within species. Hubby and Lewontin (*8*) surveyed many strains of *D. pseudoobscura* and found no electrophoretic variants of GDH, although variants of some other enzymes were common.

For this investigation, Sim's (*6*) technique was used with modifications to accommodate flat-bed electrophoresis equipment (EC Apparatus Corp.). Strips of polyacrylamide gel (5 percent acrylamide) were cast according to the method of Raymond and Wang (*9*). Before use, the gels were equilibrated with buffer of 0.025*M* tris brought down to *p*H 6.0 with H_3PO_4. Buffer in electrode vessels was 0.05*M* trisphosphate, *p*H 6.0. Flies were squashed on small squares of filter paper and inserted between cut ends of the gel. The top and bottom of the apparatus were cooled with running tap water. A potential of 24 volt/cm and a current of about 45 ma was applied across the gel for 2 to 4 hours.

The mixture to give color to regions of GDH activity contained 90 ml of tris buffer (0.05*M*, *p*H 8.5), 0.18 g of disodium dihydrogen ethylenediaminetetraacetate, 0.8 g of disodium glycerophosphate pentahydrate (α and β mixture; Calbiochem), 4 ml of nicotinamide-adenine dinucleotide solution (10 mg/ml), 4 ml of phenazine methosulfate solution (0.2 mg/ml), and 20 mg of nitro blue tetrazolium. Gels incubated in this mixture for about 1 hour at 20°C begin to show purple formazan in areas of GDH activity. One fly has sufficient enzyme activity to give dark areas. To insure that minor components were detected, the gels were incubated overnight in this mixture.

It was found that inbred strains of *D. melanogaster* may be divided into two types according to the electrophoretic mobility of their GDH. When the procedures described above are used, it is found that one type contains GDH that migrates more rapidly to the anode than the other. Canton-S, Samarkand, and Oregon-RC are examples of common wild-type strains that contain the more rapidly migrating enzyme. Swedish-c and Oregon-R have the slower migrating enzyme (see Fig. 1). There appears to be a family of enzymes in each inbred. There is a major component and two slower-moving minor components. The whole pattern is displaced when the slower and faster types are compared. Larvae, pupae, and adults have the same patterns. Whether these multiple forms are present in the living animal or are artifacts cannot be determined at this time. Sims (*6*) did not observe this heterogeneity in her experiment; however, Hubby and Lewontin (*8*) observed two areas of GDH activity in all strains of *D. pseudoobscura*.

Crosses between the two different types yield hybrids which have five visible components with GDH activity. There are three major components: the two parental ones and a hybrid with intermediate mobility (Fig. 1). The only minor components that are detectable are those of the slow-type parent. Other minor components in the hybrid are obscured by the three major components. The presence of a hybrid major component indicates that the GDH molecule contains at least two protein subunits. The parental major components contain two subunits that are alike; the hybrid contains two unlike subunits (*10*). Flies trapped from a wild population in Oak Ridge, Tennessee, are polytypic. The rapid, slow, and hybrid patterns of GDH are all found in this one population.

Genetic analysis shows that the differences in electrophoretic mobility of GDH are based on there being two alleles of a genetic locus. This locus (called Glycerophosphate dehydrogenase, symbol *Gdh*) is located on the second chromosome. On the standard linkage map of *D. melanogaster* (*11*) it has a genetic map position of about 17.8. It is between the loci of clot eye color (map position 16.5) and Sternopleural bristles (map position 22.0). Homozygotes of the allele Gdh^R have the rapid pattern. Homozygotes of the allele Gdh^S have the slow pattern of GDH. The hybrid pattern is produced by Gdh^R/Gdh^S heterozygotes.

The approximate cytological position of the *Gdh* locus is known from analysis of *Df(2L)GdhA*. This second chromosome deficiency was selected from x-irradiated chromosomes which lacked the wild-type allele of *cl* (clot eye color). In one case the irradiation induced a deficiency which included both cl^+ and *Gdh*. The electrophoretic pattern of $Df(2L)GdhA/Gdh^S$ is like that of Gdh^S homozygotes, and $Df(2L)GdhA/Gdh^R$ is like Gdh^R homozygotes. In salivary gland chromosomes there are a few bands missing. On Bridges' (*11*) salivary chromosome map the left break of *Df(2L)GdhA* is between 25E1 and 25F1. The right break is between 26B1 and 26C1. The locus of *Gdh* must therefore be between 25E1 and 26C1.

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Detergent-Solubilized RNA Polymerase from Cells Infected with Foot-and-Mouth Disease Virus

Abstract. *The foot-and-mouth disease virus RNA polymerase complex was dissociated from cellular membranes with deoxycholate in the presence of dextran sulfate. The soluble polymerase complex was active in the cell-free synthesis of virus-specific RNA; solubilization of the complex permitted direct analysis of the cell-free reaction mixtures without recourse to RNA extraction. A major RNA-containing component found early during cell-free incubation ranged from approximately 140 to 300*S. *The final major products of the cell-free system were 37*S *virus RNA, 20*S *ribonuclease-resistant RNA, and a 50*S *component containing RNA.*

Detailed studies of replication of animal virus RNA in cell-free systems have been hindered by high levels of nuclease or membrane-bound polymerase complexes, or of both (*1*, *2*). The RNA polymerase induced by the foot-and-mouth disease virus (FMDV) is reportedly bound to cellular mem-

branes in lysates of FMDV-infected baby hamster kidney cells (*3, 4*). This report concerns the dissociation of the active polymerase complex from these structures, and its activity in a cell-free system.

Direct analyses of the reaction mixtures which contain the soluble polymerase have shown a heterogeneous RNA-containing component (140 to 300*S*) and a 50*S* component containing RNA, in addition to virus-specific RNA (see *3*). The 140 to 300*S* RNA component is found before the appearance of 37*S* virus RNA.

The FMDV RNA polymerase was prepared according to Polatnick and Arlinghaus (*3*). It is known to be active in 0.5-percent deoxycholate (*3*). However, the virus-specific RNA synthesized in the presence of deoxycholate was largely degraded by contaminating nucleases, yielding 20*S* RNA resistant to ribonuclease, and 4 to 12*S* RNA fragments unless bentonite was present. By use of this deoxycholate-bentonite polymerase mixture, antibody to an antigen associated with FMDV infection (*5*) was found to inhibit FMDV-RNA synthesis by about 90 percent (*6*).

We used dextran sulfate-500 (*7*) to inhibit ribonuclease (*8*). Preliminary experiments showed that dextran sulfate at 10 to 20 μg/ml caused some stimulation of incorporation of ^{3}H-uridine triphosphate in the cell-free FMDV-polymerase system (Fig. 1 legend), whereas high concentrations (1 to 2 mg/ml) strongly inhibited incorporation. Addition of 0.25 percent of deoxycholate to an intermediate concentration of dextran sulfate (140 μg/ml) gave maximum incorporation. The sodium dodecylsulfate–extracted RNA products of the polymerase treated with deoxycholate–dextran sulfate in the cell-free synthesizing system contained all three virus-specific RNA's (*3, 9*): 37*S* virus RNA, 20*S* ribonuclease-resistant RNA, and a heterogeneous RNA.

Cell-free reaction mixtures were examined directly, without prior RNA extraction, by centrifugation on linear sucrose gradients of from 5 to 25 percent in 0.01*M* tris(hydroxymethyl) aminomethane HCl (*p*H 7.5) and 0.001*M* $MgCl_2$, (tris-$MgCl_2$) for 17 hours at 25,000 rev/min in the SW-25.1 rotor. The sucrose-gradient profile of the polymerase reaction mixture, containing neither deoxycholate nor dextran sulfate, after 60 minutes at 37°C (the time at which cell-free synthesis has stopped), showed that 90 percent of the radioactive RNA insoluble in trichloroacetic acid was in the pellet; no significant peak was seen in the gradient. The results were similar when the sample was centrifuged for only 2 hours. It was also determined that active polymerase forms pellets under the same conditions. These results indicate that the FMDV polymerase, as well as its attached RNA template, and its RNA products, are membrane-bound in cell lysates.

Direct sucrose-gradient analysis of reaction mixtures, containing polymerase and both 0.25-percent deoxycholate and dextran sulfate (140 μg/ml) gave the following results after 60 minutes at 37°C: The amount of ^{3}H-uridine triphosphate incorporated into RNA insoluble in trichloroacetic acid was 15 to 25 percent greater than in the absence of deoxycholate and dextran sulfate. The RNA products were released from the membrane, since 60 to 70 percent of the radioactive RNA insoluble in trichloroacetic acid was found in the gradient. The optical-density profile showed peaks of 18*S* and 28*S* ribosomal RNA that originated from ribosomal subparticles present in the polymerase preparation. This deproteinizing of ribosomes is attributed to the action of dextran sulfate (*4*). Three major zones were detected in the gradient (for example, Fig. 1B): a 50*S* component near the bottom of the tube, which was not found after treatment with sodium dodecylsulfate or pretreatment with either 0.01*M* ethylenediaminetetraacetate or 0.04*M* pyrophosphate; a 37*S* zone containing infectious virus RNA; and 20*S* RNA resistant to ribonuclease. No attempt has been made to demonstrate net synthesis of 37*S* infectious RNA in the cell-free system.

A minor peak of 26 to 30*S* heterogeneous RNA was always present in the reaction mixture (peak *D*; *3, 9*), but the amount varied with the activity of the polymerase (that is, activity presumably lost by denaturation of the enzyme and not by ribonuclease). The 50*S* and 37*S* zones were made soluble in trichloroacetic acid by treatment with ribonuclease (10 μg/ml) for 30 minutes at 37°C in 0.15*M* KCl and 0.1*M* tris-HCl, *p*H 7.0. Also, the infectivity of the 37*S* zone was lost on treatment with trace levels of ribonuclease. The 20*S* zone was almost completely resistant to ribonuclease, and

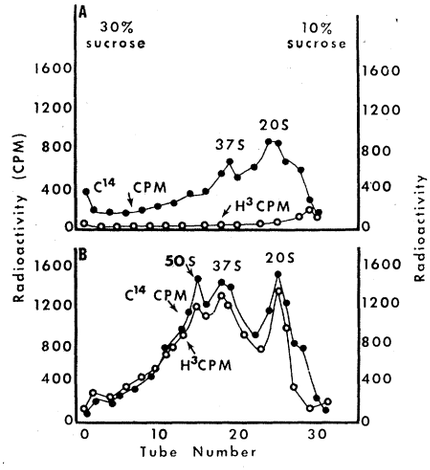

Fig. 1. Sucrose-gradient profiles of the in vitro chase of whole-cell, pulse-labeled, virus-specific RNA. Baby hamster kidney cells (6×10^8) were infected as in text. The soluble polymerase complex was isolated; it contained protein at 2.8 mg/ml and 125,000 count/min mg^{-1} protein of ^{14}C-uridine, as RNA insoluble in trichloroacetic acid, at 48-percent counting efficiency. The complete cell-free reaction mixture contained: 10 μmole of tris-HCl, *p*H 8.1 (23°C); 5 μmole of phospho(enol)-pyruvate; 20 μg of pyruvate kinase; 25 mμmole of each of adenosine triphosphate, cytidine triphosphate, uridine triphosphate, and guanosine triphosphate; 12.5 μmole of $MgCl_2$; 0.1 ml of polymerase; and water to a final volume of 0.7 ml. The mixture was incubated (see text). Casein (300 μg) and 10 ml of 5-percent trichloroacetic acid were added to each gradient fraction. After 20 minutes at 0°C, the precipitate was collected on type-B6 membrane filters (25 mm in diameter; Schleicher and Schuell), and the filter was washed five times with 5-percent trichloroacetic acid. Samples were counted in a liquid scintillation spectrometer (*9*). (A) The cell-free reaction mixture was held at 0°C for 60 minutes with 10 μc of ^{3}H-uridine triphosphate. The reaction mixture contained 100 μg of dextran sulfate, 0.05 percent deoxycholate, 0.1 ml of soluble polymerase complex, and all components of the cell-free system in a volume of 0.7 ml. The mixture was diluted to 2.2 ml with 0.01*M* tris-HCl, *p*H 7.5, prior to layering on the gradient. Two milliliters were applied, and the tube was centrifuged for 17 hours at 20,000 rev/min on a 10- to 30-percent linear sucrose gradient in tris-$MgCl_2$ in the SW-25.1 rotor. Carbon-14 at 35,344 count/min was applied to the gradient: 21,147 count/min was in the pellet; 12,336 count/min, in the gradient. (B) The reaction mixture was the same as for (A), and the tube was incubated for 60 minutes at 37°C, chilled and treated as for (A). Carbon-14 at 32,634 count/min was applied to the gradient: 5,862 count/min was in the pellet; 24,350 count/min, in the gradient.

most likely is double-stranded RNA. It has been reported that the 20*S* zone is only partially resistant to ribonuclease (*3, 9*); however, such results were obtained from RNA, extracted with sodium dodecylsulfate and phenol, that contained larger amounts of the above-mentioned heterogeneous RNA. This heterogeneous RNA (peak *D*; *3, 9*) is partially sensitive to ribonuclease, and overlaps the 20*S* double-stranded RNA (*4*).

It was of interest to examine the RNA associated with the polymerase preparation; one could then discriminate between free, unbound, virus-specific RNA and active protein-RNA complexes (polymerase-template). The latter is the structural complex that replicates virus RNA. The polymerase was harvested, 3.5 hours after infection, from FMDV-infected baby hamster kidney cells pulse-labeled with ^{14}C-uridine under such conditions that only virus-specific RNA was labeled. Actinomycin D (5 μg/ml) was added 30 minutes before harvest, and 20 μc of ^{14}C-uridine was added 15 minutes before harvest (*9*). This schedule of addition of actinomycin D caused no reduction in production of polymerase (*10*). The enzyme was prepared as usual (*3*) and made 1 mg/ml in dextran sulfate and 0.5 percent in deoxycholate before storage at −60°C; this preparation will be referred to as the "soluble polymerase complex." Activity of the soluble polymerase complex was stable for long periods at −60°C, even after repeated freezing and thawing.

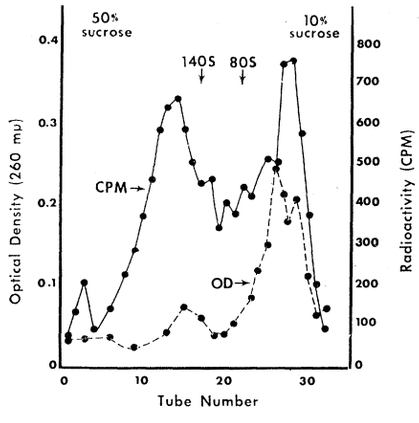

Fig. 2. Sucrose-gradient profile of the 140 to 300*S* RNA containing component of the soluble polymerase complex. The soluble polymerase complex (0.9 mg) was incubated in 2.1 ml of the complete cell-free reaction mixture (Fig. 1) for 5 minutes at 37°C, with 30 μc of ^{3}H-uridine triphosphate. The solution was rapidly chilled and adjusted to 0.1*M* tris-HCl, *p*H 8.1 (0°C). After addition of ammonium sulfate to 50 percent saturation and standing for 20 minutes at 0°C, the suspension was centrifuged for 15 minutes at 10,000*g*. The precipitate was suspended in 2.2 ml of tris-$MgCl_2$, and 2 ml was layered on a 10- to 50-percent linear sucrose gradient in tris-$MgCl_2$. The gradient was centrifuged for 17 hours at 15,000 rev/min in the SW-25.1 rotor. Tritium at 18,375 count/min was applied to the gradient; 4032 count/min was in the pellet; 12,662 count/min, in the gradient. Samples were precipitated with trichloroacetic acid as in Fig. 1.

The soluble polymerase complex labeled with ^{14}C-uridine was incubated in the complete cell-free system with ^{3}H-uridine triphosphate—specific activity, 400 μc/μmole (Fig. 1 legend). One tube was kept at 0°C; another was incubated for 1 hour at 37°C. The reaction mixture was analyzed directly (no RNA extraction) on a 10- to 30-percent linear gradient at 20,000 rev/min. The whole-cell material, pulse-labeled with ^{14}C-uridine, in soluble polymerase complex, contained both 37*S* and 20*S* virus-specific RNA, and a small amount of the 50*S* component appeared to be present (Fig. 1A). However, 65 percent of the ^{14}C radioactivity was found in the pellet (*S*-rate exceeding 100).

After incubation in the cell-free system at 37°C, nearly all the whole-cell RNA labeled with ^{14}C-uridine was found in the gradient in the three major zones (Fig. 1B). This test constituted a true chase experiment, since total counts per minute of ^{14}C in both the zero-time and the incubated sample were identical; only the distribution of radioactivity varied. This fact suggests that the pellet material (exceeding 100*S*) is a precursor to the 50*S*, 37*S*, and 20*S* components. Moreover, nearly all the polymerase complexes active in the whole cell (labeled with ^{14}C-uridine) are active in the cell-free system. The product of the cell-free synthesis of RNA (^{3}H-RNA) gave an identical profile, and 85 percent of the ^{3}H-RNA was found in the gradient (Fig. 1B).

Since the polymerase complex incorporates ^{3}H–uridine triphosphate into RNA for only 60 minutes at 37°C in the cell-free system, analysis of products formed early (after 5 to 10 minutes) should identify precursors to 37*S* virus RNA. Therefore a soluble polymerase complex was incubated for 5 minutes in the cell-free system containing dextran sulfate at 140 μg/ml. The reaction mixture was rapidly chilled to 0°C and adjusted to 50 percent of saturation with ammonium sulfate at *p*H 8.1.

Analysis of the precipitate material by zonal centrifugation gave the profile shown in Fig. 2. A peak of radioactivity is found ranging from 140 to 300*S*, and another is at the top of the tube. The 140 to 300*S* RNA component is not found after extraction with sodium dodecylsulfate; after treatment with ribonuclease at 1 μg/ml for 5 minutes at 37°C; or in reaction mixture incubated for 60 minutes. In all three instances all the RNA product occurred only at the top of the gradient. The RNA contained in the 140 to 300*S* complex (presumably of RNA and FMDV polymerase) appears to be identical with the heterogeneous RNA (peak *D*; *3, 9*). Difficulties in demonstration of the 140 to 300*S* component have been encountered because of aggregation in solutions containing magnesium. It should be noted that intact ribosomes are not detected in the soluble polymerase complex (Fig. 2). The methods of both isolation and storage of the soluble polymerase complex in dextran sulfate at 1 mg/ml and 0.5-percent deoxycholate destroy ribosomes and polyribosomes.

These results suggest that the 140 to 300*S* RNA containing component is a precursor to FMDV 37*S* RNA. Studies with poliovirus in intact HeLa cells indicated that synthesis of poliovirus RNA takes place in complex structures possessing an average *S*-rate value of 250 (*2*).

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