*THE MOLECULAR ORIGIN OF LAMBDA PROPHAGE mRNA**

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Communicated by A. D. Hershey, December 3, 1968

Abstract and Summary.—Lambda-specific RNA extracted from lysogenic bacteria hybridizes specifically with fragments of λ DNA containing 43 per cent GC (guanine plus cytosine). Therefore genes known to function in the prophage state (c_I and *rex*) lie 0.38 ± 0.08 fractional molecular length from the right end of the λ DNA molecule, according to the compositional map of Skalka, Burgi, and Hershey.

Only two functions are known to be expressed by prophage λ: immunity and exclusion of *r*II mutants of phage T4. It seems probable that only the corresponding genes c_I (ref. 1) and *rex* (refs. 2 and 3) are transcribed in lysogenic bacteria.[4] We have taken advantage of these circumstances to locate the two genes in the physical map of λ DNA (Fig. 1).

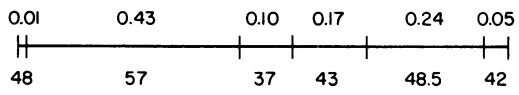

FIG. 1.—Distribution of nucleotides in λ DNA.[10] Fractional molecular lengths are indicated above the line; the GC content of each segment (in mole %) appears below. (Adapted from Fig. 8, Skalka *et al.*, *J. Mol. Biol.*, **34**, 1 (1968).)

Materials and Methods.—RNA preparations: *E. coli* 204 (F^-, gal_2^-, *str*, thy^-, rec^-), a K-12 strain of Dr. M. Meselson, was lysogenized with wild-type λ. Cultures of this lysogen yielded only 1 plaque/10^9 cells. The lysogen was grown in a medium[5] containing 2 μg/ml of thymidine and 20 μg/ml of uridine. The high concentration of uridine served to repress endogenous uridine synthesis. The culture (700 ml at 8×10^7/ml) was chilled and the cells were harvested, washed, and resuspended in 7 ml of the same medium without uridine. The suspension was aerated for 2 min at 37°C, H^3-uracil was added (500 μc, 9.65 c/mmole), and, after another 2 min, the culture was poured over an equal volume of frozen 0.9% saline. The cells were washed twice with 0.01 *M* Tris buffer (pH 7.4) containing 5×10^{-3} *M* $MgCl_2$, and then frozen in 2 ml of the same solution. After the addition of 100 μg of yeast RNA, cellular RNA was extracted as described by Skalka.[6] After phenol extraction, the aqueous phase was washed several times with ether. Uptake of H^3-uracil from the medium, measured as counts precipitable by trichloroacetic acid, was 50–80% of the input.

DNA preparations: Lambda DNA was prepared from a clear-plaque mutant[7] of that phage propagated on *E. coli* W3110. DNA of λ_{imm}^{434} (see ref. 1) was extracted from phage produced after induction of the appropriate lysogenic culture with 2 μg/ml of mitomycin C. The phages were grown in a peptone medium[6] which contained P^{32} (specific activity 1 c/gm P) and yielded DNA with about 10^5 cpm/μg.

E. coli DNA was made by the Marmur[8] procedure, and phage DNA's were prepared as described by Burgi.[9] Methods for shearing DNA and fractionating DNA-mercury complexes have been described.[10]

Membrane filters were coated with DNA or DNA fractions by the method of Gillespie and Spiegelman[11] (see legend, Fig. 2), and except for modifications employed in the preparatory steps detailed below, hybridization tests were performed as described by them.

Primary hybridization: The RNA (about 2×10^7 cpm in 2–3 ml 2 × SSC) was incubated with a membrane filter containing 50 μg λ DNA for 6 hr at 65°C. The filter was then washed by passing 100 ml 2 × SSC at 60°C through it from each side, placed in 5 ml 2 × SSC containing 20 μg/ml ribonuclease, and incubated at 30°C for 1 hr to digest nonspecifically bound RNA. The filter was washed a second time, placed in 2 ml of a solution (pH 5.3) containing 1.4 × SSC, 0.15 *M* iodoacetate, and 0.1 *M* sodium acetate, and incubated at 55°C for 40 min to inactivate the ribonuclease.[4, 12] After a third washing, the filter was dried and the radioactivity was counted. The λ-specific RNA was then eluted (93–97%) by boiling the filter in 0.01 × SSC for 10 min. The eluted material was dialyzed twice against 0.01 *M* NaCl containing 0.005 *M* Tris buffer, pH 7.4, and then treated with deoxyribonuclease.[6] The mixture was then boiled for 10 min to destroy the deoxyribonuclease and was twice dialyzed against 2 × SSC. A total of 10^5 cpm of λ-specific RNA was recovered from about 12 filters. The pooled RNA was adjusted to 10^3 cpm/ml by dilution with 2 × SSC and used in the experiments to be described.

Results.—Characterization of prophage mRNA: From 0.04 to 0.05 per cent of the labeled RNA extracted from our lysogen remained bound to λ DNA filters after the primary hybridization, as opposed to only 0.002 per cent of similarly treated labeled RNA from a nonlysogenic culture. The bound RNA was λ-specific, as verified by the results of a second annealing summarized in Table 1.

TABLE 1. *Hybridization of purified λ prophage mRNA.*

Source of DNA		RNA bound (%)
Phage λ	(3 μg)	24
Phage λ	(20 μg)	43
Phage $\lambda_{imm^{434}}$	(3 μg)	4
E. coli	(10 μg)	0.85
E. coli	(50 μg)	1.6
Phage T2	(50 μg)	<0.01

Each mixture contained about 10^3 cpm of λ-specific RNA.

The table shows that only part of the recovered RNA can hybridize with λ DNA: 43–48 per cent in various tests with filters containing 10–25 μg of unfractionated λ DNA. However, since the hybridization is specific, the counts failing to bind to λ DNA filters probably reside in impurities such as ribosomal RNA or degraded messenger.[13] The RNA hybridizing with λ DNA binds poorly to the DNA of $\lambda_{imm^{434}}$. This indicates that most of the RNA originated from the λ genes, c_I and *rex*, known to be deleted in $\lambda_{imm^{434}}$ (see refs. 1–3). The following results correlate this genetic site with a physical one.

The site of origin of prophage mRNA: Figure 2*a* presents the results of an experiment in which prophage mRNA was annealed with fragments of λ DNA that had been fractionated according to density.[10] The figure shows that the RNA binds preferentially to DNA of 43 per cent GC. Figure 2*b* shows that there is no such preferential binding to DNA of $\lambda_{imm^{434}}$. Therefore, the prophage-specific RNA originates mainly in the segments of λ DNA containing 43 per cent GC.

A considerable amount of RNA also binds to other parts of the DNA of both phages. This could reflect a low level of general transcription of the prophage, or base-sequence similarities among various genes.

Note that our main conclusion, that the characteristic prophage message con-

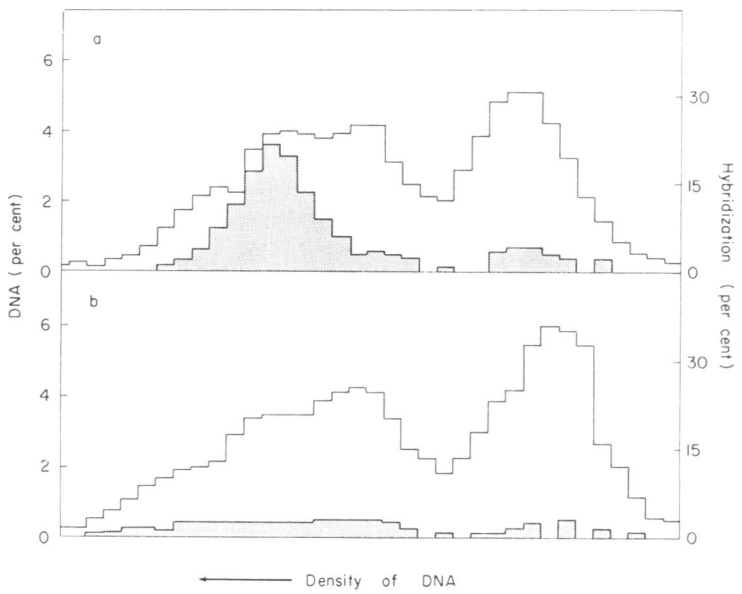

FIG. 2.—Hybridization of λ prophage mRNA with λ and λ_{imm}^{434} DNA fractions. (*a*) *Outer histogram:* Lambda DNA fragments of fractional molecular length 0.05 separated according to density in 42.5% Cs_2SO_4 containing 0.22 mole $HgCl_2$ per mole of nucleotides. The four bands, left to right, contain DNA of 37, 43, 48.5, and 57% GC. *Shaded histogram:* hybridization of λ prophage mRNA with DNA from each fraction.

The density gradient contained 100 μg of P^{32}-labeled DNA, specific activity 1.8×10^4 cpm/μg. Equal fractions were collected and diluted with 1 ml 1 *M* NaCl. Then 0.2 ml of each diluted fraction was added to separate 3-ml aliquots of 1 *M* NaCl which contained 20 μg "carrier" T2 DNA. These were then dialyzed[10] and used to prepare DNA membrane filters.[11] The filters therefore contained various amounts of λ DNA but represented equal numbers of copies of individual genes. Each annealing test included 10^3 cpm of RNA.

(*b*) The same as (*a*), except that the density gradient contained 50 μg of P^{32}-labeled λ_{imm}^{434} DNA, specific activity 1.5×10^4 cpm/μg; and 0.4 ml of each diluted fraction was used to prepare the DNA membrane filters.

sists of RNA of 43 per cent GC, could not have been reached by direct analysis because of insufficient purity of RNA and because of the practical necessity of using selective labels for RNA.

Discussion.—The results reported here indicate that the characteristic message transcribed from λ prophage contains about 43 per cent GC. DNA of this composition is found in two regions of the molecule (Fig. 1). On the basis of the genetic map of λ, we suppose that c_I and *rex* lie in the central 43 per cent region. This puts the genes in a segment beginning 0.29 and ending 0.46 molecular length from the right end of the DNA molecule.

Other estimates of this distance are somewhat less: about 0.26 (ref. 14) and 0.24 (ref. 15) for the λ_{imm} region. The discrepancy could signify inaccuracies in the map of Figure 1. For instance, it is possible that the 37 per cent-GC section is underestimated in the map or that some DNA of higher GC content lies between the 37 and 43 per cent-GC segments or interrupts the 43 per cent-GC segment. The last two alternatives are attractive in view of Inman's finding of a

preferential zone of denaturation centered at 0.27 molecular length from the right end, clearly separated from another at the molecular center.[16]

Our results show that the rate of synthesis (or labeling[17]) of λ-specific RNA in uninduced lysogens is very low. This agrees with similar observations made earlier in several laboratories.[18, 19] Furthermore, our findings, like those of others,[4] indicate that this low level of transcription originates from a particular region of the chromosome. The results verify in a direct way that repression of gene function specifically suppresses the transcription of the repressed genes.

The absence of base-sequence similarities in the immunity regions of λ and 434, evidenced by the lack of preferential binding of λ prophage messenger to λ_{imm}^{434} DNA, is consistent with the failure to detect intra-c_I recombinants in crosses between the two phages[1] and, of course, with the functional difference between the two repressor genes.

We gratefully acknowledge the advice and encouragement of Dr. A. D. Hershey and Dr. Elizabeth Burgi during the course of this work.

Abbreviations used: GC, guanine plus cytosine; SSC, 0.15 *M* NaCl, 0.02 *M* citrate, pH 7.

* This investigation was supported, in part, by USPHS research grant no. GM5876 from the National Institute of General Medical Sciences.

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