THE RIBOSOMAL-RNA SYNTHESIS FACTOR ψ_r IN ESCHERICHIA COLI INFECTED WITH RNA BACTERIOPHAGE R23

David Hunt and Mamoru Watanabe

Departments of Medicine and Biochemistry
University of Alberta
Edmonton, Alberta
Canada

Received August 30, 1971

SUMMARY

Infection of Escherichia coli with the RNA bacteriophage R23 inhibits RNA synthesis by the cell membrane-DNA-RNA polymerase complex of the host cell, and this inhibition cannot be reversed by the addition of ψ_r to the reaction mixture. Infection of E. coli with R23 does not cause a decrease in the level of the factor ψ_r present in the cell supernatant. The factor ψ_r is necessary for rRNA synthesis. Decreased availability of ψ_r is therefore probably not the cause of the reduced host RNA synthesis which follows infection with R23.

No detectable rRNA is synthesized when purified Escherichia coli RNA polymerase is used to transcribe E. coli DNA in vitro (1) in spite of the fact that up to 40% of RNA synthesis in vivo in a rapidly growing culture is rRNA synthesis (2). A factor designated ψ_r, which stimulates a purified E. coli system to synthesize rRNA in vitro, has been described in crude extracts prepared from E. coli (1).

We have previously reported that RNA synthesis by a DNA-membrane preparation from E. coli is inhibited following infection by the RNA phage R23 (3). Infection by R23 also leads to marked inhibition of rRNA synthesis in vivo (4). Kondo et al. (5) and Kamen (6) have shown that Qβ replicase comprises four different polypeptides, only one of which is coded for by the phage genome. None of the polypeptides are known subunits of the E. coli DNA-dependent RNA polymerase haloenzyme. Travers et al. (1) have shown that the factor ψ_r is a component of the RNA replicase induced in cells infected with RNA phage Qβ. In view of

the findings with the factor ψ_r and the Qβ replicase, it seemed of interest to find out whether the inhibition following R23 infection might not be the result of a competition between the E. coli DNA-dependent RNA polymerase and the R23-induced replicase for the factor ψ_r. The acquisition of the factor ψ_r by the R23-induced enzyme would inhibit overall host RNA synthesis by preventing the transcription of the rRNA genes.

MATERIALS AND METHODS

Materials. [5-^{3}H]UTP (specific activity 17.1 Ci per mmole) was purchased from Schwarz BioResearch, Orangeburg, New York. Unlabeled nucleotides were purchased from Mann Research Laboratories, Inc., New York, N.Y. Actinomycin D was purchased from Merck, Sharp and Dohme, Westpoint, Pennsylvania.

RNA polymerase (DNA-dependent nucleotide triphosphate: RNA nucleotidyltransferase, EC 2.7.7.6) was prepared from E. coli K38 by the method of Chamberlin and Berg (7). DNA was prepared from exponential phase E. coli K38 by the method of Smith (8).

Culturing. Growth media and conditions for E. coli K38 and R23 infection have been described (9, 10). Tryptone medium was used for all studies. For infected cultures, R23 was added at a multiplicity of infection of 50 phage per cell.

Preparation of Membrane Fractions. Cell lysis and the preparation of the membrane fraction have been described (3).

Preparation of ψ_r Factor. After lysis of cells and removal of the membrane fraction, the cell supernatant was centrifuged for 2 hours at 110,000 x g to remove ribosomes. One ml samples of the ribosome-free crude extract were layered onto 11 ml linear 10-30% glycerol gradients containing 0.01 M Tris-HCl, pH 7.9, 0.01 M $MgCl_2$, 0.5 M KCl, 0.1 mM EDTA and 0.1 mM dithiothreitol. The gradients were centrifuged in an SW 41 rotor for 24.5 hours at 40,000 rpm at 7° C, and 0.05 ml

samples of each fraction were assayed for ψ_r factor activity as described below.

Assay for RNA Synthesis. RNA synthesis was assayed as described previously (3) except that all the reaction mixtures contained 0.1 M KCl. A reaction mixture identical to the experimental assay except for the presence of actinomycin D (10 µg per ml) was always incubated as a control. The levels of UMP incorporation observed in these controls have been deducted in all the results presented, which thus measure actinomycin-sensitive RNA synthesis.

Assay for ψ_r Activity. Factor activity of the glycerol gradient fractions was assayed by measuring their ability to stimulate transcription of E. coli DNA by E. coli RNA polymerase. Reaction mixtures for the assay of RNA synthesis contained E. coli K38 DNA (20 µg), purified E. coli K38 RNA polymerase (12 µg), and 0.05 ml samples of each gradient fraction. The incorporation of [^{3}H]UMP was determined after 60 min of incubation.

RESULTS

When the ribosome-free extract from uninfected cells and from cells infected for 30 min with R23 was centrifuged on a glycerol gradient as described by Travers et al. (1), a fraction which stimulated RNA synthesis in vitro was found to be located in the same position as the ψ_r factor described previously by these authors. The position of the peak of stimulatory activity was the same for both extracts, and the level of activity present was the same in extracts of uninfected and R23-infected cells (Fig. 1).

In addition to their ability to stimulate RNA synthesis in vitro by a purified E. coli system, the gradient fractions containing the greatest ψ_r activity were tested for their effect on RNA synthesis by an E. coli cell membrane-DNA-RNA polymerase complex (Table I). RNA

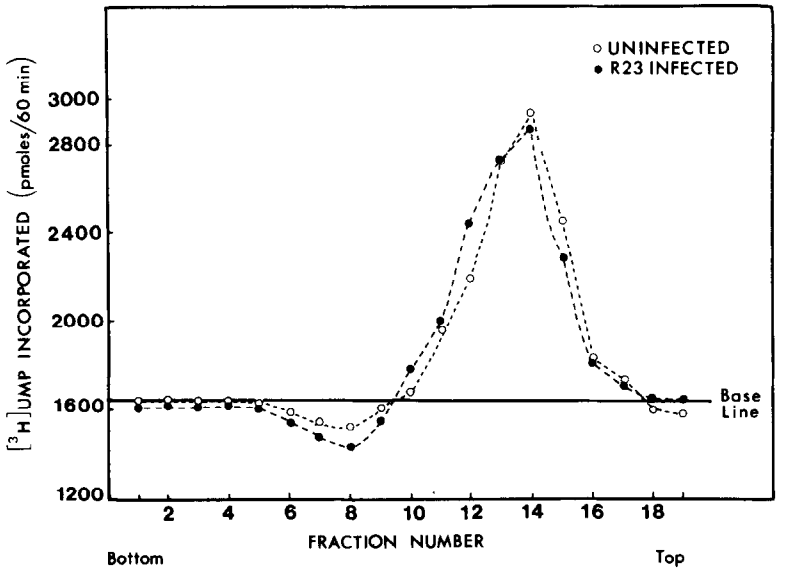

Fig. 1. ψ_r Activity in Ribosome-Free Supernatant of Uninfected and R23-Infected Cells. One ml samples of the ribosome-free supernatant from uninfected (●) cells and from cells infected for 30 min with R23 (○) were centrifuged on glycerol gradients as described in the text. The factor activity of each fraction was assayed as described in the text. The activity of the RNA polymerase without any additions is shown as a base line. Sedimentation is from right to left.

synthesis by such a complex isolated from uninfected cells and from cells infected for 30 min by R23 was assayed in the presence and absence of the gradient fractions obtained both from uninfected and from R23-infected cells. The ψ_r factor from both uninfected and R23-infected cells stimulated RNA synthesis by membrane preparations from uninfected and infected cells by approximately 15%. However, addition of ψ_r factor to infected membrane complex was unable to restore RNA synthesis to the level observed using complex from uninfected cells.

DISCUSSION

The RNA replicase induced by Qβ consists of a single phage-specific and three host-specific polypeptide chains (6). Two of the host-specific polypeptide chains almost certainly comprise the factor ψ_r, which preferentially stimulates rRNA synthesis *in vitro* (1). The

Table I

THE EFFECT OF ψ_r FACTOR ON RNA SYNTHESIS BY THE MEMBRANE FRACTION

Source of Membrane Fraction	Source of ψ_r Factor	[^{3}H]UMP Incorporated (pmoles/60 min)
Uninfected cells	None	1105
	R23-infected cells	1260
	Uninfected cells	1280
R23-infected cells	None	520
	R23-infected cells	600
	Uninfected cells	610

Actinomycin-sensitive [^{3}H]UMP incorporation was assayed as described in the text. Reaction mixtures contained membrane fraction (0.1 ml) from uninfected cells or from cells infected for 30 min with R23. Samples of the gradient fractions (0.05 ml of fraction 14) isolated from uninfected or from R23-infected cells were added to the tubes indicated. All reaction mixtures contained KCl in a final concentration of 0.1 M.

structure of R23 synthetase is at present unknown and although there may be similarities to the Qβ replicase, the possibility remains that the two enzyme structures vary since Qβ, although similar in some respects, differs in biological and physico-chemical characteristics from other RNA bacteriophages such as R23 (11). Infection of E. coli by R23 results in a marked inhibition of ribosomal RNA synthesis, with maximal inhibition occurring after 30 min of infection (4). The cell membrane-DNA-RNA polymerase complex isolated from R23-infected cells also exhibits a decreased level of RNA synthesis in vitro (3). By analogy with the Qβ system, it is therefore possible that RNA phage R23 synthetase and host DNA-dependent-RNA polymerase compete for a

factor which is necessary for both rRNA synthesis and phage RNA replication. If such a competition indeed occurs, then the inhibition of RNA synthesis in the membrane complex isolated from R23-infected cells could be at least partially explained by a reduced level of rRNA synthesis resulting directly from a decreased availability of the factor ψ_r. In addition, such a competition might be expected to reduce the level of ψ_r present in supernatant from lysed cells following infection by an RNA phage.

The inhibition of RNA synthesis by the membrane-DNA-RNA polymerase complex isolated from R23-infected cells does not appear to be the result of a reduced availability of ψ_r. There was no significant decrease in the level of ψ_r in the supernatant after R23 infection and addition of ψ_r preparations from uninfected or from R23-infected cells to the complex did not stimulate the level of RNA synthesis in infected membrane preparations to the level observed using the complex isolated from uninfected cells.

Addition of ψ_r factor to membrane fractions from uninfected cells does not stimulate RNA synthesis to the same degree as addition of the factor to purified DNA preparations. This may be due to the fact that membrane preparations do not synthesize rRNA although this seems unlikely since membrane fractions were prepared from exponentially-growing cells. It would appear more likely that enzyme molecules associated with membrane fractions contain sufficient ψ_r factor for rRNA synthesis. Hence addition of ψ_r factor results in very little stimulation of RNA synthesis.

RNA synthesis by membrane fractions from R23-infected cells is not stimulated by addition of ψ_r factor. The nature of RNA synthesized by such fractions is as yet undetermined and it is possible that nonsense RNA may be synthesized by such preparations. In such a case ψ_r factor may fail to stimulate RNA synthesis. On the other hand, R23 infection

may inhibit the association of ψ_r factor with the RNA polymerase haloenzyme.

The results also show that there is no significant difference between the levels of ψ_r present in the supernatant of uninfected cells or of cells infected for 30 min by R23. If the association between ψ_r and the product of the phage replicase gene were transitory and reversible, such a result could be explained. A rapidly reversible association seems unlikely, however, since all four polypeptides of the Qβ replicase, two of which comprise ψ_r, copurify as stable subunits of the enzyme (1).

It is possible that the phage R23 RNA synthetase uses host polypeptide factors which are synthesized following infection rather than simply relying on the pool present in the cell at the time of infection. This could account for the observation that the presence of rifampicin which inhibits DNA-dependent-RNA polymerase but not RNA replicase (12, 13), inhibits the replication of R23 (14). If de novo synthesis of the host factors comprising part of the phage synthetase molecule were sufficiently extensive, then it is possible that the level of ψ_r present in the cell supernatant would not be altered by phage infection.

ACKNOWLEDGEMENTS

This investigation was supported by Grant MA 2822 from the Medical Research Council of Canada, Ottawa, Canada.

Dr. David Hunt is a recipient of the Research Travel Grant from the Wellcome Trust (England).

Dr. Mamoru Watanabe is an Associate, Medical Research Council of Canada, Ottawa, Canada.

REFERENCES

1. Travers, A.A., Kamen, R.I., Schleif, R.F., Nature, 228, 748, 1970.
2. Kennell, D., J. Mol. Biol., 34, 85, 1968.
3. Hunt, D., Saito, Y., Watanabe, M., J. Biol. Chem., 246, 4151, 1971.
4. Watanabe, H., Watanabe, M., Canad. J. Microbiol., 17, 461, 1971.

5. Kondo, M., Gallerani, R., Weissmann, C., Nature, 228, 525, 1970.
6. Kamen, R., Nature, 228, 527, 1970.
7. Chamberlin, M., Berg, P., Proc. Natl. Acad. Sci., U.S.A., 48, 81, 1962.
8. Smith, M.G., in L. Grossman and K. Moldave (Editors), Methods in Enzymology, Vol. XII, Part A, Academic Press, New York, page 545, 1967.
9. Watanabe, M., Watanabe, H., August, J.T., J. Mol. Biol., 33, 1, 1968.
10. Watanabe, H., Watanabe, M., J. Virol., 2, 1400, 1968.
11. Watanabe, H., Watanabe, M., Canad. J. Microbiol., 16, 859, 1970.
12. di Mauro, E., Snyder, L., Marino, P., Lamberti, A., Coppo, A., Tocchini-Valentini, G.P., Nature, 222, 533, 1969.
13. Bandle, E., Weissmann, C., Biochim. Biophys. Acta, 199, 551, 1970.
14. Watanabe, H., Watanabe, M., manuscript in preparation.