ASYMMETRIC DISTRIBUTION OF THE TRANSCRIBING REGIONS ON THE COMPLEMENTARY STRANDS OF COLIPHAGE λ DNA*

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It was demonstrated in this laboratory that poly G and other guanine-rich polynucleotides show differential affinity for the two complementary strands of various DNA's, indicating asymmetric distribution of poly G-binding sites.[1,2] Furthermore, it was postulated that these sites, probably deoxycytidine(dC)-rich clusters, might act as the initiation points of the DNA-to-RNA transcription.[2,3] For DNA which contains dC-rich clusters on *both* strands, as for instance coliphage λ DNA (Fig. 1),[3,4] this hypothesis predicts that transcribing regions would be found on *both* strands. As will be shown, this prediction is confirmed for coliphage λ, which provides the first example of *in vivo* transcription from *both* DNA strands, as documented by DNA-RNA hybridization techniques.[3,5] This result agrees with the conclusions based on genetic experiments with λ phage.[6,7] In earlier studies employing other phages, only *one* DNA strand was found to hybridize with phage-specific mRNA.[8]

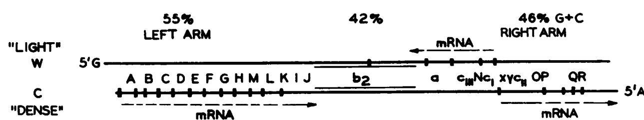

Fig. 1.—Genetic map of phage λ, including A to R *sus* markers,[9] "clear" markers c_I, c_{II}, and c_{III},[24] central b_2 region,[25,26] markers *x* and *y*,[7] and marker *a*,[27] all superimposed over λ DNA.[6,7] The base compositions (% G + C) of both arms of λ DNA and of the central b_2 region are indicated.[6,25] 5'G and 5'A identify the 5' terminal nucleotides[23] and the polarity of the *C* and *W* strands.[4] Symbol *C* (*"DENSE"*) indicates the DNA strand which is denser in the poly G-containing CsCl gradient (and "lighter" in the alkaline CsCl gradient[4,6]) than strand *W*.[2-4] The arrows (mRNA) indicate the orientation, the region, and the strand of preference for the DNA-to-RNA transcription, as discussed in this paper. The distribution of cytosine-rich clusters is indicated by the symbols (-+-), and is based on the data of Hradecna and Szybalski.[4]

Materials and Methods.—Bacterial and phage strains: *Escherichia coli* K12 strains included C600, which is permissive for λ*sus* mutants, and W3110 and W3350, which are nonpermissive for *sus* mutants.[9] These were lysogenized or infected with appropriate λ mutants as listed in Table 1. Most of the λc_I, λdg, and λ*sus* mutants and the lysogenic strains were obtained from Drs. W. F. Dove, H. Echols, A. Joyner, D. Pratt, M. Ptashne, and J. Adler. Strains T75[10] and T11 [=W3350-(λ*t*11)][7] were contributed by Dr. C. R. Fuerst. The subscript A–J indicates that genes A to J (entire left arm; Fig. 1) were deleted in λdg_{A-J} and replaced by a part of the galactose operon.[11] Biotin genes were substituted for deleted genes *a*-N or *a*-O in λdb_{a-N} (=λ*t*75) and λdb_{a-O}, respectively, the latter contributed by Dr. G. Kayajanian.

The cultivation of bacteria, infection or induction of phages, preparation of phage stocks, and purification of phages by high-low speed sedimentation and CsCl density gradient centrifugation followed the published procedures.[4,6,7,9-13] The lysogenic cultures were induced by addition of 2 μg mitomycin C/ml.[12]

Pulse-labeling and isolation of bacterial and λ-specific RNA: The method employed followed

closely the techniques described by Sly *et al.*[12] A total of 0.5 mc of H^3-uridine (8 c/mM) was added to 20 ml of the culture, which 2 min later was poured onto an equal volume of crushed ice prepared from minimal medium. This was followed by rapid sedimentation of the bacteria at 4°C, lysis by 2% sodium dodecyl sulfate (SDS), and phenol extraction of the RNA at 60°C. The upper aqueous phase was used for the "prehybridization" with λ DNA (see next section), after determination of the acid-precipitable radioactivity (total H^3-labeled RNA; see Table 1).

DNA-RNA hybridization procedure: The hybridization procedure was based on the technique described by Gillespie and Spiegelman[14] with an additional "prehybridization" step.

(a) *Prehybridization:* Denatured λ DNA (50 μg), "baked" on a 25-mm B-6 filter (Schleicher & Schuell Co.),[14] was incubated (24 hr, 60°C) with 1 ml phenol-saturated 2×SSC containing homologous H^3·RNA (total 100–500 μg RNA). The filters were exhaustively washed with 2× SSC after this and each following step. The nonspecifically bound RNA was digested with RNase (1 hr, 20°C, 4 ml of 2×SSC +20 μg RNase/ml). The residual RNase was inactivated by incubation (40 min, 55°C) of the washed filters in 2 ml of 0.15 *M* iodoacetate at pH 5 (0.1 *M* Na-acetate buffer, 2×SSC). The H^3·RNA was eluted with 1.5 ml of 1/100×SSC (15 min, 95°C, 90% recovery of H^3 count), and treated with RNase-free (iodoacetate pretreated[16]) DNase (10 μg DNase [Worthington Co.]/ml of 0.05 *M* Tris, 0.004 *M* $MgCl_2$, pH 7.4, 20 min, 37°C), which was then inactivated by 10 min heating to 95°C, as recommended by Skalka (personal communication).

(b) *Hybridization:* Denatured DNA (25 μg) or the separated λ DNA strands (3–10 μg) "baked" on a 25-mm filter[14] were incubated (24 hr, 60°C, 1 ml phenol-saturated 2×SSC) with 1,000–10,000 cpm of λ mRNA eluted from the filter in the prehybridization procedure. Following the RNase treatment (see prehybridization) and extensive washing with 2×SSC, the filters were dried (2 hr, 60°C) and transferred to toluene-2,5-diphenyloxazole (toluene-PPO) scintillation fluid for the H^3 count. An excess of hybridization sites for binding up to 2,000 cpm of "prehybridized" mRNA is provided by 3 μg of separated DNA strands, since the *C*:*W* ratios are unaffected by raising the DNA quantity to 10 or 25 μg per filter. All hybridization values are corrected for H^3·RNA counts bound by filters carrying denatured T4 coliphage DNA.

Preparative separation of the complementary DNA strands: To effect strand separation, the DNA is released, denatured, and reacted with poly IG, all three operations in a single step.[4] When the phage suspension (50 μg DNA) is heated (2 min at 90°C) in 0.5 ml 10^{-3} *M* sodium versenate (EDTA) containing 100 μg poly IG and 0.1% Sarkosyl NL 97 (Geigy Chemical Corp., New York), and is chilled and centrifuged in a polyallomer tube (2.5 ml of CsCl solution, 1.72 gm/cm³, 70 hr, 15°C, 30,000 rpm SW39 rotor), the released and denatured DNA is distributed into two symmetrical bands separated by 12–14 mg/cm³. Of the two complementary DNA strands, the one with the relatively higher content of poly IG-binding, dC-rich clusters[3, 4] (strand *C*) is found in the denser band, while the other (strand *W*) forms the less dense band (Fig. 1). After collecting the separate fractions, each of the two pooled samples containing one kind of λ DNA strand was separately subjected to self-annealing (4 hr, 65°C, 5 *M* CsCl)[4] to convert any of the contaminating opposite strands to double helices, which would be inactive in the DNA-RNA hybridization procedure. The functional purity of such "self-annealed" strands was over 99% as tested by preparing H^3·mRNA specific for one strand only (cf. (a) *Prehybridization* in *Materials and Methods*) and comparing its affinities for the two strands. The mRNA prehybridized and eluted from the *C* strands hybridized with strands *C* over 99 times more efficiently than with strands *W* (99.6:0.4 for *C*:*W*). An analogous result was obtained for strand *W*-specific mRNA or for mRNA produced by the λ*t*11 mutant (Table 1, line 11). Proof for the integrity of the separated strands at over 85% level, their properties, orientation (Fig. 1), and the details of the strand separation procedure for λ DNA were published by Hradecna and Szybalski.[4]

Results and Discussion.—Temporal control of transcription: Shortly after infection with λc_{72} phage, the amount of λ-specific mRNA rises rapidly, as previously shown by Sly *et al.*[12] This "early" λ-mRNA hybridized preferentially with the *W* strand (Fig. 1) of λ DNA (5:95 for *C*:*W*, Table 1, line 3, and Fig. 2*A*). At 30 or 40 minutes after infection, the preference for the transcription shifted to the *C* strand (85:15 for *C*:*W*, Table 1, lines 4 and 5; and Fig. 2*A*). It appears, therefore, that the transcription from strand *C* rises sharply during the development of

infectious phage, while the strand *W*-specific mRNA is synthesized at an almost constant rate (Table 1, line 3 versus 4, and 7 versus 18; Fig. 2*A*).

Chloramphenicol (*CM*) *effects:* Addition of CM before induction or infection with phage does not grossly affect the initial rate of λ-mRNA synthesis, but at all times such RNA retains the characteristics of early mRNA.[17] Temporally, we define the early λ-mRNA as that which could be produced in the absence of protein and DNA synthesis; it will be shown later that it is probably transcribed from the N-*a* and *x*-O regions. This definition is probably more restrictive than that used earlier.[12, 13, 21, 22] It appears essential to use at least 100 μg CM/ml for preparing early λ-mRNA, since the transcription from strand *C* preferentially increases at lower CM concentrations (Table 1, lines 7, 8, and 19).

Localization of the "switch sites": Transcription of both the *W* and *C* strands

TABLE 1

PERCENTAGE OF λ-SPECIFIC H^3-LABELED RNA SYNTHESIZED BY VARIOUS λ MUTANTS AND THE PROPORTION OF THIS mRNA HYBRIDIZING WITH COMPLEMENTARY STRANDS *C* AND *W*

				Hybridized with Separated Strands				
				Per Cent of Total,§ H^3-Labeled RNA (λmRNA)			Ratio (%)	
Expt. no.	mRNA donor* (host; phage)	Induction,† infection CM(μg/ml)‡	H^3-uridine pulse† (min)	*C* + *W*	*C*	*W*	*C*	*W*
1	W3110	None	2	0.001	—	—	—	—
2	W3350	None	2	0.002	0.001	0.001	50	50
3	W3110; λc_{72}	Inf.	0–2	2.0	0.1	1.9	5	95
4	W3110; λc_{72}	Inf.	28–30	8.4	7.1	1.3	85	15
5	W3350; λc_{72}	Inf.	38–40	8.1	6.9	1.2	85	15
6	W3350; λc_{72}	Inf.	38–40	8.0	7.8	0.2	97	3
				left arm				
7	W3350(λ+)	Ind. CM(100)	58–60	0.5	0.05	0.45	10	90
8	W3350(λ+)	Ind. CM(40)	58–60	0.7	0.16	0.54	23	77
9	W3350(λdg_{A-J})	Ind. CM(100)	58–60	0.4	0.08	0.32	20	80
10	W3350(λdg_{A-J})	Ind.	58–60	2.3	1.8	0.50	78	22
11	W3350(λt11)	Ind.	58–60	0.9	0.005	0.89	0.5	99.5
12	W3350(λt11)	Ind. CM(50)	58–60	0.6	0.018	0.58	3	97
13	W3350(λt11)	None	2	0.03	0.005	0.025	16	84
14	W3350(λind⁻)	None	2	0.04	0.003	0.037	8	92
15	W3350(λind⁻)	None	2	0.005	0.003	0.002	56	44
				λ*i* 434				
16	W3350(λind⁻)	None	2	0.004	0.002	0.002	47	53
				λ*i* 21				
17	W3350(λ+)	None	2	0.05	0.01	0.04	18	82
18	W3350(λ+)	Ind.	58–60	3.4	2.9	0.48	86	14
19	W3350(λ+)	Ind. CM(50)	58–60	0.6	0.1	0.5	16	84
20	T75(λdb_{a-N})	None	2	0.04	0.007	0.033	18	82
21	W3110b(λdb_{a-O})	None	2	0.02	0.012	0.008	59	41
22	W3350(λN_{53})	None	2	0.05	0.006	0.044	13	87
23	T75(λdb_{a-N})	Ind.	58–60	0.04	0.027	0.013	68	32
24	T75(λdb_{a-N})	Ind. CM(50)	58–60	0.06	0.017	0.043	28	72
25	W3110b(λdb_{a-O})	Ind.	58–60	0.02	0.011	0.009	57	43
26	W3350(λN_7)**	Ind.	58–60	0.6	0.12	0.48	21	79
27	W3350(λN_7)**	Ind. CM(50)	58–60	0.4	0.09	0.31	22	78
28	W3350(λO_{29})	Ind.	58–60	0.5	0.22	0.28	44	56
29	W3350(λO_{29})	Ind. CM(50)	58–60	0.4	0.08	0.32	20	80
30	W3350(λP_{80})	Ind.	58–60	0.6	0.34	0.26	57	43
31	W3350(λP_{80})	Ind. CM(50)	58–60	0.6	0.11	0.49	19	81

* In expts. 1 and 2, bacteria free of λ phage were used. Infection is indicated by the semicolon, whereas the lysogenic state is indicated by parentheses.

† The bacteria were infected (Inf.) at multiplicity 5 (0°C, 10^{-2} *M* Mg^{++}), and after 20 min for absorption (0°C) transferred to growth medium[12] (37°C) (= zero time). Induction (Ind.) was initiated by adding 2 μg mitomycin C/ml of growth medium.[12] The noninduced lysogens (None) were pulse-labeled for 2 min during the exponential growth phase at a cell concentration of 4 × 10^8/ml.

‡ Chloramphenicol (CM) was present from 10 min before induction to the end of the H^3-uridine pulse.

§ Percentage of total H^3 RNA which was prehybridized (50 μg λcb_2 DNA or other DNA if so specified in the column), eluted from the filter, and hybridized again with 25 μg denatured λcb_2 DNA (*C* + *W*), or with 3 μg of self-annealed *C* and *W* strands of λcb_2 DNA per filter (see *Materials and Methods*). In expt. 6, H^3-RNA was prehybridized with the "short left arm" of λc_I DNA.

** Similar results were obtained with the λN_7N_{53} double mutant.[20]

implies the presence of sites at which the λ-mRNA synthesis switches from one strand to another, either *converging* or *diverging* from such a *switch site*. A major *switch site* should be on the right arm of the DNA, since both the early (+CM) and late mRNA's produced by *E. coli* lysogenic for λdg_{A-J}, in which the whole left arm is deleted[11] (Fig. 1), hybridize with both DNA strands (Table 1, lines 9 and 10). It was previously shown that early mRNA hybridizes preferentially with the right arm of λ DNA.[18]

Employing prehybridization of H^3-labeled RNA with λdg_{A-J} DNA (Fig. 1) at various times after λc_I infection, it was possible to follow the kinetics of transcription on the right arm only, for both the *C* and *W* strands (Fig. 2*B*). The transcription on the right arm of strand *C* increases rapidly up to the 20th minute after infection and levels off around the 24th minute, whereas the transcription on the left arm of strand *C* commences at about the same time and rises sharply (Fig. 2*B*; *C*(*right*) and *C*(*left*)). The kinetics of transcription for the right arm of strand *W* (Fig. 2*B*; *W*(*right*)) are similar to those for the whole strand *W* (Fig. 2*A*; *W*), although the small and possibly fortuitous differences between these

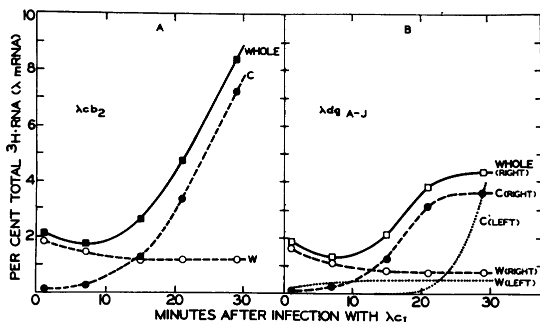

FIG. 2.—Percentage of H^3-labeled RNA (2-min pulse) specific for λcb_2 (*A*; ■) and λdg_{A-J} (*B*; □), and for the *C* (●) and *W* (○) strands of these DNA's, at various times after infection of *Escherichia coli* W3110 with λc_{72} (see second footnote in Table 1, and *Materials and Methods*; pH of growth medium, 7.7). RNA was prehybridized with 50 μg denatured DNA of λcb_2 (*A*) or λdg_{A-J} (*B*), treated with RNAse and iodoacetate (see *Materials and Methods*), eluted, and hybridized with 25 μg of denatured λcb_2 DNA (WHOLE, solid line) and with 3 μg of the separated *C* and *W* strands of λcb_2. The dashed-line values, which are fractions of the solid-line values proportional to the per cent of *C* or *W*, represent the amount of mRNA transcribed from the whole *C* or *W* strand (*A*) or from the right arms (Fig. 1) of the *C* or *W* strand (*B*). The dotted line *C* (*left*) represents the difference between the *C* and *C* (*right*) values, and indicates the kinetics of transcription from the left arm (Fig. 1) of the *C* strand. The dotted line *W* (*left*) represents the difference between the *W* and *W* (*right*) values.

values might be construed as indicative of some transcription from the left arm of strand *W* (Fig. 2*B*; *W*(*left*)). However, it was found that mRNA prehybridized with the purified left arm of λc_{72} DNA and thus containing only the mRNA transcribed by the left arm, hybridized almost exclusively with the *C* strand (Table 1, line 6). Thus, there is little reason to postulate any transcription from the left arm of strand *W*.

Where on the right arm of λ DNA is the "switch site" localized, and is it a *divergency* or *convergency* site? The most direct answer is provided by an experiment with mutant λ*t*11, in which a polar mutation in gene *x* inactivates the *x*-to-O functions[7] and at the same time blocks the transcription from strand *C* (0.5:99.5 for *C*:*W*; Table 1, line 11). This correlation indicates that genes *x*-to-O are transcribed from strand *C*, i.e., from left to right. If strand-*W*-specific mRNA is the product of region N to *a*, and thus is transcribed from right to left, a *divergency site* must be located between genes N and *x* (Fig. 1). These interpretations

are based on the known polarities of strands C and W (Fig. 1) and on the 5′-to -3′ orientation of RNA synthesis in conjunction with work on heteroduplexes in gene N.[6, 15] Since left-arm-specific mRNA seems to be transcribed from strand C, i.e., in the left-to-right direction, a *convergency site* should be located somewhere near or within region b_2 (Fig. 1). The products of the b_2 region are not included in this study, since λcb_2 DNA with the b_2 region deleted (Fig. 1) was used either for prehybridization or for the preparation of separated DNA strands. However, similar studies on the transcribing function of the b_2 region are currently being pursued in this laboratory.

Transcription from the prophage: To further localize the position of the major switch, it is necessary to determine the orientation of the transcription from gene c_I. The W3350(λind$^-$) lysogenic strain[19] was used for this purpose, since its spontaneous induction rate is very low, and thus its mRNA may consist mainly of the gene c_I product. Transcription rate from the noninduced λind$^-$ prophage (0.04% of total H^3·RNA) is up to 40 times higher than the level obtained with λ-free *E. coli* (Table 1, lines 1, 2, and 14). This mRNA hybridizes preferentially with the W strand of λcb_2 (0.037%; 8:92 for $C:W$), and has hardly any affinity for strand C (0.003%; Table 1, line 14) or for either DNA strand of λ hybrids with a heterologous c_I region (0.002–0.003%; Table 1, lines 15 and 16). The levels of λ-specific mRNA for several noninduced prophages were within relatively narrow limits (0.03–0.05%; Table 1, lines 13, 17, 20, and 22; see also Sly *et al.*[12]); all these mRNA's exhibited strong preference for strand W, with the exception of mRNA produced by the λdb_{a-0} prophage, in which gene c_I is deleted and which transcribes poorly both before and after induction (Table 1, lines 21 and 25). These data indicate that (1) in the noninduced state the bulk of λ-mRNA is the product of gene c_I, and that (2) this is transcribed from strand W, i.e., from right to left, which conclusion is similar to that based on the polar effects of the *sus*34 mutation located at the right end of gene c_I.[20] Furthermore, these data suggest that the λ repressor is a bifunctional protein (as represented by regions A and B[22]), which interacts with two operator regions (deleted in $\lambda i434$), one adjoining the left end of the c_I gene and controlling the N-a transcription from the W strand and the other located next to the right terminal of gene c_I and controlling the x-O transcription from the C strand. According to this model, the lack of host inactivation upon transient thermal induction of the c_I(B) prophage in the presence of CM (Lieb and Green[22]) can be explained by partial renaturation of the repressor with restoration of only one (x-O repression) of its two functions; temporary expression of genes N-to-a apparently has no lethal consequences.

Controls of early and late transcription: In the noninduced state, c_I mRNA seems to be the main transcription product (W strand). Immediately after infection or "early" (+CM) upon induction, the total transcription increases by factors of 10–40, with strand W being predominantly transcribed, probably in the N-a and still in the c_I regions. Strand C is transcribed to a lesser extent (5:95 to 10:90 for $C:W$), most probably from the x-y-c_{II}-O operon, since the polar mutation in gene x abolishes this early transcription from strand C. The fact that CM freezes this early stage of transcription indicates that (1) the N-a and x-O transcriptions are not mutually dependent on their protein products, and (2) that there is a need for some proteins to extend the transcription to other genes.

Which gene products are necessary for the shift to the later stages of transcription? At least two proteins, those missing in the N (as postulated by Thomas[21]) and x mutants, appear to be required; a low level of C-specific mRNA, characteristic of the early transcription pattern, persists upon induction of both the x and N mutants (Table 1, lines 11, 19, and 26). This pattern changes somewhat for the induced *sus*O and P prophages.[12, 13] The "late" transcription from strand C for both the O and P mutants increases threefold when compared with the early transcription in the presence of CM (Table 1, lines 28–31); the analogous increase in C-specific transcription for the λ^+ is 30-fold and for the right arm of λ^+ or for λdg_{A-J} is approximately 20-fold (Table 1, lines 9, 10, 18, 19; Fig. 2*B*). These results indicate either a "leaky" character for the O and P mutations, or, more probably, a shift to the next stage of transcription from strand C.

Is it necessary to invoke any special control mechanisms for the early versus late transcription from strand W, which seems to be limited to the c_I and N-to-*a* regions? In the absence of DNA synthesis the differences between the early and late transcription from the W strand are small for the x, N, O, and P mutants (Table 1, lines 11, 12, 26–31). During the normal infection (Fig. 2) or induction process (Table 1, lines 18 and 19), the progressive decrease in synthesis of the strand W-specific mRNA also appears comparatively small. This small decrease, however, would become quite precipitous if the transcription rates were divided by the corresponding numbers of λ DNA copies in the vegetative pool. These results indicate that (1) only the *parental* W strands are transcribed and at a relatively steady rate, or that (2) strands W are transcribed in *all* λ DNA molecules at a rate which is controlled by the limiting amount of the "early" RNA polymerase. In the absence of DNA synthesis, the *parental* W strands are continuously transcribed, as shown for the *sus*N, O, P and *t*11 mutants (Table 1, lines 11–12, 26–31).

Late during induction, the transcription from gene c_I seems to be repressed, as represented by the three- to fourfold decrease in the synthesis of W-specific λdb_{a-N} mRNA (Table 1, lines 20, 23, and 24); only gene c_I should be transcribed from strand W of λdb_{a-N}, since genes *a*–N are deleted.

Several recent papers discuss the control of induction and development of phage λ.[6, 7, 9, 10, 12, 13, 17, 18, 20–22, 25]

Conclusions and Summary.—Hybridization of the various λ-specific mRNA's with the separate strands of λ DNA provides a powerful new technique for determining the distribution of the transcribing regions. Several technical refinements were introduced, including (1) self-annealing of the separated DNA strands, which results in preparations of individual strands displaying a purity of over 99 per cent in hybridization tests, and (2) a highly selective two-step hybridization procedure, with prehybridization including RNAse treatment followed by inactivation of RNase by iodoacetate. With the latter method it was possible to compare the levels of λ-specific mRNA in nonlysogenic *E. coli* (0.001–0.002%), in noninduced lysogens (0.03–0.06%), and early (0.5–2.0%) or late (6–12%) in induced nondefective lysogens or in infected cells, with the simplified ratio of these figures being represented as 1 (nonlysogenic):50 (noninduced):500 ("early" induced):5000 ("late" induced).

In the noninduced state the majority mRNA is transcribed from the W strand, most probably being the product of gene c_I. Upon induction or infection two

regions adjoining the c_I gene start to be transcribed: the predominant product (90%) is copied from strand *W* in the same direction as gene c_I, through genes N-to-*a*, whereas the minority mRNA (10%) is copied in the opposite direction, i.e., from left-to-right, from strand *C* through the *x*-O operon. Both of these transcription and also translation products seem to be required to activate the further transcription of the λ genome, since the early transcription pattern could be frozen either by inhibition of protein synthesis (100 μg CM/ml) or by the nonsense or polar mutations in genes N or *x*. Within 10–20 minutes after infection the transcription changes to the "late" pattern, with over 85 per cent of the mRNA now being transcribed from the *C* strand, progressively more and more from its left arm. Thus, during the development of λ, the transcription of strand *W* decreases by only 10–30 per cent, whereas the transcription of strand *C* increases 30- to 70-fold. Transcription in the induced *sus*O and P lysogens, unable to synthesize λ DNA, does not proceed far beyond the early stage, since upon removal of CM the transcription from strand *C* increases only threefold. The hybridization pattern obtained with the λdg_{A-J} mutant, which transcribes both DNA strands although its left arm is entirely deleted, confirms that a *divergency switch* in the direction of mRNA synthesis is on the right arm of λ DNA. It is interesting to note that the segments of the individual DNA strands presently characterized as transcribing regions were independently shown to contain all the dC-rich clusters, which have been postulated by Szybalski *et al.*[3] to act as initiation points for the DNA-to-RNA transcription process. Thus, at the present level of resolution the asymmetry in the distribution of the poly IG-binding dC clusters, which permits the preparative separation of the complementary DNA strands, seems to be directly related to the asymmetric transcription pattern of mRNA and the changes in the orientation of this transcription.

The following abbreviations are employed: poly G, homopolymer of guanylic acid; poly IG, copolymer of guanylic and inosinic acids; CM, chloramphenicol; DNase, pancreatic deoxyribonuclease I; RNase, pancreatic ribonuclease; SSC, 0.15 *M* NaCl + 0.02 *M* trisodium citrate, pH = 7.4; 6×SSC, 2×SSC, 1/100×SSC, 6 or 2 times more concentrated SSC, or 100 times diluted SSC, respectively; mRNA, messenger RNA which operationally is that fraction of pulse-labeled RNA which specifically hybridizes with λ DNA (=λ mRNA); *C* and *W* strands, complementary strands of λ DNA which, when unbroken, exhibit a higher or lower affinity for poly IG and thus band at higher or lower density in the CsCl gradient, respectively (Fig. 1); SDS, sodium dodecyl sulfate; G, guanine; C, cytosine; A, adenine; T, thymine; *sus*, suppressor-sensitive mutation; dg, defective galactose transducing; db, defective biotin transducing.

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