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CSF-1-induced gene expression in macrophages: dissociation from the mitogenic response

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Early gene expression associated with the mitogenic response to colony stimulating factor-1 (CSF-1) has been examined in BAC1.2F5, a CSF-1-dependent murine macrophage cell line. Stimulation of arrested cells by CSF-1 resulted in acute, transient elevation in c-*fos* and subsequently in c-*myc* mRNA levels. Dramatic, sustained elevations were observed for JE and KC mRNAs, which are induced by platelet-derived growth factor (PDGF) in 3T3 cells. The kinetics of expression of all four messages were similar to those reported in PDGF-stimulated fibroblasts, implying a program of gene expression common to these two mitogens. Granulocyte-macrophage CSF (GM-CSF) can replace CSF-1 in stimulating the growth of 2F5 cells. It induced mRNAs for c-*fos*, c-*myc* and JE but not KC. Therefore KC expression, although correlated with mitogenesis, is not required for proliferation. The effects of CSF-1 were also examined in cells cycling continuously in its absence: 2F5 cells incubated in GM-CSF and an autonomous variant subclone of 2F5. In either case, the only detected growth effect of CSF-1 was a reduction in doubling-time. Nevertheless, all four of the mRNAs induced by CSF-1 in arrested cultures of 2F5 were strongly induced with the same kinetics in these cycling cells. Thus it would appear that the functions mediated by this early-gene program are not restricted to the mitogenic stimulation of arrested cells.

Key words: CSF-1/GM-CSF/cell proliferation/proto-oncogenes/macrophages

Introduction

Colony stimulating factor-1 (CSF-1) is a specific growth factor for mononuclear phagocytic cells (reviewed in Stanley *et al.*, 1983). It is a dimeric glycoprotein that has been purified to homogeneity and shown to be the product of a single gene (Kawasaki *et al.*, 1985). CSF-1 action is mediated by a high-affinity receptor encoded by the proto-oncogene c-*fms* (Sherr *et al.*, 1985). This receptor belongs to a tyrosine kinase family among whose members are receptors for other mitogens, including the especially closely related receptor for platelet-derived growth factor (PDGF) (Yarden *et al.*, 1986). We have sought insight into the mechanism by which CSF-1 exerts its effects by comparing early events induced by CSF-1 with those induced in potentially related systems, and by probing the relationship between these events and the growth response. In this paper, we focus on the early modulation of the expression of four genes that have previously been strongly associated with mitogenic stimulation of fibroblasts by PDGF: the proto-oncogenes c-*fos* and c-*myc* (Müller *et al.*, 1984; Greenberg and Ziff, 1984; Kruijer *et al.*, 1983), whose expression has also been correlated with mitogenesis in lymphoid, thyroid, and mononuclear phagocytic cells (Kelly *et al.*, 1983; Moore *et al.*, 1986; Colleta *et al.*, 1986; Bravo *et al.*, 1987), and the genes JE and KC (Cochran *et al.*, 1983).

The murine macrophage cell line BAC1.2F5 ('2F5') (Morgan *et al.*, 1987) strictly requires either CSF-1 or granulocyte-macrophage CSF (GM-CSF) for survival and proliferation. A number of variant subclones that have lost this requirement have been isolated (C.J.Morgan, J.W.Pollard and E.R.Stanley, unpublished results). These features open several avenues for genetic and physiological examination of the CSF-1 response. Here we use this system to demonstrate that CSF-1 induces the same program of early gene expression that has previously been observed following PDGF stimulation of fibroblasts, and the dissociability of this early-gene program from the mitogenic response to CSF-1.

Results

Mitogenesis in 2F5 cells

CSF-1 has been shown to act as a mitogen for primary macrophages rendered quiescent by overnight incubation in CSF-1-free, serum-containing medium ('starvation') (Tushinski *et al.*, 1982). We therefore began our study of CSF-1 regulated gene expression with a similar protocol applied to 2F5 cells. When growing 2F5 cultures are starved overnight, the number of cells per culture ceases to increase and the fraction of cells in S-phase drops by half (Morgan *et al.*, 1987 and data not shown). That the decrease in proportion of S-phase cells is due to arrest of the cell cycle is indicated by the inability of 40% of starved cells to be labelled by long-term incubation (50 h) in [^{3}H]thymidine (data not shown). In Figure 1, the CSF-1-induced recovery of these arrested cells is seen to follow a time-course similar to that of arrested primary macrophages (Tushinski and Stanley, 1985) and of other cell types: S-phase entry is sharply increased at about 12 h post-stimulation and a corresponding increase in mitotic figures occurs by 22 h. Long-term [^{3}H]-thymidine labelling confirms that all cells in the culture enter S-phase after CSF-1 addition (data not shown). However, the behavior of 2F5 cells is somewhat different from that of primary macrophages, in that the latter are homogeneously arrested by growth factor removal (Tushinski *et al.*, 1982), whereas about 60% of the 2F5 culture can pass through one or two cell cycles after starvation (Figure 1 and data not shown).

Rapid stimulation of c-fos and c-myc mRNA levels by CSF-1

We next examined the response of the proto-oncogenes c-*fos* and c-*myc* to CSF-1 in this system. These are the earliest genes observed to respond to PDGF in fibroblasts (Greenberg and Ziff, 1984), and they have been associated with a variety of growth-regulatory phenomena. Figure 2A shows that within 20 min of addition of CSF-1 to starved 2F5 cells, c-*fos* mRNA levels were dramatically elevated. A control probe, pCHO-C, hybridizes to a ubiquitous mRNA (Soreq *et al.*, 1980) that is not affected by CSF-1. Figure 2B (top) shows that this response is transient: a

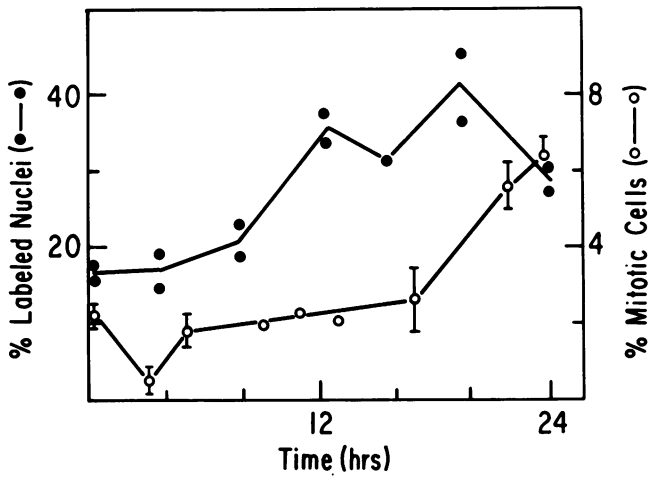

Fig. 1. CSF-1-stimulated mitogenesis. For [^{3}H]thymidine labelling (●——●), exponentially growing cultures of 2F5 were starved for 24 h, and the medium replaced with CSF-1-containing medium at time 0. At the indicated times, dishes were exposed to 10 μCi/ml of [^{3}H]thymidine (44 Ci/mmol) for 1 h, fixed in methanol:aceteic acid (3:1, v/v), and exposed to nuclear track emulsion (NTB-2, Kodak) for 8 days. Dishes were scored blind using random fields. For mitotic index determination (○——○), cultures were starved for 16–30 h, and CSF-1-containing medium was added at time 0. Cultures were exposed to 0.2 μg/ml colcemid for 1 h and then, at the indicated times, harvested by scraping. Aliquots were spun onto slides in a cytocentrifuge (500 r.p.m., 10 min) and Giemsa-stained. Slides were scored blind using random fields. Standard deviations are indicated for a minimum of five dishes.

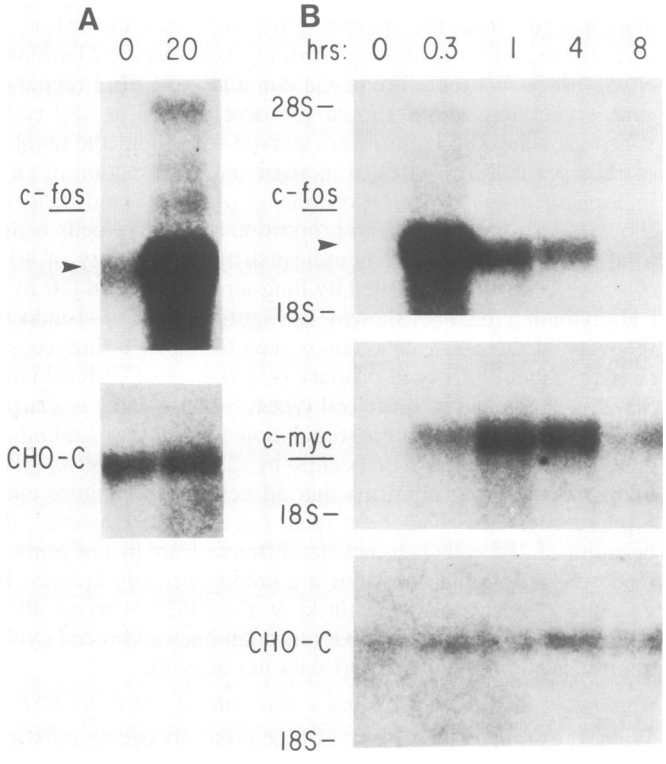

Fig. 2. Induction of c-*fos* and c-*myc* mRNAs by CSF-1. Cultures of 2F5 were grown to near confluence, starved for 18–20 h, and exposed to CSF-1 for the indicated times. Poly(A)$^+$-RNA was purified and Northern blotted. The filter in (**A**) (~10 μg RNA/lane) was hybridized sequentially to ^{32}P-labelled pfos-1 and pCHO-C. The filter in (**B**) (~7 μg RNA/lane) was hybridized to pfos-1, melted, and rehybridized to pMC413RC and then pCHO-C. Exposure times were 15–30 h, except 10 days for c-*myc* and 5 days for CHO-C in (**A**). The arrowheads indicate c-*fos* mRNA.

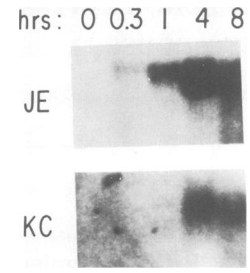

Fig. 3. Induction of JE and KC mRNAs by CSF-1. The filter in Figure 2B was rehybridized sequentially to ^{32}P-labelled pBC-JE and pBC-KC. Exposure times were 1 day (JE) or 7 days (KC). The mol. wts of the detected mRNAs are ~1.45–1.50 kb (JE) and 1.55 kb (KC).

marked decline in the c-*fos* message level was noticeable within 1 h of stimulation. The magnitude and kinetics of the c-*fos* induction and deinduction both closely resemble those reported for PDGF-stimulated fibroblasts (Muller *et al.*, 1984; Greenberg and Ziff, 1984; Kruijer *et al.*, 1984).

In Figure 2B (middle), the blot shown in Figure 2B (top) was melted and rehybridized with a probe for c-*myc*. A rapid and transient elevation of c-*myc* mRNA level was also induced by CSF-1. However, the two proto-oncogene responses have distinct kinetics: c-*fos* peaks at 20 min, whereas c-*myc* has a peak at 1 h after stimulation. This behavior precisely reproduces that reported in fibroblasts for these genes (Muller *et al.*, 1984; Greenberg and Ziff, 1984). CHO-C levels, by comparison, show little change in response to CSF-1.

A common early-gene program

The study of the modulation of c-*fos* and c-*myc* RNA levels reflects a biased interest in these genes as possible growth regulators. The conserved behavior manifested by these mRNAs in macrophages and fibroblasts may therefore not be representative of other stimulated messages in either cell type. A more stringent test of the notion of a common induced-gene repertoire among growth factors would employ genes selected only on the basis of their responsiveness. Such genes are available from cDNA libraries of PDGF-induced mRNAs. The time-courses, following CSF-1 stimulation, of two such messages, JE and KC, from a library prepared by Cochran and coworkers (Cochran *et al.*, 1983), are shown in Figure 3. Both of these mRNAs show dramatic elevation by 4 h after CSF-1 addition. Once induced, these messages remain elevated for prolonged intervals. This behavior is nearly identical to that reported in PDGF-stimulated 3T3 cells (Cochran *et al.*, 1983). In 3T3, KC message levels were reported to be maximal by 1 h. We have observed somewhat variable time-courses for this message, with maximal stimulation occurring as early as 1 h and as late as 8 h after CSF-1 addition (e.g. Figure 4D).

Taken together, the expression patterns of c-*fos*, c-*myc*, JE and KC indicate that, in the first hours of the mitogenic response, the regulation of the molecular phenotype of macrophages by CSF-1 resembles that of fibroblasts by PDGF. Six kinetic features are shared by the two responses: the four message inductions and the deinductions of c-*fos* and c-*myc*. Moreover, the 'unselected' nature of the JE and KC genes suggest that many more molecular features are shared by the two responses. We next asked whether this common 'program' of early-gene response extends to another hemopoietic growth factor, GM-CSF, which acts via a distinct receptor to support the proliferation of 2F5 cells in the absence of CSF-1 (Morgan *et al.*, 1987). Figure

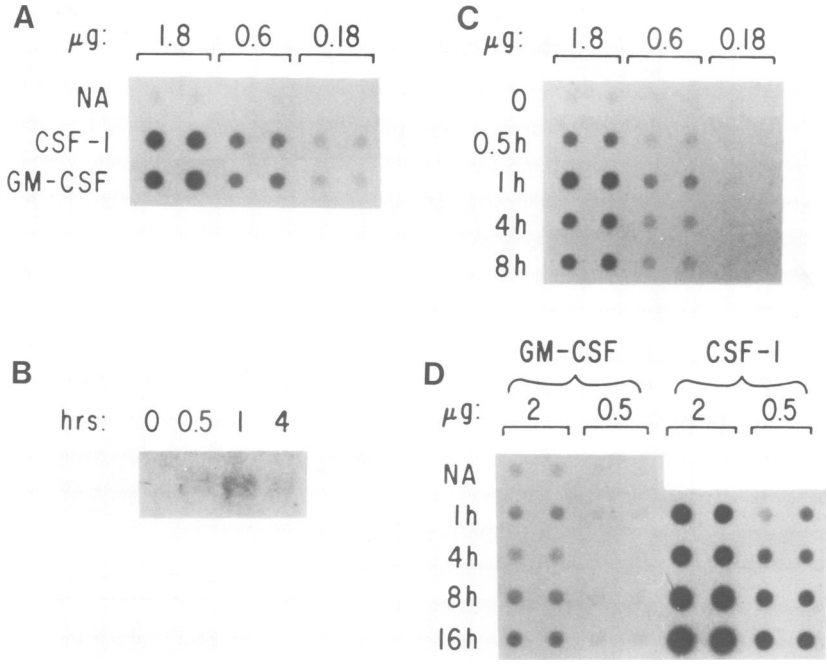

Fig. 4. Induction of early-response mRNAs by GM-CSF. 2F5 cultures were starved for 16–24 h and exposed to growth factors. Total cellular RNA was isolated and either dot-blotted (**A,C,D**) or Northern-blotted (**B**). (**A**) c-*fos*: cells were exposed for 30 min to either fresh control medium (NA) or medium containing either GM-CSF or CSF-1. The filter was hybridized to ^{32}P-labeled pfos-1 and exposed for 8 h. (**B**) c-*myc*: cells were exposed to GM-CSF for the indicated times and hybridized to ^{32}P-labelled pMC413RC. Exposure time was 3 weeks. (**C**) JE: cells were exposed to GM-CSF for the indicated times and hybridized to ^{32}P-labelled pBC-JE. Exposure time was 15 h. (**D**) KC: a parallel set of dishes was treated with either no growth factor (NA), CSF-1 or GM-CSF as indicated. The filter was hybridized to ^{32}P-labelled pBC-KC and exposed for 20 h.

4 shows that GM-CSF addition to a starved 2F5 culture stimulated c-*fos*, c-*myc*, and JE mRNA levels with kinetics very similar to those seen with CSF-1. Thus, five of the six responses common to PDGF and CSF-1 are shared by this growth factor. However, KC is not induced by GM-CSF. Although the kinetics of KC stimulation by CSF-1 are somewhat variable, we have, over the course of many experiments, always observed dramatic induction within 4 h, whereas GM-CSF reproducibly gives no stimulation even after much longer intervals (8–16 h). Thus, although KC stimulation is a conspicuous feature of the CSF-1 mitogenic response in 2F5, this event is not necessary for growth stimulation in these cells.

The early-gene program is dissociable from mitogenesis

The demonstration that induction of at least one of the 'program' genes (KC) is not required for a proliferative response suggests that some or all of these genes may have functions distinct from the mitogenic stimulation of arrested cells. One approach to this question is to examine CSF-1-induced messages under circumstances in which the growth factor is not acting as a mitogen. Figures 5 and 6 show two experiments of this kind. The first makes use of a variant subclone of 2F5 whose proliferation is independent of exogenous CSFs. This subclone, Aut1, has retained receptors for CSF-1 (C.J.Morgan, A.Orlofsky and E.R.Stanley, unpublished observations); however, the only effect observed for CSF-1 on the growth of Aut1 cultures is a small decrease in doubling-time (Figure 5A). Figure 5B shows that addition of CSF-1 to growing Aut1 cultures increased c-*fos*, c-*myc*, JE and KC mRNA production with similar kinetics and magnitude to that seen in mitogenically stimulated 2F5 (data not shown for c-*fos* deinduction). The fold induction for JE and KC is reduced in Aut1 compared to 2F5, due to substantial basal expression in untreated Aut1 cells. Cell-cycle entry was not being induced in these cultures: parallel, untreated dishes labelled with [^{3}H]-thymidine for 24 or 48 h showed 90 and 95% nuclear labelling, respectively, in the absence of CSF-1 (data not shown). Thus, the cultures were asynchronous and almost all cells were already in cycle prior to growth factor addition.

In the second experiment, we alternated the growth factor used to support exponentially growing 2F5 cells. In Figure 6, growing populations are switched from GM-CSF to CSF-1-containing medium. All four messages are seen to be elevated with magnitude and kinetics similar to those of mitogenically responding cells; however, once again, the cultures were asynchronous and almost entirely in cycle (90–96% nuclear labelling within 48 h, data not shown) before the switch. Thus two very different experimental approaches each indicate that the early-gene program can be induced independently of mitogenic stimulation.

Discussion

An increasing number of growth factors have now been shown to be individually sufficient to stimulate quiescent cells to re-enter and progress through a complete cell cycle (e.g. Gospodarowicz, 1974; Cohen *et al.*, 1975; Scher *et al.*, 1978; Tushinski *et al.*, 1982; Valente *et al.*, 1983; Kelvin *et al.*, 1986). Among the most dramatic of the many biochemical phenomena correlated with this process is an early elevation in the levels of specific mRNAs. Studies have indicated that the expression of as many as 10^2 genes may be affected within the first several hours of mitogenic stimulation (Pledger *et al.*, 1981; Cochran *et al.*, 1983; Linzer and Nathans, 1983). Only limited data, however, are available as to the extent to which these early gene responses are cell type and/or growth factor-specific. Moreover, no relationship between these responses and cell cycle progression has been established. In this paper, we show that the set of genes modulated by CSF-1

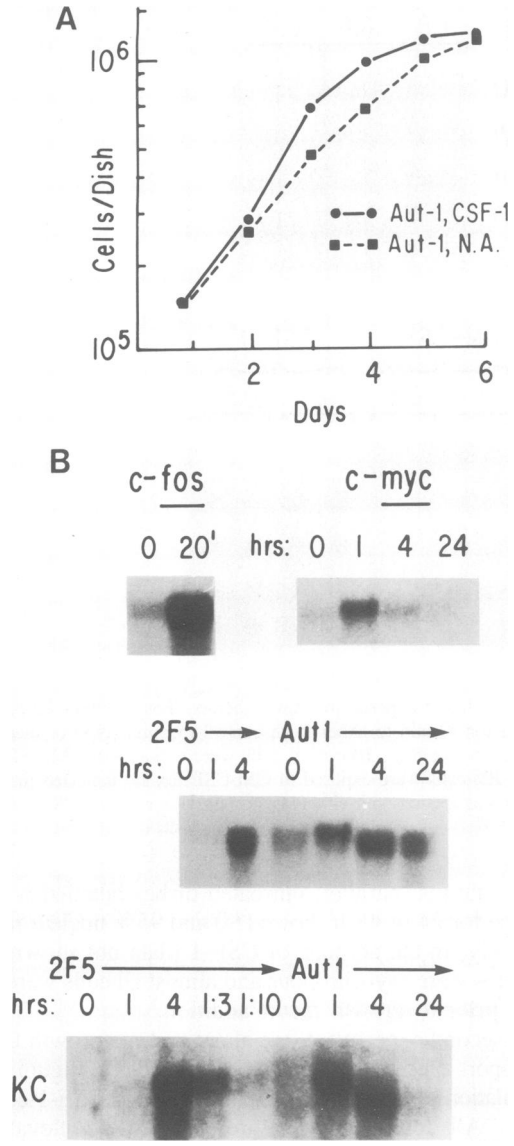

Fig. 5. (**A**) CSF-1-independence of Aut1 cells. Parallel sets of Aut1 cultures were fed daily with either control medium (■- - -■) or CSF-1-containing-medium (●——●). Each point represents the mean of three replicates. Standard deviations were not larger than the symbol size. (**B**) Induction of early-response mRNAs in growing Aut1 cells. Aut1 cultures were grown in control medium to approximately 20% of confluence (50% for c-*fos*). The medium was replaced with CSF-1-containing medium, and cells harvested at the indicated times. Poly(A)$^+$-RNA (c-*fos*) or total cellular RNA (c-*myc*, JE, KC) was isolated, Northern blotted, and hybridized with probes for the indicated mRNAs. Total cellular RNA was prepared from 2F5 cultures starved for 18 h and stimulated with CSF-1 for the indicated times. The lanes marked '1:3' and '1:10' contain 3-fold and 10-fold dilutions of 4-h 2F5 RNA, respectively. After harvest of Aut1 cultures for c-*myc*, JE and KC determination, parallel dishes in control medium were labelled with 10 μCi/ml [^{3}H]thymidine (44 Ci/mmol) for 24 or 48 h, at which time they were fixed, exposed to emulsion for 4 days, and scored for labelled nuclei (see text).

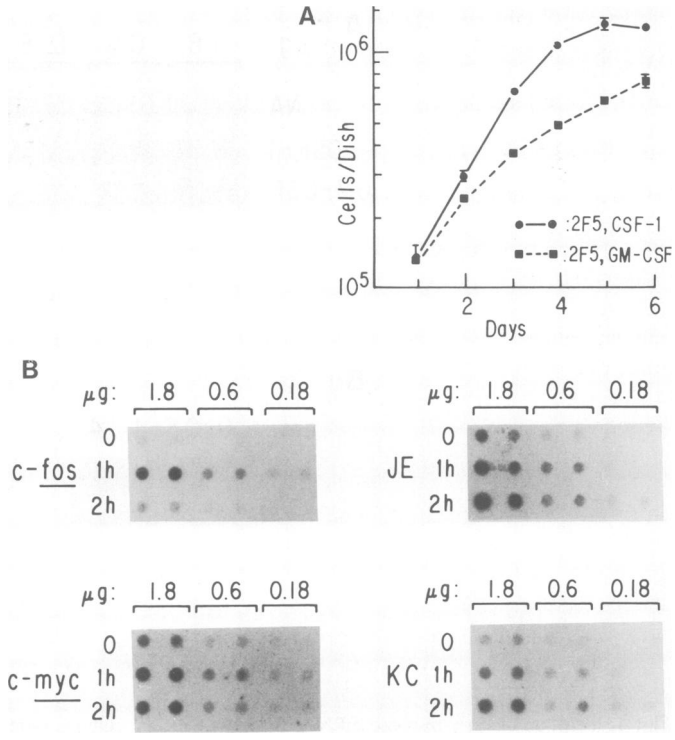

Fig. 6. (**A**) Growth of 2F5 cells in GM-CSF. 2F5 cultures were fed daily with either GM-CSF-containing-medium (150 units/ml) (■- - -■) or CSF-1-containing medium (●——●). Each point represents the mean of three replicates. Standard deviations, where not shown, were not larger than the symbol size. No growth was observed in control medium in the absence of growth factors (Morgan *et al.*, 1987). (**B**) Induction of early-response mRNAs in GM-CSF-supported 2F5 cells. 2F5 cultures were grown to approximately one third of confluence in medium containing GM-CSF. Cultures were stimulated with CSF-1 for the indicated times and total cellular RNA was isolated, dot-blotted, and hybridized to probes gainst the indicated mRNAs. At the time of CSF-1 stimulation, parallel unstimulated dishes were labelled with [^{3}H]thymidine for 24 or 48 h and scored as in Figure 5 (see text).

in macrophages is likely to be very similar to that modulated by PDGF in fibroblasts. In particular, the finding that genes selected only on the basis of PDGF responsiveness are similarly induced by CSF-1 argues for a substantial overlap between the two responses. Furthermore, the detailed similarity of the two systems with regard to the kinetics of gene expression implies that, in addition to the genes observed, the intermediate steps in the pathways that link the receptors to these genes are also likely to be conserved. Taken together, then, these findings argue for an early convergence in the pathways of the PDGF and CSF-1-mediated responses.

At the same time, our results are not consistent with the simplest scheme of convergence, in which all growth factors that stimulate these genes mediate this action via a single common intermediate (or via a single intermediate within a given cell type). Such a model is inconsistent with the finding that GM-CSF and CSF-1, although showing substantial overlap in their responses, neverthless differ qualitatively with respect to the KC gene. In addition, we have recently found evidence for a difference in the pathways used to regulate c-*fos* by these two factors: the c-*fos* deinduction subsequent to GM-CSF treatment can be reversed by CSF-1; however, in the reverse case, the deinduction is refractory to restimulation by GM-CSF (data not shown). These findings require either or both of two possible modifications of the simplest scheme: (i) the CSF-1 response diverges rapidly into two or more pathways, one of which is convergent with GM-CSF, and another of which mediates responses specific to CSF-1 (and perhaps PDGF); (ii) CSF-1 and GM-CSF act on distinct intermediate pathways, which however have overlapping but non-identical target gene specificities. It is interesting that, in fibroblasts, there is an indication that the signal generated by the PDGF receptor may diverge into two pathways each of which stimulates

c-*myc* expression (Coughlin *et al.*, 1985). Furthermore, recent evidence indicates the coexistence within macrophages of two pathways that regulate c-*fos* in distinct ways (Bravo *et al.*, 1987).

The closer similarity of the responses to PDGF and CSF-1, compared with the response to GM-CSF, may reflect a fortuitous choice of the mRNAs measured. However, there is a close evolutionary relationship between the receptors for PDGF and CSF-1 (Yarden *et al.*, 1986), and both these growth factors are active in homodimeric form (Das and Stanley, 1982; Stroobant and Waterfield, 1984; Heldin *et al.*, 1986). GM-CSF is monomeric (Burgess *et al.*, 1977; Sparrow *et al.*, 1985) (its receptor has not yet been isolated). This raises the possibility that similar receptors, even with non-overlapping tissue distributions, may share a closer functional relationship than distinct mitogenic receptors acting in the same cell.

We have addressed the relationship between the CSF-1-induced mRNAs and cell cycle regulation by examining their expression in cell populations that are synchronous and already uniformly cycling prior to the addition of growth factor. The complete program of responses was generated by CSF-1 either in the factor-independent Aut1 subclone or in wild-type cells supported by GM-CSF. Since the intensities of these responses were comparable to those generated in starved cells, it is unlikely that these results can be explained by expression occurring in a small subpopulation ($<10\%$) of synchronized, arrested cells that are being stimulated to re-enter the cell cycle. Similarly, the expression does not reflect a viability response, since these cultures are fully viable prior to growth factor addition. We conclude that the actions mediated by this gene program are not restricted to, and may perhaps not include, mitogenesis or the stimulation of cell survival.

In growing cultures of both 2F5 and Aut1 cells, addition of CSF-1 stimulates cell populations to proliferate with reduced doubling times (Figures 5A and 6A), and the gene program may perhaps function to mediate this effect. Indeed, if exponentially growing cells and stimulated arrested cells undergo a similar set of probabalistic cell cycle transitions, and if growth factors function to control the rate of one or more of these transitions (Brooks *et al.*, 1980), then mitogenesis (i.e. cell cycle re-entry by non-cycling cells) and doubling-time reduction may well be mediated by similar mechanisms. In this case, the program could serve a similar function in either starved or asynchronous (cycling) cultures. One possibility is that the program in fact mediates the probabalistic transition. However, in this case, one might expect a peak in program gene expression to recur transiently in each cell cycle. In fibroblasts grown in serum, this does not appear to be the case for either c-*fos* or c-*myc*: expression of these genes is constant throughout the cell cycle (Hahn *et al.*, 1985; Thompson *et al.*, 1985; Bravo *et al.*, 1986). In addition, 2F5 cells exponentially growing in CSF-1 contain substantial levels of these two messages (data not shown).

Perhaps a more likely possibility is that the gene program mediates the shift in transition probability rather than the transition itself. Such a 'gearshift' function need not be transient and need not recur in each cycle. A similar phenomenon may occur in fibroblasts: Bravo and co-workers have found that, in PDGF-deprived fibroblasts, PDGF can induce c-*fos* and c-*myc* expression in all phases of the cell cycle except mitosis. However, unlike the CSF-1-independent macrophages, the PDGF-deprived cells are moving toward cell cycle exit. They may thus be susceptible to a transition analogous to that which is inducible in quiescent cells (Scher *et al.*, 1980; Brooks, 1983), even if this transition is not part of the normal cell cycle.

Another possibility, not exclusive of those discussed above, is that the early-gene program may mediate a set of functions unrelated to growth. Although c-*fos* and c-*myc* have been implicated as regulators of cell proliferation (e.g. Leder *et al.*, 1983; Armelin *et al.*, 1984; Kaczmarek *et al.*, 1985; Holt *et al.*, 1986; Studzinski *et al.*, 1986), a specific role for the early transient elevation of these messages has not been established. A large variety of early responses to CSF-1 and PDGF have been observed in macrophages and fibroblasts, respectively, some of which can be separated from the control of proliferation (e.g. Hamilton *et al.*, 1980; Herman *et al.*, 1986). We have observed, for example, that the morphology and motility of Aut1 cells are altered when these cells are grown in CSF-1 (A.Orlofsky, J.W.Pollard, unpublished observations). Furthermore, our finding that KC expression is not required for growth is consistent with a non-mitogenic function for growth factor-induced mRNAs. There is, however, considerable evidence that the mitogenic signal can be carried out by two distinct pathways in the same cell (Rozengurt, 1986), and it is possible that only one of these pathways includes KC. If such multiple pathways exist, it is unlikely that correlative studies will be sufficient to define the functions of growth factor-induced mRNAs. Therefore, delineation of these functions must await further identification and characterization of the elements that make up the early growth factor response.

The results presented here indicate that a program of early-gene expression is shared by the responses to mitogens in macrophages and fibroblasts and that the functions of this program are apparently not restricted to, and therefore need not include, mitogenic stimulation of arrested cells. Furthermore, two mitogenic growth factors (CSF-1 and GM-CSF) acting on the same cell do not induce identical sets of genes in these cells. At least one of these genes (KC) is therefore not an essential component of the mitogenic response.

Materials and methods

Growth factors and plasmids

Murine CSF-1 was purified to homogeneity from L cell conditioned medium as described (Stanley, 1985). In some experiments, partially purified stage-I material was used; pure and stage-I CSF-1 produced similar growth and mRNA responses (see Note added in proof). Recombinant murine GM-CSF was a gift from Immunex Corporation. Plasmids were obtained as follows: pfos-1 (derived from v-*fos*; Curran *et al.*, 1982) from I.M.Verma; pMC413RC (a human genomic clone containing the third exon of the c-*myc* gene; Dalla-Favera *et al.*, 1983) from R.Dalla-Fevera; pCHO-C from J.E.Darnell, and pBC-JE and pBC-KC (cDNA clones of the JE and KC messages, respectively; Cochran *et al.*, 1983) from C.D.Stiles.

Cell cultures

The cell line 2F5 (Morgan *et al.*, 1987) is a clone of BAC1, an SV40-immortalized murine macrophage cell line (Schwarzbaum *et al.*, 1984). Aut1 was derived from 2F5 by mutagenesis with ethyl methane sulfonate and selection in control medium (C.J.Morgan and E.R.Stanley, unpublished results). All cells were cultured on tissue culture dishes as described (Morgan *et al.*, 1987). Control medium was alphaMEM modified as described (Morgan *et al.*, 1987) and supplemented with 15% fetal calf serum (GIBCO). For stimulation of 2F5, cells were rinsed twice in control medium and incubated in control medium overnight, prior to addition of CSF-1 (3000 units/ml; 1.3 nM) or GM-CSF (300 units/ml; 0.5 nM) (Morgan *et al.*, 1987). For growth curves, cells were harvested for counting using a zwitterionic detergent (Stanley, 1985).

Isolation of RNA, blotting, and hybridization

Total cellular RNA was purified by the guanidine thiocyanate/CsCl method essentially as described (Maniatis *et al.*, 1982), except that 12 μM EDTA was present during lysis. For poly(A)$^+$-RNA isolation, cells were rinsed rapidly in PBS and lysed in the presence of 0.5% SDS and 300–500 μg/ml of self-digested proteinase K (Sigma), in a buffer containing 0.5 M NaCl/0.01 M Tris–HCl (pH 7.4)/0.05 M $MgCl_2$/0.002 M $CaCl_2$. Lysates were incubated at 37°C for 30 min, extracted twice with phenol:chloroform and once with chloroform, and precipitated with ethanol. Poly(A)$^+$-RNA was twice purified by oligo-dT-cellulose chromatography (Maniatis *et al.*, 1982). For Northern analysis, RNA

samples were electrophoresed in 1.3% agarose at 2–3 V/cm in the presence of formaldehyde and transferred to nitrocellulose (Maniatis *et al.*, 1982). Preparation of dot-blots was essentially as described (Han *et al.*, 1986). Hybridizations were performed for 1–2 days as described (Wahl *et al.*, 1979), except that dot-blots were hybridized at 43°C in 50% formamide, and Northern blots at 45°C in 30–35% formamide. Probes were ^{32}P-labeled by nick-translation using a BRL nick-translation kit. Filters were washed after hybridization in 2 × SSC (SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 0.1% SDS, 0.05% Na pyrophosphate, first briefly at room temperature, then for several hours at 60°C, and exposed to X-ray film at −70°C with intensifying screens.

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Note added in proof

In recent experiments we have found that there are some significant differences in the intensity and/or kinetics of mRNAs induced by the partially purified CSF-1 used in Figures 2, 3, 5B and 6B, and pure CSF-1. Firstly, in Aut1 cells (Figure 5B) c-*fos*, c-*myc* and JE mRNAs are induced ~3–4-fold with pure CSF-1 and ~10-fold in the figure. Secondly, pure CSF-1 gives little or no induction of KC in Aut1 cells. Thirdly, KC induction by pure CSF-1 in starved 2F5 cells appears to be transient (absent by 4 h).