

# Inhibition of Transcription Factor Activity by Poliovirus

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## Summary

To study the poliovirus-induced inhibition of host-cell RNA synthesis, we prepared transcription extracts from mock-infected and poliovirus-infected HeLa cells. In contrast with the control extracts, poliovirus-infected cell extracts prepared 3 hr after infection were unable to transcribe specifically DNA templates recognized by RNA polymerase II. Accurate transcription by RNA polymerase III, however, was only slightly reduced. Supplementation of the infected cell extract with a crude preparation of transcription factors (S100) restored its ability to transcribe a polymerase II template specifically; supplementation with purified polymerase II had no effect. When the S100 was fractionated on a phosphocellulose column, the restoration activity eluted between 0.35 M and 1 M KCl. When we tested infected extracts for inhibitory activity by mixing uninfected and infected cell extracts, no *in vitro* inhibition of polymerase II transcription by the uninfected extract was evident. These results indicate that at least one factor required for specific transcription by polymerase II is deficient in extracts from poliovirus-infected cells.

## Introduction

Following picornavirus infection of mammalian cells, host-cell RNA synthesis is rapidly inhibited (reviewed by Martin and Kerr, 1968; Lucas-Lenard, 1979). Inhibition was first characterized in mengovirus-infected L cells, where cellular RNA synthesis decreased to less than 10% of the initial level by 1 hr after infection (Baltimore and Franklin, 1962; Franklin and Baltimore, 1962). Infection with mengovirus did not enhance RNA degradation, but it did inhibit transcription by RNA polymerase as assayed in nuclei or in a crude DNA-protein complex. Inhibitions with similar properties have also been observed in poliovirus-infected HeLa cells (Holland, 1962; Zimmerman et al., 1963; Holland and Peterson, 1964; Contreras et al., 1973) and encephalomyocarditis virus-infected mouse plasmacytoma cells (Schwartz et al., 1974).

Examination of transcription in picornavirus-infected cells has shown inhibition of all three known transcription systems. It was first shown that ribosomal RNA synthesis was inhibited during the early phase of poliovirus infection (Zimmerman et al., 1963; Darnell et al., 1967; Contreras et al., 1973). By mea-

suring  $\alpha$ -amanitin-sensitive transcription in nuclei isolated from encephalomyocarditis virus- and mengovirus-infected mouse cells, it was later shown that transcription by RNA polymerase II was rapidly inhibited (Apriletti and Penhoet, 1974; Schwartz et al., 1974). The combined activity of polymerase I and III was also inhibited, but at a slower rate.

When solubilized from infected cell extracts, however, all three RNA polymerase enzymes were active as assayed under conditions that require only nonspecific initiation (Apriletti and Penhoet, 1974, 1978; Schwartz et al., 1974). Also, no alteration in their chromatographic behavior could be detected, and the electrophoretic mobility of polymerase II subunits purified from infected and uninfected cells were indistinguishable (Apriletti and Penhoet, 1978). These results suggest that a transcriptional component other than polymerase is inactivated by viral infection.

Recently, cell-free transcription systems have been developed that give specific initiation on exogenously added DNA (Weil et al., 1979; Manley et al., 1980). We have used these systems to study poliovirus-induced inhibition of RNA synthesis and have been able to demonstrate that at least one factor needed for transcription is deficient in extracts from infected cells.

## Results

### Inhibition of Transcription

To investigate the inhibition by poliovirus of host-cell RNA synthesis, we assayed for RNA polymerase II and III activity in whole-cell extracts prepared from untreated, mock-infected and poliovirus-infected HeLa cells. Specific polymerase II activity was assayed by addition of DNA containing the major late promoter of adenovirus type 2 in an Eco RI-digested plasmid in which the Bal I E fragment of adenovirus DNA was inserted. On this DNA, polymerase II-initiated transcription produces a 2125 nucleotide runoff transcript (Manley et al., 1980). To assay for polymerase III transcription, a plasmid containing the virus-associated (VA) genes of adenovirus type 2 DNA was added. Transcription of this DNA by polymerase III produces 160 nucleotide VA RNA transcripts (Söderlund et al., 1976; Weinmann et al., 1976; Akusjarvi et al., 1980). Products were resolved by agarose gel electrophoresis following treatment with glyoxal.

The appropriate transcripts of 2125 and 160 nucleotides were observed when control and mock-infected HeLa cell extracts were assayed for both polymerase II and III (Figure 1A lanes 3 and 4). As expected, synthesis of the 2125 nucleotide product was selectively sensitive to an inhibitor of polymerase II,  $\alpha$ -amanitin (0.5  $\mu$ g/ml) (Figure 1A lane 2), and both transcripts were abolished by an inhibitor of DNA-dependent RNA synthesis, actinomycin D (5  $\mu$ g/ml) (Figure 1A lane 1).

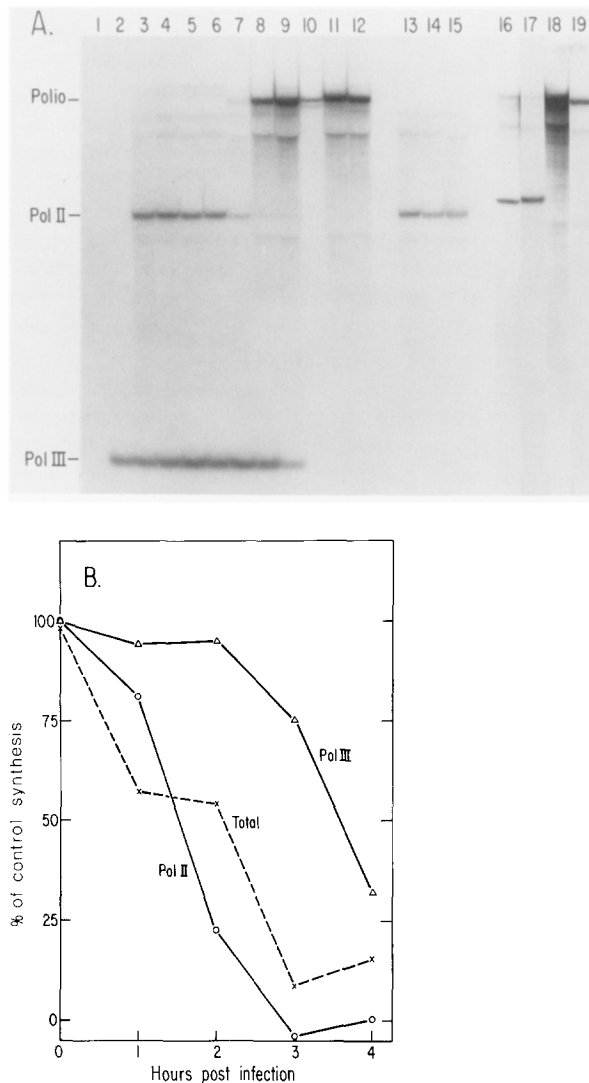


Figure 1. Inhibition of Transcription in Extracts of Poliovirus-Infected Cells

(A) Autoradiographic analysis. Reaction products were resolved by agarose gel electrophoresis following treatment with glyoxal (McMaster and Carmichael, 1977). Pol II: the 2125 nucleotide transcript. Pol III: VA RNA transcript. Polio: poliovirus RNA. (Lanes 1-3) Uninfected HeLa cell extracts were assayed for polymerase II and III activities with 5  $\mu\text{g/ml}$  actinomycin D (lane 1), 0.5  $\mu\text{g/ml}$   $\alpha$ -amanitin (lane 2) or no addition (lane 3). (Lane 4) Mock-infected cell extract. (Lanes 5-9) Poliovirus-infected HeLa cell extracts made, respectively, 0, 1, 2, 3 or 4 hr after infection. (Lane 10)  $^{32}\text{P}$ -labeled poliovirus RNA (5000 cpm, Cherenkov). (Lanes 11 and 12) 3 hr-infected extracts either without added DNA (lane 11) or with 5  $\mu\text{g/ml}$  actinomycin D (lane 12). (Lanes 13-15) Mock-infected cell extracts were incubated for 1 hr under standard reaction conditions and then processed for electrophoresis (lane 13) or incubated further for 30 min in the presence of 0.5 mM UTP and 0.5  $\mu\text{g/ml}$   $\alpha$ -amanitin with 8  $\mu\text{l}$  of mock-infected cell extract (lane 14) or 8  $\mu\text{l}$  of poliovirus-infected cell extract (lane 15). (Lanes 16-18) Assay of extracts prepared from mock-infected control cells (lane 16), mock-infected cells treated at 2 hr after mock infection with 2 mM guanidine (lane 17), cells infected for 3 hr with poliovirus (lane 18) or cells infected for 3 hr and treated at 2 hr after infection with 2 mM guanidine (lane 19). DNAs added to the various reactions were: (lanes 1-9) 25  $\mu\text{g/ml}$  pBR322-Bal I E and 5  $\mu\text{g/ml}$  pBR322-Hind III B; (lanes 12-15) 25  $\mu\text{g/ml}$  pBR322-

When poliovirus-infected HeLa cell extracts were prepared at hourly intervals following infection and assayed for polymerase activities, a rapid loss of specific polymerase II transcription, followed by a slower loss of accurate polymerase III activity, was evident (Figure 1A lanes 5-9). By quantitating the radioactivity in the bands (Figure 1B), we determined that at 2 hr after infection 75% of the polymerase II activity was lost, but VA RNA synthesis was unaffected (Figure 1A lane 7). By 3 hr, polymerase II activity was almost completely inhibited (Figure 1A lane 8), while polymerase III activity was diminished by only 25%; polymerase III activity decreased sharply thereafter.

The inhibition of total RNA synthesis was measured *in vivo* under our conditions of infection. Poliovirus- and mock-infected cells were pulse-labeled at various times for 10 min with  $^3\text{H}$ -uridine. When the amount of radioactivity incorporated into cellular RNA was determined, we found that host-cell RNA synthesis was inhibited at about the same rate as polymerase II activity; by 2 hr after infection 50% was inhibited and by 3 hr 90% was inhibited (Figure 1B).

To examine several trivial explanations for the failure of poliovirus-infected cell extracts to transcribe the polymerase II template, we investigated a number of parameters of the reaction. We varied the template DNA concentration between 5 and 50  $\mu\text{g/ml}$ , added creatine phosphokinase (8  $\mu\text{g/ml}$ ) to the reaction and increased the UTP concentration from 5 to 25  $\mu\text{M}$ . None of the changes restored transcription (data not shown). When the adenovirus DNA was replaced with other DNAs, no specific transcription of promoter-containing DNA from SV40, the human  $\beta$ -globin gene or the adenovirus type 2 early regions 1a, 1b or 3 was observed in infected cell extracts as compared with synthesis in control extracts (data not shown). To test whether the polymerase II transcript was degraded more rapidly by poliovirus-infected than mock-infected cell extracts, we synthesized labeled transcripts for 60 min from mock-infected cell extracts and then incubated them for an additional 30 min in

Bal I E; (lanes 16-19) 9.5  $\mu\text{g/ml}$  pBR322-Bal I E and 18  $\mu\text{g/ml}$  poly(dI-dC).poly(dI-dC), added to obtain optimal DNA concentration (U. Hansen, personal communication).

(B) Quantitation of inhibition. Duplicate reactions were performed with extracts from infected cells harvested at various times after infection. Synthesis was quantitated as described in Experimental Procedures. Background incorporation (reaction plus 5  $\mu\text{g/ml}$  actinomycin D) was subtracted. Radioactivity incorporated into polymerase II (O—O) and polymerase III ( $\Delta$ — $\Delta$ ) transcripts is plotted as percent of synthesis from the zero hour infected cell extract. We determined synthesis of total host-cell RNA (X—X) by labeling cells *in vivo* with  $^3\text{H}$ -uridine for 10 min. Total RNA was precipitated with trichloroacetic acid and collected on Millipore filters. Filters were dissolved in Bray's solution, and radioactivity was measured. Incorporation in the presence of 5  $\mu\text{g/ml}$  actinomycin D, representing background incorporation and poliovirus RNA synthesis, was subtracted. Incorporation into cellular RNA in infected cells is plotted as percent of synthesis in mock-infected cells.

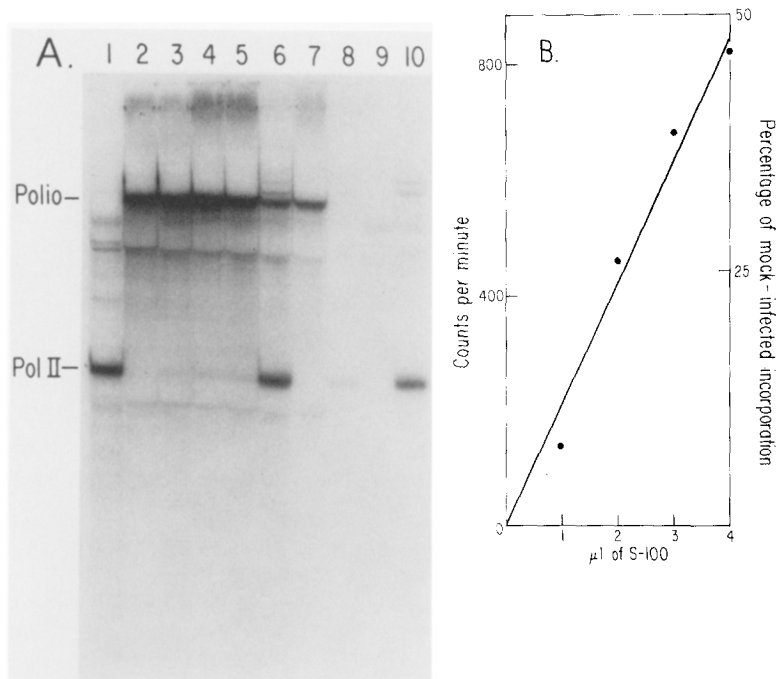


Figure 2. Restoration of Transcription

(A) Autoradiographic analysis. Extracts were assayed for transcription by polymerase II. (Lane 1) Mock-infected cell extract. (Lanes 2-7) Poliovirus-infected cell extracts were supplemented with no additions (lanes 2 and 3), 8.5 U (lane 4) or 25 U (lane 5) polymerase II, 4 μl S100 (lane 6) or 4 μl S100 and 0.5 μg/ml α-amanitin (lane 7). (Lanes 8-10) Polymerase II and S100 were assayed separately or together: (lane 8) 4 μl S100, (lane 9) 25 U polymerase II, (lane 10) 4 μl S100 and 25 U polymerase II. pBR322-Bal I E DNA was added to a concentration of 25 μg/ml in all lanes except lane 2, where it was omitted. (B) Quantitation of restoration. Infected cell extracts were supplemented with the indicated amounts of S100. Incorporation into the polymerase II transcript was quantitated, and background (synthesis by unsupplemented extract) was subtracted.

the presence of excess, unlabeled UTP and added poliovirus- or mock-infected cell extracts. Transcripts decayed at the same rate in both cases (Figure 1A lanes 13-15).

The poliovirus-infected cell extracts consistently produced a prominent, high molecular weight RNA that was not formed by mock-infected extracts (Figure 1A lanes 4 and 9). Its presence was not dependent on the addition of DNA (Figure 1A lane 11), and its synthesis was unaffected by actinomycin D (Figure 1A lane 12), suggesting that the extracts were making poliovirus RNA. By analyzing in parallel a sample of poliovirion RNA (Figure 1A lane 10), we found that the virus-specific transcript was the same size as poliovirus RNA. Furthermore, treatment of poliovirus-infected cells with guanidine (2 mM) for 1 hr before harvest substantially reduced the synthesis of the high molecular weight RNA observed *in vitro* (Figure 1A lanes 18 and 19). It has been shown that *in vivo*, poliovirus RNA synthesis is selectively inhibited by guanidine (Baltimore et al., 1963) and is resistant to actinomycin D (Reich et al., 1961). Thus these extracts must contain the poliovirus replication complex and can use the added nucleoside triphosphates to complete synthesis of poliovirus RNA molecules.

Guanidine treatment of infected cells does not prevent the virus-induced inhibition of total RNA synthesis (Bablanian et al., 1965). Likewise, guanidine treatment during the infection did not prevent inhibition of polymerase II activity (Figure 1A lane 19). Thus it appears that poliovirus RNA synthesis or the newly made RNA itself is probably not involved in the virus-induced inhibition. To examine this possibility further, viral genome RNA, double-stranded RNA and total

single-stranded RNA from 5 hr-infected cells were prepared and added to mock-infected extracts. No inhibition greater than that produced by uninfected cell RNA was caused by the viral RNAs (data not shown).

### Restoration of Transcription

Recently, *in vitro* transcription extracts have been reconstituted by mixture of a crude extract of soluble cell proteins (S100) containing transcription factors with purified RNA polymerase II (Weil et al., 1979). To determine whether a definable component of the cellular transcription system was inactivated by poliovirus, we attempted to restore transcription in poliovirus-infected cell extracts with purified polymerase II or with an S100 extract. A similar approach has been used to assay factors modified by adenovirus infection (Fire et al., 1981). To show that our enzyme and S100 were in fact active and that both were needed for transcription, we tested their activity on adenovirus DNA. The polymerase II preparation alone (25 U) gave no detectable transcription (Figure 2A lane 9), while 4 μl of the S100 gave a very small degree of transcription (Figure 2A lane 8), indicating that the S100 contained very little polymerase II. Both preparations together, however, transcribed well (Figure 2A lane 10).

When added to poliovirus-infected extracts, 425 or 1250 U/ml polymerase II did not restore the ability to transcribe the added adenovirus DNA (Figure 2A lanes 3-5). The amounts of cell extract used in these experiments ( $2 \times 10^6$  cell equivalents per reaction) should contain 150-250 U/ml polymerase II (Manley et al., 1980). In contrast, addition of 4 μl of the S100 ( $5 \times 10^5$  cell equivalents per reaction; Figure 2A lane

6) restored transcription to 50% of the mock-infected level (Figure 2A lane 1). The response to added S100 was linear with increasing concentration (Figure 2B).  $\alpha$ -Amanitin (0.5  $\mu\text{g}/\text{ml}$ ) blocked the transcription restored by the S100 (Figure 2A lane 7). These results demonstrate that polymerase II was not a limiting factor in the poliovirus-infected cell extracts, and that another component, necessary for transcription and present in the S100, was either missing or inactivated.

We used a phosphocellulose column to fractionate the S100 because of the recent successful separations achieved by Matsui et al. (1980). They found that three fractions together, flow-through, 0.35–0.6 M KCl eluate and 0.6–1 M KCl eluate, were needed to transcribe specifically exogenously added DNA in combination with purified polymerase II. An S100 extract was fractionated by the above procedure, except that protein bound to the column was eluted in two steps with 0.35 M and 1 M KCl. These two fractions and the flow-through were assayed for their ability to restore transcription in the poliovirus-infected cell extracts. Of the three fractions, the 1 M eluate preferentially stimulated the activity of the infected cell extract (Figure 3 lanes 5–7). Its restoration ability was almost that of the crude S100 from which it was made (Figure 3 lane 4). Of the activity applied to the column, 95% was recovered in the 1 M fraction with a 25-fold purification. The S100 used for purification, like the one used for the previous experiment, had little activity by itself (Figure 3 lane 2), and the poliovirus-infected extract was also much less active than the mock-infected extract (Figure 3 lanes 1 and 3). We have further fractionated the S100, and have found that the restoring activity elutes between 0.35 and 0.6 M KCl but with a poor recovery of activity (data not shown).

#### In Vitro Inhibition

Previous work has failed to demonstrate any inhibition in vitro of RNA synthesis in extracts of uninfected cells when they were mixed with extracts from picornavirus-infected cells (Holland, 1962; Martin and Kerr, 1968; Schwartz et al., 1974). We have examined our extracts for trans-inhibitory activity using two approaches. First, mock-infected (8  $\mu\text{l}$ ) and poliovirus-infected (4  $\mu\text{l}$ ) cell extracts were preincubated together and then assayed for transcription. The level of transcription was the same as that from separately preincubated extracts mixed before the assay (Figure 4 lanes 3 and 4). The actual level of transcription in this experiment was intermediate between that given by 8  $\mu\text{l}$  of mock-infected or 12  $\mu\text{l}$  of mock-infected extract alone (Figure 4 lanes 1 and 2). We surmise that some but not all of the rate-limiting transcription factors are defective in infected cell extracts, so that addition of 4  $\mu\text{l}$  of infected cell extract to 8  $\mu\text{l}$  of mock-infected cell extract (Figure 4 lanes 1 and 3) stimulates transcription but less than addition of 4  $\mu\text{l}$  of mock-

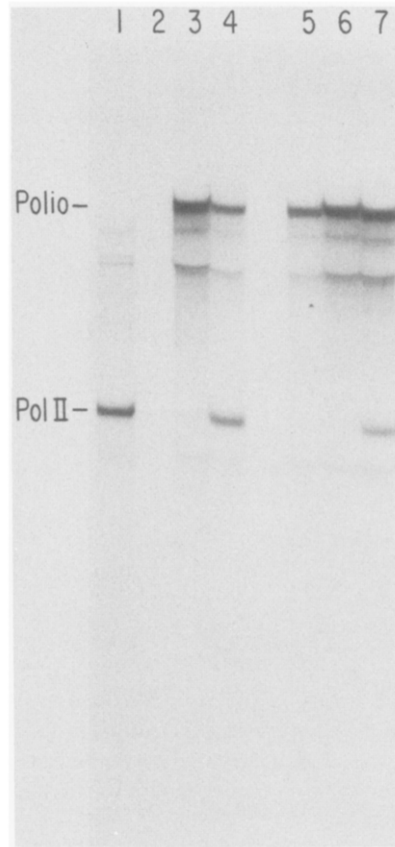


Figure 3. Fractionation of S100

S100 was separated by phosphocellulose chromatography into three fractions, which were assayed for restoration activity. (Lanes 1–4) Control reactions with mock-infected cell extract (lane 1), 4  $\mu\text{l}$  S100 (lane 2), infected cell extract (lane 3) or 4  $\mu\text{l}$  S100 and infected cell extract (lane 4). (Lanes 5–7) Infected cell extract supplemented with phosphocellulose flow-through (lane 5), 0.35 M KCl (lane 6) or 1 M KCl (lane 7) step-eluted fractions. pBR322–Bal I E DNA (25  $\mu\text{g}/\text{ml}$ ) was added to each reaction.

infected cell extract (Figure 4 lanes 1 and 2). In the second approach, the 1 M fraction from the phosphocellulose column was preincubated with the poliovirus-infected cell extract to detect any inhibition of the restoration activity. No inhibition was observed (Figure 4 lanes 5–7).

#### Discussion

We have demonstrated that in vitro transcription by RNA polymerase II, as assayed under the conditions of Manley et al. (1980), decreases following poliovirus infection of HeLa cells in parallel with the in vivo decrease of total cellular RNA synthesis. RNA polymerase III activity, measured as adenovirus VA RNA synthesis, was inhibited much later than polymerase II activity. Furthermore, a partially purified preparation of transcription factors restored specific transcription to extracts from poliovirus-infected cells. We assume that the inhibition of in vitro polymerase II activity is a consequence of the events that reduce in vivo rates

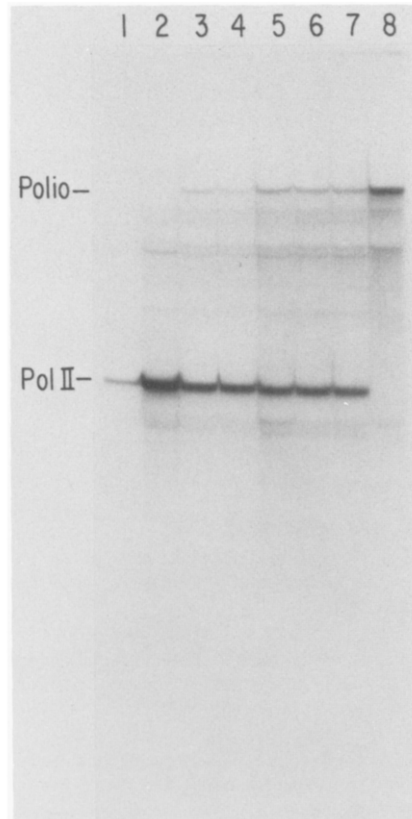


Figure 4. Transcription in Mixed Extracts

Extracts were preincubated together at 30°C for 30 min with all four ribonucleotides, or preincubated separately then mixed before the assay for polymerase II transcription. Reactions were started by addition of 10  $\mu$ Ci  $\alpha$ -<sup>32</sup>P-UTP and 25  $\mu$ g/ml template DNA (pBR322-Bal I E). (Lanes 1, 2 and 8) Control reactions with 8  $\mu$ l (lane 1) or 12  $\mu$ l (lane 2) of preincubated, mock-infected cell extract or 8  $\mu$ l of infected cell extract (lane 8). Infected cell extract (4  $\mu$ l) and 8  $\mu$ l of mock-infected cell extract were preincubated separately, then mixed (lane 3) or preincubated together (lane 4) before assaying. (Lanes 5-7) 4  $\mu$ l of phosphocellulose 1 M fraction and 8  $\mu$ l of infected cell extract were assayed together after no preincubation (lane 5), separate preincubation (lane 6) or mixed preincubation (lane 7).

of RNA synthesis, and that it is therefore the loss of activity of one or more transcription factors that explains the ability of poliovirus to inhibit host-cell RNA synthesis.

Previous work had demonstrated that RNA polymerases I, II and III were present and enzymatically active when partially purified from infected cell extracts (Apriletti and Penhoet, 1974, 1978; Schwartz et al., 1974). Our evidence supports these observations on polymerase II because we could not restore activity to extracts of infected cells by adding polymerase II. Even though the transcription factor preparations, containing little or no polymerase II, restored only half of the activity found in mock-infected extracts, a concentrated preparation of the 1 M eluate from the phosphocellulose column restored all of the activity (data not shown), suggesting that a factor (or factors) in this fraction was the only target of inhibition.

The mixing experiments indicate that the infected cell extracts cannot inactivate transcription in the uninfected cell extracts. Thus there is no free and active inhibitory factor or inactivating enzyme at work in the extracts prepared from infected cells as assayed under our conditions. We do not know that poliovirus actively inhibits a factor; the virus might cause a factor to be sequestered so it is not extractable by our methods, or it might increase the decay rate of a large number of proteins among which are transcription factors. Further work will be necessary to elucidate the mechanism of inhibition.

Since previous work demonstrated that inhibition of total RNA synthesis in HeLa cells did not begin until 2 hr after poliovirus infection (Zimmerman et al., 1963; McCormick and Penman, 1967; Contreras et al., 1973), we were surprised to find that transcription by polymerase II was inhibited 75% at 2 hr after infection. We therefore reexamined the inhibition of total RNA synthesis. We found that cellular RNA synthesis decreased at the same rate as polymerase II activity (Figure 1B). About 60% or more of the cellular RNA made during the 10 min labeling time was synthesized by polymerase II (Penman et al., 1970). Thus our *in vivo* and *in vitro* results appear to be consistent. The discrepancy between our results and previous work may be due to differences in the cell lines or to conditions of infection.

At the moment, mammalian cell preparations capable of specifically initiating transcription on exogenously added template have been divided into five required fractions (Matsui et al., 1980). RNA polymerase II is one of these; the other transcription factors have only been partially purified. It is also clear that the *in vitro* process of initiation by RNA polymerase II at a specific site is only a shadow of the more complex *in vivo* process. For example, specific DNA sequences that lie more than 50 nucleotides from the cap site are not required for *in vitro* initiation, yet are vital for initiation *in vivo* (reviewed by Breathnach and Chambon, 1981). The uncertainty of the relationship of the cellular and *in vitro* initiation reactions makes conclusive identification of transcription factors, based only on their *in vitro* activity, difficult. Proof that poliovirus infection inactivates both host-cell RNA synthesis and a factor needed for *in vitro* transcription would provide strong evidence for an *in vivo* role for at least one factor identified by *in vitro* analysis. Our system provides an assay that should permit purification of such a factor. Perhaps this approach can be applied to other viruses that modify polymerase II transcription, and thus permit identification of other transcription factors in the *in vivo* process.

#### Experimental Procedures

##### Cells and Viruses

HeLa cells were infected with poliovirus type 1 (at a multiplicity of 35) as described by Baltimore et al. (1966). After resuspension of the

cells in Joklik's modified minimal essential medium without virus, mock-infected cells were treated identically to poliovirus-infected cells. Except as indicated, infected and mock-infected cells were harvested 3 hr after infection. <sup>32</sup>P-labeled poliovirus RNA was isolated from virions as described by Ambros and Baltimore (1978).

#### Transcription System

Whole-cell extracts were prepared from HeLa cells, and transcription reactions and RNA purification were performed as described by Manley et al. (1980), except for the following modifications. Each 20  $\mu$ l reaction contained 8  $\mu$ l of cell extract. The final concentrations of ATP, GTP and CTP were 100  $\mu$ M each. The UTP concentration was 5  $\mu$ M. Creatine phosphate was added to a final concentration of 8 mM (Handa et al., 1981). To quantitate the incorporation of <sup>32</sup>P-UMP into the polymerase II and III transcripts, we dissolved the appropriate gel slice into an aqueous, saturated NaI solution and measured the number of cpm (Cherenkov radiation). The conditions for transcription reactions with S100 and purified RNA polymerase II (Weil et al., 1979) were the same as those described above. pBR322-Bal I E DNA, containing the major late adenovirus 2 promoter, was digested with Eco RI (Manley et al., 1980). The pBR322-Hind III B clone, containing the coding sequences for both VA RNAs, was a gift from S.-L. Hu.

#### S100 and Polymerase II

S100 was prepared from HeLa cells as described by Matsui et al. (1980). RNA polymerase II was purified from HeLa cells by a modification of the procedure of Hodo and Blattl (1977). Nuclei were resuspended in low salt, sonicated and clarified. Polymerase was precipitated with polyethyleneimine. The collected pellets were extracted with high salt by Dounce homogenization. Polymerase II was purified by successive chromatography on DEAE-cellulose, phosphocellulose and DEAE-Sephadex, and was then dialyzed into HEPES transcription buffer (Manley et al., 1980). We obtained 21 U/ $\mu$ l polymerase, one unit corresponding to the incorporation of 1 pmole UMP into RNA at 37°C in 20 min.

#### Chromatography of the S100

The S100 was fractionated on a phosphocellulose column as described by Matsui et al. (1980). The bound material was eluted with 0.35 M and 1 M KCl in the buffer A of Matsui et al. (1980) then dialyzed against buffer A (100 mM KCl).

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#### References

Akusjarvi, G., Mathews, M. B., Andersson, P., Vennstrom, B. and Pettersson, U. (1980). Structure of genes for virus-associated RNA<sub>1</sub> and RNA<sub>2</sub> of adenovirus type 2. *Proc. Nat. Acad. Sci. USA* 77, 2424-2428.

Ambros, V. and Baltimore, D. (1978). Protein is linked to the 5' end of poliovirus RNA by a phosphodiester linkage to tyrosine. *J. Biol. Chem.* 253, 5263-5266.

Apriletti, J. W. and Penhoet, E. E. (1974). Recovery of DNA-dependent RNA polymerase activities from L cells after Mengovirus infection. *Virology* 61, 597-601.

Apriletti, J. W. and Penhoet, E. E. (1978). Cellular RNA synthesis in normal and Mengovirus-infected L-929 cells. *J. Biol. Chem.* 253, 603-611.

Bablanian, R., Eggers, H. J. and Tamm, I. (1965). Studies on the mechanism of poliovirus-induced cell damage. *Virology* 26, 100-113.

Baltimore, D. and Franklin, R. M. (1962). The effect of Mengovirus infection on the activity of the DNA-dependent RNA polymerase of L-cells. *Proc. Nat. Acad. Sci. USA* 48, 1383-1390.

Baltimore, D., Eggers, H. J., Franklin, R. M. and Tamm, I. (1963). Poliovirus-induced RNA polymerase and the effects of virus-specific inhibitors on its production. *Proc. Nat. Acad. Sci. USA* 49, 843-849.

Baltimore, D., Girard, M. and Darnell, J. E. (1966). Aspects of the synthesis of poliovirus RNA and the formation of virus particle. *Virology* 29, 179-189.

Breathnach, R. and Chambon, P. (1981). Organization and expression of eucaryotic split genes coding for proteins. *Ann. Rev. Biochem.* 50, 349-383.

Contreras, G., Summers, D. F., Maizel, J. V. and Ehrenfeld, E. (1973). HeLa cell nucleolar RNA synthesis after poliovirus infection. *Virology* 53, 120-129.

Darnell, J. E., Girard, M., Baltimore, D., Summers, D. F. and Maizel, J. V. (1967). The synthesis and translation of poliovirus RNA. In *The Molecular Biology of Viruses*, J. Colter and W. Paranchych, eds. (New York: Academic Press), pp. 375-401.

Fire, A., Baker, C., Ziff, E. and Sharp, P. A. (1981). Transcription of adenovirus DNA in infected cell extracts. In *Developmental Biology Using Purified Genes*, ICN-UCLA Symposia on Molecular and Cellular Biology, 23, D. Brown and C. F. Fox, eds. (New York: Academic Press), in press.

Franklin, R. M. and Baltimore, D. (1962). Patterns of macromolecular synthesis in normal and virus-infected cells. *Cold Spring Harbor Symp. Quant. Biol.* 27, 175-198.

Handa, H., Kaufman, R. J., Manley, J., Geffer, M. and Sharp, P. A. (1981). Transcription of simian virus 40 DNA in a HeLa whole cell extract. *J. Biol. Chem.* 256, 478-482.

Hodo, H. G. and Blattl, S. P. (1977). Purification using polyethyleneimine precipitation and low molecular weight subunit analyses of calf thymus and wheat germ DNA-dependent RNA polymerase II. *Biochemistry* 16, 2334-2343.

Holland, J. J. (1962). Inhibition of DNA-primed RNA synthesis during poliovirus infection of human cells. *Biochem. Biophys. Res. Commun.* 9, 556-573.

Holland, J. J. and Peterson, J. A. (1964). Nucleic acid and protein synthesis during poliovirus infection of human cells. *J. Mol. Biol.* 8, 556-573.

Lucas-Lenard, J. M. (1979). Inhibition of cellular protein synthesis after virus infection. In *The Molecular Biology of Picornaviruses*, R. Perez-Bercoff, ed. (New York: Plenum), pp. 73-99.

Manley, J. L., Fire, A., Cano, A., Sharp, P. A. and Geffer, M. L. (1980). DNA-dependent transcription of adenovirus genes in a soluble whole cell extract. *Proc. Nat. Acad. Sci. USA* 77, 3855-3859.

Martin, E. M. and Kerr, I. M. (1968). Virus-induced changes in host cell macromolecular synthesis. In *The Molecular Biology of Viruses*, L. V. Crawford and M. G. P. Stoker, eds. (New York: Cambridge University Press), pp. 15-46.

Matsui, T., Segall, J., Weil, A. and Roeder, R. G. (1980). Multiple factors required for accurate initiation of transcription by purified RNA polymerase II. *J. Biol. Chem.* 255, 11992-11996.

McCormick, W. and Penman, S. (1967). Inhibition of RNA synthesis in HeLa and L cells by Mengovirus. *Virology* 31, 135-141.

McMaster, G. K. and Carmichael, G. C. (1977). Analysis of single- and double-stranded nucleic acids in polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Nat. Acad. Sci. USA* 74, 4835-4838.

Penman, S., Fan, H., Perlman, S., Rosbash, M., Weinberg, R. and

- Zylber, E. (1970). Distinct RNA synthesis systems of the HeLa cell. *Cold Spring Harbor Symp. Quant. Biol.* 35, 561–575.
- Reich, E., Franklin, R. M., Shatkin, A. J. and Tatum, E. L. (1961). The effect of actinomycin D on cellular nucleic acid synthesis and virus production. *Science* 134, 556.
- Schwartz, L. B., Lawrence, C., Thach, R. E. and Roeder, R. G. (1974). Encephalomyocarditis virus infection of mouse plasmacytoma cells. II. Effect on host RNA synthesis and RNA polymerases. *J. Virol.* 14, 611–619.
- Söderlund, H., Pettersson, U., Vennström, B., Philipson, L. and Mathews, M. B. (1976). A new species of virus-coded low molecular weight RNA from cells infected with adenovirus type 2. *Cell* 7, 585–593.
- Weil, P. A., Luse, D. S., Segall, J. and Roeder, R. G. (1979). Selective and accurate initiation of transcription at the Ad2 major late promoter in a soluble system dependent on purified RNA polymerase II and DNA. *Cell* 18, 469–484.
- Weinmann, R., Brendler, T. G., Raskas, H. J. and Roeder, R. G. (1976). Low molecular weight viral RNAs transcribed by RNA polymerase III during adenovirus 2 infection. *Cell* 7, 557–566.
- Zimmerman, E. F., Hecter, M. and Darnell, J. E. (1963). RNA synthesis in poliovirus-infected cells. *Virology* 19, 400–408.