REGULATION OF THYMIDYLATE SYNTHETASE ACTIVITY IN CULTURED MAMMALIAN CELLS

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SUMMARY
Changes in thymidylate synthetase specific activity in Don Chinese hamster cells grown in vitro have been examined during the culture cycle and after exposure of lag- and log-phase cultures to drugs which inhibit DNA, RNA, and protein synthesis. During the culture cycle enzyme activity was low during lag phase, rose 6- to 8-fold before log phase, fluctuated between 5.5 and 9 nmol dTMP/h/10^7 cells during log phase, and declined to base level during stationary phase. Puromycin prevented all increases in enzyme specific activity and caused a decrease in enzyme activity when applied to log-phase cultures. Actinomycin D prevented the initial rise in enzyme activity if applied during early lag phase but caused a pronounced increase in enzyme activity above control levels when applied during log phase.

High thymidine concentration (1 mM) stopped cell division in log-phase cultures but did not alter the log-phase plateau level of thymidylate synthetase activity. Fluorodeoxyuridine stopped cell division and depressed enzyme activity to varying degrees depending upon its concentration, but at concentrations less than 10^-4 M enzyme activity eventually returned to normal log-phase levels and cell division resumed if puromycin was not present. Methotrexate stopped cell division and caused a 3- to 4-fold increase in enzyme activity above control levels if puromycin was not present. This increase occurred in the presence of actinomycin D but was retarded by addition of thymidine when actinomycin D was not present.

These experiments suggest that the regulation of thymidylate synthetase activity in log-phase cells is complex and may involve thymidine triphosphate.

INTRODUCTION
Thymidylate synthetase catalyses the conversion of deoxyuridine monophosphate to thymidine monophosphate by facilitating the transfer of a methyl group from 5,10-methylenetetrahydrofolate to the 5 position of the pyrimidine ring of dUMP (Hartmann & Heidelberger, 1961). As has been documented previously (Conrad, 1971; Kit, Dubbs & Frearson, 1965), thymidylate synthetase activity increases when cells proliferate and decreases when they stop dividing. The same relationship between cell division and appearance of enzyme activity has been demonstrated for other enzymes associated with the synthesis of the precursors for DNA synthesis such as thymidine kinase (Brent, Butler & Crathorn, 1965; Bresnick & Thompson, 1965; Eker, 1968a, b; Hotta & Stern, 1961, 1963, 1965; Kim, Gelbard & Perez, 1967; Kit, deTorres & Dubbs, 1966; Littlefield, 1965; Maley, Lorenson & Maley, 1965; McAuslan, 1963; Nagano & Mano, 1968; Stern & Hotta, 1963; Stubblefield &

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Attempts to understand the regulation of these enzymes have focused upon the relationship between DNA synthesis and the changes in activities of related enzymes. Inhibition of DNA synthesis and cell division by such drugs as arabinofuranosylcytosine, fluorodeoxyuridine, methotrexate, hydroxyurea, or mitomycin C, or by high thymidine concentrations has been shown to elicit a variety of effects on the specific activities of different enzymes associated with DNA synthesis (Eker, 1966, 1968b; Kit et al. 1966; Littlefield, 1965; Stubblefield & Mueller, 1965). These studies suggest that the activities of the enzymes associated with DNA synthesis may be regulated individually in relation to the continuation of DNA synthesis.

Recent studies of the regulatory mechanisms which control the specific activities of glutamine synthetase (Kirk, 1965; Kirk & Moscona, 1963; Moscona & Kirk, 1965; Moscona, Moscona & Saenz, 1968), alkaline phosphatase (Cox & MacLeod, 1962, 1963; Griffin & Cox, 1966a, b), tryptophan pyrrolase (Caffery, Whichard & Irvin, 1968; Feigelson & Greengard, 1962; Garren, Howell, Tomkins & Crocco, 1964; Greengard, Smith & Acs, 1963; Schimke, Sweeney & Berlin, 1964, 1965), tyrosine transaminase (Caffery et al. 1968; Granner, Hayashi, Thompson & Tomkins, 1968; Kenney, 1967; Peterkofsky & Tomkins, 1967, 1968; Reel & Kenney, 1968; Thompson, Tomkins & Curran, 1966; Tomkins et al. 1969), and several enzymes in the cellular slime mould (Roth, Ashworth & Sussman, 1968) have suggested that while primary enzyme induction in higher organisms probably does take place at the level of DNA transcription, normal maintenance of high enzyme activity and subsequent disappearance of enzyme activity may involve other epigenetic regulatory mechanisms which operate at the level of RNA translation or subsequent protein turnover. Maley et al. (1965) have demonstrated that following partial hepatectomy in the rat thymidylate synthetase activity was inhibited by amino acid analogues but scarcely influenced at all by actinomycin D or puromycin. This suggests that thymidylate synthetase regulation may involve complex control mechanisms.

The present study of the regulation of thymidylate synthetase activity in Don Chinese hamster cells was undertaken in an attempt to examine the role of protein synthesis in changes in thymidylate synthetase activity and to investigate the effects of the interruption of DNA synthesis on the level of thymidylate synthetase activity.

MATERIALS AND METHODS

Cell culture

Monolayer cultures of Don Chinese hamster cells (Hsu & Zuaxes, 1964) were grown on Eagle's medium (Eagle, 1955) fortified with 0.1% lactalbumin hydrolysate, 10% foetal calf serum, 100 units/ml penicillin, 50 μg/ml streptomycin, and 100 μg/ml kanamycin. Preparation of medium and conditions for cell culture propagation have been described previously (Conrad, 1971). Cell cultures were tested periodically for PPLO contamination (Conrad, 1971). During the course of these studies no PPLO-contaminated cell cultures were found.

To obtain sufficient numbers of cells for a time-course analysis of changes in thymidylate
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synthetase activity under varying experimental conditions, 1·6 x 10⁴ Don cells were inoculated into 250-ml Falcon tissue culture flasks containing 20 ml medium per flask, exposed to 5% CO₂ in air for 15 s, and incubated at 37 °C. Flasks were selected according to a random number table for harvesting at the times indicated in Results.

For drug studies, stock solutions of fluorodeoxyuridine (FUDR), thymidine, methotrexate, puromycin, and actinomycin D were prepared in thrice glass-distilled water at 100 x the final concentration desired, sterilized by passage through a Millipore Sweeny Adaptor, and applied in the appropriate amounts to the test cell cultures at the times indicated in Results. Stock solutions of methotrexate also contained serine (30 mg/l.), glycine (10 mg/l.), and hypoxanthine (1·4 mg/l.).

Enzyme assay

For each enzyme sample approximately 10⁷ cells were collected by trypsinization, washed 3 times with isotonic saline, pelleted, overlaid with 0·01 M Tris-0·25 M sucrose buffer, pH 7·0, to give a final cell concentration of 2·2-2·3 x 10⁷ cells/ml, and sonically disrupted as described previously (Conrad, 1971). The sonicate was centrifuged at 27,000 g, at 0 °C, for 1 h, and the supernatant was used for determination of thymidylate synthetase activity.

Thymidylate synthetase activity was determined by the method of Roberts (1966) in which the amount of tritium released to water from the 5 position of the pyrimidine ring of deoxyuridine monophosphate upon its conversion to thymidine monophosphate (dTMP) was measured in a scintillation counter after absorption of all nucleotides by activated charcoal. Duplicate assays of individual samples by this method gave values which varied by less than 10%. The specific activity of thymidylate synthetase was expressed as the number of nmol of product formed per h per 10⁷ cells.

Chemicals

FUDR was a gift from Hoffman-LaRoch, Inc. Methotrexate was obtained from The American Cyanamide Company, puromycin from Nutritional Biochemical Corporation, thymidine from K and K Laboratories, and actinomycin D from Merck Company.

Tris, tetrahydrofolic acid, and unlabelled deoxyuridine monophosphate were obtained from Sigma Chemical Company. Tritium-labelled deoxyuridine monophosphate (4·7 mCi/mmol, 0·5 mCi/ml) was obtained from Calbiochem Company, Los Angeles, California. Activated Charcoal, N. F. Powder was purchased from Merck Company. BBOT, 2,5-bis-[2-(5-tert-butylbenzoxazolyl)]-thiophene, was obtained from Packard Instrument Company, Inc., and was prepared according to their instructions.

RESULTS

Variation in thymidylate synthetase specific activity during the culture cycle

Characteristic changes in the specific activity of thymidylate synthetase per cell occurred during the culture cycle of Don Chinese hamster cells (Fig. 1). The specific activity of the enzyme was low, about 1·1-1·5 nmol dTMP/h/10⁷ cells, during the early part of lag phase (0-10 h after subculture), but the activity rose sharply during the latter half of lag phase before any increase in cell number was observed in the cultures, reaching 7 nmol dTMP/h/10⁷ cells by 18 h after subculture. As the cells proceeded through log phase, the specific activity of thymidylate synthetase oscillated between 5·5 and 8 to 9 nmol dTMP/h/10⁷ cells. The apparent rise in enzyme specific activity in late log phase of Fig. 1 is probably not significant. Other late-log-phase cultures showed enzyme activities between 6·5 and 7·5 nmol dTMP/h/10⁷ cells. In all cases, however, when the cultures reached confluency and the cells entered stationary phase, enzyme activity declined toward basal level again in a biphasic pattern, lingering in the lower log-phase range and then decreasing to the early lag-phase level later.
Effect of inhibitors of protein and RNA synthesis on the pattern of thymidylate synthetase specific activity during the culture cycle

The initial late-lag-phase rise in thymidylate synthetase specific activity was completely prevented by addition of puromycin (10 \(\mu g/ml\) final concentration) to early-lag-phase Don cultures (Fig. 2). Moreover, cultures exposed to puromycin in early lag phase never entered the log phase of cell proliferation.

When puromycin was added to mid-log-phase cultures cell division continued at the normal rate for about 4 h, but then it ceased abruptly and did not resume over the next 18 h. Changes in thymidylate synthetase specific activity paralleled those observed when Don cultures entered stationary phase under normal confluency.
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That is, enzyme activity remained high for 3–4 h while cell division was continuing at the control rate, but then the activity decreased in a biphasic pattern as cell division ceased in the puromycin-treated cultures.

Addition of actinomycin D (5 μg/ml final concentration) to Don cells inhibited the uptake of [3H]uridine into acid-insoluble precipitates by more than 99% during the 2 h immediately following drug administration (unpublished results). When this concentration of actinomycin D was applied either at the time of cell transfer or 4–5 h after subculture, the subsequent late-lag-phase rise in thymidylate synthetase specific activity did not occur (Fig. 3), and no cell division took place in the drug-treated cultures. On the other hand, when actinomycin D was applied to mid-lag-phase cultures after the initial rise in thymidylate synthetase activity had begun, there did follow a subsequent rise in enzyme activity over the next 12 h in the absence of any cell division. However, in the drug-treated cultures the enzyme activity per cell increased in a linear fashion at a rate equivalent to that which had been achieved in the cultures at the time of drug administration, whereas enzyme activity per cell in the control cultures increased in a logarithmic fashion over the same 12-h period (Fig. 3).

On the other hand, when actinomycin D was applied to late-lag-phase or log-phase conditions (Fig. 2). That is, enzyme activity remained high for 3–4 h while cell division was continuing at the control rate, but then the activity decreased in a biphasic pattern as cell division ceased in the puromycin-treated cultures.

Fig. 2. Effect of puromycin on thymidylate synthetase activity during the culture cycle of Don cells. This is a composite graph of several separate experiments superimposed on the control enzyme activity profile taken from Fig. 1. Cultures were established as described in Methods. Puromycin (10 μg/ml final concentration) was added to some cultures at the times indicated by the arrows; the remaining cultures became untreated controls. Enzyme samples were prepared as described in Methods and assayed as described for Fig. 1. Separate controls were used for each experiment and almost exactly paralleled the composite control shown here. ○, control; ▲, with puromycin.
cultures (Fig. 3) cell division stopped, but enzyme activity continued to increase over the next 12 h, reaching levels twice as high as the normal log-phase plateau in enzyme specific activity. The maximum possible increase in enzyme activity under these conditions is not known since these experiments were terminated 12 h after addition of actinomycin D to the cultures.

![Composite graph showing effect of actinomycin D on thymidylate synthetase activity.](image)

**Fig. 3.** Effect of actinomycin D on thymidylate synthetase activity. This composite graph was compiled from experiments conducted in the same manner as those described for Fig. 2 except that at the times indicated by the arrows actinomycin D (5 μg/ml final concentration) was added to some cultures. O, control; ●, with actinomycin D.

**Effect of high concentrations of thymidine on cell growth and thymidylate synthetase specific activity**

Early log-phase Don cultures exposed to high concentrations of thymidine (1 mM) stopped cell division after an increase of about $2 \times 10^6$ cells to the population. However, the cells remained attached to the substratum for the next 24 h, and they maintained a thymidylate synthetase specific activity within the normal log-phase range of 6–9 nmol dTMP/h/10^7 cells (Fig. 4). On the other hand, when high concentrations of thymidine and puromycin were added simultaneously to Don cultures, cell division ceased after an increase in cell number of about $1 \times 10^6$ cells. The cells began to slough off the substratum by 12 h after drug administration, and thymidylate synthetase specific activity decreased to 4–4.7 nmol dTMP/h/10^7 cells, a level below the activity range normally observed in log-phase cultures of Don cells (Fig. 4).
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Fig. 4. Effect of high concentration of thymidine on thymidylate synthetase activity in log-phase cultures of Don cells. Don cells were established in cultures as described in Methods; 16 h after subculture 1 mM thymidine (final concentration) was added to some cultures, 1 mM thymidine plus 10 μg/ml puromycin (final concentrations) were added to others, and controls received no drugs. Enzyme samples were prepared as described in Methods and assayed as described for Fig. 1. ○, control; ●, with 1 mM thymidine; ▲, with 1 mM thymidine + puromycin.

Effect of FUDR on cell growth and thymidylate synthetase specific activity

FUDR is converted to fluorodeoxyuridine monophosphate when it is taken into growing cells (Harbers, Chaudhuri & Heidelberger, 1959). Fluorodeoxyuridine monophosphate is a competitive inhibitor for thymidylate synthetase and it has a very low Ki for this enzyme (Hartmann & Heidelberger, 1961; Heidelberger, Kaldor, Mukherjee & Danneberg, 1960; Reyes & Heidelberger, 1965). Thus FUDR prevents the de novo synthesis of phosphorylated thymidine in cells, thereby creating a thymidine triphosphate starvation which inhibits cell division and DNA synthesis.

Don cultures, inoculated at about 8 × 10⁴ cells/ml of medium, incubated for 24 h, and then exposed to different concentrations of FUDR during early log phase, subsequently exhibited one of 3 different cell proliferation patterns (Fig. 5): (a) at FUDR concentrations of 5 × 10⁻⁸ M or less the number of cells per culture continued to increase at a rate comparable to control cultures; (b) at FUDR concentrations of approximately 10⁻⁷ M the number of cells per culture increased by 4 × 10⁶, then remained constant for 6–8 h, and finally began to increase again at a rate equivalent to control cultures at a comparable cell density; or (c) at 10⁻⁸ M FUDR or more the number of cells per culture increased by about 4 × 10⁶, then plateaued at that cell
number and did not increase again during the course of the experiment. However, addition of $5 \times 10^{-8}$ M thymidine to cultures permanently arrested by treatment with $10^{-6}$ M FUDR allowed cell division to resume immediately at control rates (unpublished results).

The effects of these various concentrations of FUDR on the specific activity of thymidylate synthetase are illustrated in Fig. 6. At $10^{-6}$ M FUDR enzyme activity was immediately reduced to 20% of its original value where it remained throughout the course of the experiment. At $10^{-7}$ M FUDR there was an immediate but slightly less severe reduction in thymidylate synthetase specific activity which was maintained for several hours, but then the enzyme activity returned to the control level over the ensuing several hours. Cell division stopped at about 6 h after $10^{-7}$ M FUDR administration but began again when enzyme activity had regained 85% of the control level (compare time scales in Figs. 5 and 6). At FUDR concentrations of $5 \times 10^{-8}$ M or less the initial reductions in thymidylate synthetase activity were sequentially less pronounced than at the higher FUDR concentrations, and the lengths of time necessary for enzyme activity to return to within about 80% of the control level were shorter than the amount of time necessary to increase the culture populations by $4 \times 10^8$ cells (compare Figs. 5 and 6). Hence, cell division continued without interruption in cultures treated with $5 \times 10^{-8}$ M FUDR or less.

Simultaneous addition of puromycin with $5 \times 10^{-7}$ M FUDR prevented the
subsequent recovery of thymidylate synthetase activity (Fig. 7). In the absence of puromycin, however, enzyme activity reappeared at a rate sufficient to raise the specific activity by 1 nmol dTMP/h/10^7 cells. Once cell division resumed in recovered cultures, thymidylate synthetase specific activity did not increase above control log-phase activity levels.

Fig. 6. Effect of FUDR on thymidylate synthetase activity in log-phase cultures. Enzyme samples were prepared as described in Methods from cell samples obtained in the experiments described in Fig. 5. Separate controls were run for each experiment and were used for calculation of % control enzyme activity for each FUDR concentration. % control thymidylate synthetase specific activity for cultures exposed to FUDR as follows: O, 10^{-6} M; •, 10^{-7} M; ▲, 5 \times 10^{-8} M; △, 10^{-8} M; and □, 5 \times 10^{-8} M.

Effect of methotrexate on cell growth and thymidylate synthetase activity

Methotrexate inhibits dihydrofolate reductase activity (Hillcoat et al. 1967), thus limiting the intracellular supply of tetrahydrofolate, a co-factor in the thymidylate synthetase reaction. To limit the effect of methotrexate to substrate deprivation for thymidylate synthetase alone, cultures treated with this drug were also supplemented with serine, glycine, and hypoxathine (see Methods).

In log-phase Don cultures exposed to 10^{-6} M methotrexate the number of cells per culture increased by about 2–3 \times 10^8 cells. Cell division then stopped but the cells remained attached to the substratum for more than 24 h. Moreover, thymidylate
synthetase specific activity increased to 4-fold the control log-phase level in the 24 h following administration of methotrexate to the cells (Fig. 8). Again the average rate of appearance of new enzyme activity was sufficient to increase the specific activity by 1 nmol dTMP/h/10^7 cells, as it was in the recovery of enzyme activity after exposure to FUDR.

Puromycin prevented this methotrexate-stimulated increase in thymidylate synthetase specific activity when it was added to cultures concurrently with methotrexate (Fig. 8). On the other hand, actinomycin D added concurrently with methotrexate (unpublished results) or 5 h after administration of methotrexate (Fig. 8) neither prevented nor diminished the methotrexate stimulation of thymidylate synthetase specific activity.

When thymidine was added at various time intervals after administration of methotrexate to cells, cell division resumed in the cultures and the methotrexate stimulation of thymidylate synthetase specific activity was diminished in proportion.
to the length of time that thymidine had been returned to the cells (Fig. 9). If actinomycin D was present in addition to methotrexate, the subsequent addition of thymidine was not effective in restoring cell division or in reducing methotrexate stimulation of enzyme activity (Fig. 9).

DISCUSSION

The data suggest that the regulation of thymidylate synthetase activity in proliferating mammalian cells is complex. Clearly there is a structural gene for the enzyme itself, since actinomycin D inhibition of transcription during lag phase prevented the appearance of thymidylate synthetase activity. When RNA synthesis was inhibited in cells, the messenger RNA for thymidylate synthetase appeared to be stable for up to
Fig. 9. Effect of thymidine on thymidylate synthetase activity in log-phase cultures treated with methotrexate or methotrexate + actinomycin D. Cultures of Don cells were established as described in Methods; 24 h later some cultures received $10^{-6}$ M methotrexate, others $10^{-5}$ M methotrexate + actinomycin D (5 µg/ml final concentration) and controls received no drugs. At 3, 6, or 9 h thereafter $5 \times 10^{-6}$ M thymidine was added to some cultures which had received methotrexate alone and to some cultures which had received methotrexate + actinomycin D. Enzyme extracts were prepared as described in Methods starting from the time that methotrexate was added to all cultures, and assays were performed as described for Fig. 1. ○, controls; ●, with methotrexate; ▲, with methotrexate + thymidine at 3 h; ■, methotrexate + thymidine at 6 h; ▼, methotrexate + thymidine at 9 h; Δ, methotrexate + actinomycin D + thymidine at 3 h; □, methotrexate + actinomycin D + thymidine at 6 h; ▽, methotrexate + actinomycin D + thymidine at 9 h.

24 h. However, the half-life of thymidylate synthetase messenger RNA may be different under normal cellular conditions.

All increases in thymidylate synthetase specific activity were puromycin-sensitive, thus suggesting that increases in enzyme activity result from *de novo* synthesis and subsequent accumulation of additional enzyme molecules in cells. Similar data and conclusions have been drawn for other enzyme systems (Eker, 1968a, b; Feigelson & Greengard, 1962; Garren *et al.* 1964; Granner *et al.* 1968; McAuslan, 1963; Moscona *et al.* 1968; Reel & Kenney, 1968; Schimke *et al.* 1964). However, puromycin-sensitive changes in the conformational state of the enzyme molecule, such as have been demonstrated for alkaline phosphatase (Griffin & Cox, 1966b), have not been ruled out as a possible cause of increases in thymidylate synthetase activity.

The evidence regarding thymidylate synthetase turnover is contradictory. Purom-
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mycin did not appear to stabilize enzyme activity when administered alone to log-phase cells, but it did cause partial stabilization in the presence of high concentrations of thymidine and total stabilization in the presence of methotrexate. Further experimentation is required to determine whether directed turnover, such as has been described for tyrosine transaminase (Kenney, 1967; Reel & Kenney, 1968) exists for thymidylate synthetase under normal cellular conditions.

Phosphorylated thymidine appears to play a role in the regulation of thymidylate synthetase activity. Both FUDR and methotrexate stopped cell division under conditions which resulted in a depletion of thymidine triphosphate in cells. Both drugs caused puromycin-sensitive increases in thymidylate synthetase activity which were abated when the phosphorylated thymidine contents of cells were restored to levels sufficient to support cell division. On the other hand, when cell division was stopped by high concentrations of thymidine, a condition which leads to high intracellular concentrations of thymidine triphosphate (Cleaver, 1967), thymidylate synthetase specific activity did not increase. Neither thymidine monophosphate nor thymidine triphosphate feedback inhibit the biological activity of thymidylate synthetase (Hartmann & Heidelberger, 1961; Reyes & Heidelberger, 1965). Therefore, thymidine triphosphate must exert its influence on thymidylate synthetase activity by some other mechanism.

The data are consistent with the possibility that a second regulator gene is involved in the control of thymidylate synthetase activity. Actinomycin D applied during log phase stopped cell division and caused an increase in thymidylate synthetase specific activity above control levels. Analogous results have been described for other enzyme systems in higher organisms (Garren et al. 1964; Moscona et al. 1968; Reel & Kenney, 1968; Roth et al. 1968), and have been interpreted as evidence for the existence of a second regulatory RNA species whose transcription is induced concurrently with or subsequent to the initial induction of the target enzyme messenger RNA. The actinomycin-D stimulated increase in thymidylate synthetase activity would seem to result from the inhibition of transcription and not merely from the cessation of enzyme dilution by cell division. Cell division could be stopped under other conditions without causing a concomitant elevation in thymidylate synthetase activity. Since the thymidylate synthetase system locked in the 'on' condition in the presence of actinomycin D, the RNA product of such a regulatory gene would have to be more labile than thymidylate synthetase messenger RNA.

Perhaps thymidine triphosphate interacts as an effector molecule with the hypothetical thymidylate synthetase regulator RNA. A similar system has been discussed recently by Tomkins et al. (1969) for tyrosine transaminase regulation, except that in Tomkins' model the effector molecule prevents the function of the regulatory RNA, whereas in this thymidylate synthetase model the effector molecule facilitates the function of the proposed regulatory RNA system. The mechanism of action of such a thymidylate synthetase regulatory system remains to be resolved.

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REFERENCES


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