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Exhaustive Hybridization and Its Application to an Analysis of the Ribonucleic Acid Synthesized in T4-Infected Cells*

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ABSTRACT: The method of hybridization with deoxyribonucleic acid (DNA) immobilized on nitrocellulose membrane filters has been applied to the problem of "exhaustive hybridization." Under the proper conditions it is possible to take up into DNA–RNA complexes virtually all of the T4 messenger ribonucleic acid (mRNA) present in an RNA sample from infected cells; thus making this technique very useful for the assay of RNA components present in small amounts. Exhaustive hybridization has been applied to an analysis of the RNA synthesized after infection of *Escherichia coli* B with bacteriophage T4. It has been estimated that the phage-specific RNA accounts for 2.8–4.9% of the total cellular RNA. The value observed depends on the time after infection at which the sample is taken. As late as 3 min after infection a small but significant amount of the [^{3}H]uridine incorporated during a 2-min pulse is found in host-specific RNA. The relative amount of mRNA in this host-specific RNA is estimated to be considerably less than that found in a corresponding 2-min pulse of uninfected cells.

A recent refinement in hybridization methodology, which makes use of DNA immobilized on NC[1] membranes, has been shown to possess many attractive features (Gillespie and Spiegelman, 1965). We wish to report here the application of the procedure of "exhaustive hybridization" and its use in the analysis of RNA synthesis following T4 phage infection. We have coined this expression to distinguish it from "saturation hybridization" which measures the relevant DNA cistrons by saturation with their complementary species of RNA (Yankofsky and Spiegelman, 1962). The object of the exhaustive hybridization experiment is the complete uptake, into hybrid complexes, of all the RNA which is relevant (complementary) to a specific group of DNA molecules.

The previous techniques available for exhaustive hybridization were either difficult to apply to experiments involving many samples at the analytical level (McCarthy and Bolton, 1964; Bautz and Hall, 1962) or they were subject to the complication of DNA–DNA interactions (Nygaard and Hall, 1964). The latter difficulties are particularly important since exhaustive hybridizations are carried out under conditions which maximize DNA–DNA interactions. The use of DNA immobilized on membranes obviates this problem by eliminating DNA renaturation. Furthermore, at the end of the incubation removal of the DNA-bearing membrane eliminates the hybridized RNA and all of the DNA (both hybridized and unhybridized) from the reaction mixture. The unreacted RNA left behind can then be challenged with fresh filters bearing the same or a different type of DNA.

In the present investigation we have used exhaustive hybridization to analyze the RNA synthesized after infection of *Escherichia coli* B with phage T4. The experiments reported here were designed to answer specifically the following questions. What per cent of the total RNA in the infected cell is phage specific? Can the synthesis of any host-specific RNA be detected following phage infection?

Materials and Methods

Bacterial Strains and Bacteriophage. E. coli B and phage T4 were used for all of the experiments reported here. *E. coli* Q13, a mutant lacking RNase I (Gesteland,

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[1] Abbreviations used: NC, nitrocellulose; K, kieselguhr; MAK, kieselguhr coated with methylated albumin; 1X DNA, denatured DNA.

1966), was used as the source of bacterial DNA. Phage stocks were prepared according to Adams (1959) and stored in the presence of tryptophan (100 μg/ml).

Media, Buffers, and Biological Reagents. The defined medium used in all experiments was medium C of Roberts *et al.* (1957) modified to include 1% NaCl, 0.037% KCl, and 1% Casamino Acids. The phosphate concentration was lowered to 10^{-3} M and 0.1 M Tris (pH 7.3) was used for buffering. Tryptophan was present at 100 μg/ml during phage adsorption and at 20 μg/ml during growth of phage-infected cells. The doubling time in this medium is 45 min. The general buffers used and their abbreviations are as follows: Tris-magnesium (TM), 0.06 M Tris (pH 7.3) and 1×10^{-2} M $MgCl_2$; phosphate buffer (P buffer), 0.05 M sodium phosphate (pH 6.8); and saline–citrate (SSC), 0.15 M NaCl and 0.015 M sodium citrate (2X SSC, $^1/_{10}$ SSC, etc., being twice or one-tenth these concentrations, etc.). Antiphage serum was prepared according to the methods outlined by Adams (1959) and was stored frozen at −20°.

Preparation of DNA. Phage DNA was isolated from a purified phage suspension according to the method of Grossman *et al.* (1961). Bacterial DNA was extracted from *E. coli* Q13 (Gesteland, 1966) according to the method of Marmur (1961) and modified by the addition of two phenol extractions. Purified DNA preparations were denatured by raising the pH to 13 with NaOH, and after standing in ice for 5–10 min neutralizing with HCl. Denaturation was monitored by following the hyperchromic shift at 260 mμ.

Preparation of RNA. RNA was extracted from phage-infected cells immediately after harvesting as described previously (Hayashi and Spiegelman, 1961; Landy and Spiegelman, 1967). Following ethanol precipitation the RNA was taken up in 1 ml of TM buffer and incubated with electrophoretically purified DNase (Worthington) (50 μg/ml) for 15 min at 37°. The sample was then diluted 40-fold in P buffer and loaded onto a one-layer MAK column, which was prepared by mixing 1 ml of 1% methylated albumin with 8 ml of a 50% suspension (v/v) of washed kieselguhr (K) and pouring this into a column 1.5 cm in diameter. A 2-ml cap of the same K suspension was layered on top. For more than 1 mg of RNA the column size was doubled, etc. Before loading with RNA the column was washed with at least 500 ml of P buffer and after loading it was washed successively with 10 ml of P buffer and 0.2 M NaCl in P buffer. Finally, the RNA was shocked off the column in a small volume by the addition of 2.0 M NaCl in P buffer.

Protocol for Exhaustive Hybridization with Immobilized DNA. The protocol for exhaustive hybridization is based on the procedure of hybridization with immobilized DNA described by Gillespie and Spiegelman (1965). In all experiments denatured DNA was diluted to 1 μg/ml with 2X SSC and 20 ml (unless specified otherwise) was passed through an NC membrane filter which had been presoaked in 2X SSC for 12 hr. The filter was washed with 100 ml of 2X SSC, dried at room temperature for at least 4 hr, and finally dried in a vacuum oven for 2 hr at 80°. The liquid phase for hybridization in all experiments consisted of 0.4 ml of 2X SSC containing 0.5 μg of the specified RNA. The incubation was carried out in small bore vials such that when the filter was rolled and placed in the vial it was completely covered by the 0.4 ml. (Before rolling the filters for insertion into the vial it is necessary to wet them slightly with 2X SSC.) The 12-hr incubation at 65° was terminated by chilling the vials in an ice bath. The filter was removed from the hybridization liquid and each side was washed with 50 ml of 2X SSC. The filter was then incubated for 1 hr with gentle shaking at room temperature in 5 ml of 2X SSC, containing heat-treated (95° for 10 min at 2 mg/ml in 2X SSC) RNase at 30 μg/ml. Following the RNase treatment the filter was again washed on each side with 50 ml of 2X SSC, dried, and counted in a liquid scintillation spectrometer. Deviation from the mean of replicate samples was always less than 10%.

Results

RNA Purification. Interpretation of the exhaustive hybridization experiments is very much dependent upon the reliability of RNA purity. To test the efficiency of the RNA purification procedure a reconstruction experiment was performed with T4-DNA, which, because it is glucosylated, is one of the most difficult ones to eliminate. A mixture of 100 μg of purified T4 DNA and 1 μg of ^{14}C-labeled RNA was digested with DNase and chromatographed on a one-layer MAK column as described in Methods. The separation of degraded DNA products from the labeled RNA is shown in the gradient elution profile of Figure 1.

Exhaustive Hybridization of Relevant RNA. For a quantitative characterization of the RNA species present in a population of molecules, a foremost requisite is that a significant portion of the RNA input participate in the hybridization. To test the extent of RNA involvement at different DNA:RNA input ratios, NC membranes impregnated with increasing amounts of single-stranded DNA were each incubated in tubes containing a constant small amount of RNA (Figure 2). It should be noted that the usual RNase treatment (see Methods) has been deleted in this experiment. Recent studies with both *E. coli* RNA and with synthetic polymers of defined sequence indicate that RNase-sensitive hybrid contains more pairing errors, or is held together by less stable bonds, than RNase-resistant hybrid (D. Gillespie and S. Spiegelman, in preparation). Whereas in a competition experiment it is desirable to restrict scoring only to RNase resistant hybrid structures, the purpose in this experiment made it desirable to estimate the extent of total RNA involvement. Under the conditions of the present experiment 10–20% of the raw hybrid was found to be sensitive to RNase.

It is seen from Figure 2 that it is possible to achieve involvement of 90% of the input RNA in hybrid

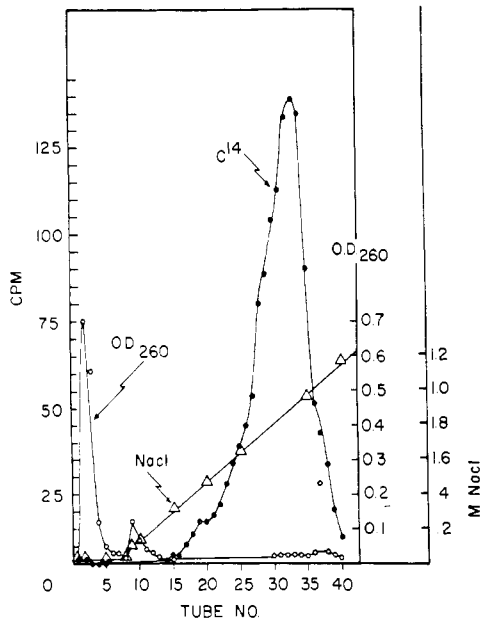

FIGURE 1: Purification of RNA by stepwise elution from MAK. T4 DNA (100 μg) in 1 ml of TM buffer was digested with electrophoretically purified DNase at 37° for 30 min. This was combined with 0.4 μg of ^{14}C-labeled RNA and loaded onto the one-layer MAK column described in Methods. An NaCl gradient (in P buffer) from 0 to 1.3 M was used to elute the material from the column. The OD_{260} and the refractive index were determined and each sample was dried on a plastic planchet which was then counted in the liquid scintillation spectrometer. All of the optical density was recovered from the column and after correcting for quenching, due to the high salt, all of the counts were also recovered.

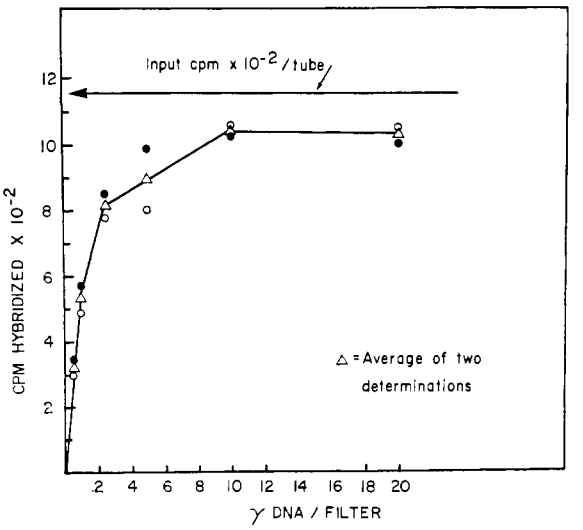

FIGURE 2: RNA uptake as a function of the amount of DNA fixed on the filter. NC filters were loaded with increasing amounts of DNA and dried. The filters were incubated in small bore tubes containing 0.5 μg of RNA in 0.4 ml of 2× SSC. The RNA used was from a T4-infected cell pulsed with [^{3}H]uridine at 13–15 min. The usual RNase treatment described in Methods was omitted in this experiment as explained in the text. The constant input radioactivity is indicated in the figure.

structures. While it is necessary to have a low absolute amount of RNA (with respect to a given quantity of DNA) the kinetics of hybridization are very much dependent upon the concentration of RNA (Nygaard and Hall, 1964). However, unlike the situation existing when both the DNA and RNA reactants are in solution, it is possible, with membrane-bound DNA, to increase the concentration of the RNA by decreasing the volume without concomitantly augmenting the DNA–DNA interaction. The exhaustive hybridization experiments in this investigation were carried out in 0.4 ml (see Methods), although considerably smaller volumes can be employed without excessive complications. The only disadvantage of these hybridization volumes is that the small loss in volume, which necessarily attends removal of the filters, becomes more significant and makes difficult the execution of successive incubations (addition of fresh filters to the same RNA solution). For this reason, when a second incubation is to be carried out, duplicate tubes are pooled following the first incubation and 0.4 ml of this is used for the second incubation.

Preparation of Labeled RNA. The questions being asked in this investigation require the differentiation between phage-specific and host-specific RNA and also a device for distinguishing between preinfection and postinfection RNA. The former is determined by the ability to hybridize with the respective DNAs, and the latter is achieved by differential isotopic labeling prior and subsequent to infection.

By the procedure outlined in Figure 3 all of the RNA, both pre- and postinfection, was labeled at a uniform specific activity with respect to ^{32}P and only that RNA made during the specified pulse period, was labeled with [^{3}H]uridine. Examination showed that in all purified RNA preparations more than 94% of the ^{32}P and more than 97% of the ^{3}H counts are made acid-soluble by treatment with RNase; both isotopes show no loss of acid-insoluble counts after incubation with DNase and more than 95.5% loss after alkali digestion.

Exhaustive Hybridization with T4 DNA. In the first experiment, T4 DNA was fixed to the filters and two consecutive hybridizations were carried out for each RNA sample (see Table I). The fact that some samples show a high percentage of hybridization in the second incubation (column D) indicates that the ability of the RNA to hybridize has not been impaired. This point will be more dramatically illustrated in Table III and the reason that not all samples show the same high figure will also be made clear.

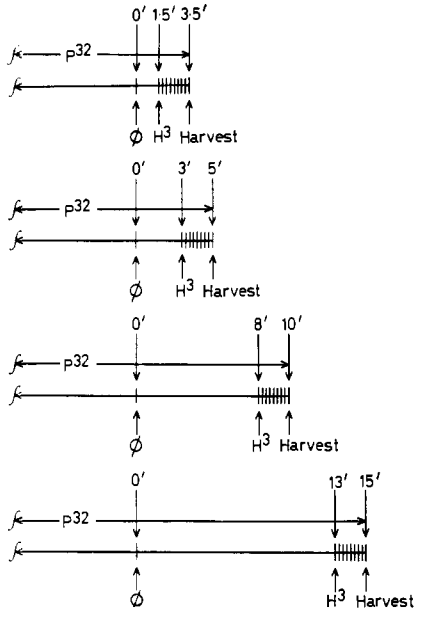

FIGURE 3: Schematic diagram of the labeling pattern of synchronously infected cells. Log-phase cells grown up in [^{32}P]orthophosphate (carrier free, 40 μc/ml with the medium phosphate reduced tenfold) are taken up in adsorption medium at 1×10^{10}/ml and immediately added to an equal volume of phage (multiplicity approximately 15). After 3-min preadsorption at 25° the phage-cell complexes are diluted tenfold into prewarmed medium (37°) and aerated vigorously (time zero). The 2-min pulse with [^{3}H]uridine (25 μc/μg ml), indicated by the hatched areas, is terminated by harvesting the cells in cups containing an equal volume of ice, NaN_3 (final 10^{-2} M), and sucrose (final 8%). Survivors measured at 1.5 min are less than 0.3%.

There are two possible ways of deriving from this experiment what per cent of the total cellular RNA (^{32}P labeled) is T4 specific. If it is assumed that all of the RNA synthesized after phage infection is phage-specific RNA, the values in the ^{3}H columns would be a measure of the hybridization efficiency of this RNA with T4 DNA, and they could be used to normalize the ^{32}P values to 100% hybridization efficiency as shown in column G. It is also possible, as shall be shown in further experiments, to determine more accurately the fraction of T4-specific RNA without resorting to normalization and its requisite assumptions.

The values for the second incubation with T4 DNA (column E) are approximately one-tenth of those for the first incubation (column B), indicating that most of the hybridizable T4-specific RNA was removed during the first incubation. As there is very little loss of hybridization efficiency, even up to the third incubation (see Table III also), the sum of the amounts hybridized in the first and second incubations (column F) should give a reasonable estimation of the total amount of RNA present which is capable of hybridizing

TABLE I: Successive Exhaustive Hybridizations with T4 DNA.[a]

RNA	Incubation I				Incubation II				Final (I plus II)		
	A		B		C		D	E	F		G
	Input (cpm)		Hybrid (% of A)		Input (cpm)		Hybrid (% of C)	Hybrid (% of A)	Hybrid (% of A)		Normalized (to 100% ^{3}H)
	^{3}H	^{32}P	^{3}H	^{32}P	^{3}H	^{32}P	^{3}H ^{32}P	^{3}H ^{32}P	^{3}H	^{32}P	^{32}P
T4 3.5	2,334	25,500	73	2.5	572	25,500	32 0.3	3.0 0.3	81	2.8	3.5
T4 5[b]	1,856	25,180	94	4.7	384	20,700	32 0.4	6.3 0.4	100	5.1	5.1
T4 10	1,233	25,700	74	3.6	152	20,200	60 0.4	7.3 0.4	631	4.0	4.9
T4 15	935	20,960	97	4.6	84	17,400	74 0.4	6.6 0.3	104	4.9	4.9

[a] Each RNA sample, labeled with isotope as described in the text (see Figure 3), was present in its respective 0.4-ml incubation mixture at 0.5 μg/tube and the NC membranes carried 20 μg of T4 1X DNA. The RNA samples are given as the time at which the 2-min [^{3}H]uridine pulse was terminated. Two successive incubations were carried out as described in the text. In the first incubation less than 0.01% of the ^{3}H input and less than 0.05% of the ^{32}P input was found on control filters containing no DNA. The values in A and B are averages of two determinations. Column C shows the input of the second incubation and is equal to the recovery of the first incubation. Column F is the sum of columns B and E. Column G shows the values obtained when the ^{32}P values in F are normalized to 100% ^{3}H. [b] This RNA sample contains a fourfold excess of cold bulk RNA from uninfected cells.

TABLE II: Exhaustive Hybridizations with DNA from *E. coli* Q13.[a]

RNA	DNA	Input (cpm)		Hybridized (cpm)		Hybridized (%)		$^3H:^{32}P$
		^3H	^{32}P	^3H	^{32}P	^3H	^{32}P	$\times 10$
T4 3.5	+	2,334	24,500	79.5	2,807	3.4	11	3.1
	−			0	8.1			
T4[b] 5	+	1,856	25,180	22.9	777	1.2	3.1	4.0
	−			0	7.1			
T4 10	+	1,233	25,700	1.7	2,753	0.1	11	0.1
	−			0	12.7			
T4 15	+	935	20,960	0.8	2,672	0.1	13	0.1
	−			0	10.8			

[a] The conditions of this experiment were identical with those described in Table I except that here 20 μg of *E. col* Q13 1X DNA was fixed to the filters instead of T4 DNA and only one incubation was carried out. The values obtained on the control filters lacking DNA are given. [b] This RNA sample contains a fourfold excess of cold bulk RNA from uninfected cells.

with T4 DNA at each of the specified times in the latent period.

Exhaustive Hybridization with E. coli DNA. In the course of exhaustive hybridization experiments with T4 DNA certain results suggested that there was a continued synthesis of host RNA for a short time after phage infection. For example, it was noted that with RNA samples taken in the first few minutes after infection, the hybridization efficiency decreased significantly during a second incubation with T4 DNA (see Table I). It appeared that, as a consequence of the first incubation, there was an enrichment for postinfection RNA not homologous to T4 DNA. That this decrease in the fraction of RNA homologous to T4 DNA is not merely a loss in the ability of the RNA to hybridize can be seen from the fact that samples labeled at later times in the lytic cycle, and which contain all of the species of RNA found at early times (Hall *et al.*, 1964; Landy and Spiegelman, 1967), do not exhibit the decrease (see Table I). Furthermore, it has been shown that after three successive incubations there is no loss in the ability of the RNA to hybridize with T4 DNA (see Table III).

The technique of exhaustive hybridization has been applied to estimating the amount of host-specific RNA which is synthesized after phage infection. The procedure and samples are similar to those described in Table I except that in place of T4 DNA the filters are loaded with *E. coli* DNA, also at 20 μg/filter. The results are presented in Table II. In this experiment, no significant host-specific RNA is detected in RNA pulse labeled with [^{3}H]uridine late in infection, however, in the preparations which were pulse labeled at 1.5–3.5 and 3–5 min after infection there is some host-specific RNA present. The estimation of how much host-specific RNA is present in the early pulse-labeled preparations is complicated by not knowing its relative mRNA content and hence its hybridization efficiency.

The values obtained in Table II for the per cent of ^{3}H-labeled RNA hybridizing to *E. coli* DNA can be interpreted quantitatively in only two cases. In the first case, if the postinfection host-specific RNA were comprised solely of mRNA then the per cent of ^{3}H-labeled RNA which was hybridized to *E. coli* DNA could be taken as roughly equal to the per cent of host-specific RNA which was present in the ^{3}H-labeled material (see Figure 2 and Table I). In the second case, if the postinfection host-specific RNA were comprised of mRNA and rRNA in proportions similar to that found in the uniformly ^{32}P-labeled preinfection RNA then the per cent of ^{3}H-labeled RNA hybridized to *E. coli* DNA could be corrected for the hybridization efficiency as measured by the per cent of ^{32}P-labeled RNA which had hybridized.

The following experiment is designed to determine which, if either, of the two cases applies. Successive exhaustive hybridizations were performed with *E. coli* DNA and the results are shown in Table III. The 75–80% hybridization efficiencies observed in the third incubation (bottom row of Table III compared with the results of Table I) prove that the residual RNA is fully capable of normal hybridization. It is further seen that the $^3H:^{32}P$ ratio in the hybrid falls after the first incubation and remains constant thereafter. The fact that the decrease was confined to the first incubation implies that (a) the ^{3}H-labeled postinfection host-specific RNA contains a higher proportion of mRNA than the uniformly ^{32}P-labeled total RNA and (b) that most of the mRNA is effectively removed during the first incubation, in concordance with the results shown in Figure 2 and Table I.

Assuming that uniformly labeled *E. coli* RNA contains approximately 3% mRNA (McCarthy and Bolton, 1964; Leive, 1965) one can calculate from the decrease in $^3H:^{32}P$ ratio that postinfection host-specific RNA contains considerably less mRNA than the 50% found in a corresponding 2-min pulse of unin-

TABLE III: Successive Exhaustive Hybridizations with DNA from *E. coli* Q13.

Incorp	RNA	DNA	Input and Recov		% Hybridized		
			^3H	^{32}P	^3H	^{32}P	$^3H:^{32}P$
I	1.5–3.5	Q13	9158	3446	3.9	16.2	0.62
		(−)			0.1	0.1	
	3–5	Q13	6496	3674	4.3	15.7	0.48
		(−)			0.1	0.1	
II	1.5–3.5	Q13	7384	2326	1.4	7.4	0.49
		(−)			0.3	0.2	
	3–5	Q13	5452	2564	1.3	6.5	0.34
		(−)			0.3	0.2	
III	1.5–3.5	Q13	5232	1560	3.2	23.6	0.48
		T4			80	4.3	
	3–5	Q13	4024	1772	1.7	8.6	0.37
		T4			75	3.0	

[a] At the outset eight identical incubations were carried out with *E. coli* DNA fixed to the filters (20 μg/filter). After the filters were removed, the samples were pooled by two's into four tubes, from each of which duplicate 0.1-ml aliquots were taken for trichloroacetic acid precipitation and 0.4 ml was used to make up a second hybridization reaction. Following the second hybridization the above procedure of pooling by two's was repeated, this time resulting in two pools. One of these was hybridized with a fresh filter as above and the other was hybridized with a filter containing T4 DNA instead of *E. coli* DNA. The experiment was performed with two samples of RNA, one from T4-infected cells pulsed with [^{3}H]uridine at 1.5–3.5 min and the other from cells infected with amN122, pulsed at 3–5 min. [In the course of this investigation the same results have always been obtained in parallel experiments using either wild-type T4 or the isogenic "early" amber mutant amN122 (Epstein *et al.*, 1963).] The results are shown as averages wherever more than one determination was obtained for any condition. The unhybridized counts recovered in the liquid after any given incubation are the same as the input for the subsequent incubation. The ratio of $^3H:^{32}P$ has been calculated from the hybridized counts, after correction for the values appearing on the control filters containing no DNA.

fected cells (Leive, 1965). To approximate the amount of postinfection host-specific RNA one must refer to the values from the second or third exhaustive incubations. Here the hybridization efficiencies of the [^{32}P]- and [^{3}H]RNA are more equal as a result of the prior exhaustion of *E. coli* mRNA. The background corrected values from the second and third incubations in Table III, when normalized to 100% hybridization efficiency, indicate that approximately 13–16% of the RNA synthesized as late as 3–5 min after phage infection, is host-specific RNA.

Discussion

Using the technique of exhaustive hybridization we have estimated that the phage-specific RNA accounts for 2.8–4.9% of the total cellular RNA, depending upon the time after T4 infection at which the sample was taken. These figures are in agreement with a previous estimation based on hybridization with DNA in solution (Nygaard and Hall, 1964) but are considerably lower than those suggested by less quantitative experiments with DNA cellulose columns (Bautz and Hall, 1962). In our determinations the amount of phage-specific RNA was obtained by summing the values of two successive exhaustive hybridizations, thus avoiding any assumptions concerning the immediacy with which host-specific RNA synthesis is turned off.

We have also carried out identical experiments with cells infected with the early amber mutant amN122 (Epstein *et al.*, 1963), which fail to synthesize many of the RNA species normally synthesized during the later portion of the wild-type infection (Landy and Spiegelman, 1967). However, no significant differences between the two infections were found with respect to the absolute amount of phage-specific RNA or the cessation of host-specific RNA synthesis.

Our results indicate that the cessation of host-specific RNA synthesis does not take place immediately. As late as 3–5 min after infection of *E. coli* B (with either wild-type T4 or with an early amber mutant) a small but significant amount of the [^{3}H]uridine incorporated during a 2-min pulse is found in host-specific RNA. The proportion of mRNA in this fraction of postinfection host-specific RNA is estimated to be considerably less than what would be found in a corresponding 2-min pulse of uninfected cells. The possibility that the hybridization observed is actually "noise" or cross-hybridization between the T4-specific RNA and *E. coli* DNA is unlikely since no host-specific RNA is detected during the late pulses, which contain both early and

late phage RNA (Hall *et al.*, 1964; Landy and Spiegelman, 1967).

Earlier experiments on RNA from phage-infected cells (Volkin and Astrachan, 1956; Nomura *et al.*, 1960; Brenner *et al.*, 1961) used procedures which could not have readily detected such small amounts of host-specific RNA. More recently the synthesis of host-specific RNA in phage-infected cells has been investigated by Nomura *et al.* (1966) who have suggested there may possibly be two mechanisms of T4 inhibition of host nucleic acid synthesis. The first is dependent on protein synthesis and inhibits host nucleic acid synthesis completely by 4–6 min. The second mechanism (which is dependent on multiplicity) is insensitive to chloramphenicol. One explanation for the relatively low mRNA content of the postinfection host-specific RNA may be that the mechanism of host inhibition which is dependent upon protein synthesis has not yet fully expressed itself while the mechanism which is independent of protein synthesis is acting preferentially on host mRNA.

Our finding of a small amount of host-specific synthesis is probably due, on one hand, to the fact that we have looked at earlier times after infection and, on the other hand, to the fact that the hybridization efficiency under our conditions of exhaustive hybridization is severalfold higher.

The biological significance of this postinfection host-specific RNA is not known. As a result of studies on β-galactosidase induction, Kaempfer and Magasanik (1967) have suggested that transcription of cistrons is not initiated after phage infection and that residual synthesis of host-specific RNA consists only of the "run-off" transcription of cistrons which were already initiated at the time of infection. The fate of this RNA, the mechanism for its turnoff, and control at the level of translation (*e.g.*, the presence of untranslated RNA later in infection (Hall *et al.*, 1964; Landy and Spiegelman, 1967)) remain interesting questions which must be considered when interpreting data relevant to the control of biological function in the phage-infected cell.

The possibility must be borne in mind that the residue of host-specific RNA synthesis observed is due to some infected cells in which the onset of phage functions was delayed. All efforts to detect delayed complexes have led to failure. However such negative evidence cannot be logically employed to eliminate the hypothesis.

The technique of exhaustive hybridization with membrane-bound DNA should prove to be a useful analytical tool for the quantitative characterization of different RNA species in a population of molecules. It provides a highly flexible procedure which is particularly well suited for the assay of minor RNA components.

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