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Regulation of Expression of Growth Arrest-Specific Genes in Mouse Fibroblasts

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The suppression of growth arrest-specific (*gas*) gene expression by serum appeared to be independent of protein synthesis, but expression in resting cells was sensitive to 2-aminopurine, an inhibitor of intracellular protein kinases. Although accumulation of *gas* gene mRNA was reduced by serum, nuclear transcription of the *gas-2*, *-3*, and *-5* genes was observed in serum-stimulated cells, indicating that posttranscriptional events may regulate mRNA levels. Growth induction by serum, on the other hand, led to suppression of transcription of the *gas-1* gene. Cell cycle regulation and the serum response of *gas-1* were lost in *ras*-transformed cells.

The majority of cells in an organism spend a considerable part of their life span in the resting state. When needed, they can reenter the cell cycle and proliferate after appropriate stimulation (25). Several growth factors that can induce cells to divide have been identified (8, 13, 39), and much effort has concentrated on identifying the individual genes activated by these factors (10, 15, 22, 24). Numerous genes are induced upon mitogenic stimulation of quiescent mammalian cells, and a cascade of gene activity from early to late times in the G1 phase has been delineated (1, 2, 5, 11, 27, 40). Some of the late genes in this cascade probably have a role in the induction of DNA synthesis (13, 14, 17, 18, 30, 31).

To understand the biochemical events that control progression through the cell cycle, it is important to investigate the mechanisms that regulate the temporal expression of cell cycle-regulated genes. Many of the immediate-early genes are transcriptionally activated upon serum stimulation of quiescent cells. Expression of the immediate-early genes into mRNA is superinduced by cycloheximide (CHX) through two mechanisms, involving stimulation of transcription and prolonged half-lives of the mRNA species (1).

The cell cycle must, on the other hand, probably possess both positive and negative controlling elements. Indeed, negative-feedback mechanisms operating during growth and negative modulators have been identified (12, 16, 19, 20, 21, 32, 38). Recently, it has been observed that serum-induced transition from the G0 to G1 phase of the mammalian cell cycle results in suppression of expression of growth arrest-specific (*gas*) mRNAs (28). Repression of expression of these genes therefore coincides with the induction of the early mitogenic response genes. Understanding the mechanisms that regulate expression of the *gas* genes may therefore provide further information about the controls operating at transition from the G0 to G1 phase.

We have therefore analyzed the regulation of expression of G0-specific genes in mouse fibroblasts at quiescence and after serum stimulation. Cell cycle-controlled expression of *gas* genes is observed in primary embryo fibroblast cells and in some other cell lines but appears at least for *gas*-1 to be constitutively expressed in *ras*-transformed cells. *gas* gene expression seems to be controlled at the posttranscriptional level except for the *gas-1* gene, which is transcriptionally regulated.

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MATERIALS AND METHODS

Cell cultures. NIH 3T3 cells were grown in Dulbecco modified Eagle medium (DMEM) supplemented with glutamine (2 mM), penicillin (100 U/ml), streptomycin (50 μg/ml), and 10% fetal calf serum (FCS). To achieve quiescence, cells were grown to confluence and then incubated in 0.5% FCS for 48 h. Serum stimulation was achieved by incubating quiescent cells with fresh medium containing 20% FCS. CHX (Sigma Chemical Co., St. Louis, Mo.) was used at 10 μg/ml, and actinomycin D (Sigma) was used at 1 μg/ml. 2-Aminopurine (2AP; Sigma) was used at 10 mM. Mouse embryo primary fibroblasts were prepared as described previously (29). K-*ras*-transformed NIH 3T3 cells were kindly provided by P. Besmer, Memorial Sloan Kettering Institute, New York, N.Y. DNA synthesis was monitored by counting [^{3}H]thymidine-labeled nuclei (29).

RNA preparation and Northern (RNA) blot analysis. RNA was isolated with guanidine isothiocyanate and purified by centrifugation over a cesium chloride cushion as described previously (9). A 20-μg sample of total RNA was denatured by heating at 65°C in a formamide-formaldehyde solution. RNA was separated on 1.3% agarose gels and transferred by blotting to GeneScreen Plus membranes (Dupont, NEN Research Products, Boston, Mass.). Filters were hybridized overnight with 1×10^6 to 2×10^6 cpm of ^{32}P-labeled DNA probes per ml. DNA probes were labeled by random priming to a specific activity of around 0.5×10^9 cpm/μg. Filters were washed at a final stringency of 0.1× SSC–0.5% sodium dodecyl sulfate at 65°C (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) Probes for the *gas* genes are described in reference 28.

Nuclear run-on transcription assay. Quiescent or serum-stimulated cultures of NIH 3T3 cells were washed with ice-cold phosphate-buffered saline. Cells were harvested with a rubber policeman and centrifuged at 1,600 × *g* for 5 min. The cell pellet was suspended in Nonidet P-40 lysis buffer (10 mM Tris [pH 7.4], 10 mM NaCl, 3 mM MgCl, 0.5% Nonidet P-40), incubated for 5 min on ice, and sheered gently through a Gilson tip. Nuclei were isolated on a 0.7 M sucrose cushion. RNA was labeled with [^{32}P]UTP and purified as described by Almendral et al. (1). Labeled RNA was hybridized to specific cDNA probes immobilized on nitrocellulose membranes. Hybridizations were performed in a solution containing 50% formamide, 0.5% sodium dodecyl sulfate, 5× SSC, and 5× Denhardt solution at 42°C for 3 days. Filters were then washed for 15 min twice in 0.1×

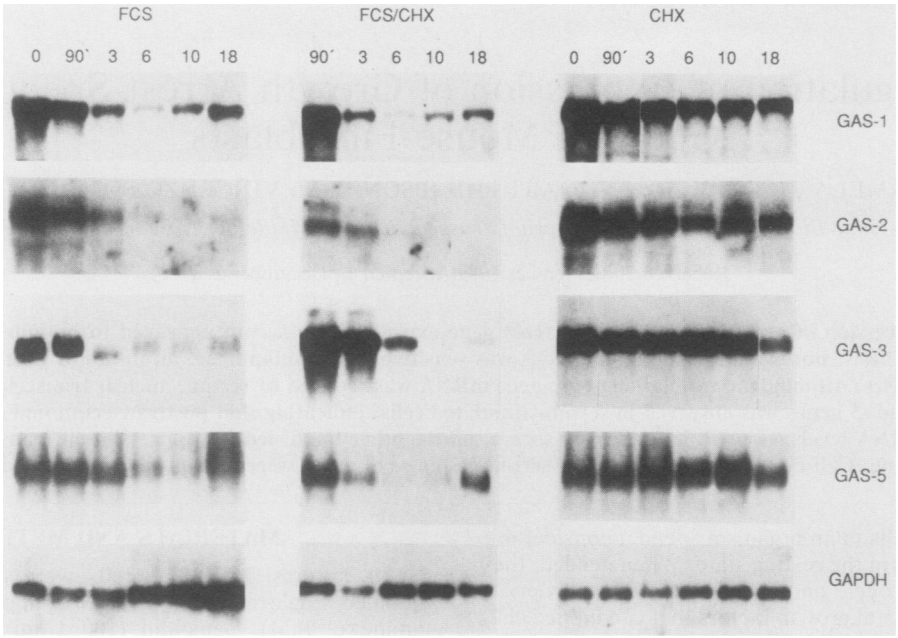

FIG. 1. Effect of CHX on *gas* gene expression. RNA was extracted from quiescent cells or cells treated with FCS, FCS plus CHX, or CHX at the indicated times (in hours). RNA was separated on agarose gels, transferred to a GeneScreen plus membrane, and hybridized with the indicated *gas* gene probes and with a GAPDH probe as a control.

SSC–0.1% sodium dodecyl sulfate at room temperature and then for an additional 30 min at 65°C.

RESULTS

Serum-induced suppression of *gas* mRNA occurs independent of protein synthesis. High levels of *gas* gene mRNA are expressed in quiescent NIH 3T3 cells. Serum stimulation results in decrease of *gas* mRNA accumulation starting after 1 to 3 h, with maximum inhibition at 6 to 10 h. The levels of *gas-1* and *gas-5* mRNA thereafter increase but never to the level observed at growth arrest. The lag period observed before suppression of *gas* mRNAs after serum stimulation may reflect the time required for events that down-regulate *gas* gene expression. We therefore first tested whether protein synthesis is necessary for serum-induced suppression of *gas* gene expression. NIH 3T3 cells were grown to confluency in DMEM medium plus 10% FCS and kept for 2 more days in 0.5% FCS to render them quiescent. Under these conditions, less than 3% of the cells incorporate [^{3}H]thymidine in the nuclei, as detected by autoradiography (not shown). Quiescent cultures were then stimulated with 20% FCS, with 20% FCS plus CHX (10 μg/ml), and with CHX alone. RNA was extracted at different time points and analyzed by Northern blotting. Filters were hybridized with the specific *gas* probes and with the GAPDH gene, which is known to be a differentially regulated housekeeping gene. In this experiment, CHX gave a 95% inhibition of protein synthesis separately assayed with [^{35}S]methionine and trichloroacetic acid precipitation (not shown). It was also ascertained that the cells could respond to growth induction and DNA synthesis after CHX treatment for 24 h, followed by washing and serum stimulation. Inhibition of protein synthesis by CHX did not prevent FCS-induced repression of *gas* gene mRNAs after serum stimulation (Fig. 1). Furthermore, CHX did not prevent the late increase in *gas-1* and *gas-5* mRNAs observed at 10 to 18 h after serum stimulation, although reappearance of *gas-3* was slightly inhibited.

In contrast to results for several serum-induced genes, CHX alone reduced the level of *gas* gene mRNA, with *gas-1* and *gas-2* being more affected than *gas-5* (Fig. 1). Expression of GAPDH mRNA was unaffected by CHX, suggesting that active protein synthesis is required for maximal accumulation of *gas* gene RNA in quiescent cells, in contrast to GAPDH mRNA and most of the serum-induced mRNAs.

Control of *gas* gene expression. Changes in the level of mRNA may be the consequence of alterations in the rate of transcription or due to posttranscriptional events. To clarify this point, several run-on transcription experiments of *gas* genes were performed in the presence and absence of CHX in quiescent and serum-stimulated cells. Transcription of *gas-2*, *-3*, and *-5* appeared unaltered after serum stimulation, although the signal was reproducibly lower than the γ-actin signal but significantly higher than that of pBR322 (Fig. 2). The variation in the accumulation of the *gas-2*, *-3*, and *-5* mRNAs may therefore be controlled at a posttranscriptional

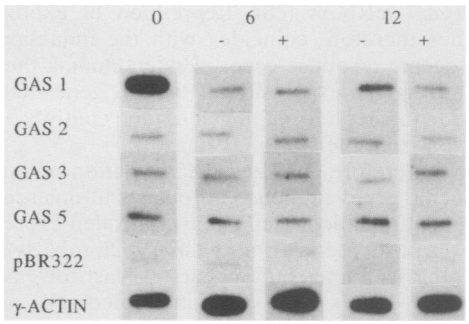

FIG. 2. Transcriptional activity of the *gas* genes in quiescent and serum-stimulated cells. Cell nuclei were isolated from quiescent cells or cells stimulated with 20% FCS in the absence (−) or presence (+) of CHX for the indicated times (in hours). Labeling of RNA and hybridization conditions are described in Materials and Methods.

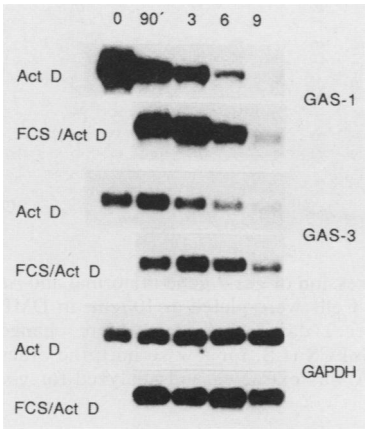

FIG. 3. Turnover of *gas* gene mRNA in quiescent and serum-stimulated cells. Quiescent cells were treated with actinomycin D (Act D) alone or with 20% FCS plus actinomycin D for the indicated times (in hours). Total RNA (20 μg) was analyzed on a gel, blotted, and hybridized against ^{32}P-labeled DNA probes.

level. On the other hand, *gas-1* gene expression is definitely modulated by serum at the transcriptional level. In isolated nuclei, CHX did not prevent repression of *gas-1* transcription. In separate experiments, it was also established that the increase in *gas-1* transcription observed at 12 to 18 h was independent of protein synthesis (not shown; cf. Fig. 1). The transcriptional regulation by serum of the *gas-1* gene therefore probably does not require de novo protein synthesis.

Stability of *gas* mRNAs. The turnover of *gas* mRNAs was determined in quiescent and serum-stimulated cells. Cells were grown to confluence, treated with actinomycin D (1 μg/ml), and then harvested at the indicated times. Figure 3 shows the decay kinetics of the *gas-1* and *gas-3* mRNAs, which had half-lives of about 2 h, as determined by several different exposures, different experiments, and densitometric tracings. Similar kinetics was observed for the *gas-2* mRNA, whereas the *gas-5* mRNA had a half-life of about 6 h (not shown). However, when turnover of the *gas* mRNAs was measured in serum-stimulated cells (treated with serum plus actinomycin D), the decay was at least twofold slower than in quiescent cells. This apparent contradiction may suggest that different posttranscriptional events regulate turnover of the *gas* mRNAs in quiescent and in serum-stimulated cells.

Protein kinases and *gas* mRNA suppression. The purine analog 2AP, which prevents phosphorylation of serine and threonine residues through cellular protein kinases, inhibits induction by serum of *fos* and *myc* mRNAs (41). To investigate whether expression of the *gas* genes in quiescent cells and the suppression by serum was sensitive to inhibitors of cellular protein kinases, we treated quiescent cultures of NIH 3T3 cells for 6 h with 2AP alone and with 2AP plus FCS. Treatment of cells with 2AP alone resulted in about 80% reduction of the level of *gas-1* mRNA, as revealed by densitometric tracing of different exposures of the film (Fig. 4). 2AP, however, did not inhibit the repression of *gas-1* mRNA by serum. On the other hand, treatment with 2AP alone was at least as efficient as treatment with serum in suppressing the mRNA levels of *gas-2*, *-3*, and *-5* in quiescent cells.

These data suggest that different pathways regulate *gas* gene expression in quiescent cells. Expression of *gas-2*, *-3*, and *-5* appears to be strongly dependent on the activity of

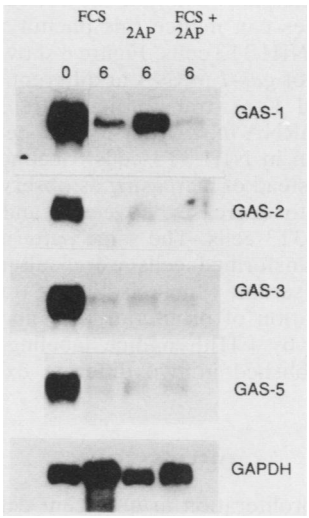

FIG. 4. Effect of 2AP on expression of *gas* genes in quiescent and serum-stimulated cells. Quiescent cells were treated with either FCS, 2AP at 10 mM, or FSC plus 2AP. Cells were harvested after 6 h, and RNA was extracted and analyzed for *gas* mRNA levels.

unknown cellular protein kinases, whereas *gas-1* expression is less sensitive. This experiment also suggests that suppression of *gas-1* mRNA is not regulated through a 2AP-sensitive pathway. In contrast, induction of *fos* and *myc* transcription appears to be 2AP sensitive (41).

Expression of *gas* mRNA in primary and transformed cells. To establish the generality of the growth arrest specificity of the *gas* genes, we analyzed their expression in primary mouse embryo fibroblasts. Expression of the *gas* genes in these cells was similar to the pattern observed in NIH 3T3 cells (Fig. 5). Neoplastic transformation is the result of

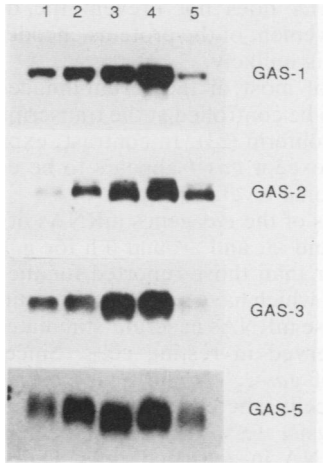

FIG. 5. *gas* gene expression in mouse embryo primary fibroblasts. Primary cells were obtained from a day 19 embryo after head and viscera were removed. Cells were used before the third passage in vitro. Confluent cultures were split in new plates at a 1:10 dilution in fresh medium containing 10% FCS. This was considered day 0. Cells were harvested after 2, 4, and 6 days (lanes 1, 2, and 3, respectively). Cells were otherwise kept for 4 days in 10% FCS and for an additional 2 days in 0.5% FCS (lane 4) and then stimulated with fresh medium containing 20% FCS for 6 h (lane 5).

deregulation in the pathways that control cell proliferation. Several oncogenes can induce this phenotype when introduced in normal NIH 3T3 cells. Figure 6 shows a time course analysis of level of *gas-1* mRNA at different days of growth in NIH 3T3 and K-*ras*-transformed NIH 3T3 cells. The levels of *gas-1* mRNA in *ras*-transformed cells were significantly lower than in NIH 3T3 cells. Second, the levels of *gas-1* mRNA, instead of increasing as observed in NIH 3T3 cells, appeared to decrease between 2 and 6 days in K-*ras*-transformed 3T3 cells. The same pattern was observed when the *ras*-transformed cells were cultured in medium containing 0.5% serum, which also in the transformed cells resulted in cessation of proliferation in about 80% of the cells, as judged by [^{3}H]thymidine labeling of the nuclei, which was established in two different experiments (not shown).

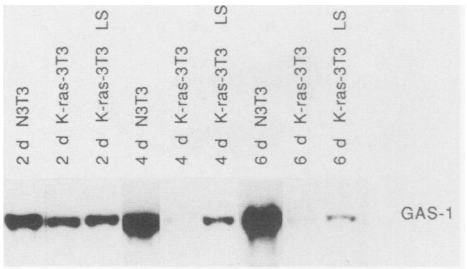

FIG. 6. Expression of *gas-1* gene in normal and *ras*-transformed NIH 3T3 cells. Cells were plated at 10^4/cm^2 in DMEM containing 10% FCS. After 1 day, some plates were changed to DMEM containing 0.5% FCS (LS, for low serum); the others were left in 10% FCS. RNA was extracted and analyzed for *gas-1* mRNA on Northern blots.

DISCUSSION

Induction of proliferation in quiescent cells is accompanied by the transcriptional activation of a large set of genes (1, 2). Although the kinetics and the magnitude of the induction vary among genes, analysis of the regulation of these genes has delineated some common mechanisms that regulate their expression, such as dependence on protein synthesis for shutoff of transcription and for maintenance of the short half-lives of the mRNAs. Here we report on the regulation of the expression of a group of genes that are negatively regulated by serum (28).

mRNAs from the *gas* genes accumulate when fibroblasts cells reach density inhibition or enter quiescence as a result of serum starvation. Serum stimulation of quiescent cells suppresses expression of these mRNAs. The decrease of the *gas* mRNAs starts after 1 h and is maximal by 6 to 10 h, corresponding to the mid- and late G1 phases. With the exception of *gas-2* and *gas-3* mRNAs, *gas* mRNAs reappear again between 12 and 18 h after serum addition. The proteins derived from early mitogen-induced genes, which temporally precede the decrease of the *gas* mRNAs, may therefore have a role in the regulation of these genes. However, since addition of CHX does not prevent the decrease in *gas* mRNAs, involvement of the proteins encoded by the serum-induced genes is unlikely.

Expression of most of the serum-induced genes in 3T3 cells appears to be controlled at the transcriptional level; one exception is proliferin (23). In contrast, expression of all of the *gas* genes except *gas-1* appears to be controlled at the posttranscriptional level.

The half-lives of the *gas* genes mRNAs of around 2 to 3 h for *gas-1*, *-2*, and *-3*, and around 6 h for *gas-5* in quiescent cells are longer than those reported for the serum-induced genes, many of which have half-lives of around 0.5 h (1). The turnover of these mRNAs in serum-stimulated cells is slower than that observed in resting cells. Since no change in transcription of *gas-2*, *-3*, and *-5* is observed after serum stimulation of cells whereas a clear decrease is observed by Northern analysis, we would have expected a shorter half-life of the mRNA in activated cells. Differences in RNA turnover, depending on the metabolic conditions of the cell, have been observed (6). A possible explanation would be that a putative RNA with a short half-life, which is absent in quiescent cells, is required for degradation of the mRNA in serum-stimulated cells. Since CHX by itself (Fig. 1) does not prevent down regulation by serum, this putative RNA does not necessarily code for a protein. Depletion of this RNA by actinomycin D in serum-activated cells would therefore stabilize the *gas* mRNAs. Involvement of an unknown RNA has been proposed to participate in the turnover of c-*myc* RNA (7). Furthermore, since the turnover of the *gas* mRNAs occurs within hours, the maintainance of a high level of mRNAs in cells treated with CHX for up to 12 to 18 h indicates that de novo protein synthesis is not essential for mRNA accumulation from these genes in quiescent cells.

Although protein synthesis is not required for accumulation of the *gas* mRNAs, expression of *gas-2*, *-3*, and *-5* is strongly sensitive to 2AP, whereas *gas-1* expression is less affected. 2AP is known to inhibit two known kinases, the heme-regulated and the double-standed RNA-dependent E1F2 α-kinases (33, 41). Although the mechanism of 2AP inhibition of cellular phosphorylation is unknown, it is thought to preferentially inhibit protein kinases, modifying serine and threonine residues.

Expression of *gas* mRNAs has been detected in several fibroblast cell lines and also in primary embryo fibroblasts (Fig. 5). They are, however, also expressed in other cell types (e.g., hematopoietic cells), in different tissues, and during embryo development. The presence of *gas* mRNA in many different cells and tissues as well as their evolutionary conservation suggest that they may serve a basic function (E. M. Coccia et al., submitted for publication).

Expression of *gas-1* mRNA in *ras*-transformed cells is also quantitatively and qualitatively different from expression in untransformed 3T3 cells (Fig. 6). In K-*ras*-transformed 3T3 cells, down regulation of *gas-1* mRNA by serum is also less efficient than in untransformed cells (not shown).

Not much is known about genes that are preferentially expressed in quiescent cells. The first one identified was the gene encoding statin, a 57-kilodalton protein that is located in the nucleus of nonproliferating human fibroblasts and not in growing or transformed cells (34, 36). Statin also disappears when arrested cells are stimulated with serum and reenter the cell cycle (35, 37). Another protein, of about 20 kilodaltons and preferentially expressed in nondividing cells, has been identified in chicken fibroblasts (3). The gene coding for this protein has recently been cloned (4). Expression of this protein is also repressed when normal cells are stimulated to divide or by *src* transformation. We do not yet know the function of the proteins encoded by the *gas* genes and of the other proteins specifically expressed in quiescence. Studies with antisense oligonucleotides are currently being performed to establish the role of these genes in the cell cycle. Once full-length clones are available, transfection experiments can establish the function of the *gas* genes and their position in the chain of events that is required for the

maintainance of growth, quiescence, and differentiation of mammalian cells.

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LITERATURE CITED

1. **Almendral, J. M., D. Sommer, H. MacDonald-Bravo, J. Burckhardt, J. Perera, and R. Bravo.** 1988. Complexity of the early genetic response to growth factors in mouse fibroblasts. Mol. Cell. Biol. **8**:2140–2148.
2. **Armelin, H. A., M. C. S. Armelin, K. Kelly, T. Stewart, P. Leder, B. H. Cochran, and C. D. Stiles.** 1984. Functional role for c-*myc* in mitogenic response to platelet-derived growth factor. Nature (London) **310**:655–660.
3. **Bedard, P.-A., Y. Yannoni, D. L. Simmons, and R. L. Erikson.** 1989. Rapid repression of quiescence-specific gene expression by epidermal growth factor, insulin, and $pp60^{v\text{-}src}$. Mol. Cell. Biol. **9**:1371–1375.
4. **Bédard, P.-C., S. D. Balk, H. S. Gunther, A. Morisi, and R. L. Erikson.** 1987. Repression of quiescence-specific polypeptides in chicken heart mesenchymal cells transformed by Rous sarcoma virus. Mol. Cell. Biol. **7**:1450–1458.
5. **Bohmann, D., T. J. Bos, T. Nishimura, P. K. Vogt, and R. Tjian.** 1987. Human proto-oncogene c-*jun* encodes a protein with antigenic and enhancer binding properties of transcription factor AP-1. Science **238**:1386–1392.
6. **Brawerman, G.** 1989. mRNA decay: finding the right targets. Cell **57**:9–10.
7. **Brewer, G., and J. Ross.** 1989. Regulation of c-*myc* mRNA stability in vitro by a labile destabilizer with an essential nucleic acid component. Mol. Cell. Biol. **9**:1996–2006.
8. **Carpenter, G.** 1987. Receptors for epidermal growth factor and other polypeptide mitogens. Annu. Rev. Biochem. **56**:881–914.
9. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. J. Biochem. **18**:5294–5299.
10. **Cochran, B. H., A. C. Reffel, and C. D. Stiles.** 1983. Molecular cloning of gene sequences regulated by platelet-derived growth factor. Cell **33**:939–947.
11. **Cohen, D. R., and T. Curran.** 1988. *fra*-1: a serum-inducible, cellular immediate-early gene that encodes a Fos-related antigen. Mol. Cell. Biol. **8**:2063–2069.
12. **Craig, R. W., and R. Sager.** 1985. Suppression of tumorigenicity in hybrids of normal and oncogene-transformed CHEF cells. Proc. Natl. Acad. Sci. USA **82**:2062–2066.
13. **Deuel, T. F.** 1987. Polypeptide growth factors: roles in normal and abnormal cell growth. Annu. Rev. Cell Biol. **3**:443–492.
14. **Gewirtz, A. M., and B. Calabretta.** 1988. A c-*myb* antisense oligodeoxynucleotide inhibits normal human hematopoeisis *in vitro*. Science **242**:1303–1306.
15. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (London) **311**:433–438.
16. **Hansen, M. F., and W. K. Cavenee.** 1988. Tumor suppressors: recessive mutations that lead to cancer. Cell **53**:172–173.
17. **Heikkila, R., G. Schwab, E. Wickstrom, S. L. Loke, D. H. Pluznik, R. Watt, and L. M. Neckers.** 1987. A c-*myc* antisense oligodeoxynucleotide inhibits entry into S phase but not progress from G0 to G1. Nature (London) **328**:445–449.
18. **Kaczmarek, L., J. K. Hyland, R. Watt, M. Rosenberg, and R. Baserga.** 1985. Microinjected c-*myc* as a competence factor. Science **228**:1313–1315.
19. **Knabbe, C., M. E. Lippman, L. M. Wakefield, K. C. Flanders, A. Kasid, R. Derynck, and R. B. Dickson.** 1987. Evidence that transforming growth factor-β is a hormonally regulated negative growth factor in human breast cancer cells. Cell **48**:417–428.
20. **Kohase, M., D. Henriksen-DeStefano, L. T. May, J. Vlicek, and P. B. Sehgal.** 1986. Induction of β_2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. Cell **45**:659–666.
21. **Lin, S. L., T. Kikuchi, W. J. Pledger, and I. Tamm.** 1986. Interferon inhibits the establishment of competence in G_0-S-Phase transition. Science **233**:356–359.
22. **Linzer, D. I. H., and D. Nathans.** 1983. Growth-related changes in specific mRNAs of cultivated mouse cells. Proc. Natl. Acad. Sci. USA **80**:4271–4275.
23. **Linzer, D. I. H., and E. L. Wilder.** 1987. Control of proliferin gene expression in serum-stimulated mouse cells. Mol. Cell. Biol. **7**:2080–2086.
24. **Müller, R., R. Bravo, J. Burckhardt, and T. Curran.** 1984. Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. Nature (London) **312**:716–720.
25. **Pardee, A. B., R. Dubrow, J. L. Hamlin, and R. F. Kletzien.** 1978. Animal cell cycle. Annu. Rev. Biochem. **47**:715–750.
26. **Roberts, A. B., and M. B. Sporn.** 1988. Transforming growth factor β. Adv. Cancer Res. **51**:107–145.
27. **Ryder, K., L. Lau, and D. Nathans.** 1988. A gene activated by growth factors is related to the oncogene v-*jun*. Proc. Natl. Acad. Sci. USA **85**:1487–1491.
28. **Schneider, C., R. M. King, and L. Philipson.** 1988. Genes specifically expressed at growth arrest of mammalian cells. Cell **54**:787–793.
29. **Sorrentino, V., and S. Bandyopadhyay.** 1989. TGFβ inhibits GO/S-phase transition in primary fibroblasts. Loss of response to the antigrowth effect of TGFβ is observed after immortalization. Oncogene **4**:569–574.
30. **Sorrentino, V., V. Drozdoff, M. D. McKinney, L. Zeitz, and E. Fleissner.** 1986. Potentiation of growth factor activity by exogenous c-*myc* expression. Proc. Natl. Acad. Sci. USA **83**:8167–8171.
31. **Sorrentino, V., V. Drozdoff, L. Zeitz, and E. Fleissner.** 1987. Increased radiation-induced transformation in C3H/10T1/2 cells after transfer of an exogenous c-*myc* gene. Proc. Natl. Acad. Sci. USA **84**:4131–4134.
32. **Sporn, M. B., and A. B. Roberts.** 1988. Peptide growth factors are multifunctional. Nature (London) **332**:217–219.
33. **Tiwari, R. K., J. Kusari, R. Kumar, and G. C. Sen.** Gene induction by interferons and double-stranded RNA: selective inhibition by 2-aminopurine. Mol. Cell. Biol. **8**:4289–4292.
34. **Wang, E.** 1986. A 57,000-mol-wt protein uniquely present in nonproliferating cells and senescent human fibroblasts. J. Cell Biol. **100**:545–551.
35. **Wang, E.** 1985. Rapid disappearance of statin, a nonproliferating and senescent cell-specific protein, upon reentering the process of cell cycling. J. Cell Biol. **101**:1695–1701.
36. **Wang, E.** 1987. Contact-inhibition-induced quiescent state is marked by intense nuclear expression of statin. J. Cell Physiol. **133**:151–157.
37. **Wang, E., and S. L. Lin.** 1986. Disappearance of statin, a protein marker for non-proliferating and senescent cells, following serum-stimulated cell cycle entry. Exp. Cell Res. **167**:135–143.
38. **Whyte, P., K. J. Bucjkovich, J. M. Horowitz, S. H. Friend, M. Raybuck, R. A. Weinberg, and E. Harlow.** 1988. Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature (London) **334**:124–129.
39. **Williams. L. T.** 1989. Signal transduction by the platelet-derived growth factor receptor. Science **243**:1564–1570.
40. **Zerial, M., L. Toschi, R. P. Ryseck, M. Schuermann, R. Müller, and R. Bravo.** 1989. The product of a novel growth factor activated gene, *fos* B, interacts with JUN proteins enhancing their DNA binding activity. EMBO J. **8**:805–813.
41. **Zinn, K., A. Keller, L. A. Whitemore, and T. Maniatis.** 1988. 2-Aminopurine selectively inhibits the induction of β-interferon, c-*fos*, and c-*myc* gene expression. Science **240**:210–213.