Ross, E. M., & Gilman, A. G. (1977) *J. Biol. Chem. 252*, 6966–6969.
Ross, E. M., & Gilman, A. G. (1980) *Annu. Rev. Biochem. 49*, 533–564.
Sternweis, P. C., & Gilman, A. G. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4888–4891.
Sternweis, P. C., Northup, J. K., Smigel, M. D., & Gilman, A. G. (1981) *J. Biol. Chem. 256*, 11517–11526.

Expression of Growth-Regulated Genes in tsJT60 Cells, a Temperature-Sensitive Mutant of the Cell Cycle†

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ABSTRACT: We have investigated the expression of growth-regulated genes in tsJT60 cells, a temperature-sensitive (ts) mutant of Fischer rat cells, which, on the basis of its kinetic behavior, can be classified as a G_0 mutant. It grows normally at 34 °C and also at 39.5 °C if shifted to the higher temperature during exponential growth. However, if the cell population is first made quiescent by serum deprivation, subsequent stimulation by serum induces the cells to enter S phase at 34 °C but not at 39.5 °C. A panel of growth-regulated genes was used that included three protooncogenes (c-fos, c-myc, and p53), several genes that are induced in G_0 cells stimulated by growth factors (β-actin, 2A9, 2F1, vimentin, JE-3, KC-1, and ornithine decarboxylase), and an S-phase gene (histone H3). The expression of these growth-regulated genes was studied in both tsJT60 cells and its parental cell line, rat 3Y1 cells. All the genes tested, except histone H3, are similarly induced when quiescent tsJT60 cells are stimulated by serum at either permissive or restrictive temperatures. These results raise intriguing questions on the nature of quiescence and the relationship between G_0 and G_1 in cells in culture.

The tsJT60 cells are a temperature-sensitive (ts) mutant of the Fischer rat cell line 3Y1 (Kimura et al., 1975) that displays some interesting characteristics. tsJT60 cells do not manifest a ts phenotype while exponentially growing, but if they enter a resting state (G_0) by serum deprivation of confluent monolayers, they cannot reenter S phase at 39.5 °C after serum stimulation, although they can do so at the permissive temperature of 34 °C (Ide et al., 1984). In this respect, tsJT60 cells behave as a G_0 mutant, i.e., a mutant that is defective in a function required for the transition from a resting to a growing stage but not necessary for cell cycle progression in cycling cells [for a review, see Baserga (1985)].

Recently, a number of genes have been reported whose cognate RNAs are markedly increased when resting (G_0) cells are stimulated to proliferate by growth factors. These include some bona fide protooncogenes like c-fos (Greenberg & Ziff, 1984; Kruijer et al., 1984; Muller et al., 1984), c-myc (Kelly et al., 1983; Campisi et al., 1984; Kaczmarek et al., 1985b), c-myb (Torelli et al., 1985), c-ras (Goyette et al., 1983, 1984; Campisi et al., 1984), and p53 (Reich & Levine, 1984). Others are genes whose products are well-known like core histones (Plumb et al., 1983; Hirschhorn et al., 1984b) and thymidine kinase (Liu et al., 1985). Finally, others have been identified as cDNA clones by differential screening of cDNA libraries (Linzer & Nathans 1983, 1984; Cochran et al., 1983; Hirschhorn et al., 1984b; Matrisian et al., 1985a,b; Edwards et al., 1985; Edwards & Denhardt, 1985; Lau & Nathans, 1985). Interestingly, some of these genes are expressed at roughly constant levels during the cell cycle (G_1 to M), and it is only in G_0 that their RNAs are not detectable or are detectable at very low levels. This is true, for instance, for c-myc (Hann et al., 1985; Thompson et al., 1985) and 2A9, a cDNA clone preferentially expressed in serum-stimulated cells (Hirschhorn et al., 1984b), which is inducible by platelet-derived growth factor and overexpressed in human acute myeloid leukemia (Calabretta et al., 1986).

Table I: List of Genes and Sequences Selected for These Studies

sequence	maximal expression	ref[a]
c-fos	G_0–G_1	Greenberg and Ziff (1984)
c-myc	G_0–G_1	Kelly et al. (1983)
KC-1	G_0–G_1	Cochran et al. (1983)
JE-3	G_0–G_1	Cochran et al. (1983)
4F1 (vimentin)	G_0–G_1	Hirschhorn et al. (1984b)
2F1	G_0–G_1	Hirschhorn et al. (1984b)
β-actin	variable	Campisi et al. (1984)
2A9	mid G_1	Hirschhorn et al. (1984b)
ornithine decarboxylase	mid G_1	Liu et al. (1985)
p53	mid/late G_1	Reich and Levine (1984)
histone H3	S phase	Plumb et al. (1983)

[a] The references given refer to papers showing the inducibility of the genes by growth factors. For simplicity, only one reference is given for each gene, but other references can be found in the text.

Since tsJT60 cells meet the criteria established for a G_0 mutant (Baserga, 1978), we thought it worthwhile to investigate the expression of a panel of growth-regulated genes

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(most of which are inducible in G_0 cells by growth factors) in quiescent tsJT60 cells serum stimulated at either the permissive or the nonpermissive temperatures. The term gene expression is used here in one of its accepted usages, i.e., as steady-state levels of specific mRNAs. Of the genes selected (Table I), at least six are genes whose RNA levels markedly increase very early after stimulation of G_0 cells. However, even 2A9 and p53 (whose maximal expression reaches a peak at later stage) should be considered primary responders for the reasons given below. Surprisingly, all the growth-regulated genes tested were expressed vigorously at either temperature, after serum stimulation of G_0 tsJT60 cells, with one exception, the S-phase gene histone H3 that was induced at 34 °C but not at 39.5 °C.

Materials and Methods

Cell Lines and Culture Conditions. 3Y1-B clone 1-6 originated from Fischer rat embryo (Kimura et al., 1975). tsJT60 is a temperature-sensitive mutant isolated from 3Y1-B clone 1-6 (Ide et al., 1984). Both cell lines were cultured in Dulbecco's modified Eagle's medium (DME medium) supplemented with 10% fetal calf serum. For tsJT60, the permissive temperature was 34 °C, and the nonpermissive temperature was 39.5 °C. The cells were made quiescent by maintaining confluent cells in serum-free DME medium for 2–3 days. Quiescent populations were stimulated with fresh DME medium containing 10% fetal calf serum prewarmed at either 34 or 39.5 °C. For short-term experiments, 15- or 30-min stimulation, the stimulated cells were placed on the water surface in a water bath with lid to equibrate the temperature quickly. The entry of cells into DNA synthesis was monitored by continuous labeling with [^{3}H]thymidine (6.7 Ci/mol, 0.5 μCi/mL, New England Nuclear) from zero time (Figure 1) or from 12 to 36 h (Table II) after stimulation followed by autoradiography. The same conditions were used for the parent cell line, 3Y1-B clone 1-6. Routine checking by autoradiography (Perez et al., 1972), uridine/uracil method (Schneider et al., 1974), and Mycotrium TC kit (Hana Media, Inc., CA) revealed no contamination with mycoplasma.

Isolation of RNA. Total cytoplasmic RNA was isolated from cells lysed with 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.9/150 mM NaCl/1.5 mM $MgCl_2$/0.65% Nonidet P-40/10 mM vanadyl ribonucleoside complex, extracted with distilled phenol/chloroform/0.2% 8-hydroxyquinoline, and ethanol precipitated (Hirschhorn et al., 1984b). The RNAs were divided by aliquots (20 μg/tube), vacuum dried, and stored at −70 °C.

RNA Blot Analysis. The RNA was dissolved in 20 μL of 50% deionized formamide/6% formaldehyde in 1× MOPS buffer [20 mM 4-morpholinepropanesulfonic acid, pH 7.0/5 mM sodium acetate/1 mM ethylenediaminetetraacetic acid (EDTA)] and heated at 65 °C for 15 min. After rapid cooling, RNA samples (20 μg/lane) were electrophoresed on formalin gels (1.1% agarose/6% formaldehyde in 1× MOPS buffer) in 1× MOPS buffer for 12–16 h at room temperature. Ribosomal RNA from AF8 cells and *Escherichia coli* were used as size markers.

Transfer of RNA to nitrocellulose filters (Schleicher & Schuell) with 20× SSC has been described (Thomas, 1980). To monitor the amount of RNA applied on each lane or blotted to nitrocellulose filter, RNA samples were sometimes ran in the presence of ethidium bromide and photographed. After the transfer, no RNA was detected in gel. Equal amounts of stained RNA were observed on nitrocellulose filters. The efficiency of hybridization was not significantly changed by ethidium bromide staining. The filters baked at 80 °C for 2 h under vacuum were hybridized with whole plasmids carrying the desired gene probes nick translated by standard procedures (Rigby et al., 1977) [sp act. (2–4) × 10^8 dpm/μg of DNA]. Oligodeoxyribonucleotide elongation procedure (Feinberg & Vogelstein, 1983) was applied to label such DNAs as fos, p53, and thymidine kinase [sp act. (2–3) × 10^9 dpm/μg of DNA]. Prehybridization (42 °C), hybridization (42 °C), and washing (52 °C) were as described (Thomas, 1980). Filters were exposed to Kodak X-ray film with an intensifying screen at −70 °C for 12–16 h. Densitomer readings of the film were done with the aid of a Zeineh soft-laser densitometer (Biomed Instruments, Fullerton, CA) in the linear range of the instrument. The accuracy of the measurements and their limits have been discussed in a previous paper (Gibson et al., 1986).

Plasmids. Plasmids carrying the selected gene probes were as follows: p2A9, p2F1, and p4F1 (carrying serum-inducible cDNA sequences from hamster ts13 cells), described by Hirschhorn et al. (1984b); pJE-3 and pKC-1 (PDGF-inducible cDNA sequences from mouse 3T3 cells, a kind gift of C. D. Stiles), described by Cochran et al. (1983); pC2SPb fos (carrying the v-fos gene in a Riboprobe vector), a gift of Dr. S. Rittling; p6p53c (carrying a mouse p53 cDNA sequence in a Riboprobe vector), a gift of Dr. L. Kaczmarek; pMc-myc54 (carrying the mouse c-myc cDNA sequence (a kind gift of Dr. Dalla-Favera), described by Stanton et al. (1983); pHF βA-1 (carrying human β-actin cDNA sequence, a kind gift of P. Gunning) described by Gunning et al., (1983); pODC934 (carrying a mouse ornithine decarboxylase cDNA sequence, a kind gift of Dr. Berger), described by Berger et al. (1984); pDHFR-11 (carrying a mouse dihydrofolate reductase sequence, a gift of R. T. Schimke), described by Chang et al. (1978) and Setzer et al. (1980); pFO422 (carrying a histone H3 genomic sequence, a kind gift of Drs. G. and J. Stein); p3.2 (carrying a Chinese hamster thymidine kinase cDNA sequence, a kind gift of J. Lewis) described by Lewis et al. (1983); pTK11 (carrying a human thymidine kinase cDNA sequence, a gift of Dr. Bradshaw), described by Bradshaw and Deininger (1984). 4F1 turns out to be a cDNA clone of human vimentin (S. Ferrari et al., unpublished results) and will therefore be referred to as such.

Results

The genes whose mRNA levels were determined in these experiments are listed in Table I with an indication of their approximate peak of expression and the original references in which their cell cycle dependency was described. The probes used for hybridization in the RNA blots are given under Materials and Methods. It should be noted that c-fos, c-myc, KC-1, JE-3, 4F1 (vimentin), 2F1, 2A9, and p53 are all inducible by growth factors in the presence of concentrations of cycloheximide that completely suppress protein synthesis (Cochran et al., 1983; Kelly et al, 1983; Rittling et al., 1985). This means that their induction does not require new protein synthesis, i.e., the products of other genes that are induced by growth factors.

Culture Conditions. tsJT60 and 3Y1 cells were arrested in G_0 as described under Materials and Methods. The time course of the entry of serum-stimulated cells into DNA synthesis is given in Figure 1. 3Y1 cells entered S phase a little faster at 39.5 than at 34 °C. The entry of tsJT60 cells into S phase was slower at 34 °C than that of 3Y1 and negligible at 39.5 °C. Table II confirms these data in cultures of cells parallel to those for RNA analysis and where the cells were exposed continuously to [^{3}H]thymidine between 12 and 36 h after serum stimulation.

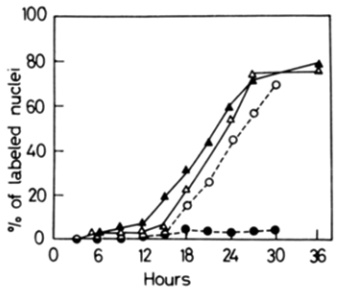

FIGURE 1: Induction of cellular DNA synthesis in 3Y1 and tsJT60 cells. Quiescent 3Y1 (△, ▲) or tsJT60 (○, ●) cells were stimulated with serum at zero time and cultured at 34 °C (open symbols) or at 39.5 °C (closed symbols). The cells were continuously labeled with [^{3}H]thymidine and processed for autoradiography.

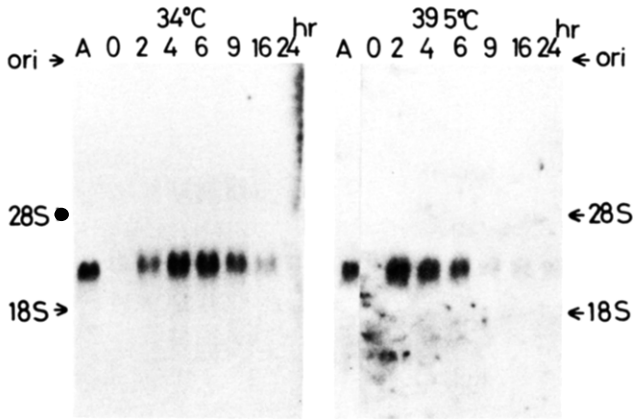

FIGURE 2: Expression of c-myc mRNA in serum-stimualted tsJT60 cells at either permissive or restrictive temperature. Total cytoplasmic RNA was extracted from tsJT60 cells stimulated with serum for the periods indicated and analyzed by Northern blotting. (Lane A) RNA from exponentially growing AF8 cells.

Table II: Frequency of Cells Synthesizing DNA between 12 and 36 h after Serum Stimulation[a]

cell line	temp (°C)	% of nuclei labeled with [^{3}H]thymidine	
		unstimulated	stimulated
tsJT60	34	2.6	60.1
	39.5	8.2	1.3
3Y1	34	11.4	65.2
	39.5	1.8	65.3

[a] Cells were made quiescent as described under Methods and Materials and subsequently stimulated with serum. All cells were labeled with [^{3}H]thymidine for a period of 24 h. These cultures were run parallel to the cultures used for RNA analysis.

RNA Levels of Growth-Regulated Genes in tsJT60 Cells at either Permissive or Nonpermissive Temperature. Total cytoplasmic RNA was collected from G_0 cells and from cells at various times after serum stimulation at either 34 or 39.5 °C and analyzed by Northern blots. Staining of RNA in gels with ethidium bromide and transfer of stained RNA revealed that the amount of RNA blotted to nitrocellulose filters was almost equal in each lane.

Figures 2 and 3 show several examples of representative blots. Figure 2 shows an RNA blot hybridized to the c-myc sequences. The amount of c-myc RNA was low or undetectable at zero time. At 34 °C, the c-myc RNA band was visible at 2 h, showed a maximal expression at 4–6 h after stimulation, and then decreased. At 39.5 °C, the appearance and disappearance of c-myc RNA were more rapid.

Figure 3 (I and II) shows the RNA levels corresponding to the c-fos sequence. As already reported in the literature, c-fos expression increased very quickly after serum stimulation

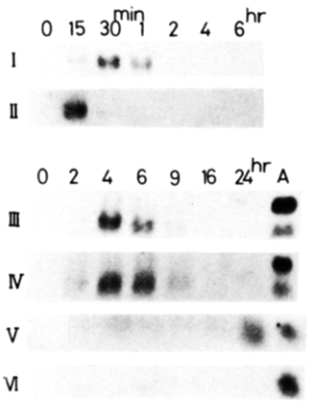

FIGURE 3: Expression of growth-regulated genes in serum-stimulated tsJT60 cells. RNA blots of RNAs extracted from cells as follows: (I) c-fos at 34 °C; (II) c-fos at 39.5 °C; (III) ODC at 34 °C, (IV) ODC at 39.5 °C, (V) histone H3 at 34 °C; (VI) histone H3 at 39.5. (A) RNA from growing AF8 cells.

(15 min is the earliest time in our experiments), and by 2 h the RNA was no longer detectable. At 39.5 °C (II), the disappearance of c-fos RNA was even more rapid, no RNA being detectable by 60 min (in the original autoradiogram, a very faint band could still be seen at 30 min). III and IV of Figure 3 are RNA blots in which the probe used was ornithine decarboxylase (ODC). At 34 °C (III), the RNA band hybridizing to the ODC probe was visible at 2 h and showed a peak at 4 h after serum stimulation, and similar results were obtained at 39.5 °C. ODC mRNA probably gives a double band, and while the upper band was very faint in rat cells, it was clearly visible in AF8 cells in agreement with other reports (Kahana & Nathans, 1984; Berger et al., 1984). The behavior of the histone H3 message is shown in Figure 3 (V and VI). At 34 °C (V), H3 RNA levels increased sharply between 16 and 24 h, while at 39.5 °C H3 RNA levels (VI) did not increase at all, confirming previous reports that, in G_0–G_1 ts mutants of the cell cycle, histones are not expressed when quiescent cells are serum-stimulated at the restrictive temperature (Hirschhorn et al., 1984a; Artishevsky et al., 1984).

We used RNA from exponentially growing tsAF8 cells as the positive control. The sizes of mRNAs from rat cell line, tsJT60, were comparable to those from the hamster cell line, AF8, although minor differences were observed in two mRNA species. The difference in ODC mRNA was described above. The sizes of 2A9 mRNAs in both species were almost identical, but a broader band was obtained in samples from rat cell lines. This was not due to degradation of mRNA during isolation from rat cells because (1) it was observed repeatedly in all samples at different times and at different temperatures and (2) other mRNA species on the same filter appeared as sharp bands. We could not detect, in rat cells and under the conditions used in the present experiments, RNA bands corresponding to DHFR and thymidine kinase.

Time Course of Gene Expression in tsJT60 Cells. Representative densitometry readings of specific RNA bands are summarized in Figures 4–6 although these experiments have been repeated at least twice. In some cases, for quantitative comparison, RNA from cells at 34 and 39.5 °C was applied on the same gel. All mRNA species except H3 could be induced following stimulation of tsJT60 cells at both 34 and 39.5 °C. The pattern of the time course of gene expression could be separated into four groups. (1) The first group contains fos, KC-1, JE-3, myc, and ODC (Figure 4). The amount of mRNA at zero time was very small or negligible. At 34 °C, RNA amounts increased quickly after serum stimulation and then decreased. At 39.5 °C, RNA amounts

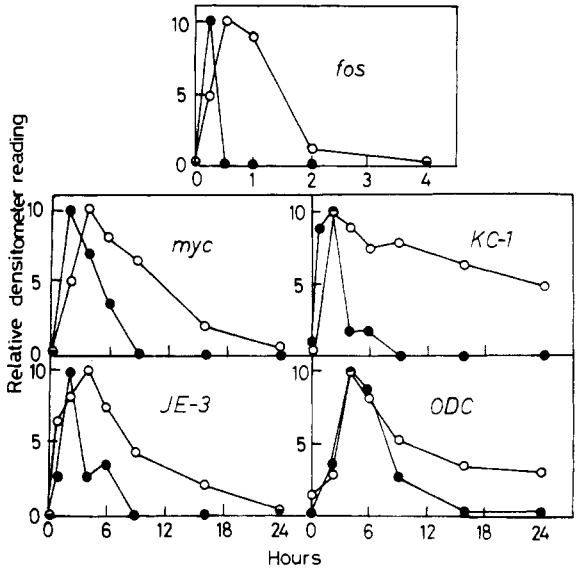

FIGURE 4: Densitometric readings of specific RNA bands from RNA blots of tsJT60 cells: (open symbols) 34 °C; (closed symbols) 39.5 °C. The abscissa is hours after stimulation. Other conditions are described under Materials and Methods.

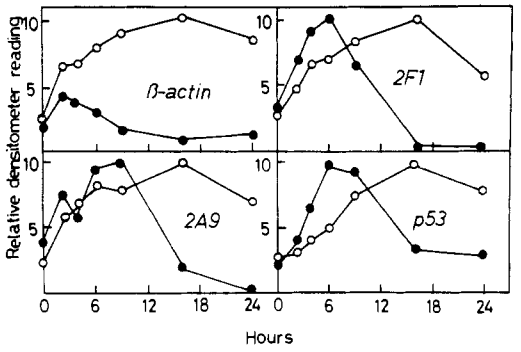

FIGURE 5: Same as in Figure 4 but for different RNAs.

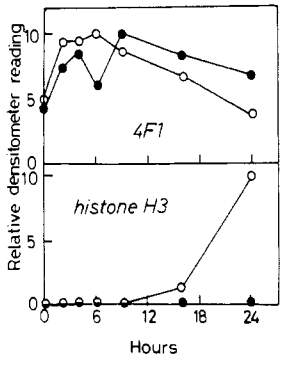

FIGURE 6: Same as in Figure 4 but different RNAs.

also increased, with a peak earlier than that at 34 °C except for ODC, and then decreased rapidly. (2) The second group contains 2A9, 2F1, p53 and β-actin (Figure 5). The respective mRNAs were detectable at zero time. At 34 °C, RNA amounts increased more slowly and then decreased toward 24 h after stimulation. At 39.5 °C, RNA amounts increased only transiently and then decreased. Whether the inability to maintain the amount of mRNA of these species at 39.5 °C is specific for tsJT60 is a point of interest. (3) The third group contains vimentin (Figure 6). The amount of vimentin mRNA (4F1) at 34 °C increased and then gradually decreased. After 9 h of stimulation, the amount of mRNA appeared to be higher at 39.5 °C than at 34 °C. Though the difference is small, this is the only one example of mRNA that was present

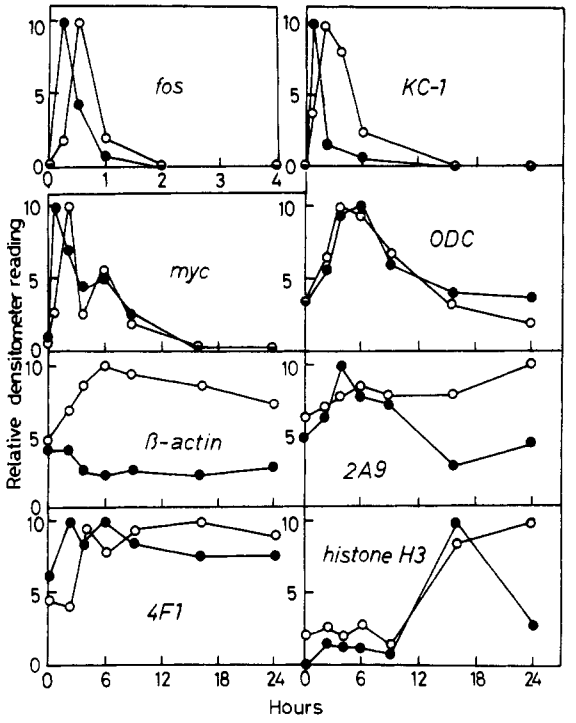

FIGURE 7: Densitometric readings of specific RNA bands from 3Y1 cells. Same conditions as in Figure 4.

in higher amounts at 39.5 °C than at 34 °C at late periods after serum stimulation. (4) The fourth group contains histone H3 (Figure 6). The amount of H3 mRNA increased in S phase at 34 °C. At 39.5 °C, cells did not enter S phase (Figure 1 and Table II) and did not have detectable amounts of H3 mRNA. This is the only example of mRNA that did not increase at 39.5 °C. We could not detect an RNA band hybridizable to the human (or Chinese hamster) cDNA of thymidine kinase or the mouse DHFR cDNA either in tsJT60 or in 3Y1 cells.

RNA Levels of Growth-Regulated Genes in 3Y1 Cells at 34 or 39.5 °C. Several examples of densitometry readings of specific RNA bands in 3Y1 cells (the parent cell line of tsJT60) are shown in Figure 7. The patterns of the time course of gene expression, except for H3, were almost identical between tsJT60 and 3Y1 cells, while minor differences were also observed. At 34 °C, mRNAs in group 1 appeared to decrease earlier in 3Y1 cells than in tsJT60. The time course of the amount of mRNA in group 2 (e.g., β-actin and 2A9) was markedly different between the two temperatures in parental 3Y1 cells and was somewhat similar to that observed in tsJT60 cells. The differences between the two temperatures in the RNA levels of vimentin (group 3) late after stimulation were too small to conclude that the pattern of the expression was different between tsJT60 and 3Y1 cells. The RNA levels of H3 in 3Y1 cells were already high at 16 h after stimulation at both temperatures and decreased at 24 h at 39.5 °C. The time course of the increase in H3 mRNA at both temperatures and the decrease at 39.5 °C was quicker in 3Y1 than that observed in tsJT60 cells at 34 °C and corresponds to the difference in time of entrance into S phase between the two cell lines (Figure 1).

Discussion

A number of growth-regulated genes have been identified in recent years, either as cellular equivalents of retroviral transforming genes or cDNA clones from differentially screened libraries or as known genes like thymidine kinase and

core histones (see the introduction). We have chosen a panel of growth-regulated genes (see Table I) that includes three oncogens (c-fos, c-myc, and p53), five genes inducible in G_0 cells by platelet-derived growth factor or serum (Cochran et al., 1983; Hirschhorn et al., 1984b), and three known genes, also inducible by growth stimuli: β-actin (Campisi et al., 1984), ornithine decarboxylase (Liu et al., 1985), and histone H3 (Plumb et al., 1983). Some of these genes were previously studied in G_1 ts mutants of the cell cycle: 2A9, 2F1 and 4F1 (Vimentin) were expressed equally well at permissive and restrictive temperatures (Hirschhorn et al., 1984b) while histone H3 and other core histones were induced only at the permissive temperature (Hirschhorn et al., 1984a).

tsJT60 cells are of interest in this respect because they do not express the ts phenotype while cycling during exponential growth but become ts if serum stimulated from quiescence. In this respect, they differ markedly from ts13 and tsAF8 cells that display the ts phenotype both while growing exponentially and when serum stimulated from quiescence (Burstin et al., 1974; Talavera & Basilico, 1977; Ashihara et al., 1978; Floros et al., 1978). Thus, from a cell kinetics point of view, the function that is temperature-sensitive in ts13 and tsAF8 cells is necessary both in G_1 and in the G_0 to S transition, while the function that is affected in tsJT60 cells is necessary only for the G_0 to S transition. Kinetically, tsJT60 cells are a G_0 mutant (Baserga, 1978; Ide et al., 1984; Baserga 1985). We hypothesized that if tsJT60 cells are ts for the G_0–S transition, the expression of some of these growth-regulated genes that are induced when G_0 cells are stimulated to proliferate could be affected at the restrictive temperature.

The hypothesis seemed even more reasonable considering that the expression of some of these genes increases very early in G_1 after serum stimulation and that at least eight of them are inducible in the presence of cycloheximide, which means that their increased expression does not require the products of other serum-inducible genes. Contrary to our expectations, all the genes tested, except histone H3, are expressed when G_0 tsJT60 cells are stimulated at either 34 or 39.5 °C. And more striking, the patterns of the gene expression in tsJT60 cells at the two temperatures were very similar to those observed in 3Y1 cells. There are, it is true, some minor differences. In general, the RNAs increase earlier and decrease earlier at 39.5 than at 34 °C, but this is probably a simple temperature effect, since it is noticeable also in the parent cell line, 3Y1 cells. The salient finding is that, although tsJT60 cells are biologically a G_0 ts mutant (Ide et al., 1984), they respond to serum similarly at either temperature in terms of the expression of several growth-regulated genes.

We think that this discrepancy between biological behavior of cells and the expression of growth-regulated genes, especially genes inducible early after serum stimulation, is of interest. Recently, one of our laboratories (Rittling et al., 1986) has shown that growth-regulated genes (roughly the same ones used in the present paper) are expressed in senescent human diploid fibroblasts just as well as in serum-stimulated young cells. Yet, senescent human diploid fibroblasts do not enter S phase after serum stimulation. In the case of human diploid fibroblasts, even S-phase genes like those for thymidine kinase and histone H_3 are expressed in senescent cells. In the case of tsJT60 cells, histone H3 is not expressed at the restrictive temperature, confirming that the cells are a ts mutant of the cell cycle, which block before S (thymidine kinase RNA could not be detected at either temperature). But tsJT60 cells are biologically a G_0 ts mutant, and yet in terms of the expression of other genes, they do not differ from G_1 ts mutants, like ts13 and tsAF8 cells (Hirschhorn et al., 1984a,b).

The results we have obtained with tsJT60 cells raise some basic questions about our present concepts of the cell cycle. The first question deals with the temporal relation between G_0 and G_1. Obviously, the two states can be distinguished [for a review, see Baserga (1985)] as confirmed also in this paper: tsJT60 are ts from G_0 but not from mitosis (Ide et al., 1984), and some RNA that are present in G_1 cells are absent from G_0 cells. Yet, the temporal relationship is more complex than a simple model in which G_0 cells enter G_1 after growth stimulation. Some G_1 features are present in tsJT60 cells stimulated at the restrictive temperature, and yet something is missing that is necessary for entry into S, something that is present in cycling cells. A possible explanation is that a gene necessary for cell cycle progression is turned off in serum-deprived cells but not in cycling cells. The ts function would not be this hypothetical gene, but another gene that controls its reactivation. In this respect, it should be noted that protein synthesis is markedly reduced in tsJT60 cells stimulated at the restrictive temperature (Ninomiya-Tsuji et al., 1986).

The second, broader question involves our concept of quiescence. It is customary to measure quiescence of cells by counting the number of cells that are labeled by [^{3}H]thymidine over a period of time. Clearly, the [^{3}H]thymidine criterion unites in one single category cells that are widely divergent in terms of expression of growth-regulated genes, as evidenced both by the findings in the present paper and by those of Rittling et al. (1986) with senescent human diploid fibroblasts. This seems also to be true with B lymphocytes, which can move out of G_0 without entering S phase (Melchers & Lernhardt, 1985), and of T lymphocytes stimulated by phytohemagglutinin in the absence of interleukin 2 (Kaczmarek et al., 1984a,b). It suggests that a cell kinetic definition of quiescence is no longer tenable. Specifically, it suggests that [^{3}H]thymidine labeling is not sufficient to define the extent of quiescence in a population of cells. It is possible for cells to leave G_0, assume some of the biochemical characteristics of G_1, and make no further progress toward S phase. This is also supported by the long-known finding that short pulses of serum are sufficient to induce cellular DNA synthesis in a small fraction of cells [for a review, see Baserga (1985)]. This observation can be interpreted as indicating that some quiescent cells may have already taken the first steps toward S phase. We conclude that other criteria, besides labeling with [^{3}H]thymidine, are necessary for the correct definition of a G_0 state, perhaps to include the mRNA and protein levels of certain growth-regulated genes.

Registry No. Ornithine decarboxylase, 9024-60-6.

REFERENCES

Artischevsky, A., Delegeane, A. M., & Lee, A. S. (1984) *Mol. Cell. Biol. 4*, 2364–2369.

Ashihara, T., Chang, S. D., & Baserga, R. (1978) *J. Cell. Physiol. 96*, 15–22.

Baserga, R. (1978) *J. Cell Physiol. 95*, 377–386.

Baserga, R. (1985) *The Biology of Cell Reproduction*, Harvard University Press, Cambridge, MA.

Berger, F. C., Szymanski, P., Read, E., & Watson, G. (1984) *J. Biol. Chem. 259*, 7941–7946.

Bradshaw, H. D., Jr., & Deininger, P. L. (1984) *Mol. Cell. Biol. 4*, 2316–2320.

Burstin, S. J., Meiss, H. K., & Basilico, C. (1974) *J. Cell. Physiol. 84*, 397–408.

Calabretta, B., Venturelli, D., Kaczmarek, L., Narni, F., Talpaz, M., Anderson, B., Beran, M., & Baserga, R. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1495–1498.

Campisi, J., Grey, H. E., Pardee, A. B., Dean, M., & Sonenshein, G. E. (1984) *Cell (Cambridge, Mass.) 36*, 241–247.

Chang, A. C. Y., Nubberg, J. H., Kaufman, R. J., Erlich, H. A., Schimke, R. T., & Cohen, S. N. (1978) *Nature (London) 275*, 617–624.

Cochran, B. H., Reffel, A. C., & Stiles, C. D. (1983) *Cell (Cambridge, Mass.) 33*, 939–947.

Cochran, B. H., Zullo, J., Verma, I. M., & Stiles, C. D. (1984) *Science (Washington, D.C.) 226*, 1080–1082.

Edwards, D. R., & Denhardt, T. (1985) *Exp. Cell Res. 157*, 127–143.

Edwards, D. R., & Parfett, C. L. J., & Denhardt, D. T. (1985) *Mol. Cell. Biol. 5*, 3280–3288.

Feinberg, A. P., & Vogelstein, B. (1983) *Anal. Biochem. 132*, 6–13.

Floros, J., Ashihara, T., & Baserga, R. (1978) *Cell Biol. Int. Rep. 2*, 259–269.

Gibson, C. W., Rittling, S. R., Hirschhorn, R. R., Kaczmarek, L., Calabretta, B., Stiles, C. D., & Baserga, R. (1986) *Mol. Cell. Biochem. 71*, 61–69.

Goyette, M., Petropoulos, C. J., Shank, P. R., & Fausto, N. (1983) *Science (Washington, D.C.) 219*, 510–512.

Goyette, M., Petropoulos, C. J., Shank, P. R., & Fausto, N. (1984) *Mol. Cell. Biol. 4*, 1493–1498.

Greenberg, M. E., & Ziff, E. B. (1984) *Nature (London) 311*, 433–438.

Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H., & Kedes, L. (1983) *Mol. Cell. Biol. 3*, 787–795.

Hann, S. R., Thompson, C. B., & Eisenman, R. N. (1985) *Nature (London) 314*, 366–369.

Hirschhorn, R. R., Marashi, F., Baserga, R., Stein, J., & Stein, G. (1984a) *Biochemistry 23*, 3731–3735.

Hirschhorn, R. R., Aller, P., Yuan, Z.-A., Gibson, C. W., & Baserga, R. (1984b) *Proc. Natl. Acad. Sci. U.S.A. 81*, 6004–6008.

Ide, T., Ninomiya, J., & Ishibashi, S. (1984) *Exp. Cell Res. 150*, 60–67.

Kaczmarek, L., Calabretta, B., & Baserga, R. (1985a) *Proc. Natl. Acad. Sci. U.S.A. 82*, 5375–5379.

Kaczmarek, L., Calabretta, B., & Baserga, R. (1985b) *Biochem. Biophys. Res. Commun. 133*, 410–416.

Kahana, C., & Nathans, D. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 3645–3649.

Kelly, K., Cochran, B. H., Stiles, C. D., & Leder, P. (1983) *Cell (Cambridge, Mass.) 35*, 603–610.

Kimura, G., Itagaki, A., & Summers, J. (1975) *Int. J. Cancer 15*, 694–706.

Kruijer, W., Copper, J. A., Hunter, T., & Verma, I. M. (1984) *Nature (London) 312*, 711–716.

Lau, L. F., & Nathans, D. (1985) *EMBO J. 4*, 3145–3151.

Lewis, J. A., Shimizu, K., & Zipser, D. (1983) *Mol. Cell. Biol. 3*, 1815–1823.

Linzer, D. I. H., & Nathans, D. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4271–4275.

Linzer, D. I. H., & Nathans, D. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4255–4259.

Liu, H.-T., Baserga, R., & Mercer, W. E. (1985) *Mol. Cell. Biol. 5*, 2936–2942.

Matrisian, L. M., Gleichenhaus, N., Gesnel, M. C., & Breathnach, R. (1985a) *EMBO J. 4*, 1435–1440.

Matrisian, L. M., Rautmann, G., Magum, B. D., & Breathnach, R. (1985b) *Nucleic Acids Res. 13*, 711–726.

Melchers, F., & Lernhardt, W. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 7681–7685.

Muller, R., Bravo, R., Burckhardt, J., & Curran, T. (1984) *Nature (London) 312*, 716–720.

Ninomiya-Tsuji, J., Goto, Y., Ishibashi, S., & Ide, T. (1986) *Exp. Cell Res. 165*, 191–198.

Perez, A. G., Kim, J. H., Gelbard, A. S., & Djorjevic, B. (1972) *Exp. Cell Res. 70*, 301–310.

Plumb, M., Stein, J., & Stein, G. (1983) *Nucleic Acids Res. 11*, 2391–2410.

Reich, N. C., & Levine, A. J. (1984) *Nature (London) 308*, 199–201.

Rigby, P. W. J., Dieckmann, M., Rhodes, C., & Berg, P. (1977) *J. Mol. Biol. 113*, 237–251.

Rittling, S., Gibson, C. W., Ferrari, S., & Baserga, R. (1985) *Biochem. Biophys. Res. Commun. 132*, 327–335.

Rittling, S., Brooks, K. M., Cristofalo, V. J., & Baserga, R. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3316–3320.

Schneider, E. L., Stanbridge, E. J., & Epstein, C. J. (1974) *Exp. Cell Res. 84*, 311–318.

Setzer, D. R., MGrogan, M., Nunberg, J. H., & Schimke, R. T. (1980) *Cell (Cambridge, Mass.) 22*, 361–370.

Stanton, L. W., Watt, R., & Marcu, K. B. (1983) *Nature (London) 303*, 401–406.

Talavera, A., & Basilico, C. (1977) *J. Cell. Physiol. 92*, 425–436.

Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.

Thompson, C. B., Challoner, P. B., Neiman, P. D., & Gourdine, M. (1985) *Nature (London) 314*, 366–369.

Torelli, G., Selleri, L., Donelli, A., Ferrari, S., Emilia, G., Venturelli, D., Moretti, L., & Torelli, U. (1985) *Mol. Cell. Biol. 5*, 2874–2877.