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The colony-stimulating factor 1 (CSF-1) receptor (c-*fms* proto-oncogene product) and its ligand

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Summary

Alterations in genes that function in normal growth and development have been linked to malignant cell transformation. The mononuclear phagocyte colony-stimulating factor (CSF-1 or M-CSF) is a polypeptide growth factor synthesized by mesenchymal cells, which stimulates the survival, proliferation, and differentiation of haematopoietic cells of the monocyte-macrophage series. Multiple forms of soluble CSF-1 are produced by proteolytic cleavage of membrane-bound precursors, some of which are stably expressed at the cell surface. The c-*fms* proto-oncogene encodes the CSF-1 receptor, which is composed of an extracellular ligand-binding domain linked by a single membrane-spanning segment to a cytoplasmic tyrosine-specific protein kinase domain. Whereas the tyrosine kinase activity of the normal receptor is stimulated by CSF-1, mutations in the c-*fms* gene can constitutively activate the kinase to provide growth-stimulatory signals in the absence of the ligand. Oncogenic activation of the c-*fms* gene product appears to involve removal of a negative regulatory tyrosine residue near the carboxyl terminus of the receptor and one or more additional mutations that may simulate a conformational change induced by CSF-1 binding. Expression of the human c-*fms* gene in mouse NIH-3T3 cells confers a CSF-1 stimulated growth phenotype, indicating that receptor transduction is sufficient for fibroblasts to respond to a haematopoietic growth factor. In contrast, the v-*fms* oncogene induces factor-independent growth and tumorigenicity in factor-dependent myeloid cell lines, and contributes to the development of proliferative disorders of multiple haematopoietic lineages when introduced into murine bone marrow progenitors. Aberrant expression of an endogenous c-*fms* gene secondary to proviral insertion and transcriptional activation has also been implicated in virus-induced myeloblastic leukaemia in mice. The c-*fms* and CSF-1 genes have been mapped on the long arm of human chromosome 5, a region that frequently undergoes interstitial deletions in certain haematopoietic disorders including acute myelogenous leukaemia. The study of CSF-1 and its receptor should provide information concerning the role of tyrosine kinases in regulating the normal growth and differentiation of haematopoietic cells and in contributing to their malignant transformation.

Introduction

The macrophage colony-stimulating factor (CSF-1 or M-CSF) is a lineage-specific haematopoietic growth factor required for the proliferation, differentiation, and viability of mononuclear phagocytes (Stanley *et al.* 1983). CSF-1 also stimulates a variety of specialized macrophage functions including tumoricidal activity (Ralph & Nakoinz, 1987) and the production of other cytokines (Metcalf & Nicola, 1985; Warren & Ralph, 1986). The growth factor is a glycosylated polypeptide homodimer (Stanley & Heard, 1977) whose pleiotropic actions on mononuclear phagocytes are mediated by binding to a single class of high-affinity receptors at the cell surface (Guilbert & Stanley, 1980, 1986). Expression of the receptor for CSF-1 is an early marker of commitment to the monocyte-macrophage series. Low numbers of CSF-1

receptors are present on immature bone marrow progenitors, and a 10-fold increase in receptor number takes place as the cells differentiate (Bartelmez *et al.* 1985). Circulating monocytes and tissue macrophages maintain high numbers of cell surface CSF-1 receptors (ca. 50 000 per cell).

The receptor for CSF-1 is encoded by the c-*fms* proto-oncogene (Sherr *et al.* 1985) and is one of a family of oncogene products and growth factor receptors, which exhibit a tyrosine-specific protein kinase activity (Hunter & Cooper, 1985). The enzymatic activity appears to be required for the generation of mitogenic signals by this class of proteins. In this paper, we will briefly review the evidence that the CSF-1 receptor is the c-*fms* gene product and focus on recent investigations of the interaction between this receptor and its ligand in normal and malignant cells.

The CSF-1 receptor is the product of the c-*fms* gene

In recent years, experimental evidence has been obtained establishing a relationship between malignant transformation and alterations of genes that are involved in normal growth and differentiation. These observations have generally followed from the study of retroviral oncogenes (v-*onc* genes) that were generated by recombination between replicating viruses and proto-oncogene (c-*onc*) sequences present in normal cellular DNA. The v-*onc* genes are responsible for the ability of these viruses to cause tumours in animals and to transform cells in culture. Our interest in the CSF-1 receptor and its ligand evolved from the study of v-*fms*, a viral oncogene transduced from the feline c-*fms* proto-oncogene.

The Susan McDonough strain of feline sarcoma virus (SM-FeSV) was isolated from a spontaneously occurring multicentric fibrosarcoma of a domestic cat (McDonough *et al.* 1971). SM-FeSV transforms established fibroblast cell lines from several species in culture and induces fibrosarcomas when re-inoculated into kittens (Sarma *et al.* 1972). Acquired cellular sequences are localized as a contiguous segment in the middle of the SM-FeSV genome, which was designated v-*fms* (Donner *et al.* 1982). Nucleotide sequence analysis of the v-*fms* gene predicted that its product was a transmembrane protein and revealed that the carboxyl-terminal domain exhibited amino acid sequence similarity with the family of tyrosine-specific protein kinases (Hampe *et al.* 1984).

The product of the v-*fms* oncogene is an integral transmembrane glycoprotein with an associated tyrosine kinase activity. The glycoprotein contains asparagine(*N*)-linked oligosaccharides, which are processed during transport to the plasma membrane (Anderson *et al.* 1982, 1984). A single membrane-spanning segment of 26 hydrophobic amino acids near the middle of the protein divides it into two domains (Hampe *et al.* 1984). The v-*fms* gene product is oriented in the plasma membrane with its glycosylated amino-terminal domain exposed at the cell surface and its carboxyl-terminal tyrosine kinase domain in the cytoplasm (Rettenmier *et al.* 1985*a*). Cell surface expression is required to elicit the transformed phenotype (Roussel *et al.* 1984; Nichols *et al.* 1985; Hadwiger *et al.* 1986). The v-*fms*-coded glycoproteins on

the plasma membrane become associated with clathrin-coated pits and are internalized in endocytic vesicles (Manger *et al.* 1984). These findings provided a basis for the hypothesis that the viral oncogene was derived from a cellular gene encoding a growth factor receptor. Indeed, precedents for a link between oncogenes and growth factors, or their receptors, were first established by the findings that the v-*sis* oncogene encodes a chain of the platelet-derived growth factor (PDGF) (Doolittle *et al.* 1983; Waterfield *et al.* 1983) whereas the v-*erb*B oncogene product represents a truncated version of the epidermal growth factor (EGF) receptor (Downward *et al.* 1984).

Although transcripts of the c-*fms* gene were first detected in mouse and human placental tissue (Müller *et al.* 1983*a*,*b*) and in human choriocarcinoma (Müller *et al.* 1983*b*) and murine myeloid leukaemia (Gonda & Metcalf, 1984) cell lines, we were initially unable to precipitate a presumptive c-*fms* protein from these sources using the then available antibodies to the v-*fms* gene product. Since the v-*fms* gene of SM-FeSV was derived from a feline cellular gene, the viral oncogene product was more likely to exhibit antigenic cross-reactivity with the c-*fms* product of the cat. Normal adult cat tissues were therefore surveyed for the presence of c-*fms* transcripts, and the highest levels of the 4·0-kb c-*fms* mRNA were present in the spleen.

When the v-*fms* gene products are precipitated with antisera and the immune complexes incubated with [γ-^{32}P]ATP in the presence of manganese ions, the glycoproteins are phosphorylated on tyrosine residues (Barbacid & Lauver, 1981). Using monoclonal antibodies raised against the viral oncogene product, the feline c-*fms*-coded glycoprotein was similarly identified in detergent homogenates of cat spleen by an immune complex protein kinase assay (Rettenmier *et al.* 1985*b*). High levels of the c-*fms* gene product were also detected in cat peritoneal inflammatory exudates. We reasoned that determination of the phenotype of cells expressing the c-*fms*-coded glycoprotein might provide an important clue to the identity of its putative ligand. Fluorescence-activated flow cytometry and cell sorting with a monoclonal antibody to a v-*fms*-coded epitope revealed that the c-*fms* product was preferentially expressed on mature feline macrophages (Sherr *et al.* 1985). The restricted distribution of the c-*fms* gene product suggested that its ligand might be the mononuclear phagocyte colony-stimulating factor CSF-1. A series of collaborative experiments was therefore initiated with Dr E. Richard Stanley who had previously purified and characterized both murine and human CSF-1.

Because antibodies to the feline v-*fms* protein did not cross-react with the mouse or human c-*fms* gene product, additional antisera were prepared by immunizing rabbits with a recombinant v-*fms*-coded polypeptide expressed in bacteria (Furman *et al.* 1986). The latter antibodies primarily recognize conserved epitopes in the cytoplasmic carboxyl-terminal domain, and specifically precipitated presumptive c-*fms* products from other species (Sherr *et al.* 1985; Rettenmier *et al.* 1986). The mouse c-*fms* gene product was identified as a glycoprotein of 165K ($K = 10^3 M_r$), in close agreement with the molecular weight of the cell surface CSF-1 receptor estimated from chemical cross-linking studies with radiolabelled ligand (Morgan & Stanley, 1984). The murine c-*fms* gene product also exhibited functional properties of the

CSF-1 receptor (Sherr *et al.* 1985). In membrane preparations, the tyrosine kinase activity of the c-*fms*-coded glycoprotein was stimulated by CSF-1. Moreover, ^{125}I-ligand–receptor complexes formed at the surface of viable macrophages were quantitatively precipitated by the antisera after detergent lysis. These results established that the c-*fms* gene product was closely related, and possibly identical, to the CSF-1 receptor.

This conclusion has been confirmed by subsequent investigations. As a consequence of the recombination event that generated SM-FeSV, the v-*fms* oncogene product retains the complete extracellular domain of its c-*fms* progenitor (Coussens *et al.* 1986; Wheeler *et al.* 1986*a*). Cells infected by SM-FeSV therefore acquire binding sites for CSF-1, and chemical cross-linking studies demonstrated that specific binding was mediated by the cell surface form of the v-*fms*-coded glycoprotein (Sacca *et al.* 1986). Direct genetic evidence that the c-*fms* gene encodes a functional CSF-1 receptor came from studies using the molecularly cloned c-*fms* and CSF-1 cDNAs (Roussel *et al.* 1987). Expression of a human c-*fms* cDNA in mouse NIH-3T3 cells enables them to form colonies in semi-solid medium when grown in the presence of recombinant human CSF-1. Moreover, co-transfection of NIH-3T3 cells with the human c-*fms* and CSF-1 genes transforms these cells by an autocrine mechanism. Although the parental cells constitutively produce low amounts of mouse CSF-1 (Sacca *et al.* 1986), the murine growth factor is not biologically active for human mononuclear phagocytes. Therefore, the requirement for human CSF-1 to stimulate the human c-*fms* gene product in this system is consistent with the known species specificity of the growth factor. These results also demonstrate that transduction of a CSF-1 receptor, which is normally expressed in mononuclear phagocytes, is sufficient to render fibroblasts responsive to the ligand. The intracellular targets for growth-promoting signals generated by the CSF-1 receptor thus appear to be shared by macrophages and fibroblasts.

Structure and function of the *fms* oncogene product

A schematic diagram depicting the structure of the v-*fms* and human c-*fms* gene products is shown in Fig. 1. The primary translation product of the human c-*fms* gene is 972 amino acids in length (Coussens *et al.* 1986). Removal of a 19-residue amino-terminal signal peptide yields a polypeptide of 953 amino acids compared to 922 in the corresponding form of the viral oncogene product (Hampe *et al.* 1984). The mature cell surface form of the v-*fms*-coded glycoprotein has an apparent molecular weight of about 140K (Anderson *et al.* 1984; Manger *et al.* 1984), although slight variations of size in different cell types are related to differences in the patterns and extent of glycosylation. The mature form of the human c-*fms* gene product is a glycoprotein of apparent molecular weight 150K (Woolford *et al.* 1985; Rettenmier *et al.* 1986). Canonical sequences for *N*-linked glycosylation (Asn-X-Ser/Thr) are clustered in the extracellular amino-terminal domain, and the carboxyl-terminal domain exhibits sequence similarity with other members of the protein-tyrosine kinase gene family. The gp140$^{v\text{-}fms}$ and human gp150$^{c\text{-}fms}$ share extensive

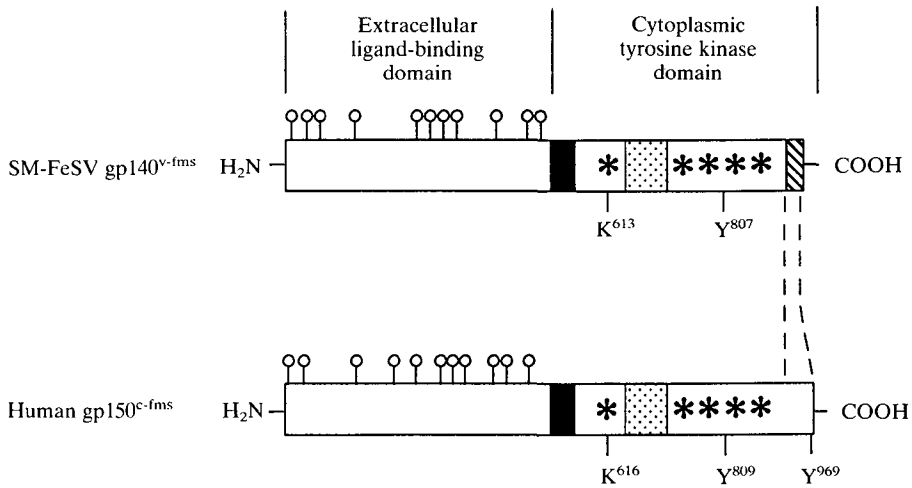

Fig. 1. Structure of the v-*fms* and c-*fms* gene products. Schematic diagrams of the mature cell surface forms of SM-FeSV gp140$^{v\text{-}fms}$ and the human gp150$^{c\text{-}fms}$ are shown. A transmembrane segment of hydrophobic amino acids (black bar) divides the proteins into an extracellular domain for ligand binding and a cytoplasmic tyrosine kinase domain. Potential sites for the addition of asparagine-linked oligosaccharides in the amino-terminal domain are marked by open circles. The portion of the carboxyl-terminal domain that exhibits sequence homology with other protein tyrosine kinases is indicated by asterisks. Within this region of each protein, the position of a critical lysine residue (K) at the ATP-binding site is noted. The segment of 70 *fms*-specific amino acids that interrupts the core kinase consensus sequence is stippled. The region of carboxyl-terminal sequence divergence between the v-*fms* and c-*fms* gene products is indicated by the cross-hatched area in gp140$^{v\text{-}fms}$. The location of a negative-regulatory tyrosine residue (Y^{969}) near the carboxyl-terminus of gp150$^{c\text{-}fms}$ is noted.

amino acid sequence identity in the extracellular (75 %) and cytoplasmic (95 %) domains but differ at their extreme carboxyl termini. As discussed below, this carboxyl-terminal region of divergence includes a single tyrosine residue (Tyr^{969}), which may play a role in modulating the response of the normal receptor to CSF-1.

The differences in amino acid sequence of the extracellular domains of the v-*fms* and human c-*fms* gene products are largely due to interspecies divergence between cats and man. The amino-terminal domain of the *fms*-coded glycoprotein lacks the cysteine-rich repeats found in the receptors for EGF and insulin (Coussens *et al.* 1986). However, the common spacing of individual cysteine residues and short segments of amino acid sequence homology in their ligand-binding domains suggest that the *fms* product and the receptor for PDGF were derived from a common ancestor (Yarden *et al.* 1986). Both CSF-1 and PDGF are dimeric growth factors, and the similarity of structure in the extracellular domains of their receptors may be related to conformational requirements for binding dimeric ligands.

The cytoplasmic domain of the *fms* gene product has sequence similarity with other protein tyrosine kinases. By analogy (Kamps *et al.* 1984), a lysine residue, located at position 613 of the viral protein and 616 of the normal human receptor, is presumed to be the critical ATP-binding site required for enzymatic activity. A

tyrosine residue at positions 807 and 809 of the respective proteins is homologous to the major site of autophosphorylation in many other tyrosine kinases (Hunter & Cooper, 1985). The v-*fms* gene product is constitutively phosphorylated on tyrosine in transformed cells (Tamura *et al.* 1986), whereas significant tyrosine phosphorylation of the CSF-1 receptor has not been detected in the absence of ligand. The *fms* gene products have a segment of about 70 amino acids interposed between the ATP-binding region and the rest of the core kinase consensus sequence. The PDGF receptor (Yarden *et al.* 1986) and the *kit* oncogene product (Besmer *et al.* 1986*a*) have a similar organization of their respective tyrosine kinase domains. Although there is very little homology among the inserted segments in the kinase domains of these three proteins, the extensive amino acid sequence similarity within their core kinase regions again suggests that they represent a family of closely related receptors. The inserted sequences may affect the activity of these kinases or their interaction with cellular substrates in the signal transduction pathway.

The v-*fms* and c-*fms* gene products differ conspicuously at their carboxyl-terminal ends where the 40 carboxyl-terminal amino acids of the normal receptor have been replaced by 11 unrelated residues in the SM-FeSV oncogene product (Hampe *et al.* 1984; Coussens *et al.* 1986). The alteration deletes a single tyrosine residue at position 969 (Tyr^{969}) which, by analogy to studies on the products of the v-*src* and c-*src* genes (Courtneidge, 1985; Iba *et al.* 1985; Cooper *et al.* 1986), may represent a negative regulatory site of tyrosine phosphorylation. Another independent isolate of feline sarcoma virus contains a v-*fms* gene encoding a different carboxyl-terminal alteration, which also lacks this terminal tyrosine residue (Besmer *et al.* 1986*b*). A chimeric v-*fms*/c-*fms* construct in which sequences for the normal human c-*fms* coded carboxyl terminus were substituted in the v-*fms* gene was more than 10-fold reduced in its transforming efficiency for NIH-3T3 cells (Browning *et al.* 1986; Roussel *et al.* 1987). A point mutation replacing Tyr^{969} with a phenylalanine residue restored the transforming activity of the chimeric construct to the level of v-*fms* itself (Roussel *et al.* 1987). NIH-3T3 cells expressing the human c-*fms* gene with either the wild-type Tyr^{969} or with the Phe^{969} mutation were not transformed. However, when plated in semi-solid medium in the presence of recombinant human CSF-1, NIH-3T3 cells expressing the receptor with the Phe^{969} mutation yielded consistently larger colonies than cells expressing the wild-type c-*fms*(Tyr^{969}) (Roussel *et al.* 1987). Moreover, in cells transformed by co-transfection of c-*fms* alleles with human CSF-1 cDNA, the efficiency of transformation induced by the wild-type c-*fms*(Tyr^{969}) was about 10-fold less than that of c-*fms*(Phe^{969}); co-transfection of the latter construct with the CSF-1 gene yielded a transforming efficiency equivalent to that of v-*fms*.

Taken together, these results suggest that Tyr^{969} plays a negative regulatory role in the activity of the CSF-1 receptor. Removal of this residue, while not an activating transforming mutation by itself, may up-regulate the receptor kinase activity, thereby enhancing the autocrine transforming efficiency of the receptor in a co-transfection assay with the CSF-1 gene. The fact that v-*fms* is a transforming gene in the absence of its ligand, whereas c-*fms*(Phe^{969}) is not, suggests that there are one or

more additional mutations in v-*fms* that contribute to its transforming activity. These latter mutations may mimic a ligand-induced conformational change in the receptor, thus rendering the kinase independent of CSF-1. It should be possible to pinpoint these mutations by comparing the sequence of v-*fms* with a cDNA of the feline c-*fms* gene and testing appropriate c-*fms* mutants for transforming activity.

The CSF-1 receptor exhibits typical properties of a growth factor receptor. Binding of CSF-1 results in autophosphorylation (Sherr *et al.* 1985; Yeung *et al.* 1987), rapid internalization, and degradation of both the ligand and the receptor (Guilbert & Stanley, 1986; Wheeler *et al.* 1986*b*; Downing *et al.* 1988). The ability to confer a CSF-1-responsive phenotype by expression of the receptor in heterologous NIH-3T3 cells will allow dissection of the components of receptor function through site-directed mutagenesis. For example, appropriate mutants can be constructed to assess the possible role of Tyr^{809} in activation of the receptor kinase and signal transduction. Reduction of a CSF-1-mediated response by substitution of a phenylalanine residue at this position would suggest that autophosphorylation of Tyr^{809} in response to ligand binding is a component of receptor activation. By analogy to results in other receptor systems, replacement of $lysine^{616}$ would be expected to abolish both the kinase activity and signal transduction without affecting ligand binding. Down-modulation of CSF-1 receptors by either CSF-1 or other compounds such as phorbol esters (Wheeler *et al.* 1986*b*) could then be assessed directly in the absence of receptor activation.

The intracellular events leading to a CSF-1 response after binding of the ligand are poorly understood. Autophosphorylation at one or more tyrosine residues is probably associated with activation of the receptor. Ligand–receptor complexes are returned to the interior of the cell *via* the endocytic pathway and rapidly degraded. The signals for receptor down-modulation and the identity of cellular substrates for the receptor kinase are not known. The production of intracellular second messengers in response to receptor activation may be involved. Cells transformed by the v-*fms* oncogene exhibit an elevated rate of phosphatidylinositol (PI) turnover (Jackowski *et al.* 1986) with cleavage of phosphatidylinositol 4,5-diphosphate (PIP_2) generating inositol-1,4,5-triphosphate to mobilize intracellular calcium and diacylglycerol, which activates protein kinase C. This is associated with increased activities of a membrane-associated PIP_2 phospholipase C (Jackowski *et al.* 1986) and a PI kinase (Kaplan *et al.* 1987). However, it has been reported that CSF-1 stimulation is not associated with PI turnover in macrophages (Whetton *et al.* 1986). Thus, a role for PIP_2-derived second messengers in signal transduction by the normal receptor has not been established.

The sequence of events leading to changes in nuclear function after CSF-1 stimulation are also unknown. As for a variety of stimuli in many other cell systems, CSF-1 rapidly induces transient high levels of expression of the c-*fos* proto-oncogene within 15 min in macrophages (Bravo *et al.* 1987), followed by a sustained increase in c-*fos* expression at lower levels 4–12 h after stimulation (Müller *et al.* 1985). The addition of CSF-1 also leads to a decrease in the rate of protein turnover within 2 h (Tushinski & Stanley, 1983) and stimulation of DNA synthesis within 8–12 h

(Tushinski & Stanley, 1985). Clearly, considerable effort will be necessary to elucidate the signal transduction mechanism initiated by binding of CSF-1 to its receptor. Working from both ends of the pathway by defining the physiologically relevant substrates for the CSF-1 receptor kinase and identifying CSF-1-inducible genes is a reasonable approach to this difficult and important problem.

Synthesis and expression of CSF-1

CSF-1 is a glycosylated polypeptide homodimer of identical subunits assembled through disulphide bonds (Stanley & Heard, 1977). The biological activity of the growth factor is dependent on its dimeric structure and is abolished by disulphide reduction. The carbohydrate component includes *N*-linked oligosaccharides, which do not appear to be required for CSF-1 function (Das & Stanley, 1982). The human growth factor has been purified from urine, and oligonucleotide probes deduced from the amino-terminal amino acid sequence were used to clone genomic sequences coding for CSF-1. Human CSF-1 is encoded by a single gene, which maps to the long arm of chromosome 5 (Pettenati *et al.* 1987). Transcripts of this gene undergo differential splicing to generate multiple mRNAs (Ladner *et al.* 1987), which specify at least two biologically active forms of the growth factor (Kawasaki *et al.* 1985; Wong *et al.* 1987). A cDNA encoding murine CSF-1 has also been isolated from the mouse L929 fibroblast cell line (DeLamarter *et al.* 1987).

A schematic diagram of two primary translation products deduced from the nucleotide sequences of various human and murine CSF-1 cDNAs is shown in Fig. 2. Analysis of the sequences suggested that soluble CSF-1 is generated by proteolytic cleavage of a membrane-bound precursor. All of the precursors contain a 32-residue amino-terminal signal peptide for membrane insertion followed by sequences of the secreted growth factor, a presumptive transmembrane segment of hydrophobic amino acids, and a short carboxyl-terminal tail. The cysteine residues and potential sites for *N*-linked glycosylation are present on the amino-terminal side of the putative membrane-spanning sequence. These features suggested that the CSF-1 precursor is synthesized as an integral transmembrane glycoprotein cotranslationally oriented in the membrane of the endoplasmic reticulum (ER) with its glycosylated amino-terminal domain in the ER cisterna and its carboxyl-terminal tail in the cytoplasm.

A 1·6-kb CSF-1 cDNA cloned from a human pancreatic carcinoma cell line encodes a 256 amino acid CSF-1 precursor (Kawasaki *et al.* 1985). The 554-residue polypeptide specified by a 4-kb human cDNA from this and other sources includes the complete coding sequence of the smaller clone, with a block of 298 additional amino acids inserted after residue 149 on the amino-terminal side of the transmembrane segment (Wong *et al.* 1987; Ladner *et al.* 1987). The two human cDNAs are generated by alternative splicing of the primary transcript (Ladner *et al.* 1987). The additional coding sequences of the 4-kb cDNA include some tryptic peptides detected in a purified form of human urinary CSF-1 (Wong *et al.* 1987). Thus, the soluble growth factors specified by these two cDNAs have different carboxyl termini.

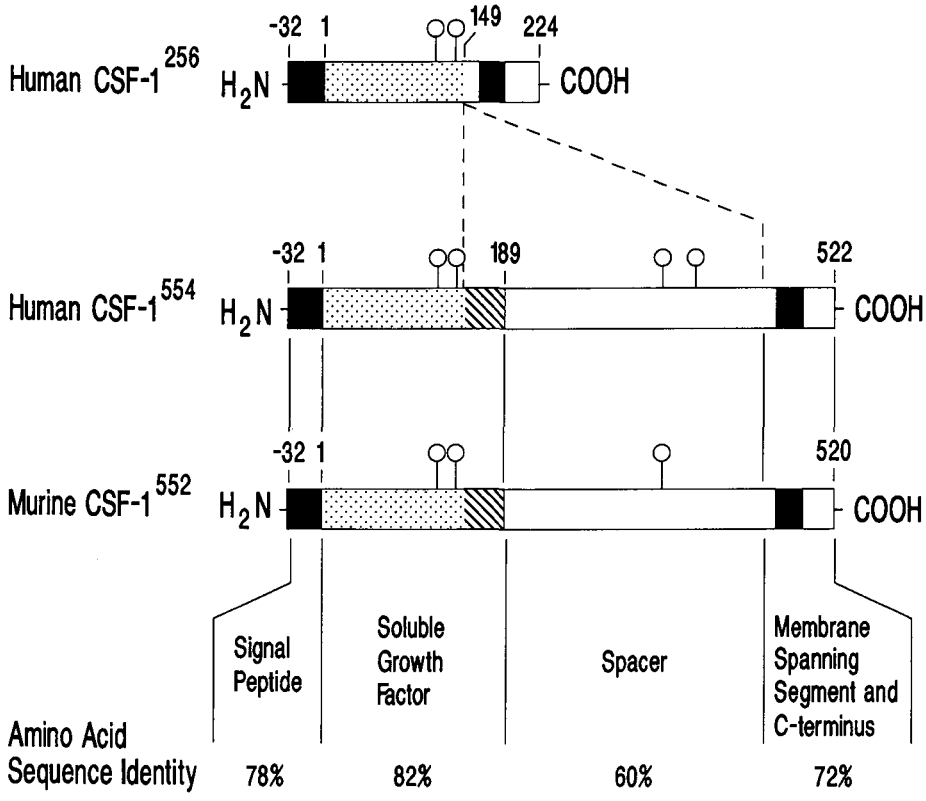

Fig. 2. Primary translation products encoded by biologically active human and mouse CSF-1 cDNAs. Each CSF-1 precursor consists of an amino-terminal signal peptide (black bar) and sequence for the secreted growth factor (stippled), a transmembrane segment (black bar), and a carboxyl-terminal tail. Canonical sequences for *N*-linked glycosylation are indicated by open circles. Human CSF-1^{256} and CSF-1^{554} are encoded by mRNAs derived by alternative splicing of the primary transcript. A contiguous segment of 298 amino acids present in human CSF-1^{554} and absent in CSF-1^{256} is delimited by the dashed lines. Polypeptide sequences from this insert, which are present in the soluble growth factor derived from CSF-1^{554}, are cross-hatched. Murine CSF-1^{552} is similar in structure to the large human CSF-1 precursor. The extent of amino acid sequence identity between the corresponding regions of the mouse and human CSF-1 precursors is indicated.

The 552 amino acid murine CSF-1 precursor encoded by a cDNA cloned from mouse L929 cells (DeLamarter *et al.* 1987) is similar in structure to the product of the 4-kb human cDNA. The sequences of the soluble human and murine growth factors (residues 1–ca. 189) exhibit more than 80% amino acid identity. The remainder of the inserted segment in the larger CSF-1 precursor shows less amino acid sequence conservation than the secreted growth factor.

Expression of CSF-1 cDNAs in viral vectors has allowed biochemical analysis of the products. The 1·6-kb human CSF-1 cDNA directs the synthesis of a membrane-bound 34K glycoprotein, which is rapidly assembled into disulphide-linked dimers

and externalized on the plasma membrane (Rettenmier *et al.* 1987). Proteolysis of this 68K homodimer at the cell surface yields soluble 44K CSF-1 dimers composed of 22K subunits. In cell culture systems, less than 10% of the precursors on the plasma membrane are recovered in the form of extracellular growth factor. The role of the membrane-spanning sequence has been directly tested by insertion of an upstream termination codon after amino acid 158 in the coding sequence of the 1·6-kb cDNA (Heard *et al.* 1987*a*). The truncated clone produces a biologically active, soluble growth factor, which is not membrane-associated and is efficiently secreted from cells. The similarity in size between the product of the truncated clone and the soluble growth factor released by cleavage of the membrane-bound precursor indicates that the termination codon was placed in the coding sequence of the 1·6-kb cDNA near the site of proteolysis.

Soluble CSF-1 encoded by the 4-kb human cDNA includes additional carboxyl-terminal amino acid residues not present in the secreted product of the smaller clone (Wong *et al.* 1987). Thus, the sites for proteolytic cleavage are different for the two CSF-1 precursors, and it appears that they are processed by different mechanisms. In contrast to the product of the 1·6-kb cDNA, which is detected at the cell surface, the CSF-1 precursor encoded by the 4-kb clone is cleaved within the cell and rapidly secreted. Differences in the mechanism of CSF-1 biosynthesis may reflect diverse physiological roles for various forms of the growth factor. Although the 4-kb mRNA is the major CSF-1 transcript detected in a variety of human cell lines, in at least one inducible system, production of biologically active human CSF-1 is correlated with the appearance of the smaller species (Ralph *et al.* 1986). In addition, two mRNAs of the corresponding sizes are differently expressed in various mouse tissues (Rajavashisth *et al.* 1987). The function of the different CSF-1 precursors *in vivo* is as yet unclear. One possibility is that product of the 4-kb cDNA is the major source of CSF-1 released into the circulation, whereas the membrane-bound form of CSF-1 encoded by the 1·6-kb clone primarily stimulates receptor-bearing cells in circumstances involving direct cell–cell contact.

CSF-1 is produced by mesenchymal cells and interacts with its receptor on mononuclear phagocytes (Tushinski *et al.* 1982). The growth factor appears in the circulation, and its concentration is determined by monocytes and macrophages, which bind and degrade the hormone after receptor-mediated endocytosis (Bartocci *et al.* 1987). The pleiotropic effects of CSF-1 include maintaining the viability of mature monocytes and macrophages and stimulating the proliferation and differentiation of committed bone marrow progenitors (Stanley *et al.* 1983). More immature haematopoietic precursors are also rendered responsive to CSF-1 in the presence of interleukin-3 (Multi-CSF) or haemopoietin-1 (Bartelmez & Stanley, 1985; Stanley *et al.* 1986). The latter molecule is now known to be the macrophage product, interleukin-1α (Mochizuki *et al.* 1987), indicating that differentiated macrophages can release mediators that stimulate the production of immature bone marrow progenitors. Positive and negative feed-back mechanisms therefore regulate the production of mononuclear phagocytes. In addition, it has recently been reported that monocytes and macrophages themselves produce CSF-1 in response to phorbol

esters and the physiological inducers, γ-interferon and granulocyte-macrophage CSF (Horiguchi *et al.* 1986, 1987; Rambaldi *et al.* 1987). This suggests that autocrine or paracrine mechanisms might also be involved in modulating the activity of these cells during inflammatory reactions.

An intriguing area for future investigations is a possible function for CSF-1 and its receptor beyond their role in the haematopoietic compartment. Transcripts of the c-*fms* gene are found in mouse and human placental tissue (Müller *et al.* 1983*a*,*b*), and the c-*fms*-coded glycoprotein has been identified in two independently derived human choriocarcinoma cell lines (Woolford *et al.* 1985; Rettenmier *et al.* 1986). The c-*fms* product in these malignant trophoblasts is indistinguishable from that detected on normal peripheral blood monocytes, and both cell types display high-affinity binding sites for CSF-1 (Rettenmier *et al.* 1986). Pregnancy results in a 1000-fold increase in the concentration of CSF-1 in the mouse uterus with the highest levels being detected at term (Bartocci *et al.* 1986). During the latter half of gestation, placental concentrations of the growth factor are higher than those detected in foetal tissues or in a variety of maternal organs. The simplest interpretation is that CSF-1 produced by stromal cells of the uterus stimulates receptors expressed on normal placental trophoblasts during embryogenesis. Thus, although CSF-1 was initially identified as a lineage-specific colony-stimulating factor in haematopoiesis, it is likely that this growth factor has additional physiological roles in other circumstances.

The role of CSF-1 and its receptor in oncogenesis

Due to its identification as the product of the c-*fms* gene, the ability of the CSF-1 receptor to induce malignant cell transformation has already been tested in a number of contexts. The v-*fms* gene was isolated from a feline fibrosarcoma, and it transforms a variety of fibroblast cell lines in culture. Because these mesenchymal cells also produce CSF-1, it was possible that transformation was mediated by an autocrine mechanism involving transduction of a competent CSF-1 receptor into cells that synthesized the corresponding growth factor. To test this hypothesis, the v-*fms* gene was expressed in murine haematopoietic cell lines that require colony-stimulating factors for their proliferation and survival in culture and do not produce CSF-1. Introduction of the v-*fms* gene induced growth factor independence and tumorigenicity in the CSF-1-dependent macrophage cell line BAC1.2F5 (Wheeler *et al.* 1986*b*) and the interleukin-3-dependent myeloid cell line FDC-P1 (Wheeler *et al.* 1987). The factor-independent cells did not synthesize CSF-1, nor did expression of the v-*fms*-coded glycoprotein alter the number or affinity of the receptors for the corresponding growth factors required by the parental cells. Thus, transformation of these cells did not involve an autocrine mechanism, and there was no evidence for transmodulation of the murine CSF-1 or IL-3 receptors by the v-*fms* gene product.

The c-*fms* gene product is expressed at high levels by mature mononuclear phagocytes, and introduction of the c-*fms* gene into NIH-3T3 cells did not induce

transformation (Roussel *et al.* 1987). These findings indicate that the v-*fms* gene product is constitutively activated to provide growth-stimulatory signals in the absence of ligand as a result of critical structural alterations. As discussed above, one of the activating mutations involves removal of a carboxyl-terminal tyrosine residue that may modulate the response of the normal receptor to ligand binding. Because the latter mutation alone is insufficient to activate the transforming potential of the c-*fms* gene, the v-*fms*-coded glycoprotein must contain one or more additional alterations that contribute to its oncogenic properties.

Expression of the human c-*fms* gene enables mouse NIH-3T3 fibroblasts to respond mitogenically to exogenously added human CSF-1 (Roussel *et al.* 1987). Constitutive co-expression of human c-*fms* and CSF-1 cDNAs transforms these cells by an autocrine mechanism. Rearrangement and expression of the endogenous murine CSF-1 gene has been implicated as a second transforming event leading to tumorigenicity in a CSF-1-dependent macrophage cell line immortalized by a retrovirus containing the c-*myc* oncogene (Baumbach *et al.* 1987). These results support the notion that unregulated expression of a growth factor and its receptor in the same cell may contribute to neoplastic progression. However, this circumstance by itself does not appear to be sufficient fully to transform all established cell lines. In the SV40-immortalized BAC1.2F5 mouse macrophage cell line (Morgan *et al.* 1987), expression of an introduced human CSF-1 gene abrogated the requirement of exogenously added CSF-1 for growth *in vitro*, but the factor-independent cells were not tumorigenic in nude mice (Roussel *et al.* 1988). Human CSF-1 is biologically active for stimulation of murine mononuclear phagocytes, and the factor-independent cells produced sufficient amounts of the growth factor to constitutively down-modulate their CSF-1 receptors. The fact that the v-*fms* gene did render these same cells factor-independent and tumorigenic (Wheeler *et al.* 1986*b*) underscores the critical differences between the viral transforming protein and the normal receptor.

The results of these studies in cell culture systems are currently being extended to expression of these genes *in vivo*. Although SM-FeSV encodes an analogue of the CSF-1 receptor, it was isolated from a mesenchymal tumour and has not been reported to induce haematopoietic neoplasms in its natural host. However, when mouse bone marrow cells containing an introduced v-*fms* gene were used to repopulate lethally irradiated recipients, diseases of multiple haematological lineages were observed (Heard *et al.* 1987*b*). After a latency period, several primary recipients developed myeloproliferative disorders, which lacked evidence of clonality and were not efficiently transplanted to secondary hosts. In contrast, other primary recipients developed in their spleens provirus-positive clones, which were transplantable to secondary, lethally-irradiated recipients and gave rise to clonal erythroleukaemias and B-cell lymphomas. Although cells expressing v-*fms* did not appear to have an obligate proliferative advantage during serial transfers, the correlation between expression of the v-*fms* gene and disease indicated that the oncogene product contributed to the development of these disorders. Clearly, similar types of experiments using molecular clones of the c-*fms* and CSF-1 genes will be of interest.

Evidence has recently been obtained for involvement of the CSF-1 receptor in the development of a naturally occurring leukaemia. The replication-competent Friend murine leukaemia virus induces myeloblastic leukaemia in mice with a latency of 6–12 months. In approximately 20% of these cases the provirus is integrated in a region upstream of the first coding exon of the c-*fms* gene in host cell DNA (Gisselbrecht *et al.* 1987). This proviral insertion results in high level expression of c-*fms* transcripts that encode the full-sized CSF-1 receptor. These myeloblasts might be unusually responsive to CSF-1 due to the high levels of receptor synthesis. Leukaemia might ultimately develop in the clonally expanded population as a result of secondary genetic changes involving either the c-*fms* gene itself or other cellular genes. The loss of the germ line c-*fms* allele in some of these tumours is consistent with the possibility that activating mutations may also have occurred in the affected c-*fms* locus to unmask the latent transforming potential of its product and provide a further proliferative advantage. Whatever the mechanism, the fact that the c-*fms* gene is a target for proviral insertion resulting in transcriptional activation indicates that this is an initiating event for the development of leukaemia in these animals.

The genes for several CSFs and cell surface receptors have been localized to the long arm of human chromosome 5, including c-*fms* at 5q33.2–33.3 (Le Beau *et al.* 1986*a*) and CSF-1 at 5q33.1 (Pettenati *et al.* 1987). Acquired interstitial deletions of 5q are a frequent cytogenetic abnormality in patients who develop acute myelogenous leukaemia after exposure to toxic chemicals (Rowley *et al.* 1981; Pedersen-Bjergaard *et al.* 1984; Le Beau *et al.* 1986*b*). Deletions of this region are also characteristic of the '$5q^-$ syndrome' (Van den Berghe *et al.* 1974), a refractory anaemia with thrombocytosis and hypolobulated megakaryocytes (Sokal *et al.* 1975; Wieniewski & Hirschhorn, 1983). Bone marrow cells from some patients with the $5q^-$ syndrome have been shown to be hemizygous for the c-*fms* gene (Nienhuis *et al.* 1985). These results raise the possibility that alterations in the structure or expression of CSF-1 or its receptor may contribute to these haematological disorders in man. A careful dissection of the genes encoding growth factors and their receptors that are clustered in this region of chromosome 5 will be required to resolve these possibilities.

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