invaginations in the small intestine known as crypts of Lieberkühn, results in
arrested postnatal development of the capillary network in the mesenchymal cores of crypt-villus units (46).

Our current findings raise questions concerning the impact of changes in the representation of ZCs and PCs the human mucosal microvasculature. For example, chronic atrophic gastritis, one outcome of infection with *Helicobacter pylori*, is associated with loss of PCs and ZCs and is an apparent precursor of gastric adenocarcinoma (47,48). PC and ZC loss occurs in late-stage Ménétrier’s disease, a disorder that results in gastric epithelial hyperproliferation (49). Correlating the degree of PC and ZC loss with the state of elaboration of the mucosal capillary network in these diseases may yield new insights about host factors that modulate their risk for progression to neoplasia. Our functional genomics studies suggest that the effects of these epithelial lineages on angiogenesis may be mediated, at least in part, by Pdgf and Vegf family members. Further genetic and pharmacologic dissection of the mechanisms by which PCs and ZCs affect the complex process of generating a mucosal microvascular network may provide new therapeutic approaches for facilitating gastric epithelial repair after injury.

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Footnotes

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1 Abbreviations used: ZC, zymogenic cell; PC, parietal cell; GEP, gastric epithelial lineage progenitor; n-LCM, navigated form of laser capture microdissection; qRT-PCR, real time quantitative reverse transcriptase-PCR; DBA, *Dolichos biflorus* agglutinin, GSII, *Griffonia simplicifolia* II lectin; AAA, *Anguilla anguilla* agglutinin; Igf, insulin like growth factor; Pdgf, platelet-derived growth factor.
Figure Legends

Fig. 1 - Organization of gastric units located in the corpus region of the stomach of a conventionally raised adult FVB/N mouse. (A) Schematic representation of the pathways of gastric epithelial lineage differentiation. (B) Visualization of the principal epithelial lineages using multi-label immunohistochemistry. A Bouin’s fixed, paraffin-embedded section of the stomach was incubated with rabbit anti-human intrinsic factor and Alexafluor 594-conjugated goat anti-rabbit Ig to visualize ZCs as red, FITC-tagged DBA to mark PCs as green, Alexafluor 647-labeled GSII lectin to tag neck cells purple, and Alexafluor 350-conjugated AAA to mark pit cells as blue. (C) Transmission EM of isthmal GEPs. Note the lack of secretory granules and the high nuclear:cytoplasmic ratio. (D) Transmission EM of two ZCs at the base of a gastric unit. (E) Transmission EM of a PC showing abundant large mitochondria and elaborate membrane folding. Bars = 25µm in B; 1 µm in C-F.

Fig. 2 - Purification of ZCs from the adult FVB/N mouse stomach. (A) Outline of GeneChip comparisons of RNAs prepared from purified cell populations. (B) Transmission EM of a ZC in the ZC+ population. (C) Transmission EM of a parietal cell in the PC+ fraction. Arrow points to the magnetic bead-DBA conjugates that bind PCs recovered by lectin panning. (D) Representative cells from the ZC-/PC- population. Insets show electron dense pit cell granules (blue box), large mitochondria in a pre-parietal cell (green box), and granules with electron-lucent cores in a neck cell (purple box). Bars = 1 µm in B-D, and in the insets to panel D.

Fig 3 - n-LCM/qRT-PCR validation of GeneChip results. (A) Outline of areas targeted for n-LCM (blue lines, pit regions of two adjacent gastric units; red, ZC-rich bases of these units; black, underlying mesenchymal tissue). The cryosection was stained with horseradish peroxidase (HRP) conjugated DBA (seen as blue after development in Vector SG) and HRP-labeled AAA
(seen as red after treatment with Vector VIP). The stained section is representative of the image templates used to guide (navigate) dissection of an adjacent eosin Y/methyl green stained section (see text for further discussion). (B) qRT-PCR studies of the efficacy of n-LCM. Mean values (± 1 SD; triplicate assays) are expressed relative to the mesenchymal region for Tff1 mRNA, and the pit region for vimentin and Gif mRNAs. (C) qRT-PCR validation of the enriched expression of transcripts from the ZC+ dataset in n-LCM ZC-enriched epithelium harvested from the base of normal gastric units. Data are expressed relative to the pit region. (D) Relative levels of expression of Pdgf mRNAs. Bar in A = 25 µm.

Fig. 4 - mRNAs in the ZC+ dataset associated with protein secretion. Gene symbols (abbreviations) are listed. See Table 6S for gene names.

Fig. 5 - Capillary networks surrounding gastric units in the corpus and antral regions of the glandular epithelium of 6-week old germ-free normal mice and their tox176 transgenic littermates. (A) H & E-stained section prepared along the cephalocaudal axis of the stomach, with false coloration of lumen to delineate forestomach, corpus and antrual regions. (B-E) Cryosections (60 µm thick) were cut from normal and tox176 mice after retro-orbital infusion of FITC-tagged high molecular weight dextran, and stained with Syto61 to visualize nuclei. Sections were serially scanned at 3 µm thick intervals using a confocal microscope. Capillaries appear green and nuclei red. Representative gastric units are shown from the indicated regions of the stomach. Three-dimensional rotating images of the capillary networks in each region can be found in the on line supplemental material http://gordonlab.wustl.edu/stomach/. (F) Quantitative analysis of the number of capillary branches surrounding well-oriented gastric units. Mean values ± SD are plotted (n = 3 mice/group). Bars in B-E = 25 µm.
### Table 1 - Real time quantitative RT-PCR analysis of mRNA levels in purified mouse gastric cell populations

| Gene Name                                      | Symbol  | ZC+ vs. PC+ | ZC+ vs. ZC-/PC- |
|-----------------------------------------------|---------|-------------|-----------------|
| Pepsinogen F                                  | Pepf    | 28±6        | -1.4±0.3        |
| Gastric intrinsic factor                      | Gif     | 293±100     | 6.2±2.1         |
| Amylase 2                                     | Amy2    | 421±97      | 35±0.2          |
| Trypsin 2/4                                   | Try2/4  | 197±65      | 10±3            |
| Alanyl (membrane) aminopeptidase (CD13)       | Anpep   | 58±11       | 3.9±0.7         |
| Secreted phosphoprotein 1 (osteopontin)       | Spp1    | 16±6        | -2.6±1.0        |
| Nucleobindin 2                                | Nucb2   | 82±26       | 4.5±1.4         |
| Feline sarcoma oncogene                       | Fes     | 21±7        | 3.2±1.0         |
| Jun oncogene                                  | Jun     | 12±3        | 2.1±0.5         |
| Jun D proto-oncogene                          | Jund1   | 10±2        | 1.5±0.3         |
| Cholecystokinin A receptor                    | Cckar   | 188±30      | 3.6±0.6         |
| Cholecystokinin B receptor (Gastrin receptor) | Cckbr   | -3±0.6      | 1.2±0.2         |
| Platelet derived growth factor A              | Pdgfa   | 10±1        | 2.5±0.8         |
| Platelet derived growth factor B              | Pdgfb   | 39±3        | 2.7±0.4         |
| Platelet derived growth factor receptor, α chain | Pdgfra | -7±2        | -17±5           |
| Platelet derived growth factor receptor, β chain | Pdgfrb | -1.4±0.2    | -2.7±0.3        |
| Gastric H⁺/K⁺ ATPase, β subunit               | Atp4b   | 3.3±0.2     | 12±0.7          |
Figure 1

A

PIT

Pit Cell

Parietal Cell

ISTHMUS

Pre-Pit Cell

Pre-Neck Cell

Stem Cell

Pre-Neck Cell

Pre-Parietal Cell

Parietal Cell

NECK

Neck Cell

Parietal Cell

BASE

Zymogenic Cell

Parietal Cell

B

C

D

E
1. Ribosome attachment/ ER translocation: Ssr4, Tram1, Rrbp1, Srpr

2. ER/Golgi function: Herpud1, Prdx4, Asns, Pycs, Galk1, Nucb2, Ero1-lb, Xbp1, Dnajc3, P4hb, Pdip, Siat10, Ugart2, Gorasp2

3. General vesicular trafficking: Gabarap, Actb, Fes, Arhq, Cckar, Cryab, Cop2, Rgl2

4. Vesicular contents: Pglyrp, B2m, Amy1, Ela2, Try2, Try4, Anpep, Furin, Pnliprp2, Srm, Hapb2, Gif, Clca3
On-line Supplemental Material

Table 1S  Counter-flow elutriation protocol, cell yield and viability

| Fraction Collected | Rotor speed (rpm) | Flow rate (ml/min) | Volume collected (ml) | Yield ($\times 10^6$ cells) | Viability (%) |
|--------------------|-------------------|-------------------|----------------------|-----------------------------|---------------|
| Before elutriation | -                 | -                 | -                    | 123±19                      | 90±3          |
| -                  | 2000              | 10                | 50                   | -                           | -             |
| 1                  | 2000              | 15                | 50                   | 35±5                        | 95±1          |
| 2                  | 2000              | 40                | 100                  | 17±2                        | 93±2          |
| 3                  | 2000              | 50                | 100                  | 15±2                        | 91±2          |
| 4                  | 2000              | 60                | 100                  | 12±2                        | 93±2          |
| 5                  | 2000              | 70                | 100                  | 5±2                         | 96±1          |

n=6 independent preparations. Mean values ± S.E.M. are presented.
Table 2S Representation of ZCs and PCs in fractions before and after DBA-magnetic bead panning

| Fraction | Before elutriation | After elutriation | After DBA panning |
|----------|--------------------|-------------------|-------------------|
|          | Zymogenic cells (%) | Parietal cells (%) | Other cells (%)   | Zymogenic cells (%) | Parietal cells (%) | Other cells (%)   |
| Before elutriation | 11 ± 3\(^a\) | 21 ± 2 | 68 ± 4 | - | - | - |
| 1        | 2 ± 1             | 5 ± 2          | 93 ± 2 | <1 | <1 | 99 ± 0 |
| 2        | 7 ± 1             | 14 ± 3         | 79 ± 3 | 7 ± 1 | 3 ± 2 | 90 ± 2 |
| 3        | 19 ± 1            | 40 ± 4         | 41 ± 5 | 39 ± 2 | 6 ± 1 | 55 ± 2 |
| 4        | 31 ± 5            | 53 ± 6         | 16 ± 3 | 73 ± 5 | 3 ± 1 | 24 ± 4 |
| 5        | 31 ± 4            | 49 ± 5         | 20 ± 2 | 71 ± 3 | 4 ± 2 | 25 ± 2 |
| DBA beads | -               | -              | -      | <1 | 84 ± 1 | 16 ± 1 |

\(^a\)Values are presented as mean values ± S.E.M.; n = 6 independent preparations.
| Gene Name                        | Symbol | Forward primer (5'-3')                           | Reverse primer (5'-3')                           | Amplicon length |
|---------------------------------|--------|-------------------------------------------------|-------------------------------------------------|-----------------|
| Glyceraldehyde-3-phosphate dehydrogenase | Gapd   | TGGCAAGTGAGATGTAAGTTGAGCC                     | AAGATGCTGATGGGCTTCCCG                          | 104             |
| Gastric intrinsic factor        | Gif    | GAAAAGTGATCTGCTGCTACTGCT                      | AGACAATAGGGCCCCCCAGGATG                       | 100             |
| Gastric H+, K+ ATPase, b subunit| Atp4b  | TCTGCTTTGCGGGAAGCTTGA                        | GGCGATTTGAGCACAGCAT                          | 72              |
| Trefoil factor 1                | Tff1   | GGCCCAAGGAGAAACATGATC                       | ACACCTGATCAAAACAGCAACCT                       | 111             |
| Amylase 2                       | Amy2   | GAGGACTGCTATTGTCACACCTGT                      | AGACCTGACACCCCTCAA                           | 100             |
| Trypsin 2/4                     | Try2/4 | CTGCTCAGAGCTGACTGTA                          | TCACCGTGGAGGAAATCTTT                         | 82              |
| Alanyl (membrane) aminopeptidase (CD13) | Anpep | ATCGCGCCCATTCCATT                           | GGAACCTTGGCACTCTGGTTTTCT                      | 82              |
| Secreted phosphoprotein 1 (osteopontin) | Spp1  | TCACCATTGGGATGACTGAT                        | TCGACTGTAGGGACGATTGG                         | 81              |
| Nucleobindin 2                  | Nucb2  | TTCAGGTGTTCAAACCTGCTTCA                      | TGCTCATCAAAGCTAAGCTGGAT                      | 82              |
| Feline sarcoma oncogene         | Fes    | CGGTTATGGAGGGGATGA                           | GGACATGGTGGAAGCAATCT                         | 84              |
| Jun oncogene                    | Jun    | CTCCAAGTGCCGGAAAAAGG                        | TCGGAGTTTTGGGCTTTCA                          | 81              |
| Jun D proto-oncogene            | Jund1  | ACACGCGAAGAAGGACTACAG                       | GCTCGGTGTTCTGGGTTTTG                        | 138             |
| Cholecystokinin A receptor      | Cckar  | CTGTAACCTCGGATGGA                           | TCAGCTGGCGACTGCGGAA                         | 81              |
| Cholecystokinin B receptor (Gastrin receptor) | Cckbr | CCAGTGAAAGCTGTTCAAACAAAT                    | GCACCCAGGCCATAACCA                           | 83              |
| Platelet derived growth factor A | Pdgfa  | AACACCAGCAGCTAAGTG                           | ACCTTGTGTTTTTTGGTCTCTTC                      | 100             |
| Platelet derived growth factor B | Pdgfb  | CTGTAATCGCGGAGTGAAGAAGA                       | CCAGGAAGTGGGGCTTGGT                         | 83              |
| Platelet derived growth factor receptor a chain | Pdgfa | ATCATGCGAGTCAACATACGT                       | CACTGAGTGGTGAGTTGTGAGGT                      | 102             |
| Platelet derived growth factor receptor b chain | Pdgfrb | ACACATAGACTGAGGGTTAAGGACTTTGG                | CCCTAGAAAGGATACGTTTTAAGGACTTG                | 107             |
| Furin                           | Furin  | GAGCTGAGATCCTGCTTTGCTATG                    | ATCTTCTGCGCTTGAGCATCA                        | 83              |
| Pepsinogen                      | Pep    | GAAGTGCCCTGGGGCTTCT                        | GGCTTTCCGGCCAGGTTTT                         | 100             |
| TC-10                           | TC-10  | GTCTTCCGACCACCTACGAGTCA                     | GATTTACCGGAAGACGATAGA                        | 154             |
| Vimentin                        | Vim    | TGCTTCTGGGACGGTCTTG                        | GGACATGCTGTTCCCTGACTG                       | 128             |
| Gene Name | Symbol | Description | GO terms |
|-----------|--------|-------------|----------|
| ATP-binding cassette, sub-family C (CFTR/MRP), member Abc3 | Abcb1 | Cytoskeleton | GO terms |
| Actin, beta, cytoplasmic | Actb | Cytoplasm | GO terms |
| AE-binding protein 1 | Aep1 | Adhesion | GO terms |
| Anterior gradient 2 (Xenopus laevis) | Agt2 | Extracellular/Cell signaling | GO terms |
| Activated leukocyte cell adhesion molecule | Alcam | Adhesion | GO terms |
| Aldolase dehydrogenase 7 family, member A1 (88% Hs.) | Aldh7a1 | Metabolism | GO terms |
| S-adenosylmethionine deacetylase 1 | Amtl | Metabolism/Polyamines | GO terms |
| Angiopin like 2 | Amsp2 | Miscellaneous | GO terms |
| Amylase 2, pancreatic | Amy2 | Enzyme/Digestive | GO terms |
| Alanyl (membrane) aminopeptidase | Anap | Membrane | GO terms |
| Adaptor-related protein complex AP-3, sigma 1 subunit | Ap3s1 | Protein/Transport | GO terms |
| Ras homolog gene family, member Q | Arrq | Intracellular signaling | GO terms |
| Arginine-rich, mutated in early stage tumors | Armt | Extracellular/Unprocessed | GO terms |
| Asparagine synthetase | Asa | Metabolism/Amino acid biosynthesis | GO terms |
| Activating transcription factor 3 | Atf3 | Nucleus/Transcription regulation | GO terms |
| Activating transcription factor 4 | Atf4 | Nucleus/Transcription regulation | GO terms |
| Beta-2 microglobulin | B2m | Defense/Immunity | GO terms |
| B-cell translocation gene 1, anti-proliferative | Btg1 | Nucleus/Transcription regulation | GO terms |
| B-cell translocation gene 2, anti-proliferative | Btg2 | Nucleus/Transcription regulation | GO terms |
| Cholera toxin A receptor | Ctkar | Membrane/Receptor | GO terms |
| Cyclic D2 | Cnd2 | Nucleus/Receptor | GO terms |
| Cd63 antigen | Cd63 | Membrane | GO terms |
| Cdkn1c | Cdkn1c | Nucleus | GO terms |
| Complement component factor I | C5 | Defense/Immunity | GO terms |
| Chloride channel calcium activated 3 | Cica3 | Transport | GO terms |
| Clusterin | Clu | Membrane | GO terms |
| Procollagen, type VII, alpha 1 | Col7a1 | Membrane | GO terms |
| Cystatin, alpha B | Cst3 | Enzyme/inhibitor | GO terms |
| Chymotrypsin-like | CltA | Enzyme/Digestive | GO terms |
| Catenin | Catc | Protein | GO terms |
| Chemokine (C-X3-C motif) ligand 1 | Cxcl1 | Defense/Immunity | GO terms |
| Cysteine rich protein 61 | Cyst61 | Cytoskeleton/Factor Binding | GO terms |
| Dolichyl-di-phosphooligosaccharide-protein glycotransferase | Dolost | Posttranslational modification | GO terms |
| DnaJ (Hsp40) homolog, subfamily A, member 1 | DnaA1 | Protein/folding | GO terms |
| DnaJ (Hsp40) homolog, subfamily B, member 1 | DnaB1 | Protein/folding | GO terms |
| DnaJ (Hsp40) homolog, subfamily C, member 3 | DnaC3 | Protein/folding | GO terms |
| Eukaryotic translation initiation factor 4E binding protein | Elf4ebp1 | Protein/Translation | GO terms |
| Elastase 2 | Ela2 | Enzyme/Digestive | GO terms |
| ELL-related RNA polymerase II, EF | Ell2 | Hypothetical/Transcription | GO terms |
| Embigin | Emb | Membrane | GO terms |
| Epithelial membrane protein 1 | Emp1 | Membrane | GO terms |
| ER-resident protein Efra85 | Efra85 | Protein/Transport | GO terms |
| Fatty acid binding protein 2, intestinal | Fabp2 | Transport/Fatty acids | GO terms |
| Felina sarcoma oncogene | Fes | Intracellular signaling | GO terms |
| Gene | Function | Description |
|------|----------|-------------|
| Fragilis | Fgl-pending | Defense/Immunity |
| FKB56 binding protein 11 | Flk11 | Protein/Transport |
| FB1 osteocaroma oncogene B | Fosb | Nuclear/Transcription regulation |
| Furin (paired basic amino acid cleaving enzyme) | Furin | Membrane/Receptor |
| FXVQ domain-containing ion transport regulator 6 | Fxyd6 | Transport/ion |
| Gamma-aminobutyric acid receptor associated protein | Gabarap | Cytoskeleton |
| Gamma-aminobutyric acid (GABA-A) transporter 1 | Gabt1 | Transport/G 
| Galactosine 1 | Gal1 | Metabolism/Glycosylation |
| Gastrin | Gastrin | Cytoskeletal/Growth factor |
| Acid beta glucosidase | Gba | Metabolism/Carbohydrate |
| Gastric intrinsic factor | Gif | Transport/Digestion |
| Golgi phosphoprotein 2 | Golph2 | Hypothetical |
| Golgi reassembly stacking protein 2 | Gorusp2 | Protein/Transport |
| H2-K region expressed gene 4 | H2-Kk4 | Transport/ion |
| H3 histone, family 3B | H3f3b | Nucleus |
| Hyaluronic acid-binding protein 2 | Halbp2 | Protein/Metabolism |
| Heparin binding epidermal growth factor-like growth factor | Hugf1 | Cytoskeletal/Growth factor |
| Homocysteine-inducible, endoplasmic reticulum stress-in-Herp1 | Proteint | Protein/Transport |
| Hairy and enhancer of split 5, (Drosophila) | Hex1 | Nucleus/Transcription regulation |
| Hepatocyte growth factor activator | Hgfac | Enzyme/Extracellular |
| Histidine triad nucleotide-binding protein | Hnt | Intracellular signaling |
| Histone 2, H2ax1 | Hist2h2a1 | Nucleus |
| Human immunodeficiency virus type I enhancer binding protein 1 | Hiv1 | Nucleus/Transcription regulation |
| Heat shock protein, 105 kDa | Hap10s | Protein/folding |
| Heat shock protein, 25 kDa | Hap25 | Protein/folding |
| Heat shock protein, 70 kDa 3 | Hap70-3 | Protein/folding |
| Heat shock 70kD protein 5 (glucose-regulated protein, 78kDa) | Hspa5 | Protein/folding |
| Immediate early response 2 | Ier2 | Miscellaneous |
| Interferon induced transmembrane protein 3-like | Ifbn3 | Defense/Immunity |
| Immunoglobulin heavy chain 6 (heavy chain of IgM) | Igh-6 | Defense/Immunity |
| Inhibin beta-C | Inhbc | Cytoskeletal/Growth factor |
| Jun oncogene | Jun | Nucleus/Transcription regulation |
| Jun proto-oncogene related gene d1 | Jund1 | Nucleus/Transcription regulation |
| Kidney androgen regulated protein | Karp | Macrophage |
| KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein n | Kdel3 | Protein/Transport |
| Keratin, type I cytoskeletal 21 (89% Rn.) | Kg21 | Cytoskeleton |
| Keratin K7, type II, epithelial (Hs. 81%) | Kk7 | Cytoskeleton |
| Laminin, beta 3 | Lamb3 | Adhesion |
| Lecin, galactose binding, soluble 3 | Lapk3 | Defense/Immunity |
| von Ebner minor salivary gland protein mRNA, complete x | LOC228801 | Hypothetical |
| Latent transforming growth factor beta binding protein 3 | Ltbp3 | Cytoskeletal/Growth factor/Folding |
| Lymphocyte antigen 6 complex, locus E | Lyle | Defense/Immunity |
| Myristoylated alanine rich protein kinase C substrate | Maca | Cytoskeleton |
| Melanoma antigen, family D, 1 | Magec1 | Receptor/Signaling |
| Melanoma antigen, family D, 2 | Magec2 | Receptor/Signaling |
| Milk fat globule-EGF factor 8 protein | Mfgf8 | Adhesion |
| Hypothetical protein | MGC28705 | Hypothetical/Enzyme/Digestive |
| Melastatin 1 | Milm1 | Transport/ion |
| Methylenedtetrahydrofolic dehydrogenase (NAD+ depend | Mthd2 | Metabolism/Mitochondrion |
| Description                                                                 | Accession | Chromosome | Location | Reference | GO terms |
|-----------------------------------------------------------------------------|-----------|------------|----------|-----------|----------|
| Suppressor of initiator codon mutations, related sequence Sui1-rs1 Protein | AA107514  | Mm.13886   | 20918    | P4824     | GO terms |
| Transforming growth factor beta 1 induced transcript 4 Tgfr1h4 Nucleus     | X61240    | Mm.20927   | 21307    | Q00992    | 13/14    |
| Transforming growth factor, beta 3 Tgfb3 Cytokines/Growth factor           | M32745    | Mm.3992    | 19020    | P17125    | 14/12    |
| Thrombospondin 1 Tiba1 Adhesion                                           | m67276    | Mm.4159    | 18060    | P35441    | 15/2     |
| T lymphoma oncogene Tim cell growth and/or maintenance                     | X53934    | Mm.20979   | 21893    | P17408    | GO terms |
| Thymosin, beta 10 Tms10 Cytoskeleton                                       | AA230778  | Mm.3532    | 18339    | P20065    | 2/2      |
| Transducer of ErbB-2.1 Tob1 Intrasubcellular signaling                     | D78382    | Mm.4218    | 60523    | P50816    | 17/-     |
| Translocating chain-associated membrane protein (93%) Tiam1 Protein       | aa390043  | Mm.20737   | 60519    | Q00992    | 8/-      |
| Trypsin 2 Enzyme/Digestive                                                 | p02574    | Mm.14419   | 60164    | P07146    | 7/-      |
| UDP-galactose translocator 2 Ugat2 Posttranslational modification         | M87950    | Mm.4593    | 34475    | Q00992    | 17/11    |
| X-box binding protein 1 Xbpl Nucleus/Transcription regulation            | AA104324  | Mm.22718   | 194355   | Q08509    | 22/11    |
| Similar to hypothetical protein MGC5976, clone MGC:548 EST                | aa276057  | Mm.19630   | 12/15    |           |
| DNA segment, Chr 6, Wayne State University 176, express D6Wsu176e EST    | w61679    | Mm.27872   | 27925    | P97805    |           |
| DNA segment, Chr 7, Wayne State University 86, express D7Wsu86e EST       | aa268073  | Mm.29703   | 27925    |           | 16/7     |
| EST                                                                         | AA261028  | Mm.19640   | N/A      |           |           |
| Highly similar to AF121863 1 sorting nexin 14 [H.sapiens] Protein/Transport| aa185262  | Mm.3154    | Q9Y5W7   | GO terms  |
| EST                                                                         | D19392    | Mm.3095    | N/A      |           |           |
| EST                                                                         | aa289002  | Mm.29940   | N/A      |           |           |
| EST                                                                         | C80919    | Mm.3640    | N/A      |           |           |
| Hypothetical protein MGC27648                                             | aa415454  | Mm.28332   | 22164    |           |
| RIKEN cDNA C13052012R gene C13052012Rik Hypothetical/Membrane              | aa402588  | Mm.30099   | 21335    | GO terms  |
| RIKEN cDNA C330016016R gene C330016016Rik Hypothetical                    | AA109042  | Mm.36723   | 103841   | 11        |
| RIKEN cDNA 2310014L17 gene C2310014L17Rik Hypothetical/Posttranslational  | c70999    | Mm.24738   | 97353    |           |
| Expresed sequence C81234 C81234                                           | c81234    | Mm.4055    | 97605    |           |
| RIKEN cDNA 061001012Rik Hypothetical                                       | w45668    | Mm.177991  | 66959    | Q9COK5    | 3/-      |
| RIKEN cDNA 1190017B18 gene 1190017B18Rik Hypothetical/Protein/Transport    | aa271049  | Mm.182194  | 48939    | Q14807    | 5/-      |
| RIKEN cDNA 1300013B24 gene 1300013B24Rik Hypothetical                     | aa217590  | Mm.45324   | 60380    | 67475     | 1/-      |
| RIKEN cDNA 1700009N14 gene 1700009N14Rik Hypothetical/Protein/Transport    | AA25993   | Mm.23522   | 75471    | GO terms  |
| RIKEN cDNA 1810045K17 gene 1810045K17Rik Hypothetical                     | aa192339  | Mm.29817   | 67890    | -3       |
| RIKEN cDNA 2210023G05R gene 2210023G05Rik Hypothetical/Transport/Membrane  | aa73986   | Mm.26580   | 72261    | N/A -8    |
| RIKEN cDNA 2310002N04 gene 2310002N04Rik Transport/Cytoskeleton            | aa604566  | Mm.41637   | 76632    | Q8D7M3    | 11/7     |
| RIKEN cDNA 0730590L21 gene 0730590L21Rik Hypothetical                     | aa017057  | Mm.181815  | 76732    | Q8C1A0    | 15/-     |
| RIKEN cDNA 9530090G24 gene 9530090G24Rik Hypothetical/Posttranslational   | aa089917  | Mm.28866   | 106687   | Q8JR2     | 2/-      |

**Totals**

| Known genes | ESTs/Uncharacterized/Hypothetical |
|-------------|----------------------------------|
| 156         | 32                      |
| Gene Name | Symbol | Description | GenBank | UniGene | OMIM | LocusLink | SwissProt/pir | Hs./Mm. | Chrom. | GO terms |
|-----------|--------|-------------|---------|---------|------|-----------|---------------|---------|--------|----------|
| Actin, beta, cytoplasmic | Actb | Cytoskeleton/Transport | trix control se | Mm.207 | 106260 | 11461 | P02570 | 7 / 5 | GO terms |
| Aldehyde dehydrogenase family 7, member A1 | Aldh7a1 | Metabolism | aai65907 | Mm.30250 | 107233 | 28121 | P45419 | 5 / 18 | GO terms |
| Arachidonate 5-lipoxygenase activating protein | Alox5ap | Defense/Immunity | aA02750 | Mm.19644 | 693700 | 11690 | P30355 | 13 / 5 | GO terms |
| Angiomotin like 2 | Amotl2 | Miscellaneous | aai599429 | Mm.21145 | 56332 | 3 / 9 | GO terms |
| Amylase 1, salivary | Amy1 | Enzyme/Digestion | v00719 | Mm.33941 | 104700 | 11722 | P04746 | 1 / 3 | GO terms |
| Amylase 2, pancreatic | Amy2 | Enzyme/Digestion | v00718 | Mm.324 | 104650 | 11723 | P00688 | 1 / 3 | GO terms |
| Alanine (membrane) aminopeptidase | Anpep | Membrane | U77083 | Mm.4487 | 151530 | 18790 | P97449 | 15 / 7 | GO terms |
| Rho GDP dissociation inhibitor (GDI) gamma | Arhgdig | Intracellular signaling | i24263 | Mm.1383 | 602844 | 14570 | Q62160 | 16 / 17 | GO terms |
| Ras homolog gene family, member Q | Arhq | Intracellular signaling | aai174954 | Mm.826 | 695857 | 104215 | Q09101 | 2 / - | GO terms |
| Acid sphingomyelinase-like phosphodiesterase 3a | Asm3a | Hypothetical/Posttranslational | y08135 | Mm.2379 | 67319 | / 10 | GO terms |
| Asparagine synthetase | Asns | Metabolism/Ammonium acid bicyclic | u38940 | Mm.2942 | 108370 | 27053 | Q61024 | 7 / 6 | GO terms |
| Beta-2 microglobulin | B2m | Defense/Immunity | v01838 | Mm.163 | 109708 | 12010 | P01887 | 15 / 2 | GO terms |
| B-cell translocation gene 1, anti-proliferative | Btg1 | Nucleus/Transcription regulator | l18646 | Mm.16590 | 109580 | 12226 | P31607 | 12 / 10 | GO terms |
| Caspase 9 | Casp9 | Metabolism/Apoptosis | AA868916 | Mm.88829 | 602234 | 13371 | Q9C3Q9 | 1 / 4 | GO terms |
| Cholecystokinin A receptor | Cckar | Membrane/Receptor | D85605 | Mm.3521 | 118443 | 12425 | Q08786 | 4 / 5 | GO terms |
| Cyclin D2 | Cond2 | Nucleus/Transcription regulator | m83740 | Mm.3141 | 128330 | 12444 | P30280 | 12 / 6 | GO terms |
| CD24a antigen | Cd24a | Defense/Membrane | v35382 | Mm.6417 | 606007 | 12484 | S15785 | 6 / 10 | GO terms |
| Creatine kinase, brain | Ckb | Metabolism | W75072 | Mm.16031 | 122380 | 13709 | P12277 | 14 / 12 | GO terms |
| Chloride channel calcium activated 3 | Clica3 | Transport | Aai51186 | Mm.6594 | 603906 | 23844 | Q9D7Z6 | 1 / 3 | GO terms |
| Coatomer protein complex, subunit zeta 2 | Copz2 | Protein/Transport | aai222096 | Mm.22144 | 601924 | 56358 | Q9Y3C3 | 17 / 11 | GO terms |
| Cisplatin resistance related protein CRRDP | Crrdp | Hypothetical/Membrane | aai580985 | Mm.30096 | 218330 | Q8BX45 | / 13 | GO terms |
| Crystallin, alpha B | Cryab | Protein folding | M73741 | Mm.178 | 123650 | 13955 | P23927 | 11 / 9 | GO terms |
| DnaJ (Hsp40) homolog, subfamily C, member 3 | Dnajc3 | Protein folding | U24823 | Mm.12616 | 601184 | 19107 | Q06873 | 13 / 14 | GO terms |
| Deleted in polyposis 1 | Dp1 | Hypothetical | U28168 | Mm.21251 | 125269 | 13476 | JC4667 | 5 / 18 | GO terms |
| Eukaryotic translation elongation factor 1 alpha 1 | Eef1a1 | Protein synthesis | W83919 | Mm.196614 | 130560 | 13627 | P10126 | 1 / 1 | GO terms |
| Epidermal growth factor | Egf | Growth factor | v00741 | Mm.1341 | 131530 | 13645 | P01132 | 4 / 3 | GO terms |
| Eukaryotic translation initiation factor 4A2 | Eif4a2 | Protein synthesis | v12507 | Mm.260284 | 601102 | 13682 | Q14240 | 3 / 6 | GO terms |
| Elastase 2 | Ela2 | Enzyme/Digestion | X04573 | Mm.21926 | 130130 | 13708 | P05208 | 19 / 10 | GO terms |
| ELL-related RNA polymerase II, EF | Ell2 | Hypothetical/Protein synthesis | Aa649990 | Mm.209627 | 209627 | Q0472 | GO terms |
| ER oxidoreductin 1-Like beta | Ero1lb | Hypothetical/Secretion | aai173000 | Mm.45224 | 603080 | 67475 | / - | GO terms |
| Feline sarcoma oncogene | Fes | Intracellular signaling | x12616 | Mm.48757 | 160030 | 14159 | P16879 | 19 / 7 | GO terms |
| Fragilis | Fgs-pending | Defense/Immunity | w12941 | Mm.141021 | 66141 | Q608L6 | / - | GO terms |
| Furin (paired basic amino acid cleaving enzyme) | Furin | Membrane/Receptor | U2648 | Mm.5241 | 136950 | 18550 | P23188 | 15 / 7 | GO terms |
| FXYD domain-containing ion transport regulator 6 | Fxyd6 | Membrane | Aa168918 | Mm.24806 | 606683 | 58695 | Q9D164 | 11 / 9 | GO terms |
| Gamma-aminobutyric acid receptor associated protein | Gabarap | Cytoskeleton | aa106790 | Mm.30064 | 605125 | 56486 | Q08765 | 17 / 11 | GO terms |
| Gamma-aminobutyric acid (GABA(A)) receptor-associated | Gabarap1 | Cytoskeleton | Z31137 | Mm.14638 | 605125 | 56486 | Q08765 | 17 / 11 | GO terms |
| Galactokinase 1 | Galk1 | Metabolism/Carbohydrate | W08486 | Mm.2820 | 604313 | 14635 | Q9RR0N | 17 / 11 | GO terms |
| Galactose-1-phosphate uridylyl transferase | Gal | Metabolism | M90286 | Mm.2420 | 604999 | 14430 | Q03249 | 9 / 4 | GO terms |
| Glutaryl-Coenzyme A dehydrogenase | Gcdh | Protein/Metabolism | U18992 | Mm.2475 | 231670 | 270978 | Q90759 | 19 / 8 | GO terms |
| Golgi associated, gamma adaptin ear containing, ARF like Gga2 | Gga2 | Transport | aai291402 | Mm.29619 | 606005 | 74105 | Q9ULU2 | 16 / 7 | GO terms |
| Gastric intrinsic factor | Gif | Transport/Digestion | L24191 | Mm.456 | 261000 | 14603 | P52787 | 11 / 9 | GO terms |
| Golgi reassembly stacking protein 2 | Garsap2 | Protein/Transport | W13835 | Mm.23836 | 70231 | 2 / 2 | GO terms |
| Hyaluronic acid-binding protein 2 | Hapsb2 | Protein/Metabolism | Aa002504 | Mm.25791 | 603924 | 15105 | Q8HXX2 | 10 / - | GO terms |
| Gene Name                                | Enzyme/Digestion/Immunity/Defense | GO terms |
|------------------------------------------|----------------------------------|----------|
| Hepcidin antimicrobial peptide           | Hamp                             | GO terms |
| Hbs1-like (S. cerevisiae)                | Hbs1                             | GO terms |
| Hepatoma-derived growth factor           | Hdgf                             | GO terms |
| Homocysteine-inducible, endoplasmic reticulum stress | Herpud1 | GO terms |
| Histone 2, H2Aa1                        | Hist2H2a1                        | GO terms |
| Human immunodeficiency virus type 1 enhancer binding | Hexp1 | GO terms |
| Immunoglobulin heavy chain 6 (heavy chain of IgM) | Igf-6 | GO terms |
| Jun oncogene                            | Jun                              | GO terms |
| Jun proto-oncogene related gene d1      | Jund1                            | GO terms |
| Lactate dehydrogenase 2, B chain        | Ldh2                             | GO terms |
| Lectin, galactose binding, soluble 1    | Lgal1                            | GO terms |
| Von Ebner minor salivary gland protein  | LOC228801                        | GO terms |
| Lipoprotein lipase                      | Lpl                              | GO terms |
| Lymphocyte antigen 6 complex, locus E   | Ly6e                             | GO terms |
| Mannosidase 1, alpha                    | Man1a                            | GO terms |
| Mitochondrial carrier homolog 1         | Mct1-pending                     | GO terms |
| Murinoglobulin 1                        | Mug1                             | GO terms |
| Esterase, probably amylase variant     | N/A                              | GO terms |
| Nicotinamide nucleotide transhydrogenase | Ntt | GO terms |
| Nucleobindin 2                          | Nucb2                            | GO terms |
| Prolyl 4-hydroxylase, beta polypeptide  | P4hb                             | GO terms |
| Platelet derived growth factor, B polypeptide | Pdgfb | GO terms |
| Protein disulfide isomerase, pancreatic (79% Hs.) | PDP | GO terms |
| Peptidoglycan recognition protein       | Pglyp                            | GO terms |
| 3-phosphoglycerate dehydrogenase        | Phgdh                            | GO terms |
| Phospholipase D3                        | Ptd3                             | GO terms |
| PMS protein                             | Pms5-pending                     | GO terms |
| Pancreatic lipase-related protein 2     | PrlP2                            | GO terms |
| Polymerase (DNA directed), delta 2, regulatory subunit | Pold2 | GO terms |
| Peroxisidex 4                           | Ptx4                             | GO terms |
| Protease, serine, 11 (lig binding)      | Pss11                            | GO terms |
| Pyrroline-5-carboxylate synthetase (glutamate gamma-Pyr) | Pycs | GO terms |
| Ral guanine nucleotide dissociation stimulator, -like 2 | Rgl2 | GO terms |
| Ribonuclease, RNA A family, 1           | Rn1                              | GO terms |
| Ribosome binding protein 1              | Rbtp1                            | GO terms |
| Selenoprotein R                         | Sepr                             | GO terms |
| Sialyltransferase 10 (alpha-2,3-sialyltransferase VI) | Stal10 | GO terms |
| Solute carrier family 3 (activators of dibasic and neutral) | Stilca32 | GO terms |
| Solute carrier family 4 (anion exchanger), member 2 | Stol4a2 | GO terms |
| Solute carrier family 6 (neurotransmitter transporter, g) | Stol6a9 | GO terms |
| Spermidine synthase                     | Srm                              | GO terms |
| Signal recognition particle 54          | Srp54                            | GO terms |
| Signal recognition particle receptor ('docking protein') | SrpR | GO terms |
| Gene Name                                      | Function                      | Accession | aa | Mm. | Chromosome | Length | GO terms                |
|-----------------------------------------------|-------------------------------|-----------|-----|-----|------------|--------|-------------------------|
| Signal sequence receptor, delta               | Ssr4                          | X90582    | 303 | 831 | 300096     | 2832   | Q96186                  |
| Synaptotagmin 7                               | Syt7                          | W50092    | 644 | 41132| 601446     | 59267  | Q9ROV7                  |
| Tumor differentially expressed 1              | Tde1                          | L29441    | 607 | 1656| 607166     | 26943  | Q8QQF9                  |
| Translocating chain-associating membrane protein 1 | Tram1                        | aa390043  | 605 | 1960| 605196     | 72256  | Q9CVJ6                  |
| Tryptsin 2                                    | Try2                          | x94574    | 601 | 1664| 601564     | 22072  | P07146                  |
| UDP-galactose translocator 2                  | Ugalt2                        | W87960    | 314 | 375 | 314375     | 110172 | X9ROV8                  |
| X-box binding protein 1                       | Xbp1                          | AA016424  | 194 | 355 | 194355     | 22433  | O08559                  |
| Hypothetical protein MGC27648                 | Hypothetical                  | MGC27648  |     |     |            |        |                         |
| EST, similar to myosin light chain            | NIA                           | W17839    |     |     |            |        |                         |
| Musculus adult male cortex cDNA, Riken full-length EST | EST                          | a408451   |     |     |            |        |                         |
| Hypothetical protein C730041J05               | Hypothetical                  | C730041J05|     |     |            |        |                         |
| Expressed sequence A428936                   | Hypothetical                  | A428936   |     |     |            |        |                         |
| Expressed sequence AL024210                   | Hypothetical                  | AL024210  |     |     |            |        |                         |
| Riken cDNA C330016O16 gene                    | Hypothetical                  | C330016O16|     |     |            |        |                         |
| Riken cDNA B430119G05 gene                    | Hypothetical/Intracellular    | B430119G05|     |     |            |        |                         |
| Riken cDNA O610010I12 gene                    | Hypothetical                  | O610010I12|     |     |            |        |                         |
| Riken cDNA 1110002E23 gene                    | Hypothetical                  | 1110002E23|     |     |            |        |                         |
| Riken cDNA 1810045K07 gene                    | Hypothetical/Intracellular    | 1810045K07|     |     |            |        |                         |
| Riken cDNA 1810073N04 gene                    | Hypothetical                  | 1810073N04|     |     |            |        |                         |
| Riken cDNA 2310014L17 gene                    | Hypothetical/Posttranslational| 2310014L17|     |     |            |        |                         |
| Riken cDNA 2310043N10 gene                    | Hypothetical                  | 2310043N10|     |     |            |        |                         |
| Riken cDNA 2610102M01 gene                    | Hypothetical                  | 2610102M01|     |     |            |        |                         |
| Riken cDNA 2900057C09 gene                    | Hypothetical                  | 2900057C09|     |     |            |        |                         |
| Riken cDNA 3100002M17 gene                    | Hypothetical                  | 3100002M17|     |     |            |        |                         |
| Riken cDNA 5730592L21 gene                    | Hypothetical/Posttranslational| 5730592L21|     |     |            |        |                         |
| Riken cDNA 9530090G24 gene                    | Hypothetical/Posttranslational| 9530090G24|     |     |            |        |                         |
| EST                                           | EST                           | X52622    |     |     |            |        |                         |
| EST, similar to gephyrin                      | Transport/Secretion           | W50073    |     |     |            |        |                         |
| EST                                           | EST                           | a000964   |     |     |            |        |                         |
| EST                                           | EST                           | AA261028  |     |     |            |        |                         |
| EST                                           | EST                           | AA154514  |     |     |            |        |                         |
| EST                                           | EST                           | AA154007  |     |     |            |        |                         |
| EST, similar to ATPase6                       | EST                           | AA108625  |     |     |            |        |                         |
| EST                                           | EST                           | AA013976  |     |     |            |        |                         |
| EST                                           | EST                           | a289002   |     |     |            |        |                         |
| EST                                           | EST                           | C76202    |     |     |            |        |                         |

**Totals**

- **Known genes**: 95
- **ESTs/Uncharacterized/Hypothetical**: 29

**ESTs**

- X52622
- EST
- EST
- EST
- EST
- EST
- EST
- EST
- EST
- EST
| Gene name | Symbol | Unigene | Links to references |
|-----------|--------|---------|---------------------|
| Actin, beta | Actb | Mm.297 | 1 Ref. |
| Aldehyde dehydrogenase 7A1 | Aldh7a1 | Mm.30250 | 4 Refs |
| Angiomotin-like 2 | Amotl2 | Mm.21145 | 4 Refs |
| Anylase 1 | Amy1 | Mm.324 | 3 Refs |
| Alanine aminopeptidase (CD13) | Anpep | Mm.4487 | 9 Refs |
| TC10 (Ras homolog Q) | Arhq | Mm.826 | 9 Refs |
| Asparagine synthetase | Asns | Mm.2942 | 5 Refs |
| Beta-2 microglobulin | B2m | Mm.163 | 8 Refs |
| Aldehyde dehydrogenase 7A1 | Aldh7a1 | Mm.30250 | 4 Refs |
| Angiomotin-like 2 | Amotl2 | Mm.21145 | 4 Refs |
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| Aldehyde dehydrogenase 7A1 | Aldh7a1 | Mm.30250 | 4 Refs |
| Angiomotin-like 2 | Amotl2 | Mm.21145 | 4 Refs |
| Anylase 1 | Amy1 | Mm.324 | 3 Refs |
| Alanine aminopeptidase (CD13) | Anpep | Mm.4487 | 9 Refs |
| TC10 (Ras homolog Q) | Arhq | Mm.826 | 9 Refs |
| Asparagine synthetase | Asns | Mm.2942 | 5 Refs |
| Beta-2 microglobulin | B2m | Mm.163 | 8 Refs |

Uncharacterized genes/ESTs

CRR9p | Mm.30099 |
LOC228801 | Mm.3783 |
1810041F13Rik | Mm.32631 |
MGC28705 | Mm.25791 |
EST | Mm.29940 |
| EST         | GenBank   |
|------------|-----------|
| 0610010I12Rik | Mm.177991 |
| 2310014L17Rik | Mm.24778  |
| 5730592L21Rik | Mm.181815 |
| C330016O16Rik | Mm.86723  |
| EST         | N/A       |
| EST         | N/A       |
| EST         | N/A       |
| EST         | N/A       |
| EST         | N/A       |
| **Totals**  |           |
| Genes with known functions | 57         |
| Uncharacterized genes/ESTs   | 14         |
Molecular characterization of mouse gastric zymogenic cells
Jason C. Mills, Niklas Andersson, Thaddeus S. Stappenbeck, Christopher C. M. Chen and Jeffrey I. Gordon

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