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Cell-cycle-specific cDNAs from mammalian cells temperature sensitive for growth

(gene expression/temperature-sensitive mutant/RNA dot blots/Southern analysis)

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ABSTRACT **A library of double-stranded cDNA was constructed from ts13 cells, a G_1-specific temperature-sensitive hamster cell line. The cDNAs, cloned into pBR322, were prepared from poly(A)$^+$ mRNA isolated from ts13 cells 6 hr after serum stimulation at the permissive temperature of 34°C. Differential screening of the library with G_1-specific and G_0-specific single-stranded cDNA probes prepared from the same cells identified five cDNA clones whose sequences were preferentially expressed in G_1. Levels of RNA complementary to these clones were 3- to 6-fold higher in G_1 than in other phases of the cell cycle. When ts13 cells were arrested in G_1 at the restrictive temperature of 39.6°C, the levels of RNA complementary to p13-2A9 and p13-4F1 were as high as 10 times that found in a resting population, while the expression of sequences complementary to p13-2A8 did not significantly change from levels found in G_0. RNA and Southern gel blot analysis suggest that these cell-cycle-specific clones represent either low copy or moderately repetitive gene sequences. Results with another ts mutant of the cell cycle, tsAF8, which is a ts mutant of RNA polymerase II, showed that these cell-cycle-specific sequences have a rapid turnover. The use of G_1-specific ts mutants of the cell cycle provides an approach to determine which cell-cycle-dependent genes are most relevant to cell-cycle progression.**

The suggestion that unique copy gene transcription was necessary for the transition of mammalian cells from a quiescent state (G_0) to a growing state (G_1 to S) was put forth over two decades ago (1), but formal evidence has remained elusive. Recently, it has been demonstrated that a G_1-specific temperature-sensitive mutant, tsAF8, which arrests in G_1 at the restrictive temperature (2), is a mutant of RNA polymerase II (3–6). In addition, the progression of cells in culture through G_1 can be inhibited by the intracellular microinjection of α-amanitin (5), a drug that at low concentrations is known to have one and only one specific site of action, the large subunit of RNA polymerase II (7). These studies conclusively showed that a functional RNA polymerase II is an absolute requirement for the transition of cells from a resting to a growing state. This requirement justifies the search for genes transcribed by RNA polymerase II that control the transition from G_0 to G_1 to S. As a first step in this direction, we have begun a search for genes whose expression is specifically increased in G_1. A cDNA library was constructed from poly(A)$^+$ mRNA isolated from serum-stimulated ts13 cells that are a G_1-specific temperature-sensitive mutant of the cell cycle (2, 8). Genes that were preferentially expressed in G_1 were identified by differential hybridization. The combination of molecular biology techniques with the use of G_1-specific temperature-sensitive mutants should ultimately allow us to select among the preferentially expressed genes those that play a major role in cell-cycle progression. This initial report describes the identification and characterization of five cDNA clones that are preferentially expressed in G_1 at the permissive temperature yet are differently affected by the temperature-sensitive block at the restrictive temperature.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. The cell lines ts13 and tsAF8 are G_1-specific temperature-sensitive mutants originally isolated from baby hamster kidney (BHK) cells (2, 8). Both lines were maintained under culture conditions described in detail (9, 10). The permissive temperature for both cell lines was 34°C, and the nonpermissive temperature was 39.6°C for ts13 and 40°C for tsAF8. Cells were made quiescent by serum deprivation [i.e., maintained 48–50 hr in Dulbecco's minimal essential medium (DME medium) supplemented with 0.5% calf serum]. Quiescent populations were stimulated with fresh DME medium containing 15% fetal calf serum. The entry of cells into DNA synthesis was routinely monitored by continuous labeling with [^{3}H]thymidine (6.7 Ci/mmol; 0.5 μCi/ml; 1 Ci = 37 GBq; New England Nuclear) followed by autoradiography.

Enzymes. All restriction endonucleases were purchased from Bethesda Research Laboratories or New England Biolabs and were used according to the manufacturers' directions. Avian myeloblastosis virus reverse transcriptase was provided by J. Beard (Life Sciences, St. Petersburg, FL). DNA polymerase I from *Escherichia coli* was purchased from Boehringer Mannheim.

RNA Isolation. Total RNA was isolated from the cytoplasm of cells lysed with 10 mM Tris·HCl, pH 7.9/150 mM NaCl/1.5 mM $MgCl_2$/0.65% Nonidet P-40/10 mM vanadyl ribonucleosides, extracted with phenol/chloroform, and ethanol-precipitated (11). Poly(A)$^+$ mRNA was selected by oligo(dT)-cellulose chromatography (12).

DNA Isolation. High molecular weight hamster DNA was isolated from ts13 cells as described (13). Recombinant plasmid DNA was isolated and purified by phenol extraction and Sepharose 2B chromatography (14). The recombinant plasmid K4E, containing the entire Kirsten murine sarcoma virus DNA, including the *ras* gene (15), was kindly provided by E. Scolnick (Merck Sharp & Dohme).

Construction of cDNA Library and Differential Screening. Double-stranded cDNA was synthesized from 5 μg of poly(A)$^+$ mRNA isolated from ts13 cells that had been serum-stimulated for 6 hr at the permissive temperature (16). After digestion with *Sau*3A1, the cDNA was ligated into the *Bam*HI site of pBR322 (17). Recombinant plasmids transformed into *E. coli* strain HB101, which were ampicillin resistant and tetracycline sensitive, were replica-plated from 96-well microtiter plates onto nitrocellulose paper in duplicate. After bacterial cell lysis (18), duplicate sets of baked filters were hybridized with ^{32}P-labeled single-stranded

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cDNA synthesized from poly(A)$^+$ RNA isolated either from quiescent (G_0) cells or from serum-stimulated (G_1) ts13 cells, using hybridization conditions described by Augenlicht and Kobrin (19).

DNA and RNA Blot Analysis. Restriction endonuclease-digested ts13 DNA (10 μg) was size-fractionated by electrophoresis through a 1% agarose gel and transferred to nitrocellulose by the method of Southern (20). Total cytoplasmic RNA (12 μg) or poly(A)$^+$ mRNA (5 μg) was denatured with 6.3% formaldehyde/50% formamide and then size-fractionated on a 1% agarose gel containing 6.6% formaldehyde (21). Blotting of RNA to nitrocellulose and RNA dot blots was done as described by Thomas (22). cDNA inserts, with flanking pBR322 sequences, were removed from recombinant plasmids by sequential digestion with *Eco*RI and *Sal* I and nick-translated (23) to high specific activity. Prehybridization, hybridization, and posthybridization washes for DNA blots, RNA blots, and RNA dot blots were essentially as described by Wahl *et al.* (24) except that glycine was not included.

RESULTS

Construction of cDNA Library and Differential Screening. Total cytoplasmic RNA was isolated from ts13 cells 6 hr after serum stimulation at 34°C. This corresponds to early-to-mid G_1 for these cells. After selection for poly(A)$^+$ mRNA, 1.4 μg of double-stranded cDNA with an average size of 800 base pairs was synthesized from 5 μg of RNA. The cDNA was inserted into the tetracycline-resistance gene of pBR322 at the *Bam*HI site after digestion with *Sau*3A1 to an average of 250–300 base pairs. The recombinant plasmids were transformed into HB101, grown, and stored in 96-well microtiter plates. Replicate filters of individual colonies were grown on nitrocellulose filters overlayed onto agar plates. After denaturation and immobilization of the recombinant plasmids, the filters were hybridized with ^{32}P-labeled single-stranded cDNA populations synthesized from poly(A)$^+$ mRNA templates isolated from either quiescent ts13 cells (G_0 probe) or ts13 cells serum-stimulated for 6 hr at 34°C (G_1 probe). After extensive washing, the extent of hybridization to the individual clones was determined by autoradiography. An example of a clone (p13-2F1) that showed preferential hybridization to the G_1 probe is shown in Fig. 1 and will be referred to as a G_1-specific clone. All hybridizations were repeated twice using independent isolations of RNA as templates for the probes. Of the first 600 clones screened, which constitutes the basis of this report, ≈0.8% were G_1 specific. The remaining experiments characterize those clones that were G_1 specific (Table 1).

Cell-Cycle Expression of G_1-Specific Clones. The expression of the G_1-specific clones selected by differential hybridization was studied by RNA dot blot analysis. Previous studies of the cell cycle in ts13 cells have shown that DNA synthesis does not begin until about 18 hr after serum stimulation, and by 21 hr after serum stimulation, ≈50% of the cells are in S phase (10). Therefore, in these cells under these conditions, 6 hr after serum stimulation is considered early G_1, 16 hr after serum addition is considered late G_1, and 24 hr after serum stimulation is considered S phase. Quiescent populations in these experiments had <10% cycling cells. Poly(A)$^+$ mRNA was isolated either from quiescent ts13 cells or from ts13 cells serum-stimulated for 6, 16, and 24 hr at 34°C. Equal amounts of RNA were dotted onto nitrocellulose and hybridized with individual nick-translated cDNA inserts. Autoradiography of these hybridizations is shown in Fig. 2. The extent of hybridization to each RNA population was quantitated by using soft-laser scanning densitometry. All of the selected cDNA clones were expressed at low levels in G_0. The kinetics of expression of the different cDNA

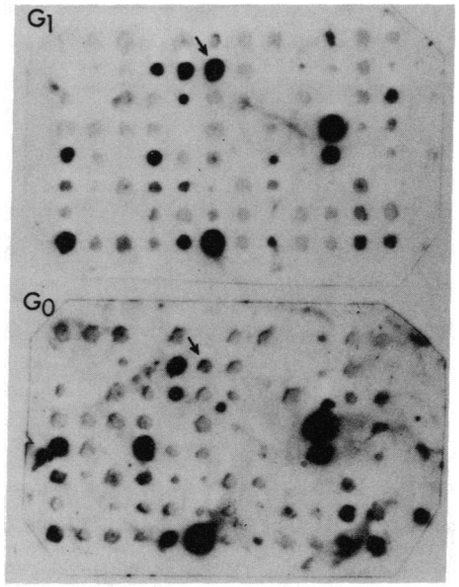

FIG. 1. Differential screening of the cDNA library. Clones grown in microtiter plates were transferred in duplicate to nitrocellulose, using a replica-plating device. After growth and lysis of the colonies, the immobilized recombinant plasmid DNA was hybridized to ^{32}P-labeled single-stranded cDNA probes synthesized from poly(A)$^+$ mRNA isolated either from serum-starved (G_0) ts13 cells or from ts13 cells stimulated for 6 hr with serum (G_1). The screening was repeated with probes synthesized from independent isolations of poly(A)$^+$ mRNA. Arrow indicates an example of a clone (p13-2F1) that repeatedly hybridized to G_1 single-stranded cDNA and not to G_0 single-stranded cDNA.

clones fall into three categories. The expression of p13-2A8 increased to its highest level 6 hr after serum stimulation and then rapidly decreased. Two of the cDNA clones, p13-2A9 and p13-2A10, did not increase significantly until 16 hr after serum stimulation (late G_1) and decreased as the cells entered S phase 24 hr after serum stimulation. The remaining two clones, p13-2F1 and p13-4F1, increased 6 hr after stimulation and remained elevated at 16 hr before they decreased at 24 hr. All clones showed a 3- to 6-fold increase in G_1 compared to G_0. K4E, a cloned Kirsten *ras* gene (15), showed little difference in expression from G_0 through G_1 and then decreased slightly at 24 hr and was included as a control. Background hybridization to pBR322 sequences was virtually nonexistent. Non-cell-cycle-dependent clones from the cDNA library showed little difference in expression across the entire cell cycle (data not shown).

Expression of the G_1-Specific cDNA Clones in ts13 Cells Stimulated at the Restrictive Temperature. ts13 cells (a G_1-

Table 1. Cell-cycle-specific cDNA clones

Clone	Insert, bp*	Size of RNA species, b†	No. of DNA fragments‡
2A8	170	No discrete size	Multiple
2A9	100	3800, 750	Several
2A10	100	450	Single
2F1	420	1500	Several
4F1	220	1550	One or two

bp, Base pairs; b, bases.
*Insert size was determined by migration of the *Eco*RI/*Sal* I fragment on a 4% acrylamide gel, and by subtraction of flanking pBR322 sequences.
†Summary of data from RNA gel blot analysis (Fig. 5).
‡Summary of data from Southern gel blot analysis (Fig. 4).

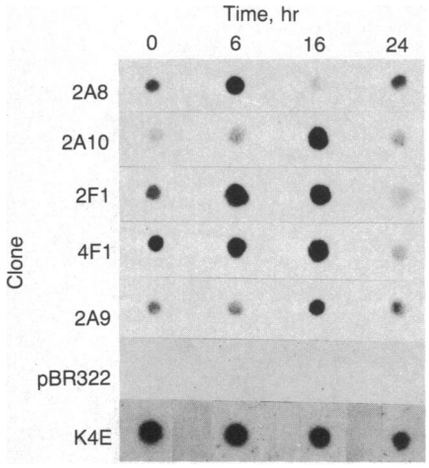

FIG. 2. Expression of the cell-cycle-specific cDNA clones after serum stimulation. Cytoplasmic poly(A)$^+$ mRNA isolated from ts13 cells at different times after serum stimulation was dotted onto nitrocellulose for hybridization. The cDNA inserts from selected cell-cycle-specific clones and controls were gel-purified after digestion with *Eco*RI and *Sal* I and then nick-translated in the presence of [^{32}P]dCTP to 1×10^8 cpm per μg. After hybridization, the filters were washed and exposed to film in the presence of an intensifying screen for 24 hr. K4E, a cloned Kirsten *ras* gene (15), and pBR322 are included as controls (see text).

specific temperature-sensitive mutant of the cell cycle) were originally chosen for these experiments because the temperature-sensitive block in G_1 offers a unique approach toward the goal of identifying, among the various genes whose expression is cell-cycle dependent, those that may be more relevant to cell-cycle progression. When ts13 cells are stimulated with 10% serum at the restrictive temperature, the growth factors in the serum are acting on the cells (25) but the cells are blocked in G_1 at a point about 3–5 hr before S phase (10). Genes involved in cell-cycle progression early after serum stimulation should still be expressed or even overexpressed in cells blocked in G_1 (see *Discussion*). Fig. 3*A* shows that the expression of two of the cDNA clones, p13-4F1 and p13-2A9, increases when ts13 cells are stimulated at the restrictive temperature considerably more than when the cells are stimulated at the permissive temperature. By densitometry, the levels of expression at 16 hr at the restrictive temperature are 3-fold and 5-fold higher than at the permissive temperature for p13-4F1 and p13-2A9, respectively.

Clone p13-2F1 showed no increase at 6 hr at the restrictive temperature, but expression increased to as high as 2.5 times the G_0 level after 16 hr at the nonpermissive temperature, which is similar to the level of expression seen at 16 hr at the permissive temperature. One of the clones (p13-2A8) showed no significant increase in expression at the nonpermissive temperature compared to G_0 expression.

Expression of the Cell-Division Cycle (*cdc*) Genes in tsAF8 Cells Stimulated at Either Permissive or Nonpermissive Temperature. tsAF8 cells are also a G_1-specific temperature-sensitive mutant of the cell cycle, derived, like ts13 cells, from Syrian hamster BHK cells (2). We thought it desirable to determine whether the expression of the *cdc* genes described above was also increased in tsAF8 cells stimulated at the permissive temperature. In addition, it has been established that tsAF8 cells are a mutant of RNA polymerase II (3–6). Since RNA polymerase II in tsAF8 cells is nonfunctional at the restrictive temperature, these cells can be used to study the turnover of the RNA sequences complementary to the inserts in the *cdc* clones. The results are shown in Fig. 3*B*.

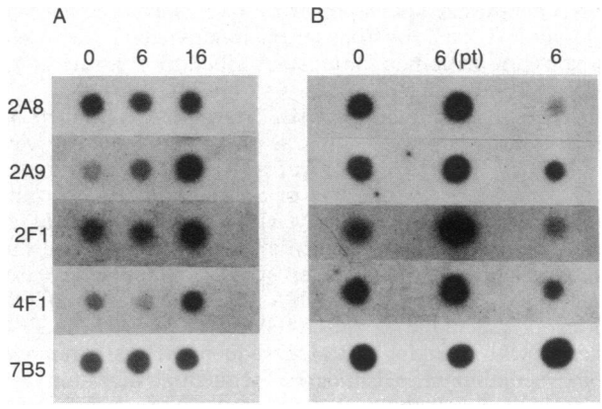

FIG. 3. Expression of cell-cycle-specific cDNA clones after serum stimulation at the nonpermissive temperature. Total cytoplasmic RNA isolated from quiescent cells or serum-stimulated cells was dotted onto nitrocellulose and hybridized to the same cDNA clones as described in the legend to Fig. 2. 7B5, a non-cell-cycle-dependent clone, is included as a control. (*A*) RNA isolated from ts13 cells 0, 6, and 16 hr after incubation at the nonpermissive temperature (39.6°C). (*B*) RNA isolated from tsAF8 cells incubated for 0 and 6 hr after serum stimulation at the permissive temperature (pt) (34°C) and 6 hr at the nonpermissive temperature (40°C).

Total cytoplasmic RNA was isolated from quiescent tsAF8 cells and from tsAF8 cells serum-stimulated for 6 hr at the permissive (34°C) or nonpermissive (40°C) temperature. After dotting equal amounts onto nitrocellulose, these tsAF8 RNA populations were hybridized with nick-translated cell-cycle-specific cDNA clones (Fig. 3*B*). All of the clones were stimulated 2- to 3-fold in tsAF8 cells incubated for 6 hr at the permissive temperature. In all of the clones tested, the levels of complementary RNA decreased at the nonpermissive temperature. This difference in expression was as much as a factor of 6 for p13-2A8, while the others decreased by a factor of 1 to 2.5. A non-cell-cycle-dependent clone selected from the library is included as a control. Notice that its level of expression does not change from G_0, whether the cells are stimulated at the permissive or the restrictive temperature.

RNA and DNA Gel Blot Analysis. Nick-translated cDNA inserts were hybridized to Southern blots (20) of genomic DNA digested with *Bam*HI, *Eco*RI, and *Hin*dIII to determine the number of complementary fragments and their size (Fig. 4; Table 1). These fell into two major categories: (*i*) cDNA clones hybridizing to one or a few bands and probably derived from a single-copy or low repeat frequency gene, and (*ii*) cDNA clones hybridizing to a multitude of fragments of widely varying sizes and probably derived from moderately repetitive sequences, or possibly multigene families. RNA blot analysis (22) revealed that p13-2A10. p13-2F1, and p13-4F1 hybridized to single transcripts with sizes of 450, 1500, and 1550 bases, respectively (Fig. 5; Table 1). Clone p13-2A9 hybridized to a major transcript of 750 bases and a minor band of 3800 bases, which may be a precursor molecule that leaked from the nucleus during the isolation procedure. The clone that appears to be derived from a moderately repetitive sequence, p13-2A8, hybridized to many different sizes of transcripts and appears as a smear. In addition, clones p13-2A10 and p13-4F1 may be present in lower abundance, as it was necessary to use poly(A)$^+$ mRNA on the gel to detect the transcripts, whereas the others could be detected with total cytoplasmic RNA. These results suggest that the five cDNA clones are unrelated, a conclusion confirmed by DNA sequencing (unpublished results).

DISCUSSION

The identification of genes whose expression is cell-cycle dependent is the first necessary step in the search for genes

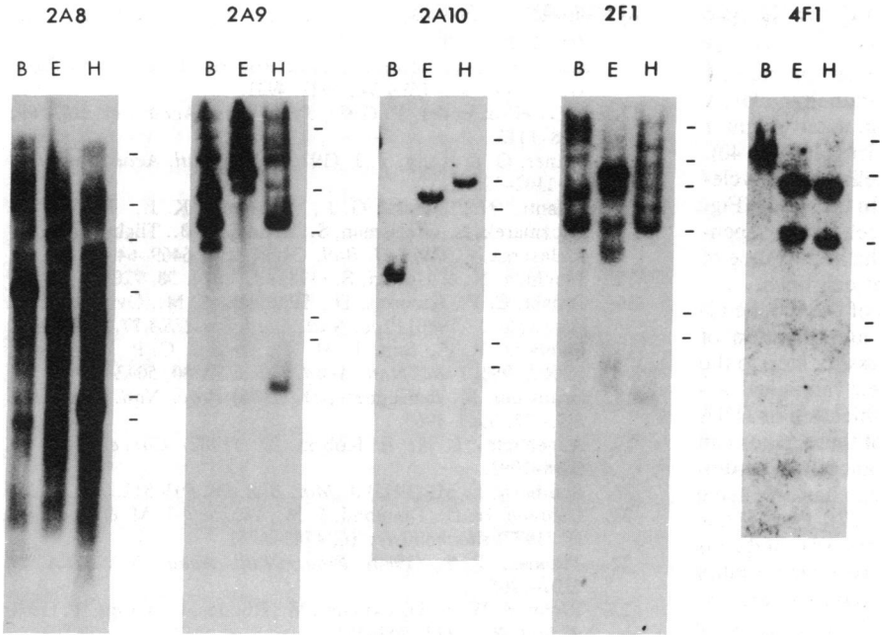

FIG. 4. Southern analysis of the cell-cycle-specific cDNA clones. High molecular weight DNA isolated from ts13 cells was digested with *Bam*HI (B), *Eco*RI (R), or *Hin*dIII (H), and was electrophoresed (10 μg per lane) on a 1% agarose gel. After transferring to nitrocellulose, the DNA was hybridized with the nick-translated probes as indicated and washed as described. Blots were exposed to x-ray film at −70°C in the presence of an intensifying screen for up to 10 days. Horizontal lines to the right of each panel indicate the position of simultaneously run DNA markers and are, from top to bottom (in kilobase pairs), as follows: 23.5, 9.7, 6.6, 4.3, 2.2, and 2.0.

that actually control cell proliferation. Although cDNA clones, whose expression is increased after stimulation with either platelet-derived growth factor (26) or serum (27), have already been reported in the literature, these cDNA clones were derived from 3T3 cells. Our cDNA clones were obtained from ts13 cells, originally derived from BHK cells (8). Much more important, however, is that we have studied the expression of cell-cycle-dependent cDNA clones in G_1-specific temperature-sensitive mutants of the cell cycle.

In all searches for genes that are preferentially expressed (whether during the cell cycle, in differentiation, or in any different physiological state), there is always the problem of distinguishing, among the preferentially expressed genes, those that regulate the transition from those that are regulated by the transition. The temperature-sensitive block in G_1 is specific and not based on the use of drugs that usually have multiple sites of action. One can expect that a G_1 block could cause overexpression of genes that regulate cell-cycle progression, much in the same way as drugs that block a certain enzymatic pathway can cause overexpression of one of the enzymes in that pathway; methotrexate and dihydrofolate reductase is a good example (28). This, indeed, appears to be the case with two of our cDNA clones, p13-4F1 and p13-2A9. A second possibility, with a temperature-sensitive block, is that the preferential expression of a gene may be abolished at the restrictive temperature, because its expression depends on some previous event that has been prevented at the restrictive temperature. cDNA clone p13-2A8 is preferentially expressed in ts13 cells stimulated at the permissive temperature, but at the restrictive temperature its level of expression in G_1 is the same as in G_0. The other clone, p13-2F1, showed increased levels of expression at either temperature. It seems, therefore, that the temperature-sensitive block can be used to separate cDNA clones whose expression is influenced by the temperature-sensitive block from those that are not. While obviously not foolproof, this manipulation gives objective support to a selection of clones for further studies. For instance, the expression of the cellular thymidine kinase gene is also cell-cycle dependent in ts13 cells, increasing at 16 hr after stimulation and reaching a peak between 24 and 30 hr. However, when ts13 cells are stimulated at the restrictive temperature, the expression of the thymidine kinase gene remains at the level of G_0 cells (unpublished results), suggesting that the temperature-sensitive block can separate genes preferentially expressed in early G_1 from those preferentially expressed in late G_1.

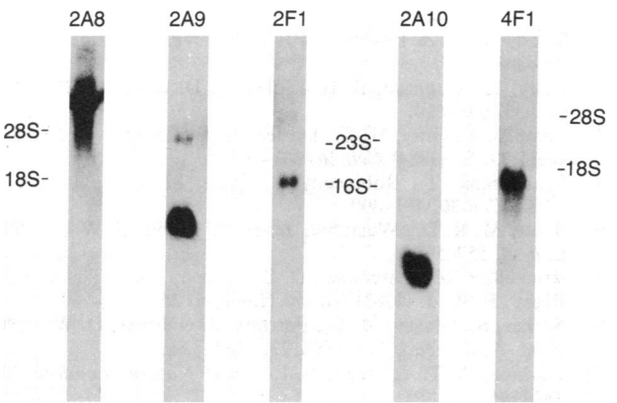

FIG. 5. RNA blot analysis of the cell-cycle-specific cDNA clones. Total cytoplasmic RNA (12 μg per lane) (labeled p13-2A8, p13-2A9, p13-2F1) or poly(A)$^+$ mRNA (5 μg per lane) (labeled p13-2A10, p13-4F1) isolated from randomly growing ts13 cells was electrophoresed on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized to nick-translated cell-cycle-specific cDNA fragments as indicated. Hybridization and washing conditions are the same as in Fig. 2. Blots were exposed to x-ray film for 3 days at −70°C in the presence of an intensifying screen. Arrows indicate the position of hamster rRNA (28S and 18S) and *E. coli* rRNA (23S and 16S).

We propose to call these genes that are preferentially expressed in specific phases of the cell cycle, cell-division-cycle genes, or briefly, *cdc* genes. This is the same nomenclature proposed by Hartwell (29) to indicate genes, defined by temperature-sensitive mutations, that are required for cell-cycle progression in yeast. It is true that our experiments as well as those mentioned above (26, 27) identify cDNA clones derived from sequences that are preferentially expressed in a phase of the cell cycle—in this case, G_1. Some of these genes are expected to regulate the progression of cells through the cell cycle, while others may not be related to cell proliferation at all. But in this context, we would like to expand the definition of *cdc* genes to all those genes whose expression is cell-cycle dependent. Some of the *cdc* genes in yeast (30) and a cell-cycle-specific temperature-sensitive mutation in mouse cells (31) have been identified as part of the

DNA synthesizing apparatus. Other genes whose expression is cell-cycle dependent include histone genes (32, 33), the dihydrofolate reductase gene (34, 35), the gene for the p53 protein (36), c-*myc* (37, 38), and actin (38). Inducible cDNA clones have also been described in cells stimulated to enter DNA synthesis by infection with simian virus 40 (39, 40). Campisi *et al.* (38) have reported in 3T3 cells a cell-cycle-dependent expression of the c-Ki-*ras* gene. In ts13 cells (Fig. 2), the c-Ki-*ras* gene is expressed in a non-cell-cycle-dependent manner. This discrepancy underlines the importance of extending these kinds of studies to different cell lines.

It is true that nothing is presently known of the biochemical function of these *cdc* genes. But the identification of preferentially expressed genes is a first necessary step to the isolation of genomic clones, testing of their biological activity by, for instance, direct manual microinjection or DNA transfection. Clues to the function of some of these genes can also be given by their sequences. For instance, the serum-inducible cDNA clone isolated from 3T3 cells has extensive homology with prolactin, a growth factor (27). One of the cDNA clones isolated from simian virus 40-transformed cells (40) has significant homology with a major histocompatibility complex antigen (41), and some of these antigens are expressed in a cell-cycle-dependent manner (42). The predicted amino acid sequence of the yeast "start" gene, *cdc* 28, has 20%–25% homology with several vertebrate oncogenes belonging to the protein kinase family (43). In this respect, preliminary studies in our laboratory have indicated that one of our cDNA clones, p13-2A8, has significant homology with a gene that is preferentially expressed in colon carcinoma (44). Two others, p13-2A9 and p13-4F1, are preferentially expressed in chronic myeloid leukemia (unpublished results) and have significant sequence homology with a feline sarcoma virus transforming gene and the human EJ bladder carcinoma oncogene, respectively (unpublished results).

In summary, while confirming previous reports that genes that are preferentially expressed in the G_1 phase of the cell cycle can be identified, we have shown that the use of temperature-sensitive mutants of the cell cycle can offer a more logical basis for determining which of those genes are relevant to cell-cycle progression.

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1. Lieberman, I., Abrams, R. & Ove, P. (1963) *J. Biol. Chem.* **238,** 2141–2149.
2. Burstin, S. J., Meiss, H. K. & Basilico, C. (1974) *J. Cell. Physiol.* **84,** 397–408.
3. Rossini, M., Baserga, S., Huang, C. H., Ingles, C. J. & Baserga, R. (1980) *J. Cell. Physiol.* **103,** 97–103.
4. Waechter, D. E. & Baserga, R. (1982) in *Genetic Expression in the Cell Cycle*, eds. Padilla, G. M. & McCarty, K. S. (Academic, New York), pp. 231–244.
5. Waechter, D. E., Avignolo, C., Freund, E., Riggenbach, C. M., Mercer, W. E., McGuire, P. M. & Baserga, R. (1984) *J. Mol. Cell. Biochem.* **60,** 77–82.
6. Shales, M., Bergsagel, J. & Ingles, C. J. (1980) *J. Cell. Physiol.* **105,** 527–532.
7. Ingles, C. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 405–409.
8. Talavera, A. & Basilico, C. (1977) *J. Cell. Physiol.* **92,** 425–436.
9. Ashihara, T., Chang, S. D. & Baserga, R. (1978) *J. Cell. Physiol.* **96,** 15–22.
10. Floros, J., Ashihara, T. & Baserga, R. (1978) *Cell Biol. Int. Rep.* **2,** 259–269.
11. Ricciardi, R. P., Miller, J. S. & Roberts, B. E. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4927–4931.
12. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
13. Ketner, G. & Kelly, T. J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1102–1106.
14. Galanti, N., Jonak, G. J., Soprano, K. J., Floros, J., Kaczmarek, L., Weissman, S., Reddy, V. B., Tilghman, S. M. & Baserga, R. (1981) *J. Biol. Chem.* **256,** 6469–6474.
15. Tsuchida, N. & Uesugi, S. (1981) *J. Virol.* **38,** 720–727.
16. Ordahl, C. P., Kioussis, D., Tilghman, S. M., Ovitt, C. E. & Fornwald, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4519–4523.
17. Feinberg, R. F., Sun, L.-H. K., Ordahl, C. P. & Frankel, F. R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5042–5046.
18. Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.
19. Augenlicht, L. H. & Kobrin, D. (1982) *Cancer Res.* **42,** 1088–1093.
20. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
21. Lehrach, H. D., Diamond, J. M., Wozney, J. M. & Boedtker, H. (1977) *Biochemistry* **16,** 4743–4751.
22. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
23. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
24. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
25. Baserga, R., Potten, C. & Ming, P. M. L. (1982) in *Cell Growth*, ed. Nicolini, C. (Plenum, New York), pp. 69–81.
26. Cochran, B. H., Reffel, A. C. & Stiles, C. D. (1983) *Cell* **33,** 939–947.
27. Linzer, D. I. H. & Nathans, D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4271–4275.
28. Hillcoat, B. L., Swett, V. & Bertino, J. R. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 1632–1637.
29. Hartwell, L. H. (1971) *J. Mol. Biol.* **59,** 183–194.
30. Conrad, M. N. & Newlon, C. S. (1983) *Mol. Cell. Biol.* **3,** 1000–1012.
31. Cohill, R. W. & Sheinin, R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4644–4648.
32. Plumb, M., Stein, J. & Stein, G. (1983) *Nucleic Acids Res.* **11,** 2391–2410.
33. DeLisle, A. J., Graves, R. A., Marzluff, W. F. & Johnson, L. F. (1983) *Mol. Cell. Biol.* **3,** 1920–1929.
34. Kaufman, R. J. & Sharp, P. A. (1983) *Mol. Cell. Biol.* **3,** 1598–1608.
35. LaBella, F., Brown, E. H. & Basilico, C. (1983) *J. Cell. Physiol.* **117,** 62–68.
36. Reich, N. C. & Levine, A. J. (1984) *Nature (London)* **308,** 199–201.
37. Kelly, K., Cochran, B. H., Stiles, C. D. & Leder, P. (1983) *Cell* **35,** 603–610.
38. Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenshein, G. E. (1984) *Cell* **36,** 241–247.
39. Schutzbank, T., Robinson, R., Oren, M. & Levine, A. J. (1982) *Cell* **30,** 481–490.
40. Scott, M. R. D., Westphal, K.-H. & Rigby, P. W. J. (1983) *Cell* **34,** 557–567.
41. Brickell, P. M., Latchman, D. S., Murphy, D., Willison, K. & Rigby, P. W. J. (1983) *Nature (London)* **306,** 756–760.
42. Sarkar, S., Glassy, M. C., Ferrone, S. & Jones, O. W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7297–7301.
43. Lörincz, A. T. & Reed, S. I. (1984) *Nature (London)* **307,** 183–185.
44. Augenlicht, L. M., Kobrin, D., Pavlovec, A. & Royston, M. E. (1984) *J. Biol. Chem.* **259,** 1842–1847.