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Review

Signal transduction via the stem cell factor receptor/c-Kit

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Abstract. Together with its ligand, stem cell factor, the receptor tyrosine kinase c-Kit is a key controlling receptor for a number of cell types, including hematopoietic stem cells, mast cells, melanocytes and germ cells. Gain-of-function mutations in c-Kit have been described in a number of human cancers, including testicular germinomas, acute myeloid leukemia and gastrointestinal stromal tumors.
Stimulation of c-Kit by its ligand leads to dimerization of receptors, activation of its intrinsic tyrosine kinase activity and phosphorylation of key tyrosine residues within the receptor. These phosphorylated tyrosine residues serve as docking sites for a number of signal transduction molecules containing Src homology 2 domains, which will thereby be recruited to the receptor and activated many times through phosphorylation by the receptor. This review discusses our current knowledge of signal transduction molecules and signal transduction pathways activated by c-Kit and how their activation can be connected to the physiological outcome of c-Kit signaling.

Key words. Stem cell factor; c-Kit; Src homology 2; receptor tyrosine kinase; signal transduction.

c-Kit and SCF

The viral oncogene v-Kit was identified in 1986 as the transforming gene of the Hardy-Zuckerman 4 feline sarcoma virus [1], and shortly thereafter its cellular homolog, c-Kit, was cloned and sequenced [2]. A few years later, it was found that c-Kit is allelic with the dominant white spotting (*W*) of mice [3, 4]. A number of naturally occurring loss-of-function mutations in c-Kit have been found in both mice and humans. Complete loss of c-Kit expression leads to death in utero or perinatally, most likely due to severe anemia. Heterozygous animals display defects in pigmentation, reduced fertility and anemia. It has been found that the severity of the phenotype correlates inversely with the tyrosine kinase activity of the receptor. Mutations in the so-called Steel (*Sl*) locus in mice give rise to a phenotype very similar to mutations in c-Kit, and it was demonstrated that the product of the Steel locus was identical to stem cell factor (SCF), the ligand for c-Kit [5, 6]. For a review on W and Sl mutations, see [7].

c-Kit alternative splicing

As a result of alternative messenger RNA (mRNA) splicing, four isoforms of c-Kit have been identified in humans and two in mice. In both mice and humans alternative splicing results in isoforms characterized by the presence or absence of a tetrapeptide sequence (GNNK) in the extracellular part of the juxtamembrane region [8, 9], and occurs due to alternate use of 5′ splice donor sites [10]. Additionally, splice variants exist that differ in the presence or absence of a single serine residue in the kinase insert region of human c-Kit, due to alternative splice acceptor site usage [9]. Furthermore, a shorter transcript of c-Kit is expressed in postmeiotic germ cells of the testis. This encodes a truncated version of c-Kit (tr-kit) consisting only of the second part of the kinase domain, thus lacking the extracellular and transmembrane domains as well as the first part of the kinase domain [11]. This isoform therefore lacks functional kinase activity. However, despite this fact, it is able to signal. Mi-

croinjection of tr-kit into mouse eggs triggers metaphase-to-anaphase transition by the sequential activation of the Src family kinase (SFK) Fyn and phospholipase C-γ1 (PLC-γ1), and their association with Sam68 [12].
Variants GNNK+ and GNNK– (also denoted Kit and KitA, respectively) are co-expressed in most tissues [8, 9], with the GNNK– form predominating. Expression of the two isoforms has been studied in human acute myeloid leukemia (AML). It was shown that among various AML cell lines the ratio of the two isoforms varied from as low as 1.3 to as high as 12 [13]. In contrast, the ratio in normal bone marrow was around 4.4–5.5. However, no relation was found between the expression of either isoform and the response to therapy or other clinical parameters [13]. However, NIH3T3 cells expressing either isoform have been shown to differ in their transforming activity [14]. In the presence of the ligand SCF, the GNNK– form induced anchorage-independent growth, loss of contact inhibition and tumorigenicity. However, no difference in ligand affinity was observed between the two isoforms. It was further demonstrated that upon ligand stimulation, the GNNK– isoform was more highly tyrosine phosphorylated and more rapidly internalized, and it activated extracellular regulated kinase (Erk) more strongly than the GNNK+ isoform. In a recent study, Voytyuk et al. [15] showed that the kinetics of phosphorylation of the adapter protein ShcA, previously demonstrated to be phosphorylated by SFKs downstream of c-Kit, was stronger and more rapid in the GNNK– form. Inhibition of SFKs by treatment with the selective inhibitor SU6656 altered the kinetics of activation of the GNNK– form of c-Kit so that they resembled those of the GNNK+ form. Thus, a very minute difference in amino acid sequence in a region with no enzymatic function or substrate binding appears to lead to dramatic differences in signaling.

c-Kit and disease

Abnormal expression or function of c-Kit is found in several human diseases. Loss-of-function mutations in c-Kit are found in the rare disorder piebaldism. These mutations lead to deafness, megacolon and defective pigmentation of the hair and skin (for review, see [7]).
c-Kit has been implicated in a number of cancer forms in humans. In a number of tumor types, autocrine loops have been found, i.e. the tumors produce both SCF and c-Kit, leading to autonomous stimulation. These include small cell lung carcinomas, colorectal carcinoma, breast carcinoma, gynecological tumors and neuroblastomas [16–19].
Interestingly, loss of expression of c-Kit has also been associated with some tumor forms, such as in thyroid cancer [20], melanoma [21] and breast cancer [22]. Loss of c-Kit expression was found to be related to malignant transformation in the female breast, but not in the male breast [23].
Gain-of function mutations in c-Kit are found in a number of cancers, including mast cell leukemia, mastocytosis [24, 25], acute myeloid leukemia [26], germ cell tumors [27] and gastrointestinal stromal tumors (GISTs) [28]. Interestingly, in most tumor types the activating mutation resides close to the activation loop, at D816, in the second part of the kinase domain of c-Kit, while in GIST the activating mutations are either deletions or insertions in the juxtamembrane region of c-Kit.

Signal transduction through c-Kit

Signaling downstream of c-Kit has been studied extensively in a variety of cell systems. Mast cells are one of the cell types most commonly used to study c-Kit signaling. A number of studies have also used transient transfection systems, such as HEK293 cells or Cos cells. In some cases, investigators have used chimeras, i.e. the extracellular part of another receptor, e.g. the epidermal growth factor (EGF) receptor fused to the intracellular part of c-Kit. Therefore, it is difficult to directly compare the results from different investigators. Discrepancies in the literature will be discussed in this review. Regarding tyrosine residues in c-Kit, the numbering in this review refers to the amino acid sequence of human c-Kit, and in cases when experiments have been performed on murine c-Kit, the numbering system used is for simplicity that of the human c-Kit sequence.

Activation of c-Kit through ligand-induced dimerization

Binding of SCF to c-Kit results in dimerization of the receptors followed by activation of its intrinsic tyrosine kinase activity [29]. It is thought that dimerization is driven by the simultaneous binding of a dimeric SCF molecule to two receptor monomers [30, 31]. The activated receptor becomes autophosphorylated on a number of tyrosine residues (see fig. 1), mainly located outside the kinase domain, which serve as docking sites for signal transduction molecules containing Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains (for review, see [32]).
The c-Kit ligand SCF is expressed as a glycosylated transmembrane protein. Alternative splicing leads to two isoforms of SCF that differ in the absence or presence of a particular proteolytic cleavage site [33]. The isoform containing the cleavage site undergoes proteolysis and becomes soluble upon release from the plasma membrane, whereas the isoform lacking the cleavage site remains cell associated. Interestingly, the two isoforms

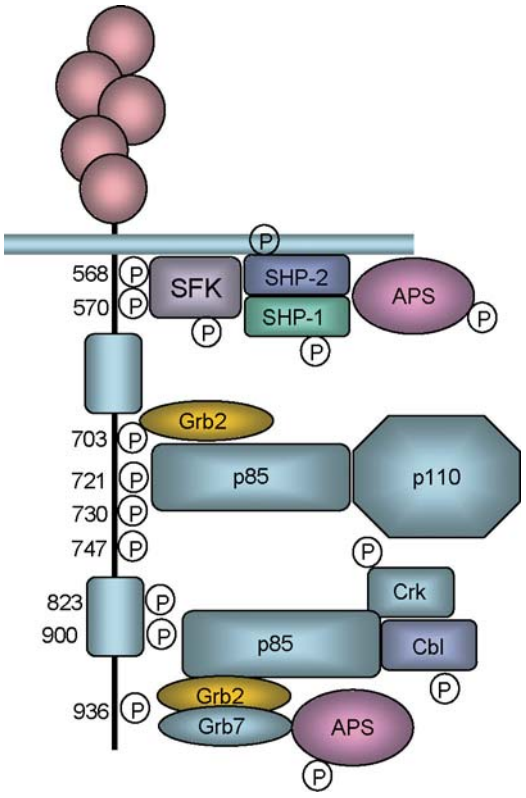

Figure 1. Signal transduction molecules binding to the activated c-Kit receptor. Upon ligand binding, c-Kit dimerizes, and its intrinsic tyrosine kinase activity is activated, leading to phosphorylation of key residues. These residues constitute high-affinity binding sites for signal transduction molecules. The numbers refer to tyrosine residues phosphorylated in c-Kit, and the corresponding signal transduction molecule is depicted.

have different abilities to transmit signals. Stimulation with the soluble isoform leads to rapid and transient activation and autophosphorylation of c-Kit, as well as fast degradation, whereas stimulation with the membrane-associated isoform leads to more sustained activation [34]. Differences also exist in signaling downstream of c-Kit. The membrane-bound ligand induced a more persistent activation of Erk1/2 and p38 mitogen-activated protein kinase (MAPK), as compared to the soluble ligand [35]. The differences in signaling might in part arise from the fact that membrane anchoring of the ligand might prevent internalization of the receptor-ligand complex. Using immobilized agonistic anti-Kit monoclonal antibodies to induce receptor dimerization in the absence of internalization, Kurosawa et al. could mimic the action of membrane-bound SCF [21].

Internalization and degradation of c-Kit

Ligand-induced downregulation of RTKs is an important phenomenon in the normal physiology of cell surface receptors. RTKs become ubiquitinated upon ligand stimulation, including c-Kit [29]. In the case of RTKs, monoubiqutination rather than polyubiqutination takes place (reviewed in [36]), targeting the receptors for internalization and degradation in the lysosomes. This is in contrast to polyubiquitination of cytosolic proteins, which targets them for degradation in the proteasomes.

Important players in the ubiquitination machinery are the ubiquitin E3 ligases, which covalently attach ubiquitin moieties to target proteins. In receptor tyrosine kinase (RTK) signaling, one of the important E3 ligases is the adapter protein Cbl, which binds to activated receptors and other tyrosine-phosphorylated proteins via its SH2 domain [37]. Cbl is activated through SFK-dependent phosphorylation [38]. Cbl is able to bind to activated c-Kit through the adapter proteins CrkL [39, 40] and APS [41] and is phosphorylated in response to SCF stimulation.

It is known from several studies that internalization of c-Kit is dependent on the activity of SFKs [15, 42, 43]. It is likely that this is due to SFK-dependent activation of Cbl and subsequent ubiquitination, but this remains to be shown.

Negative regulation of c-Kit signaling

Protein kinase C (PKC) is a family of serine/threonine kinases that are important regulators of several RTKs, including c-Kit [44]. Stimulation of c-Kit with soluble SCF results in phosphoinositide 3′-kinase (PI3-kinase)-dependent activation of phospholipase D [45], leading to release of phosphatidic acid, which can be dephosphorylated to yield diacylglycerol (DAG), an activator of PKC. The tyrosine kinase activity of c-Kit can be modulated through phosphorylation by PKC. Downmodulation of c-Kit activity by PKC occurs through dual mechanisms. Activation of PKC phosphorylates S741 and S746 in the kinase insert region of c-Kit, which leads to inhibition of kinase activity [44, 46]. Conversely, treatment of cells with the PKC inhibitor calphostin C resulted in enhanced c-Kit kinase activity and, furthermore, selectively increased activation of PI3-kinase [44]. Mutation of S741 and S746 to alanine residues resulted in a gain of function and markedly increased c-Kit tyrosine kinase activity [47]. In addition, treatment of cells with phorbol myristate acetate (PMA), an activator of PKC, results in proteolytic release of the ligand-binding domain of c-Kit, which leads to decreased responsiveness to SCF stimulation [48, 49].

The suppressors of cytokine signaling (SOCS) are a family of proteins that were originally cloned based on their ability to suppress cytokine signaling (for review, see [50]). They have a central SH2 domain flanked by an N-terminal domain of variable length and a C-terminal domain of 40 amino acids denoted the SOCS box. In a yeast

two-hybrid screen using c-Kit as bait, SOCS-1 was identified as an interactor with c-Kit [51]. Its expression is induced upon stimulation of mast cells with SCF, and it associates with c-Kit via its SH2 domain. In contrast to its function in cytokine signaling, SOCS-1 selectively suppressed c-Kit-stimulated mitogenesis, while not affecting survival signals. The mechanism does not involve inactivation of the tyrosine kinase activity of c-Kit, but through binding of Grb2 via its SH3 domain to SOCS-1, which in turn binds to Vav [51]. Interestingly, targeted deletion of SOCS-1 did not lead to enhanced c-Kit signaling in bone marrow-derived mast cells, as one might have expected, but rather a reduced proliferative response to SCF stimulation [52]. Furthermore, deletion of SOCS-1 led to increased levels of proteases, leading to degradation of signal transduction molecules.

The protein tyrosine phosphatase SHP-1 interacts with Y570 of c-Kit and negatively regulates c-Kit signaling [53, 54]. SHP-1 consists of two SH2 domains and a carboxy-terminal protein tyrosine phosphatase domain. The motheaten (*me*) mice express a loss-of-function mutation in SHP-1 and show a hyperproliferative phenotype of their hematopoietic progenitor cells [55]. However, loss of SHP-1 function did not affect SCF-induced proliferation of bone marrow-derived mast cells, suggesting that the role of SHP-1 might to some extent be cell-type specific [56].

The Ras/Erk pathway

Numerous studies have implicated the critical importance of the Ras/Erk pathway in cell division and survival (for review, see [57]). Ras is a small G-protein that can oscillate between an active GTP-bound form and an inactive GDP-bound form. Although Ras can activate a number of signal transduction molecules such as Rac or PI3-kinase [58, 59], its role in the Ras/Erk cascade is the most well characterized. RTKs activate Ras through association with Sos, a guanine nucleotide exchange factor that facilitates exchange of GDP for GTP, leading to activation of Ras. Sos exists in the cell in a preformed complex with the adapter protein Grb2, which in turn associates via its SH2 domain to phosphorylated tyrosine residues within the consensus sequence p-YXN. These tyrosine residues exist either in the receptor or in downstream signal transduction molecules such as the protein tyrosine phosphatase SHP-2 or the adapter protein ShcA [60–62]. Thus, the Grb2-Sos complex is recruited to the vicinity of the plasma membrane, where it can act on Ras. Activated Ras has the ability to interact with the serine/threonine kinase Raf-1, leading to its activation. The targets for Raf-1 kinase activity are the dual-specificity kinases Mek1 and Mek2 [63], which are activated by phosphorylation. The serine/threonine kinases Erk1 and Erk2 are activated through phosphorylation by Mek1/2 [64]. Activated Erks dimerize and are translocated to the nucleus [65], where transcription factors are phosphorylated whereby their activities are regulated, influencing gene transcription [66].

A number of studies have demonstrated the ability of SCF to activate the Ras-Erk pathway. The adapter protein Grb2 can directly associate with phosphorylated Y703 and Y936 in c-Kit [67]. In addition, Grb2 can associate with SHP-2 or ShcA following SCF stimulation [68, 69]. Furthermore, the adaptor protein Gab2 can link to the Ras/Erk pathway through association with SHP-2 [70]. Several studies have indicated an important role for SCF-induced activation of SFKs in activation of the Ras/Erk cascade [71–73]. In contrast, others have shown no effect for SFK inhibition on the activity of Erk [74]. Independence of PI3-kinase for activation of Erk in mast cells was also demonstrated. However, under certain conditions activation of Erk has been implicated to be dependent on the activity of PI3-kinase. Recently, Wandzioch et al. [75] showed that inhibition of PI3-kinase with the pharmacological agent LY294002 effectively inhibited Erk phosphorylation in a hematopoietic progenitor cell line. This is similar to findings by others showing that it was possible to inhibit PDGF-induced Erk activation by addition of a PI3-kinase inhibitor under conditions of low receptor expression, whereas cells expressing high levels of PDGF receptors were unaffected [76].

PI3-kinase

PI3-kinase is a class of lipid kinases that phosphorylate the 3′ hydroxyl group of phosphoinositides, phosphatidylinositol-4,5-bisphosphate (PIP_2) being the physiologically relevant substrate (for review, see [77]). The resulting product, phosphatidylinositol-3,4,5-trisphosphate (PIP_3), is able to physically associate with proteins containing a pleckstrin homology (PH) domain, leading to their recruitment to plasma membrane where they can be activated.

Most published work on PI3-kinase is focused on the classical class I PI3-kinases. The regulatory p85 subunit contains two SH2 domains by which it binds to target proteins, whereby conformational changes are induced and PI3-kinase enzyme is activated [78]. The p110 subunit contains enzymatic activity to phosphorylate phosphoinositides. The two subunits exist in the cell as a preformed complex. Interestingly, there is a stoichiometric excess of the p85 subunit within the cell [79], suggesting other functions of p85. It is well known that p85 also has the ability to associate with the adapter proteins Cbl, CrkII and CrkL, respectively [39, 80, 81].

Activation of PI3-kinase by c-Kit has been linked to mitogenesis, differentiation, survival, adhesion, secretion and

actin cytoskeletal reorganization [47, 74, 82–84]. In c-Kit, Y721 has been found to directly interact with PI3-kinase [82]. c-Kit promotes survival via PI3-kinase-dependent activation of Akt and phosphorylation of Bad, a pro-apoptotic molecule, at S136 in vivo. Furthermore, mutation of S741 and S746, the two serine sites involved in negative regulation by PKC, led to increased mitogenic response and increased activation of PI3-kinase, as well as enhanced Akt activation, Bad phosphorylation and survival [47]. An alternative pathway for c-Kit-mediated survival is through Akt-mediated phosphorylation and inactivation of the forkhead transcription factor FoxO3a [85].

A number of studies have implicated the importance of PI3-kinase activity in transformation through mutated c-Kit. Using immortalized murine progenitor cells transduced with the Y721F mutant D816V c-Kit, Chian et al. [86] showed that transformation by this constitutively active form of c-Kit is dependent on PI3-kinase. In another study, mutants with the cytoplasmic tyrosines of c-Kit individually mutated to phenylalanine residues were used to assess their roles in D814V transformation [87]. Two mutants severely impaired receptor activation, Y719F (in murine c-Kit) and a deletion mutant in which the two most distal tyrosines in the carboxy-terminal tail were mutated, including Tyr936 previously shown to bind to Grb2, Grb7 and APS [41, 67]. Interestingly, these mutants showed no effect on normal ligand-induced activation of c-Kit.

The physiological role of c-Kit-mediated activation of PI3-kinase was demonstrated in two studies using transgenic mice expressing c-Kit with a Y719F mutation (corresponding to Y721F in human c-Kit). Blume-Jensen et al. [88] showed that c-Kit-induced activation of PI3-kinase was essential for male fertility, while in another study Kissel et al. [89] also demonstrated an effect on female fertility. Thus, abrogated c-Kit-mediated PI3-kinase signaling may be compensated for in a number of biological processes, but appears to be critical in spermatogenesis and oogenesis. These findings together suggest that Y719 solely serves as the docking site for PI3-kinase and for no other signal transduction molecule. This, however, remains to be proven. One of the PI3-kinase association sites present in the closely related PDGF β-receptor Y751 is also known to bind to the adapter protein Nckα [90]. Thus, some of the phenotypes found using this kind of approach might be due to additional, hitherto unknown interactions.

Bone marrow-derived mast cells from mice with a targeted deletion of the p85α subunit of PI3-kinase demonstrated a dramatically reduced SCF-mediated proliferative response, compared to wild-type cells, further emphasizing the role of PI3-kinase in c-Kit signaling [91]. This effect paralleled a reduction of SCF-induced activation of JNK in p85α-deficient mast cells. Interestingly, SCF-stimulated activation of Akt was only partially impaired in p85α-deficient bone marrow-derived mast cells, and no effect on c-Kit mediated survival was observed. Therefore, additional signal transduction pathways may contribute to c-Kit-mediated survival. In addition, SCF-mediated chemotaxis has also been demonstrated to be dependent on p85α [92].

It should be noted that discrepancies do exist in results using p85α-deficient cells and Y721F mutant c-Kit. Perhaps Y721 is able to dock to signal transduction molecules other than PI3-kinase. Furthermore, p85α is known to bind to proteins other than the p110 subunit of PI3-kinase. Further studies are needed to precisely define the contribution of PI3-kinase in SCF-mediated signaling.

Apart from the classical type I forms of PI3-kinase, the type II isoform PI3KC2β was shown to physically associate with activated c-Kit and mediate part of the SCF-dependent activation of Akt in small lung carcinoma cells [93]. Interestingly, PI3KC2β association with c-Kit was ligand independent and constitutive. However, ligand stimulation of c-Kit led to tyrosine phosphorylation of PI3KC2β. The site of interaction with c-Kit is not known, although given that association seems constitutive and that other receptors previously shown to interact with the classical isoforms of PI3-kinase did not interact with PI3KC2β, it appears that the classical PI3-kinase association site is not involved.

PLC-γ

Phospholipase C-γ (PLCγ) exists as two isoforms, PLC-γ1 and PLCγ2. They both consist of two SH2 domains, one SH3 domain, one PH domain and a catalytic domain. While PLCγ1 is ubiquitously expressed, PLC-γ2 is mainly expressed in the hematopoietic system (for review, see [94]). PLC hydrolyses the phosphoinositide PIP_2, thereby generating the second messengers DAG and inositol-1,4,5-trisphosphate (IP_3). DAG is an activator of the classical and novel forms of PKC, while IP_3 binds to specific receptors present on the endoplasmic reticulum, triggering release of Ca^{2+} from internal stores. The intracellular concentration of free Ca^{2+} regulates a number of cellular processes (for review see [95]).

Although some studies have demonstrated association with and activation of PLCγ by c-Kit, others have failed to do so. It has been claimed that Y730 is the site of association of PLC-γ1. Herbst et al. [96] overexpressed EGFR-c-Kit chimeras together with PLC-γ1 in HEK293 cells and found tyrosine phosphorylation of PLC-γ1, although weaker than that seen with the EGF receptor. In a later study, the same authors saw no association between PLC-γ1 and an EGFR-c-Kit chimera unless they overexpressed PLC-γ1 [97]. However, those studies were performed using chimeric receptors with the extracellular domain of the EGF receptor fused to the intracellular part of c-Kit. Furthermore, association was only seen when the receptor

was overexpressed together with overexpressed PLC-γ1. In a more recent study, Gommerman et al. [98] studied the differential stimulation of various c-Kit mutants by membrane-bound and soluble SCF, respectively, using retrovirally transduced 32D cells. Using soluble SCF, a weak SCF-stimulated tyrosine phosphorylation of PLC-γ1 was shown in wild-type murine c-Kit-expressing cells, but in cells expressing the Y728F mutant (corresponding to Y730 in human c-Kit), no phosphorylation of PLC-γ1 was seen. Furthermore, cells expressing the Y728F mutant c-Kit did not respond with calcium mobilization following treatment with SCF. Interestingly, PLC-γ1 phosphorylation was much stronger in bone marrow-derived mast cells than in 32D infectants. In contrast, Koike et al. [99] failed to detect c-Kit-mediated activation of PLC-γ, but were able to detect SCF-dependent activation of phospholipase D. These data were confirmed by Kozawa et al. [45], who also were able to inhibit SCF-stimulated PLD activity with the PI3-kinase inhibitor LY294002. Also studying bone marrow mast cells, Huber et al. detected a robust and sustained SCF-stimulated tyrosine phosphorylation of PLC-γ2 [100]. Likewise, Triselmann et al. [101] showed that stimulation of mast cells by membrane-bound, but not soluble SCF, was dependent on PLC-γ activation. They also demonstrated ligand-induced tyrosine phosphorylation of PLC-γ2. Other studies have shown that activation of PLC-γ by c-Kit might be involved in SCF-mediated protection against apoptosis induced by chemotherapy and radiation [102, 103].

It is possible that some of the discrepancy in the findings as to whether PLC-γ is activated or not might arise from different expression levels of the two isoforms of PLC-γ in different cell types, with the cell types expressing PLC-γ2 showing stronger activation of PLC. Another possible explanation for differences in the data on activation of PLC-γ1 might be the differential signaling abilities of alternative splice forms of c-Kit. It is known that the two alternative splice forms denoted GNNK+ and GNNK– do signal at quantitatively and qualitatively different levels [14, 15]. Thus, expression of various splice forms of c-Kit might influence the outcome of studies on PLC-γ activation. Given the fact that a direct physical interaction between c-Kit and PLC-γ isoforms has not been demonstrated, except in cells overexpressing the receptor and PLC-γ1, it is possible that activation of PLC-γ might be a result of activation of other tyrosine kinases downstream of c-Kit. This might not necessarily require a direct physical association between PLC-γ and c-Kit.

The Src family of tyrosine kinases

The SFK family of tyrosine kinases is named after its prototypic family member c-Src, the cellular homolog of the transforming protein of Rous sarcoma virus, v-Src. Some members, such as Src, Yes and Fyn, are ubiquitously expressed, while others, such as Lck, Hck, Fgr, Lyn and Blk, have a more tissue-restricted expression, mainly in hematopoietic cell types (reviewed in [104]). They consist of an N-terminal sequence, which directs myristoylation, and in same cases palmitoylation, which serves to anchor the kinases to the plasma membrane. They also contain an SH3 domain, an SH2 domain and a tyrosine kinase domain. They have been implicated in a number of cellular functions, including adhesion, chemotaxis, survival, proliferation and protein trafficking.

Binding of SCF to c-Kit leads to a rapid increase in SFK kinase activity [105, 106]. A number of investigators have shown that SFKs associate primarily with phosphorylated Tyr568, while Tyr570 contributes to the overall affinity of binding by acting as an acidic determinant [72, 74, 107]. SCF-induced chemotaxis of Mo7 cells was dependent on SFK activity [42]. In another study, overexpression of a dominant negative form of Lyn in either primary hematopoietic progenitor cells or bone marrow-derived mast cells led to inhibition of both SCF-mediated proliferation and chemotaxis [108]. In Mo7e cells, activation of the SFK Lyn was demonstrated to occur during the late G1 phase of SCF-stimulated cell cycle progression [109]. Using an approach where 32D cells were transfected with chimeric c-Kit containing the extracellular domain of the M-CSF receptor, and by mutating seven tyrosine residues of the intracellular part of c-Kit, Hong et al. demonstrated a complete loss of mitogenic response of 32D cells [110]. However, by adding back Y568 and Y570 to this mutant, the mitogenic response was restored, as well as survival and migration. Furthermore, restoration of the Src binding sites also led to restored activation of the Ras/Erk pathway. This is in agreement with previous findings that SFKs play an important role in phosphorylating ShcA, thereby recruiting the Grb2-Sos complex, leading to activation of Ras [71, 72]. In addition, SCF-induced activation of other signal transduction molecules such as Rac and JNK was shown to be restored by adding back Y568 and Y570.

The function of SFKs in a more physiological context was addressed by Agosti et al. [111], who generated transgenic mice carrying c-Kit with a Y568F mutation. They found that mutation of Y568, the primary binding site of SFKs in c-Kit, led to a block in pro T cell and pro B cell development, in contrast to the Y721F mutant (affecting PI3-kinase activation), which had no effect on hematopoiesis. These data suggest that SFKs mediate a critical signal for lymphocyte development. However, one of the difficulties in interpreting these data lies in the probability that additional signal transduction molecules apart from SFKs might be involved. For example, the protein tyrosine phosphatase SHP-2 [53], the tyrosine kinase CHK [112] and the adaptor protein APS [41] have also been shown to bind to phosphorylated Y568.

The JAK/STAT pathway

The Janus kinases (JAKs) are cytoplasmic tyrosine kinases that are activated through ligand stimulation of cytokine receptors or RTKs. Downstream of JAKs are the signal transducers and activators of transcription (STATs), which are phosphorylated by JAKs. STAT proteins are a class of transcription factors with DNA binding domains, an SH2 domain and a carboxy-terminal transactivating domain. Upon tyrosine phosphorylation, STATs dimerize through phosphotyrosine interaction within their SH2 domains, and the dimerized STATs translocate to the nucleus, where they regulate expression of responsive genes (for review, see [113]). The JAK-STAT pathway is activated following SCF stimulation. c-Kit stimulates rapid and transient tyrosine phosphorylation of JAK2 [114]. JAK2 was found to be constitutively associated with c-Kit, with increased association after ligand stimulation of c-Kit [115]. Furthermore, treatment of cells with JAK2 antisense oligonucleotides resulted in a marked decrease in SCF-induced proliferation, suggesting a role for JAK2 in c-Kit-mediated signaling. In addition, SCF-induced growth of fetal liver cells was shown to be reduced in mice with a targeted deletion of JAK2 [116]. Furthermore, JAK2 was also required for differentiation of the Kit+ progenitor cells into mast cells.

Activation of c-Kit leads to physical association with and activation of STAT1α, STAT3, STAT5A and STAT5B [26, 117–119]. It has been shown that STAT3 activation is required for the constitutively active D816H mutant of c-Kit to be tumorigenic [26].

However, as is the case with the activation of PLC-γ, other investigators have failed to detect activation of the JAK/STAT pathway by c-Kit [120–122]. Reasons for this might be due to cell type-specific effects or to the experimental setup.

Other tyrosine kinases: Tec, CHK, Fer and Fes

Tec belongs to a family of tyrosine kinases that also includes the Bruton tyrosine kinase (Btk), Bmx, Itsk/Tsk and Rlk/Txk (for review, see [123]). They each contain a PH domain and a Tec homology (TH) domain in the amino-terminus followed by SH3, SH2 and tyrosine kinase domains. In contrast to the SFKs, they lack a membrane-targeting myristoylation site, but are recruited to the plasma membrane through the PH domain interacting with PIP3. Activation of Tec family kinases is thought to be mediated by members of the Src family.

Tec has been shown to become phosphorylated on tyrosine and activated upon stimulation of c-Kit with SCF [124]. It was later shown that Tec forms multiprotein complexes with Dok-1 and Lyn [125, 126]. Phosphorylation of Tec and Dok1 was dependent on recruitment to the plasma membrane through activation of PI3-kinase [125]. Both Lyn and Tec were capable of phosphorylating Dok-1, but using cells derived from animals with a targeted deletion of Lyn [126] showed that Lyn was required for SCF-dependent phosphorylation of Dok-1.

CHK (for Csk homologous kinase, also known as MATK) shows ~50% sequence similarity with Csk and phosphorylates SFKs. Similarly to SFKs, CHK were demonstrated to associate to the phosphorylated juxtamembrane region of ligand-stimulated c-Kit, specifically to Y568 [112].

Fer and Fes are structurally related cytoplasmic tyrosine kinases. They both contain an SH2 domain immediately upstream of the kinase domain. Following SCF stimulation Fer associates with c-Kit and becomes phosphorylated on tyrosine residues [127]. Using mast cells derived from mice with a kinase-inactivating mutation of Fer, Craig and Greer [128] found a requirement for Fer kinase activity for sustained p38 kinase activation and maximal chemotactic response to SCF. Fes was found to bind to c-Kit [129], although its role in c-Kit signaling remains to be shown.

Adaptor proteins

Adapter proteins are proteins with several domains that specify protein-protein interactions, and which thereby enables them to interact with several proteins simultaneously. The ability of linking proteins together through specific and many times regulated protein-protein interactions enables signaling to be spatially and sequentially regulated (for review, see [130]).

Grb2 was originally identified as a protein interacting with the phosphorylated EGF receptor [61] and found to mediate activation of the Ras/Erk pathway by RTKs. Grb2 is a ubiquitously expressed protein containing one SH2 domain and two SH3 domains. Tyrosine phosphorylated c-Kit has been shown to associate with Grb2 (see also above).

The adapter protein Gads (also denoted Mona, Grap2, GrpL or Grf40) is closely related to Grb2 and expressed in hematopoietic cells (for review, see [131]) and has been shown to interact with c-Kit in a manner similar to Grb2 [132]. Another member of the same family of adapter proteins with a very similar structure, Grap, also interacts with c-Kit [133].

ShcA is a ubiquitously expressed adapter protein that contains one SH2 domain and a PTB domain that both enable ShcA to interact with phosphorylated proteins (for review, see [134]). Phosphorylation of ShcA by RTKs, directly or indirectly via SFKs, leads to creation of high-affinity binding sites for Grb2, leading to activation of the Ras/Erk pathway. In vitro data suggest that ShcA interacts with the juxtamembrane domain of c-Kit [107].

The Grb7 family of adaptor proteins consists of Grb7, Grb10 and Grb14, which each exist in several splice form variants (for review, see [135]). Grb7 contains an SH2 and a so-called GM region (for Grb and Mig), which includes a PH domain and shows sequence homology with the *Caenorhabditis elegans* protein Mig-10, which has been implicated in embryonic migration. Grb7 interacts with activated c-Kit through Y936 in the carboxy-terminal tail of the receptor [67]. However, the role of Grb7 in c-Kit signaling remains to be elucidated. Grb10 was identified in a yeast two-hybrid screen using the constitutively active mutant D816V of c-Kit as bait [136]. The interaction between Grb10 and c-Kit is mediated through its SH2 domain, while the PH domain mediates interaction with the serine/threonine kinase Akt. It was further demonstrated that Grb10 and c-Kit are able to activate Akt in a synergistic manner.

The adaptor protein Lnk, together with APS and SH2-B, belongs to a family of closely related adapter proteins. All three proteins share common features in that they contain a conserved amino-terminal domain that includes a proline-rich stretch, a PH domain and an SH2 domain. They all contain a conserved tyrosine residue in their carboxy-termini that is presumed to be a phosphorylation site [137]. Using transgenic mice lacking the expression of Lnk, it was shown that B cell precursor cells were hypersensitive to SCF stimulation [138], leading to proportional accumulation of B cell precursors in the bone marrow and B cells in the spleen of transgenic mice. Thus, Lnk seems to have a negative regulatory role in B cell production.

APS was originally identified in a yeast two-hybrid screen using c-Kit as bait [139]. When APS is phosphorylated in its carboxy-terminal tail, it is able to physically associate with c-Cbl [140, 141]. Being a ubiquitin E3-ligase, c-Cbl is able to covalently link ubiquitin to activated RTKs, leading to their internalization and degradation. The primary association sites for APS in c-Kit have been shown to be Y568 and Y936 [41]. Mutation of both Tyr568 and Tyr936 was necessary to completely block binding of APS to c-Kit. Recently, it was shown that APS exists as a dimer [142], which might explain why both sites are needed for full binding of APS to c-Kit. Interestingly, both Y568 and Y936 are missing in the viral counterpart of c-Kit [143]. Thus, it has been speculated that loss of APS binding in v-Kit could possibly lead to reduced ubiquitination and prolonged receptor signaling, which could possibly contribute to transformation. A number of transforming mutants of RTKs have been shown to lack association sites for Cbl, leading to reduced ubiqutination and stabilization of the receptors (reviewed in [144]).

However, the physiology of mice with a targeted deletion of APS does not support a major role for APS in c-Kit signaling. The effects are mainly related to the immune system [145], although mast cells derived from APS knockout animals show a markedly augmented degranulation in response to c-Kit stimulation, as well as lower levels of F-actin [146]. In contrast, targeted deletion of either Lnk or SH2-B did not lead to any marked effect on mast cell behavior.

The Crk family of adapter proteins consists of one SH2 domain, as well as one or two SH3 domains. The family consists of four members: CrkI and CrkII (alternative splice forms of the same gene), CrkL (reviewed by [147]) and the recently discovered CrkIII [148]. SCF stimulation of c-Kit leads to stimulation of CrkL [39], which indirectly associates with c-Kit through the p85 subunit of PI3-kinase. In addition, CrkL mediates interaction with Cbl, which likely contributes to c-Kit ubiquitination and degradation. The closely related protein CrkII was also shown to be phosphorylated in response to SCF stimulation and interacted with c-Kit indirectly via the p85 subunit of PI3-kinase [81]. This interaction was dependent on phosphorylation of Y900 in the second part of the kinase domain. Y900 is not an autophosphorylation site, but is phosphorylated through the action of SFKs.

Dok-1 is an adapter protein of 62 kDa first identified as a tyrosine-phosphorylated protein associated with p120-RasGAP in fibroblasts transfected with v-Src [149]. It contains a PH domain and a phosphotyrosine binding (PTB) domain. Cells from Dok-1 knockout animals hyperproliferate in response to a number of growth factors and cytokines, suggesting a role of Dok-1 as a negative regulator of cell proliferation [150]. Dok-1 was found to associate with activated c-Kit in chronic myelogenous leukemia progenitor cells [151].

The Gab proteins are a family of scaffolding adaptors with similar overall structural organization (reviewed in [152]), containing an N-terminal PH domain, proline-rich motifs that can interact with SH3 domains and multiple tyrosine phosphorylation sites that can serve as docking sites for SH2 domains. Both Gab-1 and Gab-2 are phosphorylated in response to SCF-stimulation [153]. While Gab-1 does not seem to be essential for c-Kit signaling, Gab-2 is required for mast cell development and c-Kit signaling [154]. Bone marrow mast cells derived from Gab-2-deficient mice grew poorly in response to SCF, and both Erk and Akt activation were impaired.

Protein tyrosine phosphatases

The two closely related protein tyrosine phosphatases (PTPs) SHP-1 and SHP-2 constitute a family of proteins consisting of two amino-terminal SH2 domains, a PTP domain and a carboxy-terminal tail (reviewed in [155]). A number of SHP-binding proteins have been reported, including activated RTKs and cytokine receptors, as well as scaffolding adaptors, such as Gab proteins. Activation of

SHPs occurs through binding of the SH2 domains to tyrosine-phosphorylated peptides, in particular biphosphorylated, that open up the phosphatase structure. SHP-1 associates with phosphorylated Y570 in c-Kit [53] and is involved in negative regulation of c-Kit signaling (see also above). In contrast, SHP-2 is, despite the fact that it is a phosphatase, a positive regulator of signaling. SHP-2 physically interacts with SCF-stimulated c-Kit and becomes phosphorylated on tyrosine residues [68]. The site of interaction was shown to be Y568 in the juxtamembrane region of c-Kit [53], which also constitutes the docking site for a number of other signal transduction molecules, such as SFKs, CHK and APS. In most RTK signaling, SHP-2 activation is required for full activation of the Ras/Erk pathway, e.g. the PDGF β-receptor [156]. SHP-2 also plays an important role in mediating embryonic stem cell differentiation and hematopoiesis [157].

PTP-RO is a PTP lacking SH2 domains, but is still able to associate with the c-Kit receptor [158]. Furthermore, PTP-RO becomes phosphorylated on tyrosine residues after SCF stimulation of cells. By use of antisense oligonucleotides, the function of PTP-RO could be inhibited, which led to significantly inhibited proliferation of Mo7e cells [158].

Transcription factors

A number of genes are induced upon SCF stimulation of cells. One of these is Slug, a member of the Snail family of zinc finger transcription factors. Slug-deficient mice show pigment deficiency, gonadal defects and impairment of hematopoiesis, very much reminiscent of the phenotype of loss-of-function mutations in c-Kit [159]. It was shown that despite the expression of c-Kit, cells from Slug knockout animals were defective in SCF-induced migration, suggesting a role for Slug downstream of c-Kit. It was recently shown that Slug function in c-Kit mediated radioprotection [160].

The Mitf protein is a member of the MYC superfamily of basic helix-loop-helix leucine zipper (bHLHZip) transcription factors [161–163] and is closely related to three other bHLHZip transcription factors, Tfe3, Tfeb and TfeC. The phenotype of Mitf mutant mice shows a striking similarity to that of mice with loss-of-function mutations of c-Kit or its ligand (spotted fur color, mast cell deficiency; reviewed in [163], suggesting a functional link between the Mitf transcription factor and c-Kit and its ligand. Recent in vitro experiments indicate that the activity of the Mitf transcription factor is regulated by signaling through the c-Kit receptor tyrosine kinase. This cell signaling ultimately results in effects on the activation potential and/or stability of the Mitf protein [164–166]. SCF stimulation of c-Kit activates Erk2 and results in phosphorylation of S73 of Mitf. Co-transfection experiments have shown a significant difference in the transcriptional activation potential of a Mitf protein phosphorylated at S73 and a version containing the unphosphorylatable S73A mutation. Furthermore, Price et al. [167] have shown that only the phosphorylated version of Mitf can interact with the p300 co-activator protein. A second phosphorylation event has been shown to link the c-Kit receptor and Mitf. This is the phosphorylation of amino acid S409 by the p90/Rsk kinase, which itself is activated by Erk2, the same kinase that phosphorylates S73 of the Mitf protein. This event has been postulated to affect the stability of the protein such that Mitf protein phosphorylated on S409 is degraded more rapidly than a mutant S409A Mitf protein. This was shown to be due to increased ubiquitination of the protein and proteosome-dependent degradation [165].

Conclusions

Since the discovery of SCF as the ligand of c-Kit almost 14 years ago, numerous studies have contributed to our knowledge about the mechanism of action of c-Kit. A multitude of signaling pathways are activated by SCF, leading to diverse biological responses such as chemotaxis, proliferation, differentiation and survival. Using a number of different cell systems, investigators have many times found similar mechanisms of action of c-Kit, but sometimes also differences. The exact reason for these discrepancies is not fully understood. Some studies were conducted on transfected fibroblasts that express a different repertoire of signal transduction molecules than hematopoietic cells, possibly giving rise to activation of different signal transduction pathways. Also, the differentiation state of hematopoietic cell lines is likely to influence the response elicited by SCF stimulation. Several splice forms of c-Kit have been demonstrated to exist, with sometimes different signaling capabilities, both quantitatively and qualitatively. Very little is known about how the expression of these different splice forms is regulated during development and differentiation. It is not unlikely that differences in signaling shown in the literature might be due to differences in expression of various splice forms of c-Kit in different cell types. Furthermore, the qualitative differences in signaling of the membrane-bound versus soluble form of SCF have been demonstrated in a number of studies. The use of transgenic animals with targeted deletions of individual signal transduction molecules and the use of so-called knockin methodology to introduce specific mutants of c-Kit in animals have proven invaluable tools for our understanding of c-Kit signaling. In order to be able to study c-Kit signaling in hematopoietic development, more sensitive methods for the study of signaling in individual cells will be of utmost importance. Understanding of the mecha-

nisms of synergy between SCF and various cytokines is also a challenging field for future research. Increased knowledge of molecular mechanisms of c-Kit signaling in diseases such as cancer is paramount to the potential development of targeted therapies aiming at inhibiting specific c-Kit signaling pathways.

1 Besmer P., Murphy J. E., George P. C., Qiu F. H., Bergold P. J., Lederman L. et al. (1986) A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. Nature **320:** 415–421
2 Yarden Y., Kuang W. J., Yang-Feng T., Coussens L., Munemitsu S., Dull T. J. et al. (1987) Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J. **6:** 3341–3351
3 Chabot B., Stephenson D. A., Chapman V. M., Besmer P. and Bernstein A. (1988) The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. Nature **335:** 88–89
4 Geissler E. N., Ryan M. A. and Housman D. E. (1988) The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. Cell **55:** 185–192
5 Copeland N. G., Gilbert D. J., Cho B. C., Donovan P. J., Jenkins N. A., Cosman D. et al. (1990) Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. Cell **63:** 175–183
6 Williams D. E., Eisenman J., Baird A., Rauch C., Van Ness K., March C. J. et al. (1990) Identification of a ligand for the c-kit proto-oncogene. Cell **63:** 167–174
7 Lev S., Blechman J. M., Givol D. and Yarden Y. (1994) Steel factor and c-kit protooncogene: genetic lessons in signal transduction. Crit. Rev. Oncog. **5:** 141–168
8 Reith A. D., Ellis C., Lyman S. D., Anderson D. M., Williams D. E., Bernstein A. et al. (1991) Signal transduction by normal isoforms and W mutant variants of the Kit receptor tyrosine kinase. EMBO J. **10:** 2451–2459
9 Crosier P. S., Ricciardi S. T., Hall L. R., Vitas M. R., Clark S. C. and Crosier K. E. (1993) Expression of isoforms of the human receptor tyrosine kinase c-kit in leukemic cell lines and acute myeloid leukemia. Blood **82:** 1151–1158
10 Hayashi S., Kunisada T., Ogawa M., Yamaguchi K. and Nishikawa S. (1991) Exon skipping by mutation of an authentic splice site of c-kit gene in W/W mouse. Nucleic Acids Res. **19:** 1267–1271
11 Rossi P., Marziali G., Albanesi C., Charlesworth A., Geremia R. and Sorrentino V. (1992) A novel c-kit transcript, potentially encoding a truncated receptor, originates within a kit gene intron in mouse spermatids. Dev. Biol. **152:** 203–207
12 Paronetto M. P., Venables J. P., Elliott D. J., Geremia R., Rossi P. and Sette C. (2003) Tr-kit promotes the formation of a multimolecular complex composed by Fyn, PLCgamma1 and Sam68. Oncogene **22:** 8707–8715
13 Piao X., Curtis J. E., Minkin S., Minden M. D. and Bernstein A. (1994) Expression of the Kit and KitA receptor isoforms in human acute myelogenous leukemia. Blood **83:** 476–481
14 Caruana G., Cambareri A. C. and Ashman L. K. (1999) Isoforms of c-KIT differ in activation of signalling pathways and transformation of NIH3T3 fibroblasts. Oncogene **18:** 5573–5581
15 Voytyuk O., Lennartsson J., Mogi A., Caruana G., Courtneidge S., Ashman L. K. et al. (2003) Src family kinases are involved in the differential signaling from two splice forms of c-Kit. J. Biol. Chem. **278:** 9159–9166
16 Krystal G. W., Hines S. J. and Organ C. P. (1996) Autocrine growth of small cell lung cancer mediated by coexpression of c-kit and stem cell factor. Cancer Res, **56:** 370–376
17 Hines S. J., Litz J. S. and Krystal G. W. (1999) Coexpression of c-kit and stem cell factor in breast cancer results in enhanced sensitivity to members of the EGF family of growth factors. Breast Cancer Res. Treat. **58:** 1–10
18 Bellone G., Silvestri S., Artusio E., Tibaudi D., Turletti A., Geuna M. et al. (1997) Growth stimulation of colorectal carcinoma cells via the c-kit receptor is inhibited by TGF-beta 1. J. Cell. Physiol. **172:** 1–11
19 Inoue M., Kyo S., Fujita M., Enomoto T. and Kondoh G. (1994) Coexpression of the c-kit receptor and the stem cell factor in gynecological tumors. Cancer Res. **54:** 3049–3053
20 Natali P. G., Berlingieri M. T., Nicotra M. R., Fusco A., Santoro E., Bigotti A. et al. (1995) Transformation of thyroid epithelium is associated with loss of c-kit receptor. Cancer Res. **55:** 1787–1791
21 Kurosawa K., Miyazawa K., Gotoh A., Katagiri T., Nishimaki J., Ashman L. K. et al. (1996) Immobilized anti-KIT monoclonal antibody induces ligand-independent dimerization and activation of Steel factor receptor: biologic similarity with membrane-bound form of Steel factor rather than its soluble form. Blood **87:** 2235–2243
22 Natali P. G., Nicotra M. R., Sures I., Mottolese M., Botti C. and Ullrich A. (1992) Breast cancer is associated with loss of the c-kit oncogene product. Int. J. Cancer **52:** 713–717
23 Tsuura Y., Suzuki T., Honma K. and Sano M. (2002) Expression of c-kit protein in proliferative lesions of human breast: sexual difference and close association with phosphotyrosine status. J. Cancer Res. Clin. Oncol. **128:** 239–246
24 Longley B. J., Tyrrell L., Lu S. Z., Ma Y. S., Langley K., Ding T. G. et al. (1996) Somatic c-KIT activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. Nat. Genet. **12:** 312–314
25 Nagata H., Worobec A. S., Oh C. K., Chowdhury B. A., Tannenbaum S., Suzuki Y. et al. (1995) Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. Proc. Natl. Acad. Sci. USA **92:** 10560–10564
26 Ning Z. Q., Li J., McGuinness M. and Arceci R. J. (2001) STAT3 activation is required for Asp(816) mutant c-Kit induced tumorigenicity. Oncogene **20:** 4528–4536
27 Tian Q., Frierson H. F. Jr, Krystal G. W. and Moskaluk C. A. (1999) Activating c-kit gene mutations in human germ cell tumors. Am. J. Pathol. **154:** 1643–1647
28 Hirota S., Isozaki K., Moriyama Y., Hashimoto K., Nishida T., Ishiguro S. et al. (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science **279:** 577–580
29 Blume-Jensen P., Claesson-Welsh L., Siegbahn A., Zsebo K. M., Westermark B. and Heldin C. H. (1991) Activation of the human c-kit product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis. EMBO J. **10:** 4121–4128
30 Philo J. S., Wen J., Wypych J., Schwartz M. G., Mendiaz E. A. and Langley K. E. (1996) Human stem cell factor dimer forms a complex with two molecules of the extracellular domain of its receptor. Kit. J. Biol. Chem. **271:** 6895–6902
31 Lemmon M. A., Pinchasi D., Zhou M., Lax I. and Schlessinger J. (1997) Kit receptor dimerization is driven by bivalent binding of stem cell factor. J. Biol. Chem. **272:** 6311–6317
32 Pawson T. (2004) Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems. Cell **116:** 191–203
33 Huang E. J., Nocka K. H., Buck J. and Besmer P. (1992) Differential expression and processing of two cell associated forms of the kit-ligand: KL-1 and KL-2. Mol. Biol. Cell **3:** 349–362
34 Miyazawa K., Williams D. A., Gotoh A., Nishimaki J., Broxmeyer H. E. and Toyama K. (1995) Membrane-bound Steel

factor induces more persistent tyrosine kinase activation and longer life span of c-kit gene-encoded protein than its soluble form. Blood **85:** 641–649
35 Kapur R., Chandra S., Cooper R., McCarthy J. and Williams D. A. (2002) Role of p38 and ERK MAP kinase in proliferation of erythroid progenitors in response to stimulation by soluble and membrane isoforms of stem cell factor. Blood **100:** 1287–1293
36 Haglund K., Di Fiore P. P. and Dikic I. (2003) Distinct monoubiquitin signals in receptor endocytosis. Trends Biochem. Sci. **28:** 598–603
37 Joazeiro C. A., Wing S. S., Huang H., Leverson J. D., Hunter T. and Liu Y. C. (1999) The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. Science **286:** 309–312
38 Yokouchi M., Kondo T., Sanjay A., Houghton A., Yoshimura A., Komiya S. et al. (2001) Src-catalyzed phosphorylation of c-Cbl leads to the interdependent ubiquitination of both proteins. J. Biol. Chem. **276:** 35185–35193
39 Sattler M., Salgia R., Shrikhande G., Verma S., Pisick E., Prasad K. V. et al. (1997) Steel factor induces tyrosine phosphorylation of CRKL and binding of CRKL to a complex containing c-kit, phosphatidylinositol 3-kinase, and p120(CBL). J. Biol. Chem.**272:** 10248–10253
40 Wisniewski D., Strife A. and Clarkson B. (1996) c-kit ligand stimulates tyrosine phosphorylation of the c-Cbl protein in human hematopoietic cells. Leukemia **10:** 1436–1442
41 Wollberg P., Lennartsson J., Gottfridsson E., Yoshimura A. and Rönnstrand L. (2003) The adapter protein APS associates with the multifunctional docking sites Tyr-568 and Tyr-936 in c-Kit. Biochem. J. **370:** 1033–1038
42 Broudy V. C., Lin N. L., Liles W. C., Corey S. J., O'Laughlin B., Mou S. et al. (1999) Signaling via Src family kinases is required for normal internalization of the receptor c-Kit. Blood **94:** 1979–1986
43 Jahn T., Seipel P., Coutinho S., Urschel S., Schwarz K., Miething C. et al. (2002) Analysing c-kit internalization using a functional c-kit-EGFP chimera containing the fluorochrome within the extracellular domain. Oncogene **21:** 4508–4520
44 Blume-Jensen P., Rönnstrand L., Gout I., Waterfield M. D. and Heldin C. H. (1994) Modulation of Kit/stem cell factor receptor-induced signaling by protein kinase C. J. Biol. Chem. **269:** 21793–21802
45 Kozawa O., Blume-Jensen P., Heldin C. H. and Rönnstrand L. (1997) Involvement of phosphatidylinositol 3'-kinase in stem-cell-factor-induced phospholipase D activation and arachidonic acid release. Eur. J. Biochem. **248:** 149–155
46 Blume-Jensen P., Wernstedt C., Heldin C. H. and Rönnstrand L. (1995) Identification of the major phosphorylation sites for protein kinase C in kit/stem cell factor receptor in vitro and in intact cells. J. Biol. Chem. **270:** 14192–14200
47 Blume-Jensen P., Janknecht R. and Hunter T. (1998) The kit receptor promotes cell survival via activation of PI 3-kinase and subsequent Akt-mediated phosphorylation of Bad on Ser136. Curr. Biol. **8:** 779–782
48 Yee N. S., Langen H. and Besmer P. (1993) Mechanism of kit ligand, phorbol ester and calcium-induced down-regulation of c-kit receptors in mast cells. J. Biol. Chem. **268:** 14189–14201
49 Yee N. S., Hsiau C. W., Serve H., Vosseller K. and Besmer P. (1994) Mechanism of down-regulation of c-kit receptor. Roles of receptor tyrosine kinase, phosphatidylinositol 3′-kinase and protein kinase C. J. Biol. Chem. **269:** 31991–31998
50 Wormald S. and Hilton D. J. (2004) Inhibitors of cytokine signal transduction. J. Biol. Chem. **279:** 821–824
51 De Sepulveda P., Okkenhaug K., Rose J. L., Hawley R. G., Dubreuil P. and Rottapel R. (1999) Socs1 binds to multiple signalling proteins and suppresses steel factor-dependent proliferation. EMBO J. **18:** 904–915
52 Ilangumaran S., Finan D., Raine J. and Rottapel R. (2003) Suppressor of cytokine signaling 1 regulates an endogenous inhibitor of a mast cell protease. J. Biol. Chem. **278:** 41871–41880
53 Kozlowski M., Larose L., Lee F., Le D. M., Rottapel R. and Siminovitch K. A. (1998) SHP-1 binds and negatively modulates the c-Kit receptor by interaction with tyrosine 569 in the c-Kit juxtamembrane domain. Mol. Cell. Biol. **18:** 2089–2099
54 Yi T. and Ihle J. N. (1993) Association of hematopoietic cell phosphatase with c-Kit after stimulation with c-Kit ligand. Mol. Cell. Biol. **13:** 3350–3358
55 Shultz L. D., Schweitzer P. A., Rajan T. V., Yi T., Ihle J. N., Matthews R. J. et al. (1993) Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. Cell **73:** 1445–1454
56 Lorenz U., Bergemann A. D., Steinberg H. N., Flanagan J. G., Li X., Galli S. J. et al. (1996) Genetic analysis reveals cell type-specific regulation of receptor tyrosine kinase c-Kit by the protein tyrosine phosphatase SHP1. J. Exp. Med. **184:** 1111–1126
57 Lewis T. S., Shapiro P. S. and Ahn N. G. (1998) Signal transduction through MAP kinase cascades. Adv. Cancer Res. **74:** 49–139
58 Qiu R. G., Chen J., Kirn D., McCormick F. and Symons M. (1995) An essential role for Rac in Ras transformation. Nature **374:** 457–459
59 Rodriguez-Viciana P., Warne P. H., Khwaja A., Marte B. M., Pappin D., Das P. et al. (1997) Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. Cell **89:** 457–467
60 Rozakis-Adcock M., McGlade J., Mbamalu G., Pelicci G., Daly R., Li W, et al. (1992) Association of the Shc and Grb2/Sem5 SH2-containing proteins is implicated in activation of the Ras pathway by tyrosine kinases. Nature **360:** 689–692
61 Lowenstein E. J., Daly R. J., Batzer A. G., Li W., Margolis B., Lammers R. et al. (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell **70:** 431–442
62 Li W., Nishimura R., Kashishian A., Batzer A. G., Kim W. J., Cooper J. A. et al. (1994) A new function for a phosphotyrosine phosphatase: linking GRB2-Sos to a receptor tyrosine kinase. Mol. Cell. Biol. **14:** 509–517
63 Kyriakis J. M., App H., Zhang X. F., Banerjee P., Brautigan D. L., Rapp U. R. et al. (1992) Raf-1 activates MAP kinase-kinase- Nature **358:** 417–421
64 Crews C. M. and Erikson R. L. (1992) Purification of a murine protein-tyrosine/threonine kinase that phosphorylates and activates the Erk-1 gene product: relationship to the fission yeast byr1 gene product. Proc. Natl. Acad. Sci. USA **89:** 8205–8209
65 Khokhlatchev A. V., Canagarajah B., Wilsbacher J., Robinson M., Atkinson M., Goldsmith E. et al. (1998) Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell **93:** 605–615
66 Murphy L. O., Smith S., Chen R. H., Fingar D. C. and Blenis J. (2002) Molecular interpretation of ERK signal duration by immediate early gene products. Nat. Cell Biol. **4:** 556–564
67 Thömmes K., Lennartsson J., Carlberg M. and Rönnstrand L. (1999) Identification of Tyr-703 and Tyr-936 as the primary association sites for Grb2 and Grb7 in the c-Kit/stem cell factor receptor. Biochem. J. **341 (Pt 1):** 211–216
68 Tauchi T., Feng G. S., Marshall M. S., Shen R., Mantel C., Pawson T. et al. (1994) The ubiquitously expressed Syp phosphatase interacts with c-kit and Grb2 in hematopoietic cells. J. Biol.Chem. **269:** 25206–25211
69 Tauchi T., Boswell H. S., Leibowitz D. and Broxmeyer H. E. (1994) Coupling between p210bcr-abl and Shc and Grb2

adaptor proteins in hematopoietic cells permits growth factor receptor-independent link to ras activation pathway. J. Exp. Med. **179:** 167–175
70 Dorsey J. F., Cunnick J. M., Mane S. M. and Wu J. (2002) Regulation of the Erk2-Elk1 signaling pathway and megakaryocytic differentiation of Bcr-Abl(+) K562 leukemic cells by Gab2. Blood **99:** 1388–1397
71 Bondzi C., Litz J., Dent P. and Krystal G. W. (2000) Src family kinase activity is required for Kit-mediated mitogen-activated protein (MAP) kinase activation; however, loss of functional retinoblastoma protein makes MAP kinase activation unnecessary for growth of small cell lung cancer cells. Cell Growth Differ. **11:** 305–314
72 Lennartsson J., Blume-Jensen P., Hermanson M., Pontén E., Carlberg M. and Rönnstrand L. (1999) Phosphorylation of Shc by Src family kinases is necessary for stem cell factor receptor/c-kit mediated activation of the Ras/MAP kinase pathway and c-fos induction. Oncogene **18:** 5546–5553
73 Ueda S., Mizuki M., Ikeda H., Tsujimura T., Matsumura I., Nakano K. et al. (2002) Critical roles of c-Kit tyrosine residues 567 and 719 in stem cell factor-induced chemotaxis: contribution of src family kinase and PI3-kinase on calcium mobilization and cell migration. Blood **99:** 3342–3349
74 Timokhina I., Kissel H., Stella G. and Besmer P. (1998) Kit signaling through PI 3-kinase and Src kinase pathways: an essential role for Rac1 and JNK activation in mast cell proliferation. EMBO J. **17:** 6250–6262
75 Wandzioch E., Edling C. E., Palmer R. H., Carlsson L. and Hallberg B. (2004) Activation of the MAP-kinase pathway by c-Kit is PI-3-kinase dependent in hematopoietic progenitor/stem cell lines. Blood **104:** 51–57
76 Duckworth B. C. and Cantley L. C. (1997) Conditional inhibition of the mitogen-activated protein kinase cascade by wortmannin. Dependence on signal strength. J. Biol. Chem. **272:** 27665–27670
77 Foster F. M., Traer C. J., Abraham S. M. and Fry M. J. (2003) The phosphoinositide (PI) 3-kinase family. J. Cell Sci. **116:** 3037–3040
78 Carpenter C. L., Auger K. R., Chanudhuri M., Yoakim M., Schaffhausen B., Shoelson S. et al. (1993) Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. J. Biol. Chem. **268:** 9478–9483
79 Ueki K., Fruman D. A., Brachmann S. M., Tseng Y. H., Cantley L. C. and Kahn C. R. (2002) Molecular balance between the regulatory and catalytic subunits of phosphoinositide 3-kinase regulates cell signaling and survival. Mol. Cell. Biol. **22:** 965–977
80 Hartley D., Meisner H. and Corvera S. (1995) Specific association of the beta isoform of the p85 subunit of phosphatidylinositol-3 kinase with the proto-oncogene c-cbl. J. Biol. Chem. **270:** 18260–18263
81 Lennartsson J., Wernstedt C., Engström U., Hellman U. and Rönnstrand L. (2003) Identification of Tyr900 in the kinase domain of c-Kit as a Src-dependent phosphorylation site mediating interaction with c-Crk. Exp. Cell Res. **288:** 110–118
82 Serve H., Yee N. S., Stella G., Sepp-Lorenzino L., Tan J. C. and Besmer P. (1995) Differential roles of PI3-kinase and Kit tyrosine 821 in Kit receptor-mediated proliferation, survival and cell adhesion in mast cells. EMBO J. **14:** 473–483
83 Kubota Y., Angelotti T., Niederfellner G., Herbst R. and Ullrich A. (1998) Activation of phosphatidylinositol 3-kinase is necessary for differentiation of FDC-P1 cells following stimulation of type III receptor tyrosine kinases. Cell Growth Differ. **9:** 247–256
84 Vosseller K., Stella G., Yee N. S. and Besmer P. (1997) c-kit receptor signaling through its phosphatidylinositide-3′-kinase-binding site and protein kinase C: role in mast cell enhancement of degranulation, adhesion and membrane rufflng. Mol. Biol. Cell **8:** 909–922
85 Engström M., Karlsson R. and Jönsson J. I. (2003) Inactivation of the forkhead transcription factor FoxO3 is essential for PKB-mediated survival of hematopoietic progenitor cells by kit ligand. Exp. Hematol. **31:** 316–323
86 Chian R., Young S., Danilkovitch-Miagkova A., Rönnstrand L., Leonard E., Ferrao P. et al. (2001) Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-Kit mutant. Blood **98:** 1365–1373
87 Hashimoto K., Matsumura I., Tsujimura T., Kim D. K., Ogihara H., Ikeda H. et al. (2003) Necessity of tyrosine 719 and phosphatidylinositol 3′-kinase-mediated signal pathway in constitutive activation and oncogenic potential of c-kit receptor tyrosine kinase with the Asp814Val mutation. Blood **101:** 1094–1102
88 Blume-Jensen P., Jiang G., Hyman R., Lee K. F., O'Gorman S. and Hunter T. (2000) Kit/stem cell factor receptor-induced activation of phosphatidylinositol 3′-kinase is essential for male fertility. Nat. Genet. **24:** 157–162
89 Kissel H., Timokhina I., Hardy M. P., Rothschild G., Tajima Y., Soares V. et al. (2000) Point mutation in kit receptor tyrosine kinase reveals essential roles for kit signaling in spermatogenesis and oogenesis without affecting other kit responses. EMBO J. **19:** 1312–1326
90 Nishimura R., Li W., Kashishian A., Mondino A., Zhou M., Cooper J. et al. (1993) Two signaling molecules share a phosphotyrosine-containing binding site in the platelet-derived growth factor receptor. Mol. Cell. Biol. **13:** 6889–6896
91 Fukao T., Yamada T., Tanabe M., Terauchi Y., Ota T., Takayama T. et al. (2002) Selective loss of gastrointestinal mast cells and impaired immunity in PI3K-deficient mice. Nat. Immunol. **3:** 295–304
92 Tan B. L., Yazicioglu M. N., Ingram D., McCarthy J., Borneo J., Williams D. A. et al. (2003) Genetic evidence for convergence of c-Kit- and alpha4 integrin-mediated signals on class IA PI-3kinase and the Rac pathway in regulating integrin-directed migration in mast cells. Blood **101:** 4725–4732
93 Arcaro A., Khanzada U. K., Vanhaesebroeck B., Tetley T. D., Waterfield M. D. and Seckl M. J. (2002) Two distinct phosphoinositide 3-kinases mediate polypeptide growth factor-stimulated PKB activation. EMBO J. **21:** 5097–5108
94 Carpenter G. and Ji Q. (1999) Phospholipase C-gamma as a signal-transducing element. Exp. Cell Res. **253:** 15–24
95 Berridge M. J., Bootman M. D. and Roderick H. L. (2003) Calcium signalling: dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell. Biol. **4:** 517–529
96 Herbst R., Lammers R., Schlessinger J. and Ullrich A. (1991) Substrate phosphorylation specificity of the human c-kit receptor tyrosine kinase. J. Biol. Chem. **266:** 19908–19916
97 Herbst R., Shearman M. S., Jallal B., Schlessinger J. and Ullrich A. (1995) Formation of signal transfer complexes between stem cell and platelet-derived growth factor receptors and SH2 domain proteins in vitro. Biochemistry **34:** 5971–5979
98 Gommerman J. L., Sittaro D., Klebasz N. Z., Williams D. A. and Berger S. A. (2000) Differential stimulation of c-Kit mutants by membrane-bound and soluble Steel Factor correlates with leukemic potential. Blood **96:** 3734–3742
99 Koike T., Hirai K., Morita Y. and Nozawa Y. (1993) Stem cell factor-induced signal transduction in rat mast cells. Activation of phospholipase D but not phosphoinositide-specific phospholipase C in c-kit receptor stimulation. J. Immunol. **151:** 359–366
100 Huber M., Helgason C. D., Scheid M. P., Duronio V., Humphries R. K. and Krystal G. (1998) Targeted disruption of SHIP leads to Steel factor-induced degranulation of mast cells. EMBO J. **17:** 7311–7319
101 Trieselmann N. Z., Soboloff J. and Berger S. A. (2003) Mast cells stimulated by membrane-bound, but not soluble, steel factor are dependent on phospholipase C activation. Cell. Mol. Life Sci. **60:** 759–766

102 Maddens S., Charruyer A., Plo I., Dubreuil P., Berger S., Salles B. et al. (2002) Kit signaling inhibits the sphingomyelin-ceramide pathway through PLC gamma 1: implication in stem cell factor radioprotective effect. Blood **100:** 1294–1301

103 Plo I., Lautier D., Casteran N., Dubreuil P., Arock M. and Laurent G. (2001) Kit signaling and negative regulation of daunorubicin-induced apoptosis: role of phospholipase Cgamma. Oncogene **20:** 6752–6763

104 Abram C. L. and Courtneidge S. A. (2000) Src family tyrosine kinases and growth factor signaling. Exp. Cell Res. **254:** 1–13

105 Krystal G. W., DeBerry C. S., Linnekin D. and Litz J. (1998) Lck associates with and is activated by Kit in a small cell lung cancer cell line: inhibition of SCF-mediated growth by the Src family kinase inhibitor PP1. Cancer Res. **58:** 4660–4666

106 Linnekin D., DeBerry C. S. and Mou S. (1997) Lyn associates with the juxtamembrane region of c-Kit and is activated by stem cell factor in hematopoietic cell lines and normal progenitor cells. J. Biol. Chem. **272:** 27450–27455

107 Price D. J., Rivnay B., Fu Y., Jiang S., Avraham S. and Avraham H. (1997) Direct association of Csk homologous kinase (CHK) with the diphosphorylated site Tyr568/570 of the activated c-KIT in megakaryocytes. J. Biol. Chem. **272:** 5915–5920

108 O'Laughlin-Bunner B., Radosevic N., Taylor M. L., Shivakrupa, DeBerry C., Metcalfe D. D. et al. (2001) Lyn is required for normal stem cell factor-induced proliferation and chemotaxis of primary hematopoietic cells. Blood **98:** 343–350

109 Mou S. and Linnekin D. (1999) Lyn is activated during late G1 of stem-cell-factor-induced cell cycle progression in haemopoietic cells. Biochem. J. **342 (Pt 1):** 163–170

110 Hong L., Munugalavadla V. and Kapur R. (2004) c-Kit-mediated overlapping and unique functional and biochemical outcomes via diverse signaling pathways. Mol. Cell. Biol. **24:** 1401–1410

111 Agosti V., Corbacioglu S., Ehlers I., Waskow C., Sommer G., Berrozpe G. et al. (2004) Critical role for Kit-mediated Src kinase but not PI 3-kinase signaling in Pro T and Pro B cell development. J. Exp. Med. **199:** 867–878

112 Jhun B. H., Rivnay B., Price D. and Avraham H. (1995) The MATK tyrosine kinase interacts in a specific and SH2-dependent manner with c-Kit. J. Biol. Chem. **270:** 9661–9666

113 Kerr I. M., Costa-Pereira A. P., Lillemeier B. F. and Strobl B. (2003) Of JAKs, STATs, blind watchmakers, jeeps and trains. FEBS Lett. **546:** 1–5

114 Brizzi M. F., Zini M. G., Aronica M. G., Blechman J. M., Yarden Y. and Pegoraro L. (1994) Convergence of signaling by interleukin-3, granulocyte-macrophage colony-stimulating factor, and mast cell growth factor on JAK2 tyrosine kinase. J. Biol.Chem. **269:** 31680–31684

115 Weiler S. R., Mou S., DeBerry C. S., Keller J. R., Ruscetti F. W., Ferris D. K. et al. (1996) JAK2 is associated with the c-kit proto-oncogene product and is phosphorylated in response to stem cell factor. Blood **87:** 3688–3693

116 Radosevic N., Winterstein D., Keller J. R., Neubauer H., Pfeffer K. and Linnekin D. (2004) JAK2 contributes to the intrinsic capacity of primary hematopoietic cells to respond to stem cell factor. Exp. Hematol. **32:** 149–156

117 Deberry C., Mou S. and Linnekin D. (1997) Stat1 associates with c-kit and is activated in response to stem cell factor. Biochem. J. **327 (Pt 1):** 73–80

118 Brizzi M. F., Dentelli P., Rosso A., Yarden Y. and Pegoraro L. (1999) STAT protein recruitment and activation in c-Kit deletion mutants. J. Biol. Chem. **274:** 16965–16972

119 Ryan J. J., Huang H., McReynolds L. J., Shelburne C., Hu-Li J., Huff T. F. et al. (1997) Stem cell factor activates STAT-5 DNA binding in IL-3-derived bone marrow mast cells. Exp. Hematol. **25:** 357–362

120 O'Farrell A. M., Ichihara M., Mui A. L. and Miyajima A. (1996) Signaling pathways activated in a unique mast cell line where interleukin-3 supports survival and stem cell factor is required for a proliferative response. Blood **87:** 3655–3668

121 Pearson M. A., O'Farrell A. M., Dexter T. M., Whetton A. D., Owen-Lynch P. J. and Heyworth C. M. (1998) Investigation of the molecular mechanisms underlying growth factor synergy: the role of ERK 2 activation in synergy. Growth Factors **15:** 293–306

122 Jacobs-Helber S. M., Penta K., Sun Z., Lawson A. and Sawyer S. T. (1997) Distinct signaling from stem cell factor and erythropoietin in HCD57 cells. J. Biol. Chem. **272:** 6850–6853

123 Smith C. I., Islam T. C., Mattsson P. T., Mohamed A. J., Nore B. F. and Vihinen M. (2001) The Tec family of cytoplasmic tyrosine kinases: mammalian Btk, Bmx, Itk, Tec, Txk and homologs in other species. Bioessays **23:** 436–446

124 Tang B., Mano H., Yi T. and Ihle J. N. (1994) Tec kinase associates with c-kit and is tyrosine phosphorylated and activated following stem cell factor binding. Mol. Cell. Biol. **14:** 8432–8437

125 van Dijk T. B., van Den Akker E., Amelsvoort M. P., Mano H., Lowenberg B. and von Lindern M. (2000) Stem cell factor induces phosphatidylinositol 3′-kinase-dependent Lyn/Tec/Dok-1 complex formation in hematopoietic cells. Blood **96:** 3406–3413

126 Liang X., Wisniewski D., Strife A., Shivakrupa, Clarkson B. and Resh M. D. (2002) Phosphatidylinositol 3-kinase and Src family kinases are required for phosphorylation and membrane recruitment of Dok-1 in c-Kit signaling. J. Biol. Chem. **277:** 13732–13738

127 Kim L. and Wong T. W. (1995) The cytoplasmic tyrosine kinase FER is associated with the catenin-like substrate pp120 and is activated by growth factors. Mol. Cell. Biol. **15:** 4553–4561

128 Craig A. W. and Greer P. A. (2002) Fer kinase is required for sustained p38 kinase activation and maximal chemotaxis of activated mast cells. Mol. Cell. Biol. **22:** 6363–6374

129 Masuhara M., Nagao K., Nishikawa M., Sasaki M., Yoshimura A. and Osawa M. (2000) Molecular cloning of murine STAP-1, the stem-cell-specific adaptor protein containing PH and SH2 domains. Biochem. Biophys. Res. Commun. **268:** 697–703

130 Pawson T. and Scott J. D. (1997) Signaling through scaffold, anchoring and adaptor proteins. Science **278:** 2075–2080

131 Liu S. K., Berry D. M. and McGlade C. J. (2001) The role of Gads in hematopoietic cell signalling. Oncogene **20:** 6284–6290

132 Liu S. K. and McGlade C. J. (1998) Gads is a novel SH2 and SH3 domain-containing adaptor protein that binds to tyrosine-phosphorylated Shc Oncogene. **17:** 3073–3082

133 Feng G. S., Ouyang Y. B., Hu D. P., Shi Z. Q., Gentz R. and Ni J. (1996) Grap is a novel SH3-SH2-SH3 adaptor protein that couples tyrosine kinases to the Ras pathway. J. Biol. Chem. **271:** 12129–12132

134 Ravichandran K. S. (2001) Signaling via Shc family adapter proteins. Oncogene **20:** 6322–6330

135 Han D. C., Shen T. L. and Guan J. L. (2001) The Grb7 family proteins: structure, interactions with other signaling molecules and potential cellular functions. Oncogene **20:**: 6315–6321

136 Jahn T., Seipel P., Urschel S., Peschel C. and Duyster J. (2002) Role for the adaptor protein Grb10 in the activation of Akt. Mol. Cell. Biol. **22:** 979–991

137 Iseki M., Takaki S. and Takatsu K. (2000) Molecular cloning of the mouse APS as a member of the Lnk family adaptor proteins. Biochem. Biophys. Res. Commun. **272:** 45–54

138 Takaki S., Sauer K., Iritani B. M., Chien S., Ebihara Y., Tsuji K. et al. (2000) Control of B cell production by the adaptor protein lnk. Definition of a conserved family of signal-modulating proteins. Immunity **13:** 599–609

139 Yokouchi M., Suzuki R., Masuhara M., Komiya S., Inoue A. and Yoshimura A. (1997) Cloning and characterization of

APS, an adaptor molecule containing PH and SH2 domains that is tyrosine phosphorylated upon B-cell receptor stimulation. Oncogene **15:** 7–15

140 Wakioka T., Sasaki A., Mitsui K., Yokouchi M., Inoue A., Komiya S. et al. (1999) APS, an adaptor protein containing Pleckstrin homology (PH) and Src homology-2 (SH2) domains, inhibits the JAK-STAT pathway in collaboration with c-Cbl. Leukemia **13:** 760–767

141 Yokouchi M., Wakioka T., Sakamoto H., Yasukawa H., Ohtsuka S., Sasaki A. et al. (1999) APS, an adaptor protein containing PH and SH2 domains, is associated with the PDGF receptor and c-Cbl and inhibits PDGF-induced mitogenesis. Oncogene **18:** 759–767

142 Hu J., Liu J., Ghirlando R., Saltiel A. R. and Hubbard S. R. (2003) Structural basis for recruitment of the adaptor protein APS to the activated insulin receptor .Mol. Cell. **12:** 1379–1389

143 Herbst R., Munemitsu S. and Ullrich A. (1995) Oncogenic activation of v-kit involves deletion of a putative tyrosine-substrate interaction site. Oncogene **10:** 369–379

144 Peschard P. and Park M. (2003) Escape from Cbl-mediated downregulation: a recurrent theme for oncogenic deregulation of receptor tyrosine kinases. Cancer Cell **3:** 519–523

145 Iseki M., Kubo C., Kwon S. M., Yamaguchi A., Kataoka Y., Yoshida N. et al. (2004) Increased numbers of B-1 cells and enhanced responses against TI-2 antigen in mice lacking APS, an adaptor molecule containing PH and SH2 domains. Mol. Cell. Biol. **24:** 2243–2250

146 Kubo-Akashi C., Iseki M., Kwon S. M., Takizawa H., Takatsu K. and Takaki S. (2004) Roles of a conserved family of adaptor proteins, Lnk, SH2-B and APS, for mast cell development, growth and functions: APS-deficiency causes augmented degranulation and reduced actin assembly. Biochem. Biophys. Res. Commun. **315:** 356–362

147 Feller S. M. (2001) Crk family adaptors-signalling complex formation and biological roles. Oncogene **20:** 6348–6371

148 Prosser S., Sorokina E., Pratt P. and Sorokin A. (2003) CrkIII: a novel and biologically distinct member of the Crk family of adaptor proteins. Oncogene **22:** 4799–4806

149 Ellis C., Moran M., McCormick F. and Pawson T. (1990) Phosphorylation of GAP and GAP-associated proteins by transforming and mitogenic tyrosine kinases. Nature **343:** 377–381

150 Yamanashi Y., Tamura T., Kanamori T., Yamane H., Nariuchi H., Yamamoto T. et al. (2000) Role of the rasGAP-associated docking protein p62(dok) in negative regulation of B cell receptor-mediated signaling. Genes Dev. **14:** 11–16

151 Carpino N., Wisniewski D., Strife A., Marshak D., Kobayashi R., Stillman B. et al. (1997) p62(dok): a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells. Cell **88:** 197–204

152 Gu H. and Neel B. G. (2003) The 'Gab' in signal transduction. Trends Cell Biol. **13:** 122–130

153 Nishida K., Yoshida Y., Itoh M., Fukada T., Ohtani T., Shirogane T. et al. (1999) Gab-family adapter proteins act downstream of cytokine and growth factor receptors and T- and B-cell antigen receptors. Blood **93:** 1809–1816

154 Nishida K., Wang L., Morii E., Park S. J., Narimatsu M., Itoh S. et al. (2002) Requirement of Gab2 for mast cell development and KitL/c-Kit signaling. Blood **99:** 1866–1869

155 Neel B. G., Gu H. and Pao L. (2003) The 'Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling. Trends Biochem. Sci. **28:** 284–293

156 Rönnstrand L., Arvidsson A. K., Kallin A., Rorsman C., Hellman U., Engström U. et al. (1999) SHP-2 binds to Tyr763 and Tyr1009 in the PDGF beta-receptor and mediates PDGF-induced activation of the Ras/MAP kinase pathway and chemotaxis. Oncogene **18:** 3696–3702

157 Chan R. J., Johnson S. A., Li Y., Yoder M. C. and Feng G. S. (2003) A definitive role of Shp-2 tyrosine phosphatase in mediating embryonic stem cell differentiation and hematopoiesis. Blood **102:** 2074–2080

158 Taniguchi Y., London R., Schinkmann K., Jiang S. and Avraham H. (1999) The receptor protein tyrosine phosphatase, PTP-RO, is upregulated during megakaryocyte differentiation and Is associated with the c-Kit receptor. Blood **94:** 539–549

159 Perez-Losada J., Sanchez-Martin M., Rodriguez-Garcia A., Sanchez M. L., Orfao A., Flores T. et al. (2002) Zinc-finger transcription factor Slug contributes to the function of the stem cell factor c-kit signaling pathway. Blood **100:** 1274–1286

160 Perez-Losada J., Sanchez-Martin M., Perez-Caro M., Perez-Mancera P. A. and Sanchez-Garcia I. (2003) The radioresistance biological function of the SCF/kit signaling pathway is mediated by the zinc-finger transcription factor Slug. Oncogene **22:** 4205–4211

161 Hodgkinson C. A., Moore K. J., Nakayama A., Steingrimsson E., Copeland N. G., Jenkins N. A. et al. (1993) Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. Cell **74:** 395–404

162 Hughes M. J., Lingrel J. B., Krakowsky J. M. and Anderson K. P. (1993) A helix-loop-helix transcription factor-like gene is located at the mi locus. J. Biol. Chem. **268:** 20687–20690

163 Boissy R. E. and Nordlund J. J. (1997) Molecular basis of congenital hypopigmentary disorders in humans: a review. Pigment Cell Res. **10:** 12–24

164 Hemesath T. J., Price E. R., Takemoto C., Badalian T. and Fisher D. E. (1998) MAP kinase links the transcription factor Microphthalmia to c-Kit signalling in melanocytes. Nature **391:** 298–301

165 Wu M., Hemesath T. J., Takemoto C. M., Horstmann M. A., Wells A. G., Price E. R. et al. (2000) c-Kit triggers dual phosphorylations, which couple activation and degradation of the essential melanocyte factor Mi. Genes Dev. **14:** 301–312

166 Weilbaecher K. N., Motyckova G., Huber W. E., Takemoto C. M., Hemesath T. J., Xu Y. et al. (2001) Linkage of M-CSF signaling to Mitf, TFE3, and the osteoclast defect in Mitf(mi/mi) mice. Mol. Cell. **8:** 749–758

167 Price E. R., Ding H. F., Badalian T., Bhattacharya S., Takemoto C., Yao T. P, et al. (1998) Lineage-specific signaling in melanocytes. C-kit stimulation recruits p300/CBP to microphthalmia. J. Biol. Chem. **273:** 17983–17986