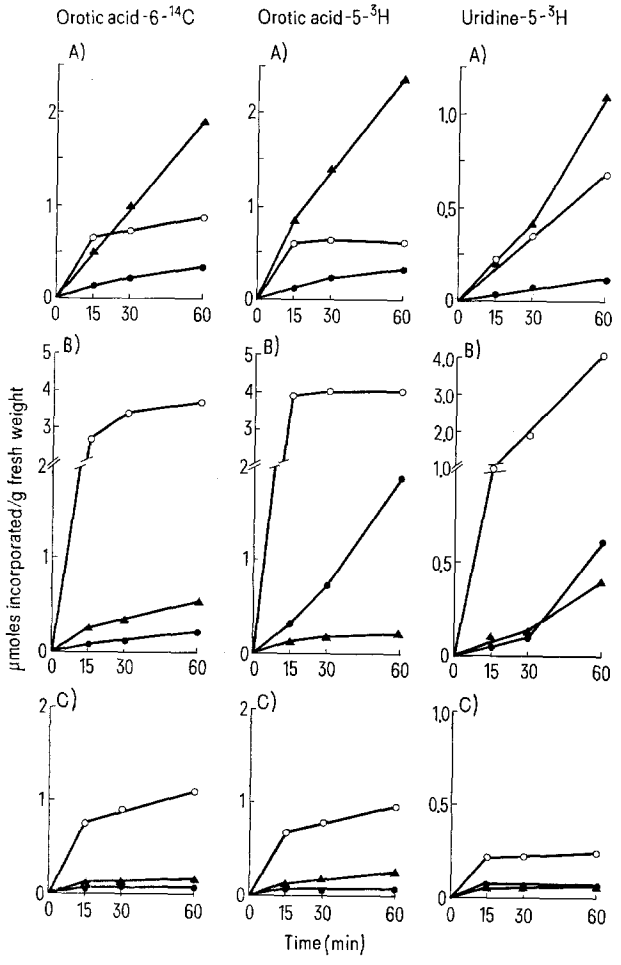
Table II. Percentual distribution of 6-^{14}C orotic acid radioactivity in the bases of RNA after 1 h poulse

	UMP	CMP	AMP	GMP
Exponentially growing cells	52.2	40.0	4.8	5.0
Cells under limited growth conditions by depletion of nitrogen source	76.2	10.0	10.3	4.0
Cells under limited growth conditions by depletion of carbon source	71.8	12.2	12.0	7.0

The data are the average of 2 experiments.



Radioactivity distribution as a function of time in the main cellular fractions of cells fed with pyrimidine precursors of RNA. A) Exponentially growing cells. B) Cells under limited growth conditions by depletion of nitrogen source. C) Cells under limited growth conditions by depletion of carbon source. ○—○, acid soluble fraction; ▲—▲, RNA; ●—●, other macromolecular fractions.

radioactivity assumed, showing that the precursors are utilized at a slower rate. In the experiments with 5-^{3}H orotic acid and 5-^{3}H uridine, 40% and 55% respectively of the total acid insoluble label is found in the fraction different from RNA; when 6-^{14}C orotic acid is used, only 28%. The distribution of the radioactivity incorporated in the macromolecular fractions different from RNA under the various experimental conditions is shown in Table I. In the experiments with 5-^{3}H uridine and 5-^{3}H orotic acid, most of the radioactivity (about 80%) of the non-RNA-insoluble fraction is equally distributed between lipids and polysaccharides, except for nitrogen source deprived cells in which more than 80% of the radioactivity of the non-RNA-insoluble fraction is found into the lipidic fraction. When 6-^{14}C orotic acid is used, the more heavily labelled fraction under all experimental conditions is that corresponding to polysaccharides (about 50% of the total radioactivity incorporated into the non-RNA-insoluble fraction).

The presence of ^{3}H label in the lipid and/or in the polysaccharides fractions is in agreement with the pattern of degradation of orotic acid and uridine through the β-alanine acetyl-CoA pathway[12]. In addition some of ^{3}H label can be lost to tritiated water in the conversion of pyrimidine precursors to TMP[13]. The distribution of the radioactivity of 6-^{14}C orotic acid can be explained by assuming that $^{14}CO_2$ deriving from its breakdown is incorporated through reductive carboxylation.

The data of Table II show the distribution of radioactivity of 6-^{14}C orotic acid in the RNA bases under all experimental conditions. The radioactivity is distributed to the same extent in uridylic acid and cytidylic acid in the RNA from exponentially growing cells, while the uridylic acid appears more heavily labelled in the RNA from cells under limited growth conditions. These findings suggest that the amount of labelled precursor incorporated in uridylic acid or cytidylic acid of the RNA depends on the nutritional condition. The data emphasize the strong limitations which are met for biochemical or autoradiographic studies in using pyrimidine precursors to RNA synthesis in an organism such as *Rhodotorula gracilis*, especially under nutritional conditions of limitation due to the deficiency of nitrogen or carbon source in the medium.

Riassunto. L'uso di precursori pirimidinici marcati in studi biochimici o autoradiografici della sintesi di RNA risulta nel lievito *Rhodotorula gracilis* fortemente limitato specie in condizioni di crescita rallentata per difetto di fonte di carbonio o di azoto.

S. M. COCUCCI and L. GIANANI

Centro di Studio per la Biologia Cellulare e Molecolare delle Piante del C.N.R., Istituto di Scienze Botaniche dell'Università, Via G. Colombo 60, I-20133 Milano (Italy), 27 September 1973.

[12] S. O. DONOVAN and J. NEUHARD, Bact. Rev. *34*, 278 (1970).
[13] H. BREMER and D. YUAN, Biochim. biophys. Acta *169*, 21 (1968).

Transcription Products Synthesized by Purified Calf Thymus DNA-Dependent RNA Polymerase on Shope Papilloma Virus DNA

The recent purification of mammalian DNA dependent RNA polymerase has provided a tool for the study of mammalian DNA viruses[1]. The in vitro transcription of the small oncogenic DNA viruses can be the first step in the elucidation of the proteins coded by these viruses. We report here on the RNA products formed by the transcription of Shope papilloma virus (SPV) DNA using purified calf thymus RNA polymerases A and B.

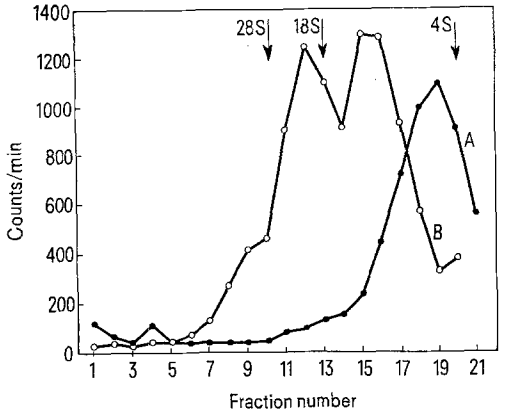


Fig. 1. Products of incubation of the two RNA polymerases and SPV-DNA II. RNA polymerase A (71 μg) was incubated for 60 min with 7.5 μg SPV-DNA II in a standard reaction mixture containing, in 125 μl: 1 μmole $MgCl_2$, 6 μmoles NaF, 600 nmoles each of ATP, GTP and CTP, 5–10 μCi of ^{3}H-UTP (22.5 Ci/nmole), 4 μmoles of phosphoenolpyruvic acid, 200 nmoles of $(NH_4)_2SO_4$, 2 μmoles of 2-mercaptoethanol, 20 μg of pyruvate kinase, 11 μmoles of *tris*-Cl (pH 7.9), enzyme and DNA primer. When the B polymerase was used, 250 nmoles of $MnCl_2$ replaced the $MgCl_2$ and the $(NH_4)_2SO_4$ was increased to 12.4 μmoles. In both assays, water was used to adjust the reaction mixture to the indicated volume. The ammonium sulfate was added after all the other components had been mixed and incubated for 2 min. This was done to facilitate binding of the enzyme to the DNA. SDS was added and the mixture layered on a 4.7 ml linear gradient of 15–30% (w/w) sucrose gradient and centrifuged for 4.5 h at 45,000 rpm in a Spinco SW 65Ti rotor. Acid precipitable RNA was collected on paper discs. ●—●—●, tritiated product of the enzyme A reaction; ○—○—○, tritiated product of the enzyme B reaction.

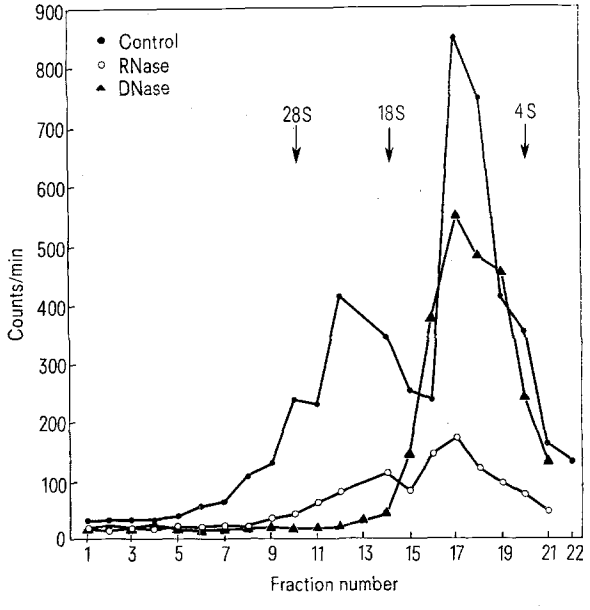


Fig. 2. The effect of DNase or RNase treatment on the products of incubation of RNA polymerase B and SPV-DNA II. RNA polymerase (42 μg) and SPV-DNA II (11.25 μg) were incubated in a standard reaction mixture as described in Figure 1 for 60 min. The mixture was divided in thirds. One part was treated with DNase I (1.35 μg), one part with RNase I (25 μg) and RNase T_1 (580 μg) and the third part with buffer. After 15 min at 37 °C, the mixtures were treated with SDS and centrifuged as described in the legend for Figure 1.

Materials and methods. Calf thymus RNA polymerases were purified by a modification of the method of CHAMBON et al.[1]. Briefly, this includes: homogenization and sonication of the glands in 0.48 *M* ammonium sulfate from high speed supernatant solutions, separation of RNA polymerases A and B on DEAE-cellulose and velocity sedimentation through 10–30% (v/v) glycerol gradients. Both A and B enzymes were free of detectable DNase and each migrated as a major band on gel electrophoresis. There was little cross contamination of A and B polymerase preparations.

SPV-DNA was isolated from isopycnically purified virus by the method of WATSON and LITTLEFIELD[2]. The superhelical DNA (Form I) was separated from the 'nicked' form (II) by velocity sedimentation on sucrose gradients[3].

All polymerase reactions were terminated by the addition of sodium deodecylsulfate (SDS) to a final concentration of 0.3% and incubation at 37 °C for 2 min followed by cooling. The samples were then analyzed on linear, 15–30% (w/w) sucrose gradients or by Cs_2SO_4 isopycnic centrifugation. Trichloracetic acid-insoluble RNA was collected and counted on filter discs.

Results and discussion. As seen in Figure 1, both RNA polymerases A and B are able to transcribe the Form I DNA. The product formed by transcription with the A enzyme sedimented as a single, heterogenous peak (4–11 s), while the B polymerase produced 2 peaks after sedimentation. Both peaks sedimented faster than the A product (4–11 s and 21–30 s). Similar results were obtained when SPV DNA II was the template.

To determine the nature of the products formed by the B polymerase, a reaction mixture containing SPV-DNA II and RNA polymerase B was divided into 3 parts after a 60 min incubation and each part treated by addition of one of the following: a) buffer + EDTA-buffer, b) DNase I (RNase free) and c) pancreatic RNase in EDTA buffer. After 15 min incubation at 37 °C, all 3 mixtures were treated with SDS and centrifuged on sucrose gradients. The results (Figure 2) show that the 18–28 s peak is sensitive to both RNase and DNase, whereas the slow

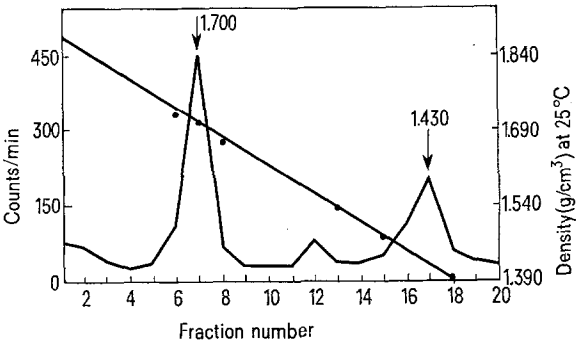


Fig. 3. Centrifugation to equilibrium of the products of incubation of RNA polymerase B and SPV-DNA II. RNA polymerase B (63.8 μg) was incubated with 22.5 μg SPV-DNA II in a standard reaction mixture as described in Figure 1 for 60 min. SDS was added to the reaction mixture which was mixed with 5 ml Cs_2SO_4 in 0.04 *M* phosphate: 0.008 *M* citrate.

[1] P. CHAMBON, F. GISSINGER, J. L. MANDEL, C. KEDINGER JR., M. GNIAZDOWKI and M. MEIHLAC, Cold Spring Harb. Symp. quant. Biol. *35*, 693 (1970).
[2] J. D. WATSON and J. W. LITTLEFIELD, J. molec. Biol. *2*, 161 (1960).
[3] L. V. CRAWFORD and E. M. CRAWFORD, Virology *21*, 258 (1963).

peak (9 s) is labile only in the presence of RNase. The residual, RNase resistant material may result from symmetrical transcription, which has been shown to occur in vivo[4].

Figure 3 shows the results of experiments in which the products of the reaction were centrifuged to equilibrium in Cs_2SO_4 after SDS treatment. Two main peaks of radioactive complementary RNA are found in the gradient at densities of 1.43 and 1.70 g/cm³. The densities indicate that the 1.70 g/cm³ peak, corresponding to the slow sucrose gradient peak, is free RNA. The labeled complementary RNA in the 1.43 g/cm³ peak is in the DNA region of the gradient and therefore is complexed to the DNA template.

The appearance of a double stranded DNA:RNA complex, resistant to 0.3% SDS treatment, appears to be unique to the SPV system. In 2 previous studies on the products of transcription of SV40 DNA by mammalian RNA-polymerases, no such species was reported[5,6]. The free RNA synthesized from SPV-DNA was also smaller than that synthesized using SV40 DNA as a template.

Differences between this study and the earlier reports on SV40 could be due to the uniqueness of the DNA's or to differences in the enzyme preparations. Further studies will be required to determine whether the hybrid formed here is a true DNA:RNA base-paired complex or if the DNA and RNA are complexed together by a protein(s) which is resistant to 0.3% SDS and high salt.

Since Shope papilloma virus has resisted all efforts to propagate it in vitro, attempts to analyze SPV products have been frustrating[6,7]. The fact that one can transcribe SPV-DNA into RNA by mammalian polymerase may be an important step towards ultimate analysis of SPV and its oncogenicity.

Zusammenfassung. Ausgehend von der DNS eines onkogenen Virus wird der Versuch gemacht, in einem in vitro System mit Warmblüter-RNS-Polymerase verschiedene RNS-Moleküle zu synthetisieren.

L. A. KAPLAN and M. E. HODES

Indiana University School of Medicine, Department of Medical Genetics, 1100 West Michigan Street, Indianapolis (Indiana 46202, USA), 21 August 1973.

[4] G. KHOURY, J. C. BYRNE and M. A. MARTIN, Proc. natn. Acad. Sci. USA *69*, 1925 (1972).
[5] J. L. MANDEL, C. KEDINGER, F. GISSINGER and P. CHAMBON, FEBS Lett. *29*, 109 (1973).
[6] M. HERZBERG and E. WINOCOUR, J. Virol. *6*, 667 (1970).
[7] J. E. MOULTON and D. LAU, Cornel Vet. *54*, 602 (1964).

Vibrationssignale bei der Paarung von *Panorpa* (Mecoptera/Insecta)

Von dem 8 Ordnungen umfassenden Komplex der Neuropteroidea (im Sinne WEBERS[1]) sind Vibrationssignale bisher nur von den Dipteren[2] bekannt. Zur Verbreitung ähnlicher Kommunikationssysteme siehe[3,4]. Neue Untersuchungen zeigen, dass diese Art der Mitteilung häufiger ist.

Der Kopulation von *Panorpa* geht ein komplizierter Einleitungsteil voraus. Paarungsbereite ♂♂ und ♀♀ suchen sich gegenseitig, indem sie hin- und herlaufen und dazwischen auffällig mit den Flügeln winken (Figur 1). Dieses rhythmische Flügelspreizen wird von lebhaften Abdomenbewegungen begleitet, deren Bedeutung bisher unerkannt war. Die raschen Auf- und Abbewegungen des Abdomenendes werden durch die Beine auf den Untergrund übertragen. Die Intensität der Signale ist äusserst gering, und die Vibrationen sind nur durch den Einsatz eines Vorverstärkers nach einem Magnetmikrophon auf einem Tonband registrierbar (Auswertung oszillographisch).

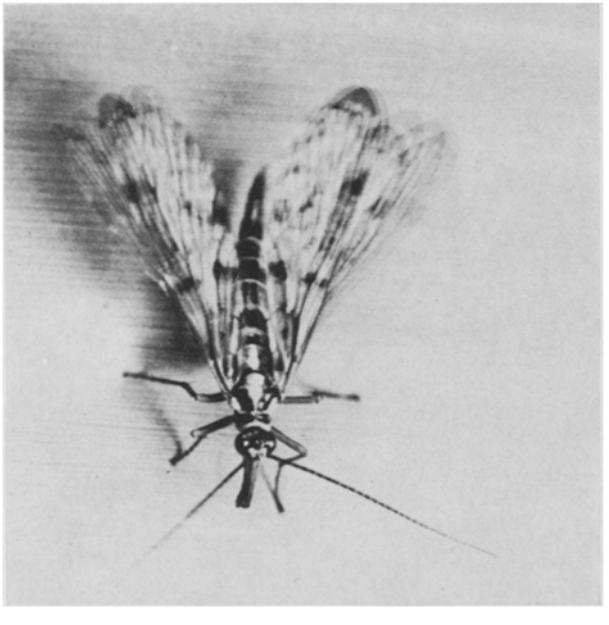


Fig. 1. Ruhig sitzendes ♀ von *Panorpa communis*, das mit den Flügeln winkt. Beleuchtung mit einem Stroboskop, Belichtung 0,5 sec.

Beobachtet wurden die Arten *Panorpa alpina* RAMBUR, *P. communis* L. und *P. germanica* L. Die Grundfrequenz der Verse liegt bei 100 Hz (in wenigen Fällen wurde bei ♂♂ von *P. communis* auch 200 [250] Hz analysiert). Die Verse dauern etwa 0,1 sec und werden im Abstand von 0,1–2 sec 3 bis 40 mal wiederholt, wobei es auch zu mehr oder weniger kontinuierlichen Vibrationen kommen kann (Figur 2). Die registrierten Erschütterungen werden nur durch die Abdomenbewegungen verursacht (im Gegensatz zu *Drosophila*[5]), da diese Vibrationen auch ohne Flügelbewegungen hervorgebracht werden. Ferner wurden keine Erschütterungen durch Flügelbewegungen registriert, wenn ohne Abdomenbewegungen gewinkt wurde. Stärker erregte ♂♂ berühren während des Vibrierens mit ihrem Abdomen auch den Untergrund und erzeugen dadurch Klopffolgen von 2–8 Hz für 1–2 sec Dauer (Figur 3).

Die Bedeutung der Vibrationssignale liegt in der Mitteilung der Fortpflanzungswilligkeit. Wie von zahl-

[1] H. WEBER, *Grundriss der Insektenkunde* (Fischer, Stuttgart 1954).
[2] J. H. MOOK and CH. BRUGGEMANN, Entomologia exp. appl. *11*, 397 (1968).
[3] F. ENGELMANN, *The Physiology of Insect Reproduction* (Pergamon Press, Oxford 1970).
[4] H. MARKL, Naturwissenschaften *56*, 499 (1969).
[5] H. C. BENNET-CLARK and A. W. EWING, New Scient. *26*, 277 (1967).