STIMULATION OF DNA SYNTHESIS BY ADENOSINE DIPHOSPHORIBOSYLATION OF HELA NUCLEAR PROTEINS DURING THE CELL CYCLE

Jerry H. Roberts, Patricia Stark and Mark Smulson

Department of Biochemistry
Georgetown University
School of Medicine--School of Dentistry
Washington, D.C. 20007

Received March 9, 1973

Summary

The inhibition of DNA synthesis by ADP-ribosylation of nuclear proteins noted in rat liver nuclei, does not occur during various phases of the cell cycle of HeLa cells. In contrast, a significant enhancement of template activity is noted for either endogenous or microbial DNA polymerases due to this nuclear modification. The greatest stimulation was noted with G2 HeLa nuclei. The stimulation is specific for the generation of the poly-ADPR polymer and transcription is essentially not affected by this modification during the cell cycle.

The enzyme poly ADPR polymerase catalyzes a unique modification of chromosomal proteins in eucaryote cells in which the ADPR moiety of NAD is covalently attached and polymerized to various species of these proteins. We have previously reported a relationship between the specific activity of poly ADPR polymerase and macromolecular events during the cell cycle (1) and during asynchronous growth of HeLa cells (2). Undoubtably the relationship between *in vitro* activity of this enzyme and *in vivo* function is quite complicated.

The work of Burzio and Koide (3,4) with rat liver nuclei have suggested that one biological function of ADP-ribosylation of nuclear proteins is to repress replication of DNA. ADP-ribosylated rat liver nuclei or chromatin have been reported to be virtually inert for DNA synthesis either with endogenous DNA polymerases or with exogenously supplemented DNA polymerase from *M. lysodeikticus* (3,4). However, as described below, in HeLa cells we have found just the opposite to be true. The biosynthesis of poly ADPR in HeLa nuclei greatly enhanced the template capacity of these nuclei when used by exogenous or

endogenous DNA polymerases. Furthermore, this enhancement seems to be related to events in the cell cycle of these cells.

Materials and Methods

NAD (adenosine-^{3}H) (1.13 Ci/mM) was obtained from NEN. Thymidine-(methyl-^{3}H) (5 Ci/mmole) and thymidine-(methyl-^{3}H)-5'-triphosphate (23.9 Ci/mmole) were purchased from Amersham Searle Co. DNA polymerase from *M. lysodeikticus* and RNA polymerase from *E. coli* K-12 were purchased from Miles Laboratories. *E. coli* DNA polymerase I was purchased from General Biochemicals.

DNA synthesis in intact nuclei was performed as described by Burzio and Koide (3) with the following modifications: each unlabeled deoxyribonucleoside triphosphate was present at a final conc. of 0.2mM, the conc. of ^{3}H-TTP was 0.025mM (160 μCi/umole), and 0.5mM ATP was added to the assay mixture.

Determination of ^{3}H-thymidine into DNA by intact cells, poly ADPR polymerase, DNA and protein were described previously (1,2).

Results and Discussion

The data in table 1 show the formation of poly ADPR in HeLa nuclei did not significantly inhibit DNA synthesis with either endogenous or exogenous DNA polymerase in contrast to data of others with rat liver (3-6). It is especially important to note that when ADPR-modified nuclei (line 4) in contrast to non-modified nuclei (line 3) or partially modified nuclei (line 5) were used as a template for *E. coli* DNA polymerase I or *M. lysodeikticus* DNA polymerase (not shown) a marked stimulation of DNA synthesis was observed. A stimulation of DNA synthesis has not been previously reported due to ADP-ribosylation of nuclear proteins.

We tested whether the inhibitory phenomenon might be a cell cycle related event since past work indicating a marked inhibition of *in vitro* DNA synthesis by poly ADP-ribose had been performed with

Table 1. Effect of ADP-ribosylation on DNA Synthesis with Endogenous and Exogenous DNA Polymerase

Experiment		Preincubation with 4mM NAD	*E. coli* DNA Polymerase	cpm	% of Control
A	1	-	-	3916	-
	2	+	-	3391	87
B	3	-	+ (1.4 units)	917	-
	4	+	+	2225	243
	5	+, plus 10mM nicotinamide	+	1513	165

A 0.5ml. reaction mixture containing 4 x 10^7 nuclei (experiment A) or 4 x 10^6 nuclei (experiment B), 50 µmoles Tris, pH 7.4, 0.5 µmoles dithiothreitol, 1 µmole $MgCl_2$ was incubated as indicated above. After 15 minutes at 25°, 3.0ml. of a solution containing 0.25M sucrose, 3mM $CaCl_2$ and 5mM $MgCl_2$ was added to the nuclei. The nuclei were collected by centrifuging at 1000xg for 5 minutes and were then resuspended in 0.5ml. of the assay mix for DNA synthesis (15 min.).

essentially non-S phase rat liver nuclei. When DNA synthesis was measured in nuclei derived from S, G2-M and G1 cells by using endogenous DNA polymerases the data in Table 2 were obtained. Note that no inhibition of *in vitro* DNA synthesis by poly ADPR was observed during the cell cycle. In fact, slight stimulation of endogenous DNA synthesis was noted by formation of poly ADPR.

Specificity of Stimulation

The marked stimulation of incorporation of TMP into DNA as catalyzed by exogenous enzyme (Table 1, line 4 and figure 1) was shown to be specific for the formation of poly ADPR in nuclei as

Table 2. Effect of ADP-ribosylation on ^{3}H-TMP Incorporation by Nuclei from Synchronized Cells

Hours after thymidine addition	Cell Phase	Preincubation with 4mM NAD	^{3}H-TMP incorporated dpm/μg DNA	dpm/μg protein
4	S	-	11.16	2.85
		+	14.68	3.74
10	G2-M	-	3.42	0.84
		+	4.05	0.99
17	G1	-	6.42	1.02
		+	7.99	1.27

The preincubation procedure and the measurement of ^{3}H-TMP incorporation were described in Table 1. Each assay tube contained 2 x 10^{7} nuclei.

evidenced by inhibition of stimulation when poly ADPR polymerase was inhibited by either nicotinamide or thymidine (7). Preincubation of nuclei with either NADH or NADP failed to cause stimulation. NAD had no effect on the *E. coli* enzyme promoted assay either with calf thymus template or with HeLa nuclei template. In addition, kinetic analysis of TMP incorporation was essentially linear for both controls and nuclei modified by ribosylation.

An attractive hypothesis might be that in intact mammalian cells, poly ADP ribose is involved in ligation of DNA fragments and that this might be contributing to the stimulation we have noted here. While NAD is known to be the substrate for polynucleotide ligase in bacteria (8), ATP has been described as the substrate for the mammalian enzyme (9). The data in Table 3 show that the forma-

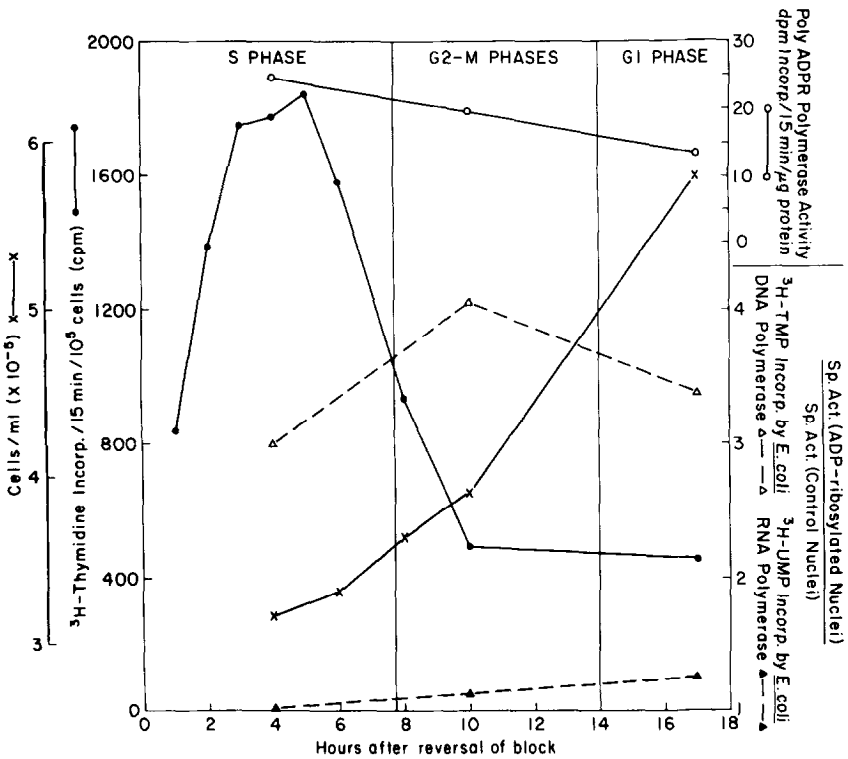

Figure 1. Extent of ADP-ribosylation of nuclear proteins during the cell cycle and effect on RNA and DNA synthesis.

A suspension culture (1500 ml) of HeLa cells was synchronized with methotrexate (13). After 16 hours, the block was reversed by addition of 5 x 10^{-6} M thymidine (time zero) and the cell number (x) and rate of ^{3}H-thymidine incorporation (●) were determined. Nuclei prepared from cells harvested 4, 10 and 17 hours after reversal of the block were used to measure: poly ADPR polymerase specific activity (○); the ratio of E. coli DNA polymerase-dependent ^{3}H-TMP incorporation utilizing ADP-ribosylated nuclei versus control nuclei (△); and the ratio of E. coli RNA polymerase-dependent ^{3}H-UMP incorporation utilizing ADP-ribosylated nuclei versus control nuclei (▲).

---

tion of poly ADPR markedly stimulates the activity of E. coli polymerase to utilize HeLa DNA even in the absence of ATP. In fact an additive effect was noted when both compounds were provided for the E. coli enzyme. Experiments to be described elsewhere (10) show that the stimulation of HeLa chromatin activation is in fact due to increased DNA nicks (as evidenced on alkaline sucrose gradients) promoted by ADP-ribosylation. We have also found that ATP ligation

Table 3. Effect of ADP-ribosylation and ATP on Template Capacity of HeLa nuclei

Preincubation with 4mM NAD	ATP	cpm
-	-	489
-	+	697
+	-	792
+	+	1122

The experiment was performed as described in experiment B of Table 1. E. coli DNA polymerase was present at a concentration of 1.4 units per assay. Where noted ATP was present at a final concentration of 0.5 mM.

of these nicked pieces during the preincubation overcomes the NAD stimulation.

To investigate whether stimulation of polymerase I-promoted incorporation of TMP into HeLa DNA was a function of event of the cell cycle, cells were synchronized (figure 1). In contrast to data we have published previously using low NAD levels in the assay (1), the specific activity of poly ADPR polymerase appeared to be highest during S phase of the cell cycle (figure 1).

Whereas the template capacity of ADP-ribosylated nuclei is markedly increased for exogenous E. coli DNA polymerase (Table 1, line 4) the data in figure 1 indicates the selective nature of this activation since transcription performed by E. coli RNA polymerase was essentially not stimulated by the modification. The data in figure 1 also show that while nuclear modification greatly stimulate the ability of E. coli DNA polymerase to incorporate TMP into DNA

throughout the cell cycle, the most marked effect (4-fold) was noted in nuclei isolated from cells in G2-M phases. The relationship between poly ADPR polymerase activity and the stimulation of TMP incorporation is not clear, however, greatest stimulation was noted when enzyme activity was intermediate between the S and G1 levels.

It is probably significant that the stimulation noted in our study was most marked with an exogenous repair type enzyme although slight relation was noted with endogenous enzyme. This might indicate that regions in HeLa cell nuclear DNA are made more accessible for an enzyme such as polymerase I by ADP-ribosylation and, in fact evidence for this will be reported elsewhere (10).

In conclusion, virtually no inhibition of DNA synthesis in HeLa nuclei could be detected due to ADP-ribosylation. It is of interest that the inhibitory effect could not be found by others using either hepatoma nuclei (11) or using rat liver nuclei and exogenous *E. coli* DNA polymerase (12).

Acknowledgement

This investigation was supported by a U.S. Public Health Services grant (CA-13195).

References

1. Smulson, M., Henriksen, O. and Rideau C., Biochem. Biophys. Res. Commun., 43, 1266 (1971).
2. Smulson, M.E. and Rideau, C., Biochim. Biophys. Acta, 111, 408 (1972).
3. Burzio, L. and Koide, S.S., Biochem. Biophys. Res. Commun., 40, 1013 (1971).
4. Burzio, L. and Koide, S.S., Biochem. Biophys. Res. Commun., 42, 1185 (1971).
5. Hilz, H. and Kittler, M., Hoppe-Seyl, Z. Physiol. Chem., 352, 1693 (1971).
6. Lehmann, A.R. and Shall, S., FEBS Letters, 26, 181 (1972).
7. Preiss, J., Schlaeger, R. and Hilz, H., FEBS Letters, 19, 244 (1971).
8. Gellert, M., Proc. Natl. Acad. Sci. U.S., 57, 148 (1967).
9. Lindahl, T. and Edelman, G.M., Proc. Natl. Acad. Sci. U.S., 61, 680 (1968).

10. Roberts, J.H. and Smulson, M.E., Manuscript in preparation.
11. Burzio, L. and Koide, S.S., FEBS Letters, 20, 29 (1972).
12. Nagao, M., Yamada, M., Mirva, M. and Sugimura, T., Biochem. Biophys. Acta, 269, 51 (1972).
13. Kidwell, W.R., Biochim. Biophys. Acta, 269, 51 (1972).