Relations between Synthesis of Deoxyribonucleotides and DNA Replication in 3T6 Fibroblasts*

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In exponentially growing 3T6 cells, the synthesis of deoxythymidine triphosphate (dTTP) is balanced by its utilization for DNA replication, with a turnover of the dTTP pool of around 5 min. We now investigate the effects of two inhibitors of DNA synthesis (aphidicolin and hydroxyurea) on the synthesis and degradation of pyrimidine deoxynucleoside triphosphates (dNTPs). Complete inhibition of DNA replication with aphidicolin did not decrease the turnover of pyrimidine dNTP pools labeled from the corresponding [3H]deoxynucleosides, only partially inhibited the in situ activity of thymidylate synthetase and resulted in excretion into the medium of thymidine derived from breakdown of dTTP synthesized de novo. These data demonstrate continued synthesis of dTTP in the absence of DNA replication. In contrast, hydroxyurea decreased the turnover of pyrimidine dNTP pools 5–50-fold. Hydroxyurea is an inhibitor of ribonucleotide reductase and stops DNA synthesis by depleting cells of purine dNTPs but not pyrimidine dNTPs. Our results suggest that degradation of dNTPs is turned off by an unknown mechanism when de novo synthesis is blocked.

A major interest of this laboratory concerns the regulation of the synthesis of dNTPs and of their utilization for DNA replication. The enzyme ribonucleotide reductase occupies a central position in this respect since it catalyzes the de novo synthesis of dNTPs from ribonucleotides (1, 2). The function and regulation of this enzyme, obtained in pure or highly purified form from Escherichia coli (3) or mammalian sources (4), have been studied in considerable detail and are reasonably well understood. However, less is known about its integration in the overall process of DNA synthesis, in particular whether its activity is regulated by or, alternatively, involved in the regulation of DNA replication. We speculated from indirect evidence that a specific product of the reductase, possibly deoxythymidine nucleotide, might provide a switch function for DNA synthesis (5, 6), and recent evidence for the existence of two separate kinetic pools of dCTP in mouse 3T6 fibroblasts may be relevant in this connection (7). Veer Reddy and co-workers (8–10) suggested a different kind of control. They presented evidence for the existence of a mammalian multienzyme complex (repilatase) involved in both dNTP and DNA synthesis. The assembly of replicatase and protein-protein interaction within the complex was proposed to have important regulatory functions.

In the present study, we use cultured mouse 3T6 fibroblasts to study relations between dNTP and DNA synthesis. The pyrimidine dNTP pools of these cells rapidly reach a dynamic isotope equilibrium with labeled deoxycytidine or thymidine of the medium (cf. Fig. 1), and it is possible to relate their turnover to the rate of DNA synthesis (7). We now investigate the effects of two inhibitors of DNA synthesis on these relations. These inhibitors act at two separate loci (Fig. 1): one, hydroxyurea, blocks de novo synthesis of all dNTPs (11–13), while the other, aphidicolin, blocks their utilization for DNA synthesis (14–16). We also investigate the effect of aphidicolin on the in situ activity of the enzyme thymidylate synthetase (17–19), the enzyme transforming dUMP to dTMP (Fig. 1). Taken together, our results demonstrate that inhibition of DNA replication by aphidicolin does not block turnover or de novo synthesis of dNTPs, while inhibition of de novo synthesis of dNTPs by hydroxyurea leads to an almost complete cessation of turnover.

MATERIALS AND METHODS

Labeled Nucleosides—[5-3H]Deoxycytidine was from ICN; [6-3H] deoxyuridine was from New England Nuclear; and [5-3H]deoxycytidine and [methyl-3H]thymidine were from Amersham Corp. All had specific activities ranging from 20 to 30 Ci/mmol and were added to the medium without dilution to a final concentration of 0.3 μM. The Packard scintillation counter used in our experiments recorded 16,000 cpm/pmol for an activity of 20 Ci/mmol. Both labeled deoxyuridines were purified by HPLC chromatography (see below) immediately before use. The labeled deoxyuridine was diluted approximately 2-fold by nonlabeled deoxyuridine excreted during growth of cells into the medium prior to addition of isotope.

Growth and Incubation of Cells—Frozen stocks of 3T6 mouse fibroblasts were prepared as follows. Cells from one clone were grown for one passage in Dulbecco's modified Eagle's medium with 5% fetal calf serum, trypsin-treated in late log phase, washed with medium, and centrifuged. After suspension in Dulbecco's modified Eagle's medium with 20% fetal calf serum and 10% dimethyl sulfoxide, aliquots of 1–2 million cells were immediately frozen in ampules at −80°C and stored in liquid nitrogen. For each experiment, one ampule was thawed, and cells were washed with medium containing 10% horse serum seeded on a 15-cm Petri dish and grown for one passage to accustom the cells to horse serum. Dishes for one experiment were then set up essentially as described earlier (7). Forty to 50 Petri dishes (5 cm) were seeded with 50,000 cells in 5 ml of medium and grown for 40–50 h to a density of 0.5–1 million cells/plate. Medium was reduced to 2 ml, and Na-Hepes buffer, pH 7.2 (final concentration 20 mM), was added. The experiment was started 3 h after further incubation at 37°C when the appropriate drug was added and the dishes were divided into two groups. One group received the labeled deoxynucleoside (final concentration 0.3 μM), while the other served as a source of conditioned medium and received an identical amount of nonlabeled deoxynucleoside. When the decay period was started (arrows in Figs. 2, 4, and 5), the labeled medium was sucked off and replaced by conditioned medium. Experiments
were carried out in a thermostated room at 37 °C with the dishes kept in boxes in an atmosphere of 5% CO₂, 95% air.

Analyses of Deoxynucleotides and DNA—At the end of incubation, the dishes were quickly transferred to an ice bath, medium was sucked off (and saved), and plates were washed three times with 2-3 ml of 50 mM Tris-HCl buffer, pH 7.2, and left tilted for about 1 min before sucking off the remaining buffer as completely as possible. Two ml of ice-cold 60% methanol were added, and the plates were scraped with a rubber policeman. Extracts were stored overnight at -20 °C and centrifuged at 22,000 × g for 45 min. The methanolic solutions were used for the analysis of deoxynucleotides, the pellet for the analysis of DNA.

Methanol extracts were evaporated to dryness in a Buchler Vortex evaporator, and the residue was dissolved in 0.2 ml of 10 mM Tris-HCl buffer, pH 7.4. Recovery in this step was better than 95%. Deoxynucleoside triphosphates were determined by the method of Skoog and co-worker (20, 21), modified to measure simultaneously the size and specific activity of the pools (22, 23).

The precipitates after centrifugation of the methanol extracts were dissolved in 0.6 M NaOH and incubated at 37 °C overnight. Aliquots were precipitated with 10% trichloroacetic acid containing 0.1 M sodium pyrophosphate after addition of 20 µg of calf thymus DNA as carrier, and the precipitate was collected on Whatman GF/C glass microfiber filters. After washing with 5% trichloroacetic acid, 0.1 M pyrophosphate and ethanol, the dried filters were counted in a scintillation counter in toluene solutions containing Permablend. Recovery of radioactivity was at least 90% compared to direct scintillation counting in Insta-Gel.

HPLC Analyses—All separations were made on 25-cm Nucleosil C₁₈ (Macherey-Nagel) columns (particle size 5 µm) with a LKB 2150 HPLC pump with a Rheodyne 7125 injector and two Shimadzu SPD-2A detectors. Separations were achieved by isocratic elution with ammonium acetate buffer, pH 5.0 (24), at a flow rate of 1 ml/min, and fractions were collected with a Pharmacia FRAC-100 fraction collector.

To determine the excretion of nucleosides from cells, proteins of the medium were precipitated with 0.3 M perchloric acid at 0 °C for 20 min and removed by centrifugation. The supernatant solutions were then neutralized with 4 M KOH, precipitated salt was removed by centrifugation, and aliquots of the supernatant solutions were chromatographed by HPLC with 0.1 M ammonium acetate, pH 5.0, containing 2% methanol to separate deoxyuridine and thymidine.

To determine the labeling of intracellular dUMP, the nucleotide pools had approached a constant specific radioactivity, the medium was replaced with nonlabeled conditioned medium and the decay of H from the pools was determined. From this decay, the turnover of the pool can be calculated (7).

In the absence of inhibitors, the incorporation of either thymidine or deoxycytidine into the acid-soluble pool reached a plateau before 30 min, indicating that a dynamic equilibrium had been established between the amount of isotope incorporated from the nucleoside into the pool and the amount of isotope leaving the pool for DNA synthesis (7). We now first investigated whether inhibition of DNA synthesis affects the attainment of such a steady state. Fig. 2 depicts the effects of aphidicolin and hydroxyurea on the time-dependent incorporation of [3H]deoxycytidine into the total intracellular acid-}

![Diagram showing the introduction to isotope into pyrimidine dNTP pools from deoxynucleosides and points of inhibition by hydroxyurea and aphidicolin.](http://example.com/diagram.png)

**FIG. 1.** Diagram showing the introduction to isotope into pyrimidine dNTP pools from deoxynucleosides and points of inhibition by hydroxyurea and aphidicolin.

**FIG. 2.** Effects of inhibitors on labeling of nucleotide pools from [5-³H]deoxycytidine and decay after change of medium. Growing 3T6 cells were treated with inhibitor for 30 min and then labeled as described in the text with change of medium at the arrow. +, control; ▲, aphidicolin (10 µM); ⊙, hydroxyurea (3 mM).

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Decay period, external cold nucleosides rapidly equilibrate. We added nonlabeled nucleosides to the medium during the decay period to dilute any isotope reincorporated into the dNTP pool in the event of a futile cycle. In the experiment depicted Fig. 2, thus, a 4-fold increase was observed with aphidicolin and a 16-fold increase with hydroxyurea.

The decay of isotope from the pool, after change to cold medium (arrow at 67 min in Fig. 2), was strongly influenced by hydroxyurea but much less so by aphidicolin. In the control and aphidicolin experiments, the half-life of the pool was approximately 5 min, while with hydroxyurea this value increased to more than 30 min. It was surprising to find a rapid turnover in the presence of aphidicolin, suggesting the possibility that dNTPs may continue to be synthesized also in the absence of DNA replication. In contrast, the half-life of the pools increased greatly in the presence of hydroxyurea, i.e. when de novo synthesis of dNTPs was blocked. This suggests a control mechanism for the coupling of synthesis and degradation of dNTPs.

One uncertainty in the interpretation of our results concerns the degree of recirculation of isotope within the acid-soluble pool, i.e. to what extent dNTPs were degraded and the nucleosides formed were reused for intracellular resynthesis of dNTPs rather than excreted into the medium. To investigate the possible effects of such a "futile cycle" (25), we added nonlabeled nucleosides to the medium during the decay period. External cold nucleosides rapidly equilibrate with intracellular labeled nucleosides (26, 27) and would be expected to dilute any isotope reincorporated into the dNTP pool in the event of a futile cycle. In the experiment depicted in Fig. 3, the intracellular pools were labeled from either deoxycytidine or thymidine, and we then investigated the effect of the corresponding cold nucleoside on their decay. Clearly the nucleosides had no effect. In both cases, the pool decay occurred with a half-life of 4–5 min in the controls and aphidicolin experiments but was much prolonged by hydroxyurea. The effect of this drug was particularly impressive in the thymidine experiment, when the half-life of the pool increased from 4 min to over 4 h (cf. also Ref. 28).

At this point it became important to understand how the addition of the two drugs and the labeled deoxycytidine influenced the size of dNTP pools. Hydroxyurea caused a severe depletion of the two purine dNTP pools, gave a moderate decrease of the dCTP pool, and increased the dTTP pool about 2-fold (Table I). These effects in 3T6 cells are similar to results observed earlier with secondary mouse embryonic cells (28). Addition of either deoxycytidine or thymidine at 3 μM to the hydroxyurea-inhibited cells slightly increased the size of both pyrimidine dNTP pools. Aphidicolin differed in that its addition resulted in an increase of both dATP and dTTP and a moderate decrease of dCTP. Addition of either deoxycytidine or thymidine did not change this picture.

The results depicted in Figs. 2 and 3 thus might reflect both changes in the size and in the specific activity of dNTP pools. To differentiate between these two effects, we determined the influence of the two drugs on the specific activities of dNTP pools and the incorporation of isotope into DNA (Figs. 4 and 5). With labeled deoxycytidine, the specific activity of dCTP reached equilibrium already after 20 min in the control and in the presence of aphidicolin, while with hydroxyurea equilibration was slower and the pool had not yet reached a plateau value after 60 min (Fig. 4). At this time point, hydroxyurea (panel C) had increased the specific activity of dCTP 30-fold and aphidicolin 10-fold (panel B), compared to the control (panel A). These effects must be corrected for in the assessment of the effects of the drugs on DNA synthesis. In the control, incorporation of isotope into DNA amounted to 2500 cpm/min, while with hydroxyurea the corresponding value was 750, i.e. 30% of the control. However, the rate of DNA synthesis was increased to 4% since hydroxyurea increased the specific activity of dCTP 30-fold.

During the second part of the experiment, after change to cold medium at 60 min (arrow in Fig. 4), the specific activity of the dCTP pool decayed with a half-life of approximately 5 min in both the control and with aphidicolin, while the decay with hydroxyurea was much slower, corroborating earlier described results with total pools.

Results from the corresponding experiment with [3H]thymidine are shown in Fig. 5. The specific activity of the dTTP pools was only marginally affected by the presence of aphidicolin or hydroxyureas (panel A). Accordingly, incorporation

**Table I**

|            | dATP | dTTP | dGTP | dCTP |
|------------|------|------|------|------|
| Control    | 39   | 40   | 25   | 149  |
| +Thymidine | 39   | 44   | 27   | 134  |
| +Deoxycytidine | 43   | 49   | 26   | 98   |
| Aphidicolin | 91   | 67   | 21   | 73   |
| +Thymidine | 98   | 72   | 22   | 81   |
| +Deoxycytidine | 85   | 70   | 19   | 75   |
| Hydroxyurea | 8    | 74   | 6    | 58   |
| +Thymidine | 4    | 96   | 5    | 69   |
| +Deoxycytidine | 4    | 105  | 5    | 78   |

**Fig. 3.** Effect of addition of nonlabeled nucleosides (0.3 μM) to the medium during the decay of radioactivity from nucleotide pools. Cells were labeled from [3H]deoxycytidine (panel A) or [3H]thymidine (panel B) in the presence of hydroxyurea. Control, control, no addition during chase; +, control with deoxynucleoside; O, hydroxyurea, no addition during chase; Δ, hydroxyurea with deoxynucleoside.
FIG. 4. Effects of hydroxyurea and aphidicolin on the incorporation of radioactivity from [3H]deoxycytidine into dCTP and DNA and the turnover of the dCTP pool. [3H]Deoxycytidine was added to growing cells 30 min after addition of inhibitor, and the specific activity of dCTP (+) and incorporation of isotope into DNA (○) were measured. At the arrow, the radioactive medium was replaced with conditioned medium with 0.3 mM nonlabeled deoxycytidine added. Panel A gives the control, panel B shows the experiment with aphidicolin, and panel C shows the experiment with hydroxyurea. Note the differences in the ordinates.

of isotope into DNA directly measures the inhibition of DNA synthesis (panel B). After change of medium, the dTTP pool in the control and with aphidicolin decayed rapidly, while with hydroxyurea the radioactivity disappeared very slowly, in line with results described in Fig. 3.

Aphidicolin Does Not Abolish in Situ Activity of dTMP Synthetase—The experiments described so far demonstrate a rapid turnover of pyrimidine dNTPs in the presence of aphidicolin, suggesting the possibility that synthesis of the nucleotides is not tightly linked to DNA replication. In these experiments, dNTPs were labeled by phosphorylation of the corresponding deoxynucleosides, and the turnover of pools labeled in this way might differ from that of pools synthesized via enzymes of the de novo pathway. We therefore proceeded to determine the effect of aphidicolin on the in situ activity of thymidylate synthetase, one enzyme of the de novo pathway.

To this purpose we used a method originally introduced by Greenberg and associates for bacteria (17) and subsequently used by others with mammalian cells (18, 19). Parallel sets of cultures were incubated with deoxyuridine labeled with 3H of equal specific activity in either the 5 or 6 positions of the pyrimidine ring. With [5-3H]deoxyuridine, the transfer of isotope to water measures the rate of methylation of dUMP to dTMP, i.e. the in situ activity of thymidylate synthetase. With [6-3H]deoxyuridine, isotope is retained during the methylation step and incorporated into the dTTP pool and DNA (cf. Fig. 1). In the latter case, we measured the specific activity of the dTTP pool, incorporation of isotope into DNA, and excretion into the medium of labeled thymidine and thymine obtained by degradation of dTTP.

The results of this experiment are summarized in Fig. 6 and Table II. Panel A of Fig. 6 demonstrates rapid labeling of the dTTP pool from [6-3H]deoxyuridine with little increase
in its specific activity after 20 min. In the presence of aphidicolin, the specific activity of dTTP was decreased from 1450 to 440 cpm/pmol. A similar drop probably occurred in the specific activity of dUMP since dTTP is formed quite directly from dUMP and should mirror its specific activity. While it was not possible to measure directly the specific activity of dUMP, we could determine the total radioactivity in the dUMP pool and the effect of aphidicolin on this parameter (Fig. 6, Panel B). The drug decreased the incorporation of [5-3H]deoxyuridine into dUMP to 20% corroborating the effect of aphidicolin on the dTTP pool shown in panel A.

The incorporation of [6-3H]deoxyuridine into DNA and the release of isotope from [5-3H]deoxyuridine into water are depicted in panels C and D of Fig. 6, respectively. After 20 min, a linear rate of incorporation was observed in the absence of aphidicolin. From a specific activity of 1450 cpm/pmol for dTTP, one can calculate that the cells incorporated 20 pmol of dTMP/min into their DNA. Assuming the same specific activity for dUMP labeled from [5-3H]deoxyuridine, the release of isotope into water corresponded to the methylation of 23 pmol of dUMP/min. Since the release of 3H from [5-3H]deoxyuridine into water was also depressed, this value amounted to as much as 11% of that found in the control. Since aphidicolin decreased the specific activity of dUMP at least 3-fold, as witnessed by the data of Fig. 6, panels A and B, this means that the rate of methylation of dUMP amounted to at least one-third of that of the control. Thus, synthesis of thymidylate nucleotides continues although DNA synthesis is blocked.

dTTP synthesized in the presence of aphidicolin cannot be used for DNA synthesis but is instead degraded and excreted as thymidine into the medium. The amount of nucleoside present at different times after addition of [6-3H]deoxyuridine to cells was calculated from the total amount of radioactivity present in thymidine isolated from the medium by HPLC and the specific activity of intracellular dTTP. In the absence of aphidicolin, only a small fraction of the dTTP pool was degraded, while with aphidicolin degradation balanced synthesis of dTTP.

Table II summarizes our calculations from the experiment with the two labeled deoxyuridines and provides a balance sheet concerning the effect of aphidicolin on the turnover of the dTTP pool. In the control, synthesis of dTTP is balanced by its incorporation into DNA, while in the presence of aphidicolin dTTP is degraded and excreted into the medium.

**DISCUSSION**

Knowledge of the properties of the enzymes involved in the production, utilization, and degradation of dNTPs has advanced far enough to make it possible to understand interrelations between these reactions by studying in intact cells the flow of isotope through dNTPs and the effects of inhibitors on this process. The complexity of the system with necessity sets a limit to the level of our interpretation. However, we believe that from our experiments two major conclusions can be drawn concerning interrelations between dNTP synthesis and DNA replication in 3T6 cells. (i) When the flow of dNTPs into DNA is stopped by inhibition of the replicase with aphidicolin, synthesis of pyrimidine dNTPs continues, albeit at a somewhat reduced rate; dNTPs accumulate only to a limited extent and are excreted into the medium after degradation; (ii) inhibition of ribonucleotide reductase by hydroxurea, i.e. a block in the production of dNTPs, stops the turnover of dNTPs.

Our conclusions rest on two kinds of experiments, both involving labeling of pyrimidine dNTPs with deoxynucleosides. In the first approach, we introduce isotope from thymidine or deoxycytidine and measure the effect of DNA inhibitors on the decay of radioactivity from the pools after removal of the labeled nucleosides. In the second approach, isotope is introduced from labeled deoxycytidine and we investigate the effect of aphidicolin on the in situ activity of thymidylate synthetase.

Let us first consider some of the possible complications inherent in labeling experiments of this kind. The labeled nucleosides added to the medium rapidly enter the cells by a process of facilitated diffusion (25, 26) and are then phosphorylated to deoxynucleotides. All three nucleosides reach a dynamic equilibrium with their corresponding dNTPs (in the case of deoxycytidine, dTTP) when dNTP synthesis is balanced by utilization for DNA replication. The level at which this equilibrium is attained differed for the three deoxynucleosides, with thymidine being most efficiently phosphorylated and deoxycytidine being the least effective precursor for its dNTP. We have identified two major effects that contribute to the dilution of isotope: (i) de novo synthesis from ribonucleotides and (ii) dilution by nucleosides accumulated in the medium by excretion from cells prior to addition of isotope. The first effect is pronounced in the case of dCTP, as witnessed by the dramatic increase in the specific activity of dCTP when de novo synthesis is blocked by hydroxurea. Dilution by the medium particularly applies to deoxycytidine which we find to be the major deoxynucleoside excreted by growing 3T6 cells, with a concentration of 0.3 μM in the medium at the time of addition of isotope.

Addition of aphidicolin expanded the dTTP pool almost 2-fold, while the dCTP pool was roughly halved (Table I). Nevertheless, isotope equilibrium was apparently attained at a normal rate. With thymidine this occurred without any major change in the specific activity of dTTP, while with deoxycytidine dCTP had a 10-fold increased specific activity and with deoxycytidine the specific activity of dTTP was decreased 3-fold. These results may be explained by allosteric effects on the two relevant deoxynucleoside kinases caused by the increased dTTP and decreased dCTP pools. While deoxycytidine kinase is inhibited by dCTP and stimulated by dTTP (29, 30), the reverse is true for thymidine-deoxycytidine kinase (31, 32). In addition, the expanded dTTP pool also leads to inhibition of ribonucleotide reductase.

Aphidicolin did not change the turnover of the labeled pools. A question that arises is whether these pools were representative of the total dNTP pools or whether they represented compartmentalized pools. In the former case, the pools must be replenished continuously, requiring de novo synthesis of dNTPs in the absence of DNA replication. In the second case, de novo synthesis need not take place. We earlier found evidence for compartmentalization of dCTP but not dTTP (7). In addition, our experiments with labeled deoxyu-
...of DNA replication was degraded and excreted as thymidine. The explanation for the discrepancy is that these authors in their conclusion, into the medium. veer Reddy and Pardee (19) from similar experiments with labeled deoxyuridine arrived at the opposite...dation does occur. In contrast, the loss of purine dNTPs is change is observed after addition of aphidicolin when degra-
dnucleotides (11–13). A detailed analysis of the effect of hydroxyurea on pyrimidine dNTP pools differs markedly from that of aphidicolin. Hydroxyurea inhibits ribonucleotide reductase and thus stops de novo synthesis of deoxyribonucleotides (11–13). A detailed analysis of the effect of hydroxyurea on dNTP pools and DNA synthesis in 3T6 cells will be presented elsewhere. For the purpose of the present investigation, it is sufficient to note that the drug causes a rapid decrease in the size of the dCTP pool to about 30% of the initial size. With deoxycytidine, the level at which an equilibrium was established increased 30-fold, with the specific activity of dUMP caused by aphidicolin. Hydroxyurea greatly slowed down the decay of the labeled deoxynucleotides (11–13). A detailed analysis of the effect of hydroxyurea on dNTP pools and DNA synthesis in 3T6... Synthesis of Deoxyribonucleotides and DNA

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REFERENCES

1. Thelander, L., and Reichard, P. (1979) Annu. Rev. Biochem. 48, 133–158.
2. Lammers, M., and Follmann, H. (1983) Struct. Bond. 54, 27–91.
3. Eriksson, S., Sjöberg, B.-M., Hahne, S., and Karlström, O. (1977) J. Biol. Chem. 252, 6132–6138.
4. Eriksson, S., Thelander, L., and Åkerman, M. (1979) Biochemistry 18, 2848–2852.
5. Bjesell, G., and Reichard, P. (1973) J. Biol. Chem. 248, 3904–3909.
6. Reichard, P. (1978) Fed. Proc. 37, 9–14.
7. Nicander, B., and Reichard, P. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1347–1351.
8. veer Reddy, G. P., and Pardee, A. B. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 3312–3316.
9. veer Reddy, G. P., and Pardee, A. B. (1982) J. Biol. Chem. 257, 12526–12531.
10. Noguchi, H., veer Reddy, G. P., and Pardee, A. B. (1983) Cell 32, 443–451.
11. Young, C. W., and Hodas, S. (1964) Science (Wash. D. C.) 146, 1172–1174.
12. Turner, M. K., Abrams, R., and Lieberman, I. (1966) J. Biol. Chem. 241, 5777–5780.
13. Krakoff, I. H., Brown, N. C., and Reichard, P. (1968) Cancer Res. 28, 1559–1565.
14. Ohashi, M., Taguchi, T., and Ikegami, S. (1978) Biochim. Biophys. Res. Commun. 82, 1084–1090.
15. Huberman, J. A. (1981) Cell 23, 647–648.
16. Nicander, B., and Reichard, P. (1981) Biochem. Biophys. Res. Commun. 103, 148–155.
17. Tomich, P. K., Chiu, C.-S., Wovcha, M. G., and Greenberg, G. R. (1974) J. Biol. Chem. 249, 7613–7622.
18. Rode, W., Scainlon, K. J., Moraes, B. A., and Bertino, J. R. (1980) J. Biol. Chem. 255, 1305–1311.
19. veer Reddy, G. P., and Pardee, A. B. (1983) Nature (Lond.) 304, 86–88.
20. Skoog, L. (1970) Eur. J. Biochem. 17, 202–208.
21. Lindberg, U., and Skoog, L. (1970) Anal. Biochem. 34, 152–160.
22. Stimac, E., Housman, D., and Huberman, J. A. (1977) J. Mol. Biol. 115, 485–511.
23. Hellgren, D., Nilsson, S., and Reichard, P. (1979) Biochem. Biophys. Res. Commun. 88, 16–22.
24. Taylor, G. A., Dady, P., and Harrep, K. R. (1980) J. Chromatogr. 183, 421–431.
25. Neesholme, E. A., Challiss, R. A. J., and Crabtree, B. (1984) Trends Biochem. Sci. 9, 277–280.
26. Plagemann, P. G. W., and Wohlhueter, R. M. (1980) J. Biol. Chem. 255, 275–280.
27. Ullman, B., Kaur, K., and Watts, T. (1983) Mol. Cell Biol. 3, 1187–1196.
28. Skoog, L., and Nordenskjöld, B. (1971) Eur. J. Biochem. 19, 81–89.
29. Momparler, R. L., and Fischer, G. A. (1968) J. Biol. Chem. 243, 4298–4304.
30. Durham, J. P., and Ives, D. H. (1970) J. Biol. Chem. 245, 2276–2284.
31. Okazaki, R., and Kornberg, A. (1964) J. Biol. Chem. 239, 275–284.
32. Bresnick, E., and Thompson, U. B. (1965) J. Biol. Chem. 240, 3967–3974.