*TRANSCRIPTION OF COMPLEMENTARY STRANDS OF PHAGE λDNA IN VIVO AND IN VITRO**

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Earlier experiments carried out in our laboratory[1, 2] and elsewhere[3] have indicated that transcription of intact native λDNA by *Escherichia coli* RNA polymerase does not occur randomly along the length of the template; nucleotide sequences located predominantly on the AT-rich (right) half of the λDNA molecule (Fig. 1) are preferentially copied throughout the duration of *in vitro* RNA synthesis. During these studies,[2] it was observed by nearest-neighbor analysis that RNA polymerase products synthesized on native λDNA contained unequal frequencies of complementary nucleotide bases (i.e., A ≠ U and G ≠ C), suggesting that both strands of the template were not being copied at the same sites. However, these experiments did not distinguish whether such asymmetric transcription of λDNA *in vitro* occurred *entirely* on *one* strand of the template or whether different sites of *both* complementary strands were transcribed.

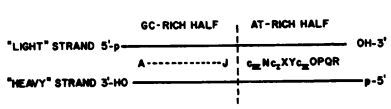

FIG. 1.—Map of the vegetative form of λDNA showing the positions of the AT-rich and GC-rich halves and the locations of certain genes and genetic regions.[6, 26] The assignment of 3′ and 5′ ends for the "heavy" and "light" strands is based on considerations discussed in the text.

Observations by Skalka[4] that frequencies of complementary nucleotide bases are unequal in λ-messenger RNA isolated after phage infection led to a similar inference of asymmetric *in vivo* transcription of λDNA. Furthermore, genetic experiments by Hogness *et al.*[5] and Eisen *et al.*,[6] indicating that the N and O cistrons of λ are transcribed in opposite directions, suggested that asymmetrical synthesis of λRNA *in vivo* reflects copying of segments of both strands of the template, since transcription is unidirectional on a single DNA polynucleotide chain.[7]

The present communication describes studies of strand selection during transcription of λDNA *in vivo* and *in vitro* carried out with physically separated, intact complementary strands of λDNA. Strands isolated by cesium chloride gradient centrifugation of denatured λDNA in the presence of polyguanylic acid were annealed with RNA polymerase products made *in vitro*, or with λmRNA pulse-labeled with tritiated uridine at various times after induction of certain λ lysogens. In contrast with results described for other bacteriophage systems, our findings indicate that portions of both strands of λDNA are copied *in vivo* and *in vitro*. Furthermore, we have observed that the pattern of transcription occurring *in vivo* can be duplicated *in vitro* by preparations of gently lysed bacteria; RNA synthesis carried out by purified *E. coli* RNA polymerase or crude bacterial sonicates appears to be less selective. Szybalski and his collaborators[8] have carried out similar studies of *in vivo* λmRNA synthesis, and have reached similar conclusions regarding the transcription of λDNA *in vivo*.

Materials and Methods.—Escherichia coli K12(λ) strain Y10 was obtained from Drs. C. Fuerst and H. Eisen. The nonpermissive *su*⁻ strains W3350 (λ *sus* 0_8) and W3350 (λ *sus* A_{11}) were supplied by Mr. M. Gefter and K12(λ t11) was obtained from Dr. D. Korn. Lysates of wild-type phage (λ⁺) were prepared by induction of Y10 by mitomycin C;[9] purification of phage, isolation of native λDNA, and separation of λDNA halves were carried out as described previously.[2]

Isolation and identification of λDNA strands: Strands of λDNA were isolated directly from suspensions of purified phage by modification of procedures described by Kubinski *et al.*[10] and Hradecna and Szybalski.[11] These procedures utilize alterations in buoyant density resulting from differential interaction of certain ribonucleotide homopolymers with DNA complementary strands to enable physical separation of the DNA strands in CsCl density gradient solutions. In the present experiments, separation of λDNA strands was accomplished with the aid of polyguanylic acid (generously supplied by Dr. M. Grunberg-Manago) as follows: 0.1 ml of a suspension of purified λ phage (containing 150–200 μg of DNA) in 6 *M* CsCl was added to 6.9 ml of a mixture containing 180–250 μg of poly G; 0.15% sodium dodecyl sarcosinate (Geigy Industrial Chemicals Corp.); 0.003 *M* Tris-HCl, pH 8.5; 0.015 *M* NaCl–0.0015 *M* sodium citrate; and ethylenediaminetetraacetate (EDTA), 0.001 *M*. The mixture was heated for 3 min at 94° and then rapidly cooled to 0°. Approximately 9.4 gm of solid CsCl (optical grade, Harshaw) were added and gently dissolved; the solution was adjusted to $\rho_{25°} = 1.721$ gm/cm³ ($\eta_{25°} = 1.4025$), and then poured into a Spinco no. 40 nitrocellulose centrifuge tube. An aliquot (approximately 0.7 ml) was removed for examination by analytical ultracentrifugation, the empty portion of the tube was filled with paraffin oil, and the denatured DNA-poly G complex was centrifuged in a Spinco no. 40 fixed-angle rotor for 65–72 hr at 37,000 rpm at 10°. Following centrifugation, fractions (6 drops each) were collected through a hole pierced in the bottom of the tube. One ml of 2 × SSC (0.3 *M* NaCl, 0.03 *M* sodium citrate) was added to each fraction, and the absorbancy was determined at 260 mμ. Pooled fractions containing each of the two principal peaks were examined by band[12] sedimentation in alkali in order to ascertain linear intactness.

Complexes of the separated strands of λDNA and poly G were subjected to hydrolysis in 0.3 *N* KOH at 37° for 16–22 hr, and the resulting monomeric guanylic acid residues were removed by dialysis against 2 × SSC containing 0.02 *M* Tris-HCl buffer, pH 7.5. The separated λDNA strands were then self-annealed in 5 × SSC for 4 hr at 65° in order to form duplex DNA molecules with any contaminating segments of the opposite strand. Since double-stranded DNA is not retained by nitrocellulose membranes,[13] such contaminating segments are therefore made unavailable for hybridization with complementary RNA.

Preparation and hybridization of RNA products: Nuclease-free[2, 14] preparations of purified RNA polymerase were prepared from *E. coli* W by a modification[14] of the procedure previously described,[15] and were a gift of Dr. U. Maitra. Crude bacterial sonicates were prepared from cell suspensions (3:1, w/v) in 0.1 *M* Tris, pH 7.5–0.005 *M* $MgCl_2$ by sonication for 30 sec at 0° using an MSE (Instrumentation Associates, Inc.) sonifier fitted with a $^3/_4$-in. probe; preparations were then centrifuged for 10 min at 12,000 *g*, and the supernatant fluid was removed and used as a source of both enzyme and template. Prior to use in certain experiments, supernatant fractions were freed from endogenous bacterial and viral nucleic acids by phase partition.[16] RNA synthesis by fractions treated in this way was stimulated tenfold by the addition of exogenous DNA. Enzymic RNA synthesis and isolation of RNA polymerase products were carried out as described previously,[2] except that the reaction mixture was extracted three times with phenol (saturated with 2 × SSC) at 60° when crude cellular preparations were used.

Extracts of gently lysed bacterial cells were prepared as described in Table 3, according to Ron *et al.*[17] Plating of such preparations indicated a viable cell count of less than 10^3 cells/ml (original cell count 2×10^{10}/ml). Cells were lysed in tubes subsequently employed for reaction mixtures in order to avoid pipetting the cellular components; radioactively labeled nucleoside triphosphates were added directly to these tubes, and RNA polymerase products were synthesized using endogenous DNA template and enzyme activity. Incubation of reaction mixtures with pancreatic DNase (20 μg/ml, electrophoretically pure, Worthington) for 15 min at 37° prior to the addition of labeled nucleotides reduced RNA synthesis more than 20-fold. RNA products were isolated as described previously[2] and subjected to three hot phenol extractions.

The procedures used for the preparation and isolation of pulse-labeled RNA synthesized *in vivo* were similar to those described by others,[4, 18] and will be described elsewhere.[19] DNA-RNA hybridization experiments were performed at saturating DNA concentrations on nitrocellulose

membranes using the method of Gillespie and Spiegelman,[13] as described previously,[2] except that the annealing solution contained 1.0% phenol that had been previously saturated with 2 × SSC.[20]

Results.—Separation and characterization of λDNA complementary strands: Preparative separation in CsCl of the complementary strands of λDNA complexed with poly G is shown in Figure 2. Two major λDNA peaks are evident. In addition, a small third peak banding at the same density as native λDNA was detected; presumably, this peak represents either renatured λDNA duplex or a small amount of DNA that was not adequately denatured. Analytical ultracentrifugation of

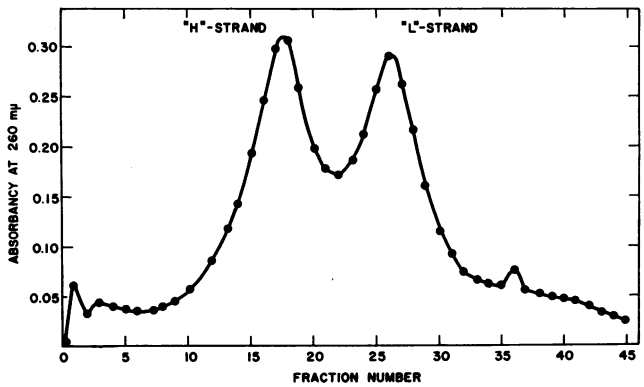

FIG. 2.—CsCl gradient ultracentrifugation of denatured λDNA complexed with poly G. Preparative centrifugation of DNA and the collection and assay of gradient fractions were carried out as described in the text. "Heavy" and "light" strands were designated on the basis of relative positions of the two main peaks of the poly G-DNA complex in relation to the bottom of the tube, which is to the left.

aliquots of the major peaks in the presence of *Cl. perfrigens* DNA density marker[19] ($\rho_{25°}$ = 1.691 gm/cm^3) indicated that both have buoyant densities greater than the density of denatured λDNA banded in the absence of poly G, demonstrating that both λDNA fractions interact with the homopolymer. The fraction showing the greater density change on interaction with poly G($\rho_{25°}$ = 1.776 gm/cm^3) was designated the "H" (heavy) strand, while the fraction whose buoyant density was altered less was designated the "L" (light) strand ($\rho_{25°}$ = 1.742 gm/cm^3).

Evidence (to be detailed elsewhere[19]) that these fractions correspond to linearly intact, complementary strands of λDNA was obtained by sedimentation velocity centrifugation of aliquots of the two DNA peaks under alkaline conditions; both DNA species showed the same sedimentation velocity ($s_{20,w}$ = 42) as unbroken native λDNA examined under the same conditions. Annealing a mixture of purified H and L strands resulted in the formation of a new band having the same buoyant density as native λDNA ($\rho_{25°}$ = 1.710 gm/cm^3); however, self-annealing of each peak produced no significant buoyant density changes. Centrifugation of physically separated λDNA strands in CsCl gradient solutions containing 0.01 *M* NaOH[21] and 0.01 *M* EDTA indicated that our L fraction corresponds to the H strand isolated by the alkaline CsCl gradient centrifugation procedure of Hogness *et al.*,[5] and that our H fraction consists of the less dense L strand purified by these investigators.

In vivo transcription of λ⁺DNA: Isolated and purified preparations of mRNA that had been pulse-labeled with H^3-uridine at various times after induction of Y10 by mitomycin C were annealed with unfractionated denatured λDNA and with each of its complementary strands (Fig. 3). In general agreement with the observations of others,[18, 20] annealing of such pulse-labeled RNA products with unfractionated λDNA indicated that λ⁺ mRNA synthesis rises above the uninduced level about 26 minutes after addition of mitomycin and that λ-hybridizable counts reach a maximum of about 11 per cent of total RNA synthesis 35–40 minutes later. Furthermore, hybridization of pulse-labeled RNA with each of the λDNA strands indicates that there is at least one major switch in the DNA strand transcription pattern during phage development. Very early λmRNA is transcribed primarily from the L strand; the rate of λmRNA synthesis on this strand increases between 26 and 30 minutes after induction and only slightly thereafter. In contrast, λmRNA made from the H strand remains at relatively low levels until 32 minutes after addition of mitomycin C. Subsequently, transcription from the H strand of λDNA rises rapidly so that about 90 per cent of "late" λ-hybridizable mRNA is made from this strand.

Preparations of mRNA synthesized after induction of Y10 were also annealed with separated λDNA halves[2] in addition to being hybridized with λDNA strands, as indicated in Figure 3; the results of these experiments are shown in the upper part of Table 1. In agreement with the findings of Naono and Gros,[3] and with the observations of Skalka[4] made following external infection of *E. coli* W3110, these experiments indicate that "early" λ-messenger RNA is transcribed predominantly from the AT-rich (*right*) half of the template. Synthesis of λmRNA on this segment of the template increases ninefold during the course of phage development, but this rise is more than matched by a marked increase (150-fold) in mRNA transcription from the GC-rich (*left*) half.

The RNA annealing patterns observed with λDNA strands and λDNA halves

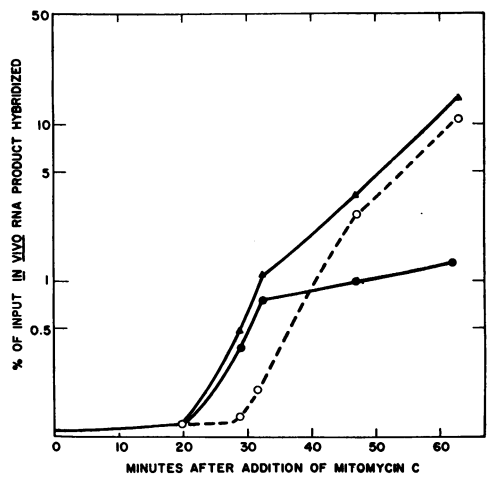

FIG. 3.—Hybridization pattern shown by λmRNA synthesized *in vivo*. RNA was pulse-labeled with H^3-uridine at various times after induction of Y10, as described in the text. Annealing was carried out in tightly closed screw-top scintillation vials for 14–18 hr at 65° as previously described,[2] using the procedure of Gillespie and Spiegelman.[13] Each nitrocellulose membrane contained 2 mμmoles (expressed in terms of nucleotide phosphorus) of the appropriate DNA species. Input RNA (sp. act. = 3–5 × 10^5 cpm) was as in Table 1. The per cent of total input RNA counts annealing with unfractionated λDNA (▲–▲–▲) and with the separated heavy (○–○–○) or light (●–●–●) strands of λDNA is indicated on a logarithmic scale. The background "noise" level of RNA counts (range 30–70 cpm) determined in each experiment by using membranes lacking DNA has been subtracted from values obtained experimentally. In parallel experiments,[19] λ-specific mRNA isolated by prior hybridization with unfractionated λDNA on agar columns was subsequently annealed on nitrocellulose membranes with separated strands of λDNA. λ-Messenger RNA isolated in this way showed an annealing pattern that was virtually identical to that observed with the direct hybridization technique illustrated in this figure.

TABLE 1

HYBRIDIZATION PATTERN OF mRNA LABELED WITH H^3-URIDINE AT VARIOUS TIMES AFTER INDUCTION OF CERTAIN λ LYSOGENS BY MITOMYCIN C

Lysogen	Time of radioactive pulse (min following addition of mitomycin C)	Cpm Annealed with Unfractionated λDNA (Cpm annealing)	Cpm Annealed with Unfractionated λDNA (% of total input RNA)	Cpm Hybridizing with H strand	Cpm Hybridizing with L strand	Cpm Hybridizing with AT-rich half	Cpm Hybridizing with GC-rich half
Y10(λ+)	26–28	452	0.4	43	431	465	41
	30–32	1,043	1.0	324	746	1,008	189
	60–62	11,514	11.5	11,223	1,350	3,879	6,973
K12(λ t_{11})	29–31	687	0.5	12	692	—	—
	58–60	5,527	3.7	67	5,591	4,764	753
W3350(λ *sus* A_{11})	29–31	467	0.5	130	355	—	—
	58–60	4,895	4.9	4,467	578	2,162	2,864
W3350(λ *sus* O_8)	29–31	844	0.4	246	584	—	—
	58–60	2,764	1.4	1,550	1,272	1,625	946

Pulse-labeling of mRNA at very early (26–28 min), early (29–31 min or 30–32 min), or late (58–30 min or 60–62 min) times was carried out as indicated in the text. Subsequent isolation and hybridization of RNA was carried out as described in the text and previously.[2] Hybridization mixtures contained 0.4–0.5 μg of RNA (sp. act. 3–5 × 10^5 cpm/μg) and a total of 10^5 cpm (λ+ and λ *sus* A_{11}), 1.5 × 10^5 cpm (λ t11) or 2 × 10^5 cpm (λ *sus* O_8).

indicate that: (1) following induction of Y10, λmRNA synthesis begins at sites present on the AT-rich half of the L strand of λDNA; (2) although RNA homologous with this λDNA strand and half continues to be made late during phage development, the majority of "late" λmRNA is transcribed from the H strand, predominantly from sites located on the GC-rich half, which contains the late-functioning cistrons A–J.[23] Recent experiments of Hogness *et al.*[5] and of Wu and Kaiser[22] demonstrate that the more dense of the two complementary λDNA strands in alkaline CsCl gradients (i.e., our L strand, as indicated above) is oriented with its 5′ terminus at the *sus* A end (Fig. 1). This finding permits assignment of the direction of transcription for both early and late λmRNA. Since DNA is read from the 3′ to 5′ direction,[7] mRNA transcribed from our L strand is necessarily polymerized from right to left on the genetic map shown in Figure 1. Similarly, RNA homologous with the H strand must be synthesized in the opposite direction.

Synthesis of mRNA by various λ lysogens: Table 1 also shows the hybridization pattern of RNA pulse-labeled with H^3-uridine at various times after induction of certain λ lysogens by mitomycin C. These experiments indicate that the switch in orientation of transcription normally observed during development of λ+ may be related to DNA replication, in accord with other data we have obtained during inhibition of DNA synthesis in λ+ by thymine deprivation.[19] Of the three λ mutants shown, only λ *sus* A_{11}, in which DNA synthesis is normal,[20] shows the sharp rise in transcription of the heavy DNA strand characteristically seen at late times after induction of λ+. Lambda *sus* O_8, which in the nonpermissive host *E. coli* W3350 is defective in DNA synthesis,[20] is deficient in transcription of λmRNA from the GC-rich half of the H DNA strand at late times; thus, λ *sus* O_8 "late" mRNA appears to be copied primarily from the AT-rich half of λDNA, and shows an almost equal homology with the two DNA strands.

Although the t11 mutant of region x^6 and λ *sus* O_8 are apparently equally defective in DNA replication,[20] substantially higher "late" mRNA synthesis was observed following induction of the former, as has been pointed out previously by Joyner *et al.*[20] As indicated in Table 1, λt11 mRNA made at late times is transcribed predominantly from the AT-rich half of the template, and almost entirely (H/L = 1/95 from the light λDNA strand. Furthermore, it can be seen that both the rate

of mRNA synthesis on the L strand of λDNA (as measured by the per cent of input counts annealing with this strand) and the relative increase of λmRNA from early to late times are greater with the t11 mutant than with λ^+. These findings suggest that the hyperproduction of exonuclease that phenotypically characterizes λ t11[6, 24] may be due to a failure of this mutant to turn off mRNA synthesis from template sequences located on the light λDNA strand.

In vitro transcription of complementary λDNA strands by purified RNA polymerase: RNA products synthesized *in vitro* by *E. coli* RNA polymerase under various conditions were annealed with unfractionated λDNA and with the separated strands of λDNA. These studies (Table 2) indicate that highly purified, nuclease-free preparations of enzyme that selectively transcribe the AT-rich half of λDNA *in vitro*[2] do *not* show a significant quantitative preference for either of the two strands under the conditions employed here. RNA products synthesized on native, denatured, or circular[2] λDNA templates all showed equal homology with both of the complementary λDNA strands. RNA products transcribed from each separated strand hybridized selectively with their own template species.[25]

In vitro transcription of complementary λDNA strands by crude bacterial extracts: The pattern of *in vitro* RNA synthesis catalyzed by certain crude preparations of *E. coli* RNA polymerase is shown in Table 3. Exogenous mature native λDNA was added to reaction mixtures as template where indicated; in other experiments, *in vitro* RNA synthesis was carried out with crude bacterial extracts using both endogenous enzyme activity and endogenous template. The results of these experiments indicate that the AT-rich half and the H strand of exogenous native λDNA are preferentially transcribed by sonicates of Y10 prepared at either early or late times after induction (cf. *in vivo* mRNA synthesis). A similar pattern of homology was seen with RNA products made by sonicates of uninduced Y10 and by sonicates of nonlysogenic bacteria.[19] The divergent patterns of DNA strand homology observed with "early" λmRNA made *in vivo* and RNA synthesized on exogenous native λDNA *in vitro* by either purified *E. coli* RNA polymerase or crude sonicates indicate that these species of RNA are not identical, although all three are transcribed predominantly from the AT-rich λDNA half.

When bacterial cells (Y10) were disrupted by either sonication or by grinding with alumina[19] at early or late times after induction, RNA polymerase products synthesized on *endogenous* DNA template *also* hybridized preferentially with the

TABLE 2

ANNEALING PATTERN OF RNA PRODUCTS SYNTHESIZED *in vitro* ON λDNA TEMPLATES BY PURIFIED *E. coli* RNA POLYMERASE

Template	Input RNA (cpm)	Cpm Hybridized with: Unfractionated λDNA	Heavy λDNA strand	Light λDNA strand
Native λDNA	3200	2222	1189	1160
Denatured λDNA	5000	1511	840	795
λ Circles (hydrogen-bonded)	2100	1331	889	737
Heavy strand of λDNA	3700	1562	1547	79
Light strand of λDNA	3900	1597	148	1521

Hybridization mixtures contained 10–30 μμmoles of RNA polymerase products which had been prepared and isolated as described previously. DNA-RNA hybrids were formed and assayed as indicated in the text, and in Fig. 3. Incubations were carried out for 50 min at 37°. Standard reaction mixtures (0.5 ml) contained: 80 mμmoles each of ATP and CTP; 40 mμmoles each of H^3-labeled (Schwarz BioResearch, Inc.) GTP and UTP (sp. act. of each = 5×10^5 cpm/mμmole); Tris-HCl buffer, pH 7.5, 25 μmoles; 2-mercaptoethanol, 4 μmoles; $MgCl_2$, 4 μmoles; *E. coli* RNA polymerase, 5 units; and 10 mμmoles of the indicated λDNA template. Separated λDNA strands employed as template were not self-annealed prior to use.

TABLE 3

ANNEALING PATTERN OF RNA POLYMERASE PRODUCTS SYNTHESIZED *in vitro* USING CRUDE BACTERIAL EXTRACTS

Source of enzyme	Post-induction interval (min)	DNA template	Input RNA (cpm × 10^3)	Cpm Hybridizing with: Unfractionated λDNA	Heavy strand	Light strand	AT-rich half	GC-rich half
A. Y10 sonicate	Uninduced	Native λ	2.2	1,385	986	603	1,001	411
	30	Endogen.	2.0	162	79	73	—	—
	60	Endogen.	25.0	1,201	828	548	886	392
B. Y10 sonicate, partitioned*	30	Native λ	2.0	1,007	743	491	749	247
	60	Native λ	2.0	1,119	703	446	782	301
C. Y10 gentle lysis	30	Endogen.	25.0	291	113	190	256	106
	60	Endogen.	10.0	1,280	1,054	150	405	941
D. K12(λ t_{11}) lysate	50	Endogen.	200	732	18	752	587	133
E. K12(λ t_{11}) lysate sonicated	50	Endogen.	300	282	124	124	215	88

* Partition of sonicates was carried out by the phase partition procedure cited in the text. Preparation of extracts A and B from appropriate lysogens and hybridization of RNA products were carried out as indicated in text. Reaction mixtures (0.5 ml) contained: 80 mμmoles each of ATP and CTP; 40 mμmoles each of H^3-labeled GTP and UTP (sp. act. = 5 × 10^5 cpm/mμmole); Tris-HCl buffer, pH 7.5, 25 mμmoles; 2-mercaptoethanol, 4 μmoles; $MgCl_2$, 2 μmoles, crude extract containing approximately 300 μg of protein; and 10 mμmoles of mature native λDNA where indicated. Extracts C, D, and E were prepared as follows: incubations were interrupted by pouring induced bacterial cultures over crushed ice at indicated times after addition of mitomycin. Cells were sedimented, washed once with one-half the original volume of 0.01 *M* $MgSO_4$, and resuspended in 0.01 *M* Tris–0.015 *M* $MgCl_2$ (4:1, v/w). Aliquots (0.045 ml) were transferred to incubation tubes, 0.005 ml of a solution of lysozyme (10 mg/ml, Worthington) was added, and the cell suspension was frozen and thawed slowly three times as described by Ron *et al.*[17] An 8% solution (0.002 ml) of sodium deoxycholate (Sigma) was added, and the incubation tubes were placed in ice while reagents required for RNA synthesis were added. In the case of extract E, 2-ml vol of *E. coli* K12(λ t11) cell suspensions were similarly frozen and thawed and treated with identical concentrations of lysozyme and sodium deoxycholate prior to sonication for 10 sec at 0°, as described in the text. Aliquots (0.05-ml) of these extracts were added to incubation tubes and used as the source of both enzyme and template. In addition to components present in extracts, reaction mixtures C, D, and E contained the reagents indicated for extracts A and B, except that 2 μmoles of $MgCl_2$ were used. Multiples of all constituents were used in certain experiments where greater quantities of RNA product were desired. *In vitro* RNA synthesis was carried out at 37° for 30 min.

AT-rich half and the H strand of λDNA. However, when gentle lysis was used to disrupt bacteria, transcription of endogenous λDNA *in vitro* by "early" or "late" extracts showed a temporal change in orientation resembling the strand-switch observed during *in vivo* mRNA synthesis. RNA synthesis catalyzed by extracts prepared from cells lysed 28 minutes after induction yielded products annealing preferentially with the L strand and the AT-rich half, while RNA made *in vitro* using "late" extracts hybridized better with the GC-rich half and showed a 7:1 preferential homology with the H strand of λDNA.

Studies of *in vitro* RNA synthesis carried out with extracts of gently lysed *E. coli* K12 (λ t11) are also summarized in Table 3. These data indicate that such extracts are capable of maintaining the pattern of predominant L strand transcription that was observed *in vivo* at both "early" and "late" times after induction. However, RNA polymerase products made with identical preparations that had been sonicated for 10 seconds following gentle lysis annealed equally with the two strands. It therefore appears that enzyme activity capable of H-strand transcription is present in such extracts, but that transcription remains essentially restricted to the L strand unless "released" by the effects of sonication.

Conclusions and Summary.—These experiments indicate that segments of both complementary strands of λDNA are transcribed *in vivo* and *in vitro*. λ-Hybridizable mRNA synthesized *in vivo* at "early" times after induction of λ^+ is transcribed (from right to left) from nucleotide sequences located predominantly on the

AT-rich half of the λDNA strand having its 5′-phosphate group at the *sus* A end of the genetic map (Fig. 1). Subsequently, there is an apparent change in orientation of transcription so that approximately 90 per cent of λmRNA made at late times after induction is synthesized in the opposite direction on the H strand of λDNA, at sites located on both the AT-rich and GC-rich halves. λRNA synthesized *in vitro* by purified RNA polymerase hybridizes equally well with both λDNA strands.

λ-Hybridizable mRNA made at late times by mutants defective in DNA replication showed persistence of the general pattern of homology normally observed with "early" λmRNA. The t11 mutant of region x appears to be characterized by hyperproduction of RNA transcribed from ("early") L strand nucleotide sequences. Our results also indicate that the patterns of transcription observed *in vivo* at "early" and "late" times are maintained *in vitro* when endogenous λDNA template is copied by extracts prepared by gentle lysis of bacteria at these same times. Loss of temporal specificity followed sonication of these extracts, suggesting that perhaps a fragile complex of the DNA template with other cellular components, or a specific structural form of λDNA,[26] is required for such specificity.

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