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# Translation of specific vaccinia virus RNAs purified as RNA-DNA hybrids on potassium iodide gradients

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ABSTRACT

A procedure has been developed for purifying specific mRNAs by hybridization to fragments of DNA and isolation of the hybrids by potassium iodide equilibrium buoyant density centrifugation. The hybrids obtained are essentially free of unhybridized RNA as well as double-stranded RNA. Moreover, the RNA in the hybrids is undamaged and can be translated in vitro. Application of this procedure to mapping vaccinia virus genes is described. A total of 34 polypeptides have been assigned to three regions of the viral genome.

## INTRODUCTION

Several different strategies for the mapping of gene products to specific regions of a large genome have been described. Isolated DNA fragments have been used as templates for sequential transcription and translation in vitro (1,2), or to specifically arrest the translation of mRNAs to which they hybridize (3), or to physically isolate hybridizable mRNAs which can then be translated. The purification of hybrids has been achieved either using DNA fragments immobilized to solid supports (4,5), or using solution hybridization followed by an isolation procedure such as hydroxyapatite chromatography (6) or gel exclusion chromatography (7).

None of the above methods have yet been applied to mapping the vaccinia virus genome. Because of its complexity (approximately 120 Mdal) and the relatively small amounts of specific DNA fragments available, solution hybridization methods appeared to be advantageous. The hybridization arrest procedure failed, however, because of the presence of self-complementary vaccinia virus late mRNAs (8,9) which annealed during hybridization and were not translated (J.A.C., unpublished). Moreover, because of the very large number of vaccinia virus gene products and the differences in relative abundance of individual RNA species (10,11), the negative approach of hybridization arrest seemed to be less desirable than the translation of physically isolated RNA

 

species. However, isolation procedures relying on the double-stranded nature of the hybridization product (e.g. gel exclusion chromatography) did not completely separate RNA-DNA hybrids from double-stranded RNA (J.A.C., unpublished). For these reasons, we investigated equilibrium buoyant density centrifugation as a means of isolating RNA-DNA hybrids for translation. The hybrids were readily separated from both unhybridized and double-stranded RNA on potassium iodide gradients, even when a considerable mass excess of RNA over DNA was used. Moreover, the RNA present in the hybrids was efficiently translated in vitro without further purification, making the procedure relatively simple. Here we demonstrate the method by comparing the translation products of RNAs hybridizing to different regions of the vaccinia virus genome.

## MATERIALS AND METHODS

Potassium iodide was analytical grade (Baker). Formamide was recrystallized at 0° (12) or purified by ion exchange resin (13).

The preparation of cytoplasmic RNA from vaccinia virus infected cells was as described (14), except that a two times plaque-purified stock of vaccinia virus (strain WR) was used. When required, approximately 2 mCi of [5,6-$^{3}$H]uridine (50 Ci/mmol) was added per liter of cells for 2 hr prior to harvesting. RNA from some preparations was selected on oligo(dT) cellulose (type T-3, Collaborative Research) as described (15). DNA was purified from vaccinia virus which had been isolated without sonication, using proteinase K treatment, phenol extraction and dialysis (16). Total DNA was used after Hind III restriction, but without separating the fragments. Treatment with Hind III and isolation of restriction fragments on sucrose gradients was as described (16), except that the peak fractions corresponding to Hind III A, B and C were pooled separately and rerun on a second set of sucrose gradients. Alternatively, fragments were isolated from 0.6% agarose gels by binding to glass powder as described (17) with minor modifications (Paterson, B., personal communication).

Hybridization of RNA to denatured vaccinia virus DNA, or to denatured purified restriction endonuclease fragments, was performed under conditions promoting the formation of RNA-DNA hybrids over DNA-DNA reannealing (18). After denaturing the DNA, the hybridization was performed at 37° in 80% formamide and 0.44 M $Na^{+}$ ions. This is only 3° above the "irreversible melting temperature" expected for a 39% (G+C) DNA (18), so higher than average G+C regions of the DNA may renature under these conditions. Nevertheless,

R-loops would be expected to form in these regions and might actually aid in the isolation of hybrids by shifting their buoyant density closer to the density of double-stranded DNA (19). Also, by keeping the temperature low, the probability of hybridizing (A+T) rich mRNA was increased.

To drive most of the high and medium abundance mRNAs into hybrids, the RNA to DNA mass ratio was fixed at 3:1 for poly(A)+ RNA, or 60:1 for total cytoplasmic RNA, which contains 4-5% poly(A)+ RNA (14). Assuming that an average vaccinia virus gene is 1 Kb (10), there are about 180 single copy genes in vaccinia virus and the mass ratio of poly(A)+ RNA to each gene becomes 540:1. Thus, individual mRNAs representing 1/1080 of the population should saturate the DNA, providing that the hybridization continues for long enough. We chose a Rot of about 4 mol sec $l^{-1}$. According to Casey and Davidson (18), the $Rot_{\frac{1}{2}}$ for a pure 1 Kb RNA in formamide is about 0.004 mol sec $l^{-1}$, independent of temperature, so even mRNAs representing only 1/1000 of the poly(A)+ RNA mass should be significantly hybridized.

The details of the protocol used were as follows. Appropriate amounts of RNA and DNA were mixed together in 1.5 ml polypropylene tubes (Brinkman) and precipitated by the addition of 0.1 M NaCl and 2.5 volumes of ethanol. After 4 hr at -20°, the nucleic acids were recovered by centrifugation for 10 min in a "microcentrifuge" (Brinkman) and dissolved using 1 µl of $H_2O$ and 8 µl of formamide for every 1 µg of poly(A)+ RNA or 20 µg cytoplasmic RNA. After heating at 100° for 2.5 min and quenching in ice/$H_2O$, to denature the DNA, 1 µl of a 10X buffer containing 4 M NaCl, 0.4 M Pipes pH 6.4, 10 mM $Na_2$ EDTA (3,18) was added, and the hybridization performed at 37° for 3.5 hr. The hybridization was stopped by diluting with ice cold water to a total volume of 0.5 ml, and a sample taken to determine the amount of labeled nucleic acids present. The hybrids were then purified using buoyant density centrifugation.

Potassium iodide gradients were used because of their high capacity (20), and because control experiments showed that nucleic acids can be ethanol precipitated from potassium iodide solutions without dialysis, and that RNA is translatable after storage in potassium iodide solutions. To each hybridization reaction, 4.5 ml of a solution prepared by dissolving 55 g of KI in 50 ml of 20 mM Tris-HCl pH 7.5, 10 mM 2-mercaptoethanol ($n_{25}$ = 1.436) was added, and the mixture centrifuged at 35,000 rpm in the SW50.1 rotor (Beckman) at 18° for 3-4 days. The gradient was tapped from the bottom to yield 20-22 fractions, of which samples were counted in 0.6 ml of 1% 2-mercaptoethanol (to prevent chemiluminescence) and 7 ml of Triton toluene

scintillant. Unhybridized RNA and double-stranded RNA banded at a refractive index of 1.45, and DNA at 1.42 (measured in the presence of components from the hybridization reaction). RNA-DNA hybrids banded as a broad peak close to DNA, at about 1.43. The 6 or 7 fractions spanning the DNA peak were pooled, diluted with a KI solution formed from 50 g KI dissolved in 50 ml of 20 mM Tris-HCl pH 7.5, 10 mM 2-mercaptoethanol ($n_{25}$ = 1.429) and centrifuged as before. The hybrid region was again pooled, diluted with 2 volumes of water and 5 volumes of ethanol and stored at -20°. If required, 25 µg of calf liver tRNA were added as carrier. The precipitate was centrifuged at 10,000 X g for 30 min, dissolved in 200 µl $H_2O$ and transferred quantitatively to a 1.5 ml tube with two 100 µl $H_2O$ washes. The hybrids were then melted at 100° for 2.5 min followed by quenching in ice. At this stage, a sample was taken to determine the radioactivity in the hybrids. The denatured hybrids were then reprecipitated with 0.1 M NaCl and alcohol, washed twice with 70% ethanol and dried under vacuum.

Normally, the entire precipitate of nucleic acid was dissolved in 10 µl of reticulocyte lysate incubation mix constructed as described (14), and incubated at 30° for 40 min. The reactions usually contained 20 µCi L-[$^{35}$S]methionine (800 Ci/mmol, Amersham). At the end of the incubation, a 2 µl sample was taken for alkaline hydrolysis and acid precipitation and the remainder treated with ribonuclease A and prepared for gel electrophoresis (14). Normally, the equivalent of 2-4 µl of the incubation was loaded on each slot of a 10-18% gradient sodium dodecyl sulfate polyacrylamide gel. Electrophoresis was performed and the gels were fluorographed or autoradiographed as described (14).

## RESULTS AND DISCUSSION

Hybridization of cellular and viral mRNA to total vaccinia virus DNA. To evaluate the usefulness of potassium iodide density gradient centrifugation for separating hybridized from unhybridized RNA, we performed RNA excess hybridizations with total cytoplasmic RNA and total Hind III cleaved vaccinia virus DNA. Because of the presence of ribosomal and transfer RNAs, as well as a mass excess of viral RNA, we expected that only a small proportion of the total RNA would bind to the DNA, providing a stringent test of the method.

Total cytoplasmic RNA was prepared from cells that had been incubated with [$^{3}$H]uridine for the previous 2 hr. As described previously (14), immediate early viral RNA (CYCLO RNA) was obtained from cells infected in

the presence of cycloheximide, an inhibitor of protein synthesis, while early RNA (CAR RNA) was obtained from cells infected in the presence of cytosine arabinoside, an inhibitor of DNA synthesis. Late viral RNA was obtained 6 hr after infection in the absence of any inhibitor. The $^{3}H$-labeled RNA was then hybridized with $^{32}P$-labeled vaccinia virus DNA under conditions favoring RNA-DNA rather than DNA-DNA annealing and purified by two cycles of KI gradient centrifugation. After the first centrifugation, peaks of radioactive material were detected at hybrid densities (Fig. 1A,C,E,G), and further purifica-

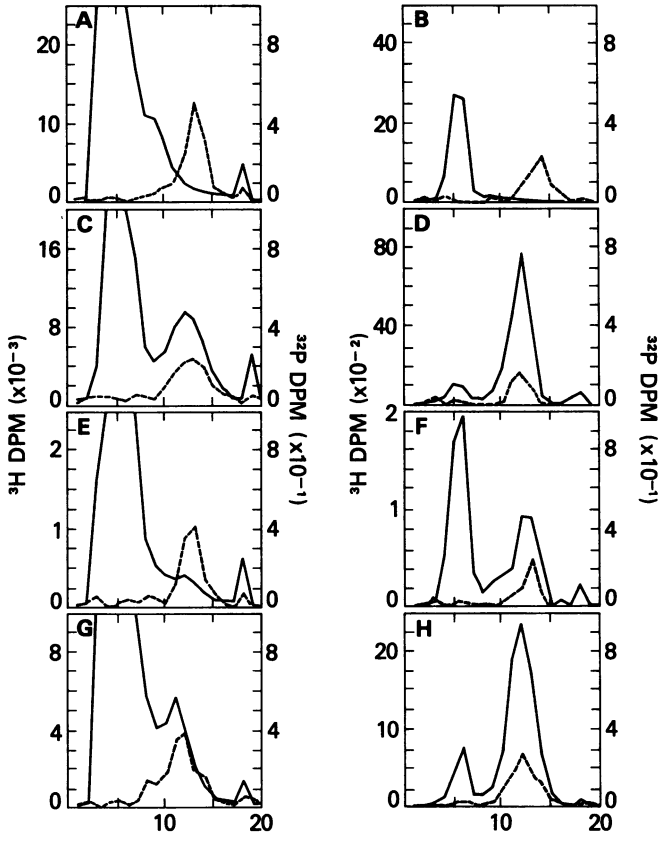


Fig. 1. Purification of RNA-DNA hybrids by KI gradient centrifugation. Total vaccinia virus DNA-labeled with $^{32}P$ was hybridized with $^{3}H$-total cytoplasmic RNA made from uninfected cells (A,B) or from cells infected in the presence of cycloheximide (C,D) or cytosine arabinoside (E,F) or from cells late in infection (G,H). The reactions were analyzed on KI gradients (A,C,E, G). Fractions 10-16 were pooled and re-centrifuged on KI gradients (B,D,F, H). Approximately 10% of each fraction was counted. Solid line: $^{3}H$ radioactivity. Broken line: $^{32}P$ radioactivity.

tion was achieved, particularly in the case of CAR RNA, by a second centrifugation (Fig. 1B,D,F,H). Control experiments showed that the small amount of uninfected cell RNA in the hybrid density region was removed during the second centrifugation (Fig. 1A,B). Although the CYCLO and LATE RNAs appeared to be substantially pure after only one cycle, two cycles of centrifugation were routinely used to ensure minimal contamination.

Quantitative analysis of this experiment (Table 1) shows that this procedure allowed recovery of about 50% of the DNA, and presumably of a similar proportion of the hybrids. When the proportion of RNA radioactivity which was recovered is corrected for the recovery of DNA (last column, Table 1) it is seen that less than 0.1% of UNINFECTED RNA hybridized to vaccinia virus DNA, but 8.4%, 0.7% or 4.1% of CYCLO, CAR or LATE RNA hybridized. The low proportion of radioactive CAR RNA which hybridized was expected because of the kinetics of viral RNA synthesis in the presence of cytosine arabinoside (21).

RNA isolated by hybridization to total vaccinia virus DNA was tested for its ability to direct protein synthesis in the message dependent reticulocyte lysate system (22). Approximately 75% of the RNA which was recovered after hybridization to 7.2 μg of DNA was added to a 10 μl incubation, and the products analyzed by gel electrophoresis (Fig. 2). Little or no translatable RNA from uninfected cells was isolated in the hybrid density region of KI gradients. The only bands seen were synthesized from endogenous RNA

Table 1. Recovery of DNA and Hybridized RNA after Two Cycles of KI Gradient Centrifugation

| RNA[a] | $^{32}$P-DNA | | | $^{3}$H-RNA | | |
|---|---|---|---|---|---|---|
| | Input | Recovered | | Input | Recovered[b] | |
| | dpm | dpm | % | dpm X $10^{-3}$ | dpm X $10^{-3}$ | % |
| UNINFECTED | 2495 | 1668 | 67 | 8980 | 5.1 | 0.06 (0.09) |
| CYCLO | 2470 | 1027 | 42 | 7260 | 256.3 | 3.53 (8.4) |
| CAR | 2745 | 1304 | 48 | 1260 | 4.2 | 0.34 (0.7) |
| LATE | 3755 | 2316 | 62 | 4320 | 110.8 | 2.56 (4.1) |

[a] 420 μg of $^{3}$H-labeled total cytoplasmic RNA was hybridized with 7.2 μg of $^{32}$P-labeled total vaccinia virus DNA. Samples were taken for scintillation counting after hybridization and after purification of hybridized RNA through two cycles of KI gradient centrifugation.

[b] The % RNA recovered, corrected for recovery of DNA, is indicated in parentheses.

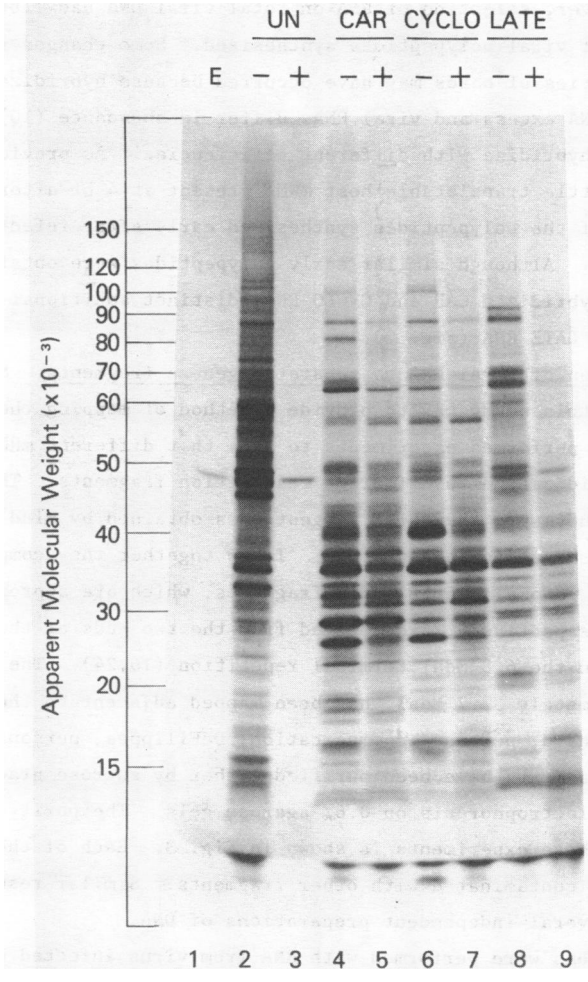


Fig. 2. In vitro translation of RNA selected by hybridization to total vaccinia virus DNA. Reticulocyte lysate incubations contained no added RNA (track 1), or 1 μg of total cytoplasmic uninfected cell, CYCLO, CAR or LATE RNA (tracks 2, 4, 6 and 8, respectively). Hybrids formed between vaccinia virus DNA and uninfected, CYCLO, CAR and LATE RNA were purified, melted and translated (tracks 3, 5, 7 and 9, respectively). Approximately equal volumes from each translation reaction were loaded on each track of a dodecyl sulphate polyacrylamide gradient gel. An autoradiograph is shown.

in the reticulocyte system. In contrast, the isolated RNA from infected cells stimulated [$^{35}$S]methionine incorporation at least as well as did 1 μg of unselected RNA, and directed the synthesis of high molecular weight poly-

peptides. Moreover, selection of RNA on total viral DNA had little effect on the pattern of viral polypeptides synthesized. Some changes in the relative intensities of bands may have occurred because hybridization was carried out in RNA excess and viral RNAs differ in abundance (10) or because different mRNAs hybridize with different efficiencies. As previously found, there is very little translatable host mRNA present at 4 hr after infection (14), and most of the polypeptides synthesized early after infection are virus coded (23). Although similar early polypeptides were obtained upon translation of hybridized CAR and CYCLO RNA, distinct additional polypeptides were formed with LATE RNA (Fig. 2).

Hybridization of viral RNA to separated genome fragments. Since the ultimate aim of this study was to provide a method of mapping the vaccinia virus genome, we performed experiments to show that different mRNAs could be purified by hybridization to different restriction fragments. The fragments used for this study were the three largest ones obtained by Hind III digestion of vaccinia virus (strain WR) DNA. Taken together they comprise about 54% of the viral genome. The B and C fragments, which are approximately 18.5 and 13.5 Mdal, respectively, are derived from the two ends of the linear genome, and contain the 6.8 Mdal terminal repetition (16,24). The A fragment, which is approximately 32.7 Mdal, has been mapped adjacent to the B fragment (Mackett and Archard, personal communication; DeFilippes, personal communication). These fragments have been purified either by sucrose gradient centrifugation or by electrophoresis on 0.6% agarose gels. The purity of the fragments used for these experiments is shown in Fig. 3. Each of the fragments is only slightly contaminated with other fragments. Similar results were obtained with several independent preparations of DNA.

Hybridizations were performed with RNA from virus infected cells and separated restriction fragments of viral DNA. In most experiments, approximately 20 X $10^{-15}$ moles of single copy genes were used, equivalent to 2.4 µg of total virus DNA, 0.65 µg of Hind III A, 0.37 µg of Hind III B or 0.27 µg of Hind III C. This represents 13 ng of a 1 Kb gene, and provides a maximum yield of 6.5 ng of a typical 1 Kb mRNA. Thus, even though the quantity of RNA and the number of different RNA species hybridized to each fragment may be approximately proportional to the size of the fragment, the quantity of each particular mRNA species should be approximately constant, and the amount of *in vitro* translation production similar.

Table 2 shows that a significant proportion of either CYCLO, CAR or LATE RNA hybridized to each of the Hind III fragments used. The amount of labeled

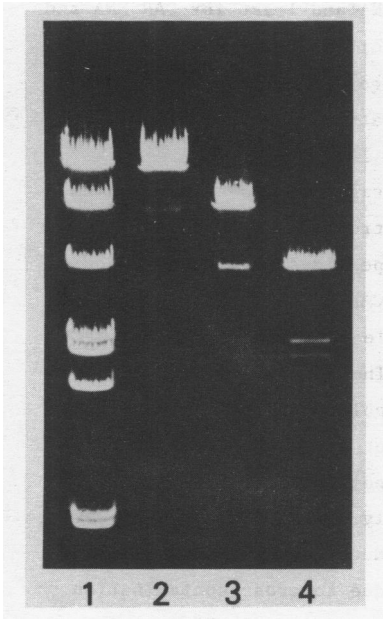


Fig. 3. Characterization of restriction endonuclease fragments used for hybridization. Fragments purified by sucrose gradient centrifugation were analyzed on a 0.6% agarose slab gel and visualized by ethidium bromide staining. Fragments in the range of 33 to 5 X $10^6$ daltons are shown. Track 1: 1.5 µg of vaccinia virus DNA, cleaved with HindIII. Tracks 2, 3 and 4: approximately 0.5 µg of HindIII A, 0.3 µg of B and 0.25 µg of C, respectively.

Table 2. Proportion of Radioactive RNA Hybridized to Fragments of Vaccinia DNA

| Expt. no. | DNA fragment | | Cytoplasmic RNA | Poly(A)+ RNA | % $^3$H-RNA cpm bound[a] | | |
|---|---|---|---|---|---|---|---|
| | | | | | CYCLO | CAR | LATE |
| | | µg | µg | µg | | | |
| 1 | HindIII A | 0.57 | – | 7 | 2.1 | nd[b] | 3.9 |
| | HindIII B | 0.30 | – | 7 | 1.5 | nd | 2.1 |
| | HindIII C | 0.27 | – | 7 | 1.2 | nd | 1.9 |
| 2[c] | HindIII A | 0.57 | – | 7 | 0.5 | nd | 4.8 |
| | HindIII B | 0.30 | – | 7 | 0.7 | nd | 1.9 |
| | HindIII C | 0.27 | – | 7 | 0.2 | nd | 1.9 |
| 3 | HindIII B | 2.3 | 420 | – | nd | nd | 2.4 |
| | HindIII C | 2.0 | 420 | – | nd | nd | 2.1 |
| 4 | HindIII A | 1.96 | 420 | – | 1.0 | 0.4 | 2.6 |
| | HindIII B | 0.92 | 420 | – | 0.5 | 0.2 | 0.8 |
| | HindIII C | 0.81 | 420 | – | 0.4 | 0.4 | 1.3 |

[a] In Expts. 1, 2 and 3, the % RNA bound was corrected for the actual recovery of DNA. In Expt. 4, this value was assumed to be 75%.

[b] Not determined because of low specific activity of the RNA used.

[c] Non-sonicated virus was used for infection. In vitro translation of this RNA before hybridization suggested that a large proportion of cellular mRNA was present in CYCLO and CAR samples.

RNA that bound was generally greatest for LATE RNA and least for CAR RNA and was proportional to the amount of DNA. As most of the viral mRNA is polyadenylated (14), selection of the RNA on oligo(dT) cellulose raised the proportion of RNA which hybridized. Using a relatively greater amount of DNA allowed more RNA to hybridize (Table 2, Expt. 3).

The RNA selected on different restriction fragments was translated in vitro and the products were analyzed by gel electrophoresis (Fig. 4). For each DNA fragment, a distinctive pattern of polypeptides was obtained and this pattern varied according to whether CYCLO, CAR or LATE RNA was used. Clearly, the hybrid isolation procedure is capable of selecting specific mRNAs from a background of unhybridized mRNAs. The 34 polypeptides detected in Fig. 4 are listed in Table 3. Most of these polypeptides have also been obtained in repeated experiments using independent preparations of hybrids. Several general features are of interest. Many polypeptides are clearly coded for by mRNA hybridizing to one fragment only. In other cases, however, polypeptides of similar molecular weight are produced using RNA hybridized to different fragments. This is unlikely to be due to cross-contamination between restriction fragments because this should effect all the translation products equally. For example, if the CAR mRNA for a 30.9 Kdal polypeptide coded by fragment B is an artefact caused by contamination of fragment B with fragment A, then this should cause contamination with other fragment A-coded mRNAs, for example, the mRNA for the major 22.5 Kdal polypeptide. In other cases, for example the 26.8 Kdal polypeptides coded by fragments A and B, the relative amounts of the different mRNAs present in CYCLO RNA and in CAR RNA are different. In the immediate-early RNA (CYCLO) there is more message hybridizing to fragment A than to B, but with early RNA (CAR) more 26.8 Kdal polypeptide message hybridizes to B than to A. There are thus two different gene products with a molecular weight of 26.8 Kdal, and the immediate early gene product (previously designated 28 Kdal [14]) is encoded on fragment A.

Also the mapping provides another "dimension" for separating gene products. The four polypeptides in the range from 34.5-35.0 Kdal, whose messages hybridize to fragments A, B and C, are difficult to resolve on the gel electrophoresis system used here. Probably other polypeptides of similar molecular weight are encoded in the rest of the genome. Thus, many of the broad bands obtained by gel electrophoresis of the translation products of total mRNA probably represent mixtures of proteins.

While distinct early and late polypeptides have been assigned to fragment A, few distinct late polypeptides appeared to be specific for fragments

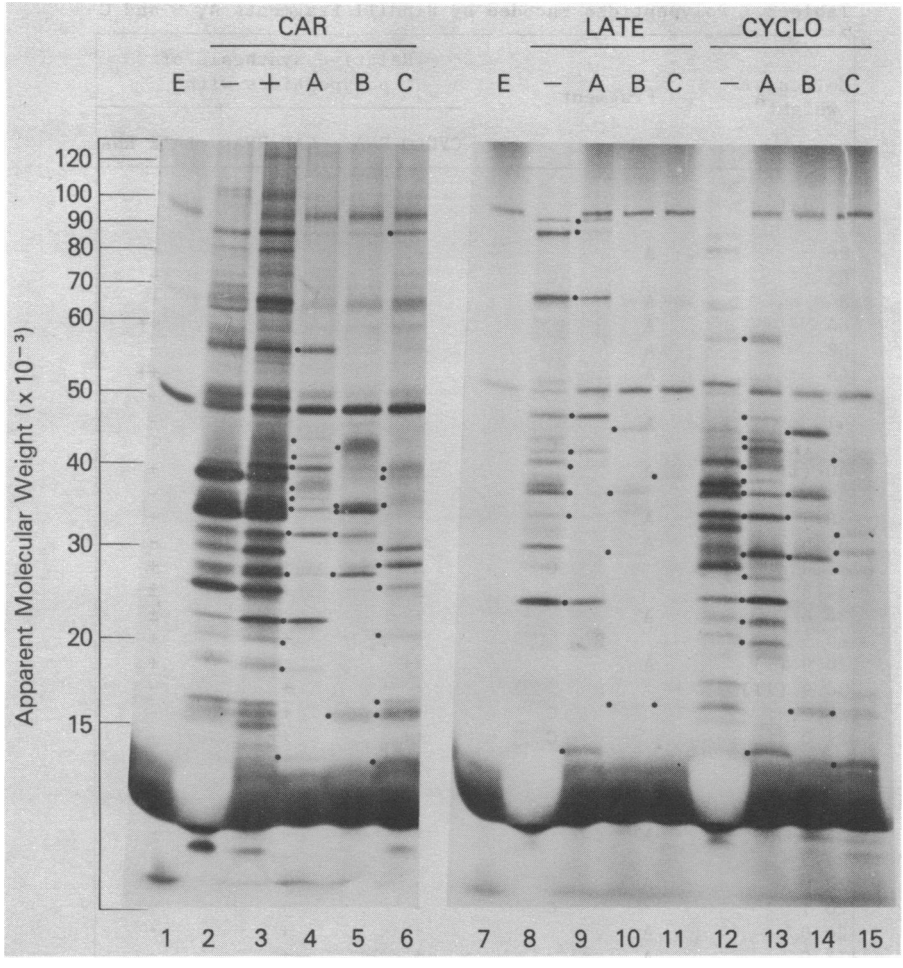


Fig. 4. In vitro translation of RNA selected by hybridization to purified fragments of vaccinia virus DNA. Reticulocyte lysate incubations contained no added mRNA (tracks 1 and 7) or 0.1 μg of poly(A)-containing CAR, LATE or CYCLO RNA (tracks 2, 8 and 12, respectively). Hybrids formed between poly(A)-containing CAR RNA and total DNA or Hind III fragments A, B or C (tracks 3, 4, 5 and 6, respectively) were purified, melted, translated and analyzed on one gel. Hybrids formed between poly(A)-containing LATE RNA and Hind III fragments A, B or C (tracks 9, 10 and 11, respectively) were purified, melted, translated and analyzed on another gel, together with hybrids similarly prepared with poly(A)-containing CYCLO RNA (tracks 13, 14 and 15). Approximately equal counts from each sample were analyzed on the gel. The spots mark polypeptides listed in Table 3.

Table 3. Polypeptides Encoded by HindIII Fragments A, B and C

| Molecular weight[a] | Fragment | Relative synthesis of polypeptides with | | |
|---|---|---|---|---|
| | | CYCLO RNA | CAR RNA | LATE RNA |
| Kdal | | | | |
| 96 | A | -[b] | - | + |
| 85 | C | - | + | - |
| 84 | A | - | - | + |
| 64 | A | - | - | ++ |
| 58 | A | ++ | ++ | - |
| 45 | A | ± | ± | ++ |
| 43 | B | ++ | ± | + |
| 41 | A | ± | + | - |
| 40 (i) | C | - | + | - |
| 40 (ii) | A | + | ++ | + |
| 39.5 | C | ± | ± | - |
| 38.5 | A | + | + | + |
| 36.5 | A | + | + | - |
| 35.0 | C | ± | ± | + |
| 34.8 | B | ± | + | ± |
| 34.6 | A | + | + | ± |
| 34.5 | B | + | ++ | + |
| 30.9 (i) | A | ++ | ++ | + |
| 30.9 (ii) | B | + | + | - |
| 29.2 | C | + | ++ | - |
| 27.0 | C | ++ | ++ | - |
| 26.8 (i) | A | ++ | ± | - |
| 26.8 (ii) | B | ++ | ++ | + |
| 24.5 | C | ± | + | - |
| 24.0 | A | + | - | - |
| 22.5 | A | ++ | ++ | - |
| 22.0 | A | - | - | ++ |
| 20.1 | C | - | + | - |
| 20.0 | A | + | + | - |
| 18.0 | A | + | + | - |
| 16.5 | C | ± | + | - |
| 16.4[c] | B C | + | ++ | + |
| 16.0 | A | ++ | + | ++ |
| 15.9 | C | + | + | - |

[a] Molecular weight values are approximate, particularly below 18 Kdal. The polypeptides listed are marked on Fig. 4.

[b] Order of increasing synthesis in vitro: -, ±, +, ++ .

[c] The gene for this polypeptide maps in the terminal repetition (data not shown).

B and C. This will have to be investigated further since Hind III fragments B and C contain the covalent cross-link present at the ends of the viral DNA

(16,25), and this may interfere with hybridization at the very end of the DNA. Secondly, late mRNA contains self-complementary sequences, and sequences complementary to early RNA (at least 15% of radioactive poly(A)+ RNA can form ribonuclease-resistant complexes [9]). This would lower the effective concentration of single-stranded RNA driving the hybridization reaction. In addition, it is likely that some of the complementary sequences do not code for polypeptides and as the RNAase resistant RNA hybridized to 25% of the genome (9), it seems likely that a considerable proportion of the late transcription products may not be translatable. Thirdly, even though a considerable proportion of radioactive late RNA was hybridized to these fragments, late RNA could have a higher specific radioactivity than early RNA because it has a shorter physical and functional half-life (26,27). Thus, the quantity of functional RNA hybridized may be relatively low.

In conclusion, we have described a procedure for isolating specific mRNAs that appears to involve minimal damage to the RNA, has high yield, requires no special techniques and yields hybrids essentially free of unhybridized or double-stranded RNA. Under some circumstances, a single equilibrium gradient centrifugation should suffice. In other cases, it may be possible to pellet most of the free RNA from a KI solution of appropriate density in place of the first of two successive equilibrium centrifugations. Using these methods, we have mapped 34 polypeptides to three regions of the vaccinia virus genome.

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ABBREVIATIONS

Mdal: $10^6$ daltons. Kdal: $10^3$ daltons. Kb: $10^3$ bases of a single-stranded or $10^3$ base-pairs of a double-stranded nucleic acid.

REFERENCES

1. Roberts, B.E., Gorecki, M., Mulligan, R.C., Danna, K.J., Rozenblatt, S. and Rich, A. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 1922-1926
2. Rozenblatt, S., Mulligan, R.C., Gorecki, M., Roberts, B.E. and Rich, A. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 2747-2751

3. Paterson, B.M., Roberts, B.E. and Kuff, E.L. (1977) Proc. Nat. Acad. Sci. U.S.A. 74, 4370-4374
4. Gilboa, E., Prives, C.L. and Aviv, H. (1975) Biochemistry 14, 4215-4220
5. Noyes, B.E. and Stark, G.R. (1975) Cell 5, 301-310
6. Lewis, J.B., Atkins, J.F., Anderson, C.W., Baum, P.R., and Gesteland, R.F. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 1344-1348
7. Woolford, J.L. and Rosbash, M. (1979) Nucl. Acids Res., in press
8. Colby, C., Jurale, C. and Kates, J.R. (1971) J. Virol. 7, 71-76
9. Boone, R.F., Parr, R.P. and Moss, B. (1979) J. Virol. 30, 365-374
10. Boone, R. and Moss, B. (1978) J. Virol. 26, 554-569
11. Paoletti, E. and Grady, L.J. (1977) J. Virol. 23, 608-615
12. Robberson, D., Aloni, Y., Attardi, G. and Davidson, N. (1971) J. Mol. Biol. 60, 473-484
13. Tibbetts, C., Johansson, K. and Philipson, L. (1973) J. Virol. 12, 218-225
14. Cooper, J.A. and Moss, B. (1979) Virology, in press
15. Aviv, H. and Leder, P. (1972) Proc. Nat. Acad. Sci. U.S.A. 69, 1408-1412
16. Garon, C.F., Barbosa, E. and Moss, B. (1978) Proc. Nat. Acad. Sci. U.S.A. 75, 4863-4867
17. Vogelstein, B. and Gillespie, D. (1979) Proc. Nat. Acad. Sci. U.S.A. 76, 615-619
18. Casey, J. and Davidson, N. (1977) Nucl. Acids Res. 4, 1539-1552
19. Thomas, M., White, R.L. and David, R.W. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 2294-2298
20. Wolf, H. (1975) Anal. Biochem. 68, 505-511
21. Woodson, B. (1967) Biochem. Biophys. Res. Commun. 27, 169-175
22. Pelham, H.R.B. and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247-256
23. Cooper, J.A. and Moss, B. (1978) Virology 88, 149-165
24. Wittek, R., Menna, A., Muller, H.K., Schumperli, D., Boseley, P.G. and Wyler, R. (1978) J. Virol. 28, 171-181
25. Geshelin, P. and Berns, K.I. (1974) J. Mol. Biol. 88, 785-796
26. Oda, K. and Joklik, W.K. (1967) J. Mol. Biol. 27, 395-419
27. Sebring, E.D. and Salzman, N.P. (1967) J. Virol. 1, 550-558