Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2341–2345, March 1991
Genetics

The c-*fms* gene complements the mitogenic defect in mast cells derived from mutant *W* mice but not *mi* (microphthalmia) mice

(receptor tyrosine kinases/mouse mutants/oncogenes/signal transduction pathways/retroviral vectors)

PATRICE DUBREUIL*†, LESLEY FORRESTER*, ROBERT ROTTAPEL*, MICHAEL REEDIJK‡, JUN FUJITA§, AND ALAN BERNSTEIN*‡¶

*Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, ON M5G 1X5, Canada; ‡Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada; and §Department of Clinical Molecular Biology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

Communicated by Salome G. Waelsch, December 26, 1990

ABSTRACT **Mutations at three loci in the mouse—*W*, Steel (*Sl*), and microphthalmia (*mi*)—can lead to a deficiency in melanocytes and mast cells. As well, *W* and *Sl* mutants can be anemic and sterile, whereas *mi* mice are osteopetrotic due to a monocyte/macrophage defect. Recent data have shown that the c-*kit* receptor tyrosine kinase is the gene product of the *W* locus, whereas *Sl* encodes the ligand for this growth factor receptor. We show here that ectopic expression of c-*fms*, a gene that encodes a macrophage growth factor receptor that is closely related to the c-*kit* receptor, complements mutations at the *W* locus in an *in vitro* mast cell/fibroblast coculture system but is unable to reverse the inability of *mi/mi* mast cells to survive under these conditions. Furthermore, mast cells expressing the c-*fms* receptor survive on a monolayer of fibroblasts homozygous for the *Sl* mutation. These results suggest that ligand binding to the c-*kit* or c-*fms* receptor activates identical or overlapping signal transduction pathways. Furthermore, they suggest that *mi* encodes a protein necessary for transducing signals mediated by way of either the c-*kit* or c-*fms* receptor.**

Interactions between cells play a major role in the determination of cell fate within the early embryo and in the differentiation of specific cell types during embryogenesis and adult life. Receptor tyrosine kinases (RTKs) appear to play key roles in this cell signaling process and hence in the regulation of embryological development, cell differentiation, and growth (1–3). The extracellular domains of these RTKs interact with specific growth factors, resulting in the activation of the intracellular kinase domain and the initiation of autophosphorylation reactions and phosphorylation of other cellular substrates.

As in other developmental systems, RTKs appear to play central roles in controlling the proliferation and differentiation of cells within the hematopoietic stem cell hierarchy. Although a large number of hematopoietic growth factors and their receptors have been described (4, 5), their biological functions *in vivo* are unclear. There is, however, compelling genetic evidence, for at least two closely related RTKs—the receptors encoded by the c-*fms* and c-*kit* genes—that they play central roles in controlling normal hematopoietic cell function. The mouse mutation osteopetrotic (*op*) is characterized by severe bone abnormalities due to the defective growth of osteoclasts from precursor macrophages (6). Recent experiments have shown that the *op* mutation resides in *mcsf*, the gene for the macrophage growth factor colony-stimulating factor 1 (CSF-1) and the ligand for the c-*fms*-receptor and results in the absence of functional CSF-1 synthesis (7, 8). Similarly, mutations at either the *W* or the Steel (*Sl*) locus can lead to severe anemia, mast cell deficiency, white spotting, and sterility (9). At the cellular level, the *W* defect appears to be intrinsic to hematopoietic stem cells, melanoblasts, and primordial germ cells, whereas the *Sl* locus appears to control some aspect of the microenvironment in which these cells develop and function (9). Consistent with these biological conclusions, genetic and molecular analyses suggest that the *W* locus encodes the c-*kit* RTK (10–16) and that the *Sl* locus encodes the ligand for the c-*kit* RTK (17–21). Thus, the osteopetrotic defect in *op* mice and the pleiotropic developmental defects in *W* and *Sl* mice result from loss-of-function mutations in the c-*fms* and c-*kit* signal transduction pathways, respectively.

The complex developmental defects manifested by *W* and *Sl* mice *in vivo* can be reproduced, in part, in a cell culture system in which mast cells are grown on a monolayer of mouse embryo fibroblasts (MEFs). Growth in this coculture system requires the presence of an intact c-*kit* signal transduction pathway. Thus, whereas mast cells derived from wild-type mice survive and proliferate in this assay, mast cells from mice homozygous for severe *W* mutations do not (22). Furthermore, the ability of mast cells derived from mice carrying various mild or severe *W* alleles to grow in this *in vitro* assay correlates with the residual levels of c-*kit* kinase activity associated with these mutations (15). In addition, MEFs derived from embryos carrying the *Sl* mutation do not support the growth and survival of +/+ mast cells (23). Thus, the survival of mast cells in this coculture system is absolutely dependent on the expression of a normal c-*kit* receptor by mast cells and a functional ligand by the fibroblast monolayer.

In addition to ligands and their receptors, which transmit and receive extracellular signals, other proteins must exist within the cell to translate these signals into a developmental program. Genetic analysis provides one approach to identify these proteins. Mutation at the microphthalmia (*mi*) locus is associated with white spotting, mast cell deficiency, and osteopetrosis (24, 25). The osteopetrosis in *mi/mi* mice results from a defect in the osteoclast (26, 27), a multinuclear cell derived from the monocyte/macrophage lineage, and can be cured by the transplantation of normal hematopoietic cells (28). These results suggest that the cellular defect in *mi/mi* mice, like the defect in mutant *W* mice, is intrinsic to cells within the hematopoietic stem cell hierarchy. Since the phenotype of *mi* mice resembles, at least in part, a summation of the phenotypes that result from mutations at the *W*, *Sl*, and *op* loci, *mi* might define a common step in the c-*kit* and c-*fms* signal transduction pathways. This notion is consistent with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RTK, receptor tyrosine kinase; MEF, mouse embryo fibroblast; *mi*, microphthalmia; CSF-1, colony-stimulating factor 1; IL-3, interleukin 3.

†Present address: Laboratoire de Hematologie et de Cancerologie Experimentale, Unite 119, Institut National de la Santé et de la Recherche Médicale, 27 b[d] Lei Roure, 13009 Marseille, France.
¶To whom reprint requests should be addressed.

the recent finding that mast cells derived from *mi/mi* mice are also unable to survive on a monolayer of MEFs (29).

To explore possible relationships between *W*, *Sl*, and *mi*, and between c-*kit* and c-*fms*, we have generated gain-of-function alterations in *W* and *mi* mast cells by introducing the c-*fms* gene into these cells. We show here that c-*fms* complements *W* but not *mi* mutations and that mast cells expressing c-*fms* are capable of growth on fibroblasts derived from *Sl* embryos. These results indicate that the c-*fms* signaling pathway is intact in mast cells and raise the possibility that c-*kit* and c-*fms* share common substrates for phosphorylation. Our results also provide genetic evidence that *mi* defines a common step in the c-*kit* and c-*fms* signal transduction pathways.

MATERIALS AND METHODS

Animals. Normal +/+ mice, $W^{41}/+$, $W^{42}/+$, and *mi*/+ mutants, all on a C57BL/6 background, and WB/Re *W*/+ mice were purchased from The Jackson Laboratory. *W/W* and W^{42}/W^{42} mouse embryos were obtained by natural matings of heterozygous animals, fertilization being assumed to occur at midnight prior to detecting vaginal plugs. Homozygous fetuses were distinguished from heterozygous littermates by their anemic appearance and verified by their deficiency in colony-forming units-erythroid. Newborn *mi/mi* mice were distinguished from their heterozygous littermates by the lack of eye pigmentation.

Cell Culture. Mast cells were derived from the bone marrow of +/+ and W^{41}/W^{41} mice, the fetal liver of *W/W* and W^{42}/W^{42} mice, and the newborn spleen of *mi/mi* animals and were maintained in Iscove's medium containing serum and a source of interleukin 3 (IL-3) (MCM), as described (15). L-cell-conditioned medium was harvested from confluent L-cell cultures, stored in aliquots at −20°C, and used as a source of CSF-1 at a concentration of 10%. The GP+E-86 ecotropic packaging cell line (ref. 30; a gift of A. Bank, Columbia, NY) was transfected as described (31) with 10 μg of plasmid DNA and selected in the presence of G418 (400 μg/ml). Colonies were screened for expression of high retroviral titer (5×10^5 G418 colonies per ml) and absence of helper virus.

Retrovirus Vectors. Helper-free replication-defective retrovirus vectors were constructed containing the dominant-selectable *neo* gene (PYN-*neo*) (32) or the *neo* gene and the murine c-*fms* gene under the control of the murine leukemia virus long terminal repeat promoter (PYN-fms/neo).

Retroviral Infection. Mast cells (10^6) were resuspended in 0.5 ml of MCM and 1.5 ml of supernatant collected from virus-producing cells. They were then plated onto an irradiated (30.0 Gy) subconfluent monolayer (35-mm plate) of virus-producing cells. An additional 2 ml of viral supernatant and 5 ml of MCM was added after 5 and 12 hr, respectively. G418 (800 μg/ml) was added after a further 24 hr, and infected mast cells were selected for at least 3 weeks before they were used in the coculture experiments. Control uninfected mast cells died between 10 and 15 days after the start of selection. Cells were maintained in MCM containing G418 by feeding every 3–4 days and passaging weekly.

Coculture Assay. Cocultures were performed essentially as described (15, 22, 23) except that, to reduce the cell numbers required, 5×10^3 embryonic fibroblasts were seeded onto 24-well plates, grown to confluency, and seeded with 2×10^5 mast cells. After the appropriate time in coculture, cells were harvested by trypsinization and counted, and cytospin preparations were stained with alcian blue to differentiate mast cells and fibroblasts. In each experiment, assays were performed in triplicate.

Protein Analysis. Procedures for metabolic labeling with [^{35}S]methionine, cell lysis, immunoprecipitation, and *in vitro* kinase assays were performed as described (15). The anti-*fms* antibody was a generous gift of L. Rohrschneider (Fred Hutchinson Cancer Center, Seattle).

RESULTS

Ectopic Expression of the c-*fms* RTK in Normal *W* and *mi* Mutant Mast Cells. The levels of c-*fms* expression in the infected mast cells were determined by immunoprecipitation of ^{35}S-radiolabeled cells with a rabbit antiserum directed against the C-terminal domain of the c-*fms* protein. As shown in Fig. 1*a*, G418-resistant mast cells infected with the *neo-fms* retrovirus vector expressed high levels of the gp165 c-*fms* protein, whereas mast cells infected with a retrovirus vector containing the *neo* gene, but not c-*fms*, did not. Furthermore, the c-*fms* receptor expressed ectopically in these cells displayed kinase activity, as determined by measuring *in vitro* autophosphorylation activity in the immunoprecipitates (Fig. 1*b*).

Growth of c-*fms*-Infected +/+ and *W* Mutant Mast Cells on MEFs. To determine whether ectopic expression of c-*fms* could complement the defect in the c-*kit* gene of mast cells from *W* mutant mice, we cocultured *W* mutant mast cells expressing c-*fms* with MEFs that naturally express CSF-1 (data not shown). The survival of mast cells from mice homozygous for various *W* mutations infected with a c-*fms* retrovirus was identical to that of +/+ mast cells, whereas control, *neo*-infected *W* mast cells died within 8 days (Fig. 2 and 3*a*). These results demonstrate that the ectopically expressed c-*fms* receptor is functional in mast cells and is fully able to transduce signals in +/+ and various *W* mutant mast cells.

Absence of Growth of c-*fms*-Infected *mi/mi* Mast Cells on MEFs. Ebi *et al.* (29) have recently demonstrated that mast cells derived from mice homozygous for the *mi* mutation are also capable of growth in IL-3 but are unable to survive on a MEF monolayer. To determine whether the c-*fms* gene could complement this defect, we analyzed the ability of *neo*-infected and *neo*/c-*fms*-infected *mi/mi* cells to survive on MEFs. As shown in Fig. 3*b*, in contrast to the results described above for *W* mutants, c-*fms* did not complement the *mi* defect in this *in vitro* assay, even though these cells were also expressing high levels of the c-*fms* protein (Fig. 1*a*) that is functionally active in an *in vitro* kinase assay (Fig. 1*b*).

CSF-1 Dependence of c-*fms*-Infected Cells. To determine whether ectopic expression of c-*fms* in mast cells would induce specific CSF-1 dependence, the infected mast cells were analyzed for their ability to survive and grow in the presence of CSF-1. As shown in Fig. 4*b*, c-*fms*-expressing mast cells from either +/+ or various *W* mutant mice survived after 8 days in medium containing CSF-1, whereas the uninfected or *neo*-infected mast cells did not. There was no significant difference in the growth rates of *neo*- or *neo*/c-*fms*-infected cells in the presence of IL-3 (Fig. 4*a*). These results demonstrate that CSF-1/c-*fms* interactions can fully replace the interactions between the c-*kit* receptor expressed on mast cells and the c-*kit* ligand expressed by MEFs. In contrast, introduction of the c-*fms* gene into mast cells derived from *mi/mi* mice did not overcome the inability of these cells to survive in CSF-1 (Fig. 4*b*). The *mi/mi* mast cells expressing c-*fms* did, however, retain their ability to respond to IL-3 (Fig. 4*a*). In this experiment *mi/mi* cells grew at a faster rate than +/+ and *W/W* cells but there was still no significant difference between *neo*- and *neo*/c-*fms*-infected cells. The results in Figs. 3*b* and 4*b* indicate that *mi/mi* mast cells display an intrinsic inability to respond to the CSF-1 and c-*kit* mitogenic signals.

Mast Cells Expressing c-*fms* Are *Sl* Independent. MEFs derived from mutant Sl/Sl^d embryos are unable to support the growth of +/+ or *W* mast cells (23). These observations provide further evidence for the suggestion that the *Sl* locus

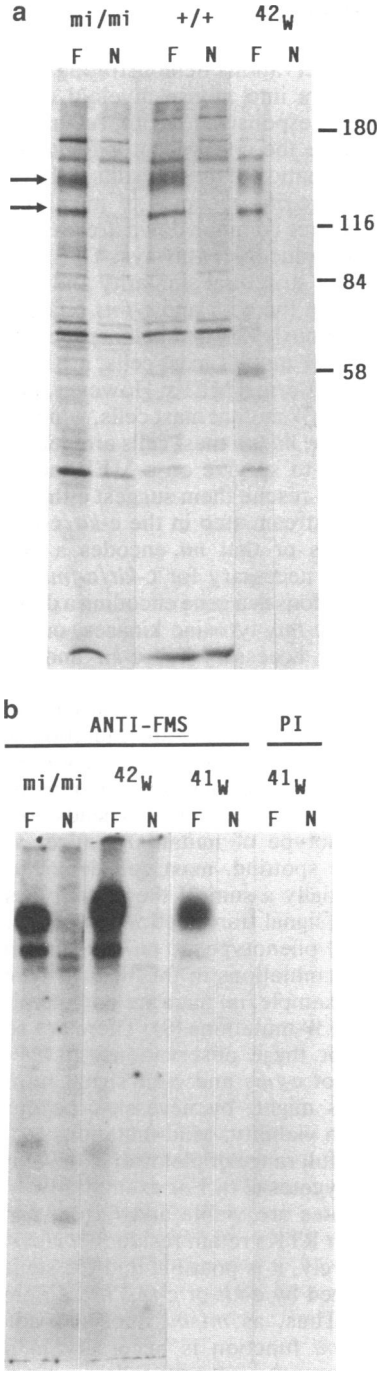

FIG. 1. (*a*) *neo/fms* (F)- and control *neo* (N)-infected mast cells derived from *mi/mi*, +/+, and W^{42}/W^{42} (^{42}W) mice were metabolically labeled with [^{35}S]methionine, and cell lysates were immunoprecipitated with anti-*fms* antiserum prior to SDS/7.5% PAGE and autoradiography. c-*fms*-specific immunoprecipitated proteins (gp165 and gp130) are indicated. Molecular weights are given as $M_r \times 10^{-3}$. (*b*) *In vitro* kinase assays using [γ-^{32}P]ATP were performed on immunoprecipitates of *neo/fms* (F)- and *neo* (N)-infected mast cells derived from *mi/mi*, W^{42}/W^{42} (^{42}W), and W^{41}/W^{41} (^{41}W) mice. Anti-*fms* (ANTI-*FMS*) or preimmune (PI) serum was used to immunoprecipitate cell lysates prior to SDS/7.5% PAGE and autoradiography.

directly encodes the ligand for c-*kit* (10, 17–21) rather than a protein involved in presenting ligands to RTKs (33, 34). As shown in Fig. 5, mast cells expressing c-*fms* were able to survive on Sl/Sl^d MEFs, whereas mast cells that did not express c-*fms* did not survive. Thus, mast cells expressing

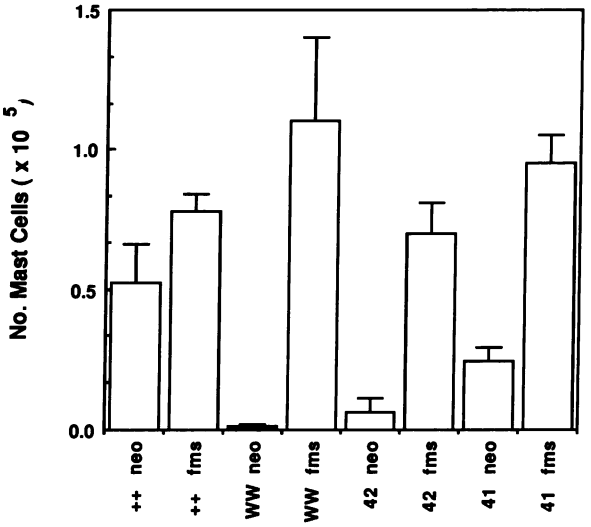

FIG. 2. Survival of *neo*- and *neo/fms*-infected mast cells derived from +/+, *W/W*, W^{42}/W^{42} (42), and W^{41}/W^{41} (41) mice after 8 days on a feeder layer of +/+ embryonic fibroblasts. Values are expressed as mean ± SD.

c-*fms* are *Sl* independent, indicating that the defect in Sl/Sl^d fibroblasts does not impair their ability to synthesize and present a functional CSF-1 growth factor to the c-*fms* receptor.

DISCUSSION

The results presented in this paper demonstrate that introduction and expression of the c-*fms* receptor into either normal or mutant *W* mast cells are sufficient to confer a CSF-1-responsive phenotype in these cells. From these ob-

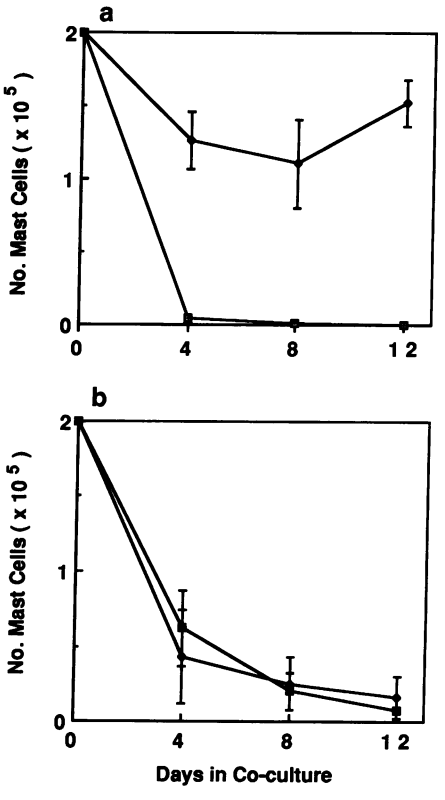

FIG. 3. Survival of *neo*- (□) and *neo/fms*-infected (◆) *W/W* (*a*) and *mi/mi* (*b*) mast cells after 4, 8, and 12 days on a feeder layer of +/+ MEFs. Values are expressed as mean ± SD.

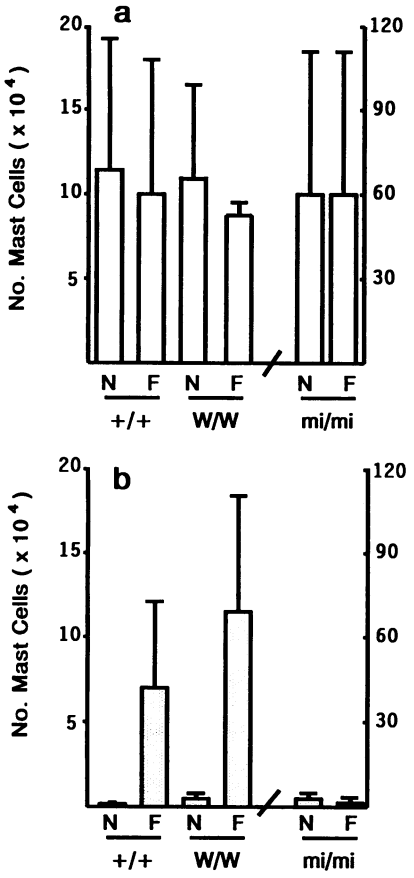

FIG. 4. Survival of *neo* (N)- and *neo/fms* (F)-infected +/+, *W/W*, and *mi/mi* mast cells after 8 days in Iscove's modified Dulbecco's medium containing 1% IL-3 (*a*) and 10% L-cell-conditioned medium (*b*). The results are the mean ± SD of two experiments in which each assay was performed in triplicate. Large standard deviations reflect differences between the two experiments rather than within each experiment.

servations, we conclude that the immediate physiological substrates for tyrosine phosphorylation by the c-*fms* receptor, and other downstream elements of the c-*fms* signaling pathway necessary for mast cell survival and mitogenesis, are

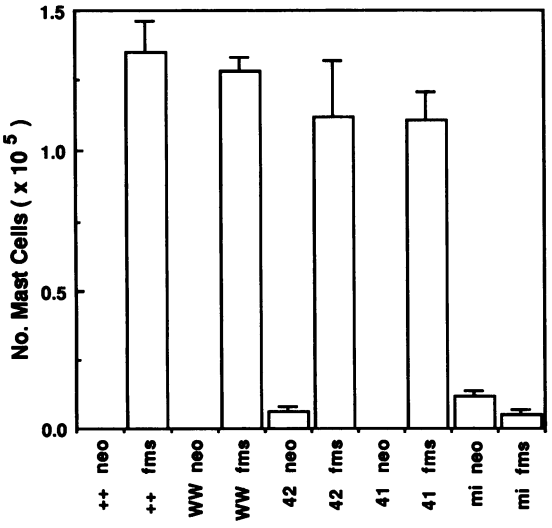

FIG. 5. Survival of *neo*- and *neo/fms*-infected mast cells derived from +/+, *W/W*, W^{42}/W^{42} (42), W^{41}/W^{41} (41), and *mi/mi* (mi) mice after 8 days on a feeder layer of Sl/Sl^d embryonic fibroblasts. Values are expressed as mean ± SD.

present in a cell population that normally does not express c-*fms*. These observations extend to primary hematopoietic cells previous observations demonstrating that introduction of CSF-1 receptors into mouse myeloid cell lines renders these cells CSF-1 responsive (35, 36). As mast cells normally express and utilize the c-*kit* receptor for their survival and growth, one explanation of these results is that the productive utilization of the c-*fms* receptor in mast cells is possible because c-*fms* shares common downstream effector elements with the c-*kit* transduction pathways. This is consistent with the high degree of structural similarity and overall sequence homology between the c-*kit* and c-*fms* receptors (1, 2).

As shown previously (29) and in this paper, mast cells from *mi/mi* mice, like *W* mutant mast cells, are unable to survive on a monolayer of normal MEFs. However, in contrast to the observations with *W* mutant mast cells, which are rescued by the c-*fms* receptor, *mi/mi* mast cells are not. The inability of *mi/mi* mast cells to survive on a MEF monolayer and the failure of c-*fms* to rescue them suggest either that *mi* defines a common downstream step in the c-*kit*/c-*fms* signal transduction pathways or that *mi* encodes a component of a parallel pathway necessary for c-*kit*/c-*fms* function. Loss-of-function mutations in a gene encoding a downstream target of the c-*kit* and c-*fms* tyrosine kinases, or that is part of a parallel pathway necessary for c-*kit* and c-*fms* function, would be expected to mimic some or all aspects of the phenotype of mutations that lie upstream in these signal transduction pathways. Germ-line mutations directly in the c-*kit* receptor (*W*) or in its ligand (*Sl*) lead to dominant white spotting and mast cell deficiency, whereas mutation in the ligand for the c-*fms* receptor (*op*) leads to osteopetrosis. Thus, the phenotype of mutant *mi* mice, which includes dominant white spotting, mast cell deficiency, and osteopetrosis, is partially a sum of the phenotypes of mutations that affect both signal transduction pathways.

Although the phenotype of *mi/mi* mice resembles that associated with mutations in the *W*/c-*kit* receptor, it is not identical. For example, *mi* mice are not anemic, a phenotype associated with *W* mutations (9). There are several possible explanations for these observations. If *mi* controls some central aspect of c-*fms* and c-*kit* signal transduction, then most *mi* alleles might, by necessity, be quite mild to be compatible with viability. Mild mutations at the *W* locus do not exhibit the full range of pleiotropic development defects, even as homozygotes (37). For example, W^{41}/W^{41} and W^{57}/W^{57} homozygotes are viable and fertile, and, correspondingly, their c-*kit* RTKs retain residual *in vitro* kinase activity (15). Alternatively, it is possible that the signal transduction pathways utilized by c-*kit* or c-*fms* are different in different cell lineages. Thus, as *mi/mi* mice are not anemic, it is possible that *mi* function is necessary in mast cells and macrophages but not erythroid cells.

Mutations at the *W* locus all appear to be loss-of-function mutations in the c-*kit* RTK (14–16). Structural mutations in the c-*kit* receptor (e.g., W^{37}, W^{41}, W^{42}) are strongly dominant negative in mice heterozygous for that allele, whereas regulatory mutations that result in a reduction in the levels of c-*kit* expression (e.g., W^{57}, *W*) have only mild effects in heterozygotes. These results indicate that expression of mutant forms of the c-*kit* receptor interfere with signal transduction by the wild-type protein (15). The results in this paper show that the c-*fms* receptor is able to rescue mast cells homozygous for either the W^{41} or W^{42} mutations, indicating that dominant-negative mutations in the c-*kit* gene do not interfere with signal transduction by the closely related c-*fms* receptor.

The binding of growth factors such as CSF-1 or Steel to their cognate receptors activates the intrinsic tyrosine kinase activity of these receptors (2). The result is ligand-induced tyrosine phosphorylation of cellular proteins that ultimately contributes to a mitogenic or developmental response. The

biologically relevant substrates for RTKs remain poorly characterized, although a number of molecules, phosphorylated rapidly in response to the addition of ligand, have been identified. The results presented here do not provide direct molecular evidence that *mi* encodes one of the downstream targets for phosphorylation by the activated c-*fms* or c-*kit* receptors. However, the molecular analysis of certain developmental mutations such as *W*, *Sl*, and *op* in the mouse, and torso, torso-like, lethal polehole, and sevenless in *Drosophila*, suggests that genetic approaches may provide the most direct way to identify and isolate the important components of cell signaling pathways in multicellular organisms (3).

We thank Larry Rohrschneider for the generous gift of the *fms* reagents, Arthur Bank for the packaging cells, and Carol Steeg for critical reading of the manuscript. This work was supported by grants from the National Institutes of Health, Medical Research Council, and National Cancer Institute of Canada. L.F. is a recipient of a European Molecular Biology Organization Postdoctoral Fellowship and R.R. is supported by a fellowship from the Medical Research Council.

1. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 42–52.
2. Yarden, Y. & Ullrich, A. (1988) *Annu. Rev. Biochem.* **57**, 443–478.
3. Pawson, T. & Bernstein, A. (1990) *Trends Genet.* **6**, 350–356.
4. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F. & Look, A. T. (1985) *Cell* **41**, 665–676.
5. Clark, S. C. & Kamen, R. (1987) *Science* **236**, 1229–1237.
6. Marks, S. C., Jr. (1984) *Clin. Orthop. Relat. Res.* **189**, 239–263.
7. Yoshida, H., Hayashi, S. I., Kunisada, T., Ogawa, M., Nishikawa, S., Okamura, H., Sudo, T., Shultz, L. D. & Nishikawa, S. I. (1990) *Nature (London)* **345**, 442–444.
8. Wiktor-Jedrzejczak, W., Bartocci, A., Ferrante, A. W., Ahmed-Ansari, A., Sell, K. W., Pollard, J. W. & Stanley, E. R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4828–4832.
9. Russell, E. S. (1979) *Adv. Genet.* **20**, 357–458.
10. Chabot, B., Stephenson, D. A., Chapman, V. M., Besmer, P. & Bernstein, A. (1988) *Nature (London)* **335**, 88–89.
11. Geissler, E. N., Ryan, M. A. & Housman, D. E. (1988) *Cell* **55**, 185–192.
12. Nocka, K., Majumder, S., Chabot, B., Ray, P., Cervone, M., Bernstein, A. & Besmer, P. (1989) *Genes Dev.* **3**, 816–826.
13. Bernstein, A., Chabot, B., Dubreuil, P., Reith, A., Nocka, K., Majumder, S., Ray, P. & Besmer, P. (1989) *Ciba Found. Symp.* **148L**, 158–172.
14. Tan, J. C., Nocka, K., Ray, P., Traktman, P. & Besmer, P. (1990) *Science* **247**, 209–212.
15. Reith, A. D., Rottapel, R., Giddens, E., Brady, C., Forrester, L. & Bernstein, A. (1990) *Genes Dev.* **4**, 390–400.
16. Nocka, K., Tan, J. C., Chiu, E., Chu, T. Y., Ray, P., Traktman, P. & Besmer, P. (1990) *EMBO J.* **9**, 1805–1813.
17. Williams, D. E., Eisenman, J., Baird, A., Rauch, C., Van Ness, K., March, C. J., Parks, L. S., Martin, U., Mochizuki, D. Y., Boswell, H. S., Burgess, G. S., Cosman, D. & Lyman, S. D. (1990) *Cell* **63**, 167–174.
18. Copeland, N. G., Gilbert, D. J., Cho, B. C., Donovan, P. J., Jenkins, N. A., Cosman, D., Anderson, D., Lyman, S. D. & Williams, D. E. (1990) *Cell* **63**, 175–183.
19. Flanagan, J. G. & Leder, P. (1990) *Cell* **63**, 185–194.
20. Zsebo, K. M., Williams, D. A., Geissler, E. N., Broudy, V. C., Martin, F. H., Atkins, H. L., Hsu, R.-Y., Birkett, N. C., Okino, K. H., Murdock, D. C., Jacobsen, F. W., Langley, K. E., Smith, K. A., Takeishi, T., Cattanach, B. M., Galli, S. J. & Suggs, S. V. (1990) *Cell* **63**, 213–224.
21. Huang, E., Nocka, K., Beier, D. R., Chu, T.-Y., Buck, J., Lahm, H.-W., Wellner, D., Leder, P. & Besmer, P. (1990) *Cell* **63**, 225–233.
22. Fujita, J., Nakayama, H., Onoue, H., Ebi, Y., Kanakura, Y., Kuriu, A. & Kitamura, Y. (1988) *Blood* **72**, 463–468.
23. Fujita, J., Onoue, H., Ebi, Y., Nakayama, H. & Kanakura, Y. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2888–2891.
24. Lyon, M. F. & Searle, A. G. (1989) *Genetic Variants and Strains of the Laboratory Mouse* (Oxford Univ. Press, Oxford).
25. Silvers, W. K. (1979) *The Coat Colours of Mice* (Springer, New York).
26. Murphy, H. D. (1973) *Calcif. Tissue Res.* **13**, 19–26.
27. Thesingh, C. W. & Scherft, J. P. (1985) *Bone* **6**, 43–52.
28. Walker, D. G. (1975) *Science* **190**, 784–785.
29. Ebi, Y., Kasugai, T., Seino, Y., Onoue, H., Kanemoto, T. & Kitamura, Y. (1990) *Blood* **75**, 1247–1251.
30. Markowitz, D., Goff, S. & Bank, A. (1988) *J. Virol.* **62**, 1120–1124.
31. Ellis, J. & Bernstein, A. (1989) *Mol. Cell. Biol.* **9**, 1621–1627.
32. Brady, G., Williams, R. L., Nordstrom, K., Vennstrom, B. & Iscove, N. N. (1988) *Oncogene* **3**, 687–689.
33. Morrison-Graham, K., West-Johnsrud, L. & Weston, J. A. (1990) *Dev. Biol.* **139**, 299–307.
34. Morrison-Graham, K., Bork, T. & Weston, J. A. (1990) *Dev. Biol.* **139**, 308–313.
35. Rohrschneider, L. R. & Metcalf, D. (1989) *Mol. Cell. Biol.* **9**, 5081–5092.
36. Kato, J.-Y. & Sherr, C. J. (1990) *Blood* **75**, 1780–1787.
37. Geissler, E. W., McFarland, E. C. & Russell, E. S. (1981) *Genetics* **97**, 337–361.