Stem cell factor (also known as mast cell growth factor and kit-ligand) is a transmembrane growth factor with a highly conserved cytoplasmic domain. Basolateral membrane expression in epithelia and persistent cell surface exposure of stem cell factor are required for complete biological activity in pigmentation, fertility, learning, and hematopoiesis. Here we show by site-directed mutagenesis that the cytoplasmic domain of stem cell factor contains a monomeric leucine-dependent basolateral targeting signal. N-terminal to this motif, a cluster of acidic amino acids serves to increase the efficiency of basolateral sorting mediated by the leucine residue. Hence, basolateral targeting of stem cell factor requires a mono-leucine determinant assisted by a cluster of acidic amino acids. This mono-leucine determinant is functionally conserved in colony-stimulating factor-1, a transmembrane growth factor related to stem cell factor. Furthermore, this leucine motif is not capable of inducing endocytosis, allowing for persistent cell surface expression of stem cell factor. In contrast, the mutated cytoplasmic tail found in the stem cell factor mutant Mgf

1 The abbreviations used are: SCF, stem cell factor; CSF-1, colony-stimulating factor-1; TGN, trans-Golgi network; GFP, green fluorescent protein; EGFP, enhanced green fluorescent protein; Tac, a-chain; MDCK, Madin-Darby canine kidney; PACS, phosphofurin acidic cluster-sorting.

Received for publication, September 12, 2000, and in revised form, December 28, 2000
Published, JBC Papers in Press, January 10, 2001, DOI 10.1074/jbc.M008357200

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Stem cell factor (SCF)1 belongs to the family of cell surface-anchored growth factors with highly conserved cytoplasmic domains, which includes the related colony-stimulating factor-1 (CSF-1) (1). SCF is expressed as two alternatively spliced membrane-bound forms (M1 and M2), distinguished by an exon containing a proteolytic cleavage site in the M1 form. This site is used to generate soluble growth factor from the M1 membrane-bound precursor. The membrane anchor of SCF is required for its biological activity in vivo because the expression of only the extracellular receptor binding domain leads to the loss of SCF-dependent cells affecting skin pigmentation, fertility, hematopoiesis, and learning (2–4). Furthermore, a point mutation, which results in the skipping of the exon coding for the cytoplasmic tail of mouse SCF (Mgf1117H), leads to an altered cytoplasmic sequence that abrogates coat pigmentation and male fertility and reduces hematopoiesis (5–7). In this mouse mutant, cell surface expression of SCF is reduced, and basolateral sorting in epithelial tissues is lost (8). Hence, the cytoplasmic tail of SCF harbors information required for efficient cell surface presentation and basolateral targeting of SCF, functions that are absolutely required to fulfill its function in vivo.

Polarized epithelial cells exhibit an apical and basolateral surface with distinct protein compositions. Basolateral sorting of transmembrane proteins takes place in the trans-Golgi network (TGN) or endosomal compartments and is mediated by clathrin-coated vesicles (9). Selective incorporation of proteins into these transport vesicles is accomplished by adaptor complexes (10). Short cytoplasmic targeting sequences frequently containing either a tyrosine or di-leucine motif have been identified in the sorted proteins and are required for the interaction with adaptor complexes and for basolateral transport of the proteins (11). Recently a tyrosine-based targeting motif has been shown to bind to an epithelial specific AP1 subunit that is required for basolateral transport (12). When the tyrosine or the di-leucine sorting domains are removed from the proteins, apical instead of basolateral sorting occurs, mediated by N-linked carbohydrates or by association with lipid rafts (13–15). Some basolateral sorting signals resemble endocytic signals used to incorporate membrane proteins into clathrin-coated pits at the plasma membrane, suggesting that basolateral sorting and endocytosis are regulated by similar mechanisms. For example, the macrophage Fc receptor and the invariant chain of the class II major histocompatibility complex contain a di-leucine-based determinant that is used for basolateral sorting as well as endocytosis (16, 17). Furthermore, many membrane proteins carry several different targeting determinants, which enables them to shuttle between the basolateral plasma membrane and endosomes (18, 19).

Although SCF does not have typical tyrosine or di-leucine sorting sequences in its cytoplasmic tail, it is delivered directly to the basolateral cell surface in epithelial cells and does not accumulate in an intracellular compartment (8). Consequently, SCF remains at the cell surface until the extracellular domain is proteolytically shed within 0.5 (M1) to 5 (M2) depending on the respective splice form (20). Because basolateral sorting is critical for the proper biological function of SCF, we tried to identify the possibly novel basolateral targeting determinant in the cytoplasmic tail of SCF. To do so, we used reporter constructs consisting of extracellular green fluorescent protein (GFP)-tagged SCF or chimeras of the extracellular domain of the interleukin-2 receptor a-chain (Tac) fused to the transmembrane and cytoplasmic sequences of SCF. In these chimeras the normal intracellular domain of SCF is left intact, allowing optimal interaction of the latter with the sorting machinery of...
polarized cells and the identification of critical targeting domains by mutagenesis.

EXPERIMENTAL PROCEDURES

SCF Chimeras and Site-directed Mutagenesis—cDNA for SCF and Tac were kindly provided by Drs. John Flanagan (Harvard Medical School, Boston) and Pierre Conson (University of Geneva, Switzerland), respectively. Mouse CSF-1 and mouse tyrosinase cDNAs were kindly provided by Dr. Willy Hofstetter (MMI, Bern, Switzerland) and Friedrich Beermann (ISREC, Lausanne, Switzerland), respectively. SCF-GFP chimeras were constructed in pcDNA3 (Invitrogen, Groningen, The Netherlands) by inserting the enhanced GFP sequence (CLONTECH Laboratories, Palo Alto, CA) together with a Myc tag 5′ into the exon 5/6 junction of SCF (SSTLGP/DESRV), which resulted in the following sequence: SSTLGP/KEQISEEDLQGG/SV... (enhanced GFP)... YK/TQPEK/DSRV (single letter amino acid code; the sequence of the Myc tag is underlined). To prevent translation at internal start sites producing cytoplasmic GFP, we replaced the start codon of GFP with nucleotides coding for a CacI site. A unique PinAI site was introduced C-terminal to the GFP sequence to swap wild-type and mutant cytoplasmic tail sequences at this site.

To generate the Tac-CSF chimeras (all in pcDNA3), the transmembrane and cytoplasmic domains of SCF were swapped at a unique BgII site in Tac located at a homologous leucine (L) and glutamine (Q) residue upstream of the transmembrane sequences of SCF and Tac. This resulted in the sequence: . . . SIFTTDLQWTAMALP... . . . at this position (conserved LQ is bold and transmembrane residues of SCF are underlined).

The Tac-CSF-1 and Tac-tyrosinase (Tac-tyr) chimeras were constructed in a similar way. CSF-1 and tyrosinase transmembrane and cytoplasmic sequences were polymerase chain reaction amplified with a BgII site containing the forward primers (CSF-1: AACAGATCTCCA-GATCCCTGAGTCTG; tyrosinase: AACAGATCTCCAAGCAGCTCG-TATCGG) at a common glutamine residue (Q) and swapped with the Tac sequence of this region creating the respective junctional sequences: . . . SIFTTDLQETVESJHIV... . . . and Tac-tyr: . . . SIFTTDLQASRIWPWLL... (the common glutamine residue (Q) is bold, and the respective transmembrane region is underlined). Tac-EGFP was cloned by polymerase chain reaction amplification of EGFP with a HindIII-containing primer and inserted at a unique HindIII site at the extreme C terminus of Tac (TIQASSStop) resulting in the new junctional sequence (TIQASTMV... EYKGGF/EGFP).

Site-specific mutagenesis of the cytoplasmic tail of SCF was performed using polymerase chain reaction overlap extension. Two overlapping polymerase chain reaction fragments containing a specific mutation were amplified with external primers (containing either the PinAI or BgII site for SCF-GFP or Tac-SCF chimeras, respectively) and swapped with the wild-type sequence of the cytoplasmic tail. All constructs were verified by dideoxy sequencing. A list of primers used to generate the different constructs listed in Fig. 2: can be provided upon request.

Cell Culture, Live Fluorescence Microscopy, and Immunocytochemistry—MDCK II cells were kindly provided by Dr. Karl Matter (University of Geneva, Switzerland) and cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Paisley, Scotland) supplemented with 10% fetal calf serum (Inotech, Dottikon, Switzerland). Cells at 60% confluence were transfected using calcium phosphate as described (21), and stable clones were selected with 0.6 mg ml−2. Mutants lacking the last eight C-terminal amino acids (d36, d29) still localized to the basolateral membrane and cytoplasmic domains of SCF were swapped at a unique BgII site in Tac located at a homologous Leucine 26 Is Required for Basolateral Targeting of SCF—To identify the motif in the cytoplasmic tail of SCF responsible for basolateral sorting, we created various cytoplasmic SCF mutants of the membrane-bound (M2) form of GFP-tagged SCF (SCF-GFP) (Fig. 2A). Mutants lacking the last eight C-terminal amino acids (d36, d29) still localized to the basolateral membrane. However, when 15 or more amino acids were deleted (d22, d12), SCF-GFP was located on the apical membrane cadherin. After washing, bound antibodies were revealed with Texas Red-coupled anti-mouse antibodies (Southern Biotechnoloy Associates Inc., Birmingham, AL). Fluorescence was subsequently analyzed on a confocal microscope as indicated above. Contrast enhancement was performed in Photoshop (Adobe Systems Inc., San Jose, CA).

RESULTS

Leucine 26 Is Required for Basolateral Targeting of SCF—GFP was inserted into the alternatively spliced extracellular domains of both membrane-bound variants of SCF and transfected into polarized epithelial MDCK II cells (Fig. 1). Confocal microscopy revealed that both wild-type constructs accumulated in basal and lateral membranes where they colocalized with E-cadherin, a marker for the lateral membrane compartment in polarized epithelial cells (shown for M2 variant; Fig. 1).

To identify the motif in the cytoplasmic tail of SCF responsible for basolateral sorting, we created various cytoplasmic SCF mutants of the membrane-bound (M2) form of GFP-tagged SCF (SCF-GFP) (Fig. 2A). Mutants lacking the last eight C-terminal amino acids (d36, d29) still localized to the basolateral membrane. However, when 15 or more amino acids were deleted (d22, d12), SCF-GFP was located on the apical membrane...
Basolateral Targeting Determinant of SCF

and showed no basolateral expression. The critical region for basolateral sorting was demonstrated to reside within the sequence E19K because an internal deletion mutant (d21–28) also localized to the apical membrane. Interestingly, to be functional, it appeared that this sequence must be considerably separated from the membrane; deletion of intervening amino acids proximal to the membrane (d5–20) interfered with basolateral sorting. Increasing the distance of the motif from the membrane by reinserting amino acids 5–11 (d12–20) only partially rescued basolateral sorting, suggesting that other amino acids important for basolateral targeting are present N-terminal to the motif (see below).

Sequence comparison among human, mouse, chicken, and salamander SCF (24) revealed the residues SML as being completely conserved within the NEISMLQQ28 motif (Fig. 2B). This sequence encompasses a serine at position 24 as well as a di-hydrophobic methionine-leucine at positions 25 and 26, respectively (see above). To test whether a portion of this motif was required for basolateral sorting of SCF, we replaced serine 24 by alanine (S24A), or methionine 25 and leucine 26 to a double alanine (M25A/L26A), showing that replacement of leucine 26 to alanine (L26A), or methionine 25 and leucine 26 to a double alanine (M25A/L26A), did not alter basolateral localization of the constructs. Below each panel a corresponding Z-scan is shown. The bar in F corresponds to 24 μm.

**Fig. 2.** Cytoplasmic tail sequences of wild-type and mutant SCF. A, alignment of sequences of wild-type and cytoplasmic tail mutants of mouse SCF and their respective steady-state distribution in MDCK II cells. Construct 1, the name of the constructs represents the site of amino acid deletions (marked with a dotted line) or point mutations (bold and underlined). Polarity 2, steady-state localization of GFP and Tac SCF chimeric proteins in polarized MDCK II cells (wt, wild-type; BL, basolateral; AP, apical). E, Clustal W alignment of different SCF cytoplasmic tail sequences. GenBank accession numbers are M59964 (human), M57647 (mouse), D13516 (chicken), and AF119044 (salamander).

**Fig. 3.** Leucine 26 is required for basolateral targeting of SCF-GFP constructs in polarized MDCK II cells. Confocal microscopy (fluorescence isothiocyanate channel) of live wild-type (A) and mutant SCF-GFP-M2 (B–F) expressing confluent MDCK II cells. Basolateral staining is lost upon mutation of leucine 26 to alanine (B), or methionine 25 and leucine 26 to a double alanine (D). Replacement of serine 24 by alanine (C) or aspartic acid (E), as well as the change of glutamic acid 19 to lysine (F), did not alter basolateral localization of the constructs. Below each panel a corresponding Z-scan is shown. The bar in F corresponds to 24 μm.

**Fig. 4.** Extracellular SCF Sequences Are Not Required for Basolateral Targeting—Dimer formation involving the extracellular domain of SCF or lateral association of the extracellular and/or intracellular portions of SCF with other proteins that contain targeting information may in fact be responsible for the polarized expression of SCF. Therefore, to test the ability of the cytoplasmic targeting sequence of SCF to mediate polarized expression independently of the extracellular domain, we replaced the latter with the extracellular domain of Tac (Fig. 4A) (22). Wild-type Tac as well as Tac with a C-terminally fused EGFP accumulated apically when expressed in MDCK II cells (Fig. 4A). In contrast, Tac-SCF chimeras expressing the wild-type cytoplasmic domain of SCF localized to basolateral membranes in a manner identical to the SCF-GFP wild-type constructs (Fig. 4B). Likewise, constructs involving extracellular Tac with deletion mutations of the cytoplasmic tail of SCF (d29, basolateral, Fig. 4C; d22, apical, Fig. 4D; d21–28, apical, Fig. 4E; d5–20, apical (not shown); and d12–20, basolateral/ apical, Fig. 4F), showed identical basolateral sorting behaviors compared with the mutant SCF-GFP constructs. This indicates that the extracellular domain of SCF is not required for basolateral targeting and that the basolateral targeting motif of SCF contained within its cytoplasmic portion is sufficient to direct the Tac extracellular domain basolaterally. Moreover, sequences N-terminal to methionine 25 and leucine 26 removed in the d12–20 mutation influence the efficiency of basolateral
targeting (Fig. 4F).

Efficient Basolateral Targeting Is Mediated by an Acidic Cluster N-terminal to the Monomeric Leucine Determinant—Although it is evident that the leucine residue at position 26 is critical for basolateral targeting it is not known whether a second hydrophobic residue (methionine 25) as found in all di-leucine-like determinants is equally required for basolateral sorting of SCF. Moreover, the region N-terminal to the ML motif which is also required for efficient basolateral targeting \((^{13}ENIQ\text{NEED}^{20})\) bears a domain important for SCF sorting as well.

To address the first question, we replaced methionine 25 with an alanine residue and analyzed the distribution of the Tac-SCF construct at steady-state conditions. In contrast to the leucine 26 to alanine mutation, the change of methionine 25 to alanine did not affect basolateral targeting of Tac-SCF (Fig. 5, A and B). Moreover, replacement of methionine by leucine in an attempt to create a classical di-leucine determinant led to intracellular and apical localization of Tac-SCF (not shown).

Analysis of sequences N-terminal to leucine 26 which are absent in the d12–20 mutation reveal an unusually high concentration of acidic amino acids. An acidic cluster N-terminal to an FI motif has recently been identified as a basolateral targeting signal in the furin protease (25). To test whether the acidic cluster in SCF contributes to basolateral sorting or whether other acidic amino acid residues localized closely to the leucine residue are critical, we mutated glutamic acid 22 \((^{22}\text{EED}^{20})\) to alanine (Fig. 5C). In addition, we replaced glutamic acid 19 with a lysine to destroy the acidic cluster formed by residues \(^{18}\text{EED}^{20}\) (Fig. 5F). Neither modification had any effect on basolateral targeting of Tac or of the GFP chimeric SCF constructs. However, the replacement of all three acidic residues 18, 19, and 20, with alanine residues \((^{22}\text{EXX}^{20})\) did alter basolateral sorting of the Tac-SCF chimeras. In clones expressing relatively low amounts of the Tac-E-D18A-A chimera, basolateral targeting was still efficient; however, in clones expressing higher amounts of mutant Tac-SCF, both basolateral and apical surface staining was detected (Fig. 5D). Anti-Tac staining of these clones strongly resembled the phenotype already seen with the d12–20 mutation (Fig. 4F). These data suggest that the removal of the acidic cluster \((^{12}\text{EED}^{20})\) is the cause of the phenotype of the d12–20 mutation which results in a reduced efficiency of basolateral transport mediated by the monomeric leucine determinant.

Comparison of the Basolateral Targeting Domain of SCF with That of CSF-1—SCF belongs to a large family of transmembrane growth factors that play important roles during development, tissue homeostasis, and hematopoiesis. Based on

Fig. 4. The basolateral targeting determinant in SCF acts independently of the extracellular domain. Confocal microscopy of anti-Tac antibody-stained fixed MDCK II cells stably transfected with Tac-EGFP (A) and wild-type (B) or mutant (C–F) Tac-SCF chimeric constructs is shown. A scheme representing the Tac-SCF chimera is shown above the panels. The fusion protein consists of the extracellular domain of Tac and the transmembrane and cytoplasmic sequence of SCF. A, unmodified Tac with C-terminal EGFP fusion of which the anti-Tac is antibody-stained. B, Tac-SCF chimera with wild-type SCF sequences. C, deletion of the last 8 amino acids from the cytoplasmic tail of SCF does not alter basolateral targeting of the Tac hybrid (d29). However, removal of the last 15 amino acids (d22) (D) or amino acids 21–28 (E) resulted in an apical localization of Tac-SCF. The deletion of amino acids N-terminal to the leucine 26-containing region (d12–20) resulted in basolateral as well as apical localization of the chimeric proteins (F). Below each panel, a corresponding Z-scan is shown. SP, signal peptide of SCF and Tac, respectively; ED, extracellular domain. The bar in F corresponds to 24 μm.

Fig. 5. An acidic cluster-assisted monomeric leucine-dependent basolateral targeting determinant. Confocal microscopy of anti-Tac antibody-stained fixed MDCK II cells stably transfected with Tac-SCF point mutations of hydrophobic and acidic amino acids is shown. A, apical localization of the Tac-SCF chimera carrying a double alanine substitution of methionine 25 and leucine 26 (M25A/L26A). B, the single point mutation at methionine 25 to alanine did not alter basolateral targeting. Similarly, the point mutation of glutamic acid 22 to alanine (E22A) did not influence basolateral targeting (C). Alanine substitution of the acidic cluster \(^{18}\text{EED}^{20}\) (E-D18A-A) resulted in basolateral as well as apical accumulation of Tac chimeric proteins (D). Below each panel, a corresponding Z-scan is shown. The bar in D corresponds to 24 μm.
The distribution of Tac-CSF-1 on basolateral as well as apical surfaces gave the impression that this construct is not sorted. To determine whether the homologous leucine in CSF-1 can interact with the sorting machinery of the cell, we mutated leucine 24 of CSF-1 to alanine. The respective Tac chimera (Tac-CSF-L24A) accumulated apically (Fig. 6D'), similar to Tac-CSF-L26A (Fig. 6D'), suggesting that the leucine at the respective position in CSF-1 is nevertheless recognized as a basolateral sorting signal but that the efficiency of basolateral transport is lower compared with that of wild-type SCF. This difference may depend on the presence of the acidic cluster in SCF which is absent from CSF-1.

In Contrast to Wild-type, the Mutant Cytoplasmic Tail of Mn817 Tac SCF Induces Constitutive Endocytosis—Many basolateral sorting determinants have been shown to induce endocytosis, for example the basolateral targeting motif (ML) in the invariant chain of the major histocompatibility complex II also mediates endocytosis of the respective proteins (11, 28). Therefore, we tested whether the wild-type cytoplasmic tails of SCF, expressed as a Tac chimera (Tac-CSF), are able to internalize Tac-CSF-anti-Tac complexes in nonpolarized COS-7 cells. Anti-Tac antibodies were bound to transfected cells in the cold. Subsequently, Tac-CSF-anti-Tac antibody complexes were allowed to internalize at 37 °C and visualized after acid removal of cell surface remaining anti-Tac antibodies. Wild-type Tac-CSF-expressing cells (Fig. 7B) as well as cells expressing various C-terminal deletions encompassing the basolateral sorting signal showed a similar low amount of internalized anti-Tac antibodies (not shown). This suggests that the mono-leucine determinant in SCF does not induce endocytosis, a finding that is consistent with the persistent cell surface expression of membrane-bound SCF.

In contrast to wild-type SCF, GFP and Tac-CSF chimeras containing the cytoplasmic tail of the Mn817 Tac SCF mutation accumulated in intracellular vesicular structures (Tac-CSF-17H, Fig. 7D; see also Ref. 8). This intracellular accumulation of the mutant constructs could be the result of retention of newly synthesized chimeric proteins in the endoplasmic reticulum as suggested by Briley and colleagues (29) or of endocytosis of cell surface SCF. To determine, whether the intracellular steady-state localization of Tac-CSF-17H in polarized MDCK II was the result of endocytosis (Fig. 7D), we compared the localization with that of Tac-tyr. Tyrosinase is a protein that carries an established signal for endocytosis and lysosomal/melanosomal targeting and is therefore constitutively internalized from the cell surface (30). Interestingly, in polarized MDCK II cells, Tac-tyr localized to intracellular vesicular structures (Fig. 7G), resembling the staining seen for the Tac-CSF-17H construct (Fig. 7D). Sequence analysis of the cytoplasmic domain of Mn817 Tac (KYAA TERERISG VIVDVSTLLPSHSNWG; Ref. 5) revealed a sequence homologous to the signal for endocytosis and lysosomal/melanosomal targeting, identified in tyrosinase, LIMP II and CD3 γ (D17LL22) (30–32). Furthermore, mutation of the leucine residues (Leu21-Leu22), which are part of this putative motif in Mn817 to alanines, resulted in the loss of intracellular but led to apical accumulation of Tac-17H-LLAA in polarized MDCK cells (Fig. 7J). In addition, using the anti-Tac internalization assay in COS-7 cells, we tested whether the intracellular localization of the Mn817 Tac mutant was caused by increased endocytosis of surface-expressed Mn817 Tac chimeras. Indeed, compared with wild-type Tac-CSF, significantly more Tac-CSF-17H anti-Tac complexes were internalized (Fig. 7E), and a similar intracellular staining pattern was observed as for the Tac-tyr construct (Fig. 7H). Further study is needed to determine whether the localization of Mn817 Tac is mediated by an endocytosis signal in the cytoplasmic tail of SCF.
corresponds to 24 Mgf endocytosis. Therefore, the antibody was not removed from the cell surface (illustrated by staining of parallel cultures from which the anti-Tac SCF constructs were initially expressed on the COS-7 cell surfaces as bound anti-Tac antibodies (K)). Comparable levels of the different Tac-Hnalized efficiently (E). However, the di-leucine mutation in Tac-SCF-large intracellular vesicles (E). Likewise, Tac-tyr constructs were inter-

However, internalization of the Tac-SCF-17H chimera was blocked by the di-leucine mutation (17H-LLAA; Fig. 7K). This suggests that the reduced amount of cell surface SCF observed in the Mgf SL17H mutation (8, 33) is caused by constitutive removal of the Mgf SL17H mutant SCF from the cell surface by endocytosis. Therefore, the Mgf SL17H mutation represents a molecular gain of function mutation with respect to the endocytosis of SCF.

**DISCUSSION**

An Acidic Cluster Assists the Leucine-dependent Basolateral Targeting Signal in SCF. We identified here—a novel motif in a transmembrane growth factor that is used for basolateral targeting but not for endocytosis. Both an acidic cluster and a critical leucine residue are required for efficient basolateral targeting of SCF. Although the leucine is indispensable for basolateral transport, the presence of the acidic cluster enhances the efficiency of basolateral sorting. Because the acidic cluster is not absolutely required for basolateral targeting, it is unlikely that the two motifs form a single sorting determinant. Interestingly, a basolateral targeting motif has been described for the polymeric Ig receptor, which does not belong to the family of tyrosine or di-leucine determinants and which does not mediate endocytosis. This critical targeting domain consists of a single valine located in a β-turn and two critical residues 3 and 4 amino acids N-terminal to it. Mutation of valine to alanine reduces basolateral targeting and destabilizes the β-turn (34). In addition, the amino acids N-terminal to the valine which do not participate in the β-turn are also required for efficient basolateral sorting and form a second, valine-

![Endocytosis of MgfSL17H mutant Tac-SCF by a lysosomal targeting signal.](image1)

![Multiple biological effects of cytoplasmic mutations in SCF.](image2)
independent, functional targeting domain (35). Based on these similarities, it is possible that leucine 26 of SCF is part of a β-turn or loop, exposing its hydrophobic side chain in such a way that it could bind to the adaptor complex of clathrin-coated vesicles. In addition, many di-leucine sorting motifs have been described which require critical N- or C-terminally located acidic residues as described for the furin protease (25), the low density lipoprotein receptor (16) and the invariant chain of major histocompatibility complex class II (17). In contrast to these determinants, in which the acidic amino acids are essential for basolateral targeting, the acidic cluster in SCF is partially dispensable serving however to increase the fidelity of the basolateral sorting process. Members of the recently identified phosphofurin acidic cluster-sorting (PACS) family of adaptor proteins, which bind to clusters of acidic amino acids, are involved in directing TGN localization and plasma membrane sorting (18, 19). Interestingly, intracellular sorting of the furin protease by PACS is regulated by the phosphorylation of critical serine residues adjacent to a cluster of acidic amino acids. The same PACS binding, acidic cluster which directs TGN localization, is also required for basolateral sorting of furin (25). Although PACS may bind to the acidic amino acid cluster in SCF and thereby increasing the fidelity of the basolateral sorting process in the TGN, there is no indication that this is a phosphorylation-dependent interaction involving the conserved serine residue at position 24. However, in the absence of such acidic clusters as in the cytoplasmic tail of CSF-1, reduced protein recognition at the level of the TGN could affect the fidelity of basolateral targeting compared with SCF. Consequently this inefficiency of basolateral sorting might lead to the apical accumulation of CSF-1 by an N-glycan-dependent apical targeting pathway (13).

MgfSl17H, a Gain of Function Mutation Leading to Constitutive Endocytosis of Mutant SCF—Many basolateral targeting signals resemble those for coated pit localization and endocytosis. In contrast to the protease furin or the invariant chain, wild-type SCF is expressed at the cell surface and is not endocytosed. Interestingly, the cytoplasmic tail of SCF found in the MgfSl17H mutation has a high capacity for inducing endocytosis when expressed as a Tuc chimera. Analysis of the cytoplasmic tail of the MgfSl17H mutant reveal a match of sequence between KYAATERERISRGVIVDVSTLLPSHSGW (5), and the signal for endocytosis or lysosomal/melanosomal/vacular targeting (DXXXXLL). This sequence was found in CD3γ (DXXXLL) (31) and in related form in the invariant chain (DDQXLL; EXXXML) (17, 28, 36), Vam3p (EXXXLL) (37), LIMP II (EE-XXXLL) (32), and tyrosinase (D/EEXXXLL) (30). In all these proteins, the endocytotic activity is critically dependent on the presence of the di-leucine motif and is lost after alanine mutation of the di-leucine motif and is lost after alanine mutation of the MgfSl17H mutation. Therefore the MgfSl17H mutation may represent a gain of function in respect to endocytosis and lysosomal targeting of SCF. As a consequence, only a limited amount of mutant SCF would be available on the cell surface to stimulate responsive, c-Kit-expressing neighboring cells. This could be the cause for the reduced amount of peripheral SCF-dependent mast cells and a limited capacity to support hematopoiesis as observed in the MgfSl17H mutant animals (6, 7). In contrast, based on our results, wild-type SCF lacks a signal for endocytosis, and this is consistent with the role of the cytoplasmic tail of SCF for continuous presentation and signaling of the noncleavable form of SCF toward responsive cells.

The Biological Role of Intracellular Targeting of SCF and Related Transmembrane Growth Factors—Our results suggest multiple roles for the cytoplasmic tail of SCF. First, SCF is targeted to the cell surface in a polarized fashion, being expressed basolaterally and not at the apical surface. Second, after reaching the surface, SCF is retained at the plasma membrane. The first function of the cytoplasmic tail would be important in cells within polarized tissues, such as keratinocytes, Sertoli cells, and neurons, whereas the second function would be relevant to all SCF-expressing cells (Fig. 8). We suggest that the absence of basolateral delivery of SCF leads to the death of melanocytes and male germ cells, which normally require basal delivery of SCF from polarized keratinocytes and Sertoli cells, respectively, as illustrated by the MgfSl17H mutation (8). In addition to the loss of pigmentation and fertility, the absence of spatial learning has been demonstrated in a mouse mutant of SCF (MgfSid4), which lacks the transmembrane and cytoplasmic domain (4). In contrast to wild-type SCF, such mutant forms of SCF are secreted from the apical surfaces of polarized epithelia (8). Cell surface expression of SCF in unpolarized stromal cells of the bone marrow is required for hematopoiesis (8). Consequently, constitutive endocytosis resulting in reduced cell surface expression of SCF would lead to a hematopoietic defect comparable to that of the MgfSid17H mutant mice (6, 7).

Based on functional similarities and sequence comparison with other transmembrane growth factors such as CSF-1, we propose that the basolateral determinant and associated functions are not unique to SCF. Because of the absence of mouse mutations affecting the cytoplasmic tail of CSF-1 it is not known whether the role of its cytoplasmic tail is equally important as that of SCF. In op/op mice that lack CSF-1, CSF-1-dependent macrophages are absent from epithelial as well as mesenchymal tissues. Intravenous injection of soluble CSF-1 rescues only mesenchymal macrophages but not epithelial cells as well as mesenchymal epithelial tissues. Intravenous injection of soluble CSF-1 may lead to a hematopoietic defect comparable to that of the MgfSid17H mutant mice (6, 7).

Clearly defined targeting determinants in SCF and other transmembrane growth factors may offer possibilities for altering polarized presentation and cell surface expression of these factors. This may lead to new therapeutic approaches for treatment of pathological conditions such as allergies, chronic inflammation, osteoporosis, or hyperpigmented lesions caused by overexpression or mutations of these factors.

Acknowledgments—We thank Marie-Claude Jaquier for excellent technical assistance and Caroline Johnson-Léger, Monique Wehrle-Haller, Claes Wollheim, Pierre Cosson, and James Weston for discussions and critical reading of the manuscript. We give special thanks to Robert Kelsh for suggesting a gain of function mutation as the cause of the MgfSid17H phenotype. We thank Willy Hofstetter, Friedrich Beermann, and Karl Matter for providing cDNAs for CSF-1, tyrosinase, and MDCK II cells.

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J. Biol. Chem. 2001, 276:12667-12674.
doi: 10.1074/jbc.M008357200 originally published online January 10, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M008357200

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