BLOOD

The Journal of
The American Society of Hematology

VOL 75, NO 1 JANUARY 1, 1990

REVIEW ARTICLE

Colony-Stimulating Factor-1 Receptor

By Charles J. Sherr

COLONY-STIMULATING factor-1 (CSF-1), also called macrophage colony-stimulating factor (M-CSF), is a lineage-specific hematopoietin that stimulates proliferation and supports differentiation and survival of cells of the mononuclear phagocyte series.[1,2] Its activity was originally detected in biological fluids, including serum[3] and urine,[4] in various tissues,[5] and in the medium conditioned by certain established cell lines.[6-8] The purified growth factor is a 70- to 90-Kd homodimeric glycoprotein synthesized primarily by fibroblasts,[9,10] but its synthesis can be induced in endothelial cells[11] and in monocytes and macrophages[12-15] in response to other cytokines. CSF-1 induces formation of pure macrophage colonies from individual bone marrow (BM) precursors plated in semisolid medium and can stimulate proliferation of macrophages isolated from peritoneal and pleural exudates, alveoli, lymph nodes, and spleen.[16] It also potentiates the ability of mature mononuclear phagocytes to perform their differentiated functions by enhancing their ability to kill infectious microorganisms[17,18] and tumor cells[19,20] and by regulating release of macrophage cytokines, such as interferon, tumor necrosis factor (TNF), interleukin-1 (IL-1), and granulocyte-CSF (G-CSF),[21-25] as well as inflammatory modulators, such as prostaglandins, biocidal oxygen metabolites, thromboplastin, and plasminogen activator.[26-31] Biologically active, secreted, and membrane-bound forms of CSF-1[32-35] are encoded by alternatively spliced messenger RNAs (mRNAs) transcribed from a single gene[36,37] that maps to the long arm of human chromosome 5 at band q33.1.[38] CSF-1 differs in its physicochemical properties and spectrum of action from other known hematopoietins, including granulocyte-macrophage CSF (GM-CSF) and IL-3, each of which stimulates proliferation of immature myeloid cells, including the progenitors of macrophages.[39,40]

Although originally defined through its activities on blood cells, CSF-1 may have important functions outside the context of hematopoiesis. The growth factor is produced by uterine epithelium during pregnancy,[41-43] and the levels of uterine CSF-1 production increase more than a thousand-fold during gestation and are highest at parturition.[41] Detection of CSF-1 receptors on placental trophoblasts and on choriocarcinoma cell lines[43-45] suggests that CSF-1 may be an important modulator of placental development. The possibility that other cell types might also respond to CSF-1 has not been well-studied.

In the hematopoietic system of adult animals, CSF-1 exerts its pleiotropic effects by binding to a single class of high-affinity receptors[46,47] expressed predominantly on monocytes, macrophages, and their committed BM precursors.[48] The CSF-1 receptor (CSF-1R) is encoded by the c-*fms* protooncogene[49] and is a member of a family of growth factor receptors that exhibit ligand-induced tyrosine-specific protein kinase activity.[50,51] Activation of the receptor kinase triggers a cascade of biochemical events, some of which convey signals from the plasma membrane to the cell nucleus that affect the transcription of CSF-1–responsive genes and lead to cell division. Critical mutations in the c-*fms* gene, such as those observed in the transduced feline retroviral v-*fms* oncogene, can unleash their latent oncogenic potential by altering CSF-1R so as to upregulate its kinase activity in a ligand-independent manner.[52-54] Expression of "activated" *fms* oncogene products in susceptible cells provides sustained signals for cell growth in the absence of appropriate environmental cues, leading to cell transformation and tumorigenesis.

STRUCTURE AND TRANSCRIPTION OF THE CSF-1R GENE (C-*FMS*)

The c-*fms* protooncogene maps near the CSF-1 locus on human chromosome 5 at band 5q33.3[55-57] in relative proximity to other growth factor and receptor genes that play

From the Howard Hughes Medical Institute, Department of Tumor Cell Biology, St Jude Children's Research Hospital; and the Department of Biochemistry, University of Tennessee College of Medicine, Memphis, TN.

Submitted August 1, 1989; accepted September 8, 1989.

Supported in part by Grant No. R35-CA47064 (C.J.S.) from the National Institutes of Health (NIH), Bethesda, MD, NIH Cancer Center Support Grant No. P30-CA21765, and by the American Lebanese Syrian Associated Charities of St Jude Children's Research Hospital. C.J.S. is an Investigator of the Howard Hughes Medical Institute and Herrick Foundation Chairman of the Department of Tumor Cell Biology, St Jude Children's Research Hospital.

Address reprint requests to Charles J. Sherr, MD, PhD, Howard Hughes Medical Institute, Department of Tumor Cell Biology, St Jude Children's Research Hospital, 332 N Lauderdale, Memphis, TN 38105.

© 1990 by The American Society of Hematology.

0006-4971/90/7501-0039$3.00/0

important roles in hematopoiesis. These include the GM-CSF, IL-3, IL-4, and IL-5 genes,[57-63] as well as the gene encoding the B isoform of the platelet-derived growth factor receptor (PDGF-R_B), previously mapped to 5q31-32.[64] Molecular cloning of a PDGF-R_B cDNA established that it shared a relatively high degree of amino-acid sequence homology to CSF-1R, particularly in its kinase domain.[64]

Although cytogenetic assignments had suggested that the CSF-1R and PDGF-R_B genes were at least one megabase apart, we found that the two genes were juxtaposed in a tandem head-to-tail array.[65] By aligning the CSF-1R and PDGF-R_B cDNA sequences and knowing the positions of splice junctions within the c-*fms* gene,[66] it was possible to predict correctly the organization of PDGF-R_B exons.[65] Together, these results imply that CSF-1R and PDGF-R_B were derived from an ancestral gene that underwent duplication and subsequent evolutionary divergence. Recently, the A isoform of PDGF-R was cloned and assigned to human chromosome 4.[67,68] The same region of chromosome 4 harbors the c-*kit* protooncogene,[69] which encodes a structurally related receptor for an as-yet-uncharacterized ligand, raising the possibility that the c-*kit* and PDGF-R_A genes might also be organized in a tandem array.

A schematic diagram of the CSF-1R and PDGF-R_B loci is shown in Fig 1. Transcription of CSF-1R RNA appears to be initiated at two independent promoters that function in a tissue-specific manner.[71] In placental trophoblasts, transcripts include 5′ sequences originating from c-*fms* exon 1,[65,70,71] which specifies only nontranslated sequences of c-*fms* mRNA and is separated by a 26-kilobase (kb) intron from the remaining 21 exons encoding the receptor itself. The location of c-*fms* exon 1 is less than 0.5 kb from the polyadenylation signal of the PDGF-R_B gene, suggesting that transcription of PDGF-R_B might influence the activity of a putative c-*fms* promoter mapping between the two genes. Thus, the tandem organization of PDGF-R_B and CSF-1R might underlie a mechanism for their mutually exclusive expression in different cells of the developing placenta. In monocytes, however, c-*fms* transcripts are initiated from an independent promoter[71] located directly upstream of exon 2, which specifies the amino-terminal signal peptide of CSF-1R.[66] The exact locations of the two c-*fms* promoters are presently unknown, as are the positions of putative tissue-specific enhancer elements that regulate expression of alternative CSF-1R mRNAs in placenta and mononuclear phagocytes.

The size of the c-*fms* gene makes it unlikely that its mRNA levels can be rapidly regulated by transcriptional mechanisms. The minimal length of genomic CSF-1R coding sequences is 32 kb, so that the formation of its unspliced mRNA precursors should require more than 30 minutes, based on the estimated rate of transcription by RNA polymerase II and barring attenuation of RNA synthesis within the gene.[66] This means that receptor synthesis is more likely to be governed by posttranscriptional mechanisms involving RNA stabilization or translation. Although the steady-state levels of c-*fms* mRNA gradually increase as precursors of mononuclear phagocytes differentiate and mature,[72] the gene is continuously transcribed during cell maturation and the half-life of its mRNA in mature monocytes is regulated by inducers of CSF-1R expression.[73]

STRUCTURE AND BIOSYNTHESIS OF CSF-1R

Human CSF-1R is an integral transmembrane glycoprotein 972 amino acids long.[70] Including the signal peptide, it consists of a 512 amino-acid *N*-terminal extracellular segment containing the ligand-binding domain, a hydrophobic 25 amino-acid membrane-spanning region, and a 435 amino-acid intracellular domain that includes all the sequences necessary for tyrosine kinase activity (Fig 2). Similar structures are predicted for the mouse[74] and feline[52] CSF-1 receptors. These show 75% and 84% overall homology to human CSF-1R, with the greatest degree of sequence conservation occurring between their intracellular kinase domains. Based on a consideration of conserved structural motifs as well as its genomic organization, CSF-1R is most closely related to the A and B isoforms of PDGF-R,[64-68] the c-*kit* protooncogene product,[69] and the recently cloned receptor for basic fibroblast growth factor (FGF-R).[75] In contrast, CSF-1R shows much less overall homology to other receptors of the protein tyrosine kinase (PTK) gene family, such as the epidermal growth factor (EGF-R), insulin (I-R), and insulin-like growth factor-1 (IGF-1R) receptors.[50,51]

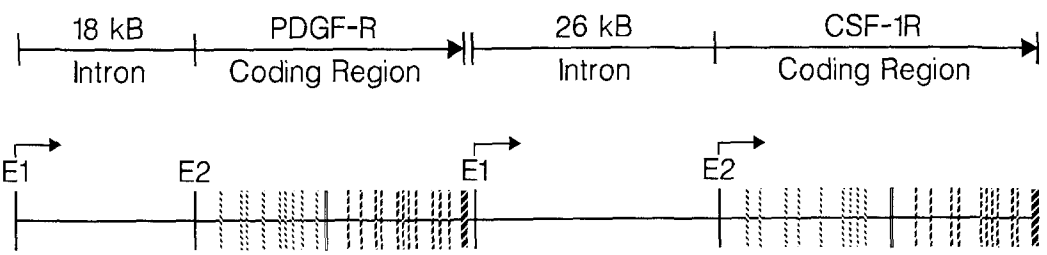

Fig 1. Genomic organization of the human CSF-1R and PDGF-R_B genes. The CSF-1 and PDGF-R_B genes are aligned in a head-to-tail array on the long arm of human chromosome 5. Both are shown in 5′ to 3′ orientation (coding strand); their orientation with respect to the centromere is unknown. Each gene is approximately 60 kb long and contains coding sequences organized into small exons (vertical bars) separated by longer introns (horizontal lines). The positions of two c-*fms* promoters are indicated by arrows positioned just upstream of exons 1 and 2 (E1 and E2). The unstippled exon indicated by a double vertical bar in the middle of each gene encodes the transmembrane segment of the receptor. Exons 5′ and 3′ to these segments encode the ligand-binding and kinase domains of the receptors, respectively. Data regarding organization of CSF-1R were derived from a previously reported genomic nucleotide sequence[66]; data concerning PDGF-R were derived from our unpublished observations and from reference 65.

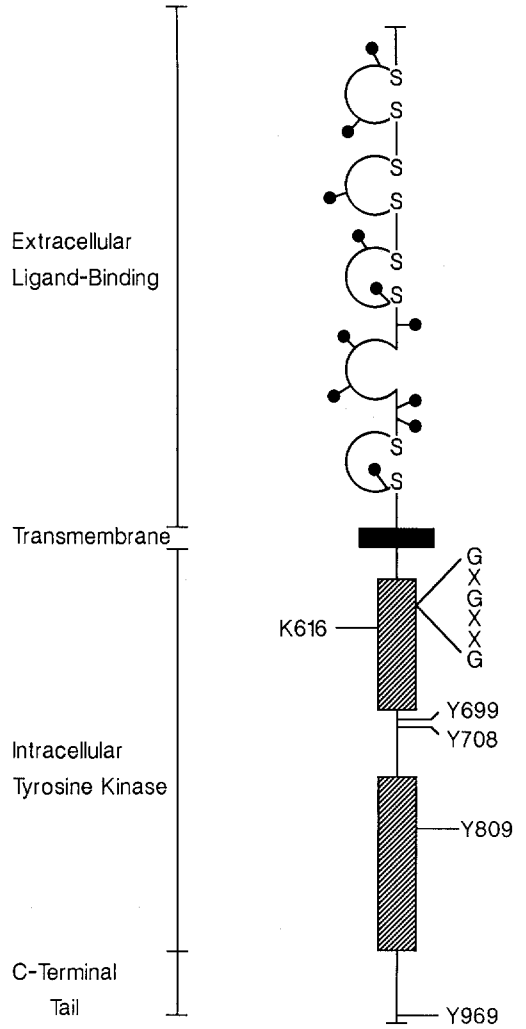

Fig 2. Predicted structure of human CSF-1R. The extracellular ligand-binding portion is organized into five immunoglobulin-like domains, four of which are stabilized by disulfide bonds (S-S). All such domains are homologous to sequences of the C2-SET.[76] Positions of asparagine-linked oligosaccharide chains are indicated (●). The intracellular kinase domain (stippled bars) is interrupted by spacer sequences containing two predicted sites of receptor phosphorylation at tyrosines (Y) 699 and 708. A third phosphorylation site (Y809) occurs in the distal core consensus sequences. The membrane-proximal kinase segment includes a glycine-rich signature sequence (G-X-G-X-X-G) characteristic of kinases in general. This is followed by the ATP binding site at lysine (K) 616. The *C*-terminal tail contains a single tyrosine residue (Y969) whose removal upregulates receptor kinase activity.[131] The data are taken from a previously reported human c-*fms* cDNA sequence.[70] Unpublished information regarding sites of phosphorylation within the murine receptor was provided by Drs P. Tapley, A. Kazlauskas, and L. R. Rohrschneider and was aligned with the human amino-acid sequence.

The extracellular segment of CSF-1R is predicted to include a series of disulfide-bonded loops, each representing motifs characteristic of members of the immunoglobulin gene superfamily (Fig 2).[76] Analogous immunoglobulin-like domains have been predicted for the two PDGF receptors and FGF-R, but not for EGF-R, I-R, or IGF-1R, which instead contain interspaced blocks of cysteine-rich sequences also found in the EGF precursor and low-density lipoprotein receptor.[51] The extracellular domain of CSF-1R is heavily glycosylated and contains 11 sites of addition of asparagine-linked (but not *O*-linked) oligosaccharide chains. The presence of these carbohydrate side chains contributes approximately 45 Kd to the molecular mass of the mature 150-Kd human glycoprotein.[45,77]

The intracellular portion of CSF-1R contains a characteristic sequence consisting of a gly-X-gly-X-X-gly motif at amino acids 589 to 594, followed by a lysine residue at position 616, which was predicted to be the site of ATP binding.[78] Indeed, mutation of lysine 616 to a methionine residue abolishes receptor tyrosine kinase activity and renders CSF-1R incapable of transducing mitogenic signals.[79] Sequences flanking the ATP binding site show a high degree of amino acid similarity to other members of the PTK gene family, as does a second, more extended cluster of amino acid residues closer to the receptor C-terminus (Fig 2).[50] As compared with other prototypic tyrosine kinases, such as EGF-R, I-R, IGF-1R, and members of the *src* protooncogene family, the intracellular kinase domain of CSF-1R contains an additional 72 amino-acid "spacer" or "kinase insert" sequence that separates sequences surrounding the ATP binding site from the remainder of the PTK consensus sequences. These spacer sequences are conserved among the human, mouse, and feline CSF-1 receptors, but are unrelated to analogous spacer elements of different lengths found in PDGF-Rs, FGF-R, and the c-*kit* product, suggesting that they may function in specific substrate recognition.[69]

CSF-1R is synthesized as an integral transmembrane glycoprotein, oriented with its *N*-terminal domain in the cisternae of the rough endoplasmic reticulum (ER) and its kinase domain in the cell cytoplasm.[54] The 105-Kd polypeptide is cotranslationally glycosylated, acquiring *N*-linked oligosaccharides of the high-mannose type, so that the immature intracellular glycoprotein has an apparent molecular mass of 130 Kd. During intracellular transport of the glycoprotein to the plasma membrane, the *N*-linked carbohydrates undergo modification in the Golgi complex, resulting in an increase in the apparent molecular mass of human CSF-1R to 150 Kd. Variations in molecular masses of mature receptors in different species result in part from differences in processing of the carbohydrate side chains. Transport of the receptor from the ER to the plasma membrane occurs efficiently, so that newly synthesized molecules can be detected at the cell surface within 30 minutes of synthesis. Several lines of evidence suggest that expression of the receptor at the cell surface is necessary for biologic activity,[80-82] implying that physiologic substrates for the receptor kinase may also be preferentially disposed at the inner surface of the plasma membrane.

DOWNREGULATION AND TRANSMODULATION OF CSF-1R

In the absence of ligand, the mature cell-surface form of CSF-1R is relatively stable, with a half-life of 3 to 4 hours.[33] The receptors can move into clathrin-coated pits,[83] and the possibility that a proportion are continuously being internalized into peripheral endosomes and reshuttled back to the cell surface has not been well studied. On CSF-1 binding,

however, ligand–receptor complexes are rapidly internalized, targeted to lysosomes, and degraded,[33,47,84] a process known as downregulation. Complete receptor degradation occurs within 15 minutes of stimulation with saturating concentrations of CSF-1, so that cells become completely refractory to restimulation by ligand until they resynthesize and reaccumulate new receptors at the cell surface. Although this process can attenuate the duration of ligand-induced PTK activity, it is unlikely that complete receptor downregulation occurs in vivo, because mononuclear phagocytes do not normally encounter saturating concentrations of CSF-1, nor do they require them for biologic responses to ensue. Kinase-inactive CSF-1R mutants do not undergo accelerated ligand-induced turnover, indicating that PTK activity is required for directing ligand–receptor complexes to the degradative pathway.[79] The extent of receptor downregulation and the relative "strength" of PTK signals can also be influenced by other cell-surface proteins (eg, by stabilizing CSF-1R at the cell surface, the bovine papillomavirus E5 protein can potentiate the CSF-1–induced mitogenic response, leading to cell transformation).[85]

A loss of CSF-1 binding sites,[86,87] resulting from accelerated turnover of mature CSF-1R molecules,[79,84] also occurs after treatment of macrophages with phorbol esters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), or with other physiologic inducers of protein kinase C (PKC) such as bacterial lipopolysaccharide (LPS).[88] Several lines of evidence suggest that the mechanisms of ligand- and TPA-induced receptor turnover are different. First, kinase-inactive receptor mutants remain sensitive to TPA-induced, but not ligand-induced, degradation. Conversely, if cells are chronically treated with phorbol esters so that PKC is itself downregulated, CSF-1R molecules reexpressed at the cell surface are sensitive to ligand- but not phorbol ester-induced, degradation.[79] Thus, PKC activity is not required for ligand-induced receptor degradation. Third, the turnover of the feline v-*fms*–coded glycoprotein is not accelerated by TPA treatment,[84] indicating that mutational differences which distinguish the v-*fms*– and c-*fms*-coded molecules (described below) must affect their sensitivity to PKC-induced degradation. Chimeric receptors containing half of the extracellular domain of human CSF-1R fused to the remaining portion of the feline v-*fms* gene product are rapidly downregulated in response to human CSF-1, but not by phorbol esters, further demonstrating that these two properties can be genetically segregated from one another.[89]

Activation of PKC by phorbol esters induces proteolytic cleavage of CSF-1R in its extracellular domain at a site(s) near the membrane-spanning sequence.[79] This results in release of the glycosylated 100-Kd ligand-binding domain from the cell and the simultaneous appearance of a cell-associated 50-Kd fragment representing the CSF-1R tyrosine kinase domain, which then undergoes rapid intracellular degradation. Because receptor cleavage can occur in response to a variety of physiologic inducers of PKC and does not appear to involve receptor internalization through the endocytic pathway, this process has been termed "transmodulation." CSF-1R is phosphorylated only on serine residues in the absence of ligand stimulation, and its transmodulation by PKC is not accompanied by a detectable increase in receptor serine or threonine phosphate or by phosphorylation at new sites. Thus, PKC does not directly modify and "target" the receptor for cleavage, but rather activates a protease that recognizes CSF-1R as a substrate. The 50-Kd tyrosine kinase fragment produced by this reaction lacks phosphotyrosine, the hallmark of an activated enzyme (described below), and therefore is unlikely to be functional. The receptor ligand-binding domain released from the cells retains the ability to bind CSF-1 (S. Morris, J.R. Downing, C.J. Sherr, unpublished observations), enabling it to compete with cell-surface receptors for ligand.

The significance of CSF-1R transmodulation may be to regulate the inflammatory response. Physiologic inducers of PKC, such as the macrophage activator LPS, would be expected to inhibit CSF-1–dependent cell proliferation and survival by this mechanism. Moreover, unlike its inductive effects in HL-60 cells, TPA treatment of mature monocytes does not increase c-*fms* transcription but instead destabilizes c-*fms* mRNA,[73] thereby inhibiting new receptor synthesis. Because macrophage activation and proliferation are likely to represent antagonistic processes, transmodulation of CSF-1R might not only enhance macrophage differentiated functions but would also limit subsequent survival of activated phagocytes at sites of inflammation. PKC induces transcription of many genes, one of which is the CSF-1 gene itself.[12,15] Although activated macrophages should release CSF-1, transmodulation of CSF-1R and concomitant diminution in levels of CSF-1R mRNA would make such cells refractory to autocrine stimulation. Perpetuation of the inflammatory response might therefore necessitate recruitment of naive macrophages in response to local CSF-1 production.

RECEPTOR SIGNAL TRANSDUCTION

CSF-1 binding alters the receptor's conformation in such a way as to upregulate PTK activity. The nature of these conformational changes is poorly understood, but work in other systems suggests that the receptors form dimers or higher order aggregates in response to ligand, thereby activating the receptor kinase through an intermolecular mechanism.[90-93] Induction of PTK activity leads to phosphorylation of the receptor itself on tyrosine, possibly owing to transphosphorylation by aggregated CSF-1R subunits. Three major sites of tyrosine phosphorylation within the CSF-1R kinase domain have been identified. One of these appears to be tyrosine 809 (Fig 2), whose position and context are analogous to those of the major sites of tyrosine phosphorylation in the *src* gene product and insulin receptor. Two other sites have been mapped within the kinase insert region of murine CSF-1R (P. Tapley, A. Kazlauskas, L.R. Rohrschneider, personal communication). In contrast to the ligand-induced phosphorylation of EGF-R which occurs at multiple sites clustered within the receptor *C*-terminal tail, the CSF-1R *C*-terminus contains only a single tyrosine residue at position 969 (Fig 2), which is not detectably phosphorylated either in the absence or presence of ligand (J.R. Downing and C.J. Sherr, unpublished observations). It is not known whether "autophosphorylation" of CSF-1R on

tyrosine plays any direct role in regulating its PTK activity, determining its specificity for heterologous protein substrates or governing its rate of ligand-induced internalization and degradation. Recent studies with PDGF-R_B suggest that phosphorylation within its kinase insert sequences can affect its interaction with certain substrates.[94]

Activation of CSF-1R PTK activity leads to rapid phosphorylation of heterologous protein substrates within seconds of CSF-1 stimulation.[95-97] With use of antibodies to phosphotyrosine for immunoblotting analysis, several tyrosine-phosphorylated polypeptides can be detected in stimulated macrophages after CSF-1 treatment, but none of these proteins has yet been purified or characterized in detail. That CSF-1 can elicit a pleiotropic response that includes both rapid and delayed biochemical events strongly suggests that multiple physiologic substrates for the receptor kinase will be identified. CSF-1 induces rapid effects on membrane ruffling and vacuolization,[98] immediate cytoplasmic alkalinization through Na^+/H^+ exchange,[99] increased hexose transport,[100] and induction of a family of "immediate early response" genes, including the protooncogene c-*fos*,[101,102] within minutes of stimulation. These rapid events appear insufficient for mitogenesis, because CSF-1 is required throughout the entire G1 phase of the cell cycle to ensure a proliferative response.[103] Thus, an evolution of different substrates for the receptor kinase may occur as the cells progress toward S phase. Moreover, submitogenic doses of the growth factor can support the viability of nonproliferating cells that become growth arrested in early G1,[103] indicating that the family of CSF-1–responsive genes must include those that support cell metabolism as well as those that regulate cell growth.[102]

A major challenge, then, is to pinpoint tyrosine phosphorylated substrates that lie along the "mitogenic pathway" and act to relay signals from the receptor to the cell nucleus. Excitement was generated by recent observations that ligand-stimulated EGF-R and PDGF-R_B could bind and phosphorylate phospholipase C-γ (PLC-γ),[104-108] an enzyme that cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) to yield soluble inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG). Both IP3 and DAG act as "second messengers" in signal transduction, the former in mobilizing calcium from intracellular stores and the latter in activating PKC.[109] Thus, phosphorylation of PLC-γ by EGF-R and PDGF-R_B could affect the activity or topology of PLC-γ so as to increase second messenger production. However, in parallel studies we were not able to demonstrate that PLC-γ is a direct substrate of the CSF-1R kinase,[110] consistent with a lack of evidence for rapid mobilization of soluble phosphoinositides in macrophages responding to CSF-1 stimulation.[111] Although our studies have not ruled out the possibility that CSF-1R can bind and phosphorylate other PLC isoforms, the simplest interpretation is that CSF-1R uses alternative second-messenger pathways.

Other protooncogene products that function "downstream" of CSF-1R along the mitogenic pathway may also represent, or communicate with, candidate substrates; eg, stimulation of fibroblasts by PDGF or their transformation by the v-*fms* oncogene induces phosphorylation of the c-*raf* protooncogene product,[112,113] itself a serine/threonine protein kinase.[114] The kinase activity of the c-*raf* protein is encoded in its *C*-terminal moiety, implying that the *N*-terminal half of the polypeptide plays a modulatory role. Because *N*-terminally truncated versions of the c-*raf* gene product can transform cells, this domain appears to regulate *raf*-coded kinase activity negatively. Receptor-mediated tyrosine phosphorylation of its *N*-terminal domain might provide one mechanism by which the c-*raf*–coded enzyme is physiologically regulated.

Microinjection of monoclonal antibodies (MoAbs) to the Harvey c-*ras* gene product (p21*ras*) inhibits growth of fibroblasts transformed by the retroviral v-*fms* oncogene, but not by v-*raf*, suggesting that the activity of a *ras*-coded protein might be independently coupled to CSF-1R.[115] The mechanism by which antibodies to p21*ras* suppress v-*fms* action and, by extension, normal CSF-1R function, is not understood. By analogy to prototypic guanine nucleotide-binding (G) proteins that function in signal transduction,[116] *ras* gene products require bound GTP for activity. Hydrolysis of bound GTP to GDP is mediated by the intrinsic GTPase activity of p21*ras* itself, thereby attenuating its signal. Conversely, oncogenic p21*ras* mutants which fail to hydrolyze GTP provide sustained signals for cell proliferation and induce cell transformation and tumorigenesis.[117] In eukaryotes, a GTPase-accelerating protein (GAP) that binds to p21*ras*–GTP complexes is required for maximal levels of GTP hydrolysis,[118] implying that inhibition of GAP activity might also modulate p21*ras*-induced mitogenic signals. Candidate regulators of *ras* proteins and/or GAP activity include certain phospholipids.[119] If substrates of the CSF-1R kinase that upregulate p21*ras* or inhibit GAP activity could be identified, inhibition of v-*fms* transformation by anti-*ras* MoAbs might be explained.

The physical association of a novel phosphatidylinositol (PI)-3′ kinase with both the PDGF-R_B receptor and the v-*fms* gene product, and the possibility that this form of PI kinase is activated by tyrosine kinases,[120-122] indicate an alternative mechanism by which synthesis of phospholipids might be regulated by growth factor receptors. Mutant PDGF-R_B molecules lacking their kinase insert sequences are impaired in triggering fibroblast mitogenesis, although they retain PTK activity.[121,123] Such mutants fail to regulate either the c-*raf* product or with PI kinase,[113] reinforcing the view that each of these putative substrates may play a critical role in signal transduction. Analogous kinase insert deletion mutants of murine CSF-1R retain both their PTK activity and mitogenic potential when expressed in fibroblasts,[124] but their signal-transducing capability in macrophages has not yet been examined.

Prostaglandins of the E series (PGE_1 and PGE_2) can negatively regulate mononuclear phagocyte proliferation,[26,29,125,126] suggesting that elevated levels of cAMP, induced through the action of the PGE_2 receptor, might attenuate CSF-1 responsiveness. In the SV40-immortalized, CSF-1-dependent mouse macrophage cell line, BACl.2F5,[127] PGE_2 inhibited CSF-1–induced DNA synthesis and cell division; its effects were mimicked by dibutyryl-cAMP and potentiated by 1-methyl-3-isobutyl xanthine, an inhibitor of cAMP phosphodiesterase (S. Jackowski, C.W. Rettenmier,

C.O. Rock, personal communication). CSF-1 activates a pertussis toxin-sensitive GTP-binding protein in monocyte membranes,[128] suggesting that it might limit cAMP levels through an effect on a G protein coupled to adenyl cyclase. In BACl.2F5 cells, however, CSF-1 does not attenuate the rise in cAMP levels induced by PGE_2. Instead, BACl.2F5 cells produced PGE_2 when starved for CSF-1 but terminated release on stimulation with the growth factor. In the presence of both CSF-1 and PGE_2, nondividing BACl.2F5 macrophages remained viable, whereas those starved for CSF-1 did not (S. Jackowski, C.W. Rettenmier, C.O. Rock, personal communication). That PGE_2 did not abrogate functions of CSF-1 necessary for cell survival indicates that CSF-1R signal transduction continues in the presence of elevated cAMP concentrations and that cAMP interdicts some but not all downstream signals.

How does second-messenger synthesis ultimately lead to alterations in the transcription of target genes? Posttranslational modification of preexisting transcriptional regulatory factors in the cytoplasm and their transduction to the nucleus represent basic requirements for activation of several early response genes.[129,130] Efforts to identify and clone candidate transcriptional regulatory factors and to delineate modifications that regulate their functions represent topics of investigation for many laboratories and are likely to pinpoint critical connections along the mitogenic pathway.

TRANSDUCTION OF THE CSF-1R GENE

Expression of c-*fms* cDNA under the control of retroviral vectors has enabled receptor function to be evaluated in cells that normally do not express CSF-1R. When human CSF-1R was expressed in naive mouse NIH-3T3 fibroblasts, the cells were able to form colonies in semisolid medium in response to physiologic concentrations of human recombinant CSF-1. Cotransfection of the same cells with a second vector encoding human CSF-1 closed an autocrine loop and led to cell transformation and tumorigenicity.[131] Thus, CSF-1R can interact functionally with elements of the mitogenic pathway, even in cells that normally do not respond to the growth factor. NIH-3T3 cells expressing CSF-1R, like the $CSF\text{-}1R^-$ parental line, required PDGF and IGF-1 (or insulin) for growth in chemically defined medium, but in the presence of CSF-1 their requirements for exogenous PDGF and IGF-1 were abrogated.[132] Although the doubling times of the cells in CSF-1 or in PDGF plus insulin were identical, cells grown in CSF-1 were no longer contact inhibited and appeared morphologically transformed. That their growth response was qualitatively altered in response to CSF-1 suggests that PDGF-R and CSF-1R might differentially interact with substrates that regulate contact-mediated growth arrest.

Insertion of human CSF-1R into Chinese hamster lung fibroblasts (CCL39) also enabled them to respond mitogenically to CSF-1.[133] In these cells, CSF-1 stimulated Na^+/H^+ exchange and DNA synthesis in the absence of PI breakdown, consistent with previous observations in macrophages.[111] Pertussis toxin weakly inhibited CSF-1–induced proliferation, again suggesting that the receptor can communicate with a signal-transducing G protein.[128] In the absence of CSF-1, receptor-bearing CCL39 cells exhibited a higher basal level thymidine incorporation and decreased anchorage independence as compared with parental cells.[133] Both processes were inhibited by an MoAb that abrogates signal transduction by human CSF-1R.[134] The observation that "relaxation" of growth control depended on receptor expression suggested that basal levels of receptor kinase activity per se might provide certain cells with a proliferative advantage.

The mouse myeloid cell line FDC-P1 does not normally express CSF-1R and requires IL-3 for proliferation and survival.[135] Introduction of human CSF-1R under the control of an inducible metallothionein promoter enabled the cells to grow in CSF-1.[136] In the absence of CSF-1 or IL-3, a low percentage of receptor-bearing (but not parental) cells remained able to proliferate and their frequency was increased when receptor expression was further induced by heavy metal treatment. Again, an MoAb that inhibits signal transduction through human CSF-1R was able to arrest the growth of both CSF-1–dependent and factor-independent receptor-bearing cells. These results raise the possibility that unscheduled expression of CSF-1R in immature myeloid cells might not only alter their factor dependence but might also relax their growth factor requirements. Introduction of the murine c-*fms* gene into FDC-P1 cells induced monocytic characteristics in response to CSF-1, further suggesting that CSF-1R–mediated signals can directly trigger the expression of genes differentially expressed in mononuclear phagocytes.[163]

TRANSFORMING POTENTIAL OF THE C-*FMS* GENE

The v-*fms* gene of the Susan McDonough strain of feline sarcoma virus (SM-FeSV) was derived by recombination between a feline leukemia virus and c-*fms* protooncogene sequences of the domestic cat.[137] The v-*fms* oncogene can transform cultured cat, mouse, rat, canine, and mink fibroblasts to anchorage-independent, tumorigenic derivatives[54] and can abrogate the growth factor requirements of both CSF-1–dependent and IL-3–dependent murine myeloid cells, rendering them tumorigenic in nude mice.[84,138] When SM-FeSV–infected BM cells were used to reconstitute lethally irradiated mice, the transplanted recipients developed a variety of hematopoietic proliferative disorders, including clonal erythroleukemias and B-cell lymphomas.[139] Recently, v-*fms* was shown to recapitulate effects of the v-*abl* oncogene in transforming murine pre-B cells in long-term BM cultures.[140] Thus, although it was derived from a lineage-specific receptor, the v-*fms* gene product is promiscuous in its transforming activity and can contribute to tumorigenesis in a variety of settings.

Although analogous in structure to CSF-1R, the v-*fms*–coded glycoprotein can act as a ligand-independent tyrosine kinase. The oncogene product is itself constitutively phosphorylated on tyrosine[77,141] and induces phosphorylation of a series of cellular proteins on tyrosine in the absence of CSF-1,[142] thereby providing sustained signals for cell growth. The SM-FeSV v-*fms* and feline c-*fms* gene products differ by only nine scattered point mutations and by a *C*-terminal truncation in which 50 amino acids of the c-*fms*–coded *C*-terminal tail were replaced by 11 unrelated v-*fms*–coded residues.[52] Truncations, mutations, or sequence substitutions

engineered within the *C*-terminus of human CSF-1R appear to disrupt a negative regulatory domain and upregulate its ligand-dependent receptor kinase activity.[131,143] However, these genetic alterations in themselves are insufficient to activate c-*fms* as an oncogene.[131] In contrast, specific amino acid substitutions in the receptor extracellular domain act as potent "activating mutations" that enable mutant CSF-1R to transform cells in the absence of CSF-1.[52,53] When mutant feline receptors were assayed in rat-1 cells, point mutations at codons 301 and 374, as well as the *C*-terminal truncation, were required to generate a fully transforming phenotype.[52] When programmed into human CSF-1R, the single substitution of serine for leucine at codon 301 enabled the receptor to convert NIH-3T3 cells to a tumorigenic phenotype[53]; mutation of codon 374 had no independent or additive effect in this sytem (M.F. Roussel and C.J. Sherr, unpublished observations). However, human receptors bearing an activating mutation at codon 301 together with cooperating *C*-terminal modifications, such as substitution of tyrosine 969 by phenylalanine, were more active in transformation,[53] showing that in either system multiple genetic events occurring within c-*fms* can cooperate to augment its transforming efficiency incrementally.

Although the activating mutation at codon 301 is located in the receptor extracellular domain, it does not perturb the ligand-binding site[53]; indeed, cells transformed by v-*fms* show a further enhancement in their growth rate in the presence of CSF-1.[89,144] As expected, equilibrium binding studies performed with ^{125}I-labeled human recombinant CSF-1 and cell lines expressing either activated forms of human CSF-1R or the feline v-*fms* product showed that the kd for binding was 1 to 4 $\times$ 10^{-10} mol/L, similar to that calculated for the wild-type receptor.[53,144] In recent studies, we found that certain amino acid substitutions at codon 301 other than serine are effective in activating the transforming potential of human CSF-1R, whereas others had no effect.[145] The activating mutations must therefore induce a specific conformational change that mimics an effect of ligand binding on receptor kinase activity. The ability of an MoAb directed to an extracellular receptor epitope to inhibit the growth of cells transformed by human CSF-1R mutants[132,134] is consistent with the possibility that the activating mutations alter the aggregation state of the receptor at the cell surface, thereby enhancing receptor dimer formation in the absence of ligand. Similar effects have recently been observed with the product of the *neu* (*erb*B-2, HER-2) oncogene,[146] which is activated by specific point mutations within its transmembrane domain.[147,148]

POSSIBLE ROLES FOR C-*FMS* IN HUMAN NEOPLASIA

Activating mutations within the c-*fms* gene in myeloid cells might contribute to human hematopoietic malignancies involving receptor-positive cells of the mononuclear phagocyte series.[149,150] Sophisticated methodologies and available reagents should permit rapid evaluation of these possibilities. It is now possible to screen human leukemic blasts quickly for activating mutations at c-*fms* codon 301 by polymerase chain reaction techniques. An initial survey of leukemic blasts from 20 cases of receptor-positive acute myeloid leukemia has not shown the presence of such mutations (S. Kramer, M.F. Roussel, C.J. Sherr, unpublished observations). The experimental results to date do not preclude the possibility that point mutations elsewhere in the receptor might similarly activate its oncogenic potential, however. Apart from SM-FeSV, c-*fms* sequences were independently transduced in only one other acutely transforming mammalian retrovirus, the HZ-5 FeSV strain.[151] Nucleotide sequencing of the HZ-5 v-*fms* gene showed no activating mutation at codon 301, but rather the presence of mutations elsewhere in the gene (M.F. Roussel, P. Besmer, C.J. Sherr, unpublished observations). Assuming that the latter mutation(s) can also be shown to convert human CSF-1R to a ligand-independent kinase, the search for c-*fms*–activating mutations in human leukemias would at least need to be extended to these codons as well.

Alterations in the developmental timing of c-*fms* expression could also contribute to disease development. In mice, integration of the Friend strain of murine leukemia virus (F-MuLV) 5′ to exon 2 of the c-*fms* gene (Fig 1) results in greatly increased levels of c-*fms* transcription and CSF-1R expression in immature myeloid cells.[152] Based on experiments with cultured myeloid cell lines programmed to express CSF-1R (already described), high levels of receptor expression might not only alter the growth factor dependence of such cells but might also provide them with an intrinsic proliferative advantage.[136] Indeed, after F-MuLV infection, cells bearing viral integrations within the c-*fms* locus proliferate as dominant clones in the spleen, even before they can be successfully explanted into culture or have the potential to induce transplantable leukemias in mice. Thus, although c-*fms* activation appears to act as an initiating carcinogenic event, progression to frank malignancy must involve secondary genetic perturbations. Theoretically, chromosomal rearrangements affecting c-*fms* promoter/enhancer sequences might contribute in an analogous fashion to human myeloid malignancies. With MoAbs as probes for CSF-1R expression, at least 30% of human acute myeloid leukemia cells expressed detectable levels of the receptor at their cell surface irrespective of their French-American-British (FAB) classification.[149] Results obtained with more sensitive assays for c-*fms* mRNA indicated that the incidence of cells transcribing the gene is even higher.[150] The apparent lack of concordance between CSF-1R expression and other characteristics of monocytic differentiation in leukemic cells could reflect aberrant gene regulation in certain instances. No rearrangements of the c-*fms* gene have yet been detected, however, in an initial survey of both pediatric and adult leukemic cells.[149] A more precise characterization of the c-*fms* promoters and the elements that determine their developmental and tissue-specific regulation is needed to define the mechanisms governing c-*fms* expression by human myeloid leukemic blasts.

Normal monocytes produce CSF-1 in response to certain physiologic inducers.[12-15] Assays of c-*fms* and CSF-1 mRNA levels in human myeloid leukemic cells have indicated that both genes may be simultaneously and stably expressed in some cases,[153,154] suggesting that the growth of such cells might be enhanced through an autocrine mechanism. Rearrangements of the CSF-1 gene, such as have been observed in

a case of murine c-*myc*-induced monocytic leukemia,[155] might therefore facilitate disease progression. Despite the attraction of autocrine models for cell transformation, however, GM-CSF expression in transgenic mice[156] or retrovirus vector-mediated IL-3 expression in BM progenitors in vivo[157-159] induced profound hematologic proliferative disorders but failed to yield leukemias. Similarly, introduction of the human CSF-1 gene into a CSF-1–dependent, SV40-immortalized mouse macrophage cell line abrogated their dependence on exogenous CSF-1 but did not render them tumorigenic in nude mice.[160] In contrast, expression of v-*fms* in these same cells induced both factor independence and tumorigenicity.[84] Therefore, the v-*fms* and c-*fms* gene products may elicit qualitatively different growth-promoting responses owing to mutations in the v-*fms* product that affect kinase activity, substrate recognition, or receptor turnover.

FUTURE EXPECTATIONS

Despite progress arising from application of molecular biological techniques to studies of CSF-1 and its receptor, many fundamental questions remain. For example, human CSF-1 paradoxically stimulates formation of fewer macrophage colonies from human than from mouse BM precursors,[10,22,134,161] perhaps suggesting that its actions need be potentiated by other cytokines or that its primary role is to regulate the survival and effector functions of mature mononuclear phagocytes in the inflammatory response. Alternatively, despite its discovery as a hematopoietin, the dominant physiological actions of CSF-1 might well be exerted on other cell types, such as placental trophoblasts. Emerging genetic techniques now provide novel avenues for testing the function of CSF-1 in different cell lineages. As one approach, homologous recombination in embryonic stem cells[162] can now be used to create transgenic strains of mice bearing defective or mutant CSF-1 and CSF-1R genes. A better understanding of CSF-1 activity should also derive from animal experiments and from human clinical trials with the purified recombinant growth factor and, possibly, from in vivo studies with MoAbs that interfere with ligand–receptor interactions or with drugs that inhibit receptor tyrosine kinases. Although major challenges lie ahead, such information will not only contribute to the way in which we conceptualize disease processes but should also yield novel approaches for manipulating CSF-1–dependent responses.

REFERENCES

1. Stanley ER, Guilbert LJ, Tushinski RJ, Bartelmez SH: CSF-1—A mononuclear phagocyte lineage-specific hemopoietic growth factor. J Cell Biochem 21:151, 1983
2. Sherr CJ, Stanley ER: Colony stimulating factor-1, in Sporn MB, Roberts AB (eds): Peptide Growth Factors and Their Receptors. Heidelberg, FRG, Springer-Verlag (in press)
3. Robinson W, Metcalf D, Bradley TR: Stimulation by normal and leukemic mouse sera of colony formation in vitro by mouse bone marrow cells. J Cell Physiol 69:83, 1967
4. Stanley ER, McNeill TA, Chan SH: Antibody production to the factor in human urine stimulating colony formation in vitro by bone marrow cells. Br J Haematol 18:585, 1970
5. Sheridan JW, Stanley ER: Tissue sources of bone marrow colony stimulating factor. J Cell Physiol 78:451, 1971
6. Bradley TR, Sumner MA: Stimulation of mouse bone marrow colony growth in vitro by conditioned medium. Aust J Exp Biol Med Sci 46:607, 1968
7. Worton RG, McCulloch EW, Till JE: Physical separation of hemopoietic stem cells from cells forming colonies in culture. J Cell Physiol 74:171, 1969
8. Mauel J, Defendi V: Regulation of DNA synthesis in mouse macrophages. I. Sources, action and purification of the macrophage growing factor (MGF). Exp Cell Res 65:33, 1971
9. Stanley ER, Heard PM: Factors regulating macrophage production and growth. Purification and some properties of the colony stimulating factor from medium conditioned by mouse L cells. J Biol Chem 252:4305, 1977
10. Das SK, Stanley ER: Structure-function studies of a colony stimulating factor (CSF-1). J Biol Chem 257:13679, 1982
11. Seelentag WK, Mermod J-J, Montesano R, Vassalli P: Additive effects of interleukin 1 and tumor necrosis factor-alpha on the accumulation of the three granulocyte and macrophage colony-stimulating factor mRNAs in human endothelial cells. EMBO J 6:2261, 1987
12. Horiguchi J, Warren MK, Ralph P, Kufe D: Expression of the macrophage specific colony stimulating factor (CSF-1) during human monocytic differentiation. Biochem Biophys Res Commun 141:924, 1986
13. Horiguchi J, Warren MK, Kufe D: Expression of the macrophage-specific colony-stimulating factor in human monocytes treated with granulocyte-macrophage colony-stimulating factor. Blood 69: 1259, 1987
14. Oster W, Lindemann A, Horn S, Mertelsmann R, Herrmann F: Tumor necrosis factor (TNF)-alpha but not TNF-beta induces secretion of colony stimulating factor for macrophages (CSF-1) by human monocytes. Blood 70:1700, 1987
15. Rambaldi A, Young DC, Griffin JD: Expression of the M-CSF (CSF-1) gene by human monocytes. Blood 69:1409, 1987
16. Stanley ER, Chen DM, Lin H-S: Induction of macrophage production and proliferation by a purified colony stimulating factor. Nature 274:168, 1978
17. Karbassi A, Becker JM, Foster JS, Moore RN: Enhanced killing of *Candida albicans* by murine macrophages treated with macrophage colony stimulating factor: Evidence for augmented expression of mannose receptors. J Immunol 139:417, 1987
18. Lee MT, Warren MK: CSF-1-induced resistance to viral infection in murine macrophages. J Immunol 138:3019, 1987
19. Wing EJ, Waheed A, Shadduck RK, Nagle LS, Stephenson K: Effect of colony stimulating factor on murine macrophages. Induction of anti-tumor activity. J Clin Invest 69:270, 1982
20. Ralph P, Nakoinz I: Stimulation of macrophage tumoricidal activity by the growth and differentiation factor CSF-1. Cell Immunol 105:270, 1987
21. Moore RN, Oppenheim JJ, Farrar JJ, Carter CS Jr, Waheed A, Shadduck RK: Production of lymphocyte-activating factor (interleukin 1) by macrophages activated with colony-stimulating factors. J Immunol 125:1302, 1980
22. Motoyoshi K, Suda T, Kusumoto T, Takaku F, Miura Y: Granulocyte-macrophage colony stimulating and binding activities of purified human urinary colony stimulating factor to murine and human bone marrow cells. Blood 60:1378, 1982
23. Moore RN, Larsen HS, Horohov DW, Rouse BT: Endogenous regulation of macrophage proliferative expansion by colony stimulating factor-induced interferon. Science 223:178, 1984
24. Metcalf D, Nicola A: Synthesis by mouse peritoneal cells of G-CSF, the differentiation inducer for myeloid leukemia cells:

Stimulation by endotoxin, M-CSF, and multi-CSF. Leuk Res 9:35, 1985

25. Warren MK, Ralph P: Macrophage growth factor CSF-1 stimulates human monocyte production of interferon, tumor necrosis factor, and colony stimulating activity. J Immunol 137:2281, 1986

26. Kurland JI, Bockman R: Prostaglandin E production by human blood monocytes and mouse peritoneal macrophages. J Exp Med 147:952, 1978

27. Lin H-S, Gordon S: Secretion of plasminogen activator by bone marrow-derived mononuclear phagocytes and its enhancement by colony-stimulating factor. J Exp Med 150:231, 1979

28. Hamilton JA, Stanley ER, Burgess AW, Shadduck RK: Stimulation of macrophage plasminogen activator activity by colony-stimulating factors. J Cell Physiol 103:435, 1980

29. Moore RN, Pitruzzello FJ, Larsen HS, Rouse BT: Feedback regulation of colony stimulating factor (CSF-1)-induced macrophage proliferation by endogenous E prostaglandins and interferon-α/β. J Immunol 133:541, 1984

30. Wing EJ, Ampel NM, Waheed A, Shadduck RK: Macrophage colony-stimulating factor (M-CSF) enhances the capacity of murine macrophages to secrete oxygen reduction products. J Immunol 135:2052, 1985

31. Lyberg T, Stanley ER, Prydz H: Colony stimulating factor-1 induces thromboplastin activity in murine macrophages and human monocytes. J Cell Physiol 132:367, 1987

32. Wong GG, Temple PA, Leary AC, Witek-Giannotti JS, Yang Y-C, Ciarletta AB, Chung M, Murtha P, Kriz R, Kaufman RJ, Ferenz CR, Sibley BS, Turner KJ, Hewick RM, Clark SC, Yanai N, Yokota H, Yamada M, Saito M, Motoyoshi K, Takaku F: Human CSF-1: Molecular cloning and expression of 4 kb cDNA encoding the human urinary protein. Science 235:1504, 1987

33. Rettenmier CW, Roussel MF, Ashmun RA, Ralph P, Price K, Sherr CJ: Synthesis of membrane-bound colony-stimulating factor-1 (CSF-1) and downmodulation of CSF-1 receptors in NIH 3T3 cells transformed by cotransfection of the human CSF-1 and c-*fms* (CSF-1 receptor) genes. Mol Cell Biol 7:2378, 1987

34. Rettenmier CW, Roussel MF: Differential processing of colony-stimulating factor-1 precursors encoded by two human cDNAs. Mol Cell Biol 8:5026, 1988

35. Manos MM: Expression and processing of a recombinant human macrophage colony-stimulating factor in mouse cells. Mol Cell Biol 8:5035, 1988

36. Kawasaki ES, Ladner MB, Wang AM, Van Arsdell J, Warren MK, Coyne MY, Schweickart VL, Lee MT, Wilson KJ, Boosman A, Stanley ER, Ralph P, Mark DF: Molecular cloning of a complementary DNA encoding human macrophage-specific colony stimulating factor (CSF-1). Science 230:291, 1985

37. Ladner MB, Martin GA, Noble JA, Nikoloff DM, Tal R, Kawasaki ES, White TJ: Human CSF-1: Gene structure and alternative splicing of mRNA precursors. EMBO J 6:2693, 1987

38. Pettenati MJ, Le Beau MM, Lemons RS, Shima EA, Kawasaki ES, Larson RA, Sherr CJ, Diaz MO, Rowley JD: Assignment of *CSF*-1 to 5q33.1: Evidence for clustering of genes regulating hematopoiesis and for their involvement in the deletion of the long arm of chromosome 5 in myeloid disorders. Proc Natl Acad Sci USA 84:2970, 1987

39. Metcalf D: The granulocyte-macrophage colony stimulating factors. Science 229:16, 1985

40. Sachs L: The molecular control of blood cell development. Science 238:1374, 1987

41. Bartocci A, Pollard JW, Stanley ER: Regulation of colony-stimulating factor 1 during pregnancy. J Exp Med 164:956, 1986

42. Pollard JW, Bartocci A, Arceci R, Orlofsky A, Ladner MB, Stanley ER: Apparent role of the macrophage growth factor, CSF-1, in placental development. Nature 330:484, 1987

43. Regenstreif LJ, Rossant J: Expression of the c-*fms* proto-oncogene and of the cytokine, CSF-1, during mouse embryogenesis. Dev Biol 133:284, 1989

44. Müller R, Slamon DJ, Adamson ED, Tremblay JM, Müller D, Cline MJ, Verma IM: Transcription of c-*onc* genes c-*ras*^{ki} and c-*fms* during mouse development. Mol Cell Biol 3:1062, 1983

45. Rettenmier CW, Sacca R, Furman WL, Roussel MF, Holt JT, Nienhuis AW, Stanley ER, Sherr CJ: Expression of the human c-*fms* proto-oncogene product (colony-stimulating factor-1 receptor) on peripheral blood mononuclear cells and choriocarcinoma cell lines. J Clin Invest 77:1740, 1986

46. Guilbert LJ, Stanley ER: Specific interaction of murine colony-stimulating factor with mononuclear phagocytic cells. J Cell Biol 85:153, 1980

47. Guilbert LJ, Stanley ER: The interaction of ^{125}I-colony-stimulating factor-1 with bone marrow-derived macrophages. J Biol Chem 261:4024, 1986

48. Byrne PV, Guilbert LJ, Stanley ER: Distribution of cells bearing receptors for a colony-stimulating factor (CSF-1) in murine tissues. J Cell Biol 91:848, 1981

49. Sherr CJ, Rettenmier CW, Sacca R, Roussel MF, Look AT, Stanley ER: The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell 41:665, 1985

50. Hanks SK, Quinn AM, Hunter T: The protein kinase family: Conserved features and deduced phylogeny of the catalytic domains. Science 241:42, 1988

51. Yarden Y, Ullrich A: Growth factor receptor tyrosine kinases. Annu Rev Biochem 57:443, 1988

52. Woolford J, McAuliffe A, Rohrschneider LR: Activation of the feline c-*fms* proto-oncogene: Multiple alterations are required to generate a fully transformed phenotype. Cell 55:965, 1988

53. Roussel MF, Downing JR, Rettenmier CW, Sherr CJ: A point mutation in the extracellular domain of the human CSF-1 receptor (c-*fms* proto-oncogene product) activates its transforming potential. Cell 55:979, 1988

54. Sherr CJ: The *fms* Oncogene. BBA Reviews on Cancer. Amsterdam, The Netherlands, Elsevier, 1988, p 225

55. Roussel MF, Sherr CJ, Barker PE, Ruddle FH: Molecular cloning of the c-*fms* locus and its assignment to human chromosome 5. J Virol 48:770, 1983

56. Groffen J, Heisterkamp N, Spurr N, Dana S, Wasmuth JJ, Stephenson JR: Chromosomal localization of the human c-*fms* oncogene. Nucleic Acids Res 11:6331, 1983

57. Le Beau MM, Westbrook CA, Diaz MO, Larson RA, Rowley JD, Gasson JC, Golde DW, Sherr CJ: Evidence for the involvement of GM-CSF and *fms* in the deletion (5q) in myeloid disorders. Science 231:984, 1986

58. Le Beau MM, Epstein ND, O'Brien SJ, Nienhuis AW, Yang Y-C, Clark SC, Rowley JD: The interleukin 3 gene is located on human chromosome 5 and is deleted in myeloid leukemias with a deletion of 5q. Proc Natl Acad Sci USA 84:5913, 1987

59. Huebner K, Isobe M, Croce CM, Golde DW, Kaufman SE, Gasson JC: The human gene encoding GM-CSF is at 5q21-q32, the chromosome region deleted in the 5q$^-$ anomaly. Science 230:1282, 1985

60. Sutherland GR, Baker E, Callen DF, Campbell HD, Young IG, Sanderson CJ, Garson OM, Lopez AF, Vadas MA: Interleukin-5 is at 5q31 and is deleted in the 5q$^-$ syndrome. Blood 71:1150, 1988

61. Yang Y-C, Kovacic S, Kriz R, Wolf S, Clark SC, Wellems TE, Nienhuis A, Epstein N: The human genes for GM-CSF and IL-3 are closely linked in tandem on chromosome 5. Blood 71:958, 1988

62. Le Beau MM, Lemons RS, Espinosa R, Larson RA, Arai N, Rowley JD: Interleukin-4 and interleukin-5 map to human chromo-

some 5 in a region encoding growth factors and receptors and are deleted in myeloid leukemias with a del(5q). Blood 73:647, 1989

63. van Leeuwen BH, Martinson ME, Webb GC, Young IG: Molecular organization of the cytokine gene cluster, involving human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5. Blood 73:1142, 1989

64. Yarden Y, Escobedo JA, Kuang WJ, Yang-Feng TL, Daniel TO, Tremble PM, Chen EY, Ando ME, Harkins RN, Francke U, Fried VA, Ullrich A, Williams LT: Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature 323:226, 1986

65. Roberts WM, Look AT, Roussel MF, Sherr CJ: Tandem linkage of human CSF-1 receptor (c-*fms*) and PDGF receptor genes. Cell 55:655, 1988

66. Hampe A, Shamoon B-M, Gobet M, Sherr CJ, Galibert F: Nucleotide sequence and structural organization of the human *fms* proto-oncogene. Oncogene Res 4:9, 1989

67. Matsui T, Heideran M, Miki T, Popescu N, La Rochelle W, Kraus M, Pierce J, Aaronson SA: Isolation of a novel receptor cDNA establishes the existence of two PDGF receptor genes. Science 243:800, 1989

68. Claesson-Welsh L, Eriksson A, Westermark B, Heldin C-H: cDNA cloning and expression of the human A-type platelet-derived growth factor (PDGF) receptor establishes structural similarity to the B-type PDGF receptor. Proc Natl Acad Sci USA 86:4917, 1989

69. Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu TJ, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A: Human proto-oncogene c-*kit*: A new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 6:3341, 1987

70. Coussens L, Van Beveren C, Smith D, Chen E, Mitchell RL, Isacke CM, Verma IM, Ullrich A: Structural alteration of viral homologue of receptor proto-oncogene *fms* at carboxyl terminus. Nature 320:277, 1986

71. Visvader J, Verma IM: Differential transcription of exon 1 of the human c-*fms* gene in placental trophoblasts and monocytes. Mol Cell Biol 9:1336, 1989

72. Sariban E, Mitchell T, Kufe D: Expression of the c-*fms* proto-oncogene during human monocytic differentiation. Nature 316:64, 1985

73. Weber B, Horiguchi J, Luebbers R, Sherman M, Kufe D: Post-translational stabilization of c-*fms* mRNA by a labile protein during human monocyte differentiation. Mol Cell Biol 9:769, 1989

74. Rothwell VM, Rohrschneider LR: Murine c-*fms* cDNA: Cloning, sequence analysis, and retroviral expression. Oncogene Res 1:311, 1987

75. Lee PL, Johnson DE, Cousens LS, Fried VA, Williams LT: Purification and complementary DNA cloning of a receptor for basic fibroblast growth factor. Science 245:57, 1989

76. Williams AF, Barclay AN: The immunoglobulin superfamily—Domains for cell surface recognition. Annu Rev Immunol 6:381, 1988

77. Woolford J, Rothwell V, Rohrschneider L: Characterization of the human c-*fms* gene product and its expression in cells of the monocyte macrophage lineage. Mol Cell Biol 5:3458, 1985

78. Kamps MP, Taylor SS, Sefton BM: Direct evidence that oncogenic tyrosine kinases and cyclic AMP-dependent protein kinase have homologous ATP-binding sites. Nature 310:589, 1984

79. Downing JR, Roussel MF, Sherr CJ: Ligand and protein kinase C downmodulate the colony stimulating factor 1 receptor by independent mechanisms. Mol Cell Biol 9:2890, 1989

80. Roussel MF, Rettenmier CW, Look AT, Sherr CJ: Cell surface expression of v-*fms*-coded glycoproteins is required for transformation. Mol Cell Biol 4:1999, 1984

81. Nichols EJ, Manger R, Hakomori S, Herscovics A, Rohrschneider LR: Transformation by the v-*fms* oncogene product: Role of glycosylational processing and cell surface expression. Mol Cell Biol 5:3467, 1985

82. Hadwiger A, Niemann H, Käbisch A, Bauer H, Tamura T: Appropriate glycosylation of the *fms* gene product is a prerequisite for its transforming potency. EMBO J 5:689, 1986

83. Manger R, Najita L, Nichols EJ, Hakomori S-I, Rohrschneider L: Cell surface expression of the McDonough strain of feline sarcoma virus *fms* gene product (gp140^{fms}). Cell 39:327, 1984

84. Wheeler EF, Rettenmier CW, Look AT, Sherr CJ: The v-*fms* oncogene induces factor independence and tumorigenicity in a CSF-1 dependent macrophage cell line. Nature 324:377, 1986

85. Martin P, Vass WC, Schiller JT, Lowy DR, Velu TJ: The bovine papillomavirus E5 transforming protein can stimulate the transforming activity of EGF and CSF-1 receptors. Cell 59:21, 1989

86. Chen BD-M, Lin HS, Hsu S: Tumor-promoting phorbol esters inhibit the binding of colony stimulating factor (CSF-1) to murine peritoneal exudate macrophages. J Cell Physiol 116:207, 1983

87. Guilbert LJ, Nelson DJ, Hamilton JA, Williams N: The nature of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) stimulated hemopoiesis. Colony stimulating factor (CSF) requirement for colony formation and the effect of TPA on ^{125}I-CSF-1 binding. J Cell Physiol 115:276, 1983

88. Guilbert LJ, Stanley ER: Modulation of receptors for the colony-stimulating factor, CSF-1, by bacterial lipopolysaccharide and CSF-1. J Immunol Methods 73:17, 1984

89. Roussel MF, Downing JR, Ashmun RA, Rettenmier CW, Sherr CJ: Colony-stimulating factor 1-mediated regulation of a chimeric c-*fms*/v-*fms* receptor containing the v-*fms*-coded tyrosine kinase domain. Proc Natl Acad Sci USA 85:5903, 1988

90. Yarden Y, Schlessinger J: Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. Biochemistry 26:1443, 1987

91. Böni-Schnetzler M, Kaligian A, Del Vecchio R, Pilch PR: Ligand-dependent intersubunit association within the insulin receptor complex activates its intrinsic kinase activity. J Biol Chem 263:6822, 1988

92. Glenney JR, Chen WS, Lazar CS, Welton GM, Zokos LM, Rosenfeld MG, Gill GN: Ligand-induced endocytosis of the EGF receptor is blocked by mutational inactivation and by microinjection of anti-phosphotyrosine antibodies. Cell 52:675, 1988

93. Heldin C-H, Ernlund A, Rorsman C, Rönnstrand L: Dimerization of B type PDGF receptors occurs after ligand binding and is closely associated with receptor kinase activation. J Biol Chem 264:8905, 1989

94. Kazlauskas A, Cooper JA: Autophosphorylation of the PDGF receptor in the kinase insert region regulates interactions with cell proteins. Cell 58:1121, 1989

95. Downing JR, Rettenmier CW, Sherr CJ: Ligand-induced tyrosine kinase activity of the colony stimulating factor-1 receptor in a murine macrophage cell line. Mol Cell Biol 8:1795, 1988

96. Sengupta A, Liu W-K, Yeung YG, Yeung DCY, Frackelton AR, Stanley ER: Identification and subcellular localization of proteins that are rapidly phosphorylated in tyrosine in response to CSF-1. Proc Natl Acad Sci USA 85:8062, 1988

97. Jubinsky PT, Yeung Y-G, Sacca R, Li W, Stanley ER: Colony stimulating factor-1 stimulated macrophage membrane protein phosphorylation, in Kudlow JE, Maclennan DH, Bernstein A, Gottlieb AI (eds): Biology of Growth Factors: Molecular Biology, Oncogenes, Signal Transduction, and Clinical Applications. New York, Plenum, 1988, p 75

98. Tushinski RJ, Oliver IT, Guilbert LJ, Tynan PW, Warner JR, Stanley ER: Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. Cell 28:71, 1982

99. Vairo G, Hamilton JA: Activation and proliferation signals in murine macrophages: Stimulation of Na^+, K^+-ATPase activity by hematopoietic growth factors and other agents. J Cell Physiol 134:13, 1988

100. Hamilton JA, Vairo G, Lingelbach SR: Activation and proliferation signals in murine macrophages: Stimulation of glucose uptake by hemopoietic growth factors and other agents. J Cell Physiol 134:405, 1988

101. Bravo R, Neuberg M, Burckhardt J, Almendral J, Wallich R, Müller R: Involvement of common and cell type-specific pathways in c-*fos* gene control: Stable induction by cAMP in macrophages. Cell 48:251, 1987

102. Orlofsky A, Stanley ER: CSF-1-induced gene expression in macrophages: Dissociation from the mitogenic response. EMBO J 6:2947, 1987

103. Tushinski RJ, Stanley ER: The regulation of mononuclear phagocyte entry into S phase by the colony stimulating factor CSF-1. J Cell Physiol 122:221, 1985

104. Wahl MI, Daniel TO, Carpenter G: Antiphosphotyrosine recovery of phospholipase C activity after EGF treatment of A431 cells. Science 241:968, 1988

105. Wahl MI, Nishibe S, Suh P-G, Rhee SG, Carpenter G: Epidermal growth factor stimulates tyrosine phosphorylation of phospholipase C-II independently of receptor internalization and extracellular calcium. Proc Natl Acad Sci USA 86:1568, 1989

106. Wahl MI, Olashaw NE, Nishibe S, Rhee SG, Pledger WJ, Carpenter G: Platelet-derived growth factor induces rapid and sustained tyrosine phosphorylation of phospholipase C-γ in quiescent Balb/c 3T3 cells. Mol Cell Biol 9:2934, 1989

107. Meisenhelder J, Suh P-G, Rhee SG, Hunter T: Phospholipase C-γ is a substrate for the PDGF and EGF receptor protein-tyrosine kinases in vivo and in vitro. Cell 57:1109, 1989

108. Margolis B, Rhee SG, Felder S, Mervic M, Lyall R, Levitzki A, Ullrich A, Zilberstein A, Schlessinger J: EGF induces tyrosine phosphorylation of phospholipase C-II: A potential mechanism for EGF receptor signaling. Cell 57:1101, 1989

109. Berridge MJ: Inositol trisphosphate and diacylglycerol: Two interacting second messengers. Annu Rev Biochem 56:159, 1987

110. Downing JR, Margolis BL, Zilberstein A, Ashmun RA, Ullrich A, Sherr CJ, Schlessinger J: Phospholipase C-γ, a substrate for PDGF receptor kinase, is not phosphorylated on tyrosine during the mitogenic response to CSF-1. EMBO J 8:3345, 1989

111. Whetton AD, Monk PN, Consalvey SD, Downes CP: The haemopoietic growth factors interleukin 3 and colony stimulating factor-1 stimulate proliferation but do not induce inositol lipid breakdown in murine bone-marrow-derived macrophages. EMBO J 5:3281, 1986

112. Morrison DK, Kaplan DR, Rapp UR, Roberts TM: Signal transduction from membrane to cytoplasm: Growth factors and membrane-bound oncogene products increase *raf*-1 phosphorylation and associated protein kinase activity. Proc Natl Acad Sci USA 85:8855, 1989

113. Morrison DK, Kaplan DR, Escobedo JA, Rapp UR, Roberts TM, Williams LT: Direct activation of the serine/threonine kinase activity of *raf*-1 through tyrosine phosphorylation by the PDGF β-receptor. Cell 58:649, 1989

114. Raff UR, Cleveland JL, Bonner TI, Storm SM: The *raf* oncogene, in Reddy EP, Skalka AM, Curran T (eds): The Oncogene Handbook. Amsterdam, Elsevier, 1988, p 213

115. Smith MR, De Gudicibus SJ, Stacey DW: Requirement for c-*ras* proteins during viral oncogene transformation. Nature 320: 540, 1986

116. Gilman AG: G proteins: Transducers of receptor-generated signals. Annu Rev. Biochem 56:615, 1987

117. Barbacid M: *ras* Genes. Annu Rev Biochem 56:779, 1987

118. Trahey M, McCormick F: A cytoplasmic protein stimulates normal N-*ras* p21 GTPase, but does not affect oncogenic mutants. Science 238:542, 1987

119. Yu C-L, Tsai M-H, Stacey DW: Cellular *ras* activity and phospholipid metabolism. Cell 52:63, 1988

120. Kaplan DR, Whitman M, Schaffhausen B, Pallas DC, White M, Cantley L, Roberts TM: Common elements in growth factor stimulation and oncogenic transformation: 85 kDa phosphoprotein and phosphatidylinositol kinase activity. Cell 50:1021, 1987

121. Coughlin SR, Escobedo JA, Williams LT: Role of phosphatidylinositol kinase in PDGF receptor signal transduction. Science 243:1191, 1989

122. Auger KR, Serunian LA, Soltoff SP, Libby P, Cantley LC: PDGF-dependent tyrosine phosphorylation stimulates production of novel polyphosphoinositides in intact cells. Cell 57:167, 1989

123. Escobedo JA, Williams LT: A PDGF receptor domain essential for mitogenesis but not for many other responses to PDGF. Nature 335:85, 1988

124. Taylor GR, Reedijk M, Rothwell V, Rohrschneider L, Pawson T: The unique cellular and viral *fms* protein tyrosine kinase domain is dispensible for enzymatic and transforming activities. EMBO J 8:2029, 1989

125. Kurland JI, Bockman R, Broxmeyer HE, Moore MAS: Limitation of excessive myelopoiesis by the intrinsic modulation of macrophage derived prostaglandin E. Science 199:552, 1978

126. Hamilton JA: Glucocorticoids and prostaglandins inhibit the induction of macrophage DNA synthesis by macrophage growth factor and phorbol ester. J Cell Physiol 115:67, 1983

127. Morgan C, Pollard JW, Stanley ER: Isolation and characterization of a cloned growth factor dependent macrophage cell line, BACl.2F5. J Cell Physiol 130:420, 1987

128. Imamura K, Kufe D: Colony-stimulating factor-1 induced Na^+ influx into human monocytes involves activation of a pertussis toxin-sensitive GTP binding protein. J Biol Chem 263:14093, 1988

129. Mitchell PJ, Tjian R: Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. Science 245:371, 1989

130. Lenardo MJ, Baltimore D: NF-KB: A pleiotropic mediator of inducible and tissue-specific gene control. Cell 58:227, 1989

131. Roussel MF, Dull TJ, Rettenmier CW, Ralph P, Ullrich A, Sherr CJ: Transforming potential of the c-*fms* protooncogene (CSF-1 receptor). Nature 325:549, 1987

132. Roussel MF, Sherr CJ: Mouse NIH-3T3 cells expressing human colony-stimulating factor 1 (CSF-1) receptors overgrown in serum-free medium containing human CSF-1 as their only growth factor. Proc Natl Acad Sci USA 86:7924, 1989

133. Hartmann T, Seuwen K, Roussel MF, Sherr CJ, Pouysségur J: Functional expression of the human receptor for colony-stimulating factor 1 (CSF-1) in hamster fibroblasts: CSF-1 stimulates Na^+/H^+ exchange and DNA-synthesis in the absence of phosphoinositide breakdown. Growth Factors, in press

134. Sherr CJ, Ashmun RA, Downing JR, Ohtsuka M, Quan SG, Golde DW, Roussel MF: Inhibition of colony-stimulating factor-1 activity by monoclonal antibodies to the human CSF-1 receptor. Blood 73:1786, 1989

135. Dexter TM, Garland J, Scott D, Scolnick E, Metcalf D: Growth of factor-dependent hemopoietic precursor cell lines. J Exp Med 152:1036, 1980

136. Kato J-Y, Roussel MF, Ashmun RA, Sherr CJ: Transduction of human colony stimulating factor-1 receptor into interleukin-3-dependent mouse myeloid cells induces both CSF-1-dependent and factor-independent growth. Mol Cell Biol 9:4069, 1989

137. Donner L, Fedele LA, Garon CF, Anderson SJ, Sherr CJ: McDonough feline sarcoma virus: Characterization of the molecu-

larly cloned provirus and its feline oncogene (v-*fms*). J Virol 41:489, 1982

138. Wheeler EF, Askew D, May S, Ihle JN, Sherr CJ: The v-*fms* oncogene induces factor-independent growth and transformation of the interleukin-3-dependent myeloid cell line FDC-P1. Mol Cell Biol 7:1673, 1987

139. Heard JM, Roussel MF, Rettenmier CW, Sherr CJ: Multilineage hematopoietic disorders induced by transplantation of bone marrow cells expressing the v-*fms* oncogene. Cell 51:663, 1987

140. Borzillo GV, Sherr CJ: Early pre–B-cell transformation induced by the v-*fms* oncogene in long-term mouse bone marrow cultures. Mol Cell Biol 9:3973, 1989

141. Tamura T, Simon E, Niemann H, Snoek GT, Bauer H: $Gp140^{v\text{-}fms}$ molecules expressed at the surface of cells transformed by the McDonough strain of feline sarcoma virus are phosphorylated in tyrosine and serine. Mol Cell Biol 6:4745, 1986

142. Morrison DK, Browning PJ, White MF, Roberts TM: Tyrosine phosphorylations in vivo associated with v-*fms* transformation. Mol Cell Biol 8:176, 1988

143. Browning PJ, Bunn HF, Cline A, Shuman M, Nienhuis AW: "Replacement" of COOH-terminal truncation of v-*fms* with c-*fms* sequences markedly reduces transformation potential. Proc Natl Acad Sci USA 83:7800, 1986

144. Lyman SD, Park L, Rohrschneider LR: Colony stimulating factor-1 induced growth stimulation of v-*fms* transformed fibroblasts. Oncogene 3:391, 1988

145. Roussel MF, Downing JR, Sherr CJ: Transforming activities of human CSF-1 receptors with different point mutations at codon 301 in their extracellular domains. Oncogene (in press)

146. Weiner DB, Liu J, Cohen JA, Williams WV, Greene MI: A point mutation in the *neu* oncogene mimics ligand induction of receptor aggregation. Nature 339:230, 1989

147. Bargmann CI, Hung M-C, Weinberg RA: Multiple independent activations of the *neu* oncogene by a point mutation altering the transmembrane domain of p185. Cell 45:649, 1986

148. Bargmann CI, Weinberg RA: Oncogenic activation of the *neu*-encoded receptor protein by point mutation and deletion. EMBO J 7:2043, 1988

149. Ashmun RA, Look AT, Roberts WM, Roussel MF, Seremetis ST, Ohtsuka M, Sherr CJ: Monoclonal antibodies to the human CSF-1 receptor (c-*fms* protooncogene product) detect epitopes on normal mononuclear phagocytes and on human myeloid leukemic blast cells. Blood 73:827, 1989

150. Dubreuil P, Torres H, Courcoul M-A, Birg F, Mannoni P: c-*fms* Expression is a molecular marker of human acute myeloid leukemias. Blood 72:1081, 1988

151. Besmer P, Lader E, George PC, Bergold PJ, Qiu F-H, Zuckerman EE, Hardy WD: A new acute transforming feline retrovirus with *fms* homology specifies a C-terminally truncated version of the c-*fms* protein that is different from SM-feline sarcoma virus v-*fms* protein. J Virol 60:194, 1986

152. Gisselbrecht S, Fichelson S, Sola B, Bordereaux D, Hampe A, André C, Galibert F, Tambourin P: Frequent c-*fms* activation by proviral insertion in mouse myeloblastic leukaemias. Nature 329: 259, 1987

153. Wakamiya N, Horiguchi J, Kufe D: Detection of c-*fms* and CSF-1 RNA by in situ hybridization. Leukemia 1:518, 1987

154. Rambaldi A, Wakamiya N, Vellenga E, Horiguchi J, Warren MK, Kufe D, Griffin JD: Expression of the macrophage colony stimulating factor and c-*fms* genes in human acute myeloblastic leukemia cells. J Clin Invest 81:1030, 1988

155. Baumbach WR, Stanley ER, Cole MD: Induction of clonal monocyte-macrophage tumors in vivo by a mouse c-*myc* retrovirus: Rearrangement of the CSF-1 gene as a secondary transforming event. Mol Cell Biol 7:664, 1987

156. Lang RA, Metcalf D, Cuthbertson RA, Lyons I, Stanley E, Kelso A, Kannourakis G, Williamson DJ, Klintworth GK, Gonda TJ, Dunn AR: Transgenic mice expressing a hemopoietic growth factor gene (GM-CSF) develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage. Cell 51:675, 1987

157. Wong PMC, Chung SW, Nienhuis AW: Retroviral transfer and expression of the interleukin-3 gene in hemopoietic cells. Genes Dev 1:358, 1987

158. Wong PM, Chung SW, Dunbar CE, Bodine DM, Ruscetti S, Nienhuis AW: Retrovirus-mediated transfer and expression of the interleukin-3 gene in mouse hematopoietic cells result in a myeloproliferative disorder. Mol Cell Biol 9:798, 1989

159. Chang JM, Metcalf D, Lang RA, Gonda TJ, Johnson GR: Nonneoplastic hematopoietic myeloproliferative syndrome induced by dysregulated multi-CSF (IL-3) expression. Blood 73:1487, 1989

160. Roussel MF, Rettenmier CW, Sherr CJ: Introduction of a human colony stimulating factor-1 gene into a mouse macrophage cell line induces CSF-1 independence but not tumorigenicity. Blood 71:1218, 1988

161. Waheed A, Shadduck RK: Purification of colony stimulating factor by affinity chromatography. Blood 60:238, 1982

162. Frohman MA, Martin GR: Cut, paste, and save: New approaches to altering specific genes in mice. Cell 56:145, 1989

163. Rohrschneider LR, Metcalf D: Induction of macrophage colony stimulating factor-dependent growth and differentiation after introduction of the murine c-*fms* gene into FDC-P1 cells. Mol Cell Biol (in press)