Rous Sarcoma: Current Research. I.

Rous Sarcoma: Current Research. I.

Papers by
Peter Duesberg, Warren E. Levinson, Howard M. Temin, John P. Bader, Anthony Faras, Axel-Claude Garapin, Lois Fanshier, Saiji Yoshii, Alice Golde, Paul P. Hung, Christina M. Scheele, R. L. Stolfi, Clifford J. Bellone, Frieda K. Roth, G. F. Rabotti, Richard G. Olsen et al.

MSS Information Corporation
655 Madison Avenue, New York, N. Y. 10021

Library of Congress Cataloging in Publication Data
Main entry under title:

Rous sarcoma: current research.

1. Rous sarcoma. I. Duesberg, Peter.
[DNLM: 1. Sarcoma, Experimental--Collected works.
2. Rous sarcoma--Collected works. QZ 345 R863]
RC262.R68 616.9'94 72-10910
ISBN 0-8422-7063-9

TABLE OF CONTENTS

Rous Sarcoma: Current Research. I.

Papers by
Peter Duesberg, Warren E. Levinson, Howard M. Temin, John P. Bader, Anthony Faras, Axel-Claude Garapin, Lois Fanshier, Saiji Yoshii, Alice Golde, Paul P. Hung, Christina M. Scheele, R. L. Stolfi, Clifford J. Bellone, Frieda K. Roth, G. F. Rabotti, Richard G. Olsen et al.

MSS Information Corporation
655 Madison Avenue, New York, N. Y. 10021

Library of Congress Cataloging in Publication Data
Main entry under title:

Rous sarcoma: current research.

1. Rous sarcoma. I. Duesberg, Peter.
[DNLM: 1. Sarcoma, Experimental--Collected works.
2. Rous sarcoma--Collected works. QZ 345 R863]
RC262.R68 616.9'94 72-10910
ISBN 0-8422-7063-9

TABLE OF CONTENTS

CREDITS AND ACKNOWLEDGMENTS

Bader, John P., "Synthesis of the RNA of RNA-Containing Tumor Viruses," *Virology*, 1970, 40:494-504.

Bader, John P.; and Nancy R. Brown, "Induction of Mutations in an RNA Tumour Virus by an Analogue of a DNA Precursor," *Nature*, 1971, 234:11-12.

Bellone, Clifford J.; and Morris Pollard, "A Transient Cytotoxic Host Response to the Rous Sarcoma Virus-Induced Transplantation Antigen (34851)." *Proceedings of the Society for Experimental Biology and Medicine*, 1970, 134:640-643.

Duesberg, Peter; Klaus V. D. Helm; and Eli Canaani, "Comparative Properties of RNA and DNA Templates for the DNA Polymerase of Rous Sarcoma Virus," *Proceedings of the National Academy of Science*, 1971, 68:2505-1509.

Duesberg, Peter; Klaus V. D. Helm; and Eli Canaani, "Properties of a Soluble DNA Polymerase Isolated from Rous Sarcoma Virus," *Proceedings of the National Academy of Sciences*, 1971, 68:747-751.

Duesberg, Peter; G. Steven Martin; and Peter K. Vogt, "Glycoprotein Components of Avian and Murine RNA Tumor Viruses," *Virology*, 1970, 41:631-646.

Fanshier, Lois; Axel-Claude Garapin; Jerome McDonnelo; Anthony Faras; Warren Levinson; and J. Michael Bishop, "Deoxyribonucleic Acid Polymerase Associated with Avian Tumor Viruses: Secondary Structure of the Deoxyribonucleic Acid Product," *Journal of Virology*, 1971, 7:77-86.

Faras, Anthony; Lois Fanshier; Axel-Claude Garapin; Warren Levinson; and J. Michael Biship, "Deoxyribonucleic Acid Polymerase of Rous Sarcoma Virus: Studies on the Mechanism of Double-Stranded Deoxyribonucleic Acid Synthesis," *Journal of Virology*, 1971, 7:539-548.

Golde, Alice, "Radio-Induced Mutants of the Schmidt-Ruppin Strain of Rous Sarcoma Virus," *Virology*, 1970, 40:1022-1029.

Hung, Paul P.; Harriet L. Robinson; and William S. Robinson, "Isolation and Characterization of Proteins from Rous Sarcoma Virus," *Virology*, 1971, 43:251-256.

Levinson, Warren E.; J. Michael Bishop; Nancy Quintrell; Jean Jackson; and Lois Fanshier, "Synthesis of RNA in Normal and Rous Sarcoma Virus-Infected Cells: Effect of Bromodeoxyuridine," *Virology*, 1970, 42:221-224.

Levinson, Warren E.; Harold E. Varmus; Axel-Claude Garapin; and J. Michael Bishop, "DNA of Rous Sarcoma Virus: Its Nature and Significance," *Science*, 1971, 175:76-79.

Olsen, Richard G.; James R. McCammon; Joseph Weber; and David S. Yohn, "Cutaneous Skin Test for Delayed Hypersensitivity in Hamsters to Viral Induced Tumor antigens," *Canadian Journal of Microbiology*, 1971, 17:1145-1147.

Rabotti, G. F.; and F. Blackham, "Immunological Determinants of Avian Sarcoma Viruses: Presence of Group-Specific Antibodies in Fowl Sera Demonstrated by Complement-Fixation Inhibition Test," *Journal of the National Cancer Institute*, 1970, 44:985-991.

Roth, Frieda K.: Paul Meyers; and Robert M. Dougherty, "The Presence of Avian Leukosis Virus Group-Specific Antibodies in Chicken Sera," *Virology*, 1971, 45:265-274.

Scheele, Christina M.; and Hidesaburo Hanafusa, "Proteins of Helper-Dependent RSV," *Virology*, 1971, 45:401-410.

Stolfi, R. L.; Ruth A. Fugmann; J. J. Jensen; and M. M. Sigel, "A C1-Fixation Method for the Measurement of Chicken Anti-Viral Antibody," *Immunology*, 1971, 20:299-308.

Temin, Howard M.; and Satoshi Mizutani, "RNA-dependent DNA Polymerase in Virions of Rous Sarcoma Virus," *Nature*, 1970, 226:1211-1213.

Yoshii, Saiji; and Peter K. Vogt, "A Mutant of Rous Sarcoma Virus (Type O) Causing Fusiform Cell Transformation (35039)," *Proceedings of the Society for Experimental Biology and Medicine*, 1970, 135:297-301.

PREFACE

The first oncogenic virus to be discovered, RSV remains a major focus of interest for those concerned with the etiology of cancerous growth. At present, RSV represents the best experimentally accessible model of an oncogenic RNA virus whose effects can be observed both *in vivo* and *in vitro*.

The present two-volume collection includes papers published from 1970–1972 on host cell surface changes induced by RSV, on the enzymic machinery contained in the virion as well as on the viral nucleic acids. Current research on RSV mutants or genetic variants is also presented.

NUCLEIC ACIDS OF ROUS SARCOMA VIRUS

Comparative Properties of RNA and DNA Templates for the DNA Polymerase of Rous Sarcoma Virus

PETER DUESBERG, KLAUS V. D. HELM, AND ELI CANAANI

Indirect evidence suggests that RNA tumor-virus replication requires virus-specific DNA early in infection (1, 2). This virus-specific DNA is thought to be transcribed from viral RNA by the virus-associated DNA polymerase (3, 4). The ubiquity of this enzyme in all known RNA tumor-viruses (3–5), its ability to transcribe *in vitro* most or all viral RNA (6, 7), and the presence of at least one enzyme per virion (8) are compatible with its role of a RNA–DNA transcriptase essential for virus replication. Very different kinds of nucleic acids, however, were reported to be templates for virus-associated DNA polymerase, perhaps indicating that Rous Sarcoma Virus (RSV) DNA polymerase is not specific for virus replication. Unpurified DNA polymerase of RNA tumor-viruses was shown to accept exogenous natural DNA (9–11), synthetic DNA, DNA–RNA hybrids, or double-stranded RNA as templates (10, 12) besides the endogenous viral RNA (4, 3, 6). Synthetic homopolymer RNAs, however, were found to be poor templates (12, 10), except when present with a complementary oligodeoxynucleotide primer (13). A purified DNA polymerase of RSV that was free of endogenous template responded to both natural DNA and single-stranded RNA templates, and preferred among natural RNAs the 60–70S RNA of RSV (8).

Abbreviations: RSV, Rous sarcoma virus; TMV, tobacco mosaic virus.

The present report describes quantitative comparisons of the template activities of various nucleic acids in the presence or absence of oligodeoxynucleotides for the purified RSV DNA polymerase. Further, the question of whether RNA and DNA templates compete for the same RSV DNA polymerase was investigated.

MATERIALS AND METHODS

[^{3}H]dCTP, [^{3}H]dTTP, 14–20 Ci/mmol; [^{3}H]dATP, 6 Ci/mmol were purchased from New England Nuclear, [^{3}H]dGTP 3 Ci/mmol from Amersham, deoxynucleotide triphosphates from Sigma or CalBiochem, and oligodeoxyribonucleotides (of chain length 12–18) from Collaborative Research Inc., Waltham, Mass. Poly(dAT) was a gift of Dr. M. Chamberlin.

Viruses. Prague RSV of subgroup (14) C was used to prepare (8) 60–70S RNA and DNA polymerase, unless otherwise indicated. DNase treatment of RSV RNA (8) was in 5 mM $MgCl_2$–5 mM KCl–50 mM Tris (pH 7.4). Schmidt–Ruppin RSV of subgroup A, myeloblastosis-associated virus of subgroup B, and avian leukosis MC-29 virus of subgroup A were all originally obtained from Dr. P. K. Vogt (14) and grown as described (8).

Standard DNA Polymerase Assay. Purification of the soluble RSV DNA polymerase (8) was in a 20–40% (v/v) glycerol gradient. The enzyme was stored at −20°C in the gradient solution after the addition of glycerol to a final concentration of 50%.

Standard assays were at 40°C for 2 hr in 100 μl of solution containing 50 mM KCl, 50 mM Tris (pH 8.0), 5 mM dithiothreitol, 6 mM $MgCl_2$, three unlabeled dNTPs at 0.1 mM, and one [^{3}H]dNTP at 1.25 μM (8). The assay contained 15 μl of enzyme solution. Given a 20-fold purification of the enzyme, based on the total soluble protein of the virus (8) and the assumption that 2 A_{260} (measured in 0.2% sodium dodecylsulfate) of purified virus corresponds to 1 mg of protein (8), the protein content of 15 μl of enzyme solution was 50–75 ng, which was operationally defined as one protein unit.

RESULTS

Dependence of DNA synthesis on enzyme and template concentration

Constant concentrations of 60–70S RSV RNA, heat-dissociated RSV RNA (15), influenza RNA, or tobacco mosaic virus RNA were incubated with different concentrations of RSV DNA polymerase. Maximal [^{3}H]DNA synthesis was obtained in all cases with 2 protein units of enzyme (Fig. 1), 5 times more DNA was synthesized at saturating

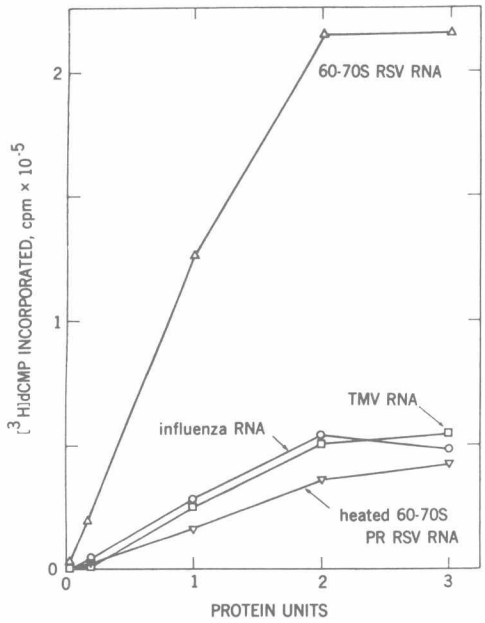

FIG. 1. Dependence of RNA-primed [^{3}H]DNA synthesis by RSV DNA polymerase on enzyme concentration. 1 μg of the indicated RNA was incubated under standard assay conditions, but with different amounts of protein units (*Methods*) of purified enzyme.

concentrations of enzyme with 60–70S RSV RNA than when heat-dissociated RSV RNA or other RNAs were used as templates. This result is compatible with the hypothesis that a defined structure of the RNA template determines the rate of [^{3}H]dCTP incorporation by RSV DNA polymerase.

Saturation quantities of a constant concentration (half-saturating for 1 μg of RNA, see Fig. 1) of RSV DNA polymerase with several nucleic acids are shown in Fig. 2. It appears that all nucleic acids reach, or at least approach, plateaus of maximal DNA synthesis at concentrations of 1–5 μg. Particularly with TMV RNA, but also with 28S ribosomal RNA and influenza RNA (Fig. 2*B*), the saturation curves suggest that there is inhibition of the enzyme by high concentrations of RNA templates. Heating of ribosomal RNA to 100°C, followed by sedimentation, did not affect its template activity, (not shown). Among the RNAs, 60–70S RSV RNA stimulates incorporation of [^{3}H]dCTP 5- to 10-times better than all single-stranded RNAs tested, including heat-dissociated RSV RNA. The template activity of denatured salmon DNA (8) was about 1.3 times, and that of poly (dAT) about 3 times (see below), higher than that of 60–70S RSV RNA.