Nucleotide Distribution and Functional Orientation in the Deoxyribonucleic Acid of Phage $\phi 80$

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The distribution of nucleotides in the deoxyribonucleic acid (DNA) of $\phi 80$ was determined by density analysis of molecular fragments of known length and origin. One half of the molecule includes a long, fairly homogeneous segment that contains 55% guanine plus cytosine (GC). DNA in the other half contains about 50% GC, except for a short stretch near the molecular end where the GC content is higher. Transcription of $\phi 80$ DNA was studied by ribonucleic acid-DNA hybridization tests with isolated molecular halves. At times early in the growth cycle, messenger is synthesized at a constant, relatively low rate and originates almost exclusively from DNA in the lower-GC half. At later times, messenger represents both halves and is synthesized at a greatly increased rate. The DNA of $\phi 80$ is closely analogous, physically and functionally, to λ DNA. The similarity is most striking in the high-GC half, which is defined here as "left" since its molecular end contains a cohesive site homologous to that at λ 's left end. The left halves of the DNA of the two phages contain primarily late-functioning genes, and possess similar nucleotide distributions and some similar base sequences. The right halves of the two DNA molecules are less similar. Both contain the early genes, but they differ considerably in GC content (45% in λ , 51% in $\phi 80$) and do not strongly cross-react in hybridization tests. The DNA of $\phi 80$ lacks the central 37%-GC segment found in λ .

The deoxyribonucleic acid (DNA) molecule of the temperate coliphage λ contains three main sections: one of 57% guanine plus cytosine (GC) on the left, which includes all the late genes in the left third of the genetic map; one of 46% GC on the right, which includes mostly early genes; and a short section of 37% GC near the molecular center, which probably contains the b_2^+ determinant but no known genes (18). These facts suggested a direct relationship between nucleotide composition and gene function that might be expected to hold also for other phages, particularly those related to λ .

The bacteriophage $\phi 80$ is one of several temperate coliphages with cohesive sites that cross-react with those of λ (1, 21). In addition, $\phi 80$ DNA and λ DNA possess base-sequence similarities in other regions (3, 10), a fact consistent with the ability of the two phages to undergo genetic exchange (16). Phages $\phi 80$ and λ are, nevertheless, distinct species (12, 13), and their DNA molecules are distinguishable. The $\phi 80$ molecule is shorter (15) and denser (22) than that of λ .

This paper includes a report of the distribution of nucleotides in $\phi 80$ DNA, and a summary of some studies that bear on the relation between

structure and function in this molecule. The results do not support the idea that GC content influences gene function.

MATERIALS AND METHODS

Phage $\phi 80$ was obtained from Naomi Franklin. *Escherichia coli* W3110 was grown in 1% peptone (Difco) selected for low P content (0.9 mg/g), which contained 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.4), 0.01 M MgSO_4 , 0.085 M NaCl, and 0.1% glucose. The cells were harvested (at titer about 3×10^8), washed in 0.02 M MgSO_4 , and suspended in $\phi 80$ adsorption medium (13) at about 3×10^{10} /ml. From 7 to 15 infectious particles of $\phi 80$ were added per bacterium, and the mixture was warmed for 15 min at 37 C. The suspension was then diluted with warm peptone medium at what will be called the "time of infection" and was aerated vigorously. For labeling of the DNA, the mixture was diluted 100-fold and ^{32}P (final specific activity, 0.1 c/g of P) was added immediately, or ^3H -uracil (final concentration 1.5 $\mu\text{C}/\text{ml}$) was added in three portions at 30, 32, and 33 min after infection. The culture was allowed to lyse (about 2 hr) and DNA was isolated from the purified phage (2). For pulse-labeling of ribonucleic acid (RNA), the infected cells were diluted 10-fold with warm peptone medium after a 20-min adsorption period at 1 C (on ice). At specified times after dilution, ^{32}P was added (final specific

activity, 30 to 50 c/g of P); 2 min later, the culture was poured over crushed ice containing 0.1 M NaCl and 0.01 M phosphate buffer (pH 7). The RNA was extracted as has been described (17).

The rate of synthesis of $\phi 80$ messenger RNA (mRNA) was measured in terms of the fraction of RNA that can hybridize with $\phi 80$ DNA (7). The assumptions required by such measurements have been discussed (17, 19). Our use of the filter technique for preparative purposes has also been described (19).

Full details of our methods for shearing DNA and for density analysis of mercury complexes of DNA have been published (18).

RESULTS

Analysis of $\phi 80$ DNA. The distributions of mercury complexes of intact $\phi 80$ DNA molecules and λ half-molecules are compared in Fig. 1a. The position of the band of intact $\phi 80$ molecules indicates 52.8% GC. Yamagishi, Yoshizako, and Sato (22) reported 53% GC.

Half-molecules of $\phi 80$ form two bands (Fig. 1b); one is sharp and corresponds in density to 51% GC, the other, in most experiments, is somewhat broader and about 54% GC.

Fragments of $\phi 80$ DNA that originated from purified half-molecules were tested for specificity of joining with left and right cohesive sites of λ . Figure 2 shows the results when fragments originating from the 54%-GC halves were annealed with right and left λ halves and then sedimented in sucrose gradients. When annealed with the λ right halves, about 25% of the $\phi 80$ fragments (and some of the λ halves) sedimented in a separate band at the rate predicted for a mixed dimer (Fig. 2a). Since the average size of the fragments was 0.25 fractional length, only half of them contained cohesive sites. The efficiency of joining was therefore about 50%. No mixed dimers were detected if these fragments were annealed with λ left halves (Fig. 2b), or (Fig. 2c) if a sample of the mixture annealed with λ right halves was reheated and quickly cooled before centrifugation.

Analogous tests (*not shown*) with fragments originating from the 51%-GC halves gave the complementary results; end-to-end mixed dimers were formed only with λ left halves, with about 50% efficiency. Thus, λ and $\phi 80$ have like cohesive sites attached to their high-GC ends. The 54%-GC half of $\phi 80$ is hereafter called left, and the 51%-GC half, right, in analogy with the designations for λ .

Analysis of smaller fragments of $\phi 80$ DNA gave additional information. DNA originating from whole molecules that had been broken into 0.05 fractional-length subsections yielded two poorly resolved bands, as shown by the distribution of the "marker" in Fig. 3. The denser band, which was relatively broad, contained 0.46 of the DNA

FIG. 1. Density distribution of mercury complexes. (a) Unbroken $\phi 80$ DNA, solid line; and λ half-molecules, broken line. (b) Half-molecules of $\phi 80$, solid line; and λ half-molecules, broken line. The denser band of λ DNA contains right half-molecules; the less dense (and broader) band contains lefts. In each gradient, 2 μ g of ^3H -labeled $\phi 80$ DNA (2,500 counts per min per μ g) and 1 μ g of ^{32}P -labeled λ DNA (2,900 counts per min per μ g) were banded with unlabeled T2 DNA carrier in 41.7% Cs_2SO_4 with HgCl_2 at R_f (mole/mole nucleotide) 0.35. The $\phi 80$ DNA was broken by stirring for 30 min at 42.4 $\mu\text{g}/\text{ml}$ and 4,420 rev/min.

and centered at the position of 50% GC DNA. The other band, which contained 0.54 of the DNA, centered at the 55%-GC position. These compositions were verified in other experiments by nucleotide analyses of ^{32}P -labeled DNA isolated from the center of each band.

Terminal segments of fractional lengths 0.1 and 0.3 were analyzed in a similar way. They were isolated by annealing DNA from appropriately

FIG. 2. Specificity of joining of λ and $\phi 80$ cohesive sites. Mixtures containing about 10 μg of DNA from the 54%-GC $\phi 80$ half (10^8 ^3H counts per min per μg) and about 10 μg of either of the purified λ halves (5×10^8 ^{32}P counts per min per μg) in 0.5 ml of 0.6 M NaCl and 0.05 M phosphate buffer, pH 7, were annealed as described by Hershey and Burgi (8). Samples of 0.05 ml were analyzed on 5 to 20% sucrose gradients which contained 0.6 M NaCl and 0.01 M Tris buffer, pH 7.4. In (a) the mixture contained λ right halves, in (b) it contained left halves. The broken lines indicate the reference marker positions of λ left (a) and right (b) halves. In (c), a sample of the same mixture used in (a) was heated (to disjoin dimers) before analysis (8).

sheared ^{32}P -labeled $\phi 80$ whole molecules with ^3H -labeled right or left halves of λ and selecting the joined fraction. The selected segments were then broken to average length 0.05 and banded in $\text{Hg}-\text{Cs}_2\text{SO}_4$.

Results of the analysis of left molecular ends are given in Fig. 3a. They show that small fragments from left ends of fractional length 0.3 contain 55% GC, except for about 15% of them that tail into the 50%-GC region. Small fragments from left ends of fractional length 0.1 form a wide symmetrical band, suggesting equal amounts of 50%-GC and 55%-GC DNA. Therefore, the left third of the molecule contains 55% GC, except for a stretch of fractional length 0.05, at or near the molecular end, that contains about 50% GC.

Results of the analysis of right ends are given in Fig. 3b. They show that small fragments from right ends of length 0.3 are diverse in composition, averaging about 52% in GC content. Right ends of length 0.1 yield mainly 50%-GC fragments, with some tailing into the 55%-GC region. Fragments from the rest of the end third (as seen by the difference in patterns of terminal fragments of the two lengths) distribute about equally between the 50%-GC and the 55%-GC positions. Thus, right molecular thirds contain 50% GC, except for a subterminal (possibly uninterrupted) 10% that contains about 55% GC.

The compositions of terminal segments were determined independently by nucleotide analyses (11, 20). The results were compatible with those from density analyses. The left-terminal 0.1 and 0.3 segments contain 53.4% and 54.4% GC, respectively; the right 0.1 and 0.3 segments are 50.3% and 51.9%, respectively.

Base-sequence similarities between $\phi 80$ DNA and λ DNA. According to the results just summarized, left molecular halves of $\phi 80$ DNA closely resemble left halves of λ DNA; both contain long stretches of similarly high GC content. The $\phi 80$ and λ right halves, however, differ both in average base composition and in the degree of heterogeneity among 0.05 fractional-length segments. These facts suggest that the partial base-sequence similarities known to exist in the two DNA molecules (10) should be found in their left halves.

The results of hybridization tests with $\phi 80$ and λ halves are shown in Table 1. Left halves interact strongly; right halves interact weakly. Hybridization of left halves of $\phi 80$ is specific for DNA from the analogous region of λ . Such specificity is not seen in the weak reaction of $\phi 80$ right halves. There is no reaction of either half of $\phi 80$ with DNA from the central, high-AT region of λ .

Thus, $\phi 80$ and λ left halves are similar in com-

FIG. 3. Density distribution of fragments of length 0.05 originating from terminal segments of $\phi 80$ DNA. (a) Fragments from left terminal segments; (b) fragments from right terminal segments. Results from two analyses (one for terminal tenths and one for terminal thirds) were combined by superimposing the marker patterns. The marker DNA, 0.05 fractional length subsections originating from $\phi 80$ whole molecules, expressed as ^3H counts per min per fraction, is represented by a broken line. Fragmented terminal thirds, expressed as percentage DNA per fraction, are shown by solid lines. The shaded areas show the contribution of terminal tenths to the distribution of fragmented terminal thirds.

TABLE 1. Hybridization tests of $\phi 80$ halves with λ DNA fractions^a

Test DNA	Hybridization (%) with						
	λ Centers (3 μg)	λ Lefts (3 μg)	λ Rights (3 μg)	Unfraction- ated λ (50 μg)	Unfraction- ated $\phi 80$ (6 μg)	T2 DNA (6 μg)	No DNA
$\phi 80$ right half-molecules	0.5	1.0	1.4	4.6	81.0	0.5	0.4
$\phi 80$ left half-molecules	<0.6	12.0	<0.6	18.0	71.0	0.7	0.6

^a Each mixture contained 0.5 to 0.6 μg (about 10^8 ^3H count/min) of sonically degraded test DNA. ^{32}P -labeled λ DNA fractions were isolated from the centers of three bands formed by mercury complexes of molecular tenths (3.5×10^8 daltons) in Cs_2SO_4 density gradients. "Centers" come from the short 37% GC region near the center of the molecule; "lefts" and "rights" from either side of this region (9, 10). The fractions were affixed to membrane filters according to Denhardt (4).

position and nucleotide distribution and possess some similar base sequences.

Temporal control of transcription in $\phi 80$. The rates of synthesis of $\phi 80$ -specific mRNA measured at various times during phage growth are shown in Fig. 4. The data show that synthesis starts immediately and is constant in rate for about 15 min; then the rate increases. The rate at 40 min, the latest time measured, is more than six times that at 15 min.

The results summarized in Table 2 show that the $\phi 80$ messenger synthesized at early times comes almost exclusively from the right half of the DNA. At later times, both halves are transcribed. The data are presented graphically in Fig. 4, which shows that synthesis of left mRNA, barely measurable during the early phase, dominates the late phase.

The base compositions of $\phi 80$ messenger synthesized at various times after infection are shown in Table 3. The data reveal no striking trends in

composition, although the earliest messenger is lower in GC content than later messenger. Even at early times, however, the GC content is relatively high. This may reflect some preferential transcription of the high-GC stretches in the right half. At all times the messenger is rich in purines (particularly guanine), a fact showing that, as with λ (17), pyrimidine-rich sequences (particularly those containing cytosine) are preferentially transcribed.

DISCUSSION

Figure 5a shows the simplest structural model of the $\phi 80$ chromosome consistent with all the known facts. In places where exact details are not known, components have been arranged to make the map resemble that of λ (18). The model shows a very short region (0.04 fractional length) of 50% GC at the left terminus, followed by a rather long region (0.41) of 55% GC. The rest of

FIG. 4. Rate of labeling of $\phi 80$ mRNA. ($\blacktriangle$) Total messenger labeled in a 2-min period. Broken line, long dashes, the proportion originating in the right half; broken line, short dashes, the proportion originating in the left half. Proportions were calculated from relative percentages of Table 2. About $20 \mu\text{g}$ of RNA (1.5×10^5 to 1.2×10^6 counts/min) and $50 \mu\text{g}$ of unfractionated $\phi 80$ DNA were used in each test for total messenger. The percentage of pulse-labeled RNA from uninfected cells that hybridized with the $\phi 80$ DNA is shown as the 0 time point. Blanks (percentages bound to membranes containing no DNA) varied from 0.04 to 0.3%. Other experiments in which points intermediate between 2 and 15 min were assayed verified that there was no significant change in rate during this period.

the chromosome is 50% GC, except for one short stretch (0.11), near the right end, that is 55% GC.

The lengths of various segments were calculated from compositions summarized in Fig. 5b. It was assumed for simplicity that the DNA is uniformly either 50 or 55% GC. Whereas this assumption is probably valid for the high-GC segment in the left half (0.05 subsections form a fairly sharp 55%-GC band), other regions may be less homogeneous, and neither the lengths nor the compositions of segments are accurately known. Certain details were suggested by the following facts. (i) A small percentage of the 0.05 fragments from the right terminal 0.1 segment were less dense than 50%-GC DNA. It seemed reasonable, therefore, to place one boundary of the right high-GC segment close to the terminal 0.1 segment. (ii) The band of $\phi 80$ left halves is relatively broad. This is also observed, in a more exaggerated version, with λ left halves. It was assumed that in $\phi 80$, as in λ (18), this pattern indicates a boundary between segments of unlike composition lying to the left of the molecular center. For this reason the left high-GC section is shown uninterrupted, as it is known to be in λ .

TABLE 2. Hybridization of $\phi 80$ -specific RNA with halves of $\phi 80^a$

Time of synthesis (min)	Actual percentage ^b		Relative percentage	
	Rights	Lefts	Rights	Lefts
0-2	50.5	8.9	85	15
5-7	29.1	7.5	80	20
10-12	46.7	11.1	81	19
15-17	49.3	17.4	74	26
22-24	32.8	42.3	44	56
30-32	24.5	39.6	37	63
34-36	22.0	39.9	35	65
40-42	33.5	47.9	40	60

^a Pulse-labeled RNA was from the same extracts used in the experiments described in Fig. 4. The $\phi 80$ mRNA was purified by annealing with membranes. Hybridized RNA was eluted and tested in a second annealing with membranes containing $3 \mu\text{g}$ each of the purified $\phi 80$ halves. About 10,000 to 20,000 counts/min of the purified RNA was used in each test.

^b Expressed as the percentage of purified labeled RNA that hybridized with the DNA. Differences in the total $\phi 80$ -specific fraction of individual RNA preparations are due to differences in the success with which nonspecific RNA was removed during purification.

TABLE 3. Base composition of $\phi 80$ -specific RNA (mole %)^a

Time of synthesis (min)	C	A	G	U	GC	Purines
0-2	23.32	26.98	28.98	20.72	52.30	55.96
15-17	29.33	25.40	27.48	17.80	56.81	52.48
22-24	24.95	24.94	29.09	21.02	54.04	54.03
30-32	24.87	24.90	28.70	21.51	53.57	53.60
40-42	24.07	25.26	30.89	19.76	54.96	56.15

^a After counting, the membranes used in the experiment described in Fig. 4 were removed from scintillation fluid, washed with toluene, and dried. The hybridized RNA was eluted from the filters and the base compositions were determined as has been described (18).

The model of Fig. 5a was constructed from data for halves and shorter terminal fragments. It agrees with two additional data: whole molecules contain 52.8% GC (52.6% according to the model); and they yield small fragments of which 0.54 (0.52 according to the model) contain 55% GC. Other features could be checked by analysis of additional fragments.

In most respects $\phi 80$ DNA resembles that of λ . Their left halves are clearly similar. Both $\phi 80$ and λ right halves are more diversified than the lefts. The main difference is that the range in GC content, for all molecular fragments studied, is nar-

FIG. 5. Distribution of nucleotides in $\phi 80$ DNA. (a) A structural model of the $\phi 80$ chromosome. (b) Compositions of segments of $\phi 80$ DNA. Terminal tenths and terminal thirds were analyzed directly. Figures in parentheses were calculated from these results and from the compositions of halves estimated from the data of Fig. 1. The values above the lines indicate fractional lengths; figures below the lines indicate GC content (mole %).

rower in $\phi 80$ than in λ . Another striking difference deserves special mention. The DNA of $\phi 80$ does not contain the AT-rich central segment which probably includes the b_2^+ determinant in λ . Since $\phi 80$ must possess a similar determinant, a large segment of high AT content does not seem to be essential.

Results of independent studies by Cowie and Falkow (3) verify some of the present findings. These authors observed that the temperature required to elute $\phi 80$ DNA from a λ DNA-agar column was considerably higher than that required for the elution of $\phi 80$ DNA from a $\phi 80$ column. They suggested, therefore, that the reacting segments in the two molecules are contained in regions where the GC content is higher than the average in either DNA. The present studies show that the reacting segments can be assigned almost entirely to the GC-rich left molecular halves. Falkow and Cowie (5) also found that $\phi 80$ DNA is denatured in two thermal phases, as if 45% of the molecule contained 46% GC and the remainder 57% GC. These estimates of the base compositions of the two regions differ from those presented here, but, as Falkow and Cowie have stated, their methods involve several assumptions that ours do not.

Similar chromosome structure in λ and $\phi 80$ was suggested by the isolation of λ - $\phi 80$ hybrids presumed to have exchanged left and right arms (6). Sato and co-workers (14) have recently shown that the gene arrangement in $\phi 80$ is similar to that in λ ; the cluster of head genes is found at one end of the vegetative map, followed by tail genes. Presumed early genes are located at the other end of the map. I find that $\phi 80$ transcription closely resembles that of λ . The right half is transcribed first, then both halves. In addition,

with $\phi 80$ as with λ , early messenger is synthesized at a slow and constant rate, late messenger at a faster, accelerating rate. These similarities reflect similar functional organization and also suggest similar control mechanisms.

Although the modes of transcription are similar for the two phages, $\phi 80$, unlike λ , shows only small differences in base composition between the two molecular halves and even smaller differences in composition between early and late messenger. The GC content of $\phi 80$ messenger is always high, and there seems to be no necessity for early genes to be coded in AT-rich sequences. For these reasons, it now seems unlikely that nucleotide composition itself influences the expression of early genes (18).

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LITERATURE CITED

1. Baldwin, R. L., P. Barrard, A. Fritsch, D. Goldthwait, and F. Jacob. 1966. Cohesive sites on the deoxyribonucleic acids from several temperate coliphages. *J. Mol. Biol.* 17:343-357.
2. Burgi, E. 1963. Changes in molecular weight of DNA accompanying mutations in phage. *Proc. Natl. Acad. Sci. U.S.A.* 49:151-155.
3. Cowie, D. B., and S. Falkow. 1967. Nucleotide sequence relationships among phages $\phi 80$ and λ , and *E. coli*. *Carnegie Inst. Wash. Year Book* 66:95-106.
4. Denhardt, D. T. 1966. A membrane-filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.* 23:641-646.
5. Falkow, S., and D. B. Cowie. 1968. Intramolecular heterogeneity of the deoxyribonucleic acid of temperate bacteriophages. *J. Bacteriol.* 96:777-784.
6. Franklin, N. C., W. F. Dove, and C. Yanofsky. 1965. The linear insertion of a prophage into the chromosome of *E. coli* shown by deletion mapping. *Biochem. Biophys. Res. Commun.* 18:910-923.
7. Gillespie, D., and S. Spiegelman. 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. Mol. Biol.* 12:829-842.
8. Hershey, A. D., and E. Burgi. 1965. Complementary structure of interacting sites at the ends of lambda DNA molecules. *Proc. Natl. Acad. Sci. U.S.A.* 53:325-328.
9. Hershey, A. D., and E. Burgi. 1966. Internal structure of lambda DNA. In A. D. Hershey (ed.), *Structure and function of phage DNAs*. Carnegie Inst. Wash. Year Book 65:559-562.
10. Ingraham, L., R. Ehring, and A. D. Hershey. 1966. Base-sequence homologies. In A. D. Hershey (ed.), *Structure and function of phage DNAs*. Carnegie Inst. Wash. Year Book 65:562-564.
11. Markham, R., and J. D. Smith. 1952. The structure of ribonucleic acids. I. Cyclic nucleotides produced by ribonuclease and by alkaline hydrolysis. *Biochem. J.* 52:552-557.
12. Matsushiro, A. 1963. Specialized transduction of tryptophan markers in *Escherichia coli* K12 by bacteriophage $\phi 80$. *Virology* 19:475-482.

13. Matsushiro, A., K. Sato, and S. Kida. 1964. Characteristics of the transducing elements of bacteriophage $\phi 80$. *Virology* **23**:299-306.
14. Sato, K., Y. Nishimune, M. Sato, R. Numich, A. Matsushiro, H. Inokuchi, and H. Ozeki. 1968. Suppressor-sensitive mutants of coliphage $\phi 80$. *Virology* **34**:637-649.
15. Shinagawa, H., Y. Hosaka, H. Yamagishi, and Y. Nishi. 1966. Electron microscopic studies on $\phi 80$ and $\phi 80$ pt₁ phage virions and their DNA. *Biken's J.* **9**:135-148.
16. Signer, E. R. 1964. Recombination between coliphages λ and $\phi 80$. *Virology* **22**:650-651.
17. Skalka, A. 1966. Regional and temporal control of genetic transcription in phage lambda. *Proc. Natl. Acad. Sci. U.S.* **55**:1190-1195.
18. Skalka, A., E. Burgi, and A. D. Hershey. 1968. Segmental distribution of nucleotides in the DNA of bacteriophage lambda. *J. Mol. Biol.* **34**:1-16.
19. Skalka, A., B. Butler, and H. Echols. 1967. Genetic control of transcription during development of phage λ . *Proc. Natl. Acad. Sci. U.S.* **58**:576-583.
20. Wu, R., and A. D. Kaiser. 1967. Mapping the 5'-terminal nucleotides of the DNA of bacteriophage λ and related phages. *Proc. Natl. Acad. Sci. U.S.* **57**:170-177.
21. Yamagishi, H., K. Nakamura, and H. Ozeki. 1965. Cohesion occurring between DNA molecules of temperate phages $\phi 80$ and λ or $\phi 81$. *Biochem. Biophys. Res. Commun.* **20**:727-732.
22. Yamagishi, H., F. Yoshizako, and K. Sato. 1966. Characteristics of DNA molecules extracted from bacteriophages $\phi 80$ and $\phi 80$ pt₁. *Virology* **30**:29-35.