INHIBITION OF DNA-PRIMED RNA SYNTHESIS DURING POLIOVIRUS INFECTION OF HUMAN CELLS*

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It was shown previously that poliovirus infection of HeLa cells leads within several hours to a drastic change in the base ratios of newly-synthesized RNA, and it was suggested that the synthesis of normal cell RNA is inhibited (Holland, 1961). Data in press indicate that most RNA synthesis during poliovirus infection is virus-directed (Holland, 1963a) and that host cell-controlled RNA synthesis is in fact suppressed up to 90% during poliovirus infection (Holland, 1963b). The present report concerns the mechanism by which virus suppresses normal cell RNA synthesis. It will be demonstrated that DNA extracted from infected cells is as capable of priming RNA synthesis with E. coli RNA polymerase as normal cell DNA. Even the "native" deoxyribonucleoproteins of gently-disrupted normal and infected HeLa cells are equal in priming ability for the E. coli RNA polymerase described by Chamberlin and Berg (1962). However, "aggregate enzyme" (DNA-protein complex containing RNA polymerase [Weiss, 1960]) extracted from infected cells shows only a small fraction of the RNA polymerizing activity of similar preparations extracted from normal cells.

Baltimore and Franklin (1962) have recently reported a similar depression of aggregate enzyme activity in Mengo virus infection of mouse cells.

Methods: HeLa cells were grown as monolayers in glass bottles in a medium consisting of 10% calf serum, 0.1% yeast extract, and 0.1% proteose peptone #3 (Difco) in Hanks balanced salt solution. Cells were infected by a 10 minute exposure to a high multiplicity of type 1 poliovirus (Mahoney) (effective multiplicity > 10). Normal or infected cells were removed from glass by

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treatment with 0.05% trypsin to prepare cell suspensions for extraction of nucleic acid, nuclei, and aggregate enzyme. Cells were washed twice to remove residual trypsin.

HeLa cell nuclei were prepared by gentle grinding of cells at 0° in a Potter-Elvehjem homogenizer with a loose-fitting pestle in a medium containing 10^{-3} M tris buffer pH 7.2, and 2 x 10^{-3} M β mercaptoethanol. Aggregate enzyme was prepared from nuclei according to Weiss (1960) and tested for RNA polymerase activity in the high ionic strength reaction mixture of Goldberg (1961).

E. coli RNA polymerase was purified from E. coli strain K12 as described by Chamberlin and Berg (1962), who used E. coli strain B. The purified enzyme behaved as described by Chamberlin and Berg except for the absence of detectable polyadenylic acid formation in the K12 preparations. The reaction mixture of Chamberlin and Berg was employed for all studies using E. coli polymerase, and ATP (labelled with P^{32} in the phosphorous atom proximal to ribose) was used as the radioactive nucleotide. Incorporation of ATP into acid-insoluble polynucleotide was determined after about 10 x precipitation of the reaction mixture with 0.3 N perchloric acid and alcohol, and boiling in 1% sodium dodecyl sulfate for 2 minutes to release non-specifically bound ATP^{32} from acid-alcohol insoluble material. Product RNA was hydrolyzed by treatment with KOH for 18 hr at 37° (Davidson and Smellie, 1952) and individual nucleotides separated by paper ionophoresis (Markham and Smith, 1952).

ATP^{32} was prepared by incubating 5' AMP^{32} with a crude E. coli kinase preparation (based on purification method of Lehman et al., 1958), phosphoenolpyruvate and pyruvate kinase (obtained from Calbiochem). AMP^{32} was synthesized enzymatically using 4-6 millicuries P^{32}-labelled orthophosphate, adenosine, and a crude enzyme extract from brewers yeast. Following chromatography on Dowex 1 x 2 (Lehman, et al., 1958), over 10 μM ATP^{32} were usually obtained with a specific activity between 2 and 6 x 10^7 CPM/μM.

DNA was prepared by shaking HeLa cells with equal volumes of reagent grade liquid phenol and 2% sodium dodecyl sulfate in 1 M NaCl. DNA was precipitated from the aqueous phase with ethanol and reprecipitated several times.

Results and Discussion: In order to determine whether DNA from infected HeLa cells was capable of priming RNA synthesis the E. coli polymerase of Chamberlin and Berg (1962) was used. Infection did not significantly alter the amounts (about 1μM/10^7 cells) of DNA that were present in normal cells and in cells infected for 5, 7 and 10 hr. Table 1 shows that DNA from normal and infected cells primed equally well at limiting concentrations of DNA. Similar results have been obtained when DNA was extracted by a mild procedure (ethanol precipitation from 1 M NaCl). Furthermore, even deoxyribonucleoprotein (DNP) from disrupted HeLa cell nuclei showed no differences in priming ability following poliovirus infection.

TABLE 1. ABILITY OF DNA OR DNP FROM POLIOVIRUS-INFECTED HeLa CELLS TO PRIME RNA SYNTHESIS WITH E. COLI RNA POLYMERASE

Nature and Quantity of HeLa Cell DNA primer	Incorporation of ATP^{32} (mμM)
200 mμM normal cell DNA	3.7
200 mμM normal cell DNA, denatured at 100°	3.7
200 mμM DNA from HeLa cells infected 6 hr	3.8
200 mμM DNA from HeLa cells infected 10 hr	4.0
no DNA	< 0.02
25 mμM normal cell DNA	0.78
25 mμM DNA from HeLa cells infected 6.5 hr	0.86
normal HeLa cell nuclear DNP*	0.42
nuclear DNP from HeLa cells infected 5 hr*	0.44
minus E. coli polymerase	< 0.02
minus UTP, CTP, GTP	< 0.02

Reaction mixture of Chamberlin and Berg (1962) contained in 0.25 ml total: 10 μM tris, pH 8.0; 0.25 μM $MnCl_2$; 1.0 μM $MgCl_2$; 100 μM each GTP, UTP, CTP; 100 μM α labelled ATP^{32}; 3.0 μM β mercaptoethanol; and 100 units E. coli RNA polymerase. DNA or DNP supplied as indicated. Reaction allowed to proceed 10 min in 37° water bath.

* Nuclear DNP is the insoluble DNP sedimented at 4000 x g after disrupting normal or infected HeLa cell nuclei by repeated cycles of freezing and thawing in 0.15 M NaCl. The yield from approximately 10^7 cell nuclei was used in each reaction mixture.

Table 2 shows that the RNA product had approximately the same nearest neighbor to adenine relationships (Josse et al., 1961), whether DNA from normal or infected cells was used as template. This indicates no major changes in the composition of the RNA synthesized.

TABLE 2. NEAREST NEIGHBOR TO ADENYLATE RELATIONSHIPS IN NUCLEOTIDES OF RNA SYNTHESIZED USING DNA FROM NORMAL AND POLIOVIRUS-INFECTED HeLa CELLS AS PRIMER FOR E. COLI RNA POLYMERASE

Primer	Total CPM in RNA	Ratio of P^{32} counts in each nucleotide after KOH hydrolysis of RNA*			
		U	G	A	C
normal HeLa cell DNA	27,000	1.00	0.82	1.28	0.90
DNA from HeLa cells infected 7 hr	26,000	0.98	0.85	1.29	0.88

RNA polymerase reaction conditions as in Table 1.
* Ratios of nucleotide radioactivities calculated to an arbitrary total of 4.

It appears, therefore, that supression of host cell RNA synthesis during poliovirus infection is not due to breakdown of DNA, nor to permanent alterations preventing DNA priming, nor even to firm masking of DNA by protein in some manner to prevent contact with polymerase, since nuclear DNP from infected cells behaves like normal cell DNP. However, E. coli RNA polymerase may differ greatly from HeLa cell RNA polymerase in this respect. The nuclear DNP from both normal and infected HeLa cells was very inefficient in priming RNA synthesis, as compared to purified DNA. This agrees with the finding of Huang and Bonner (1962) who used pea seedling chromosomal DNP.

Next, the activity of "aggregate enzyme" RNA polymerase from normal cells was compared to that from infected cells. Table 3 shows that RNA polymerase activity was very low with "aggregate enzyme" from infected HeLa cells as compared with aggregate enzyme from an equal number of normal cells. Since the DNA (or DNP) templates of infected cells retain priming ability at least for E. coli RNA polymerase (Table 1), it is probable that the suppression of RNA polymerase activity seen following poliovirus infection (Table 3) is due either to interference with or destruction of RNA polymerase enzymatic activity, or with its

access to nuclear DNA or else to suppressed synthesis of RNA polymerase. These alternatives could not be tested directly since it has not yet proved possible to isolate mammalian cell RNA polymerase quantitatively and reproducibly in soluble form free from DNA. However, by treating normal cell nuclei or aggregate enzyme with concentrated lysates of poliovirus-infected HeLa cells, it could be shown that infected cells did not contain any soluble substance capable of blocking RNA polymerase activity under the conditions used for *in vitro* assay (Table 4). Furthermore, nuclei of normal cells yielded normal levels of aggregate enzyme activity when they were suspended in infected cell lysates before extraction of aggregate enzyme.

TABLE 3. ABILITY OF AGGREGATE ENZYME FROM NORMAL HeLa CELLS AND FROM POLIOVIRUS-INFECTED HeLa CELLS TO SYNTHESIZE RNA *IN VITRO*

Source of aggregate enzyme	mμM ATP^{32} incorporated			
	Experiment number			
	1	2	3	4
normal HeLa cells	0.7	0.67	0.74	0.85
HeLa cells infected 5 hr	0.04	0.05	0.10	0.04

RNA polymerase reaction mixture modified from Goldberg (1961) contained in a total volume of 0.5 ml: 50 μM tris buffer, pH 8.0; 1.5 μM $MnCl_2$; 0.05 ml of saturated $(NH_4)_2 SO_4$, pH 8.0; 5 μM β mercaptoethanol; 0.4 μM UTP, CTP, GTP; 0.4 μM ATP^{32}; and "aggregate enzyme" containing about 0.5 mg protein (obtained from equal numbers of infected or normal cells in each experiment). Incubated at 37° for 20 min.

It is unlikely that virus depression of normal RNA synthesis is due to inhibition of the synthesis of a labile RNA polymerase, for if normal HeLa cell protein synthesis is inhibited for 5 hours with 100 μg/ml puromycin, only a 50% to 70% reduction of RNA synthesis is observed (unpublished observations). Therefore, it appears that HeLa cell RNA polymerase is fairly stable, and failure of polymerase synthesis cannot alone explain the rapid repression of normal RNA synthesis in poliovirus infected cells. Further, increased ribonuclease activity cannot explain this depression of RNA synthesis. Ribonuclease activity

toward HeLa cell RNA has been found to be nearly identical in both normal and infected HeLa cell homogenates at least in the pH range between 6 and 8 (unpublished data). It is interesting that an RNA animal virus causes inhibition of DNA-primed RNA synthesis, but without the breakdown of host DNA observed following DNA phage infection.

TABLE 4. FAILURE TO DEPRESS IN VITRO RNA POLYMERASE ACTIVITY OF NORMAL HeLa CELL EXTRACTS BY EXPOSURE OF AGGREGATE ENZYME OR INTACT NUCLEI TO LYSATES OF POLIOVIRUS-INFECTED HeLa CELLS

Treatment of normal HeLa cell extracts	mμM ATP^{32} incorporated
none, untreated aggregate enzyme	0.65
aggregate enzyme treated with normal HeLa cell lysate*	0.59
aggregate enzyme treated with 5 hr infected cell lysate*	0.68
none, aggregate enzyme from untreated nuclei	0.57
aggregate enzyme from nuclei treated with 5 hr infected cell lysate‡	0.58

RNA polymerase reaction mixture as in Table 3.

* Normal cell aggregate enzyme was suspended in lysate of 10^8 HeLa cells in 1 ml polymerase reaction mixture minus nucleotides for 20 min at 0°, then for 2 min at 37°, before transfer to complete reaction mixture for incubation.

‡ Normal cell nuclei were suspended in lysate of 10^8 infected HeLa cells in 1 ml 0.25 M sucrose containing 2×10^{-3} M mercaptoethanol and 10^{-3} M $MgCl_2$ for 20 min at 0° and 2 min at 37° before preparation of aggregate enzyme.

Normal and infected HeLa cells were disrupted by 15 cycles of freezing and thawing in reaction mixture or sucrose solution, and insoluble components were removed by centrifugation at 4,000 x g before addition of nuclei or aggregate enzyme.

Although the findings reported here demonstrate that the depression of normal RNA synthesis by poliovirus cannot be attributed to DNA breakdown, nor to firm masking of the priming ability of DNA, the production of a loosely bound material capable of masking DNA cannot be ruled out. It is possible that in poliovirus-infected cells DNA-primed RNA polymerase is in some way prevented from contacting DNA and from being extracted with DNA "aggregate." The reproducible isolation and quantitation of mammalian cell DNA-primed RNA polymerase free of DNA would greatly facilitate the further study of this problem, and work is continuing along these lines.

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