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Mast cell transcriptional networks

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Abstract

Unregulated activation of mast cells can contribute to the pathogenesis of inflammatory and allergic diseases, including asthma, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis (1;2). Absence of mast cells in animal models can lead to impairment in the innate immune response to parasites and bacterial infections(3–5). Aberrant clonal accumulation and proliferation of mast cells can result in a variety of diseases ranging from benign cutaneous mastocytosis to systemic mastocytosis or mast cell leukemia(6). Understanding mast cell differentiation provides important insights into mechanisms of lineage selection during hematopoiesis and can provide targets for new drug development to treat mast cell disorders,. In this review, we discuss controversies related to development, sites of origin,, and the transcriptional program of mast cells.

Keywords

Mast cells; transcription factors; GATA; PU.1; Mitf

Origins of Mast Cells

Like other granulocytes (neutrophils, eosinophils and basophils), mast cells are derived from hematopoietic stem cells in the marrow; however, they are unique with regard to the tissue compartments in which they traffic and reside. The gastrointestinal mucosa is the major compartment occupied by committed mast cell precursors in the mouse (7). Trafficking to these compartments is dependent on the expression of the alpha4 beta7 integrin and the chemokine receptor CXCR2.(8;9) Mast cell progenitors migrate to the connective tissue of the skin, lung, and the mucosa of other gastrointestinal tissues where they mature under the influence local

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environmental and developmental cues. Unlike other hematopoietic cells, mature mast cells do not circulate in the blood.

Transplantation studies in mice indicate that mast cells are derived from hematopoietic stem cells of marrow (or spleen)(10;11). Studies using prospectively identified hematopoietic precursors have reported different precursor cells that give rise to the committed mast cell progenitor. One study found that the committed mast cell progenitor (MCP) arose from an early multipotential progenitor (MPP) that is distinct from the common myeloid progenitor (12). However, using a similar strategy to prospectively isolate hematopoietic precursors from mouse marrow, another study found that the MCP was derived from either the common myeloid progenitor or the granulocyte-monocyte progenitor(13) (Figure 1). Plasticity may permit other committed progenitor cell populations to be “reprogrammed” into the mast cell lineage by altering the expression of selected transcription factors. Isolated common lymphoid precursors that are retrovirally-transduced with the transcription factor GATA-2 can convert into basophil-mast cell precursors(14). Differentiation into mast cells is negatively regulated by CCAAT/enhancer-binding protein alpha (CEBP α) since expression of CEBP α in basophil-mast cell precursors drives basophil development at the expense of mast cell formation. Another study has shown that forced expression of the T-cell specific factor, GATA-3, into fetal thymocytes reprograms them to develop into mast cells(15). Reduction of transcription factor expression can also alter the differentiation capacity of multipotential progenitors. Megakaryocyte-erythroid progenitors derived from the common myeloid progenitor are restricted to develop into either megakaryocytes or erythroid cells. However, in GATA-1 “knockdown”/GATA-1 “low” mutant mice, in which GATA-1 expression is markedly reduced(16), megakaryocyte-erythroid progenitors also can develop into mast cells(17). Similarly, a conditional deletion of the stem cell leukemia gene (*SCL*) also results in promiscuous development of mast cells from the megakaryocyte-erythroid progenitors population, suggesting that *SCL* and GATA-1 participate in regulating megakaryocyte-erythroid progenitors differentiation into mast cells (18) (Figure 1). These studies illustrate plasticity of progenitor cells permitting development into mast cells; the influence of transcription factor expression on mast cell differentiation is dependent on the cellular context and other dynamic factors.

Transcriptional Regulators of Mast Cell Development

Current models of hematopoietic development suggest that lineage-specific transcription factors are expressed at low levels in uncommitted precursors(19–21). During differentiation, sequential programs of transcription factor expression are activated, resulting in specified sets of dominantly co-expressed transcription factors in committed cells. These lineage-specific sets may positively regulate critical gene targets, but they may also function to inhibit the activation of other lineage programs(22). The transcription factors GATA-1, GATA-2, PU.1 and the microphthalmia-associated transcription factor (*Mitf*) play essential roles in mast cell development. These factors are co-expressed in committed mast cells, and are required for proper development of the mast lineage (figure 2).

Microphthalmia-associated transcription factor—*Mitf* is a basic helix-loop-helix leucine zipper factor (bHLH-zip); mice with mutations in *Mitf* display defects in diverse tissues (23;24). Melanocytes are deficient, resulting in abnormalities of coat color, and the pigmented retinal epithelium does not develop normally resulting in microphthalmia. Dominant negative mutations affect osteoclast function and result in an osteopetrotic phenotype. *Mitf* mutant mice also show a severe mast cell deficiency. The connective tissues of deficient mice are severely devoid of mature mast cells(25;26). The marrow of *Mitf* deficient mice display a defect in terminal mast cell differentiation in culture. A number of mast cell target genes have been identified(27), and an analysis of the *Mitf* transcriptome revealed that *Mitf* regulates a number of proteases, cell surface receptors, signaling molecules and transporters critical for mast cell

function(28). There are cell specific isoforms of *Mitf*(29;30) that appear to be controlled by mast cell-specific regulatory elements. Elucidation of the genomic organization and specific DNA sequences that control the expression of the mast cell isoforms of *Mitf*, as well as the downstream *Mitf* targets, should provide insights into the transcriptional circuitry in mast cells.

GATA-1 and GATA-2

GATA-1 and GATA-2 are zinc finger transcription factors that play important roles in mast cell development. GATA-1 was cloned from erythroid cells and is required for erythroid differentiation, as well as for normal development of the megakaryocyte and eosinophil lineages. Mast cells derived from GATA-^{-/-} embryonic stem cells appear to differentiate normally in culture(31); however, mice in which an upstream enhancer of the GATA-1 gene is replaced with a neomycin selection cassette display a reduced GATA-1 expression (GATA-1 “low”). Mast cell precursors from these mice proliferate abnormally and display defects in apoptosis (32;33). In addition, committed mast cell progenitors from these mice cannot be detected in the marrow, but mast cells can be derived from the megakaryocyte-erythroid progenitor population(34). A deletion of the proximal erythroid promoter of GATA-1 (GATA-1 “enfeebled”) also results in reduced GATA-1 expression(35); these mice display reduction in mature mast cells in the skin(36). Mast cell target genes have been identified for GATA-1, including the alpha and beta chains of *FcεRI*(37;38), carboxypeptidase A(39), interleukin-4(40), and interleukin-13(41). GATA-2 is essential for the maintenance of early hematopoietic progenitors as well as mast cell precursors. A targeted deletion of the GATA-2 gene in mice results in embryonic lethality(42); however, GATA-2 deficient embryonic stem cells are incapable of differentiating into the earliest mast cell progenitors(43). Thus, both GATA-1 and GATA-2 are important for the proper specification of the mast cell lineage, but GATA-2 appears to be required at a much earlier stage of commitment.

PU.1

PU.1 is a member of the ets transcription factor family and it is essential for the development of myeloid and lymphoid cells. Conditional deletion of PU.1 in murine hematopoietic cells established a role for PU.1 in the maintenance of the hematopoietic stem cell compartment as well as for generation of common lymphoid progenitors and common myeloid progenitors(44; 45). PU.1 has also been shown to play an indispensable role in mast cell development. PU.1 ^{-/-} mouse fetuses lack dermal mast cells; in addition, PU.1 ^{-/-} fetal liver cells do not differentiate into mast cells when cultured in IL3 and SCF. Rescue of mast cell differentiation of these cultured fetal liver cells require both PU.1 and GATA-2 expression(46). Lineage selection between the myeloid and lymphoid fates is determined in part by the dosage of PU.1 expression. Low levels of PU.1 expression promote B-cell lymphocyte development, while enforced expression of high levels of PU.1 drives macrophage differentiation at the expense of B-cells(47). In support of this notion, retroviral expression of PU.1 into murine hematopoietic cells from marrow promotes monocyte development in culture conditions that usually support mast cell differentiation(48). Furthermore, enforced expression of PU.1 into differentiated marrow derived mast cells will give rise to cells with the morphologic appearance and the gene expression profile characteristic of monocytes(49). However, the mechanisms by which graded expression of PU.1 might mediate cell fate decisions in mast cells are not well understood.

Signal transducer and activator of transcription

The signal transducer and activator of transcription (STAT) family of transcription factors transmit extracellular signals to the nucleus. STAT5 is activated in response to c-Kit signaling, which is a receptor tyrosine kinase that provides critical mast cell growth and survival functions. STAT5A/B deficient mice lack tissue mast cells in vivo. Furthermore, marrow-

derived mast cells from STAT5A/B deficient mice have defects in maturation, survival and proliferation(50). This mast cell phenotype is similar to that of mice with mutations in c-Kit or its ligand, stem cell factor, (SCF)(51), indicating that STAT5 is a critical downstream regulator of c-Kit signaling. Mice with mutations in a number of other signaling molecules downstream of c-Kit also display defects in mast cells. Targeted deletions of the p85 α subunit of PI-3 kinase(52), the PI-3 kinase adapter protein Gab2(53), and Jak3(54) each result in mast cell deficiency and/or dysfunction. These studies support the critical role of c-Kit signaling in mast cell development, but also suggest a functional connection between the c-Kit signaling pathways and the transcription factors discussed in this review.

CCAAT/enhancer-binding protein alpha

Transcription factors that suppress mast cell development have also been identified. CEBP α is required for granulocyte development. This factor plays a role in the determination of the basophil and mast cell fates. Forced expression of the CEBP α into the basophil or mast cell precursors drives basophil development while suppressing that of mast cells. Conversely, cells deficient in CEBP α differentiate into mast cells and cannot develop into basophils or eosinophils(55;56). Similar to PU.1, the dosage of CEBP α expression affects the cell fate decisions between mast cells and the other granulocyte lineages(57).

Interaction of transcription factors

How transcription factors cooperate to participate in a network that regulates mast cell differentiation, trafficking, and function is a central question in the study of mast cell biology and hematopoiesis. PU.1 and GATA-1 are major regulators of the myeloid and erythroid lineages, respectively. They are not abundantly co-expressed in these separate lineages and, furthermore, a number of studies have shown that they antagonize each other's function through direct physical interactions(58–60). In contrast, PU.1 and GATA-1 are both abundantly co-expressed in mast cells, and both are required for normal mast cell development. Several mast cell target genes are co-regulated by both the GATA factors and PU.1 including interleukin-4(61) and interleukin-13(62), suggesting the functional cooperation between these factors in the expression of mast cell genes. In further support of this notion is that restoration of both GATA-2 and PU.1 expression into PU.1 deficient cells is required for mast cell differentiation(63). The mechanism by which the GATA factors and PU.1 are co-expressed and cooperate in mast cells, but not other hematopoietic cells is unknown.

Transcription factor networks in mast cells

Mast cell specific isoforms of transcription factors

Distinct sets of transcription factors are expressed in different cell types. Mitf, GATA-1, GATA-2, and PU.1 are coordinately regulated in mast cells during development (figure 2). The coordinated regulation of these factors in a lineage-specific manner is required for proper specification of distinct cell types. One possible mechanism that might underlie the coordinated regulation of these factors is the existence of cell-specific transcription factors isoforms, which expression is controlled by lineage-restricted cis-regulatory elements. An example of this specificity is the transcription factor Mitf. Mast cells express specific isoforms of Mitf; these isoforms arise from alternatively spliced first exons which join with the common body of the gene(64). The mRNA isoforms encode for unique protein domains, which may regulate distinct biological functions(65). The expression of these isoforms is regulated by tissue-specific promoters, indicating the presence of mast cell regulatory elements. The GATA-2 gene also expresses distinct mRNA isoforms that arise from alternative splicing and are regulated differentially (66). One of the isoforms is expressed preferentially in hematopoietic cells (incorporating the distal first exon, IS), while the other isoform is expressed widely

(incorporating the proximal first exon, IG). These two GATA-2 isoforms are regulated by distinct promoters. Another example of a transcription factor whose expression is under control of lineage-restricted promoters is SCL/Tal-1. This transcription factor is in the family of basic helix-loop-helix factors and is essential for early hematopoietic development. It is also expressed in the erythroid, megakaryocyte, and mast cell lineages. A mast cell-specific promoter for SCL/Tal-1 has been identified(67), providing insights into mast cell-specific regulation by Ets factors including PU.1(68). We have identified a variant isoform of GATA-1 from mast cells. This isoform arises from a non-coding first exon and is preferentially expressed in mast cells. We are currently investigating the cis-regulatory elements that control its expression in mast cells.

Regulation of the GATA-1 locus in mast cells

In spite of its important role in mast cell development, relatively little is known about the regulation of GATA-1 in mast cells. However, a number of regions critical for GATA-1 expression in erythroid cells and megakaryocytes have been identified by transgenic reporter experiments and targeted deletions in mice, and are summarized in figure 3. A genomic region that includes DNase 1 hypersensitive site lies approximately 3.5 kilobases upstream of the IE exon and can direct expression in primitive and definitive erythroid precursors and megakaryocytes, and has been referred to as an upstream enhancer(69–71). The large GATA-1 first intron contains exon IB and is important for definitive hematopoiesis, particularly in specifying expression in erythroid cells and megakaryocytes(72;73). Mast cell expression was not examined with transgenes containing deletions of the GATA-1 first intron. A targeted replacement of the upstream enhancer region of the GATA-1 gene with a neomycin selection cassette results in reduced expression of GATA-1, however, and has been termed a GATA-1 “knockdown”(74;75) or “GATA-1 low(76).” These mice have anemia and thrombocytopenia but normal eosinophil counts(77). In addition, they have also been shown to have a mast cell defect associated with decreased GATA-1 expression in mast cells; this mast phenotype was characterized by hyperproliferation of early mast cell precursors and a susceptibility to apoptosis(78;79). Aberrant insertion of a neomycin cassette between GATA-1 promoter regulatory elements and the GATA-1 IE exon also results in low GATA-1 expression(80). These mutant mice have lower GATA-1 levels and more severe anemia and thrombocytopenia (81). In addition, there is a reduction in the mature mast cells that normally populate the skin (82). A specific deletion of a GATA palindromic binding site region upstream of the neomycin insertion site and the IE exon resulted in a selective defect in eosinophil development(83). These mice were noted to have normal mast cell development. Taken together, these results suggest that the IE promoter and upstream enhancer play a role in regulating GATA-1 expression and differentiation in mast cells, but that the cis-regulatory elements that control GATA-1 in mast cells remain to be characterized.

As a first step in this direction, we used chromatin immunoprecipitation (ChIP) to evaluate the chromatin structure of the murine GATA1 locus in primary primitive and definitive erythroid cells (figure 4). Similar results have been published previously for definitive erythroid hematopoiesis(84). We observed that hyperacetylated histone H3 and H4 generally associated with open chromatin(85) at the previously identified DNase I hypersensitive sites in both definitive (fetal liver) and primitive (yolk sac) erythroid cells (figure 4C). The DNase I hypersensitive sites represent erythroid and megakaryocyte enhancers of the GATA-1 locus (86–89). An unexpected result in one of the controls of our analysis, however, was the finding of hyperacetylated histone H3 and H4 chromatin in the GATA-1 large first intron in the M1 myeloid cell line (figure 4B). Western blots were performed to evaluate GATA-1 protein expression in these cells and demonstrate that only in the erythroid cells, and not the M1 myeloid cells, are high levels of GATA1 expressed. In the fibroblast control cell line 3T3, only background acetylation was observed with these same ChIP probes (figure 4B), and no

GATA-1 protein expression was detected (data not shown). Evaluation of the role of GATA-1 intron sequences in the function of GATA-1 expression in hematopoietic cells may shed some light on this observation. A DNase I hypersensitive site has been described within the GATA-1 intron in assays of multipotential hematopoietic cells, FDCP-mix(90). An alternatively spliced first exon (IB) of GATA-1 has been reported in this region(91). GATA-1 isoforms containing this exon have been detected in primary murine myeloid cells(92), eosinophils (93) and mast cells (Takemoto et al, manuscript submitted). Taken together, these data are consistent with the possibility that this intron hypersensitive region may be the first region of open chromatin in the GATA-1 locus in myeloid progenitors, and thus be potentially regulating the granulocyte-monocyte, eosinophil, mast cell, and erythroid-megakaryocyte lineage determination decisions in competition with the upstream IE promoter and other regulatory elements in such progenitors. We performed a TESS Transfac (<http://www.cbil.upenn.edu/>) computational analysis in this region and identified many potential myeloid PU.1 and erythroid GATA transcription factor binding sites. Extending this analysis to the entire GATA-1 locus reveals several hundred putative GATA and PU binding sites (data not shown). Scanning ChIP (94), ChIP on Chip(95), and more robust computational methods are being applied to this problem to identify the true in vivo transcriptional regulators of the GATA-1 locus in mast cells, including GATA factors and PU.1 (Takemoto and McDevitt, personal communication).

Mechanisms of transcription factor cooperation

In light of the general antagonism observed between GATA-1 and PU.1 transcription factors in hematopoietic cells, a critical question can be posed: how might GATA factors and PU.1 cooperate to regulate essential downstream targets in mast cells? We have found that PU.1 and GATA-2, required for early mast cell commitment, bind to potential regulatory elements of the GATA-1 gene (Takemoto et al, manuscript submitted). These findings support a transcriptional hierarchy in which PU.1 and GATA-2 may function in concert to activate GATA-1 expression in mast cells (figure 5); however, the mechanism of cooperation between PU.1 and GATA-2 is not clear. One hypothesis is that GATA-2 and PU.1 form transcriptional complexes, possibly with recruitment of additional transcriptional regulators. GATA-2 and PU.1 have been shown to interact in vitro(96); however, an in vivo interaction in mast cells between GATA-2 and PU.1 has not been demonstrated. If GATA-2 and PU.1 do interact in mast cells, we would predict that the nature of the interaction differs from that between PU.1 and GATA-1, given that GATA-2 and PU.1 appear synergistic in mast cells, while GATA-1 and PU.1 are antagonistic in other lineages. Alternatively, GATA-2 and PU.1 may not physically interact, but may recruit transcriptional co-factors to regulate critical mast cell genes. GATA-2 has been shown to partner with a number of proteins, including the retinoic acid receptor alpha (RARα)(97), the promyelocytic leukemia protein (PML)(98), and the zinc finger proteins PLZF and FAZF(99). The GATA cofactor, Friend of GATA-1 (FOG-1) binds GATA-1 as well as GATA-2(100). However, while FOG-1 is required for GATA-1 dependent megakaryocyte and erythroid development, it is dispensable for mast cell development(101). Putative co-regulatory proteins that interact with GATA-2 in mast cell have not yet been identified. Similarly, a number of protein partners have been identified for PU.1, but complexes in mast cells have not been reported. Intriguingly, however, Mitf and c-Jun, transcription factors abundantly expressed in mast cells, have been shown to bind with PU.1(102–104), however the biological functions mediated by these interactions in mast cells is unknown.

GATA-2 and PU.1 may synergistically regulate critical mast cell targets by occupying distinct and physically separate regulatory regions. The interleukin (IL)-4 enhancer is regulated by both PU.1 and GATA-2 and mast cells(105). The binding sites for these two factors are separated by over 60 base pairs. We are currently investigating whether this spatial arrangement is common to other critical mast cell genes that are regulated by both PU.1 and GATA-2. Alternatively, PU.1 and/or GATA-2 may control chromatin configuration to regulate access

by other regulatory factors. PU.1 regulates the expression of the *c-fms* gene by remodeling chromatin to allow access and assembly of transcription factor complexes(106). Similarly, GATA-2 appears to participate in the regulation of granulocyte-monocyte colony stimulating factor in mast cells by directing nucleosome positioning within the genes enhancer elements (107). These findings indicate that PU.1 and GATA-2 cooperativity in mast cells also involves chromatin remodeling in a cell-context specific manner.

Thus, a number of transcription factors play critical roles in the differentiation of mast cells. The mechanisms by which these factors coordinately participate in development and function are yet to be determined. Identification of a comprehensive list of mast cell target genes for these transcription factors, such as GATA-1, GATA-2, PU.1, and *Mitf*, will provide an important database to elucidate transcriptional networks in mast cells (figure 5). Further areas of investigation related to the identification of protein partners and other potential novel mechanisms of regulating tissue-specific transcription factor function and expression, such as regulation through microRNAs, will be required to fully clarify mechanisms of cooperativity. Uncovering the components of this transcriptional network will lead to a greater understanding of mast cell development in normal physiology and disease.

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Figure 1. Ontogeny of the mast cell precursor

The committed mast cell progenitor (MCP) has been identified by analysis of prospectively isolated hematopoietic precursors. Chen et al. determined that the MCP is derived from the multipotential progenitor (MPP)(123), while Arinobu and colleagues found that the MCP comes from the common myeloid progenitor (CMP) and the granulocyte/monocyte progenitor (GMP)(124). The normal physiologic pathways to the MCP are shaded in yellow. MCPs have been shown to be derived from other hematopoietic progenitors with altered expression of transcription factors. Forced expression of GATA-2 into the common lymphoid progenitor (CLP)(125), or forced expression of GATA-3 into early T-cell precursors (pro-T) can drive development to the MCP(126). Deficient expression of GATA-1 in the GATA-1 “low” mice results in megakaryocyte/erythroid progenitors (MEP) with MCP potential. Mice with a conditional deletion of the stem cell leukemia (SCL) gene also result in MEP with capacity to develop to MCPs. Altered pathways to the MCP are denoted by dashed red arrows. HSC—hematopoietic stem cell; B—B lymphocyte; T—T lymphocyte; MΦ--macrophage; PMN--neutrophil; MK—megakaryocyte; E--erythroid

Figure 2. Distinct transcription factor sets expressed in mast cell development

Northern blot analysis demonstrates coordinated induction of Mitf, GATA-1, GATA-2 and PU.1 expression during mast cell differentiation of murine embryonic stem cells. On the right, a northern blot shows the co-expression of these transcription factors in the mast cell line, C57. These transcription factors are expressed in other cell lines, but they are not coordinately expressed. Below, cytopsin preparations of embryonic stem cells at different stages of differentiation stained with Wright-Geimsa.

Figure 3. Summary of transgenic studies and targeted deletions of the GATA-1 locus

Schematic representation of the GATA-1 genomic locus with exons denoted by boxes is shown above. IT, IE and IB represent alternative promoters with non-coding first exons that are spliced to exon II, which contains the translational start codon. The IT exon is expressed primarily in testis while the IE exon is expressed primarily in erythroid cells. The IB exon is expressed in early myeloid cells, mast cells and eosinophils. The double asterisk (**) represent a conserved GATA palindromic binding site. HS denotes previously identified DNase I hypersensitivity sites. A) Transgene reporter studies demonstrate the in vivo capacity of GATA-1 genomic regions for expression in various tissues. The upstream enhancer region (HS UE) can direct expression during primitive erythropoiesis in the yolk sac (ys). The HS UE requires the presence of the GATA-1 first intron (between exon IE and II) for expression during definitive erythropoiesis (def ery) and in megakaryocytes (meg). B) Targeted deletion studies demonstrate the in vivo requirement of GATA-1 genomic regions for expression in various tissues. Disruption of the HS UE with a neomycin cassette (loxP neoΔHS) results in abnormal megakaryocyte and mast cell development as well as decreased erythroid production. Cre-mediated deletion of the site (loxP ΔneoΔHS) restores erythroid development, but abnormal megakaryocyte phenotype persists. Disruption of a region between a GATA palindromic binding site (**) and the IE exon with a neomycin cassette (neo) results in defects in erythroid, megakaryocyte and mast cell development. A deletion of the GATA palindromic binding site region yields a selective deficiency of eosinophils. Ery—erythroid; plt—platelet; eos—eosinophil; mast—mast cell. ND—not determined; nl—normal; abnl—abnormal. References for transgenics and targeted deletions are listed.

Figure 4. Intronic GATA-1 sequences exhibit open chromatin in murine myeloid M1 cells, as well as in primary primitive and definitive erythroid cells and erythroid cell lines

A) Schematic representation of the GATA-1 genomic locus with alternative promoters indicated with arrows. Exons denoted by red boxes. Regions spanned by the ChIP probes are shown below in brown bars. Two GATA palindrome binding sites are indicated by the purple stars. One palindromic site is in the IE promoter (IE) and the other is in the GATA-1 intron (P). B) Chromatin Immunoprecipitation (ChIP) was performed in cell lines and show open chromatin around the GATA-1 promoters. Note that in the myeloid M1 cell line, only the region around the IB promoter is open. PCR primers to localize specific GATA-1 regions include IT (testis exon), IE (erythroid exon I), P (intronic GATA palindrome region), and IB (myeloid

and mast cell exon I). Primers for m13 are a negative control. The cell lines 3T3, M1, MEL, and J2E represent well characterized murine fibroblast, myeloid, and erythroid cell lines respectively. C) ChIP performed on yolk sac and fetal liver cells show open chromatin around the GATA-1 promoters. Yolk sac stage (E11.5, E12.5) and fetal liver stage (E13.5, E14.5) primary erythroid cells were harvested from mice for analysis. ChIP was carried out as described by Boyd et al(127). Antibodies were obtained from Upstate. Detailed methods and PCR primer sequences are available upon request.

Figure 5. Proposed transcription hierarchy linking the PU.1 and GATA factors in mast cell development

Published studies suggest that PU.1 and GATA-2 participate in the early commitment of the mast cell lineage and cooperate to regulate the expression of downstream target genes. Mitf and GATA-1 regulate later mast cell developmental programs and are critical for full maturation. We propose that PU.1 and GATA-2 directly activate the Mitf and GATA-1 genes, and upregulate the expression of mast cell specific isoforms of Mitf and GATA-1. The molecular mechanisms of such PU.1 and GATA-2 activation are currently under investigation. MPP—multipotent progenitor; MCP—mast cell precursor; MC—mast cell.

Table 1

In vivo and ex vivo mast cell phenotypes of transcription factor mutant mice.

Transcription factor	In vivo	Ex-vivo
GATA-1	GATA-1 "low" mice display hyperproliferation of early mast cells with increased apoptosis in (108;109). in GATA-1 "enfeebled" mice have reduction in mature skin mast cells (110).	GATA-1 $-/-$ Embryonic stem cells differentiate normally(111); GATA-1 "low" marrow cells lack mast cell progenitors, but display aberrant development of mast cells from megakaryocyte-erythroid progenitors(112).
GATA-2	GATA-2 $-/-$ mice are embryonic lethal of (113)	GATA-2 $-/-$ embryonic stem cells fail to differentiate into mast cells(114)
PU.1	PU.1 $-/-$ mice are embryonic lethal(115) or die in the neonatal period of infection(116); PU.1 $-/-$ fetuses lack skin mast cells (117)	PU.1 $-/-$ fetal liver cells fail to differentiate into mast cells(118)
Mitf	Mitf null or recessive mutations display a deficiency of tissue mast cells; Mitf dominant mutations result in a more severe deficiency of tissue mast cells(119)	Mitf mutant marrow mast cells display impaired differentiation, deficient expression of Mitf target genes, and poor response to stem cell factor(65;120)
STAT5A/B	STAT 5A/B $-/-$ mice display an absence of tissue mast cells in (121)	Impaired survival and proliferation of marrow mast cells(122)