I. INTRODUCTION

The v-kit oncogene was identified in 1986 as transforming gene in the Hardy-Zuckerman 4 feline sarcoma virus (25), and the cellular counterpart c-Kit soon thereafter (306). c-Kit was found to be encoded by the W locus in which there are more than 30 known mutations (reviewed in Ref. 164). The ligand for c-Kit, SCF, was found to be the product of the Sl locus and also in this locus are there naturally occurring mutations (reviewed in Ref. 164). The analysis of mice with mutations in the W or Sl loci has given much information about the in vivo function of c-Kit, highlighting its importance in hematopoiesis, pigmentation, and fertility.
II. STEM CELL FACTOR

A. Gene and Protein Structure

Stem cell factor (SCF, also called Steel factor or Kit ligand) is a growth factor that exists both as a membrane-bound and soluble form. It is expressed by fibroblasts and endothelial cells throughout the body, promoting proliferation, migration, survival, and differentiation of hematopoietic progenitors, melanocytes, and germ cells. It maps to chromosome 10 in the mouse and to chromosome 12 in humans (232). In both human, mouse, and rat, SCF is encoded by nine exons (183).

Whether membrane-bound or soluble SCF will be produced is regulated both at the mRNA level and at the protein level. SCF exists as two alternative splice forms, one soluble isoform and one membrane-bound isoform (FIGURE 1; Ref. 6). They differ only in exon 6. Both isoforms encode membrane-bound SCF, containing an extracellular domain, a transmembrane domain, and an intracellular region. The longer splice form is rapidly cleaved to generate a 165-amino acid soluble SCF. The shorter transcript, lacking exon 6, remains membrane-bound. Both membrane-bound and soluble SCF bind to c-Kit and activate its intrinsic tyrosine kinase activity, but with both qualitative and quantitative differences in how they signal. The proteases that have been suggested to be responsible for cleavage of membrane-bound SCF include matrix metalloprotease-9 (102), chymase-1 (173), as well as several members of the ADAMs family including ADAM17 and ADAM33 (317). Interestingly, ADAM19 was found to be a negative regulator of SCF shedding (51). In total, six alternative transcripts of SCF have been found in humans, out of which four encode protein (http://www.ensembl.org). In the mouse, only four transcripts have been found, which all code for protein. However, the two above-mentioned alternative transcripts are the ones mainly expressed, and most literature is dealing with those transcripts.

![FIGURE 1](http://physrev.physiology.org/). Schematic representation of stem cell factor (SCF) splice forms and protein processing. A: SCF protein is produced as two transmembrane forms due to alternative splicing of exon 6, SCF220, and SCF248. In SCF248, exon 6 is kept and encodes a proteolytic cleavage site, generating the soluble SCF165. B: SCF220, lacking the cleavage site, forms membrane-bound SCF dimers (mSCF), and SCF248 is processed to SCF165 that forms soluble SCF (sSCF). Dashed lines indicate that the SCF dimers are held together by noncovalent interactions.
The regulation of SCF expression on the gene level is very complex. Bedell et al. (17) described the gene structure of SCF. The SCF encoding mRNA consists of a short 5’ untranslated region, a 0.8-kb open reading frame, and a long 3’ untranslated region. In the 5’ region, there are three ATG motifs where the third serves translation initiation site. Twenty-eight bases upstream of the transcription initiation sites resides a TATA box consensus sequence (TATAAA) and three overlapping GCCGGG motifs. These are binding sites for the transcription factors TFIID and SP1, respectively. It has been reported that high mobility group A1α upregulates the SCF promoter in, e.g., MCF-7 breast cancer cells and in OCC1 ovarian cancer cells (277). Furthermore, HIF-1α upregulates the expression of SCF in response to hypoxia as well as to growth factor receptor activation (93, 152). Since ligand stimulation of c-Kit is also known to stabilize and upregulate the expression of HIF-1α (218), a positive feedback loop can be suggested, which could contribute to angiogenesis in tumors even in the absence of hypoxia. The POU-homeodomain transcription factor POU3F2 that is expressed both in neurons and in melanoma cells regulates the SCF promoter via clusters of four closely spaced binding sites located in the proximal promoter (139). UVB light is also known to induce expression of SCF in human epidermis both on the mRNA level and as soluble as well as membrane-bound SCF (12, 90). The mechanism of induction of SCF gene expression by UVB is, however, unknown. In Sertoli cells, SCF expression is upregulated by treatment with follicle stimulating hormone (FSH). It is known that this effect is mediated by increase in cAMP (272).

Soluble SCF exists in homodimeric conformation as two monomers interacting head-to-head to form a slightly bent, elongated dimer. Dimerization of SCF is a dynamic process that might play a regulatory role in the dimerization and activation of c-Kit (315). Stem cell factor expressed in mammalian cells is N-glycosylated and a majority of sugar is attached to Asn120, while Asn65 and Asn93 seem to be glycosylated at lower stoichiometry (315). SCF expressed in Chinese hamster ovary (CHO) cells contains ~30% carbohydrate, which is both of the N-linked and the O-linked type. However, the presence or absence of carbohydrates in SCF does not seem to influence its biological activity (9).

Early studies on developing mouse embryos demonstrated widespread expression of SCF mRNA, many times coinciding with c-Kit mRNA expression (132).

### III. STEM CELL FACTOR RECEPTOR, c-Kit

A. Gene Structure and Regulation of c-Kit mRNA Expression

The viral oncogene v-c-Kit was in 1986 identified as the transforming gene of Hardy-Zuckerman 4 feline sarcoma virus (hence its name c-Kit as in kitten) (25). Shortly thereafter, the cellular homolog, c-Kit, was cloned and sequenced (306). Within a few years time, it was discovered that c-Kit is allelic with the dominant white spotting locus (W) of mice (47, 82). Mutations in the so-called Steel (Sl) locus in mice give rise to a phenotype very much resembling the phenotype of mice with loss-of-function mutations in the W locus, and it was soon demonstrated that the product of the Steel locus was identical to the ligand for c-Kit, stem cell factor (SCF) (56, 296). For a review on the W and Sl locus mutations, see Reference 164.

The gene for c-Kit was cloned and found to be located on chromosome segment 4q11 in humans (59, 306) and is comprised of 21 exons, spanning more than 34 kb of DNA. The first exon encodes the translational initiation codon and the signal peptide. The remainder of the extracellular part of c-Kit is encoded by exons 2–9. The transmembrane region is encoded by exon 10, while the remaining exons encode the intracellular part of the receptor.

The promoter region of murine and human c-Kit has been thoroughly investigated (303, 307). A major transcription initiation site was found 58 bp upstream of the translation initiation codon. Deletion studies of the 5’ flanking region led to the identification of three short regulatory regions. This region does not include CCAAT or TATA boxes, but consensus binding sites for AP-2, basic helix-loop-helix proteins, Sp1, Ets, and Myb. In a more recent study of the human c-Kit promoter (227), a region of ~139 nucleotides upstream from the translation initiation site was demonstrated to be critical for promoter activity. This sequence was found to contain functional binding sites for the transcription factors Myb and Ets-2, which serve as regulators of c-Kit expression in hematopoietic cells. Park et al. (212) demonstrated the importance of Sp1 for maximal activity of the c-Kit promoter. The c-Kit promoter contains three functional AP-2 binding sites (112), and loss of c-Kit expression in malignant melanomas occurs due to loss of AP-2 expression. Finally, the basic helix-loop-helix transcription factor microphthalmia associated transcription factor (MITF) is an important regulator of c-Kit expression in mast cells (279) and melanocytes (209). MITF binds to a CACCTG motif in the c-Kit promoter. Interestingly, recent data suggest that there is also an inverse regulation, in that c-Kit signaling regulates MITF expression through miRNAs miR-539 and miR-381 (157).

Apart from regulation of expression levels by transcription factors, c-Kit has also been reported to be regulated miRNA [miR-193b in leukemic cells (80) and mir-221 in melanoma cells (117)], miR-221 and miR-222 have been described as regulators of c-Kit expression in hematopoietic cells and have also been reported to be potential regulators of c-Kit expression in gastrointestinal stromal tumors (73, 140).
B. The c-Kit Protein Structure

The c-Kit receptor is a type III receptor tyrosine kinase and is closely related to the platelet-derived growth factor receptors, the macrophage colony stimulating factor receptor, and Flt3. The class III receptor tyrosine kinases are characterized by the presence of five immunoglobulin-like domains and the presence of a kinase insert sequence of 70–100 amino acids that resides in the middle of the kinase domain (FIGURE 2). In the case of c-Kit, the kinase insert region is ~80 amino acids long. The receptor tyrosine kinase encoded by the KIT gene is a transmembrane protein with an extracellular domain comprised of five immunoglobulin-like domains followed by a single spanning transmembrane region. The intracellular part of c-Kit starts with the juxtamembrane region, a region of great importance for regulation of c-Kit kinase activity (see below). The kinase domain is comprised of two subdomains, tyrosine kinase domain 1 and 2, which is interrupted by a kinase insert sequence. Finally, the COOH-terminal tail ends the protein. Most of the phosphorylation sites that occur upon ligand-stimulated activation of c-Kit reside either in the juxtamembrane region, the kinase insert, or the COOH-terminal tail.

C. Alternative Splicing of the c-Kit

Alternative splicing of mRNA that encodes c-Kit leads to the occurrence of at least four isoforms of c-Kit in humans and two in mice (FIGURE 2). Two of these isoforms, in both human and mice, differ from each other by the presence or absence of a tetrapeptide sequence (GNNK) in the extracellular juxtamembrane region of the encoded protein (58, 230, 316). The alternative splicing occurs as a result of alternative usage of the 5' splice donor sites (98). Additional splice variants exist that differ by the presence or absence of a single serine residue in the kinase insert region of c-Kit. In this case, the altered splicing occurs as a result of alternative splice acceptor usage (58). Postmeiotic germ cells express a shorter transcript of c-Kit that encodes a truncated version of c-Kit (tr-Kit). This form contains only the second part of the kinase domain and the COOH-terminal tail of c-Kit. This results in a receptor without kinase activity (234). Despite that, tr-Kit is able to signal presumably by acting as a scaffolding protein. Microinjection of tr-Kit into mouse eggs was shown to trigger activation of the Src family kinase Fyn, which in turn led to the phosphorylation and activation of phospholipase C-γ, and their association with the adapter protein Sam68 (214). These signaling events triggered the metaphase-to-anaphase transition of the egg.

In malignant cells of various types, expression of the alternative splice forms of c-Kit could be different from its normal counterpart. The above-mentioned tr-Kit is frequently expressed in advanced stages of prostate carcinoma where it drives Src family kinase activation (213).

The most well-studied pair of alternative splice forms of c-Kit are the two GNNK+ and GNNK− isoforms (also denoted c-Kit and c-KitA, respectively). They are coexpressed in most tissues, but the GNNK− isoform usually predominates (58, 230, 316). Whether the expression of either isoform is connected to cancer is somewhat debated. While one study suggest that coexpression of GNNK− and GNNK+ correlates with worse prognosis in acute myeloid leukemia (89), other studies have suggested that there is no correlation between the expression of either isoform and the response to therapy or other clinical parameter (221). In mast cells from patients suffering from systemic mast
cell disease expression of GNNK− dominated over the GNNK+ isoform, compared with mast cells from healthy volunteers (195). However, most of those studies were performed on a rather small series of patients, and thus larger scale studies are needed to firmly establish these findings. In testicular germ cell tumors, a predominance of the GNNK− was seen in all cases examined (236).

Despite these findings, using NIH3T3 cells as a model system, the expression of either isoform was shown to possess differences in their transforming ability (46). The GNNK− form induced loss of contact inhibition, anchorage-independent growth and tumorigenic in mice, while the GNNK+ did not. The affinity for ligand is identical between the two isoforms, so differences must have another explanation. Upon ligand stimulation, the GNNK− isoform was more rapidly and strongly phosphorylated, and it was more rapidly internalized and degraded. Furthermore, activation of the extracellular regulated kinase (ERK) was stronger in the GNNK− expressing cells, compared with cells expressing the GNNK+ isoform, while the phosphorylation of Akt was of the same intensity in cells expressing either splice form. This effect seems to be cell dependent. Using the proB cell line Ba/F3, which differs from fibroblasts in that it expresses higher levels of the adapter protein GAB2, a stronger Akt phosphorylation was induced following SCF stimulation in cells expressing the GNNK− splice form, as opposed to cells expressing the GNNK+ (262). It turned out that the effect is mediated through stronger Src activation by the GNNK− isoform, leading to elevated phosphorylation of GAB2 and subsequently stronger recruitment of PI3-kinase to GAB2 and stronger Akt phosphorylation.

D. Posttranslational Processing

c-Kit is a protein consisting of 976 amino acids, making the core protein size ~110 kDa. Heterogeneous N-linked glycosylation results in a mature protein of between 145 and 160 kDa (226, 306). There are up to nine N-glycosylation sites, most of which are concentrated in extracellular domain closest to the plasma membrane. Additional known posttranslational modification of c-Kit include phosphorylation on both tyrosine residues and serine residues, both constitutive as well as ligand-induced serine phosphorylation (29) that in some cases have been shown to function in fine tuning of receptor responses. Finally, ligand-induced ubiquitination of c-Kit is known to regulate both internalization and degradation of c-Kit (184, 260, 312).

E. Expression Pattern

The expression pattern of c-Kit and SCF during mouse embryogenesis suggests that they are involved in migration of cells of the hematopoietic, germ cell, and melanoblast lineages, as well as in the differentiation and proliferation of these cells (132, 186, 210). It should be noted that the expression pattern of c-Kit and SCF does not completely overlap with the phenotypic effects of loss-of-function mutations of either c-Kit or SCF. Thus expression data also suggest that c-Kit signaling may have important roles in the nervous system, placenta, heart, lung, and midgestational kidney. Using a transgenic mice expressing LacZ at the KIT locus, Bernex et al. (24) could study both the importance of c-Kit for the development of various embryonal structures, but also where c-Kit is normally expressed during embryogenesis. With the use of WlacZ/+ and WlacZ/WlacZ embryos, it was possible to distinguish between tissues that are dependent or independent on c-Kit expression for their development. Interstitial cells of Cajal express c-Kit during embryogenesis but are not dependent on c-Kit for their function during embryogenesis. C-Kit expression was also found at places, such as endothelial, epithelial, and endocrine cells, that were not expected based on the phenotype of c-Kit knockout animals.

F. Receptor Activation

Activation of growth factor receptors universally requires dimerization or oligomerization of monomeric receptor molecules (159). In some cases the dimer is formed independently of ligand (e.g., insulin- and insulin-like growth factor receptors), but most frequently dimerization is provoked by ligand binding.

The kinetics of c-Kit activation is rapid, and dimers can be detected already within minutes after addition of ligand (39). During the last 10 years there has been a substantial increase in structural data that in combination with biochemical studies provides a detailed description of the c-Kit activation process which is summarized in FIGURE 3. Early biochemical investigations argued that SCF is a homodimer, and c-Kit dimerization is driven by its ability to simultaneously interact with two c-Kit monomers (158). Furthermore, it was proposed that only the first three Ig-like domains were required for SCF binding. These interpretations of biochemical data have since been confirmed by structural studies on SCF (315) and the complex between SCF and c-Kit (310). The first three Ig-like domains in c-Kit have a complementary shape and charge to allow tight binding of SCF, and after binding to each other, no major structural changes occur (310). Furthermore, in addition to bringing two c-Kit monomers together into a dimeric complex, ligand binding also induced a conformational change that enabled homotypic interactions between Ig-like domains 4 and 5 in two adjacent c-Kit molecules (310). Mutation of key residues involved in Ig-like domain 4-mediated receptor-receptor interaction dramatically reduced tyrosine phosphorylation but did not influence the SCF-induced dimerization of c-Kit. A consequence of the SCF-induced conformational change of the c-Kit extracellular domain is
that the transmembrane regions of c-Kit move into close proximity of each other (15 Å); this potentially allows protein-protein interactions between the transmembrane regions as well as positioning the intracellular tyrosine kinase domains close to each other to facilitate their activation and subsequent transphosphorylation. The latter possibility is consistent with the observation of reduced kinase activity after mutation of residues providing binding between Ig-like domain 4 (310). The idea of stabilizing interactions between two transmembrane regions is supported by data from the platelet-derived growth factor (PDGF) receptor in which the transmembrane regions display a strong affinity for each other potentially by forming a leucine-zipper-like structure (205). Since c-Kit and PDGF receptors are closely related, it is likely that a similar situation also occurs for c-Kit. In addition, homotypic interactions between Ig-like domains 4 and 5 in two proximal c-Kit molecules may also influence the relative positions of the intracellular kinase domains within the dimer in a fashion optimal for transphosphorylation. In fact, such coupling between the transmembrane- and intracellular kinase domains had been proposed earlier for the to c-Kit closely related PDGF receptor. Using a simplified transmembrane domain in which a dimerization motif had been introduced and scanned through the transmembrane domain in a manner that allowed controlled rotation of the kinase domain relative the transmembrane domain of PDGF receptor, Donoghue and co-workers (19) showed that the orientation of the two intracellular kinase domains in the receptor dimer influences receptor activation.

The structure of the c-Kit kinase domain had been solved for both the inactive and active state (193, 194). Overall, the kinase domain of c-Kit conforms to the general kinase fold with an NH₂-terminal lobe and COOH-terminal lobe with the active site located in their interface. Biochemical
studies using a synthetic peptide derived from the amino acid sequence of the c-Kit juxtamembrane domain indicated that this region can interact with the c-Kit kinase domain and by doing so suppresses its kinase activity (48). The concept of an inhibitory juxtamembrane domain in c-Kit kinase was confirmed by the structure of the inactive state of the kinase domain (193). It was found that the juxtamembrane domain was positioned between NH₃- and COOH-terminal lobes of the kinase domain and as a consequence of this affects the regulatory control (αC)-helix, phosphate binding loop (P-loop), and the DFG motif containing activation loop in a manner that is not compatible with enzymatic activity (193). Furthermore, in the inactive conformation, tyrosine residue 823 (Tyr-823) in the activation loop interacts with active site aspartic acid residue 792 suggestive of a pseudo-substrate function. The juxtamembrane domain contains two tyrosine residues, Tyr-568 and Tyr-570, which become tyrosine phosphorylated after SCF treatment, and this releases the juxtamembrane domain from its inhibitory configuration and allows the kinase domain to adopt a configuration that enables catalytic function (193, 194). It is likely that the change in structure of the juxtamembrane domain is the major regulatory mechanism, since Tyr-823 in the activation loop was not phosphorylated in the active kinase structure (194) and furthermore mutation of Tyr-823 to phenylalanine did not affect c-Kit kinase activity (65). The function of Tyr-823 is not clear, and it is possible that it interacts with signaling molecules, but no such interaction partners have yet been identified. Another possibility is that phosphorylation of Tyr-823 though not necessary for activation may contribute to stabilization of the active kinase conformation. Typically receptor tyrosine kinases have tyrosine residues located within the activation loop segment, but their role in kinase activation is not conserved; the activation loop tyrosine is not necessary for EGF receptor activation (87), whereas activation loop phosphorylation is essential for insulin receptor activation (114). As expected from the critical role of the juxtamembrane in regulating kinase activity, the two tyrosine residues (Tyr-568 and Tyr-570) in this region are the first to be phosphorylated in the activation process (65, 194). DiNitto et al. (65) performed phosphopeptide mapping of the autoactivated c-Kit kinase domain and found that phosphorylation proceeded in an orderly manner with the juxtamembrane being phosphorylated first followed by kinase insert and the activation loop was not phosphorylated until the end of the activation process. Eight in vivo tyrosine phosphorylation sites have been identified in c-Kit (Tyr-568, -570, -703, -721, -730, -823, -900 and -936) (161) and two additional sites found on in vitro activated kinase domain (Tyr-547 and -553) (65). Out of the in vivo identified phosphorylated tyrosine residues, seven, including those in the juxtamembrane region involved in regulation of kinase activity, act as docking sites for signaling molecules with Src homology 2 (SH2) domains, which in turn initiate intracellular signal transduction pathways. However, as mentioned above, Tyr-823 in the activation loop has no known interaction partner.

In summary, c-Kit adopts a canonical kinase structure with two lobes with the ATP-binding and active site in their interface. In the inactive state, the juxtamembrane region forms a hairpin loop that inserts into the active site and disrupts the regulatory αC-helix and ATP-binding P-loop, thereby suppressing kinase activity. c-Kit dimerization process is driven by ligand-induced dimerization that is further stabilized by interactions between Ig-like domains 4 and 5 in two proximal receptor molecules and possibly interactions between the transmembrane regions. The proximity of two kinase domains within the dimer enables trans-phosphorylation to occur. Initially, this occurs in the juxtamembrane region, thereby relieving the autoinhibitory conformation this domain adopts in the inactive state. Next, autophosphorylation proceeds on other tyrosine residue in an orderly manner. The role of the activation loop tyrosine in c-Kit is not clear; however, it is not necessary for activation of the kinase domain but it may contribute to stabilization of the active conformation.

G. Downregulation Process

Attenuation of c-Kit signaling is important to obtain a suitable intensity and duration of signal transduction to meet the biological needs. There are at least three levels of c-Kit downregulation that function in concert: 1) removal from the cell surface and intracellular degradation, 2) inactivation of the kinase domain by serine phosphorylation, and 3) tyrosine dephosphorylation.

Attachment of single ubiquitin moieties (monoubiquitination) onto lysine residues in cell surface receptors has been associated with their internalization through clathrin-coated pits (91). In regard to receptor tyrosine kinases, the E3 ubiquitin ligase c-Cbl seems particularly important for proper ubiquitination, although it is likely that also other ubiquitin ligases contribute depending on cell type and the specific receptor involved. It has been found that c-Cbl can associate directly with SCF-activated c-Kit via phosphorylated tyrosine residues 568 and 936 (184) and in addition indirectly through Grb2 to tyrosine residues 703 and 936 (260). Interestingly, the adaptor protein APS interacts with c-Cbl and has been shown to interact with tyrosine residues 568 and 936 in c-Kit, highlighting the importance of these regions in c-Kit for the interaction with c-Cbl (298). Moreover, there are also studies describing an interaction between c-Kit and c-Cbl mediated by p85 subunit of phosphatidylinositol 3-kinase (PI3-kinase) or the adaptor protein Crkl. (241, 297). Thus c-Cbl can be recruited to c-Kit both directly and indirectly, and this may have functional consequences as has been shown for the EGFR where a direct interaction with c-Cbl is necessary for its degradation (88). To perform ubiquitination of c-Kit, c-Cbl has to be phos-
IV. SIGNALING DOWNSTREAM OF c-KIT

In this section we review the vast amount of information available on signal transduction downstream of c-Kit (FIGURE 4), with emphasis on the well-established signaling routes PI3-kinase, Src family kinases, mitogen-activated protein kinase pathways, and phospholipases. However, it is important to note that these pathways do not operate in isolation and that they are integrated into a signaling circuit, although for clarity they are described as separate pathways below. Furthermore, the information obtained is derived from different kinds of experiment such as cell lines with endogenous or transfected components, cells in different differentiation states, cells from different species, as well as genetically modified animals or cells derived from them. All these parameters may influence the results and should be kept in mind when interpreting the data.

A. Phosphatidylinositol 3′-Kinase Signaling

There are multiple classes of phosphatidylinositol 3′-kinases (PI3′-kinases); however, growth factor receptors predominantly activate class Iα, and subsequent discussion will be limited to this group. Autophosphorylated receptors interact with the Src homology 2 (SH2) domains of the p85 subunit (p85α, p50α, p55α, p85β, and p55γ), resulting in a conformation change in the associated enzymatic p110 subunit (p110α, p110β, and p110δ), leading to its activation (138). Furthermore, the translocation from the cytoplasm to the activated receptor complex at the plasma membrane positions PI3′-kinase in close proximity of its lipid substrates, one important example being phosphatidylinositol 4,5-bisphosphate (PIP2), which is converted to the second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3). An important function of PIP3 is to allow membrane docking of proteins containing pleckstrin homology (PH) domains, such as the serine/threonine kinase Akt (also denoted PKB). Akt is a key molecule downstream of PI3′-kinase that promotes cell survival by interfering with the initiation of apoptosis (60). Interestingly, there appear to be more p85 in a cell than p110 (282), and p85 has been demonstrated to interact with the adaptor protein CrkL as well as the ubiquitin ligase c-Cbl (96, 241). This indicates that p85 recruitment to phosphorylated tyrosine residues may have functions besides regulating p110 location and activity, although this remains to be firmly established.

PI3′-kinase is activated by SCF both through direct binding to Tyr-721 in c-Kit (165, 247) and indirectly through binding to the tyrosine phosphorylated adaptor protein GAB2 (202, 309). GAB2 becomes tyrosine phosphorylated through Grb2-dependent recruitment to the receptor followed by Src-mediated phosphorylation (262, 309). The contribution of GAB2 to SCF-induced PI3′-kinase activation allows for increased complexity, since the expression of GAB2 may differ between cell types and the c-Kit splice forms GNNK− and GNNK+ differentially phosphorylate GAB2 (262). In the hematopoietic cell line Ba/F3, GNNK− provoked a more efficient GAB2 phosphorylation that correlated with stronger Akt phosphorylation compared with cells expressing GNNK+ isoform. In contrast, in transfected NIH3T3 fibroblasts, both c-Kit isoforms were equally potent in promoting Akt phosphorylation (262, 287).

The serine/threonine kinase Akt is located downstream of PI3′-kinase and is a molecule in survival signaling in response to SCF. Akt was initially discovered as a viral oncogene (258), and subsequent work has shown that there are

phosphorylated by Src family kinases (SFK) and indeed interfering with SFK activity inhibits c-Kit internalization (40, 121, 184, 287, 312). In line with this, it has been observed that a c-Kit mutant devoid of kinase activity did not efficiently internalize (308), likely due to the inability of the unphosphorylated receptor to recruit and promote c-Cbl phosphorylation. Furthermore, overexpression of mutant c-Cbl resulted in inhibition of c-Kit ubiquitination and internalization and led to a prolonged signaling and a hyperproliferative phenotype (13). Recently, another E3 ubiquitin ligase complex containing SOCS6 has been shown to interact with tyrosine residue 568 in c-Kit and to be involved in its downregulation (16, 311). Once internalized, the activated c-Kit is targeted for proteolytic degradation that occurs both in lysosomes and proteasomes (192, 312). In addition, it is clear that internalization is not only a way to remove activated receptors but is also important for proper signaling, since certain complexes are assembled and activated at different intracellular locations (109). Following downregulation, c-Kit repopulates the cell’s surface through synthesis of new protein (253).

Negative feedback regulation is common in most signal transduction pathways and can be found also at the level of c-Kit kinase activity. A consequence of c-Kit activation is increased PKC activity, which has been shown to negatively regulate c-Kit kinase activity by phosphorylating serine residues 741 and 746 in the kinase insert region (16, 311). Once internalized, the activated c-Kit is targeted for proteolytic degradation that occurs both in lysosomes and proteasomes (192, 312). In addition, it is clear that internalization is not only a way to remove activated receptors but is also important for proper signaling, since certain complexes are assembled and activated at different intracellular locations (109). Following downregulation, c-Kit can be released from the cell surface in response to PKC stimulation (308).

The tyrosine phosphorylation of most receptor tyrosine kinases is suppressed by protein tyrosine phosphatases, which can be seen by treatment of cells with phosphatase inhibitors even in the absence of ligand. In this regard, only SHP1 tors which often lead to increased receptor phosphorylation even in the absence of ligand. In this regard, only SHP1 can be seen by treatment of cells with phosphatase inhibitors are suppressed by protein tyrosine phosphatases, which often lead to increased receptor phosphorylation even in the absence of ligand. In this regard, only SHP1 can be seen by treatment of cells with phosphatase inhibitors (144, 216). Moreover, the extracellular domain of c-Kit can be released from the cell surface in response to PKC stimulation (308).

The tyrosine phosphorylation of most receptor tyrosine kinases is suppressed by protein tyrosine phosphatases, which can be seen by treatment of cells with phosphatase inhibitors even in the absence of ligand. In this regard, only SHP1 can be seen by treatment of cells with phosphatase inhibitors (144, 216). Furthermore, the extracellular domain of c-Kit can be released from the cell surface in response to PKC stimulation (308).
three human Akt isoforms with 80% sequence homology between each other (36). Akt contains an NH2-terminal pleckstrin homology (PH) domain that interacts with the PI3'-kinase product PIP3, a serine/threonine kinase domain, and a COOH-terminal regulatory domain. PI3'-dependent colocalization of Akt and its upstream activator PDK-1 leads to activation through PDK-1-mediated phosphorylation of Thr-308 in the Akt activation loop (5). However, full Akt activation also requires mTor complex 2 (mTORC2)-mediated Ser-473 phosphorylation in the regulatory COOH-terminal domain (240). Akt is inactivated through dephosphorylation by phosphatases such as PH-domain leucine-rich repeat protein phosphatase 1 (PHLPP1) and PHLPP2 (37, 79). Activated Akt promotes cell survival in different ways including phosphorylation of Bad, Foxo, and activation of nuclear factor kappa-light chain enhancer of activated B cells (NF-κB). Bad is a protein involved in the control of cytochrome c release from the mitochondria, an initial event in the activation of the caspase cascade. In the absence of survival signals, Bad heterodimerizes and thereby neutralizes the anti-apoptotic proteins Bcl-XL or Bcl-2 (305). In response to SCF treatment, Akt is activated in a PI3'-kinase-dependent manner resulting in Bad phosphorylation on Ser-136 (27), which disrupt the interaction between Bad and Bcl-XL, and Bad is sequestered by 14–3-3 proteins (313). Bcl-XL can then antagonize the pro-apoptotic Bax protein in a manner blocking cytochrome c release and consequently apoptosis. In U2-OS cells transfected with a mutant c-Kit unable to interact with PI3'-kinase (Tyr721 to Phe mutation), SCF could only partially protect from starvation-induced apoptosis compared with the wild-type receptor (27). Moreover, in mast cells and cortical neurons, SCF promoted an increase in Bcl-2 and Bcl-XL protein levels, which at least in cortical neurons was dependent on Akt and ERK signaling, and this will further protect the cell from apoptosis (63, 189). Furthermore, the increase in Bcl-2 and Bcl-XL expression was associated with activation of NF-κB transcription factor. NF-κB contains two subunits, p50 and p65, and is kept in the cytoplasm through interaction with IkB; phosphorylation of IkB results in its proteasomal degradation and thereby release of NF-κB which then enters the nucleus and drives gene expression (283). Consistent with NF-κB activation, Dhandapani et al. (63) observed SCF-
induced IkBα phosphorylation. Akt also promotes increased cell viability by phosphorylating and thereby negatively regulating forkhead transcription factors (42). In absence of phosphorylation, the forkhead transcription factors (FoxO1, FoxO3, and FoxO4) bind to DNA and promote expression of pro-apoptotic genes, while after phosphorylation they translocate to and are retained in the cytoplasm. It has been demonstrated that Akt-mediated phosphorylation of FoxO3 is important for SCF’s ability to promote survival of hematopoietic progenitor cells (71). Moreover, expression of a mutant FoxO3 that cannot be phosphorylated by Akt promoted apoptosis even in the presence of SCF, suggesting that in these cells Akt-mediated FoxO3 phosphorylation is more important than that of Bad in maintaining cell survival. Similar results have been observed in mast cells, in which SCF has an important survival function, and in this case it was found that Akt-mediated FoxoO3a phosphorylation reduced the expression of the pro-apoptotic Bcl-2 family member Bim (200). Furthermore, existing Bim proteins are phosphorylated in response to SCF treatment in a fashion sensitive to both PI3’-kinase and Mek/ERK inhibition. Phosphorylation of Bim has been connected to its proteosomal degradation (166, 175).

The serine/threonine kinase mammalian target of rapamycin (mTor) is the catalytic subunit in two distinct multiprotein complexes, mTor complex 1 (mTORC1; mTor, Raptor, GbL, PRAS40, and Depotor) and complex 2 (mTORC2; mTor, Rictor, mSin1, Protor-1, mLST8, and Depotor) (153). mTORC1 is located downstream of Akt and mTORC2 upstream. mTORC1 phosphorylates and activates S6-kinase, which in turn promotes translation by phosphorylating elongation factors and ribosomal subunits. SCF has been demonstrated to activate mTor and S6-kinase in a PI3’-kinase-dependent manner (74, 133). Functionally, inhibition of mTor by rapamycin resulted in inhibition of SCF-induced proliferation and cyclin D3 expression in spermatogonia cell cultures (74) and in mast cells inhibition of cytokine production, chemotaxis, and survival (133). It has also been found that melanocyte proliferation and migration in response to SCF stimulation was dependent on PI3-kinase (125). In this study it was found that ERM proteins, which link the actin cytoskeleton to the plasma membrane, were threonine phosphorylated in a PI3-kinase-dependent manner and in addition that PI3-kinase was involved in Rac1 activation. Many signaling pathways are dependent on the production of reactive oxygen species (ROS), which will cause a transient inactivation of phosphatases and allow for efficient signal transduction. Treatment of megakaryocytic cell line Mo7e cells with SCF results in an increased ROS production, which in these cells is associated with increased glucose uptake (76, 242). The molecular mechanism by which c-Kit promotes ROS production is not clear, but for the structurally related PDGF receptor, this has been shown to involve a PI3’-kinase-dependent translocation of cytosolic NAD(P)H oxidase subunits to the plasma membrane (44).

Activation of c-Kit promotes activation of the Tec family members Btk and Tec in a PI3’-kinase-dependent manner. Tec and Btk contain a PH domain and can therefore be translocated to the plasma membrane by PI3P (211). At the plasma membrane, Btk is activated through phosphorylation, presumably by Src family kinases. In hematopoietic cell lines, SCF induced Tec activation in a PI3’-kinase- and Lyn-dependent manner, and this was important for the tyrosine phosphorylation of the Dok-1 adaptor protein (284). Functionally, it has been observed that activation of Btk can cooperate with other receptor systems; in mast cells, it has been observed that the SCF-induced Btk activation potentiates the FcεRI-promoted mast cell activation, and in erythroid progenitors, SCF activates Btk and in this system Btk interacted with the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-receptor 1 in a manner possibly promoting survival (244).

B. Src Family Kinase Signaling

The Src family of tyrosine kinases (SFK) contains eight cytoplasmic kinases, some of which are ubiquitously expressed (Src, Yes, and Fyn), whereas others have more restricted, often hematopoietic expression (Lck, Hck, Lyn, Fgr, and Blk). The domain structure of SFK is from the NH2 terminus; a unique membrane targeting domain, SH3, SH2, tyrosine kinase domain and a negatively regulating COOH-terminal tail. In the inactive state, SFK form a closed conformation where the SH3 and SH2 domain interact with the kinase domain in a manner that does not allow efficient catalysis. The closed conformation of SFK is stabilized by interactions between the SH3 domain and a proline-rich stretch in the amino acid sequence between the SH2 and kinase domain, as well as an intermolecular binding of the SH2 domain and the phosphorylated COOH-terminal tail. SFK are activated by events that release these negatively regulatory systems, e.g., binding of a high-affinity phosphotyrosine residue to the SH2 domain or strong interaction between a proline-rich sequence and the SH3 domain. Moreover, dephosphorylation of the COOH-terminal tail will also increase SFK activity. The activity of SFK is often increased in tumors, although mutations are not so often found. However, certain colon cancers contain activating mutations in Src (120).

In response to SCF stimulation, c-Kit becomes phosphorylated on two residues in the juxtamembrane region (Tyr-568 and Tyr-570) (146, 160, 170, 225). These phosphorylation sites interact with the SH2 domain in SFK (160), thereby displacing the COOH-terminal tail of SFK from the intermolecular interaction with the SH2 domain resulting in an opening of the structure and increased catalytic activity. It has become clear that the phosphatase SHP2 plays an
important role for SFK activation downstream of tyrosine kinase receptors, possibly by dephosphorylating the transmembrane scaffolding protein PAG and consequently blocking the recruitment of the negative SFK regulator Csk to the membrane where SFK are normally located (314). Whether SHP2 also plays an important role in c-Kit-mediated SFK activation remains to be clarified, but SHP2 has been shown to dock to the juxtamembrane region of c-Kit where SFK also binds (144).

Activated SFK have been shown to contribute to activation of several signal pathways downstream of c-Kit. Several groups have implicated SFK in SCF-induced ERK1/2 mitogen-activated protein (MAP) kinase activation (34, 160), possibly by promoting the phosphorylation of the Shc adapter protein (160). The JNK MAP kinase pathway is activated by c-Kit in a manner that requires both SFK and PI3-kinase acting on Rac1 (276). For more information on the role of SFK in MAP kinase activation, please refer to the section below dealing with MAP kinase signaling. There are also reports proposing that SFK activation in lipid rafts by c-Kit is important for Akt phosphorylation (10). Although one commonly groups different members of the SFK family together, it is clear that different family members have distinct functions. This was clearly illustrated in two studies using bone marrow-derived mast cells with Lyn or Fyn genes deleted; one study showed that Lyn was important for SCF-induced JNK and Stat3 activation and negatively regulated Akt (254), whereas another report showed defects in SCF-induced SHP2 and p38 phosphorylation but normal Stat3 and Akt phosphorylation in cells lacking Fyn (237). As described above, alternative splicing of the mRNA encoding c-Kit generates two splice variants that differ by four amino acids in the extracellular juxtamembrane region. Studies on these two splice forms revealed that they differ dramatically in their abilities to signal, where the form of c-Kit lacking the tetrapeptide sequence in general produces a stronger and a more rapid response (46, 287). The reason for these differences was shown to a large extent to depend on the ability to recruit and activate SFK (287).

Several studies have implicated activation of SFK in c-Kit-induced proliferation. For example, in a megakaryocytic cell line Mo7e, it was demonstrated that Lyn promotes the G1/S phase transition and proliferation and that the SFK inhibitor PP1 could interfere with this (170, 197). The same group also showed that mast cells lacking Lyn expression displayed a reduced proliferation, as well as migration, compared with control cells (204). These results suggest that the SFK member Lyn is important for c-Kit-driven proliferation in hematopoietic cells. Furthermore, Lck was found to interact with c-Kit and to be important for proliferation of the small cell lung cancer cell line HS26 (146). In mast cells it was demonstrated that SCF-induced activation of SFK and PI3-kinase converged on the Rac1-JNK signaling and that this pathway was necessary for SCF-induced proliferation (276). Recently it was found that in c-Kit promoted proliferation in melanocytes was dependent on the transcription factor MITF and that several pathways downstream of c-Kit, including SFK, were important for MITF activation (220). In contrast, using endothelial cells transfected with c-Kit lacking the SFK binding site Tyr-568, no obvious effect on proliferation could be noted (160).

Activation of c-Kit protects many cells from apoptosis; this effect has to a large extent been ascribed to PI3-kinase signaling. However, SFK also contributes to the survival effect. Treatment of erythroid precursor cells with SCF is able to suppress Fas-mediated apoptosis, and this effect was abolished by the SFK inhibitor PP2 (203). In mast cells, both SFK and PI3-kinase were shown to be important for SCF-mediated protection form apoptosis (276).

Several studies have implicated SFK in promoting cell migration, and this can occur through different routes, e.g., SFK can interact with and phosphorylate FAK which has a well-established function in regulating focal adhesions or directly phosphorylate the focal adhesion protein paxillin (84, 255, 274). SFK have also been implicated downstream of c-Kit in promoting cell migration, e.g., in mast cells where the SFK member Lyn was knocked-out (204). In another study using a panel of c-Kit mutants in which individual tyrosine residues had been mutated to phenylalanine, it was found that activation of SFK was involved in p38 MAP kinase activation and Ca\(^++\) influx, which promoted ERK1/2 activation and cell migration, and in addition, Ca\(^++\) influx was also stimulated by PI3-kinase activation (281). Furthermore, by mutating all autophosphorylation sites in c-Kit to phenylalanine residues and then adding back individual tyrosines, it was found that by allowing SFK to bind to c-Kit the receptor was able to promote migration, survival, and to some extent the proliferative responses (110).

To study the importance of the SFK binding site in c-Kit in vivo, knock-in mice were produced that expressed c-Kit\(^{Y567F}\) and c-Kit\(^{Y567/569F}\) in place of wild-type c-Kit (1, 134). c-Kit\(^{Y567F}\) mice showed defects in maintaining normal numbers of pro-T and pro-B cells in older animals (1). In mice with both Y567 and 569 mutated, there were also defects in mast cells and pigmentation, and the mice displayed splenomegaly (134). The difference in phenotype between mice carrying c-Kit\(^{Y567F}\) and c-Kit\(^{Y567/569F}\) is interesting since in vitro it has been found that the single and double mutants are equally incompetent in activating SFK; however, only the double mutant was unable to support a proliferative response (160).

A complication in interpreting data obtained using c-Kit, where the SFK binding site in the juxtamembrane region have been mutated, is that these sites have been found to interact with other signaling proteins as well and that this
region of the receptor is involved in regulating its kinase activity. Furthermore, studies using low-molecular-weight inhibitors have to be interpreted with care since molecules targeting SFK kinase activity may also affect c-Kit, as has been demonstrated for the drug PP1 initially described as a SFK inhibitor but later shown to also effectively target c-Kit (270).

As described above, it is clear that SFK are an integral part of the signaling systems mediating most c-Kit-driven responses, proliferation, survival, and migration. Furthermore, the nature of the SFK involvement in c-Kit-driven responses is different in various cell types, which probably is a reflection of which SFK members and substrate proteins are expressed in a given cell type. There exists mutant forms of c-Kit (D816V) that can drive tumor progression, and it has been shown that c-Kit<sup>D816V</sup> does not depend on activation of SFK as the wild-type c-Kit for its ability to promote survival and proliferation (261). Biochemical analysis of the c-Kit mutant revealed that its substrate specificity was changed from that of normal c-Kit to more resemble that of Abl and SFK, hence explaining the reduced need of SFK (261). This illustrates that the need of a particular signal molecule can be changed by a single point mutation.

C. MAP Kinase Pathways

MAP kinases are activated downstream of most types of cell surface receptors and hence play central roles in a multitude of biological processes, both under normal and pathological conditions. The MAP kinase pathway has the architecture of a three-layered kinase module that is initiated at the plasma membrane and reaches all the way to the nucleus where it regulates gene expression often by phosphorylating transcription factors. However, also processes occurring in the cytoplasm, for example, translation and cell migration, can be regulated by MAP kinases. The biological consequence of MAP kinase activation is connected to the magnitude as well as the duration of MAP kinase phosphorylation (182, 304). All eukaryotic cells have at least one type of MAP kinase and human cells contain four major groups: ERK1 and -2 (ERK1/2), ERK5, p38, and JNK.

Among the MAP kinases, ERK1/2 have been studied in great detail. Receptor tyrosine kinases often engage ERK1/2 by recruiting the RAS guanine exchange factor Sos to plasma membrane. However, there are also other guanine exchange factors for RAS such as Vav, which have been found to function downstream of c-Kit in hematopoietic cells (3). Sos is constitutively associated with the Grb2 adaptor protein that can interact with phosphorylated receptors directly or indirectly through additional proteins such as Shc or possibly SHP2. However, it has been observed that the phosphatase activity of SHP2 is important for ERK1/2 activation, suggesting that SHP2 may promote ERK1/2 activation by dephosphorylation of regulatory proteins, e.g., Sprouty (94). Bringing Sos into the proximity of the small GTPase RAS provokes a nucleotide exchange from GDP to GTP, which induced a conformational change in RAS that allows it to interact with its downstream effectors. One downstream effector of active RAS is the serine/threonine kinase Raf, which through the interaction with active RAS translocate to the plasma membrane where Raf becomes activated through a process involving both phosphorylation and dephosphorylation (53). Next, Raf phosphorylates and activates Mek1/2, which in turn phosphorylates and activates ERK1/2. Many of the proteins downstream of ERK1/2 are transcription factors, including c-Fos and Elk-1, but there are also cytoplasmic substrates such as Rsk. Furthermore, for a signal to flow efficiently through the MAP kinase pathways, it is well appreciated that scaffolding proteins holding the different components together are of great importance, e.g., KSR (41).

Stimulation of c-Kit has been shown to activate ERK1/2, p38, JNK, and ERK5, although most studies addressing activation mechanisms have been performed on ERK1/2. With the use of mutant receptors deficient in Src family kinase (SFK) activation, it has been demonstrated that activation of SFK by c-Kit is important for Shc phosphorylation and ERK1/2 activation (160). This study suggests that the Grb2-Sos complex is recruited to c-Kit not primarily through direct binding of Grb2 to the receptor, but rather indirectly by interacting with tyrosine-phosphorylated Shc. Interestingly, there are direct interaction sites for Grb2 in c-Kit, i.e., Tyr-703 and -936 (275), and the relative importance of these versus indirect Grb2 binding via Shc is not clear. Other reports using mutant receptors have also indicated a critical role of SFK in ERK1/2 activation (110, 134). In one study, all tyrosine residues in the intracellular region of c-Kit were mutated to phenylalanine and then different tyrosine residues were added back; it was clear that adding back the SFK binding site in c-Kit restored ERK1/2 activation despite the absence of Grb2 binding sites (110). In mast cells in which the PTPr<sub>i</sub> gene had been deleted, SCF was impaired in its ability to activate the SFK member Fyn and ERK1/2 as well as the phosphorylation of the GAB2 adaptor protein (238). SCF-mediated activation of ERK1/2, p38, and JNK was found to be negatively regulated by the adaptor protein Lnk (256). Interestingly, the SH2 domain of Lnk associates with Tyr-568, the major SFK binding site in c-Kit restored ERK1/2 activation despite the absence of Grb2 binding sites (110). In mast cells in which the transition factor of c-kit had been deleted, SCF was impaired in its ability to activate the SFK member Fyn and ERK1/2 as well as the phosphorylation of the GAB2 adaptor protein (238). SCF-mediated activation of ERK1/2, p38, and JNK was found to be negatively regulated by the adaptor protein Lnk (256). Interestingly, the SH2 domain of Lnk associates with Tyr-568, the major SFK binding site in c-Kit, and since SFK have been implicated in at least ERK1/2 and JNK activation, the inhibitory effect of Lnk may be due to interference with SFK activation by c-Kit. However, the requirement of SFK in ERK1/2 activation appears to be cell type specific since a study using erythroblasts expressing a c-Kit mutant unable to bind SFK did not display a defective ERK1/2 phosphorylation, but rather defective JNK activation (2) and other studies in using mast cells have also implicated SFK in activation of JNK (254, 276). It is tempting to speculate that in cells relying on Shc for c-Kit coupling to ERK1/2 SFK would be important, whereas if Grb2/Sos di-
rectly interacted with the receptor this would occur in an SFK-independent manner, although this has not been established. Furthermore, the mechanism of ERK1/2 activation downstream of c-Kit appears to be different cell types or dependent on their state of differentiation; in primitive hematopoietic cells, ERK1/2 activation is dependent on PI3'-kinase signaling and not on RAS, whereas in mature mast cells ERK1/2 stimulation went through RAS (288). One possible way in which PI3'-kinase can activate ERK1/2 and bypass the requirement for RAS is through activation of p21-activated kinase 1 (PAK1). PAK1 is activated downstream of Rac, which in turn is activated in a PI3'-kinase-dependent manner (33). In mast cells from PAK1 knockout mice, it was seen that SCF-induced Mek1/2 and ERK1/2 phosphorylation were strongly suppressed, implicating PAK1 in the activation process (188). In a recent study using bone marrow-derived mast cells in which the KSR gene had been deleted, it was found as expected that the ERK1/2 phosphorylation was reduced, but also p38 and PAK phosphorylation (50). Furthermore, in KSR−/− cells, SCF displayed reduced ability to promote cell proliferation and migration. Previous studies have shown that mast cells depend on p38 for their ability to migrate toward SCF (264).

p38 has also been described in several studies to be activated in response to c-Kit activation. Mast cells deficient in the SFK member Lyn were defective in JNK activation. In line with this finding, it has been demonstrated that mast cells lacking the SFK member Fyn have a defective JNK as well as p38 activation (237). Furthermore, in PTPα knockout mast cells, Fyn and p38 activation were reduced compared with normal cells (238). In mast cells lacking the Fes tyrosine kinase, SCF was unable to promote sustained p38 activation, and this correlated with impaired cell migration (257). Furthermore, SCF-induced Fes phosphorylation was dependent on Fyn. In addition, the kinase PAK1 has also been suggested to be crucial for activation of p38 downstream c-Kit (188). Several studies have implicated p38 in the signaling circuitry regulating cell migration, and inhibition of p38 inhibits this pathway (147, 188, 264). Ueda et al. (281) found that SCF-induced p38 and PI3'-kinase activation was important for Ca^{2+} influx, which in turn activated ERK1/2, and promoted cell migration.

Also, the JNK pathway is induced by SCF treatment. It has been demonstrated that activation of c-Kit results in a SFK-dependent phosphorylation of GAB2 which then recruits SHP2, and this is critical for SCF-induced Rac/JNK but also partially for RAS activation (309). Moreover, in Fyn knockout mast cells, SHP2 was found not to be phosphorylated, which was correlated with defective p38 and JNK activation (237). Also in PTPα null mast cells, it was shown that SCF-induced Fyn and JNK activation, and GAB2 phosphorylation was reduced (238). Consistent with the requirement of GAB2 for normal SCF-induced RAS activation (309), ERK1/2 activation has been found to be decreased in mast cells lacking GAB2 expression (202). Similarly, PTPα knockout cells that have reduced Fyn and GAB2 phosphorylation were deficient in SCF-induced activation of ERK1/2, p38, and JNK (238). Moreover, overexpression of GAB2 in leukemic cells selectively increased ERK1/2 signaling (66).

In response to c-Kit activation, ERK5 is activated in a manner sensitive to PI3'-kinase inhibition, and the active ERK5 translocates to the nucleus (155). In mast cells it was found that activation of c-Kit (as well as FceR1) activates Mekk2, which in turn can promote JNK and ERK5 activation that are important for cytokine production (81).

**D. Phospholipases C and D Signaling**

There are six families (β, γ, δ, ε, ζ, and η) of phospholipase C (PLC) that generate 13 isoforms in human cells. PLC enzymes can be found in all eukaryotic cells and function by hydrolyzing the polar head group from the membrane phospholipid PIP2 generating the membrane-bound second messenger diacylglycerol (DAG) and soluble inositol 1,4,5-trisphosphate (IP3). DAG interacts with effector proteins, e.g., certain PKC isoforms thereby promoting their activation, whereas IP3 leads to mobilizing of Ca^{2+} from internal stores in the endoplasmic reticulum. The PLC-γ family contains SH2 domains that allow them to interact with activated tyrosine kinase receptors. In regard to c-Kit signaling, most work has been done with PLC-γ, and hence our discussion will focus on this subtype. Another lipase that has been found to be important in c-Kit signaling is phospholipase D (PLD), of which there are two mammalian isoforms. The enzymatic function of PLD is to hydrolyze the membrane lipid phosphatidylcholine into phosphatidic acid and soluble choline. Phosphatidic acid is then rapidly hydrolyzed to DAG by phosphatidic acid hydrolase.

It has been found that PLC-γ can interact with tyrosine residue 730 in c-Kit, and interestingly, cell proliferation induced by membrane-bound SCF has been shown to depend on PLC-γ association with and activation by c-Kit (85, 278). Furthermore, soluble SCF was shown to be unable to activate PLC, but did in contrast activate PLD in a PI3-kinase-dependent manner (141, 143). Activated PLD produces phosphatidic acid, which is further metabolized into DAG, which can participate in PKC activation; this may be the dominating way for c-Kit to activate PKC in response to soluble SCF. Furthermore, it was found that DAG produced by PLD activation was necessary for the release of arachidonic acid from mast cells (142).

The available data suggest that PLC-γ plays a minor role downstream c-Kit, and this was supported by an add-back approach where intracellular tyrosine residues in c-Kit were mutated and then added back one at a time. It was found
that adding back the PLC-γ binding site in c-Kit (Tyr-730) did not have a major impact on SCF-induced proliferation or migration (110). However, it may be that PLC-γ signaling is more important for cell stimulation by membrane-bound SCF as some data suggest (85, 278).

It has been observed that c-Kit can protect cells from the effects of radiation. Activation of PLC-γ has been found to be important for the radioprotective effect. For example, in cells expressing a mutant c-Kit unable to bind and activate PLC-γ or in cells treated with U73122 that interfere with PLC-γ function, stimulation of SCF could not confer radioprotection (179). Maddens et al. (2002) showed that SCF-induced PLC-γ activation blocked the production of ceramide which can promote apoptosis (179). Dependence for PLC-γ-mediated suppression of ceramide production and apoptosis following SCF stimulation was found in daunorubicin-treated cells, using the U73122 inhibitor (224).

A truncated cytoplasmic form of c-Kit (tr-Kit) that lacks kinase activity has been found to block and activate PLC-γ or Ca2+ chelator (248). Furthermore, microinjection of tr-Kit in metaphase II-arrested mouse eggs leads their transition into anaphase, which can be blocked by either U73122 or Ca2+ chelator (248). Furthermore, it has been found that the SFK Fyn is important in this process by causing phosphorylation of tr-Kit and PLC-γ (250). A likely scenario is that the sperm brings the tr-Kit to the oocyte upon fertilization and that Fyn interacts with tr-Kit and promotes its phosphorylation, thereby allowing it to interact with and activate PLC-γ. The activated PLC-γ then triggers increased intracellular calcium, which in turn is necessary for oocyte activation. In addition, tr-Kit also promotes the interaction between the RNA binding protein Sam68 and both Fyn and PLC-γ (214). In a recent publication from Mucciaccia et al. (198), the authors found a correlation between tr-Kit expression and the DNA integrity and hence sperm quality although the underlying mechanism remains elusive.

E. Adaptor Proteins

Adaptor proteins contain several interaction domains that promote specific interactions but have no enzymatic function. Since they contain multiple interaction domains, they can interact with several other proteins or lipids simultaneously, thereby linking them together, often in a regulated manner. p85 (the regulatory subunit of PI3-kinase), Grb2, Gab2, and Shc are examples of adaptor proteins that already have been described above in the context of their involvement in certain signaling pathways.

The adaptor protein Grb7 contains one SH2 domain, PH domain, and a proline-rich sequence. It has been shown that Grb7 can interact with its SH2 domain with Tyr-936 in the COOH-terminal tail of c-Kit (275). The function of Grb7 downstream of c-Kit has not been elucidated, but there are several reports implicating Grb7 in cell migration in other systems (reviewed in Ref. 92). A related adaptor is Grb10 that also interacts with c-Kit through its SH2 domain (122). Grb10 was found to constitutively associate with Akt, suggesting that Akt is translocated to the plasma membrane when Grb10 interacts with c-Kit, thus facilitating its subsequent PI3-kinase-dependent activation (122).

APS and Lnk are two related adaptor proteins containing SH2 and PH domains that have been shown to interact with c-Kit, and for APS this binding has been mapped to Tyr-568 and Tyr-936 in c-Kit (298). With the overexpression of Lnk in the MC9 mast cell line, it was proposed that it serves to attenuate proliferative signaling from c-Kit (266). The decreased proliferation correlated with reduced SCF-induced GAB2 phosphorylation and activation of the ERK1/2 pathway. In contrast, using bone marrow-derived mast cells from lnk−/−, APS−/−, and SH2-B−/− mice did not display any impact in SCF-induced proliferation (148). It is possible that this discrepancy in SCF-induced proliferation between these studies is due to the different methods used; Lnk overexpression can compete with other proteins sharing the same binding site in c-Kit as Lnk. Alternatively, in the knockout cells, the other family members can compensate for loss of a single member. Consistent with a role of Lnk as a negative regulator of c-Kit signaling, lnk−/− mice had an enhanced hematopoiesis and the B-cell lineage was increased, which was suggested to be due to an increased sensitivity of c-Kit expressing precursor cells to SCF (266, 267).

The Crk family of adaptor proteins, CrkI, CrkII, CrkL, and CrkIII, consists of one SH2 domain and one or two SH3 domains. It has been found that activation of c-Kit induced phosphorylation of CrkI and an indirect association through p85 adaptor protein (241). CrkII is also phosphorylated by SCF and was shown to interact with Tyr-900 in c-Kit, which is not an autophosphorylation site but instead a Src kinase phosphorylation site (163). The functional consequence of Crk binding to c-Kit is not clear, but it has been seen that CrkI can interact with the ubiquitin E3 ligase c-Cbl, indicating a possible role in c-Kit downregulation (241). Also, the nucleotide exchange protein C3G can interact with Crk, providing a possible link to JNK activation (269).

Dok-1 is an adaptor related to IRS-1 that contains PH and PTB domains as well as several tyrosine phosphorylation sites. Activation of c-Kit results in binding of Dok-1 to the juxtamembrane region of c-Kit and subsequent Lyn kinase-dependent tyrosine phosphorylation of Dok-1, but Tec kinases are probably also involved in the phosphorylation process (168, 284). Furthermore, translocation of Dok-1 to the plasma membrane was dependent on PI3-kinase activity. A possible scenario is that upon activation of c-Kit Dok-1 is translocated to the membrane through interaction
with phosphorylated c-Kit via its PTB domain as well as with PI3-kinase-generated PIP3 through its PH domain. Following translocation, Dok-1 can be phosphorylated by Lyn and Tec. Phosphorylated Dok-1 could interact with several signaling proteins, e.g., Abl, CrkL, SHIP, and PLCγ1, whereas Vav and Shc failed to interact with Dok-1 (284). Functionally, Dok-1 has been shown to negatively regulate SCF-induced proliferation (64).

**F. Interaction With Other Types of Receptors**

It is well established that c-Kit acts synergistically with many cytokines, including IL-3, IL-7, granulocyte-macrophage colony stimulating factor (GM-CSF), and Epo, in promoting proliferation of hematopoietic cells, and for at least Epo, IL-7, IL-33, and GM-CSF, this has been shown to involve direct interactions between these receptors and c-Kit (67, 123, 162, 300). In some cases, but not all, these types of heterotypic receptor interactions have been shown to correlate with cross-phosphorylation, e.g., SCF stimulation may promote tyrosine phosphorylation of the Epo or IL-7 receptor (123, 300). In a study from Kapur’s laboratory, it was demonstrated that mutation of seven tyrosine residues in the intracellular region of c-Kit abolished the ability of c-Kit to cooperate with Epo receptor, but interestingly adding back only the Src family kinase binding site in c-Kit restored cooperativity (111). Moreover, adding back the Src family binding site in combination with the PI3'-kinase binding site also allowed cooperatively between c-Kit and Epo receptor; however, this was inhibited by restoring both Src and PLCγ binding (111). Also, the synergistic interaction between c-Kit and GM-CSF receptor may involve transphosphorylation; GM-CSF by itself did not promote c-Kit phosphorylation, but concurrent treatment with both SCF and GM-CSF did result in a stronger c-Kit phosphorylation compared with SCF by itself (127), although other studies have failed to demonstrate this (162). Besides tyrosine phosphorylation, it has been suggested that activation of c-Kit can promote a protein kinase C-dependent serine phosphorylation of the IL-3 receptor (172), although the importance of this is not understood. Another possible mechanism lying behind synergistic signaling is that signals through one receptor promote the expression of the other, and this has also been demonstrated in situations involving c-Kit; IL-2, IL-15, and GM-CSF promote c-Kit expression (20, 127), and vice versa, c-Kit activation has been demonstrated to increase Epo receptor expression (130). Yet another fashion that different receptors combine and potenti ate the biological responses is by synergistically enhancing the activity of a common signal transduction pathway. G-CSF and SCF synergistically activate Stat3, and in this case, it has been shown that G-CSF promotes tyrosine phosphorylation of Stat3 and is necessary for its dimerization and nuclear translocation, whereas SCF induces Ser-727 phosphorylation that is important for the transactivation potency of Stat3 (69). A pathway that is commonly seen to be synergistically activated by c-Kit together with other receptors, including receptors for IL-2, IL-15, Epo, and GM-CSF, is the ERK1/2 pathway (20, 156, 162, 217, 259), which is important for cell proliferation, thus explaining why SCF often synergize with cytokines to promote hematopoietic cell expansions. Furthermore, it was recently shown that activation of the human G protein-coupled melancortin 1 receptor (MC1R) leads to ERK1/2 phosphorylation by a mechanism involving Src-mediated transphosphorylation of c-Kit, but independent of cAMP production (105).

**V. SIGNALING FROM ONCOGENIC c-KIT MUTANTS**

Expression of c-Kit with oncogenic mutations in cells has shown that these mutations cause SCF-independent receptor phosphorylation and can support proliferation. The majority of oncogenic c-Kit mutations are located in the juxtamembrane region (e.g., c-KitV560G) or within the kinase domain (e.g., c-KitD816V). The exact mechanism behind the constitutive activation is not fully understood. One possibility is that the mutation within the kinase domain results in a structural change that relieves autoinhibitory mechanisms; however, no crystal structure of the kinase domain of c-Kit with an activating mutation has yet been published. Recently, an in silico analysis was made on the structural consequences of D816V mutation. This modeling suggested that mutation of D816 caused a structural change in the activation loop, but also weakened the binding of the juxtamembrane region to the kinase domain (150). Consequently, the juxtamembrane region, which has an inhibitory effect on the kinase activity, is no longer able to efficiently suppress the enzymatic activity of c-Kit. In addition, it was proposed that the extended juxtamembrane region potentially could make contact with another c-Kit receptor, thereby promoting dimerization in the absence of ligand (150). In support of this hypothesis, it has previously been found that recombinant kinase domain with the related D816Y mutation, but not the wild-type kinase domain, formed dimers in solution (151). Furthermore, with the use of recombinant kinase domains, it has been suggested that c-KitD816V has an increased affinity for ATP and specific activity, compared with wild-type c-Kit kinase domain (151). Studies on the V560G juxtamembrane domain mutant showed, using chemical cross-linking, that this mutant receptor undergoes SCF-independent dimerization that was not the case for c-KitD816V (137). However, the lack of ligand-independent c-KitD816V dimers in this study may relate to the low surface expression of this mutant receptor (see discussion below). It is interesting to note that the juxtamembrane region, which is the major negative regulator of c-Kit kinase activity, is affected by both mutational hotspots in c-Kit, directly by mutations in this region or indirectly by mutations in the kinase domain that have long range effects on the juxtamembrane region as proposed by molecular modeling. An important observa-
tion is that c-Kit^{D816V} is resistant to kinase inhibitor ima-
tinib, whereas wild-type c-Kit and c-Kit^{V560G} are more sen-
sitive (78).

Several groups have compared the ability of wild-type and
oncogenic mutants of c-Kit to induce signal transduction
and found that they differ not only quantitatively but also
qualitatively. This is potentially important since it suggests
that there might be ways to selectively target oncogenic
signaling with less impact on the normal situation. The
reason for the different signaling abilities can stem from
changes in intracellular localization of the mutant c-Kit,
 altered substrate specificity, or a combination of both.

In most cells one can detect two forms of c-Kit, one ~125
kDa precursor and the mature ~145 kDa protein with com-
plex N-glycosylation pattern. Treatment with SCF leads to
phosphorylation of the 145-kDa but not the 125-kDa form,
suggesting that the smaller protein is not expressed on the
cell surface (26). It has been reported that c-Kit with activ-
ating mutations in the juxtamembrane region does not
normally mature to the 145-kDa surface-exposed form; in-
stead, it remains to a larger extent as a precursor protein
localized to the endoplasmatic reticulum (ER) and Golgi
compartments (265). c-Kit with gain-of-function mutations
in the kinase domain display reduced cell surface expres-
sion, and inhibition of kinase activity in both c-Kit with
juxtamembrane or kinase domain mutations restored cell
surface expression of the mutant receptor (35, 265). An-
other study demonstrated that c-Kit^{D816V} was primarily
localized to and could transmit oncogenic signals from the
Golgi apparatus, whereas c-Kit trapped in the ER could not
do this (302). In an attempt to elucidate the role of local-
ization on signaling, cells expressing activating mutations of
c-Kit (or the related RTK Flt3) were treated with brefel-
din A, which causes protein accumulation in the ER, and
then subjected to analysis of various signaling pathways; it
was found that certain proteins such as Akt and ERK1/2
were not activated in brefeldin A-treated cells, whereas
wild-type c-Kit required Src activity for these processes (160, 184). Coherent with
the Src-like substrate preference of c-Kit^{D816V}, it was shown
that both the GNNK− and GNNK+ splice forms of mutant
c-Kit behaved similar in signaling, whereas the wild-type
GNNK+ is less efficient in activating Src which leads to
different signaling compared with GNNK− splice form of
c-Kit (219, 287). This suggests that regardless of in which
splice form the oncogenic mutation occurs, the transform-
ing ability of the resulting oncoprotein is similar.

Several studies have tried to identify signaling pathways
critical for the transforming abilities of mutant c-Kit. One
study showed that the tyrosine kinase Fes was important for
negative regulation of Stat and positive regulation of mTor
phosphorylation downstream of c-Kit^{D816V} (285). Furth-
more, silencing of Fes expression also led to a partial reduc-
tion in c-Kit^{D816V}-driven proliferation. It has been demon-
strated that PI3-kinase binding to c-Kit is necessary for the
oncogenic properties of c-Kit^{D816V} or the corresponding
mutation in murine receptor D814V (52, 97). The import-
ance of PI3-kinase signaling was confirmed in a transplan-
tation experiment where bone marrow cells expressing or
lacking p85α were transduced by c-Kit^{D814V}; normal bone
marrow cells were transformed, but not cells lacking p85α
(199).

VI. NORMAL FUNCTION OF c-KIT

Numerous loss-of-function mutations in W and the Sl loci
have been described in mice. These loci encode c-Kit and
SCF, respectively. These naturally occurring mutations
comprise a spectrum of defects ranging from minor defects
in the tyrosine kinase activity of c-Kit to a complete loss of
its kinase activity, resulting in the corresponding degree of
severity in the phenotype displayed by these mice (for re-
view, see Ref. 164). These mutations have given us hints on

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the normal physiological function of c-Kit during embryogenesis and adulthood. The vast number of various loss-of-function mutations in this receptor/ligand system suggests crucial functions in the hematopoietic system, during gametocyte development, pigmentation, intestinal motility, as well as in the nervous system (132, 164, 235). Data from other models have also suggested a function in the immune system including inflammation (given its expression in both dendritic cells and mast cells) and in the regulation of vasculogenesis (reviewed in Refs. 103, 190, 228).

### A. Hematopoiesis

Hematopoietic stem cells (HSC) are characterized by their ability to self-renew and to be able to differentiate into all hematopoietic lineages. The process of lineage commitment results in a diminished ability to self-renew, while their proliferative capacity is increased, which led to expansion in cell numbers. With few exceptions, c-Kit can be detected in early hematopoietic cells (including stem cells and progenitor cells), and its expression is lost during their differentiation (38, 206, 207). Early hematopoietic cells are dependent on c-Kit-mediated signals for their proliferation and survival. This occurs by and large in synergy with other cytokines and factors. In two cases, c-Kit expression is not lost during maturation, namely, the mast cell and the dendritic cell. They express high levels of c-Kit even as fully differentiated cells, and they are dependent on c-Kit for their proliferation, survival, and function (reviewed in Refs. 228 and 191). The W and Sl mice show defects in the development of erythrocytes, megakaryocytes, and mast cells (164, 235). Such defects can be rescued by transplantation of wild-type HSC, which indicates that the lethality of these mice is due to anemia. However, there is not a total dependency of SCF for erythropoiesis. Transgenic mice expressing erythropoietin can rescue the homozygotic W mice and support hematopoiesis in the absence of functional c-Kit (293).

### B. Pigmentation

The phenotypic observation that mice defective in c-Kit function have defective pigmentation is believed to be linked to the role of c-Kit in proliferation, survival, and haptotactic migration of melanocytes from the neural crest to epidermis during embryogenesis (178, 245, 294). The exact pathways linking c-Kit to pigment production are not fully known. Kimura et al. (134) demonstrated that Y568 and Y570 in the juxtamembrane region of c-Kit, known to be involved in binding and activation of Src family kinases, are required for normal pigmentation. Activation of Src family kinases is essential for the activation of the RAS/ERK pathway by c-Kit (160) and has also been described to be important for activation of the transcription factor MITF (220). The transcription factor MITF has been described as an important player in c-Kit-mediated melanocyte action. Activation of MITF by c-Kit has been claimed to occur due to ERK- and Rsk-mediated phosphorylation of MITF at S73 and S409, respectively (104, 301). However, recent data from transgenic mice suggest that additional phosphorylation events are involved in MITF activation (15).

### C. Reproduction

The sterility seen in W and Sl mutants is most likely linked to the loss of c-Kit-mediated protection of germ cells from apoptosis and also due to defects in c-Kit-mediated migration and proliferation (174). One of the pathways that seem to be important for gametocyte function is the PI3-kinase/Akt pathway(27). Knock-in mouse studies where mice carried a mutant c-Kit (Y719F, the binding site for the p85 subunit of PI3-kinase in mice) resulted in defective PI3-kinase activation and male sterility (28, 136). In the work by Kissel et al. (136), also female mice displayed reduced fertility. Collectively, these studies suggest that c-Kit is involved in processes of oogenesis, folliculogenesis, and spermatogenesis. The function of c-Kit in germ cells is strictly dependent on the ability to activate PI3-kinase. However, it is striking that these mice did not exhibit any other phenotype, suggesting that other cell types have a system for compensating for the loss of the direct binding of PI3-kinase to the receptor. One such possible compensatory mechanism could be PI3-kinase activation through the binding of PI3-kinase to tyrosine phosphorylated GAB2. It has been shown that in hematopoietic Ba/F3 cells that express GAB2, PI3-kinase activation is dependent on both the direct binding to Y721 in c-Kit as well as binding to tyrosine phosphorylated GAB2 (262). Finally, another caveat is that mutation of the direct binding site of p85 subunit of PI3-kinase, Y721, might not only affect PI3-kinase. It has been described that there is a stoichiometric excess of p85 over p110 in the cell (14, 282) and that p85 can serve as an adaptor also for other proteins. The observed phenotype could also, at least in part, be due to blocked recruitment of other proteins that bind to c-Kit via p85. Several proteins have been described to associate with p85 in a manner that is not dependent on the SH2 domain of p85, including CrkL, Crk (129, 163, 241), and the Src family kinase Fyn (129). In addition to full-length c-Kit, there exists a truncated form of c-Kit (tr-Kit) that is produced by an alternative intronic promoter which is active during spermatogenesis (233). Despite the fact that it lacks kinase activity, it can activate the murine egg if microinjected. This occurs in a Src family kinase and PLC-γ-dependent manner (249, 250). It is likely that tr-Kit becomes phosphorylated by Src family kinases and then serves as a scaffolding protein for the recruitment of downstream signaling molecules. However, the exact mechanism is not known to date. Paronetto et al. (213) found tr-Kit ectopically expressed in certain types of human prostate cancer. Thus further studies are needed to investigate its potential role in carcinogenesis.
D. Gastrointestinal Tract

In humans suffering from a loss-of-function mutation of c-Kit, so-called piebaldism, one of the symptoms is constipation (83) due to loss of the interstitial cells of Cajal (ICC). These are c-Kit positive cells that govern the motility of the gut. A complete loss of ICC has also been described in other motility disorders such as idiopathic intestinal pseudo-obstruction and slow transit constipation (124, 176). The ICC regulate the gut movement by their ability to communicate with both nerve cells and smooth muscle cells. ICC express c-Kit, and in mice with loss-of-function mutations of the c-Kit system, there is a constipation phenotype that suggests an important role of c-Kit in ICC development and function (115, 290, 292). Furthermore, treatment of mice with an inhibitory c-Kit antibody led to a loss of ICC and a subsequent impaired gut motility (180).

E. Nervous System

Studies on mice carrying loss-of-function mutations in either c-Kit or SCF have established a role for c-Kit signaling in the spatial learning function of the hippocampal region of the brain (131, 196). Furthermore, c-Kit has been found to be expressed in neuroproliferative zones in the adult brain and in neuronal cultures (126). Administration of SCF in animals leads to proliferation of primitive neurons. c-Kit plays an important role in the migration of neuronal stem- and progenitor cells to sites of injury in the brain (263). Further investigation is needed to clarify the exact role of c-Kit in development and function of the nervous system.

F. Cardiovascular System

c-Kit has a number of functions in the cardiovascular system both under normal physiology and under pathological conditions. For a review on the role of c-Kit in vasculogenesis, see Reference 103. It may be involved in atherosclerosis, since it has been shown in animal models that SCF attenuates vascular smooth muscle cell (VSMC) apoptosis and increases intimal hyperplasia following vascular injury (289). There is also an upregulation of c-Kit expression in VSMCs. These effects are reversed in mice with loss-of-function mutations in either c-Kit or SCF. Using a closed-chest model of murine cardiac infarction/reperfusion, it could be shown that bone marrow activation of c-Kit in response to released soluble SCF was necessary for bone marrow progenitor cell mobilization after ischemic cardiac injury (72). It was further shown that release of SCF and activation of c-Kit required the activity of matrix metalloproteinase 9 within the bone marrow compartment. It was also shown that mice carrying loss-of-function mutations in either c-Kit or its ligand, SCF, developed cardiac failure after myocardial infarction, but that this could be rescued by bone marrow transplantation. It has also been shown that c-Kit is important for cardiac stem cell differentiation and regulation of cardiomyocyte terminal differentiation (167). In a study on the role of c-Kit and membrane-bound SCF in endothelial progenitor cell recruitment by microvascular endothelial cells, inflammation induced the expression of membrane-bound SF and that recruitment of endothelial progenitor cells depended on this interaction between ligand and receptor (62). On the other hand, another study demonstrated a downregulation of c-Kit expression in HUVEC cells following inflammation (149).

Matsui et al. (185) demonstrated that SCF stimulation of c-Kit promoted survival, migration, and capillary tube formation of HUVEC cells. This effect was dependent on both intact ERK activation and Akt activation. SCF binding to c-Kit leads to stabilization of hypoxia-inducible factor 1α under normoxia (171, 218). This in turn leads to activation of the VEGF promoter and enhanced angiogenesis.

Thus the SCF-c-Kit system is involved in maintaining the vasculature at several levels, and the presence of constitutively active c-Kit mutants in several tumor types might contribute to angiogenesis in a hypoxia-independent manner.

G. Lung

Recent data suggest that c-Kit has an important function in maintaining the integrity of lung tissue. With the use of mouse models, it has been shown through genome-wide linkage analysis that there is a region on mouse chromosome 5 that is connected to susceptibility to airspace enlargement (229). One candidate gene located on chromosome 5 is c-Kit. It could be shown that mice deficient in c-Kit signaling spontaneously developed airspace enlargement and that static lung compliance increased significantly over time in such mice (169). Residual volume and ex vivo lung compliance was also significantly increased in mice deficient in c-Kit signaling. One potential mechanism for this could be that c-Kit regulates the epithelial progenitor cell expansion, and thus protects the lung tissue from destruction. These data are important for our understanding of the mechanisms of how chronic obstructive lung disease develops.

VII. c-KIT AND SCF IN CANCER

c-Kit has been implicated in numerous human malignancies, including small cell lung carcinoma, malignant melanomas, colorectal cancer, and in more than 80% of cases of gastrointestinal stromal tumors. In these cancers mutations in the c-Kit gene are commonly seen. More than 500 different mutations of c-Kit have been described in human tumors (Sanger Institute Catalogue of Somatic Mutations in Cancer; http://www.sanger.ac.uk/genetics/CGP/cosmic/).
However, most of these mutations are likely to be passenger mutations rather than driver mutations. One can assume that those mutations that are very rarely found are more likely to be passenger mutations, while those repeatedly found are more likely to be driver mutations. Figure 5 summarizes the mutations found in c-Kit in human malignancies. As a semiquantitative measure of their relative abundance in human tumors, the number of citations in the literature regarding a particular mutation is shown on the y-axis. It can be clearly seen that the most common mutations cluster in exon 11 and exon 17.

### A. Gastrointestinal Stromal Tumors

Gastrointestinal stromal tumors (GISTs) are the most common tumor type of mesenchymal origin in the gastrointestinal tract. However, they account for only 1% of all tumors in the gastrointestinal tract (reviewed in Ref. 57). They arise from the interstitial pacemaker cells of Cajal. Approximately 20 individuals in a million get the disease each year. Although it is a malignant tumor, it rarely metastasizes to the lymph nodes. Almost all GISTs express c-Kit, and ~80–85% carry activating mutations in c-Kit. Figure 5 shows some of the c-Kit mutations most commonly found in human malignancies. Mutations of c-Kit in GISTs are most commonly located in exon 11, which encodes the juxtamembrane region of c-Kit (108). This region associates in wild-type c-Kit with the kinase domain and suppresses its kinase activity. Mutations in this region lead to a release of this suppression and activation of the tyrosine kinase (193). Less common mutations also occur in exon 9 (encoding the extracellular part of c-Kit) and exon 17 (encoding regions around the A loop of the kinase domain). However, the most common c-Kit mutation seen in other malignancies, D816V, is rarely detected in GISTs. It is not known why exon 17 mutations are found in hematological malignancies and testicular carcinomas, while exon 11 mutations are found in GISTs. It might be that local production of SCF plays a role. While the hematological malignancies do not reside in solid tissues, local production of SCF in solid tissues (either in the tumor cells or by the surrounding stroma) might contribute to the transforming phenotype and might select for the less strongly activating mutations in exon 11. It is known that a majority of GISTs produce soluble SCF (273).
B. Melanoma

c-Kit is important for the normal development of the melanocytes and an important mitogen for melanocytes. Early studies showed that c-Kit expression is lost during the progression of melanomas to a highly metastatic state (154, 201) and that forced expression of c-Kit in such metastatic melanomas led to death of the melanoma cells (113). However, during the last couple of years it has become increasingly clear that melanomas are highly heterogeneous, and while a majority of human malignant melanomas carry mutations in BRAF (more than 80%), a subset of malignant melanomas carry activating mutations of c-Kit (reviewed in Ref. 299), in particular melanomas occurring on the foot soles and the palm of the hands (so-called acral melanomas) ~20–25% of the patients carry activating mutations in c-Kit. In the much rarer melanoma type mucosal melanomas (accounting for ~1% of all melanomas), activating c-Kit mutations have also been described. Some mutations have been repeatedly found in these types of melanomas, including L576P in exon 11 and K642E in exon 13. D816X mutations have also been described in melanomas, but they do not seem to be as common as the two mutations described above.

C. Small Cell Lung Carcinoma

Small cell lung carcinoma (SCLC) constitutes 13% of human lung carcinomas and is strongly associated with tobacco smoking (reviewed in Ref. 77). Overexpression of c-Kit has been reported in 70% of SCLC patients (107, 246) and also the presence of concomitant expression of both c-Kit and SCF resulting in an autocrine loop (106). However, it is still debated whether c-Kit expression has any prognostic significance for SCLC (reviewed in Ref. 77).

D. Testicular Carcinoma

Testicular germ cell tumors are the most common tumor type in young men. This group of tumors can be subdivided into seminomas and nonseminomas. About half of the testicular germ cell tumors are seminomas. One of the most common mutations found in seminomas is activating mutations of c-Kit in exon 17 (~25% of seminomas) (for review, see Ref. 208). The by far most common mutation is D816X (where X can be either V or H). Although initially claimed to be a marker of bilateral disease, the D816 mutation has in larger patient material been shown not to be a biomarker for bilateral disease (55).

E. Mastocytosis

Mastocytosis is a disease that is characterized by an accumulation of mast cells and mast cell precursors in patients and overactivation of mast cells (reviewed in Ref. 11). The clinical spectrum is quite wide ranging, from a disease in children that is most likely to go away without treatment to a more severe, progressive disease in adults. According to the World Health Organization classifications, mastocytosis can be divided into three major categories: 1) cutaneous mastocytosis. This is a benign disease primarily seen in children and with a tendency to heal by itself with time. 2) Systemic mastocytosis (SM) can be further subdivided into three categories. Indolent SM, which primarily affects the bone marrow and skin, is the most common form. There are also SM with an associated nonmast cell clonal hematological disease, aggressive SM which affects areas other than the skin, and mast cell leukemia. 3) Localized extracutaneous mast cell neoplasms are the final category. Activating mutations of c-Kit are found in almost all adult patients with SM. More than 90% of patients with SM that carry mutations of c-Kit carry the D816V. In contrast, in children with systemic mastocytosis, a much smaller proportion of the patients carry the D816V mutation (~40%) while another 40% carry other mutations in c-Kit that reside outside exon 17 (32). Many mutations were found to be located in the fifth Ig-like domain of c-Kit (encoded by exons 8 and 9). It has been shown that the fifth Ig-like domain of c-Kit is of great importance for stabilizing the receptor dimers formed upon SCF binding (310) which might explain why these mutations lead to constitutive activation of c-Kit.

The symptoms of mastocytosis (skin rash, urticaria, etc.) occur due to the release of mast cell mediators in the tissues. Although the disease is usually slowly progressing, it can in rare cases develop into highly malignant mast cell leukemia.

F. Acute Myeloid Leukemia

Expression of c-Kit has been found in ~85% of AML cells (99). c-Kit is many times found constitutively phosphorylated in AML cells. This could be achieved in several ways. Stem cell factor stimulates the proliferation of many AML cells in vitro, and coexpression of c-Kit and its ligand has been described in AML blasts, thus providing a possible autocrine loop (118, 119, 128, 223). The other option is to activate c-Kit through activating mutations. Although the most commonly mutated receptor tyrosine kinase in acute myeloid leukemia (AML) is the closely related FLT3 that has been found to be mutated and constitutively active in ~30% of patients with AML. However, in certain subtypes of AML, FLT3 is not mutated and instead c-Kit is mutated and activated. This occurs in particular in AML with inv (16) or t(8;21), i.e., so-called core factor binding AML (reviewed in Ref. 239). Mutations in the KIT gene occur in 20–25% of t(8;21) cases and in ~30% of cases with inv (16) (215). Most of the activating mutations in this type of leukemia reside in exon 17 of KIT (D816X, N822K). Interestingly, in this type of AML, some cases of internal tandem duplication of the juxtamembrane region of c-Kit have been found, similar to the ITD mutations in FLT3 (18). It has
been shown that the D816V mutation of c-Kit is a negative prognostic factor correlating higher incidence of relapse and a lower overall survival rate in adult patients (45) as well as in children (232).

VIII. c-KIT AND SCF IN ASTHMA AND ALLERGY

Allergic diseases such as allergic rhinitis, allergic asthma, and atopic dermatitis are a result of an immune response which in part involves the generation of allergen-specific IgE antibodies that leads to allergen-induced activation of mast cells and basophils. These cells, together with the leukocytes they recruit, are important for both the early acute response, the late response (4–24 h after antigen exposure), as well as the chronic phases of allergic inflammation. Although most hematopoietic cells lose c-Kit expression as they mature, both mast cells and dendritic cells express c-Kit as mature cells. Mast cells have their origin in hematopoietic CD13+/CD34+/CD117+ progenitors in the bone marrow. They migrate to the periphery and circulate in the vessels as immature progenitors (135, 191). This is by and large regulated by SCF that is produced by adjacent cells. Apart from its role in mast cell development, SCF-c-Kit signaling is also important for mast cell survival and its attachment to the submucosa, so-called homing. It is also important for mast cell degranulation and antigen-induced responses such as cytokine production. Given the role of mast cell in allergic responses, the SCF-c-Kit system is an attractive system for intervention. In several animal models, inhibition of the c-Kit signaling system has improved allergic asthma and other types of allergy. Treatment of mice exposed to cockroach antigen with anti-SCF antibodies inhibited the antigen induced airway hyperreactivity (22). Intranasal administration of SCF antisense oligonucleotides in a mouse ovalbumin-sensitized asthma model was shown to suppress lung inflammation as indicated by reduced interleukin (IL)-4 production and reduced eosinophil infiltration in the airways (75). Although these effects on asthma through inhibition of c-Kit signaling suggest an involvement of mast cells in this process, there is a possibility that additional c-Kit expressing immune cells at least in part could contribute to the effects seen.

The dendritic cell is a cell that in recent years has gained increased attention in relation to c-Kit effects. Krishnamoorthy et al. (145) demonstrated, using a mouse model, that the allergen house dust mite or the mucosal adjuvant cholera toxin promoted cell surface expression of c-Kit and SCF on dendritic cells. This led to sustained activation of c-Kit that promoted secretion of IL-6. Dendritic cells expressing functionally inactive c-Kit were unable to induce a robust T_{H}2 or T_{11}17 response. Those dendritic cells also showed a diminished allergic airway inflammation response when adoptively transferred into mice.

To summarize, these data collectively suggest that the c-Kit system (either c-Kit/SCF or downstream signaling molecules such as p110δ) could be a suitable target for pharmacological intervention in patients suffering from various allergic conditions. There are several animal studies published, where the c-Kit inhibitor imatinib (Gleevec) has been used to inhibit c-Kit, which resulted to attenuate allergic reactions. Animals who received a single dose of imatinib mesylate by oral gavage 30 min prior to challenge with cockroach allergen, demonstrated decreased pulmonary cytokine levels (21, 23). However, it is unclear whether these effects are due to c-Kit inhibition or inhibition of other proteins affected by imatinib. Although initially claimed to be rather specific, imatinib has been demonstrated to inhibit several tyrosine kinases (ABL, ARG, BCR-ABL, c-Kit, PDGFR, DDR1) as well as the nonkinase enzyme NAD(P)H dehydrogenase (95). Thus it is difficult to unequivocally conclude that the effects of imatinib are due to inhibition of c-Kit. When considering treatment of patients with these kinds of inhibitors, one might have to consider the risks that inhibitor treatment for extended periods of time might affect the immune system. In older mice, treatment with imatinib mesylate led to defects in the development of pro-B and pro-T cells (1).

IX. TARGETED INHIBITION OF c-KIT IN DISEASE

The fact that tyrosine kinases often are found mutated and constitutively active in various forms of human malignancies has prompted the development of selective inhibitors of tyrosine kinase activity. A multitude of such inhibitors have been developed and are in clinical trials or already in the clinic. The strategy of treating mutated, constitutively active receptor tyrosine kinases with antibodies are usually not successful since a great proportion of the expressed receptors reside inside the cell and are thus not accessible to the antibodies in the extracellular space. However, in cases of overexpression of wild-type receptors, therapeutic antibodies have proven to be a successful concept (e.g., Herceptin in the treatment HER2 overexpressing breast carcinoma). Most small molecule kinase inhibitors that exist are ATP competitive. The question of whether to use selective inhibitors or multitargeted inhibitors has been somewhat debated. On one hand one might aim for a selective inhibitor to really prove that a certain tumor is dependent on a particular kinase and to diminish the possible side effects. On the other hand, a multitargeted inhibitor might inhibit several signal transduction pathways that coexist in a given tumor, and thus provide a higher efficacy.

The first tyrosine kinase inhibitor to be used in the clinic, Gleevec (imatinib), was initially developed as an inhibitor of the BCR-ABL fusion protein as well as the platelet-derived growth factor receptor (68) and proved to be very effective in treating CML patients carrying the BCR-ABL
oncogenic fusion protein. It was later found also to inhibit c-Kit (101) and to be an efficient drug for treatment of patients with GIST (280). While imatinib is an effective inhibitor of wild-type c-Kit and some of the exon11 mutants found in GIST, several other c-Kit mutants, such as D816, are resistant to imatinib treatment (TABLE 1; Ref. 78). This discrepancy can be explained by the ability of imatinib to only inhibit the kinase in its inactive conformation. Apart from this type of primary resistance to imatinib, a number of patients develop with time resistance to imatinib. This can occur through several mechanisms, but one of the common mechanisms involves mutation of certain key residues in the kinases. This was originally described in imatinib-resistant CML where this type of mutation affects residues that are in close contact with the imatinib binding site and therefore interferes with binding of imatinib. This type of mutation includes the commonly observed mutation of the so-called gatekeeper residue, T670 in the case of c-Kit, which forms a hydrogen bond with imatinib within the ATP binding pocket (86, 268). A second class of mutations that render the kinases insensitive to imatinib involves residues that are important for determining the activation state of the kinase. Since imatinib binds to the inactive form of the kinase, mutations that either destabilize the inactive conformation or stabilize the active conformation are likely to display resistance against imatinib. Thus most of the mutations found in GISTs are located in the juxtamembrane region and sensitive to imatinib. However, upon development of resistance, secondary mutations occur in other parts of the receptor (such as exon 17) that renders the tyrosine kinase resistant to the inhibition of imatinib (7). One of the mutations resistant to imatinib treatment is D816V that is also commonly found in mastocytosis patients and certain types of AML.

To overcome primary as well as secondary resistance to imatinib in several tumor types, novel drugs targeting the kinase activity of c-Kit have been developed. Due to limitations in space, only the most well-studied and well-characterized inhibitors will be discussed. For a review on kinase inhibitors targeting c-Kit, see Reference 187. Dasatinib (Sprycel) and nilotinib (Tasigna) were developed as second-generation dual-specificity Src/Abl inhibitors (251, 295). In contrast to imatinib, they bind to their target kinases also when they are in the active conformation, which means that, e.g., the imatinib-resistant mutant D816V is inhibited by both dasatinib and nilotinib (243, 286). Interestingly, in contrast to what was initially thought, the various D816X mutants do not exhibit the same inhibitor profile. While wild-type c-Kit has an IC50 value of 93 nM for inhibition by dasatinib, the D816V mutant of c-Kit has a very similar value, 132 nM, but the D816H mutant is much more sensitive, displaying an IC50 value of only 2.6 nM (231). This suggests that although the various D816X mutants all lead to autoactivation of c-Kit, they are distinctive molecular entities with quite different characteristics.

It has been debated whether to use selective kinase inhibitors or multitargeted kinase inhibitors for the treatment of patients. Both approaches have, at least theoretically, both pros and cons. While selective kinase inhibitors might reduce the risk of unwanted side effects, many times tumors are dependent on more than one pathway, and the therapy against a single target might not be so effective. On the other hand, a kinase inhibitor that targets multiple targets might give rise to more unwanted side effects, while it might be more efficient since it targets several pathways of importance for the tumor. It should be noted that several tyrosine kinases that we originally described as dual specificity Src/Abl inhibitors, such as dasatinib and nilotinib, have proven to have a somewhat wider specificity. It has been shown that imatinib and nilotinib have a rather selective profile, only inhibiting six enzymes, while specificity is very broad. More than 20 tyrosine kinases have been described to be targeted by dasatinib (reviewed in Ref. 95). Interestingly, even a nonkinase enzyme, NADP(H) dehydrogenase (NQO2), was identified as a target of both imatinib and nilotinib but not dasatinib.

There is also a potential use for inhibitors targeting c-Kit in other diseases than cancer, such as asthma and allergy. In a patient study the c-Kit inhibitor masitinib was shown to improve disease control in severe corticosteroid-dependent asthmatics (116).

Novel approaches of designing inhibitors of c-Kit have been described in recent years. By exploiting the fact that direct contact between Ig-like domains 4 and 5 are needed for full activation of c-Kit, inhibitors are being developed that interfere with this interface (181).

**X. CONCLUSION**

Since the c-Kit/SCF system was discovered more than 20 years ago, a considerable body of knowledge on the biolog-

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**Table 1. Sensitivity of various oncogenic mutants of c-Kit for imatinib and dasatinib**

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Imatinib</th>
<th>Dasatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>V560D</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L576P</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K642E</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D816V</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D816F</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D816Y</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D816H</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N822K</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

In particular, mutations in exon11 behave similarly to wild-type c-Kit in that they are inhibited by both imatinib and dasatinib. In contrast, mutants with mutations in exon17 are resistant to imatinib inhibition, but are inhibited by dasatinib. The table is based on data found in References 8, 49, 100, 177, 243, 287.
ritical role of SCF and c-Kit has accumulated. We know much more about the mechanisms of signal transduction, the basis of cell type specific signaling, and the role of alternative splice forms of both SCF and c-Kit. Most of our knowledge on the signaling downstream of c-Kit is based on cell lines. Transgenic mice with the individual signaling molecules knocked out, or with the specific tyrosine residues in c-Kit mutated to phenylalanine residues, have provided us with more detailed information on the role of c-Kit in the physiological setting, but more work remains to provide us with a better picture of the physiological role of these pathways. While two of the alternative splice forms of c-Kit have been shown to show dramatic differences in signaling in cell lines, both in qualitative as well as in quantitative terms, no data on the role of alternative splicing in animals exist to date. The role of membrane-bound versus soluble SCF in c-Kit signaling as well as the mechanisms of synergy between SCF and other cytokines deserve a deeper investigation. Synergy between SCF and erythropoietin has been described in erythropoiesis, but does it also occur in other physiological settings? Some types of breast cancer express both c-Kit and the erythropoietin receptor. Does synergy between the two receptors play a role in breast cancer? Signals of proliferation and survival transmitted through c-Kit are likely to at least partially contribute to the initiation and progression of many human malignancies. However, activated c-Kit has also been shown to stabilize HIF-1α, thus contributing angiogenesis. Thus, to summarize, c-Kit is a suitable target for future drug development for the treatment of a multitude of human malignancies. The propensity of tumors to develop resistance to inhibition by c-Kit inhibitors emphasizes the importance of a deeper understanding of the c-Kit signaling machinery in cells expressing oncogenic mutants of c-Kit. With this knowledge, novel target molecules for pharmacological intervention can be discovered and novel therapeutic modalities can be developed. If one can target the specific pathways that are utilized by the oncogenic mutants of c-Kit, the risk of side effects should be reduced. It is also likely that simultaneous targeting of different signaling molecules will lead to synergistic anticancer effects and circumvent or at least delay the emergence of drug resistance.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


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