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Breakthroughs and Views

Signaling by Kit protein-tyrosine kinase—The stem cell factor receptor ☆

Robert Roskoski Jr. *

Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, 1100 Florida Avenue, New Orleans, LA 70119, USA

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Abstract

Signaling by stem cell factor and Kit, its receptor, plays important roles in gametogenesis, hematopoiesis, mast cell development and function, and melanogenesis. Moreover, human and mouse embryonic stem cells express Kit transcripts. Stem cell factor exists as both a soluble and a membrane-bound glycoprotein while Kit is a receptor protein-tyrosine kinase. The complete absence of stem cell factor or Kit is lethal. Deficiencies of either produce defects in red and white blood cell production, hypopigmentation, and sterility. Gain-of-function mutations of Kit are associated with several human neoplasms including acute myelogenous leukemia, gastrointestinal stromal tumors, and mastocytomas. Kit consists of an extracellular domain, a transmembrane segment, a juxtamembrane segment, and a protein kinase domain that contains an insert of about 80 amino acid residues. Binding of stem cell factor to Kit results in receptor dimerization and activation of protein kinase activity. The activated receptor becomes autophosphorylated at tyrosine residues that serve as docking sites for signal transduction molecules containing SH2 domains. The adaptor protein APS, Src family kinases, and Shp2 tyrosyl phosphatase bind to phosphotyrosine 568. Shp1 tyrosyl phosphatase and the adaptor protein Shc bind to phosphotyrosine 570. C-terminal Src kinase homologous kinase and the adaptor Shc bind to both phosphotyrosines 568 and 570. These residues occur in the juxtamembrane segment of Kit. Three residues in the kinase insert domain are phosphorylated and attract the adaptor protein Grb2 (Tyr703), phosphatidylinositol 3-kinase (Tyr721), and phospholipase Cγ (Tyr730). Phosphotyrosine 900 in the distal kinase domain binds phosphatidylinositol 3-kinase which in turn binds the adaptor protein Crk. Phosphotyrosine 936, also in the distal kinase domain, binds the adaptor proteins APS, Grb2, and Grb7. Kit has the potential to participate in multiple signal transduction pathways as a result of interaction with several enzymes and adaptor proteins.

Keywords: Akt; APS; Bad; Cancer; Cbl; Dok1; Erk; Fyn; Grb2; Grb7; Hematopoiesis; Leukemia cell lines; Lyn; MAP kinase; Mast cell; Oncogene; Piebaldism; Protein kinase; Protein phosphatase; Proto-oncogene; Ras; Shp1; Shp2; Src family kinase

Besmer and co-workers [1] identified the viral oncogene of the Hardy–Zuckerman 4 feline sarcoma virus in 1986 and arbitrarily designated the oncogenic transforming factor as v-Kit. Subsequently, Yarden et al. [2] cloned the gene and deduced the sequence of Kit, the normal cellular homologue of v-Kit. Kit is a receptor protein-tyrosine kinase. The ligand for Kit was identified and variously named—in alphabetical order—(a) Kit ligand [3], (b) mast cell growth factor [4], (c) Steel factor (where Steel is a mouse mutant) [5], and (d) stem cell factor [6].

Stem cell factor (SCF) and Kit, its receptor, play pivotal roles in cell differentiation, proliferation, and survival. Kit is encoded by the mouse White locus (*W*)

☆ *Abbreviations:* Flk, fetal liver kinase; Flt, FMS-like tyrosyl kinase; GST, glutathione *S*-transferase; KDR, kinase insert domain receptor; PDGF, platelet-derived growth factor, PH, pleckstrin homology; PI 3-kinase, phosphatidylinositol 3-kinase; PLC, phospholipase C; pTyr, phosphotyrosine; SCF, stem cell factor; SH2, Src homology 2; SH3, Src homology 3; VEGF, vascular endothelial growth factor.

* Fax: +1 504 619 8775.
E-mail address: biocrr@lsuhsc.edu.

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[7], while the stem cell factor is encoded by the mouse Steel locus (*Sl*) [8]. Mutations in these loci generate deficiencies of several cell types. The complete absence of SCF or Kit kinase activity leads to death in utero or perinatally as a result of anemia [9,10]. Partial loss-of-function mutations in the White or Steel locus in mouse lead to a deficiency of mast cells, hypopigmentation (white fur color), and sterility.

Kit signaling is important in erythropoiesis, lymphopoiesis, mast cell development and function, megakaryopoiesis, gametogenesis, and melanogenesis [11]. Kit is expressed in hematopoietic stem cells, dendritic, erythroid, megakaryotic, and myeloid progenitor cells, and pro-B and pro-T cells [12]. Kit expression is lost during cell differentiation with the exceptions of mature mast cells, melanocytes, and the intestinal interstitial cells of Cajal. Deficiency of the latter cells leads to decreased gut motility. The adult mouse oocyte expresses Kit while the surrounding granulosa cells express SCF. Defects in Kit function can produce abnormal peripheral nerve regeneration and spatial learning memory deficits [13].

Loss-of-function mutations in humans lead to autosomal-dominant piebaldism [14]. This syndrome is characterized by abnormal pigmentation of the hair and skin, deafness, and megacolon. Gain-of-function mutations occur in a percentage of human neoplasms including gastrointestinal stromal tumors (>90%), mastocytomas (>70%), sinonasal T-cell lymphomas (17%), and seminomas/dysgerminomas (9%) [15]. Moreover, paracrine or autocrine activation of Kit has been postulated in numerous other human malignancies including ovarian neoplasms and small-cell lung cancer [15,16].

Stem cells in both embryos and adults have the unique ability to balance self-renewal and differentiation such that mature cells necessary for the function of specific organ systems can be generated and replaced without depletion of the stem cell pool. The origin of the term stem cell factor initially referred to its role in survival, self-renewal, and differentiation of hematopoietic stem cells. However, a recent work indicates that the pluripotent R1 mouse embryonic stem cell line expresses Kit transcripts and functional protein [17]. Using gene arrays and reverse-transcriptase polymerase chain reaction, Palmqvist and co-workers found that Kit transcripts decrease to 20% of their initial value 72 h after the removal of leukemia inhibitory factor (LIF) as cells lose their pluripotency and become differentiated. Similar results were observed in J1 and EFC mouse embryonic stem cell lines. The authors suggest that differentiated cell types emerge later that re-express Kit. The R1 embryonic stem cell line does not express SCF, but irradiated mouse embryo fibroblasts that nourish the cell line in culture express SCF transcripts [17].

Properties of stem cell factor

Stem cell factor is widely expressed during embryogenesis and can be detected in brain, endothelium, gametes, heart, kidney, lung, melanocytes, skin, and the stromal cells of the bone marrow, liver, and thymus [10]. Stem cell factor acts synergistically with hematopoietic colony-stimulating factors such as granulocyte-macrophage colony-stimulating factor, interleukin 3, and erythropoietin [18].

SCF exists as membrane-anchored and soluble isoforms that arise from alternative RNA splicing and proteolytic processing; both isoforms contain initially an extracellular domain, a transmembrane segment, and an intracellular component [19]. The precursor for isoform 1, from which the soluble factor is derived, contains 273 amino acids. Residues 1–25 comprise the signal sequence, residues 26–214 make up the extracellular domain, residues 215–237 represent the transmembrane segment, and residues 238–273 constitute the intracellular component (the residue numbers in this article correspond to those of human proteins). Following the removal of the signal sequence, additional processing leads to the generation of the soluble form of SCF (residues 26–189).

The enzyme that catalyzes the release of soluble SCF from isoform 1 is most likely matrix metalloprotease-9 [20]. Cleavage occurs after Ala189 in the following sequence: Pro-Val-Ala-Ala-Ser (187–191). Matrix metalloprotease-9 prefers proline at the P_3 position, a hydrophobic residue at the $P_{1'}$ position, and a serine or threonine at the $P_{2'}$ position [21]. Isoform 2 contains 28 fewer amino acids because exon 6 is omitted as a consequence of alternative splicing. In humans, isoform 2 lacks the metalloprotease-9 cleavage site and is chiefly membrane anchored. In mice, isoform 2 can be cleaved at an alternative site to release a soluble SCF, but it is liberated at a slower rate than isoform 1. SCF functions as a non-covalent homodimer. The structure of each protomer is a short-chain four-helix bundle [19].

Structure of Kit, the stem cell factor receptor

Kit is a type III receptor protein-tyrosine kinase [2] (see [22] for a description of types I through IX receptor protein-tyrosine kinases). The type III class also includes the platelet-derived growth factor receptor (α- and β-chains), the macrophage colony-stimulating-factor receptor (CSF-1), and the Fl cytokine receptor (Flk2/Flt3). Receptor protein-tyrosine kinases all share the same topology: an extracellular ligand-binding domain, a single transmembrane segment, and a cytoplasmic kinase domain. The class III receptors are characterized by the presence of five immunoglobulin-like domains in their extracellular portion. SCF binds to the second

and third immunoglobulin domains while the fourth domain plays a role in receptor dimerization [19]. The structure of the class III receptors differs from that of other receptor tyrosyl kinases by the insertion of 70–100 amino acids near the middle of the kinase domain. In human Kit, the kinase insert domain is about 80 residues in length. The vascular endothelial growth factor (VEGF) receptor family contains seven immunoglobulin-like extracellular domains and a kinase insert like the PDGF receptor family [22].

There are four isoforms of human Kit and two isoforms of murine Kit that are produced by alternative splicing. Both human and mouse Kit mRNAs can be spliced to yield a form lacking a Gly-Asn-Asn-Lys (510-GNNK-513) sequence in the extracellular domain near the transmembrane segment. In both species, the $GNNK^-$ sequence is more abundant than the $GNNK^+$ sequence in cells isolated from various tissues [23]. Humans contain two additional isoforms that contain or lack a serine residue at position 715 in the kinase insert domain with the Ser^+ form predominating. The GNNK isoforms behave differently in signaling as described later, but possible differences in the serine isoforms have not been investigated. Mouse Kit lacks the residue corresponding to human Ser715 (although it contains several nearby serine residues).

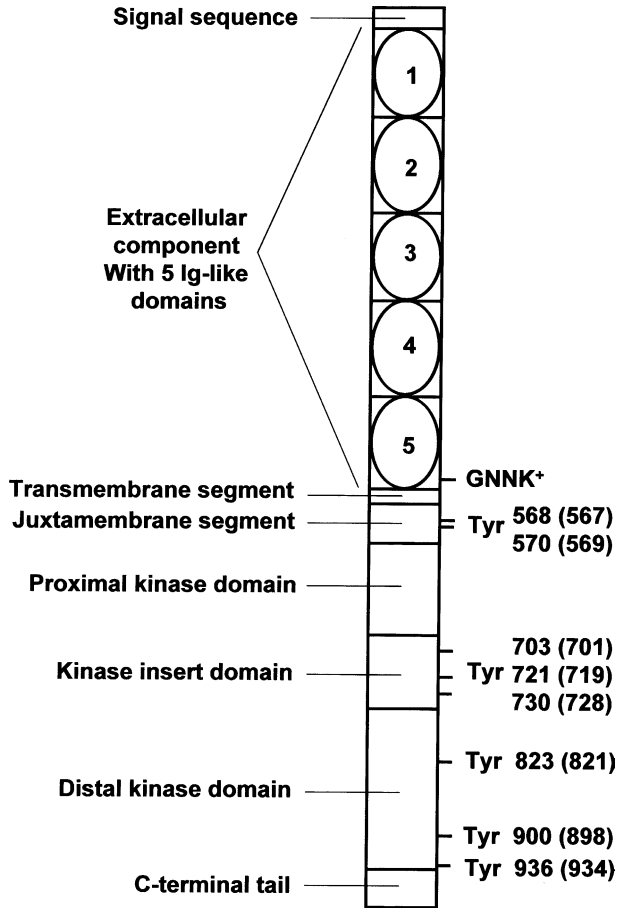

Fig. 1. Tyrosine phosphorylation sites and organization of Kit. The relative length of the domains is to scale. The human (mouse) numbering system is displayed. G refers to glycine; N, asparagine; K, lysine; and Ig, immunoglobulin.

Binding of SCF to Kit leads to receptor dimerization and activation of protein kinase activity [24]. SCF forms a non-covalent dimer that binds to two Kit monomers and promotes Kit dimer formation. The receptor becomes autophosphorylated at tyrosine residues during activation; the resulting phosphotyrosine residues serve as docking sites for signal transduction molecules containing SH2 and other phosphotyrosine-binding domains. The initial residues that undergo autophosphorylation, which are in the juxtamembrane segment, include Tyr568 and Tyr570. Other residues undergo phosphorylation, including three in the kinase insert domain (Fig. 1). Activated Kit also catalyzes the phosphorylation of substrate proteins.

Kit and Src family kinase activation

Src and Src family protein kinases play key roles in cell differentiation, motility, proliferation, protein trafficking, and survival. From the N- to C-terminus, Src family kinases contain an N-terminal 14-carbon myristoyl group, a unique segment, an SH3 domain, an SH2 domain, a protein-tyrosine kinase domain, and a C-terminal regulatory tail [25,26]. Src family kinases are controlled by integrin, G-protein coupled, antigen- and Fc-coupled, cytokine, and steroid hormone receptors, and receptor protein-tyrosine kinases including Kit. Members of the Src family of protein kinases include Blk, Brk, Fgr, Frk, Fyn, Hck, Lck, Lyn, Src, Srm, and Yes. Fyn, Src, and Yes are expressed in all cell types [25]; Blk, Fgr, Hck, Lck, and Lyn are expressed primarily in hematopoietic cells.

Linnekin and co-workers studied the role of stem cell factor in the regulation of Lyn in a human megakaryoblastic leukemia cell line (Mo7e), a human erythroleukemia cell line (MB-02), and human fetal liver cells [27]. They demonstrated that SCF treatment increases Lyn tyrosine phosphorylation in both cell lines and in normal progenitor cells isolated from human fetal liver. Furthermore, measurements in vitro show that SCF treatment increases Lyn autophosphorylation and the ability of Lyn to phosphorylate enolase, a non-physiological tyrosyl kinase substrate.

Linnekin et al. prepared GST fusion proteins containing the juxtamembrane segment, the proximal kinase domain, the kinase insert, the kinase activation segment from the distal kinase domain, or the C-terminal tail of Kit. Studies with these GST fusion proteins demonstrate that Lyn interacts with phosphotyrosine residues 568 and 570 in the Kit juxtamembrane segment [27]. In contrast, Lyn fails to

bind to the other tyrosine-phosphorylated GST fusion proteins. Lyn also fails to bind to the unphosphorylated GST-juxtamembrane segment fusion protein. Linnekin and co-workers suggest that Lyn interacts with phosphotyrosines 568 and 570 by its SH2 domain. The association of Lyn with Kit raises the possibility that one or both of the enzymes may catalyze the phosphorylation of the other. SCF-induced activation of Lyn and the association of Lyn with Kit indicate that Lyn plays a role in SCF signal transduction. To provide evidence for this concept, studies with antisense oligonucleotides demonstrate that a decrease in Lyn content leads to a reduction of SCF-induced human Mo7e cell proliferation [27].

To more precisely determine the Kit residues that bind to Src family kinases, Timokhina et al. [28] transfected mouse bone-marrow-derived mast cells with tyrosine to phenylalanine mutants corresponding to human Kit residues 568, 570, or both. They measured the binding of the GST-Fyn SH2 domain to immunoprecipitated mouse Kit after mast cell treatment with SCF. They found that the wild type and the Kit Tyr570Phe mutant co-immunoprecipitate with GST-Fyn SH2 whereas the Kit Tyr568Phe mutant and the Tyr568/570Phe double mutant do not co-precipitate. These observations point to Kit pTyr568 as the chief binding site for Src family kinase members. Using a complementary approach, Lennartsson et al. [29] created porcine aortic endothelial cells that expressed wild type human Kit and the Tyr568Phe, the Tyr570Phe, and the corresponding double mutant of human Kit. Following SCF stimulation, cells were lysed and treated with antiserum that precipitates Src, Fyn, and Yes. Src protein kinase activity was increased in immunoprecipitates from cells containing wild type and the Tyr570Phe mutant Kit. There was no Src kinase activity in immunoprecipitates prepared from stimulated cells expressing the Tyr568Phe mutant or the double mutant thereby confirming that Kit pTyr568 is necessary for the association with and activation of Src kinases.

Shivakrupa and Linnekin [30] studied the role of Lyn (a Src family kinase member) in the regulation of several signal transduction pathways in bone-marrow-derived mast cells prepared from wild type and Lyn-deficient mice. They found that SCF-induced proliferation is reduced in Lyn-deficient mast cells. Lyn thus contributes to SCF-induced proliferation. They report that Kit expression is increased in Lyn-deficient cells. However, tyrosine phosphorylation at residues 568 and 570 (the juxtamembrane segment), at residues 703, 721, and 730 (the kinase insert domain), and at residue 823 (the activation loop of Kit) is diminished in Lyn-deficient cells. The precise role of Lyn in Kit signaling is unclear. Lyn may stimulate Kit activity allosterically or by catalyzing the phosphorylation of regulatory residues. Alternatively, activation may be indirect and involve other proteins. Additional work will be required to decipher this regulatory mechanism.

Lennartsson et al. studied the phosphorylation of human Kit expressed in porcine aortic endothelial cells. They found that Tyr900 of Kit is phosphorylated following SCF treatment [31]. They report that the Tyr568Phe mutant Kit fails to exhibit Tyr900 phosphorylation after SCF treatment. Because pTyr568 is responsible for binding Src family kinases, they surmised that Tyr900 is phosphorylated by one of these enzymes. This hypothesis is supported by the finding that SU6656 (a Src family kinase inhibitor) blocks Tyr900 phosphorylation in the endothelial cells. They also report that Tyr900 is phosphorylated in vitro in cell extracts containing Src and Kit, but Tyr900 is not phosphorylated in cell extracts lacking Src.

Lennartsson et al. [31] characterized proteins that bind to phosphorylated peptides corresponding to the Tyr900 phosphorylation site. They found that the p85 regulatory subunit of PI 3-kinase (see below) and Crk form a complex with pTyr900-modeled peptides. Crk contains an N-terminal SH2 domain followed by two SH3 domains and has the potential to interact with many signal transduction proteins. They found that Crk and p85 are tyrosine phosphorylated in NIH3T3 cells expressing the human wild type Kit but not in cells expressing the Tyr900Phe mutant. They also report that Crk does not bind directly to Kit pTyr900; rather, Crk binds to p85 which in turn binds to pTyr900 of Kit. To provide support for the physiological importance of these findings, these investigators showed that SCF stimulation of [^{3}H]thymidine incorporation is decreased 75% in NIH3T3 cells that express the Tyr900Phe mutant when compared with cells that express wild type Kit [31].

Binding of APS to Kit

APS is an adaptor protein that contains a PH and an SH2 domain (Table 1). APS was originally cloned using a yeast two-hybrid system with the constitutively active oncogenic Asp816Val mutant of human Kit as bait. Using immobilized phosphopeptides corresponding to various autophosphorylated tyrosine residues in Kit, Wollberg et al. [32] demonstrated that APS preferentially associates with pTyr568 and pTyr936. pTyr568 is the binding site for Chk, Shc, Shp2, and Src family kinases (Table 2). These workers demonstrated that Tyr936 is an autophosphorylation site involved in binding the adaptor proteins Grb2 and Grb7. Moreover, stimulation of Kit, the PDGF β-receptor, the erythropoietin receptor, the nerve growth factor receptor (TrkA), or the insulin receptor leads to APS binding to each receptor and APS tyrosine phosphorylation by the receptor. APS phosphorylation occurs at a single site in its C-terminus

Table 1
Selected protein domains

Name	Description/function	Interpro accession no.
PH	Pleckstrin homology domains consist of about 100 amino acid residues that recognize phosphoinositides; they target their host protein to the plasma and internal membranes where phosphoinositides occur	IPR011993
SH2	The Src homology 2 domain consists of about 100 amino acid residues first identified in Src protein kinase. SH2 domains interact with phosphotyrosine-containing targets where they recognize between 3 and 6 residues C-terminal to the phosphorylated tyrosine; binding is strictly phosphorylation-dependent	IPR000980
SH3	Src homology 3 domains consist of about 50 amino acid residues that bind proline II helices. Such helices usually contain Pro-Xxx-Xxx-Pro segments	IPR001452

Table 2
Proteins interacting with Kit phosphotyrosine residues

Human residue	Protein	Function/ comments	Receptor source	Biological effect	Citations
568	APS	Adaptor containing PH and SH2 domains	Human	Kit degradation	[32]
568	Shp2	SH2 domain-containing phosphatase 2 (pTyr specific)	Mouse	Inhibits proliferation	[37]
568	Src kinases	A non-receptor protein-tyrosine kinase family	Human, mouse	Proliferation	[27,28,36]
568, 570	Chk	Csk homology kinase where Csk is C-terminal Src kinase	Human	Not reported	[36]
568, 570	Shc	SH2-containing transforming protein C1; binds Grb2	Human	Not reported	[36]
570	Shp1	SH2 domain-containing phosphatase 1 (pTyr specific)	Mouse	Inhibits proliferation	[37]
703	Grb2	Growth factor receptor-bound protein 2; binds RasGEF	Human	Not reported	[53]
721	p85	Regulatory subunit of PI 3-kinase	Human, mouse	Adhesion, survival, proliferation	[40,41]
730	PLCγ	Phospholipase Cγ	Mouse	Survival, proliferation	[47]
823		Kit activation loop tyrosine phosphorylation site	Human	Proliferation	[41]
900	p85	Regulatory subunit of PI 3-kinase	Human	Proliferation	[31]
900	Crk	Adaptor protein (pronounced crack) that contains one SH2 and two SH3 domains that binds to p85 and indirectly binds to residue 900	Human	Proliferation	[31]
936	APS	Adaptor containing PH and SH2 domains	Human	Kit degradation	[32]
936	Grb2	Growth factor receptor-bound protein 2	Human	Not reported	[53]
936	Grb7	Growth factor receptor-bound protein 7	Human	Not reported	[53]

thereby creating a pTyr-binding site for Grb2 and Cbl (the action of Cbl is described in the next section).

APS promotes a positive signaling response that leads to enhanced mitogenicity in the case of the insulin receptor and TrkA [32]. APS promotes a negative signaling response that leads to degradation of the erythropoietin receptor, Kit, and the PDGF β-receptor. A possible explanation for the disparity in APS-mediated signaling among the different receptor tyrosyl kinases lies in the mode of interaction with the receptor. With the insulin receptor and TrkA, where APS mediates an increased effect, APS interacts with phosphorylated tyrosine residues in the activation loop of the tyrosyl kinase domain. The binding of APS to pTyr residues in the activation loop may stabilize the active conformations of the insulin receptor and the TrkA protein kinases, accounting for the augmented response. Such an interaction fails to occur with the erythropoietin receptor (not a protein kinase and lacking an activation loop), Kit, or the PDGF β-receptor. The insulin receptor and TrkA activation loops contain multiple phosphotyrosine residues while the Kit and PDGF-β activation loops contain a single phosphotyrosine.

Binding of Cbl to Kit

Cbl functions as a negative regulator of many signaling pathways that start from receptors at the cell surface. The *c-Cbl* proto-oncogene was first discovered as the cellular homologue of *v-Cbl*, a viral transforming gene from a Cas murine retrovirus that causes pre-B cell lymphomas in mice (Cas refers to the Lake Casitas region in California where the virus was isolated) [33]. Cbl family members are components of the ubiquitin ligation machinery involved in the targeting and degradation of proteins. Cbl acts as an E3 ubiquitin-protein ligase, which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes, and then transfers it to substrates promoting their degradation by the proteasome [34]. The N-terminus of Cbl family proteins contains a conserved tyrosyl kinase-binding (TKB) domain which

is composed of a four-helix bundle, a Ca^{2+}-binding EF hand, and a variant SH2 domain. A linker region connects this N-terminal domain with a really interesting new gene (RING) finger domain. The C3HC4 type zinc-finger (RING finger) is a cysteine-rich domain of about 40 residues that coordinates two zinc ions; C3HC4 refers to three cysteines, one histidine, and four cysteines. The RING finger domain recruits a ubiquitin-conjugating enzyme (E2), and the N-terminal tyrosyl kinase-binding domain mediates the conjugation, or covalent attachment, of ubiquitin to target proteins. At the C-terminus of Cbl is a ubiquitin-associated (UBA) domain of approximately 40 amino acid residues that is found in diverse proteins involved in the ubiquitin/proteasome pathway and cell signaling via protein kinases. Humans contain two other related genes: *Cbl-b* and *Cbl-c*/*Cbl-3*.

Zeng et al. [34] studied human kidney 293 cells transfected with human Kit with or without Cbl or Cbl-b. When cells were treated with SCF, there is a marked decrease of Kit in Cbl co-transfected cells compared to Cbl non-transfected cells. They found that anti-Kit leads to the co-precipitation of Cbl or Cbl-b with Kit in the presence or absence of SCF treatment. Cbl-b containing loss-of-function mutations of the RING finger is ineffective in mediating Kit degradation in transfected 293 cells. Tyrosine to phenylalanine mutation of residues 568 and 570 abolishes SCF-induced Cbl phosphorylation and Kit degradation. The authors suggest that Src family kinases, which bind to pTyr568 and secondarily to pTyr570, are required for Cbl phosphorylation and Kit degradation. However, Hu and Hubbard [35] report that Src family kinase-mediated phosphorylation of APS generates a Cbl-binding site. After Cbl docks with APS, Cbl becomes activated. Thus, additional work will be required to determine whether Cbl activation by Kit and Src family kinase is direct, requires APS as an intermediary, or involves both processes.

Binding of Chk to Kit

Csk (C-terminal Src kinase), a cytoplasmic protein-tyrosine kinase, catalyzes the phosphorylation of the C-terminal regulatory tyrosine of Src which is inhibitory [26]. Chk (Csk homology kinase) also catalyzes the phosphorylation of the inhibitory tyrosine of Src family kinases. Csk is expressed in all mammalian cells, whereas Chk is limited to breast, hematopoietic cells, neurons, and testes. Csk and Chk consist of an SH3, SH2, and kinase domain. Price et al. [36] demonstrated that Chk, but not Csk, binds to human Kit. Phosphopeptide inhibition of the interaction of the GST-Chk SH2 fusion protein with Kit from SCF-treated Mo7e human megakaryoblastic leukemia cells in vitro indicates that two sites of Kit are able to bind to the Chk SH2 domain; these sites are the tyrosine 568 and 570 diphosphorylated sequence and phosphorylated tyrosine 721. They also showed that Chk from cell extracts binds to heptapeptides (linked to Affi-Gel 15) corresponding to protein sequences encompassing (a) phosphotyrosines 568 and 570, and (b) phosphotyrosine 721. In addition, the purified GST-Chk SH2 fusion protein binds to the same peptide beads. However, site-directed mutagenesis of full-length human Kit cDNA, followed by transient transfection, indicates that the Chk SH2 domain binds to phosphotyrosines 568 and 570 but not phosphotyrosine 721. Further experiments have demonstrated that the Chk SH2 domain binds to this site directly and not through phosphorylated intermediates such as Fyn or Shc [36].

A scheme for the regulation of Src kinase by Chk involves the SCF-stimulated dimerization of Kit, one monomer bearing Src kinase and the other monomer bearing Chk. This dimerization would bring Src in proximity to Chk; phosphorylation of Src by Chk would lead to inhibition of Src kinase activity. Even the non-covalent association of Chk with the activated and autophosphorylated form of Src is inhibitory [26].

Regulation of Kit signaling by Shp1 phosphatase

Shp1 is a cytosolic phosphotyrosyl phosphatase containing two tandem SH2 domains, a phosphatase domain and a C-terminal tail. Shp1 occurs primarily in hematopoietic and epithelial cells, and it is a negative regulator of growth factor signaling. Besides inhibiting Kit signaling, Shp1 diminishes the growth-promoting properties of the colony-stimulating factor 1, erythropoietin, and interleukin 3 receptors, an effect mediated either directly by receptor dephosphorylation or indirectly by dephosphorylation of receptor-associated protein-tyrosine kinases [37]. Shp1 plays a role in the control of signaling cascades that couple growth factor receptors to hemopoietic cell differentiation. The N-terminal-SH2 domain of Shp1 blocks its catalytic domain and keeps the enzyme in an inactive conformation [26]. One plausible notion for enzyme regulation involves the recruitment and binding of this Shp1 SH2 domain to target phosphotyrosine residues with concomitant phosphatase activation.

Kozlowski et al. [37] generated GST-Kit fusion proteins that contain the mouse Kit juxtamembrane segment, the kinase insert, or the C-terminal tail. Analysis of Shp1 interactions with phosphorylated forms of these fusion proteins and with phosphorylated peptides encompassing the phosphotyrosine sites within Kit led to the identification of pTyr570 within the juxtamembrane segment as the binding site for Shp1. Consistent with these observations, mouse Kit and Shp1 are not co-immunoprecipitated from the SCF-treated Ba/F3-

Kit Tyr570Phe cells, but they are co-immunoprecipitated from stimulated cells expressing the wild type or the Tyr568Phe mutant. Moreover, analysis of Ba/F3 cells retrovirally transduced to express mouse Kit, the Tyr570Phe mutation disallows the binding of Shp1 to Kit, a disruption that leads to hyperproliferation following SCF treatment. This finding demonstrates that Shp1 is a negative regulator of Kit signaling. Ba/F3 is a murine, interleukin 3-dependent, Kit negative pro-B lymphoid cell line.

Regulation of Kit signaling by Shp2 phosphatase

Shp2, like Shp1, is a cytosolic phosphotyrosyl phosphatase containing two tandem SH2 domains, a phosphatase domain, and a C-terminal tail. Shp2, in contrast to Shp1, occurs in many types of cells. The SH2 domains of Shp2 target this enzyme to phosphotyrosines in a variety of growth factor receptors and other signaling molecules [26].

The ability of Shp2 to associate with activated Kit in Ba/F3-Kit transfectants is markedly reduced by the Tyr568Phe mutation but is unaffected by the Tyr570Phe mutation [37]. Moreover, expression of Kit bearing phenylalanine substitutions for either Tyr568 or Tyr570 is associated with markedly enhanced proliferation of Ba/F3 cells in response to SCF. These increases in proliferation are specifically related to interactions between SCF and the mutant Kits because proliferative responses to interleukin 3 in cells bearing these Kit mutants are comparable to those detected in wild type Ba/F3 cells. These observations indicate that pTyr570 mediates the association of Kit with Shp1 while pTyr568 mediates the association of Kit with Shp2 in vivo. Thus, Shp1 and Shp2 can negatively modulate Kit signaling by interacting with these specific phosphotyrosine residues.

Kit and phosphatidylinositol 3-kinase activation

Phosphatidylinositol 3-kinase (PI 3-kinase) represents a class of lipid kinases that catalyze the phosphorylation of the 3-hydroxyl group of the inositol ring of phosphatidylinositol derivatives. The preferred substrate in vivo is phosphatidylinositol 4,5-bisphosphate, and the product is phosphatidylinositol 3,4,5-trisphosphate. PI 3-kinase was initially identified through its association with two viral oncoproteins: v-Src and the middle T antigen of polyoma virus [38]. The PI 3-kinases are heterodimers consisting of regulatory and catalytic subunits. There are three classes of PI 3-kinase (I, II, and III), and the first class is divided into two groups (IA, IB). The class IA enzyme is most closely associated with intracellular signaling by receptor protein-tyrosine kinases. The class IA PI 3-kinase is a heterodimer that consists of a regulatory (p85) and a catalytic (p110) subunit [38]. These subunits are each encoded by three distinct human genes each with a number of splice variants [39]. From the N- to C-terminus, the typical class IA regulatory subunit contains an SH3 domain, a Rho-GTPase activating protein (Rho-GAP) homology domain, and two SH2 domains. From the N- to C-terminus, the typical catalytic subunit contains a Ras-binding domain, a phosphatidylinositol kinase accessory (PIKa) domain, and a catalytic domain. PI 3-kinase is implicated in many aspects of cell physiology including cell survival, chemotaxis, DNA synthesis, and receptor trafficking.

Treatment of cells with stem cell factor [40,41] and a variety of growth factors [38] leads to the activation of PI 3-kinase. The p85 regulatory subunit binds to phosphotyrosine residues of activated protein-tyrosine kinase receptors by one or both of its SH2 domains, and p85–p110 interaction leads to the allosteric activation of the catalytic subunit. Using Chinese hamster ovary cells transfected with carboxyterminally truncated or wild type human Kit, Lev et al. [40] showed that treatment with SCF leads to the rapid (within minutes) association of PI 3-kinase with phosphorylated Kit based upon their co-precipitation from cell lysates treated with anti-Kit. In contrast, mutant Kit lacking the kinase insert domain precipitates without PI 3-kinase. Moreover, they showed that a GST fusion protein containing a phosphorylated kinase insert domain, but not the unphosphorylated fusion protein, binds to PI 3-kinase. Their work indicates that phosphorylated tyrosine residues 721, 730, or both are the sites that bind PI 3-kinase.

To determine more precisely which residue of the kinase insert domain binds to PI 3-kinase, Serve et al. [41] examined the association and activation of mutant forms of mouse Kit transiently expressed in COS-1 cells. They prepared tyrosine to phenylalanine mutants corresponding to residues 703, 721, 730, and 747. They showed that wild type and mutant Kits were readily autophosphorylated. Moreover, they demonstrated that PI 3-kinase associates with the wild type, and the Tyr703Phe, Tyr730Phe, and Tyr747Phe mutants after SCF treatment. Although SCF treatment of cells expressing the Tyr721Phe receptor exhibit Kit autophosphorylation, this mutant receptor fails to bind PI 3-kinase. They also showed that kinase-dead Kit fails to undergo autophosphorylation and fails to bind PI 3-kinase. These findings indicate that phosphotyrosine 721 within a pTyr-Met-Asp-Met (721–724) sequence plays a key role in binding to PI 3-kinase. This segment corresponds to a p85 SH2 consensus-binding site (pTyr-Met/Val-Xxx-Met) [41].

Binding and activation of PI 3-kinase occur at or near the plasma membrane where the PI 3-kinase substrates are located. Akt (protein kinase B), a protein-serine/threonine kinase that was initially characterized in an AKR mouse-derived thymoma, is downstream from PI

3-kinase and promotes cell survival [42]. The 3-phosphoinositides activate phosphatidylinositol-dependent protein kinase (a protein-serine/threonine kinase) that leads to the phosphorylation and activation of Akt. Moreover, Akt contains a PH domain that binds to 3-phosphoinositides that arise from the action of PI 3-kinase. This binding brings Akt near phosphoinositide-dependent protein kinase, 3-phosphoinositides, and PI 3-kinase. One substrate of Akt is Bcl2 antagonist of cell death (Bad), a pro-apoptotic protein that promotes cell death. Following phosphorylation, Bad no longer promotes apoptosis.

Blume-Jensen et al. [42] demonstrated that the human Kit Tyr721Phe mutant expressed in a human osteosarcoma cell line (U2-OS) is unable to protect against Bad-induced apoptosis. This mutant is associated with a reduction in SCF-induced activation of Akt. The physiological role of Kit-mediated activation of PI 3-kinase was demonstrated in studies using transgenic mice harboring a Kit Tyr721Phe mutation. These mutations decrease both male and female fertility (reviewed in [11]). Sterility in males is due to a block in spermatogenesis with decreased proliferation and extensive apoptosis; decreased fertility in females is due to impaired follicle development. Moreover, Akt plays an important role in the survival and growth of primordial germ cells [43]. Activation of the PI 3-kinase/Akt pathway may explain in part how activating mutations of Kit participate in neoplastic transformation.

Lyn (a Src family kinase) plays a role in the regulation of the PI-3 kinase pathway [30]. While SCF treatment leads to the association of PI 3-kinase with wild type Kit in mouse bone-marrow-derived mast cells, PI 3-kinase is constitutively associated with Kit in Lyn-deficient cells. This finding suggests that Lyn negatively regulates PI 3-kinase association with Kit. As noted above, the regulatory subunit of PI 3-kinase binds to Kit via pTyr721, but the extent of phosphorylation of this residue is decreased (not increased) in Lyn-deficient cells. The mechanism for the increase in the association of PI 3-kinase with Kit in Lyn-deficient cells, which most likely involves interactions that are independent of pTyr721, is unresolved.

Kit and phospholipase Cγ activation

Phospholipase C (PLC) represents an enzyme family that catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate and diacylglycerol. Inositol trisphosphate promotes the elevation of intracellular Ca^{2+}; diacylglycerol activates protein kinase C (a protein-serine/threonine kinase family of isozymes). A myriad of cell functions are regulated by phospholipase C and Ca^{2+} including cell differentiation, division, motility, survival, egg fertilization, and the immune response [44].

The phospholipase C family consists of four subtypes including four PLCβ isoforms, two PLCγ isoforms, four PLCδ isoforms, and one PLCε isoform. Of these, the β-isoforms are regulated by G-protein coupled receptors and the γ-isoforms by receptor and non-receptor protein-tyrosine kinases [45]. The mechanism of regulation of the δ- and ε-isoforms is unclear. The overall architecture of the PLC family is similar. Human PLCγ1 consists of 1290 amino acids. From the N- to C-terminus, it contains a PH domain, a PLC X box, two SH2 domains, an SH3 domain, a PLC Y box, and a C2 domain. One part of a split PH domain occurs proximal to the first SH2 domain and the second part occurs distal to the SH3 domain. The PLC X and Y boxes form the catalytic site [45].

Rottapel et al. [46] investigated the association of signaling proteins with Kit in murine mast cells in culture. They identified PI 3-kinase and phospholipase C in anti-Kit immunoprecipitates from SCF-stimulated but not unstimulated cells. To identify the precise residue that interacts with PLCγ, Gommerman et al. [47] studied the activation of wild type and mutant mouse Kit by SCF using retrovirally transduced 32D cells. 32D is the designation of a murine myelomonocytic, interleukin 3-dependent, Kit-negative cell line. SCF stimulates PLCγ phosphorylation with the wild type receptor but not with the Tyr730Phe mutant Kit. Tyrosine 730 of Kit is followed by a consensus sequence that binds to the SH2 domain of phospholipase C [47]. Furthermore, cells expressing the Tyr730Phe mutant Kit fail to show increased Ca^{2+} in response to SCF treatment.

When COS-1 cells that transiently express the $GNNK^-$ or $GNKK^+$ isoforms of human Kit were treated with SCF, both isoforms become associated with PI 3-kinase and PLCγ. However, the two isoforms behave differently in the absence of SCF. The $GNNK^-$ variant, but not $GNNK^+$, shows constitutive autophosphorylation that is accompanied by a low level of association with PI 3-kinase and PLCγ [48]. When experiments with human Kit were performed in NIH3T3 cells, the $GNNK^-$ isoform shows more rapid and extensive autophosphorylation and faster internalization [49]. Moreover, the $GNNK^-$ isoform promotes more mitogen-activated protein kinase phosphorylation.

Gommerman et al. [47] showed that treatment with soluble SCF leads to an increase in [^{3}H]thymidine incorporation into DNA in 32D cells containing the wild type, the Tyr721Phe, and the Tyr730Phe mutant mouse Kit. They observed a different response when the cells were treated with membrane-anchored SCF. The Tyr730Phe mutant, in contrast to the Tyr721Phe mutant, shows only a minor increase in [^{3}H]thymidine incorporation. This observation suggests that activation of PLCγ is required for membrane anchored, but not soluble, SCF signaling.

Trieselmann and co-workers prepared mouse bone-marrow-derived mast cells. They showed that stimulation of mast cell [^{3}H]thymidine incorporation by membrane-bound, but not soluble, stem cell factor was dependent upon PLCγ activation based upon the use of the following PLCγ antagonists: U73122, neomycin sulfate, and oleic acid [50]. However, these antagonists do not significantly interfere with Kit phosphorylation or PLCγ recruitment. Treatment of mast cells with soluble SCF leads to transient Kit phosphorylation, followed by rapid internalization, endocytosis, and receptor degradation. In contrast, treatment with membrane-bound receptor leads to persistent Kit phosphorylation. The attachment of SCF to the plasma membrane of an adjacent cell apparently disallows endocytosis of the receptor and its bound ligand. Whether differences in Kit endocytosis explain fully the requirement for PLCγ recruitment for membrane-anchored SCF signaling but not for soluble SCF signaling is unclear.

Matsui et al. [51] examined soluble SCF signaling pathways in human umbilical vein endothelial cells. They found that SCF promoted survival, migration, and capillary tube formation. SCF leads to tyrosine phosphorylation of Kit, Akt, and Erk1/2, but does not lead to the tyrosine phosphorylation of PLCγ. Extracellular signal-regulated kinase (Erk) is a member of the prototypical mitogen-activated protein kinase cascade; it is a protein-serine/threonine kinase. VEGF treatment of these cells leads to the phosphorylation of KDR (a VEGF receptor), Akt, and Erk1/2. VEGF, but not SCF, promotes proliferation of these cells and PLCγ phosphorylation. STI571, an inhibitor of Kit kinase [52], blocks phosphorylation of Kit, Akt, and Erk1/2 ordinarily produced by SCF treatment. This observation is consistent with the idea that Akt and Erk1/2 activation are downstream from Kit.

Shivakrupa and Linnekin [30] studied the contribution of Lyn (a Src family kinase) in the regulation of Jnk and Erk in bone-marrow-derived mast cells prepared from wild type and Lyn-deficient mice. Jnk refers to Jun N-terminal protein kinase where Jun is a transcription factor. Jnk is a downstream member of a mitogen-activated protein kinase cascade that occupies a position comparable to that of Erk; Jnk is a protein-serine/threonine kinase. They found that SCF leads to the activation of Jnk in wild type mast cells measured in immune complex assays with GST-Jun as substrate. Both basal and SCF-treated Lyn-deficient cells exhibit less Jnk activity than the corresponding wild type cells. These findings are consistent with the notion that Jnk can be activated by Lyn-dependent and -independent pathways. However, complete SCF-mediated activation of Jnk in mouse bone-marrow-derived mast cells requires Lyn. In contrast, the activity of Erk in SCF-treated wild type and Lyn-deficient cells is the same [30]. Thus, Lyn participates in the regulation of Jnk but not Erk1/2 in mouse mast cells.

Binding of Grb2 and Grb7 to Kit

Grb2 is an adaptor protein that contains one SH2 domain between two SH3 domains. It links receptor tyrosyl kinases including the insulin and epidermal growth factor receptor to the Ras/mitogen-activated protein kinase pathway. Grb2 forms a complex with the Ras guanine nucleotide exchange factor (Ras-GEF, or Sos), and the complex binds to phosphotyrosine residues. Grb7 is an adaptor protein that contains a Ras-associating domain, a PH domain, and an SH2 domain. Grb7 binds to the phosphorylated forms of ErbB2 (epidermal growth factor receptor family), the PDGF β-receptor, Shc (an adaptor protein), and Shp2 (a phosphotyrosyl phosphatase).

Thommes et al. [53] studied the association of Grb2 and Grb7 GST fusion proteins with human Kit expressed in porcine aortic epithelial cells. The binding site for Grb2 has an asparagine two residues C-terminal to phosphotyrosine; pTyr703 and pTyr936 represent potential binding sites. Cells treated with SCF were incubated with radiophosphate and lysed. Kit was then immunoprecipitated and digested with trypsin. The digest was treated with antisera to target peptides that were then degraded using the Edman procedure and radioactivity was measured. These studies were consistent with the radiophosphate labeling of Tyr703 and Tyr936. These findings were confirmed by first immunoprecipitating Kit and then performing the phosphorylation in vitro. The authors state that the result is due to autophosphorylation of Kit. However, it is conceivable that the Kit immunoprecipitate contained other kinases that catalyze the phosphorylation of either of the target tyrosines.

Using a GST-fusion protein containing the SH2 domain of Grb2, Thömmes et al. showed that this construct binds to human Kit in SCF-stimulated cells. To demonstrate specificity, they showed that phosphopeptides corresponding to the sequence encompassing Tyr703 and Tyr936 inhibit GST-Grb2 SH2 binding to Kit, whereas the unphosphorylated peptides fail to inhibit binding. They also showed that the GST-Grb7 SH2 fusion protein binds to Kit from lysates prepared from cells treated with SCF. They found that the peptide corresponding to pTyr703 fails to inhibit the GST-Grb7 SH2 fusion protein from binding to stimulated Kit, whereas the peptide corresponding to pTyr936 inhibits binding [53]. The authors conclude that Grb7 binds to pTyr936 and not to pTyr703. These experiments suggest that Grb2 and Grb7 participate in Kit-mediated signal transduction pathways. The authors note that the binding of Grb2 to Kit may link SCF signaling to the Ras/mitogen-activated protein kinase pathway.

Binding of Dok1 to Kit

Dok represents a family of six human docking proteins that contains an N-terminal PH domain, a central phosphotyrosine-binding (PTB) domain, and a C-terminal tail containing many tyrosine phosphorylation sites. Phosphotyrosine-binding domains consist of about 100 residues that bind to phosphotyrosine and to phosphoinositides. Dok proteins function as scaffolds to organize signaling complexes. Asn-Pro-Xxx-pTyr represents a consensus Dok-binding sequence. Dok1 is phosphorylated in response to a variety of growth factors including granulocyte-macrophage colony-stimulating factor, insulin-like growth factor, PDGF, SCF, and VEGF [54]. The resulting phosphotyrosines act as docking sites for SH2-containing proteins. Dok1 is a negative regulator of cell proliferation.

Liang et al. [54] found that wortmannin (a PI 3-kinase inhibitor) blocks Dok1 phosphorylation in SCF-treated Mo7e human megakaryoblastic leukemia cells. This finding suggests that SCF-stimulated Dok1 phosphorylation is mediated by PI 3-kinase. These cells were treated with SCF; then cell lysates were immunoprecipitated with antibodies against Dok1, Lyn (a Src family kinase), or Tec. Tec is a non-receptor protein-tyrosine kinase that is highly expressed in many hematopoietic cell lines; Tec contains an N-terminal PH domain, an SH3 domain, an SH2 domain, and a C-terminal protein kinase domain. Following SCF treatment, increases in all three proteins in immunoprecipitates occur with antibody against each of them, suggesting that complexes of Dok1, Lyn, and Tec form in response to SCF. GST fusion proteins were phosphorylated with Lyn and used to identify the portion of Kit that interacts with Dok1. These experiments indicate that both the phosphorylated juxtamembrane segment and the phosphorylated C-terminal tail of Kit bind to Dok1; the non-phosphorylated fusion proteins fail to bind. When Lyn or Fyn (Src family kinases) are co-expressed in COS-1 cells expressing human Kit, SCF treatment leads to 10- to 20-fold greater Dok1 phosphorylation when compared with cells not co-expressing the kinases [54].

Liang et al. [54] propose that SCF treatment of cells leads to the activation of PI 3-kinase and the generation of phosphoinositides. These attract Dok1 via its PH domain so that Dok1 binds at or near the juxtamembrane segment and C-terminal tail of Kit; this places it near an activated Src family kinase associated with Kit. Their work indicates that Lyn is required for the association of Dok1 and Kit; they suggest that Lyn catalyzes the phosphorylation of sites on Kit that promote Dok1 binding. They also suggest that Lyn may catalyze the phosphorylation and activation of Tec, leading to the phosphorylation of Dok1. Note that additional work will be required to determine which residues in the juxtamembrane segment and C-terminal tail of Kit bind to Dok1.

Epilogue

Mice with mutations in the White locus (discovered in 1927) and in the Steel locus (discovered in 1956) exhibit a deficiency of melanocytes, a macrocytic anemia, and sterility [7,8]. No morphologic or hematologic characteristic can be used to definitely distinguish between an adult W/W^v mutant mouse and an adult Sl/Sl^d mutant mouse [9]. Physiological studies in vivo and in vitro suggested that deficits in *W* mutant mice are expressed by the affected cells and deficits in *Sl* mutant mice are expressed by auxiliary cells necessary for the normal development of affected lineages [10]. The discovery that the *W* locus is allelic with Kit, and the *Sl* locus is allelic with stem cell factor confirmed the notion that *W* encodes a receptor and *Sl* encodes the corresponding ligand [1–9].

SCF and Kit are expressed in numerous cell types during embryogenesis. The chief phenotypic changes that arise from mutations in the Steel locus or the White locus center on blood cells, gametocytes, interstitial cells of Cajal, mast cells, and melanocytes. Brain lesions were also observed in a small proportion of embryos in the initial description of the White locus [7]. Subsequent work showed that SCF and Kit are widely expressed during embryogenesis and development [10]. These observations indicate that there are redundant pathways that parallel SCF-Kit signaling; otherwise, the phenotype of *W* and *Sl* mutations would involve many more organ systems. Redundancy increases the experimental difficulty in mapping signaling pathways, but it is physiologically advantageous owing to the existence of multiple mechanisms for achieving specific endpoints.

Kit signaling is intricate. Signals are transmitted from Kit to Lyn and Fyn (Src family kinases); moreover, signaling from the Src kinases back to Kit is likely. Several adaptor proteins bind to Kit phosphotyrosines. It is plausible that Src family kinases and the adaptor proteins Grb2 and Shc participate in Ras and mitogen-activated protein kinase activation. Shp2 phosphatase binds to the same phosphotyrosine that binds to Src family kinases. It is difficult to sort out the effects related to competition of these proteins to the same Kit-binding site. One rule of signal transduction is that protein phosphorylation and dephosphorylation are exactingly regulated.

The association of Shp1 and Shp2 phosphatases near or at the pTyr568 that binds to Src family kinases (Table 2) leads conceptually to the formation of signaling complexes. There are many possible outcomes for these interactions. Shp1 and Shp2 catalyzed dephosphorylation of Kit inhibits SCF action. However, Shp1 and Shp2 catalyze the dephosphorylation of

inhibitory phosphotyrosines of the Src family kinases [26]; such dephosphorylation promotes SCF functioning in those situations where Src family kinases are activated by Kit. Moreover, the localization of Shp1 and Shp2 near the Src family kinase phosphorylated products has the potential to reverse Src action. The binding of Chk at or near the Src-kinase-binding residue can lead to the inhibition of Src kinase activity by Chk-mediated phosphorylation thereby inhibiting SCF action.

When the actions of the two isoforms of SCF are compared, differences in signaling by the soluble and membrane-anchored products are observed [50]. Membrane-anchored SCF, but not soluble SCF, requires the action of PLCγ in order to enhance [^{3}H]thymidine incorporation in mouse bone-marrow-derived mast cells [50]. Furthermore, Akt phosphorylation is impaired following treatment by membrane anchored but not soluble SCF. The explanation for these differences in the mechanism of action of the two isoforms is unclear and warrants further investigation. Most experimental studies are performed using the recombinant soluble isoform because it is more difficult to perform co-culture experiments with the membrane-anchored SCF in one cell type stimulating the Kit receptor in another cell type.

Membrane-anchored SCF participates in short-range signaling transmitted by cell–cell contacts whereas soluble SCF participates in longer-range signaling transmitted by diffusion through the extracellular medium. It is unclear whether the membrane-anchored isoform 1 functions as a signaling molecule or whether it must be cleaved to yield the soluble SCF to exhibit physiological activity in vivo. However, it is apparent from cell culture experiments that membrane-anchored and soluble SCF are active [50]. As a result of alternative splicing, isoform 2 of SCF lacks the preferred matrix metalloprotease-9 cleavage site and is chiefly membrane anchored. In mice, but not humans, isoform 2 can be cleaved to generate a second soluble SCF [55]. In 40-day-old mice, isoform 1 is the predominant transcript in bone marrow, brain, and thymus. Both isoform 1 and isoform 2 transcripts are observed in heart, kidney, lung, placenta, spleen, and testis [56].

Mice homozygous with the Steel–Dickie allele (Sl^d/Sl^d) are severely anemic, infertile, and non-pigmented. This allele encodes only a soluble SCF that is derived from a precursor lacking the transmembrane and intracellular domains [57]; this mutant thus lacks the precursors corresponding to isoforms 1 and 2. This severe but viable phenotype indicates that soluble SCF cannot substitute for membrane-anchored SCF. Note, however, that this soluble SCF is produced by exocytosis and not by liberation from a membrane-anchored form. The aberrant process of SCF production in Steel–Dickie homozygous mice, which bypasses the normal mechanisms of regulation, may add to the severity of the phenotype.

It is uncertain, moreover, whether membrane-anchored SCF is able to compensate for a deficiency of the soluble form. Besmer and co-workers addressed this important question. They generated a mouse mutant lacking exon 6 of SCF so that only isoform 2 is expressed [58]. However, the blood plasma of these mice contained 69% of the concentration of immunoreactive SCF when compared with wild type mice. The latter result is consistent with the observation that mouse membrane-anchored isoform 2 of SCF is liberated by using an alternative proteolytic cleavage site to yield a soluble factor [55]. Although the homozygous mutant mice exhibited normal fertility, hematopoiesis, and pigmentation, the quantity of mast cells in the peritoneum and skin was reduced by more than 90% [58]. Despite the generation of a soluble SCF in these mice, exclusive expression of membrane-anchored SCF isoform 2 was unable to substitute for the expression of both isoforms. Although it is reported that membrane-associated SCF is the relevant physiological form [57,59], it may be that all forms are required to support a normal state.

The differentiation of hematopoietic stem cells depends upon signaling by numerous pathways including SCF, various cytokines, and several growth factors. Numerous studies indicate that such signaling is greater than additive [18,59]. For example, erythropoietin, granulocyte-macrophage colony-stimulating factor, interleukin 3, and interleukin 7 function synergistically with SCF. Considerable effort has been expended to determine the mechanisms responsible for this enhanced response. These studies have focused on receptor–receptor interaction and on downstream signaling events not included in this survey such as (a) the Janus kinase/signal transducers and activators of transcription (Jak/STAT) pathways and (b) the suppressors of cytokine signaling (SOCS) pathways.

The observation that mouse embryonic stem cells express Kit transcripts and protein promises to increase interest in Kit signaling during the earliest stages of development. Helgason and co-workers [17] suggest that Kit represents a candidate marker for embryonic stem cells owing to its good correlation with functional measures of pluripotency. At least one study indicates that Kit transcript expression is greater in human embryonic stem cell lines when compared with differentiated cells [60]. The role of SCF and Kit in embryogenesis and development continues to be an area of active and fruitful research.

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