increased significantly when transducing mixtures containing the wild type phage are plated with antiphage serum. The second is that transduction by ts mutants at the permissive temperature continues to be more efficient than that by wild type. Since different adsorption rates of the phages may be ruled out by experiment, one is left with the possibility that wild type and mutant lysates produced under parallel conditions contain different fractions of transducing particles.

Considering the protective function of the capsule, the question remains to be answered how infection of cells which were not artificially decapsulated is possible. Experiments of Warren and Gray indicate that streptococci which produce hyaluronic acid also elaborate hyaluronidase. Coexistence of the enzyme and its substrate leads to disappearance of hyaluronic acid on continued incubation. Since cells to be transduced came from the stationary phase of growth, they are likely to possess no pro-


As for the temperature dependence of the process that results in the formation of transducing particles, further work is necessary to find out the underlying mechanism. Among others, the following hypothesis is attractive. As the temperature decreases from 37° to 26°, the reproduction and/or maturation of progeny phage is increasingly delayed (see Figs. 2 and 3) in favour of either a genetic interaction between vegetative phage and host genome or some kind of phenotypic mixing, as a result of fragmentation of the bacterial chromosome to pieces of proper size for incorporation into phage heads.

I thank Drs R. M. Cole and A. E. Colón who generously provided bacterial strains and phages and critically read the manuscript. The dependable technical help of Mrs. I. Hoffmann is gratefully acknowledged.

Separation of poliovirus specific RNA structures by analytical gelelectrophoresis

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Die analytische Gelelektrophorese an Agarose-Polyacrylamiglden gestattet eine Auftrennung der poliovirus-spezifischen RNS-Strukturen: Einzelstrang-RNS, doppelsträngige replikative Form (RF) und replikative Intermediatform (RI). Unter den verwendeten Bedingungen werden die RNS-Arten nach ihrer Molekülgröße getrennt. Auch in dieser Trennmethode zeigt sich die Inhomogenität der mehrsträngigen RI-Struktur.

Three types of virus specific RNA have been found in poliovirus infected cells: a single stranded viral RNA with a molecular weight of $2 \times 10^6$, a double stranded replicative form (RF) with a molecular weight of $4 \times 10^6$ and a replicative intermediate form (RI) with a molecular weight of $4 - 8 \times 10^6$.

This communication reports about the application of the gel electrophoresis by W. Davies for the separation of RNA structures from the Qβ-bacteriophage, Reovirus RNA and Influenza virus. The method proves useful for a qualitative and quantitative analysis of the three types of poliovirus specific RNA. Similar results by Noble et al. appeared recently. In their case RI did not enter the 2.25% acrylamide gel used and then would be separated from the two other and smaller viral structures. We also observed this behaviour of RI. In order to analyze the expected inhomogeneity of RI

3 D. BALTIMORE, J. molecular Biol. 32, 359 [1968].
by gelelectrophoresis, it appeared derivable to reduce the gel concentration to permit migration of this structure.

Owing to the relatively high molecular weights of the RNA structures in question the concentration of acrylamide and the degree of polymerization had to be rather low (1% acrylamide and about 0.1% \( N,N'\)-Bismethyleneacylamide). The resulting very soft polymers were stabilized by the addition of agarose according to Peacock and Dingman\(^\text{10}\).

In order to prevent any interaction between proteins and nucleic acids the gels, the buffer and the samples to be analyzed contained sodium dodecyl sulfate (SDS) and ethylenediaminetetraacetic acid (EDTA)\(^6,7\). (For details see legends.) The experimental samples consisted of cytoplasmic extracts of poliovirus infected HeLa-Cells. The virus specific RNA was pulse labelled with \(^{14}\)C- or \(^{3}\)H-Uridine in the presence of actinomycin D. Residual cellular RNA synthesis amounts for about 5—8% in infected cells under these conditions. According to Zimmermann et al.\(^\text{11}\) this residual cellular RNA sediments drastically slower than the 18 S viral RF and was not pointed out in these experiments. Half of each preparation was treated with pancreatic RNase. Both aliquots were then adjusted to 1% SDS and 0.01 M EDTA. The samples were analyzed by gelelectrophoresis as well as by sucrose gradient centrifugation. Fig. 1 depicts the electropherogram of the untreated (solid line) and RNase treated (broken line) cell extracts. The material without RNase distributed itself in several peaks named A, B and C. After preincubation of the extract with RNase only Peak B remained. Since pancreatic RNase exclusively digests single-stranded RNA the material in peak B was considered to represent the double-stranded RF. This was confirmed by sucrose gradient analysis. As shown in Fig. 2, the RNase treated material sedimented in three peaks. One of them is 18 S RNA (broken line), which is characterized physicochemically by other authors in the literature as RF\(^1\). The other peaks of slower sedimenting RNA, most likely degradation products, could not be detected by electrophoresis because they migrated under the conditions used out of the gel.

\(^{10}\) A. C. Peacock and C. W. Dingman, Biochemistry 7, 668 [1968].

\(^{11}\) F. F. Zimmermann, M. Heeter, and J. E. Darnell, Virology 19, 400 [1963].

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![Fig. 1. Electrophoresis of cytoplasmic extracts from polio infected HeLa cells before (○—○) and after (●—●) treatment with RNase. Electrophoresis was performed for 150 min. (for details see text below); 20 µl of material were applied to each gel. Extracts were prepared in the following way: 2.4×10⁶ HeLa S3 cells in 10 ml GBI-Spinner medium without serum containing 1 mmolar Guanidine and 5 µg/ml actinomycin D were exposed for 45 min to poliovirus type 1 Mahoney at an input multiplicity of 50. The cells were then washed with warm GBI-Spinner medium containing 5% calf serum and transferred into 100 ml of the same medium. 5 µg/ml of actinomycin D were added and the suspension kept at 37° degrees under magnetic stirring, 225 min after infection a 30 min-pulse of \(^{14}\)C-Uridine (10 µC, 510 mC/mole) was given. The cytoplasmic extracts were prepared as described by Baltimore et al.\(^\text{13}\) Half of the extract was treated with RNase (5 µg/ml for 5 min at 37°). SDS (1% final concentration) and EDTA (0.01 M final concentration) were added to both the RNase treated and untreated material. Preparation of gels: according to Peacock and Dingman\(^\text{10}\): 1% acrylamide; 0.075% \( N,N'\)-bis-methylene-acylamide; 0.01 M EDTA; 1.5% agarose; 0.0005 M TEMED (\( N,N',N',N'\)-Tetramethylethylenediamine), 0.5 m of a 1.6% solution of \((\text{NH}_4)_2\text{SO}_4\) was added to 15.5 m of the gel mixture and polymerization was allowed to proceed for 16 hours at 20°C. Size of the gels: 70×4.5 mm. Electrode buffer: 0.1 m sodium phosphate buffer (pH 7.2) containing 0.1% SDS and 0.01 M EDTA. Electrophoresis: 3 V/cm and 12 mA/gel at 20°C for various times as indicated in the figures. Analysis of the gels: after electrophoresis the gels were frozen at −70°. After extraction from the glass tubes they were cut into 2 mm fractions by means of a multiblade cutter. The fractions were transferred into glass vials as used in the Packard liquid scintillation counter and slightly agitated overnight with 0.5 ml of water. After addition of Bray’s solution \(^\text{14}\) the radioactivity in the individual samples was counted in the liquid scintillation spectrometer.

The majority of labelled RNA in cell extracts was found in the faster moving peak A. This material was destroyed by RNase. In keeping with these features peak A was considered to represent the single-stranded viral RNA.

\(^{13}\) D. Baltimore, M. Girard, and J. E. Darnell, Virology 29, 179 [1966].

\(^{14}\) G. A. Bray, Analytic. Biochem. 1, 279 [1960].
Fractions of Gradient

Fig. 2. Sucrose gradient centrifugation of extracts from polio infected cells (see text Fig. 1) untreated (o—o) and after treatment with RNase (●—●). The arrows indicate the positions of cellular ribosomal RNA (OD 260 m). Gradients: 15—30% sucrose in SDS-buffer (0.01 m tris-HCl, pH 7.4; 0.1 m NaCl, 0.01 m EDTA; 0.5% SDS). Centrifugation: the gradients were centrifuged for 16 hours at 28 °C and 19000 rpm in a Spinco L 2 ultracentrifuge, rotor SW 25.1. Analysis: 0.55 ml fractions were collected. 0.1 ml of each fraction was precipitated with TCA after addition of serum albumine as described 15. The washed precipitates were counted in the liquid scintillation spectrometer in POPOP/PPO-toluene.

As shown in Fig. 3, the 35 S peak material derived from sucrose gradient centrifugation moved exactly to the position of peak A in electrophoresis. Therefore this peak represents single-stranded viral RNA.

The RNA in peak C (see Fig. 1) was also sensitive to RNase in that it moved together with peak B after RNase treatment of the extracts as indicated by the increase of material moving in position B although the absolute amounts applied for the two gels were equivalent. This resembles the characteristics described for the replicative intermediate form (RI) of poliovirus RNA.

In a separate experiment we investigated whether or not material C is actually identical with RI. The pulse labelled viral RNA was isolated from cell extracts by means of cold phenol. From the resulting aqueous phase the single-stranded RNA and the RI were precipitated by 2 m LiCl according to BALTIMORE 13.

![Fig. 3. Gel electrophoresis of extracts from polio infected cells (see text Fig. 1): full circles●●●●; single strand RNA derived from the sucrose gradient (see 35-S Fig. 2, fract. 12): open circles o—o. Time of electrophoresis: 140 min, other conditions were equal to those described in Fig. 1. 20 μl of samples were applied to each gel.](image)

![Fig. 4. I: Gelelectrophoresis of 3 H-labelled RI as isolated by precipitation in 2 m LiCl (●—●). II. Gelelectrophoresis of a mixture of 3 H-labelled RI as above and 14 C-labelled RF. The counter was set to register 14 C exclusively in one channel (o—o) and both 14 C and 3 H in the other (●—●). Electrophoresis was allowed to proceed for 200 min; all other conditions were the same as described in Fig. 1. The RF was isolated from extracts after RNase treatment (see Fig. 1). 3 H-labelled RI was obtained in the following way: 3.5 x 108 HeLa S 3-cells (see Fig. 1) were pulse labelled for 2.5 min at 225 min after infection with 3 H-Uridine (0.25 mC with a spec. act. of 6700 mC/mmole). Cytoplasmic extracts were prepared according to BALTIMORE 13. The extract was centrifuged for 30 min at 39000 rpm (Spinco L 2, rotor SW 39). The pellet obtained after centrifugation was resuspended in 3 ml of buffer (0.15 m NaCl, 5 m tris-HCl, pH 7.5) and SDS was added to 0.1%. The solution was extracted at 4 ° with buffer-saturated phenol. After residual phenol was removed from the aqueous phase, single-strand RNA and RI were precipitated by 2 m LiCl as described by BALTIMORE and GIRARD 1. The pellet obtained after centrifugation was resuspended in the buffer described above (1% SDS) and used for electrophoresis. When 3 H-RI was run alone in the gel 20 μl of the sample were used. When running the mixture (Fig. 4, II) 10 μl each of RF 14 C and 3 H (RI + single-strand-RNA) were applied to the gel. At the conditions used, the single-stranded RNA Peak A migrated out of the gel: Fig. 4, therefore, only shows the first 30 fractions collected.](image)
MORE and Girard, while the double-stranded RF remained in solution. The resulting mixture of single-stranded viral RNA and RI was then analyzed by gelelectrophoresis (Fig. 4, I). Under the conditions chosen the material of peak A (single-stranded RNA) moved out of the gel. The RI migrated slowly to give an irregular profile at a position corresponding to that of material C of the cell extracts (see Fig. 1). In order to demonstrate that the 18 S RF was not part of this material an artificial mixture of ³H-labelled RI and ¹⁴C-labelled RF was investigated by means of gelelectrophoresis.

To distinguish the two isotopes in the resulting fractions the liquid scintillation counter was set to register exclusively ¹⁴C in one channel and both ¹⁴C and ³H in the other. It is obvious that the ¹⁴C-labelled RF (peak B) moves ahead of the majority of ³H-labelled RI (peak C) and thus was not identical with material C as contained in cell extracts.

Note in proof: After this paper had been submitted for publication similar observations were published in a very interesting article by M. Girard and Louise Marty, Bull. Soc. Chim. biol. 51, 31 [1969].

The RI complex proved inhomogenous in electrophoretic mobility as well as in its sedimentation behaviour. This is to be expected on theoretical grounds since the molecular weights of RI vary between 4—8 × 10⁶. The sedimentation constants were found to range between 70—18 S. The technique of gelelectrophoresis as applied here separates structures mainly according to molecular size

The RI structures contained in the cell extracts and prepared as described in this communication were predominantly of the smaller variety because the differences in molecular weights between RI (peak C) and RF (peak B) were minor and thus the two structures were not fully separated by means of gelelectrophoresis.

The separation was, however, sufficient to distinguish between them and to assess their relative amounts.

I thank Prof. Dr. E. Wecker for his support and discussions and Miss A. Pomp for her excellent technical assistance. This work was partially supported by the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk.