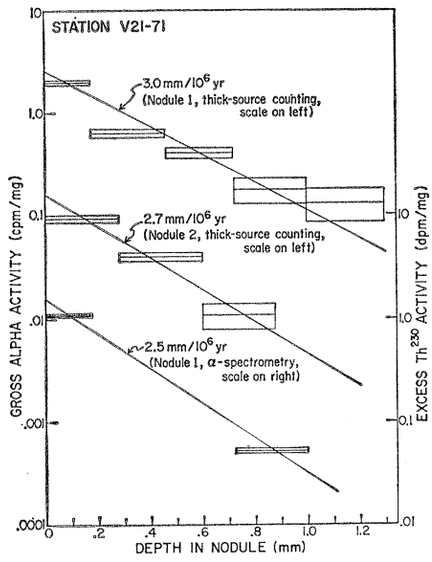

Fig. 3. Accumulation rate for Mn nodules from station V-21-71, northwestern Pacific.

the clay fraction and by substracting this from the total manganese content (Table 1). The striking feature of these results is that, as expected on the basis of the above arguments, sedimentation rates for authigenic manganese are remarkably constant. This finding is true for very different types of sediment in which manganese content and accumulation rates differ by an order of magnitude.

The manganese profiles for V-21-71, V-16-75, and V-10-95 are noteworthy. Concentration of manganese oxide in each of these cores increases substantially at a depth corresponding to an extrapolated age of about 10^6 years; if this increase reflects primary differences in the manganese : sediment ratio of accumulation, then either total sedimentation has been faster during the last 10^6 years or the manganese deposition rate has been much lower during this period. These possibilities may be resolved by other dating methods (such as by Be^{10}) that promise to extend the chronology of sedimentation. If, in addition to areal constancy, the sedimentation rate of manganese has been constant through time, measurement of manganese concentrations in sediments will provide a useful tool for analyzing sedimentation rates.

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Gene-Specific Messenger RNA: Isolation by the Deletion Method

Abstract. *Messenger RNA molecules, homologous to a small portion of the genome of bacteriophage T4, have been isolated. RNA fragments specific to the rII A and the rII B cistrons have been separated by hybridization with DNA isolated from appropriate deletion mutants. An RNA species homologous in nucleotide sequence to a defined part of the rII A cistron has been identified.*

The separation of a gene-specific messenger RNA (mRNA) species from a multitude of structurally similar, but informationally different, messengers can be accomplished in principle by either of two procedures. In some instances, a limited number of genes can become integrated into the genome of a transducing phage, and as such be separated from the bacterial genome; this DNA can be made to form complexes selectively with its homologous RNA (*1*). In those cases, where an episomal element is not available for the selection of a particular segment of DNA, a deletion mutant lacking the gene to be studied can be used to remove, by hybridization, all but the desired mRNA species.

The method of complex formation between mRNA and episomal DNA is straightforward once the selection of the proper DNA piece has been achieved. This selection is, however, so far limited to only three experimental systems (*1*). Since there are many more genes known for which deletion mutants are available, the method of removal by hybridization would provide a wider range of application. The experimental difficulty encountered here is that the first step of hybridization, namely, elimination of all undesired mRNA species, has to be quantitative.

A previous attempt (*2*) has revealed the feasibility of purifying RNA, specific for the rII region of bacteriophage T4, by this approach. In this report we describe a general procedure for the isolation and the detection of gene-specific mRNA. The procedure applies the observations that nitrocellulose binds single-stranded DNA (*3*) and that the bound DNA is still capable of forming complexes with homologous RNA (*4*).

DNA was extracted by shaking concentrated bacteriophage stocks three times with phenol saturated with 0.01*M* tris (*p*H 7.2) and then by precipitating the DNA from the aqueous layer with two volumes of ethanol. The DNA was collected on a glass rod and stored as dried fibers. Before use a portion of the DNA was dissolved in low-salt buffer (*5*) and denatured by heat at a concentration of 0.3 mg/ml; the denatured DNA was cooled rapidly, adjusted to high-salt buffer, and pipetted into a suspension of nitrocellulose powder in high-salt buffer; this mixture was then rapidly stirred for 5 minutes. The suspension of nitrocellulose (type RS, Hercules Powder Co.) was prepared by grinding it in high-salt buffer in a mortar, passing the slurry through a 40-mesh stainless-steel sieve, decanting to remove fine particles, and washing with high-salt buffer for 2 hours at room temperature. A slurry of 10 mg of DNA of bacteriophage T4r+ (wild type) on nitrocellulose was poured into a jacket column of 15 mm diameter to yield a column height of 12 cm. A small amount of plain nitrocellulose was packed on top of it, and on this layer was placed a 12-cm column of nitrocellulose containing 10 mg of DNA of the mutant phage r1272 [a

deletion spanning the entire rII region, (*6*)]. The three layers were visibly divided by small plugs of glass wool inserted between them. With these precautions, cross contamination between the two types of DNA was avoided.

Tritiated T4 mRNA was obtained by extraction of *Escherichia coli* B cells that had been infected with bacteriophage T4r+ and labeled with uracil-H^3 (*2*). The phage mRNA was then adjusted to high-salt buffer and applied to the top of the column; its passage (18 hours at 60°C) through the entire column was controlled by a continuous-flow pump which delivered a portion of the high-salt buffer to the top of the column at regular intervals of 30 minutes or 1 hour. After removal of unhybridized RNA from the column by extensive washing with high-salt buffer, the three layers were extruded from the column, collected separately, and the two layers containing the DNA's from T4r+ and the T4r1272 were repacked into two different columns and eluted with low-salt buffer at 65°C. Table 1 shows the relative amounts of RNA-H^3 recovered from the bottom layer. Portions of the RNA fractions thus obtained were incubated with nitrocellulose filters containing 100 μg of either T4r+ DNA or T4r1272 DNA (*4*). RNA specified by the rII genes should be recognized by this test, for this RNA is expected to find no complementary sequences in the DNA of the deletion mutant r1272. As shown in Table 1, more radioactivity was adsorbed by the filter containing T4r+ DNA in the first experiment, but not in the other two where either the columns were inverted or an RNA preparation from cells infected with mutant r1272 was applied. Thus the

Table 1. Fractionation of T4r+ RNA-H^3 by passage through DNA nitrocellulose. RNA, pulse labeled with uracil-H^3 (specific activity 2.7 c/mole) for 3 minutes after infection of *E. coli* B with T4r+ or with mutant T4r1272, was passed through a double column as indicated. Portions of RNA eluted from the bottom column with low-salt buffer were incubated in high-salt buffer with nitrocellulose filters containing 100 μg of T4r+ or T4r1272 DNA for 9 hours at 65°C, washed, and counted (*4*).

Infecting phage	DNA in column		RNA input (10^5 count/min)	Hybrid recovered from bottom column (10^4 count/min)	Total hybrid (%)	Radioactivity on filters (count/min)		Fraction of radioactivity bound to r+ DNA exclusively
	Top	Bottom				T4r+	T4r1272	$\frac{r^+-r1272}{r^+}$
T4r+	T4r1272	T4r+	7.1	2.614	4.8	607	358	0.41
T4r+	T4r+	T4r1272	3.5	0.292	1.3	131	141	0
T4r1272	T4r1272	T4r+	9.12	2.518	3.0	759	800	0

Table 2. Fractionation of H^3-labeled T4r+ RNA on DNA from deletions of different sizes and map locations. The RNA, labeled with uridine-H^3 (specific activity 8 c/mole) from 6 to 8 minutes after infection of *E. coli* B with T4r+, was fractionated first on a column containing T4r1272 DNA (top) and T4r+ DNA (bottom). A sample of the RNA eluted from the part of column I containing T4r+DNA was applied to column II. A portion of the RNA eluted from the top portion of this column, containing T4r638 DNA, was then fractionated on column III. Before passage through columns II and III, the RNA samples were treated with deoxyribonuclease (20 μg/ml) for 20 minutes at 37°C in $10^{-2}M$ Mg^{++}. Deoxyribonuclease was removed with phenol, and the RNA was dialyzed against high-salt buffer.

Column number	DNA in column		Input (10^3 count/min)	Hybrid recovered (count/min)		Fraction of total hybrid	
	Top	Bottom		Top	Bottom	Top (%)	Bottom (%)
I	T4r1272	T4r+	3450.00	290×10^4	4.02×10^4	98.6	1.4
II	T4r638	T4r+	14.53	7384	2561	74.2	25.8
III	T4r1231	T4r+	2.74	700	605	55.1	44.9

Table 3. Filter test of RNA fractions eluted from column I and II of Table 2. Portions of the RNA were incubated with the filters as described in Table 1. The efficiency of binding to the T4r+ DNA on the filters varied from 60 to 75 percent of the input.

RNA eluted from column	Treated with deoxyribonuclease	Filters (count/min)				Fraction of radioactivity bound to T4r+ DNA exclusively	
		T4r+	T4r638	T4r1272	Blank	$\frac{r^+-r1272}{r^+}$	$\frac{r^+-r638}{r^+}$
I—T4r1272	—	20029	20158	21593	5165	0	
I—T4r+	—	1324	855	249	182	0.81	
I—T4r+	+	1300	575	101	19	0.92	
II—T4r638	—	523	389	103	66		0.25
II—T4r638	+	305	278	37	0		.10
II—T4r+	—	239	40	22	28		.83
II—T4r+	+	199	8	12	4		.96

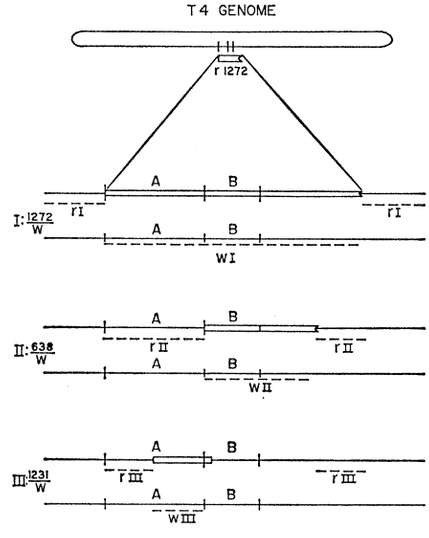

Fig. 1. Schematic presentation of the results of the runs described in Table 2. Tritiated RNA isolated from T4r+ infected, labeled *E. coli* B cells was passed through column I of Table 2. The two RNA fractions obtained, shown as dashed lines, are rI, the fraction eluted by low-salt buffer from the upper layer containing T4r1272 DNA, and wI, that eluted from the lower layer containing T4r+ DNA. RNA fraction wI was applied to column II which yielded RNA fractions rII, eluted from the layer containing T4r638 DNA; and wII, eluted from that containing T4r+ DNA. Fraction rII was then applied to column III, and RNA fraction rIII and wIII were obtained from layers containing T4r1231 and T4r+ DNA's. The length of extension of the deletions into the cistron to the right of the B cistron is arbitrary for mutant r638, and only approximately to scale for mutant r1272.

Table 4. Filter test of RNA fractions eluted from column III of Table 2; the experimental details are as described in Tables 1 and 3. The RNA was not treated with deoxyribonuclease.

RNA eluted from column	Filters (count/min)		$\frac{r^+-r1231}{r^+}$
	T4r+	T4r1231	
III—T4r1231	152	148	0.03
III—T4r+	138	18	0.87

RNA fraction of experiment 1 (first line of Table 1), which was adsorbed to the portion of the column containing the r+ DNA, represents some 40 percent of RNA homologous to the DNA region deleted by mutant r1272.

In experiment 1, the purified rII RNA fraction still contains a considerable number of sequences nonspecific for the rII region. In order to trap the nonspecific RNA more effectively in the upper layer of the column, and to minimize its adsorption to the bottom layer, we prepared a column with an upper layer 20 cm long containing 12 mg of T4r1272 DNA and a lower layer, 5 cm in length, containing only 3 mg of T4r+ DNA. Passage of a preparation of T4r+ RNA through this column produced an RNA fraction which appears to be specific by more than 90 percent for the DNA region absent in mutant r1272, but present in T4r+ DNA (Tables 2 and 3). This RNA was fractionated further by hybridization with DNA of mutant r638, which lacks cistron B, but possesses cistron A. RNA molecules corresponding to the two cistrons could thus be separated (Table 3 and Fig. 1).

Since the right ends (that is, those distal to the A cistron) of both mutants, r1272 and r638, are genetically not defined [r1272 extends some ten recombination units beyond the B cistron (*7*)], we attempted to purify an RNA fraction not accepted by the DNA of a deletion mutant terminating on both sides within the rII region. For this purpose RNA eluted from the upper layer, containing T4r638 DNA, of the column in run II (638/W) in Fig. 1 was passed through a column containing T4r1231 DNA and T4r+ DNA. The RNA excluded by the r1231 DNA but bound by the r+ DNA was expected to be equivalent to the right half of the rII A cistron. Hybridization tests with filters containing T4r+ and T4r1231 DNA's confirmed this expectation (Table 4).

When nitrocellulose filters not containing DNA were incubated with RNA eluted from either column a considerable amount of labeled material resistant to ribonuclease became attached to the filters. Treatment of the RNA with deoxyribonuclease (electrophoretically purified, Worthington) before incubation diminished most of the activity in the control filters. This observation suggests that, upon elution from the DNA-nitrocellulose column, some DNA was released and became adsorbed to the test filters and was then able to bind some of the RNA to the filters.

The high purity of the RNA specific for the rII region (Table 3) suggests that we would have been able to identify RNA specified by a similar-sized region in a genome at least ten times as large as the genome of phage T4. Therefore it seems likely that this deletion method might be successfully applied to the isolation of operon- or even gene-specific bacterial messengers with but little improvement of our technique.

The smallest segment of the T4 genome whose complementary RNA we have succeeded in purifying is that of approximately half a cistron with an estimated length of some 1000 nucleotides. Preliminary evidence indicates that RNA molecules of even smaller sizes can be fractionated almost equally well. If so, the deletion method would provide a unique opportunity to place unequivocally small fragments of messenger molecules on the genetic map according to their sites of transcription. With the availability of such a genetically well-defined system as the rII region in bacteriophage T4, the deletion method meets the first of two principal requirements for the elucidation of the primary structure of an mRNA, namely, the orientation in a linear fashion of RNA fragments of the size of transfer RNA. Whether the deciphering of these fragments, obtainable in extremely small quantities only, will ever become possible, remains to be shown (*8*).

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Thyrocalcitonin Inhibition of Bone Resorption Induced by Parathyroid Hormone in Tissue Culture

Abstract. *Added thyrocalcitonin greatly diminished parathyroid-hormone–induced resorption of bone in tissue culture. The results indicate that bone is a primary site of action of thyrocalcitonin.*

Thyrocalcitonin, the hypocalcemic, hypophosphatemic principle of mammalian thyroid gland discovered by Hirsch *et al.* (*1*) is now generally recognized as a hormone of major importance in the regulation of the amount of calcium in plasma. Bone was suspected as its principal site of action because of the hypocalcemic effect of thyrocalcitonin in rats even after parathyroidectomy (*1*), nephrectomy (*2*), or removal of the gastrointestinal tract (*3*) and because of the parallellism of the observed hypocalcemia and hypophosphatemia (*2*). We now report that thyrocalcitonin added in vitro to bone undergoing resorption in tissue culture under the influence of parathyroid hormone acts directly on isolated bone and we suggest that bone is a principal site of action of the newly recognized thyroid hormone.

The essentials of the procedure for the bone cultures (*4*) are as follows. Calvariae of Swiss albino mice of the Webster strain, aged 4 to 5 days, were removed aseptically and attached to coverslips with a mixture of chicken plasma and chicken embryo extract