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Regulation of the Proliferating Cell Nuclear Antigen Cyclin and Thymidine Kinase mRNA Levels by Growth Factors*

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The enzymes of the DNA synthesizing machinery constitute a group of gene products that are generally expressed co-ordinately at the G_1/S boundary of the cell cycle. We have investigated how growth factors regulate the steady-state mRNA levels of two of these genes, the PCNA (proliferating cell nuclear antigen)/cyclin and the thymidine kinase genes. To detect the PCNA/cyclin mRNA, we isolated a cDNA clone from a human library. Two different cell lines were used for these studies: BALB/c3T3 cells, which are exquisitely sensitive to growth factors, and ts13 cells, a temperature-sensitive (ts) mutant of the cell cycle, which arrests in G_1 at the restrictive temperature. The steady-state levels of the RNAs for these two genes under different growth conditions were also compared with the levels of histone H3 RNA which are good indicators of the fraction of cells in S phase. Both PCNA/cyclin and thymidine kinase genes share two fundamental characteristics, *i.e.* they are not inducible in a G_1-specific ts mutant of the cell cycle at the restrictive temperature and their expression is inhibited by cycloheximide, indicating that unlike early growth-regulated genes, they require the previous expression of other growth-regulated genes. However, the two genes also show differences, the most notable being that PCNA/cyclin is inducible by epidermal growth factor alone, while thymidine kinase is not.

The proliferating cell nuclear antigen (PCNA)[1] was originally described as a nuclear protein whose appearance correlated with the proliferative state of the cell (1, 2). A similar nuclear protein was described by Bravo and co-workers (for a review see Ref. 3) and called "cyclin." PCNA and cyclin share similar properties and were eventually shown to be identical (4). Recently, PCNA/cyclin was shown to be required for SV40 DNA replication *in vitro* (5), and it was identified as the auxiliary protein of DNA polymerase δ (6, 7).

Thymidine kinase is a member of a group of enzymes involved in the synthesis of cellular DNA. The activity of thymidine kinase, as well as that of other enzymes of the DNA synthesizing machinery, increases sharply at the G_1-S boundary of the cell cycle and remains elevated throughout the S phase (8–10). Steady-state levels of thymidine kinase mRNA also increase sharply as cells enter S phase (11–13).

Since the products of both the PCNA/cyclin and the thymidine kinase genes are part of the complex group of proteins involved in cellular DNA synthesis, we investigated whether or not the genes share similar regulatory mechanisms. There is substantial evidence in the literature that the proteins of the DNA synthesizing machinery are co-ordinately expressed at the G_1/S boundary (for a review on animal cells, see Ref. 14, and for yeast, Ref. 15). Some information is also available on the mRNA levels for some of these genes (11–13, 16, 17). However, little is known on how mRNA levels are regulated by specific growth factors. The finding of common regulatory mechanisms among genes that are part of the DNA synthesizing machinery could be of considerable importance, since it would indicate that the control of the onset of S phase could be reduced to a common denominator. To test this hypothesis we have compared, as a first step, how the PCNA/cyclin and thymidine kinase genes respond to individual growth factors in BALB/c3T3 cells, which are strictly regulated by growth factors (18, 19). We have also investigated the expression of the cyclin/PCNA gene in ts13 cells, a G_1-specific temperature-sensitive (ts) mutant of the cell cycle (20). In this study we have limited our interest to the growth factor regulation of the steady-state levels of the cytoplasmic mRNAs for PCNA/cyclin and thymidine kinase. The results indicate that there are both differences and similarities in the way the PCNA/cyclin and thymidine kinase genes respond to growth factors. The manuscript also describes the isolation of a PCNA/cyclin cDNA clone from an Okayama-Berg library (21). The insert from this clone is 41 base pairs longer than the cDNA insert of PCNA/cyclin previously described (16).

MATERIALS AND METHODS

Cell Lines—BALB/c3T3 cells are grown routinely in our laboratory, as described previously (22). The cells are made quiescent by incubating them for 5 days in 1% fetal calf serum. Quiescent cells were subsequently stimulated in a variety of ways. With fetal calf serum and horse plasma, the medium of quiescent cells was completely replaced by fresh growth medium containing either 10% fetal calf serum or 5% horse plasma. With individual growth factors, or combinations of them, the desired growth factors were added to the medium of quiescent cells.

The other cell lines used were ts13 cells (20), which are G_1-specific ts mutants of the cell cycle originally derived from baby hamster kidney cells and therefore of Syrian hamster origin, and tK^-ts13 cells, which are tK^- mutants of ts13 cells (23). ts13 cells and their derivatives are grown at 34 °C; the nonpermissive temperature is 39.6 °C. They were made quiescent by serum deprivation.

Growth Factors—Unless otherwise stated, the following concentrations of growth factors were used. Platelet-derived growth factors, 1 ng/ml; epidermal growth factor (EGF), 200 ng/ml; insulin 10 μg/ml. Platelet-derived growth factor was a kind gift of Dr. Russell Ross, University of Washington, Seattle. EGF was purchased from Collaborative Research and insulin from Sigma. Horse plasma (Hyclone)

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[1] The abbreviations used are: PCNA, proliferating cell nuclear antigen; TK, thymidine kinase (Miniprint); FCS, fetal calf serum; HP, horse plasma; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; ODC, ornithine decarboxylase.

has been used interchangeably with platelet-poor plasma (22). Neither horse plasma nor platelet-poor plasma support the growth of BALB/c3T3 cells. However, the quality of horse plasma varies from batch to batch: in these experiments, we only used a batch that did not support the growth of 3T3 cells.

RNA Extraction and RNA Blots—Total cytoplasmic RNA was extracted from cells by two different methods: from BALB/c3T3 by the method of Rittling *et al.* (24) and from ts13 cells by the method of Chomczynski and Sacchi (25). RNA blots were carried out by standard procedures (26). In all experiments, 10 μg of cytoplasmic RNA were applied for each lane, and the conditions of hybridization and exposure were kept as constant as possible so that the intensity of the bands in the various figures could be compared, at least semiquantitatively. The amount of RNA was monitored as described previously (24). Radioactive probes were prepared by the random priming method (27). The following probes were used for hybridization: 1) a cDNA clone of cyclin (see below), isolated in our laboratory: 2) a partial length cDNA clone of cyclin, a kind gift from Rodrigo Bravo, EMBO Laboratory, Heidelberg, Federal Republic of Germany; 3) pTK11 which contains a full-length cDNA of human thymidine kinase. It was isolated from an Okayama-Berg library (21) by Bradshaw and Deininger (28); 4) the plasmids used to isolate probes for histone H3 (29), v-*myc* (30) and β-actin (31) have been described previously.

Isolation of a PCNA/Cyclin cDNA Clone—In order to isolate a full-length cDNA clone of human cyclin, we first isolated a human genomic clone of cyclin and then with the cyclin probe we screened a human cDNA library of SV40 transformed fibroblasts (21).

Screening of a Genomic Library—A human genomic library of placental DNA cloned in vector Charon 28 was a kind gift of Dr. P. Leder (Harvard University). Fifty-thousand recombinants were screened with two ^{32}P-end-labeled oligodeoxynucleotides 37 base pairs in length, each synthesized on the basis of the published sequence of a human cyclin cDNA clone (16). The two oligodeoxynucleotides were derived from the 5′ region of human cyclin cDNA beginning immediately after the putative first codon. The hybridization procedure was essentially as described by Maniatis *et al.* (32) except that the hybridization took place in relatively low stringency conditions (35% formamide at 37 °C) and posthybridization washings were done at 42 °C in 1 × SSC for 1 h and then 52 °C in 1 × SSC for an additional hour. The DNAs of two positive individual clones were purified after the tertiary screening and digested with various restriction enzymes. A *Pst*I fragment 1.2 kilobase pairs in length devoid of human repetitive sequences and positive with both cyclin oligodeoxynucleotides even after washing the filters at 60 °C for 1 h in 1 × SSC was hybridized in Northern blot experiments to total RNA from several human myeloid cell lines (HL-60, U-937, ML3, and HEL). An abundant mRNA species of a size corresponding to that described for human cyclin was detected.

Screening of a cDNA Library—The 1.2-kilobase pair *Pst*I fragment labeled at high specific activity (5–7 × 10^8 cpm/μg) by random oligonucleotide priming (27) was used to screen 500,000 recombinants of the Okayama-Berg library derived from SV40-transformed human fibroblasts (21). The library was screened at high density according to the procedure described by Hanahan and Meselson (33). 25 positive clones were identified in duplicate filters of the primary screening. 8 cDNA clones were isolated after secondary and tertiary screening and two of them with the longest *Bam*HI inserts (~1.7–1.8 kilobase pairs) were grown in large scale for further analysis.

Sequencing Strategy—To demonstrate that our longest cyclin cDNA was longer than the one described by Almendral *et al.* (16) we took the following steps: 1) digestion of our cDNA cloned in the Okayama-Berg vector with the restriction enzyme *Stu*I; 2) isolation of a ~350-base pair *Stu*I fragment encompassing the 5′ region of the cyclin clone and the 40 nucleotides of SV40 that precedes in the Okayama-Berg vector the *Pst*I cloning site; 3) cloning of the blunt-end *Stu*I fragment at the *Sma*I restriction site of the plasmid pGEM 1; 4) sequencing of the *Stu*I fragment according to the sequence protocol of Promega-Biotec.

RESULTS[2]

DISCUSSION

The three components of the cell cycle that are present in all animal cells are doubling in size of the cell, DNA replication, and mitosis (14). There are cells that do not have a G_1 (40) or a G_2 (14), but in all cycling animal cells, in culture or in the intact animal, there is a discrete period of the cell cycle in which DNA is replicated. The beginning of this period, the S phase, is rather sudden, and DNA synthesis itself is accompanied by a cohort of other events (14) among which is the simultaneous appearance of enzymes that are part of the DNA synthesizing machinery. There is also evidence that the levels of the mRNAs coding for some of these enzymes increase abruptly at the G_1/S boundary (11–13, 16, 17, 41), although exceptions have been reported (11, 42, 43). It is certainly desirable to determine how the genes coding for the proteins of the DNA synthesizing machinery are regulated and whether there is uniformity or variety in the mechanisms of regulation. We have selected for this purpose two genes, the PCNA/cyclin and the thymidine kinase genes, both of which can be considered as part of the DNA synthesis apparatus of the cell, the former as a cofactor for DNA polymerase δ (6, 7), and the latter as an enzyme in the salvage pathway of thymidine. To carry out these experiments, we first isolated a PCNA/cyclin probe from an Okayama-Berg cDNA library (21). Two cDNA clones contained inserts longer than the cDNA clone reported by Almendral *et al.* (16). Sequencing revealed that our cDNA clones were 41 base pairs longer (Fig. 1). It is impossible, at present, to say whether this is a full-length cDNA clone, a problem that will be solved only with a structural analysis of the gene. For the moment, we can say (combining our results with those of Almendral *et al.* (16)) that PCNA/cyclin mRNA has a long 5′-untranslated region, at least 159 base pairs.

As a first step to study the regulation of S phase genes, we have investigated the mRNA levels of PCNA/cyclin and thymidine kinase under different growth conditions. The levels of PCNA/cyclin and thymidine kinase mRNAs (and other mRNAs) were determined on the same filters so that they are strictly comparable to each other. In addition, the extent of cellular proliferation in the cultures used was monitored directly on the same filters with the histone H3 probe. The levels of histone H3 RNA parallel exactly the percentage of cells that can be labeled by [^{3}H]thymidine (data not shown). The behavior of c-*myc*, ornithine decarboxylase, and β-actin constantly in agreement with the literature (24, 34, 35, 41, 44–48) indicates that the growth conditions used in the present experiment were not anomalous.

The results presented in this paper indicate that there are similarities but also differences in the regulation of the PCNA/cyclin and thymidine kinase mRNA levels by growth factors.

There are kinetic differences in the induction of the respective mRNAs by serum, PCNA/cyclin mRNA being detectable earlier than thymidine kinase mRNA. This difference may not be biologically significant. PCNA/cyclin mRNA is obviously more abundant than thymidine kinase mRNA, and that may account for its earlier appearance on RNA blots. In addition, there is substantial evidence that the time of increase in RNA or even protein levels does not correlate well with the time at which a particular gene product is required in the cell cycle (reviewed in Ref. 14).

There are also some slight differences between PCNA/cyclin and thymidine kinase in their response to platelet-derived growth factor (either alone or in combination with other growth factors), but the most striking difference is that PCNA/cyclin responds to EGF, while thymidine kinase, under the same conditions, does not. At variance with the data of Bravo and co-workers (3, 37, 49), in BALB/c3T3 cells PCNA/cyclin mRNA can be induced (by EGF) even in the

[2] Portions of this paper (including "Results" and Figs. 1–7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

absence of a stimulation of cellular DNA synthesis (no histone H3 RNA could be detected in the experiments of Fig. 5).

We have previously shown that thymidine kinase mRNA is not detectable in two G_1-specific ts mutants (ts13 and tsAF8 cells) serum-stimulated at the nonpermissive temperature (11), although it is clearly detectable at 34 °C. We show here that PCNA/cyclin behaves in the same way. This is an important consideration, since most of the growth-regulated genes that are inducible early after growth factor stimulation (often called the "competence" genes) are still inducible in G_1-specific ts mutants serum-stimulated at the restrictive temperature (48, 50). These early genes are also inducible (in terms of RNA levels) in the presence of concentrations of cycloheximide that completely inhibit protein synthesis (47). Indeed, some of these genes are superinduced in the presence of cycloheximide (34). This indicates that the expression of these genes does not require the previous synthesis of other growth factor-inducible proteins. Thymidine kinase mRNA, on the contrary, is exquisitely sensitive to even low concentrations of cycloheximide (13). So is the mRNA for PCNA/cyclin, as indicated in Fig. 6. A reasonable explanation for such behavior is that both the thymidine kinase and the PCNA/cyclin genes requires *at least* two inducers, one of which is not available if the cell is blocked in G_1. Since all growth factors, under these conditions, are present in the environment (10% fetal calf serum), the second regulation should depend not directly on a growth factor, but on some intracellular modulator. If we take the cycloheximide experiments as a model, we could venture to say that to be induced PCNA/cyclin and thymidine kinase require the previous expression of other growth factor-inducible genes (or gene).

In conclusion, our experiments have established in BALB/c3T3 cells the effect of growth factors on the mRNA levels of two genes that are part of the DNA synthesizing machinery: the PCNA/cyclin and the thymidine kinase genes. There are some differences in their response to growth factors, but experiments with ts mutants of the cell cycle and with cycloheximide indicate that both of them have a complex regulation that is dependent on the action of products from other growth factor-regulated genes.

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SUPPLEMENTAL MATERIAL TO REGULATION OF THE PCNA CYCLIN AND THYMIDINE KINASE mRNA LEVELS BY GROWTH FACTORS

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RESULTS

The characterization of the cDNA clones isolated in our laboratory showed that they are authentic PCNA/cyclin cDNA clones and that they are slightly longer than the one described by Almendral et al. (16). A restriction enzyme analysis of the two cyclin cDNA clones was undertaken. Our restriction analysis confirmed the presence of the restriction sites indicated by Almendral et al. (16), in the their published cyclin cDNA clone. We found from 3' to 5' the restriction sites Hpa I, Kpn I, EcoRI, EcoRV and Stu I. However, our cDNA clones are longer in the 5' region than the one published by R. Bravo group (16). This was confirmed by sequencing. Fig. 1 shows the sequence extending from the Stu I site of the vector into the 5' region of the PCNA/cyclin sequence of Almendral et al. (16). There are 41 new bases, after which our sequence is exactly the same as that of Almendral et al. (16). Finally, the identity of our clones as (presumably) full-length cDNAs of human cyclin was established by direct hybridization of our cDNA clones to a DNA insert derived from a partial-length cyclin cDNA clone kindly provided by Dr. R. Bravo (Heidelberg).

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Stu I site
I
-------------------------------------------------
CCT GTA CGG AAG TGT TAC TTC TGC TCT AAA AGC TGC TGC

-----------------------------------------
AGG GGG GGG ATT AAA CGG TTG CAG GCG TAG AGA GTG GTC

--------------------------------------------
GTT GTC TTT CTA GGT CTC AGC CGG TCG TCG CGA CGT TCG

-----------------------------
CCC GCT CGC TCT GAG GCT CCT


-------- pcD vector sequence

-------- NEW 41 BASES

======== Bravo's PCNA/Cyclin cDNA sequence
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Fig. 1 Partial sequence of the 5' region of the PCNA/cyclin cDNA. The sequence (see Methods and Material) is based on cDNA clones isolated in our laboratory from an Okayama-Berg cDNA library (21).

Expression of PCNA and TK mRNAs after Stimulation with Serum or Horse Plasma.

In these experiments, BALB/c3T3 cells were made quiescent as described in Methods and Materials. At 0 time, they were stimulated with 10% FCS or 5% HP. RNA was extracted at different times after stimulation, RNA blots were prepared, and the same filter was successively hybridized to different radioactive probes. Figure 2 is a composite picture of the results obtained with probes for PCNA-cyclin, and TK. PCNA/cyclin mRNA is detectable in G_0 cells, its levels are already increased by 4 hrs, reaching a peak between 16 and 24 hrs. Interestingly, faint bands are visible at 4 and 8 hrs after HP, but they are no longer visible at later times. TK mRNA becomes detectable only at 16 hrs after stimulation with FCS which, under these conditions, is the time when 3T3 cells begin to enter S phase, confirming results from our own and other laboratories (11,12,13). Faint bands are visible at 16 and 24 hrs after HP. We interpret these bands as indicating the small fraction of BALB/c3T3 cells that are induced to enter S phase by HP. These results indicate that the kinetics of expression (mRNA levels) are different (under the same exact conditions) for PCNA-cyclin and TK. Since the conditions of hybridization and exposure were kept as constant as possible, the intensities of the bands can be compared in a semi-quantitative way. Fig. 2 indicates that in serum-stimulated cells in S phase, the mRNA for PCNA/cyclin is more abundant ($\sim$4 folds) than the TK mRNA.

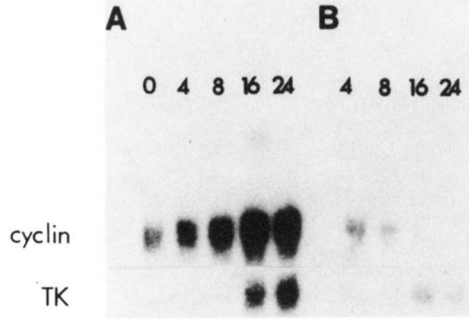

Fig. 2 Expression of the PCNA cyclin and thymidine kinase genes in BALB/c3T3 cells. RNA was prepared from BALB/c3T3 cells that were quiescent (0 time), or stimulated for the indicated times (in hours) either with 10% fetal calf serum (panel A), or with horse plasma (panel B). The blots were hybridized first with a probe for TK and subsequently the same filter was hybridized to a cyclin probe.

The c-myc probe (Fig. 3) served as a control, confirming what is already well known, namely that c-myc RNA, undetectable in G_0 cells, increases quickly after stimulation with serum (34,35) while HP (the equivalent of platelet-poor plasma) has no effect. Since it is important, in this context, to establish clearly the response of BALB/c3T3 cells to serum or plasma, we have included three other probes (always used on the same filter). Figure 3, shows that ODC mRNA levels are increased by 10% FCS as early as 4 hrs after stimulation but that a modest increase also occurs with HP. β-actin mRNA levels are increased by both serum and plasma but, with the latter, the increase is not sustained. Finally, histone H3 levels monitor the extent of entry into S phase of these populations of cells under these conditions. Histone H3 mRNA is clearly detectable at 16 and 24 hrs after serum-stimulation; a weaker band is also visible at 24 hrs after HP, confirming that a small fraction of cells was induced to enter S phase by HP. There is, in fact, an excellent correlation between expression of TK mRNA and histone H3 mRNA.

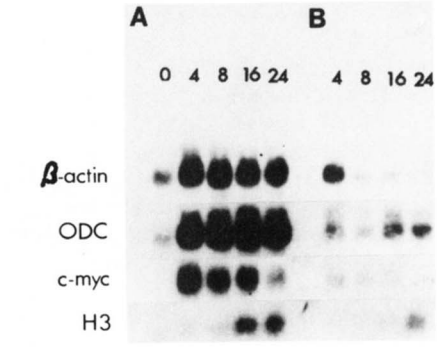

Fig. 3 Expression of the genes for β-actin, ornithine decarboxylase, c-myc and histone H3 in BALB/c3T3 cells. RNA was prepared from quiescent BALB/c3T3 cells and from cells stimulated with calf serum, or horse plasma, as described in Fig. 1. This is the same filter used in Fig. 1 that was subsequently hybridized to probes for β-actin, ornithine decarboxylase, c-myc, or histone H3.

Expression of PCNA and TK mRNA after exposure of quiescent cells to PDGF.

Again, BALB/c3T3 cells were made quiescent as described in Materials and Methods. At 0 time, PDGF alone or a combination of PDGF with other growth factors were added and RNA blots were prepared from cells at different times afterwards.

Fig. 4 shows that PDGF alone is effective in increasing PCNA/cyclin mRNA levels as is any combination of PDGF with other growth factors (insulin, or EGF, or insulin plus EGF). TK mRNA levels are only modestly increased by PDGF, while the combination of PDGF, EGF and insulin results in maximal expression, comparable to that obtained with 10% FCS. The levels of histone H3 mRNA are elevated at 24 hrs in all conditions, the PDGF-EGF combination being a little weaker. TK and histone H3 expression in these experiments correlate when PDGF is combined with EGF or insulin, but not when PDGF is used alone. These results indicate that the response to PDGF differs between the TK gene and the PCNA/cyclin gene, in agreement with the results obtained with proteins (8,9,10,36,37). TK activity is not induced by PDGF only (19), while cyclin can be detected by immunofluorescence even after exposure of cells to PDGF only (37). c-myc mRNA levels, as already well known (34,35) are increased by PDGF only (data not shown) early after exposure. In fact, c-myc mRNA is detectable at 4 and 8 hrs, but not at later times, regardless of growth conditions. The same comments apply to ODC and β-actin mRNAs.

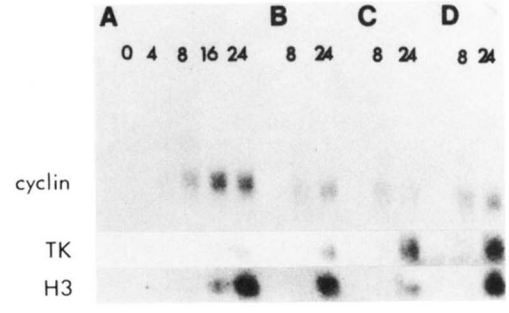

Fig. 4 Expression of the PCNA cyclin, thymidine kinase and histone H3 genes in BALB/c3T3 cells stimulated with platelet derived growth factor. The cells were made quiescent (0 time) and then they were stimulated for the indicated times (in hours) with either PDGF (panel A), PDGF plus insulin (panel B), PDGF plus EGF (panel C), and PDGF plus EGF plus insulin (panel D). The filter was subsequently hybridized to probes for thymidine kinase, cyclin or histone H3.

Expression of PCNA/cyclin and TK mRNAs after exposure to different growth factors.

Differences between TK and PCNA/cyclin expression are also apparent when quiescent BALB/c3T3 cells are stimulated with other individual growth factors (Figure 5). Cyclin mRNA levels are not increased by insulin alone, but they are increased by EGF or by a combination of insulin and EGF. Instead, TK mRNA levels are not detectable after exposure of BALB/c3T3 cells to these growth factors. Interestingly, the increase in PCNA/cyclin mRNA levels occurs early after EGF or EGF+insulin, but the increase is not sustained, as it happens instead with 10% FCS (Fig. 2). Stimulation of DNA synthesis in these experiments was again monitored with the histone H3 probe. Neither insulin, nor EGF nor a combination of both had any stimulatory effect on the entry of 3T3 cells into S phase (data not shown). ODC RNA was also induced by EGF (or a combination of EGF and insulin, Fig. 5), while c-myc was not induced at all (not shown).

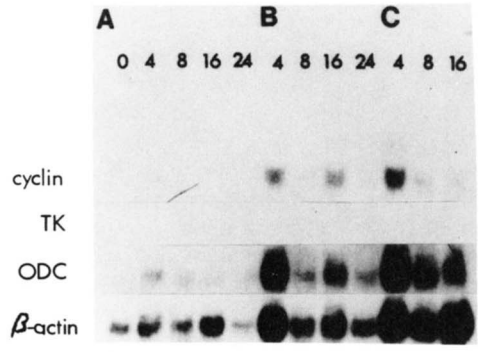

Fig. 5 Expression of the PCNA cyclin, thymidine kinase, ornithine decarboxylase, and β-actin genes in BALB/c3T3 cells. Quiescent cells (0 time) were stimulated for the indicated hrs with individual growth factors, namely: insulin (panel A), EGF (panel B), or EGF plus insulin (panel C). The isolated RNA's were blotted and successively hybridized with probes for cyclin, thymidine kinase, ornithine decarboxylase, or β-actin.

Effect of cycloheximide on the expression of the PCNA/cyclin gene.

It has been previously shown that cycloheximide, added to the cultures 8 hrs after serum stimulation, effectively inhibits the induction of TK mRNA by serum (13). Fig. 6 shows that, under the same conditions, cycloheximide (100 ng/ml, added 8 hrs after serum stimulation, effectively inhibits the increase in PCNA/cyclin mRNA levels that occurs 24 hrs after serum stimulation of BALB/c3T3 cells (lanes B and C). The levels of histone H3 mRNA also are reduced by exposure of the cells to cycloheximide (Fig. 5), and so are the levels of TK mRNA (data not shown, since they are identical to those of Coppock and Pardee, 13).

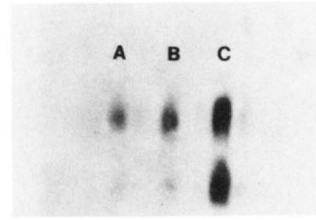

Fig. 6 Effect of Cycloheximide on the expression of the PCNA/cyclin gene. BALB/c3T3 cells were made quiescent (lane A) and were subsequently stimulated with 10% fetal calf serum for 24 hrs, either in the presence (lane B) or absence (lane C) of cycloheximide. The drug (100 ng/ml) was added 8 hrs after serum. The RNA blot was hybridized to probes for PCNA/cyclin (upper band) and histone H3 (lower band).

Expression of PCNA/cyclin mRNA in a G_1-specific ts mutant of the cell cycle.

G_1-specific ts mutants of the cell cycle are cell lines that grow normally at 34°, but arrest in G_1 at the restrictive temperature, usually 39.5°. These ts mutants become arrested in G_1, whether they come from mitosis or from G_0 (38). It is already well established that when G_1-ts mutants are stimulated by serum at the restrictive temperature, there is no increase in TK enzyme activity. This is true for more than one G_1-specific ts mutant, as for instance, tsAF8 cells (11), ts13 cells (11) and others (39). There is also no increase in TK mRNA levels after stimulation of quiescent ts13 or tsAF8 cells at the restrictive temperature, although TK mRNA is readily detectable in the same cells serum-stimulated at 34° (11).

PCNA/cyclin mRNA levels in quiescent and proliferating ts13 and tK^-ts13 cells are shown in Fig. 7. mRNA levels are lower in quiescent than in serum-stimulated tK^-ts13 cells, (lanes A and B) and barely detectable in cells incubated at the restrictive temperature (lane D). PCNA/cyclin mRNA levels increase in ts 13 cells serum-stimulated at 34°, (Fig. 7, lanes F,G,H) but not in cells stimulated at the restrictive temperature (Fig. 7 lanes I, L, M). If tK^-ts13 cells at 39.6° are shifted-down to 34° in 10% FCS, cyclin mRNA levels increase again (Fig. 7, lane E). In ts13, the expression of TK followed exactly the same behaviour as PCNA/cyclin (not shown here since the results are the same as those already published by Liu et al 11). In tk^-ts13 cells, TK mRNA is not detectable.

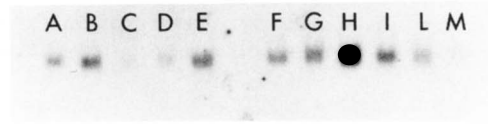

Fig. 7 Expression of the PCNA/cyclin gene in G_1 specific temperature sensitive mutants of the cell cycle. Two ts mutants were used, ts13 cells and tK^-ts13 cells. RNA blots were hybridized to a probe for PCNA/ cyclin. The lanes are as follows: tK^-ts13 cells serum deprived (A), and stimulated for 24 hr with serum at 34° (B), tk^-13 cells, serum deprived for 4 days (C), stimulated for 24 hrs at the nonpermissive temperature (D), serum deprived for 24 hrs at the restrictive temperature and subsequently stimulated for 24 hrs with serum at the permissive temperature (E). Lanes F to M are ts 13 cells. (F) Serum deprived at permissive temperature. (G) stimulated for 16 hrs with 10% serum at the permissive temperature. (H) Stimulated for 24 hrs with serum at the permissive temperature. (I) Serum deprived at the nonpermissive temperature for 24 hrs. (L) Stimulated for 16 hrs with serum at the nonpermissive temperature. (M) Stimulated for 24 hrs with serum at nonpermissive temperature.