within the gene could conceivably determine the degree of interaction with other molecules in a manner which influences the activity of specific regions of the chromosome.

References

Aktipis, S., and Kindelis, A. (1973a), *Biochemistry 12*, 1213.

Aktipis, S., and Kindelis, A. (1973b), *Fed. Proc., Fed Amer. Soc. Exp. Biol. 32*, 2047.

Aktipis, S., and Martz, W. W. (1970), *Biochem. Biophys. Res. Commun. 39*, 307.

Blake, A., and Peacocke, A. R. (1967), *Biopolymers 5*, 383.

Dalgleish, D. G., Fujita, H., and Peacocke, A. R. (1969), *Biopolymers 8*, 633.

Dalgleish, D. G., Peacocke, A. R., Fey, G., and Harvey, C. (1971), *Biopolymers 10*, 1853.

Elliott, W. H. (1963), *Biochem. J. 86*, 562.

Fasman, G. D., Lindblow, E., and Grossman, L. (1964), *Biochemistry 3*, 1016.

Fuller, W., and Waring, M. J. (1964), *Ber. Bunsenges. Phys. Chem. 68*, 805.

Gersch, N. F., and Jordan, D. O. (1965), *J. Mol. Biol. 13*, 138.

Giacomoni, P. U., and LeBret, M. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 29*, 227.

Holcomb, D. N., and Tinoco, Jr., I. (1965), *Biopolymers 3*, 121.

Hoogsteen, K. (1959), *Acta Crystallogr. 12*, 822.

Lee, H. C., Chang, C. T., and Wetmur, J. G. (1973), *Biopolymers 12*, 1099.

LePecq, J. B., and Paoletti, C. (1967), *J. Mol. Biol. 27*, 87.

LePecq, J. B., and Paoletti, C. (1965), *C. R. Acad. Sci. Paris 260*, 7033.

Löber, G., and Achtert, G. (1969), *Biopolymers 8*, 595.

Mahler, H. R., Kline, B., and Mehrotra, B. D. (1964), *J. Mol. Biol. 9*, 801.

Michelson, A. M., and Monny, C. (1966), *Proc. Nat. Acad. Sci. U. S. 56*, 1528.

Peacocke, A. R., and Skerett, J. N. H. (1956), *Trans. Faraday Soc. 52*, 261.

Pritchard, N. J., Blake, A., and Peacocke, A. R. (1966), *Nature (London) 212*, 1360.

Rich, A. (1958), *Nature (London) 181*, 521.

Steiner, R. F., and Beers, R. F. (1961), Polynucleotides, Amsterdam, Elsevier, p 186.

Tomchick, R., and Mandel, H. G. (1964), *J. Gen. Microbiol. 36*, 225.

Waring, M. J. (1964), *Biochim. Biophys. Acta 87*, 358.

Waring, M. J. (1965a), *Mol. Pharmacol. 1*, 1.

Waring, M. J. (1965b), *J. Mol. Biol. 13*, 269.

Waring, M. J. (1966), *Biochim. Biophys. Acta 114*, 234.

Warner, R. C. (1957), *J. Biol. Chem. 229*, 711.

Wells, R. D. (1971), Progress in Molecular and Subcellular Biology, New York, N. Y., Springer-Verlag, p 21.

Distribution of Pyrimidine Oligonucleotides in Complementary Strand Fractions of *Escherichia coli* Deoxyribonucleic Acid†

Rivka Rudner* and Mary LeDoux

ABSTRACT: The distribution of pyrimidine oligonucleotide clusters (isostichs) in complementary fractions L and H of *Escherichia coli* DNA separated by MAK chromatography has been determined. Preparations of native and single-stranded DNA were degraded with diphenylamine in formic acid, and the released isostichs under the general formula of Py_nP_{n+1} were separated on DEAE-cellulose according to chain length. Eleven isostichs were recovered from strand fractions L and H in unequal proportions. Each isostich fraction was subfractionated according to base composition on DEAE-cellulose at pH 3.0. Fifty-nine different nonisomeric pyrimidine oligonucleotides were separated from both strand fractions. The findings show an asymmetric distribution of pyrimidine clusters between the L and H strand fractions, with a predominance of oligonucleotides of length 6–11 in the H fraction. The distribution bias between the fractions involves both the cytosine-rich and thymine-rich oligonucleotides to the same extent. Unlike *Bacillus subtilis* DNA where the asymmetry between the strands is extensive and follows certain regularities, in *E. coli* the bias is more limited and less regular. The findings can be correlated with the extent of asymmetric transcription in these two bacterial species.

Alkali-denatured DNA preparations from *Bacillus subtilis* (Rudner *et al.*, 1968a) and from several microbial species (Rudner *et al.*, 1969) can be separated by a technique of *intermittent gradient* elution from an MAK column into two distinct components designated by their buoyant densities, as light (L) and heavy (H). On the basis of several criteria such as transforming activity, temperature–absorbance behavior, nucleotide composition, and hybridization to RNA, it was previously concluded that the two MAK fractions isolated from denatured *B. subtilis* DNA represent two families of strand fragments, each derived from one of the original chains (Rudner *et al.*, 1968a,b, 1969; Karkas *et al.*, 1968, 1970;

† From the Department of Biological Sciences, Hunter College of the City University of New York, New York, New York 10021. *Received August 6, 1973.* Supported by Research Grant GM 16059 from the National Institute of General Medical Sciences and City University of New York Faculty Research Award 1593.

Margulies *et al.*, 1970, 1971; Rudner and Remeza, 1973a,b). Similar MAK fractions derived from denatured *Escherichia coli* DNA were only characterized with respect to their nucleotide composition and hybridization to RNA components (Rudner *et al.*, 1969; Karkas *et al.*, 1970; Margulies *et al.*, 1970, 1971). The base composition analyses have shown that the MAK fractions isolated from DNA of seven bacterial species exhibit varying degrees of *chemical asymmetry*, namely, that the L-strand fraction is purine rich and the H-strand fraction is pyrimidine rich. Physical characterization of the MAK fractions from *B. subtilis* DNA have shown that they also differ in molecular size, configuration, and secondary structure (Rudner and Remeza, 1973a,b). On the basis of these findings we proposed that during isolation and denaturation of DNA from *B. subtilis*, the purine-rich L strands are more susceptible to breaks than the pyrimidine-rich H strands, yielding a size bias in the population of strand fragments which contributes to strand resolution by MAK chromatography.

The chemical bias between the complementary strands of *B. subtilis* DNA was further investigated by nucleotide cluster analyses on depurinated MAK fractions (Rudner *et al.*, 1972). We reported an asymmetric distribution of pyrimidine clusters (*isostichs*) between the complementary fractions, with a predominance of long clusters in the H fraction. Fifteen pyrimidine oligonucleotides of isostichs 10 and 11 were found only in the H fraction and not in the L fraction. The bias in the distribution of purines and pyrimidines between the complementary strands was correlated with the extent of asymmetric transcription *in vivo* in this bacteria. We have reported that in *B. subtilis*, 85–90% of mRNA and all the stable RNA components (ribosomal RNA (rRNA) and tRNA) are transcribed from the pyrimidine-rich H strand (Margulies *et al.*, 1970, 1971). In *E. coli*, on the other hand, asymmetric transcription is more limited, namely, 30–35% of mRNA and 30% of tRNA species are transcribed from the L strand (Margulies *et al.*, 1970, 1971). We were naturally interested in ascertaining the generality of this correlation in bacterial systems. *E. coli* was therefore selected for the comparison with *B. subtilis* since its complementary MAK fractions exhibit a limited chemical asymmetry (Rudner *et al.*, 1969; Karkas *et al.*, 1970).

In this paper, we report the distribution of pyrimidine oligonucleotide clusters in fractions L and H of denatured *E. coli* DNA obtained by MAK chromatography. These fractions were also characterized with respect to their self-annealing ability by temperature–absorbance measurements and hydroxylapatite chromatography. The method of chemical depurination by a solution of diphenylamine in formic acid (Burton and Petersen, 1960), which is specific and quantitative for the release of pyrimidine oligonucleotides from DNA, was used, coupled with improved analytical procedures described by Petersen and Reeves (1966) and Cerny *et al.* (1968). The findings show that although there is an asymmetric distribution of pyrimidine clusters between the L- and H-strand fractions of *E. coli* DNA, the distribution bias is more limited and less regular as compared to the *B. subtilis* strand fractions. The same oligonucleotides found exclusively in the H fraction of *B. subtilis* DNA are distributed in both strand fractions of *E. coli* DNA. There is no regularity in the preferential distribution of cytosine-rich and thymine-rich clusters in the H-strand fraction as was observed in *B. subtilis* (Rudner *et al.*, 1972). The overall H/L distribution ratio of the long pyrimidine oligonucleotides can be correlated with the extent of asymmetric transcription in *E. coli*.

Materials and Methods

Preparation of ^{32}P-Labeled DNA. DNA was prepared from *E. coli* B_{23} grown in a low-phosphate medium (Grossman, 1967). As soon as the cells entered logarithmic growth, 10 μCi of [^{32}P]orthophosphate/ml (New England Nuclear) was added, and the culture was incubated at 37° until bacterial growth reached the stationary phase. DNA was prepared according to Thomas *et al.* (1966). The specific activity of the purified DNA preparations ranged from 1.5 to 2.7 $\times$ 10^5 dpm/μg. Radioactive phosphorylated contaminants were removed by a further purification on a hydroxylapatite column in the case of native DNA and on a MAK column in the case of denatured DNA.

Strand Separation. Fractionation of the L and H strands, after alkali denaturation, by chromatography on MAK columns has been described in detail (Rudner *et al.*, 1968a, 1969). About 0.5–1 mg of alkali-denatured [^{32}P]DNA was applied to a MAK column (1.9 $\times$ 4.0 cm) containing one-quarter of the standard quantities of adsorbent (Mandell and Hershey, 1960), and eluted by means of an *intermittent salt* gradient (0.6–1.5 M NaCl in 50 mM sodium phosphate (pH 6.7), total volume, 340 ml). The absorbance of the eluates, collected in portions of 5 ml, was monitored at 254 nm with an ISCO ultraviolet analyzer. As soon as the first peak (L) began to emerge, the gradient was interrupted until the monitor indicated the beginning of a decline just after maximal absorbance was attained by this fraction. At this point, the gradient was reestablished and maintained until the end of the fractionation. The extent of secondary structure produced after a self-annealing treatment of these fractions was established by hydroxylapatite chromatography (Figure 3) and by temperature–absorbance measurements (Figure 2) as described previously (Rudner and Remeza, 1973a). Native DNA and MAK fractions L and H were dialyzed exhaustively against distilled water prior to a pyrimidine clusters analysis.

DNA Degradation and Oligonucleotide Separation, Identification, and Quantitation. Dialyzed ^{32}P-labeled native DNA and MAK fractions L and H (0.4–0.5 mg) were hydrolyzed in formic acid–diphenylamine (Burton and Petersen, 1960) according to the modified procedure of Spencer *et al.* (1969). Equal amounts (75 mg) of the hydrolysate of calf-thymus DNA were added as a carrier to each of the hydrolyzed radioactive sample. Diphenylamine and formic acid were removed as described by Spencer *et al.* (1969) with the addition of three ether extractions after the pH of the hydrolysates reached 3.7. The hydrolysates were fractionated according to chain length (n) on DEAE-cellulose at pH 5.5 in 7 M urea (Figure 4) following the techniques of Petersen and Reeves (1966) and Cerny *et al.* (1968). Each isostich fraction with the general formula of Py_np_{n+1} was subfractionated on DEAE-cellulose at pH 3.0 according to Cerny *et al.* (1968). Individual oligonucleotides separated according to base composition (Figure 5) were identified from the position of the absorbance peaks along the DEAE elution profile of the carrier DNA and from their ultraviolet (uv) spectral ratios in acid (Spencer and Chargaff, 1963). For quantitation of the individual radioactive oligonucleotides, the fractions corresponding to each component were pooled, the volume was measured, and 1.0-ml samples were mixed with scintillation mixture (Bray, 1960) and counted in a liquid scintillation spectrometer (Tri-Carb, Packard Instrument). Pooled fractions with levels of radioactivity below 50 cpm were not included in the analyses. The counts per minute were converted to disintegrations per minute, and the results were expressed as moles of pyrimidine

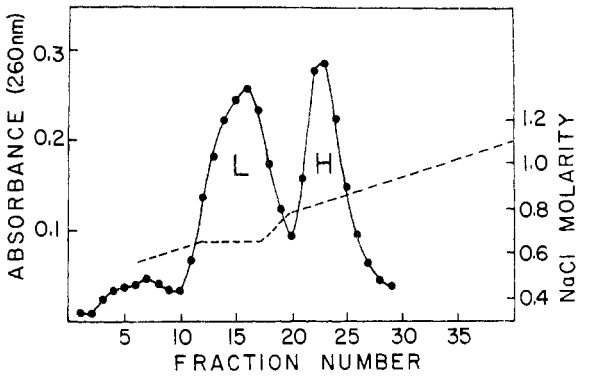

FIGURE 1: Separation of complementary fractions L and H of *E. coli* DNA by chromatography on an MAK column. Alkali-denatured ^{32}P-labeled DNA (0.75 mg) was applied to a MAK column (1.9 × 4.0 cm) and eluted with an *intermittent salt* gradient between 0.6 and 1.5 M NaCl in 50 mM sodium phosphate (pH 6.7) (total volume, 340 ml). The gradient was interrupted at tube 12 and reestablished at tube 17. Recovery (as per cent of input DNA) was: total, 93; strand fraction L, 41; and strand fraction H, 44. Fractions 12–16 were pooled for the L and fractions 22–26 were pooled for the H. Two such fractionations were combined for each pyrimidine cluster analysis, with a total radioactivity in fractions L and H for 6.5 × 10^7 and 5.8 × 10^7 dpm, respectively.

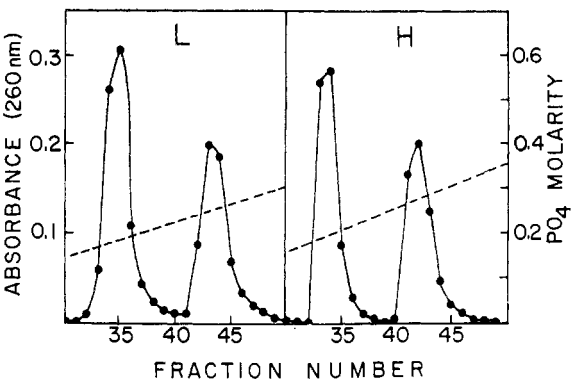

FIGURE 3: Hydroxylapatite chromatography of self-annealed L and H MAK fractions of *E. coli* DNA. Pooled L and H fractions (see Figure 1) were self-annealed (68°, 4 hr) at the salt concentration of the MAK eluates and dialyzed against 50 mM sodium phosphate buffer (pH 6.7). To hydroxylapatite columns (1.3 × 5.0 cm) equilibrated with the same buffer, 345 μg of fraction L and 320 μg of fraction H were loaded. Elution was carried out with a linear molarity gradient of sodium phosphate buffer (pH 6.7) (0.05–0.5 M; total volume, 300 ml). Recoveries (as per cent of input DNA) in the L column: total, 94; fraction L_I, 55; fraction L_{II}, 39; in the H column: total, 98; fraction H_I, 47; fraction H_{II}, 41.

per 100 g-atoms of DNA phosphorus with respect to the initial amount of DNA used.

Results

Fractionation of Complementary Fractions by MAK Chromatography. Figure 1 shows a typical MAK chromatogram of alkali-denatured *E. coli* DNA obtained through the use of the *intermittent gradient* elution technique. The L and H MAK fractions were further characterized with respect to their self-annealing ability by temperature–absorbance measurements and hydroxylapatite chromatography. As shown in Figure 2A, although the renaturation product of the mixture L plus H produced a recovery of 71% of the secondary structure of native DNA, the individual fractions contained a considerable amount of self-annealing material. Estimations of recovered helical structures based on the hyperchromic rise between 80 and 95° amounted to 37 and 43% (of native DNA) for self-annealed L and H, respectively. Similarly, analysis on hydroxylapatite columns showed that self-annealing of L and H produced 39 and 41% "native-like" DNA, respectively (see peak II in Figure 3). The melting profiles after self-annealing and cross-annealing of the hydroxylapatite fractions (I and II) are presented in Figures 2B and 2C. These profiles show that although the hydroxylapatite purified strand fractions (L_I and H_I) no longer contain "residual hydrochromicities," the restoration of secondary structure in the cross-annealed mixture (L_I plus H_I) amounted to only 37% of native DNA (Figure 2B). Cross-annealing denatured peak II from hydroxylapatite (L_{II} plus H_{II}) did not yield additional secondary structures (Figure 3C). Kinetic studies of the self-annealing reaction which affects only the H strand in *B. subtilis* have shown that the restoration of secondary structure is rapid and independent of DNA concentration as compared to the kinetics of duplex formation in the cross-annealing reaction (Rudner and Remeza, 1973a). Removal of the self-annealing species by hydroxylapatite chromatography decreases the hybridization efficiencies to the opposite strand fraction (Figure 2B) and to RNA components (rRNA and mRNA; Galloway and Rudner, 1973). On the basis of similar investigations to be reported at a later date, we concluded that the observed self-annealing ability of the *E. coli* MAK fractions is not due solely to cross-contamination but rather to the formation of intrastrand secondary structures. Since these structures may have functional significance and since the self-annealed portion represents 40% of the original denatured DNA, we subjected the original MAK

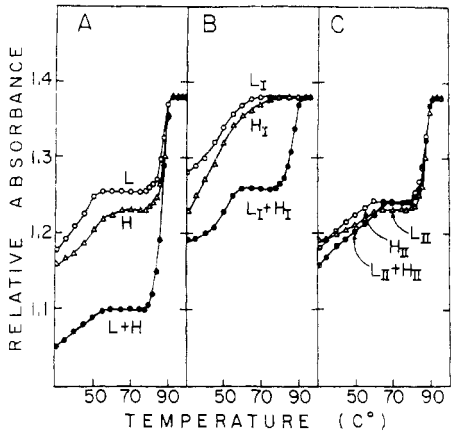

FIGURE 2: Melting profiles of self-annealed and cross-annealed *E. coli* L- and H-strand fractions before and after hydroxylapatite chromatography. (A) Melting curves of the initial MAK fractions, L, H, and L + H after annealing. The recovery of secondary structure calculated from the hyperchromic shift at 80–95° corresponded to (as per cent of native DNA) 37, 43, and 71 for L, H, and L + H, respectively. (B) Melting curves of fraction I after hydroxylapatite chromatography of the self-annealed L and H MAK fractions. The recovery of secondary structure in L_I, H_I, and $L_I + H_I$ corresponded to (as per cent of native DNA) 0, 0, and 37, respectively. (C) Melting curves of fraction II after hydroxylapatite chromatography. The recovery of secondary structure in L_{II}, H_{II}, and $L_{II} + H_{II}$ corresponded to (as per cent of native DNA) 46, 42, and 44, respectively. The concentration of DNA during annealing was 20 μg/ml in 2 × SSC. After annealing at 68° for 4 hr, the samples were cooled, diluted with 1 vol of water (final concentration 10 μg of DNA/ml in 1 × SSC), and subjected to a temperature–absorbance measurement recorded on a Gilford automatic recording spectrophotometer.

TABLE I: Composition of Native DNA and of Separated L- and H-Strand Fractions of *Escherichia coli* and *Bacillus subtilis*.

Bacterial Species	DNA Preparation	Composition[a] (Mol %)				Molar Ratios		
		A	G	C	T	Pu/Py	A + T/ G + C	6-Am/6K[b]
E. coli	L	24.4	27.3	24.3	24.0	1.07	0.94	0.95
	H	23.8	24.9	27.0	24.3	0.95	0.93	1.03
	L + H	24.1	26.1	25.7	24.2	1.01	0.93	0.99
	Native	24.6	25.5	25.6	24.3	1.00	0.96	1.01
	Py ratio H/L = 1.06							
B. subtilis	L	30.1	23.7	20.1	26.1	1.16	1.28	1.01
	H	27.3	19.6	23.2	29.8	0.88	1.33	1.02
	L + H	28.6	21.7	2.17	28.0	1.01	1.30	1.01
	Native	28.2	21.9	21.6	28.3	1.00	1.30	0.99
	Py ratio H/L = 1.14							

[a] Base composition determined by acid hydrolysis (7.5 N $HClO_4$) and paper chromatography of unlabeled DNA preparations were previously reported (Rudner *et al.*, 1968b, 1969). [b] 6-Amino (A + C)/6-keto (G + T).

fractions to a pyrimidine cluster analysis rather than the hydroxylapatite purified strand fractions.

Fractionation of Isostichs According to Chain Length. The base analyses of the *E. coli* and of the *B. subtilis* fractions which were reported earlier (Rudner *et al.*, 1969) are presented in Table I. As shown, the purine/pyrimidine ratios in *E. coli* are 1.07 and 0.95 for fractions L and H, respectively. Unlike the *B. subtilis* strands which show a bias in the distribution of both purines and pyrimidines, in the *E. coli* fractions the total distribution bias appears limited to guanine and cytosine. The overall pyrimidine ratio of H/L in the total MAK fractions is 1.06 in *E. coli* and 1.14 in *B. subtilis*. These values will serve as references for all subsequent evaluations of the degree of bias between the strand fractions and for the comparison between the two bacterial species. Fractionation of pyrimidine oligonucleotides according to chain length released by hydrolysis in formic acid–diphenylamine from native DNA and strand fractions L and H is shown in Figure 4. Eleven isostich fractions were recovered in all three hydrolysates. The chromatographic profiles show a slight disparity in the relative distribution of radioactivity between the L- and H-strand fractions. The quantitative analyses of these fractionations, summarized in Table II, further establish this disparity. As shown in Table II, the isostich distribution ratio of H/L gradually increased from 0.99 to 2.50 over the range of chain length 1–11. With the exception of isostich 3, the H/L ratios did not correspond to the overall pyrimidine ratio of H/L = 1.06 determined directly on the total MAK fractions (see Table I). Similar to the findings in *B. subtilis* (Rudner *et al.*, 1972), the observed change in the isostich distribution ratio

TABLE II: Distribution of Pyrimidine Isostich–Phosphorus in Native DNA and in Strands L and H of *E. coli*.[a]

Pyrimidine Isostich	Mol of Pyrimidine/100 g-atoms of DNA-P for				Isostich Distribution Ratio of H/L	
	L Strand	H Strand	Native DNA	(L + H)/2	*E. coli*	*B. subtilis*[b]
1	14.04	13.92	13.52	13.98	0.99	0.93
2	15.41	15.52	15.37	15.47	1.01	0.99
3	7.97	8.55	8.32	8.26	1.07	1.07
4	4.81	5.23	5.09	5.02	1.09	1.23
5	2.90	3.47	3.21	3.19	1.20	1.32
6	1.43	1.96	1.85	1.70	1.37	1.41
7	0.80	1.11	1.19	0.96	1.39	1.69
8	0.47	0.76	0.78	0.62	1.62	1.77
9	0.25	0.40	0.36	0.33	1.60	1.86
10	0.16	0.23	0.18	0.20	1.44	3.06
11	0.06	0.15	0.12	0.11	2.50	82.44
Total	48.30 ± 1.82	51.30 ± 1.69	50.00 ± 0.85	49.84		
Uncorrected total	46.57	47.29	47.92			
% P_i recovered	25.98	24.05	24.48			

[a] The values for the native DNA and the L and H strands represent the mean of two separate analyses. The differences from the mean are given in the uncorrected totals. The values for the L strand have been adjusted to 48.30 and for the H strand to 51.30 as determined from direct base analysis (see Table I). The pyrimidine ratios H/L from the direct analysis were 1.06 and 1.14 for *E. coli* and *B. subtilis*, respectively. [b] The H/L ratios for *B. subtilis* DNA were reported in a previous publication (Rudner *et al.*, 1972).

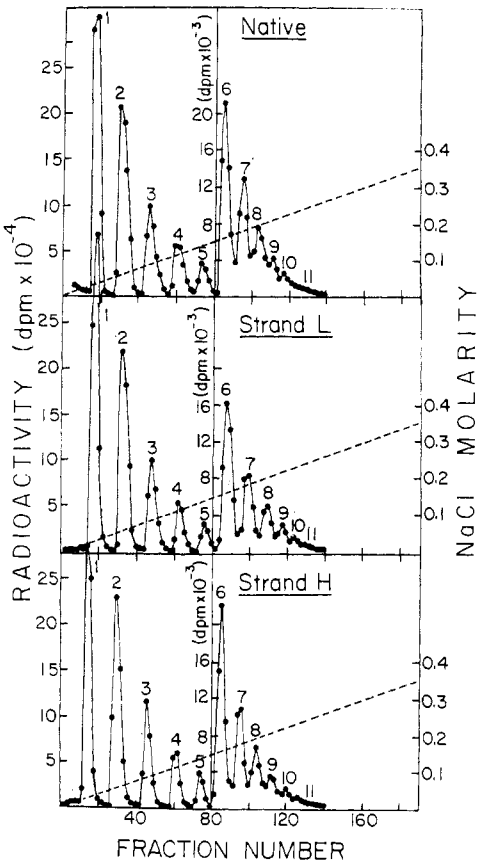

FIGURE 4: Chromatography of diphenylamine–formic acid hydrolysates of ^{32}P-labeled native DNA and fractions L and H of *E. coli* on DEAE-cellulose (5.0 g, 325 mesh, 1.5 × 23 cm, chloride form). The hydrolysate ^{32}P-labeled DNA, 75 mg of calf-thymus DNA carrier (67% formic acid, 2% diphenylamine, 30°, 18 hr), was applied to the column after diphenylamine and formic acid were removed. The column was washed with 50 mM sodium acetate buffer in 7 M urea (pH 5.5) to remove purines and orthophosphate and then eluted with a linear gradient of NaCl from 0 to 0.35 M in 0.1 M sodium acetate buffer in 7 M urea (pH 5.5) (total volume, 2000 ml). The absorbance of the carrier DNA eluates was recorded by a continuous flow ultraviolet monitor (ISCO UV analyzer). Fractions of 10 ml were collected, and 1.0-ml samples were mixed with 3.0 ml of scintillation mixture (Bray, 1960) and counted in a liquid scintillation spectrometer (Tri-Carb, Packard Instrument). The total radioactivity of native DNA was 7.8 × 10^7 dpm and of fractions L and H as indicated in the legend of Figure 1.

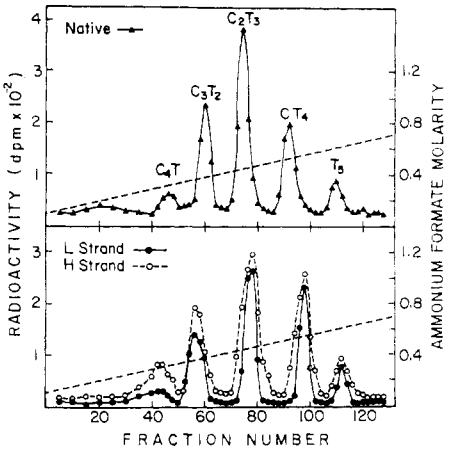

FIGURE 5: Chromatography of pyrimidine isostich 5 from ^{32}P-labeled native DNA and strand fractions L and H on DEAE-cellulose (5.0 g, 325 mesh, 1.5 × 23 cm, formate form). The pooled isostich fractions, diluted with distilled water, were applied to the columns. The column was washed with 0.1 M formic acid and then eluted with a linear gradient of ammonium formate from 0 to 1.0 M, pH 3.0 (total volume, 1000 ml). Fractions (5 ml) were collected and samples (1.0 ml) counted as described in Figure 4.

of H/L indicates that the bias between the strands is not of a uniform nature. Short isostichs of length 1–2 are distributed about equally in L and H fractions. From isostich 3, there occurs a gradual increase in the number of long isostichs in the H fraction. The amounts of the mono- and dinucleotides observed in the strand fractions of *E. coli* DNA (Table II) were consistently larger than the amounts reported for *B. subtilis* (Rudner *et al.*, 1972). For example, in the H-strand fraction of *E. coli* the amounts (in moles of pyrimidine) of isostich 1 and 2 were 13.92 and 15.52, respectively, as compared to 10.81 and 11.67 respectively, for *B. subtilis*. The reverse pattern was observed for the long isostichs, namely the amounts of isostichs 4–11 were lower in the *E. coli* fractions as compared to *B. subtilis*. The overall phosphorus balance shows that recoveries were high in the range of 93–96% (Table II). The amount of released inorganic phosphorus, which originates from the degradation of the purine clusters, was slightly unequal between the strand fractions (Table II). As expected, the L fraction released slightly more orthophosphate (25.98 mol %) than the H fraction (24.05 mol %). Finally, Table II shows that the values computed for (L + H)/2 agree with the isostich distribution pattern determined in the native *E. coli* DNA. The results of our native analyses also agree with the distributions reported by Cerny *et al.* (1968) who compared four methods of analysis in *E. coli* DNA.

Fractionations According to Base Composition. Each isostich fraction was pooled and subfractionated according to base composition on DEAE-cellulose at pH 3.0. Figure 5 illustrates a typical chromatographic profile obtained by refractionating isostich 5 from native DNA and fractions L and H. The results of these analyses are summarized in Table II. In addition to the mole concentration of the individual components, Table III lists the number of tracts per molecule (single or double stranded) of *E. coli* DNA (calculated on the basis of a mol wt of 2.5 × 10^9 daltons or 4 × 10^6 base pairs). In order to assess the degree of asymmetry between the strand fractions, we also include the individual component ratio of H/L for *E. coli* and for *B. subtilis* taken from an earlier report (Rudner *et al.*, 1972). The individual component ratio of H/L should be compared to the overall pyrimidine ratio H/L in the total MAK fractions presented in Table I. As shown in Table III, 59 different nonisomeric pyrimidine oligonucleotides were separated, identified, and quantitated in both strand fractions. Up to isostich 4, all the theroretically possible pyrimidine oligonucleotides were present in both strand fractions. From isostich 5 to 11, the missing components in both strand fractions were consistently oligocytidylate and cytosine-rich clusters. From isostich 9 to 11, oligothymidylate was also absent in both strand fractions. Finally, the summations of the numbers of oligonucleotides in fractions L + H presented in the last column of Table III agree with the numbers determined directly in native *E. coli* DNA. The values obtained for isostichs 1–6 agree better than the ones obtained for isostichs 7–11 (Table III).

The results in Table III indicate that the asymmetry between the strand fractions is not uniform, but rather the bias is restricted to the long isostichs. First, as mentioned earlier, short isostichs of length 1–5 (with the exception of T$_5$p,

C_4Tp_6, and T_5p_6) are distributed about equally between the strand fractions. Long isostichs of length 6–11 are distributed in larger amounts in the H-strand fraction. The greatest asymmetry between the strand fractions is seen in isostich 11 where the average H/L component ratio is 2.50. Second, unlike the pyrimidine sequence patterns reported for *B. subtilis* (Rudner *et al.*, 1972), there is no distinctive difference in the relative distribution of cytosine-rich and thymine-rich clusters between the strand fractions. We observed in the L strand of *B. subtilis* DNA a drastic reduction in the number of cytosine-rich clusters as compared to a gradual reduction in the number of thymine-rich clusters (Rudner *et al.*, 1972). This disparity was not apparent in the *E. coli* L fraction. As shown in Table III, the fluctuations in the H/L component ratio reflect no such regularity.

The distribution of homooligomer sequences of cytidylate and thymidylate between the strand fractions is of special interest since they may have some functional significance. Earlier investigations of the distribution of pyrimidine isostichs in native microbial DNA preparations demonstrated that oligocytidylate sequences of length 4 or 5 are very scarce, whereas oligothymidylate sequences are found in amounts often exceeding random predictions (Rudner *et al.*, 1966). Table III shows the same relative scarcity of oligocytidylate clusters in both strand fractions of *E. coli* DNA and as reported in the *B. subtilis* strands (Rudner *et al.*, 1972). We did not detect cytidylate sequences longer than 4 in *E. coli* and longer than 5 in *B. subtilis*. Longer, naturally occurring cytosine oligonucleotides of chain lengths 6 and 7 have been reported in the DNA of bacteriophages T7 and λ, respectively (Mushynski and Spencer, 1970a,b). In contrast, unusually long thymine oligonucleotides were found in both *B. subtilis* and *E. coli* strand fractions (Rudner *et al.*, 1972, and Table III). Here again the two bacterial species differ; namely, the *E. coli* strand fractions do not exhibit the same distribution pattern of thymine oligonucleotides as was found in *B. subtilis*. Table IV summarizes the comparison in the distribution of thymine oligonucleotides between the strand fractions of *B. subtilis* and *E. coli* DNAs. In *B. subtilis*, as shown in Table IV, thymine clusters of length 1–3 are preferentially distributed in the L strand. Clusters of length 4–6 are distributed about equally in both strands; clusters of length 7–9 are distributed predominantly and clusters of length 10 and 11 are distributed exclusively in the H strand. In *E. coli* on the other hand, this pattern was not observed; instead, the distribution of mono- and dithymidylates is about equal between the strand fractions followed by a gradual increase in the number of thymine clusters in the H-strand fraction. Unlike *B. subtilis*, thymine oligonucleotides of length 9–11 were not detected in *E. coli* DNA.

Discussion

The present studies deal with certain features of the chemical asymmetry that exist in complementary fractions of *E. coli* DNA. The significance of the analytical data presented here is largely dependent upon the effectiveness of strand separation by MAK chromatography. Unlike the MAK fractionated strands of *B. subtilis* whose complementarity has been proven beyond any reasonable doubt (Rudner *et al.*, 1968a,b; Karkas *et al.*, 1968; Rudner and Remeza, 1973a,b), the degree of complementarity of the *E. coli* fractions must be qualified. In this paper we have shown that although the annealed mixture of L plus H produced duplex DNA effectively (*ca.* 70% of the secondary structure of native DNA), the individual L and H fractions retain a high degree of self-annealing ability. On the basis of temperature–absorbance measurements and from hydroxylapatite chromatography, we have estimated that *ca.* 40% of the L and H fractions of *E. coli* DNA can produce secondary structures upon a self-annealing treatment. Higher values of 70–90% have been recently reported for MAK fractions of denatured *Salmonella typhimurium* DNA (Udvardy and Venetianer, 1973). As mentioned before, the observed self-annealing ability of the *E. coli* MAK fractions is probably due in part to the formation of intrastrand secondary structures. More compelling evidence for strand resolution was obtained from the DNA hybrids formed by mixing the hydroxylapatite purified fractions and from hybridization to purified ribosomal RNA. The hydroxylapatite purified L and H fractions devoid of self-annealing ability produced duplex DNA (*ca.* 40% of native DNA) when cross-annealed. Hybridization and hybrid-competition tests with purified rRNA in *E. coli* and *S. typhimurium* have clearly demonstrated that the DNA sequences coding for rRNA can be separated into complementary strands by MAK chromatography (Margulies *et al.*, 1970, 1971; Udvardy and Venetianer, 1971, 1973). In conclusion, one may roughly estimate that fractionation by MAK chromatography of alkali-denatured *E. coli* DNA leads to separation of at least 40–60% of the complementary sequences including those which code for rRNA.

The nucleotide cluster analyses reported here show that the differences between the L and H fractions are found predominantly in the long isostichs of length 6–11. Since both complementary fractions of *E. coli* DNA were analyzed separately, the pyrimidine distribution in one strand fraction should be a mirror image of the purine distribution in the second strand fraction. Thus, the results give an account of the majority of the purine and pyrimidine oligonucleotides in both complementary fractions. These analyses do not provide information regarding the distribution of all possible sequential isomers of each component. It is more than likely that greater differences between the strand fractions might have been detected if such isomers could have been separated and quantitated. There are some distinct differences between the pyrimidine oligonucleotide catalogs reported here for the *E. coli* fractions and those reported previously for the *B. subtilis* complementary strands (Rudner *et al.*, 1972). First, all 59 nonisomeric components of isostichs 1–11 occur in both strand fractions of *E. coli* DNA. In *B. subtilis*, on the other hand, 15 components of isostichs 10 and 11 were found exclusively in the H strand (Rudner *et al.*, 1972). Second, the limited asymmetry between the strand fractions of *E. coli* DNA involves both the cytosine-rich and the thymine-rich clusters to the same extent, while in *B. subtilis*, the chemical asymmetry involves the cytosine-rich clusters to a greater extent than thymine-rich clusters. Third, the distribution of thymine oligonucleotides in the *E. coli* strand fractions does not follow the same unique pattern as was found in the *B. subtilis* strands (see Table IV). Finally, the following components, C_5p_6, T_9p_{10}, $T_{10}p_{11}$, and $T_{11}p_{12}$, were only detected in *B. subtilis* DNA, while the components C_6Tp_8 and $C_7T_4p_{12}$ were only detected in *E. coli* DNA.

Similar differences in the distribution of pyrimidine oligonucleotides can be seen in the catalogs reported for the strands of T7 and λ DNAs (Mushynski and Spencer, 1970a,b). For example, a dozen components of isostichs 11–13 found exclusively in strand r (corresponding to H in the present study) of T7 DNA are distributed in both strands of λ DNA (Mushynski and Spencer, 1970a,b). There are definite similarities in the asymmetric distribution of pyrimidine isostichs in the complementary strands of DNA from viral and bacterial

TABLE III: Distribution of Pyrimidine Isostich Components in the DNA of *E. coli* and in Strands L and H.[a]

Isostich *n*	Component	Mol of Pyrimidine/100 g-atoms of DNA-P			Component Ratio of H/L		No. of Tracts/DNA Molecule[b]			
		L Strand	H Strand	Native DNA	*E. coli*	*B. subtilis*[c]	L Strand	H Strand	Native DNA	Strands L + H
1	Cp_2	7.78	7.18	7.21	0.92	0.94	311,200	287,200	576,800	598,400
	Tp_2	6.26	6.74	6.31	1.08	0.92	250,400	269,600	504,800	520,000
2	C_2p_3	4.65	4.37	4.59	0.94	1.11	93,000	87,400	183,600	180,400
	CTp_3	6.91	7.65	7.19	1.11	1.02	138,200	153,000	287,600	291,200
	T_2p_3	3.85	3.50	3.59	0.91	0.86	77,000	70,000	143,600	147,000
3	C_3p_4	1.32	1.18	1.23	0.89	1.56	17,600	15,732	32,800	33,332
	C_2Tp_4	2.88	2.72	2.70	0.94	1.15	38,400	36,266	72,000	74,666
	CT_2p_4	2.59	2.93	2.87	1.13	1.07	34,532	39,066	76,533	73,598
	T_3p_4	1.18	1.71	1.51	1.45	0.89	15,732	22,800	40,266	38,532
4	C_4p_5	0.383	0.380	0.340	0.99	1.34	3,830	3,800	6,800	7,630
	C_3Tp_5	0.945	0.980	0.896	1.04	1.52	9,500	9,800	17,920	19,300
	$C_2T_2p_5$	1.49	1.59	1.78	1.07	1.53	14,900	15,900	35,600	30,800
	CT_3p_5	1.31	1.53	1.37	1.17	0.98	13,100	15,300	27,400	28,400
	T_4p_5	0.681	0.746	0.708	1.10	1.08	6,810	7,460	14,200	14,270
5	C_4Tp_6	0.202	0.381	0.298	1.89	1.56	1,616	3,048	4,768	4,664
	$C_3T_2p_6$	0.688	0.750	0.699	1.09	1.66	5,500	6,000	11,184	11,500
	$C_2T_3p_6$	0.960	1.06	1.15	1.10	1.45	7,680	8,480	18,400	16,160
	CT_4p_6	0.773	0.825	0.707	1.07	1.08	6,184	6,600	11,312	12,784
	T_5p_6	0.277	0.418	0.356	1.51	1.05	2,216	3,344	5,696	5,560
6	C_5Tp_7	0.087	0.110	0.090	1.26	1.56	580	733	1,200	1,313
	$C_4T_2p_7$	0.174	0.271	0.265	1.56	1.27	1,160	1,807	3,533	2,967
	$C_3T_3p_7$	0.417	0.562	0.497	1.35	1.59	2,780	3,747	6,627	6,527
	$C_2T_4p_7$	0.398	0.489	0.505	1.23	1.34	2,653	3,260	6,733	5,913
	CT_5p_7	0.240	0.361	0.353	1.50	1.61	1,600	2,407	4,707	4,007
	T_6p_7	0.114	0.167	0.140	1.46	1.01	760	1,113	1,866	1,873
7	C_6Tp_8	0.031	0.055	0.042	1.77	—	177	314	480	491
	$C_5T_2p_8$	0.071	0.092	0.079	1.30	1.58	406	526	903	932
	$C_4T_3p_8$	0.150	0.177	0.200	1.18	1.53	857	1,011	2,286	1,868
	$C_3T_4p_8$	0.242	0.302	0.334	1.25	1.74	1,368	1,726	3,817	3,109
	$C_2T_5p_8$	0.201	0.264	0.304	1.31	1.68	1,149	1,509	3,474	2,658
	CT_6p_8	0.069	0.162	0.166	2.35	1.81	394	926	1,897	1,320
	T_7p_8	0.036	0.058	0.065	1.61	1.62	206	331	743	537
8	$C_6T_2p_9$	0.019	0.031	0.043	1.63	3.21	95	155	430	250
	$C_5T_3p_9$	0.053	0.080	0.078	1.51	1.95	265	400	780	665
	$C_4T_4p_9$	0.101	0.178	0.170	1.76	2.34	505	890	1,700	1,401
	$C_3T_5p_9$	0.127	0.214	0.206	1.69	1.46	635	1,070	2,060	1,705
	$C_2T_6p_9$	0.099	0.149	0.158	1.51	1.34	495	745	1,580	1,240
	CT_7p_9	0.050	0.073	0.078	1.46	2.23	250	365	780	615
	T_8p_9	0.021	0.035	0.047	1.67	2.18	105	175	470	280
9	$C_6T_3p_{10}$	0.029	0.037	0.034	1.28	1.94	129	164	302	293
	$C_5T_4p_{10}$	0.039	0.072	0.051	1.85	2.18	173	320	453	493
	$C_4T_5p_{10}$	0.057	0.099	0.082	1.74	2.35	253	440	729	693
	$C_3T_6p_{10}$	0.062	0.091	0.093	1.47	1.74	276	404	827	680
	$C_2T_7p_{10}$	0.039	0.064	0.060	1.64	1.58	173	284	533	457
	CT_8p_{10}	0.024	0.039	0.040	1.63	1.47	107	173	356	280
10	$C_7T_3p_{10}$	0.016	0.025	*d*	1.56		64	100	*d*	164
	$C_6T_4p_{10}$	0.021	0.030	*d*	1.43		84	120	*d*	204
	$C_5T_5p_{10}$	0.033	0.045	*d*	1.36		132	180	*d*	312
	$C_4T_6p_{10}$	0.037	0.052	*d*	1.41		148	208	*d*	668
	$C_3T_7p_{10}$	0.029	0.038	*d*	1.31		116	152	*d*	268
	$C_2T_8p_{10}$	0.014	0.022	*d*	1.57		56	88	*d*	144
	CT_9p_{10}	0.010	0.018	*d*	1.80		40	72	*d*	112
11	$C_7T_4p_{11}$	0.005	0.012	0.009	2.40		18	44	65	62
	$C_6T_5p_{11}$	0.008	0.015	0.013	1.86		29	55	95	84
	$C_5T_6p_{11}$	0.012	0.030	0.028	2.50		44	109	203	153
	$C_4T_7p_{11}$	0.013	0.035	0.026	2.69		47	127	189	174
	$C_3T_8p_{11}$	0.011	0.027	0.021	2.45		40	98	152	138
	$C_2T_9p_{11}$	0.006	0.019	0.013	3.17		22	69	95	91
	$CT_{10}p_{11}$	0.005	0.012	0.010	2.40		18	44	73	62

Footnotes to Table III

[a] The values represent the means of two separate experiments. The amounts were corrected according to the values in Table I. [b] The number of tracts were calculated for a mol wt of *E. coli* DNA of 2.5×10^9 daltons, equivalent to 4×10^6 base pairs. [c] The H/L ratios for *B. subtilis* DNA were reported in a previous publication (Rudner *et al.*, 1972). [d] Unfortunately isostich 10 of native DNA was lost in both experiments.

TABLE IV: Distribution of Homooligomer Sequences of Thymidylate in Strands L and H of *B. subtilis* and *E. coli* DNAs.

	B. subtilis[a] Mol of Pyrimidine/100 g-atoms of DNA-P for			*E. coli* Mol of Pyrimidine/100 g-atoms of DNA-P for		
Component	L Strand	H Strand	H/L	L Strand	H Strand	H/L
Tp_2	6.00	5.51	0.92	6.25	6.74	1.08
T_2p_3	3.52	3.01	0.86	3.85	3.50	0.91
T_3p_4	2.12	1.89	0.89	1.18	1.71	1.45
T_4p_5	0.842	0.911	1.08	0.681	0.746	1.10
T_5p_6	0.452	0.474	1.05	0.277	0.418	1.51
T_6p_7	0.248	0.250	1.01	0.114	0.167	1.46
T_7p_8	0.124	0.201	1.62	0.036	0.058	1.61
T_8p_9	0.045	0.098	2.18	0.021	0.035	1.67
T_9p_{10}	0.026	0.051	2.27			
$T_{10}p_{11}$		0.041				
$T_{11}p_{12}$		0.027				

[a] These values were reported in a previous publication (Rudner *et al.*, 1972).

systems. For example, when all isostichs of length 6–13 in the strands of λ DNA or 6–11 in the L and H fractions of *E. coli* DNA containing over four cytosine residues are included, the H/L ratio is 1.50 and 1.51, respectively. Similarly, when isostichs of length 6–13 in the strands of T7 DNA or 6–11 in the strands of *B. subtilis* DNA containing over four cytosine are included, the H/L ratio of H/L is 2.36 and 2.55 respectively. In conclusion, bacteria, like viruses, exhibit a highly suggestive correlation between the asymmetric distribution of pyrimidine-rich sequences and the transcribing function of the complementary strands of DNA. In cases where the asymmetry in transcription is either total like T7 (Summers, 1969) or highly preferential like *B. subtilis* (Margulies *et al.*, 1970, 1971), the chemical asymmetry between the strands is extensive. On the other hand, in cases where the extent of asymmetric transcription is more limited like λ (Taylor *et al.*, 1967) or *E. coli* (Margulies *et al.*, 1970, 1971), the chemical asymmetry between the strands is much less pronounced. These observations are consistent with the proposal that specific long pyrimidine oligonucleotides which are cytosine-rich comprise promoter regions (Szybalski *et al.*, 1966). Although the present analyses do provide indirect support to the theory that pyrimidine runs may serve as general signals for the initiation of transcription, further experimental refinements are required to prove it.

References

Bray, G. A. (1960), *Anal. Biochem. 1*, 279.

Burton, K., and Petersen, G. B. (1960), *Biochem. J. 75*, 17.

Cerny, R., Mushynski, W. E., and Spencer, H. J. (1968), *Biochim. Biophys. Acta 169*, 439.

Galloway, D., and Rudner, R. (1973), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 32*, 663.

Grossman, L. (1967), *Methods Enzymol. 12, A*, 700.

Karkas, J. D., Rudner, R., and Chargaff, E. (1968), *Proc. Nat. Acad. Sci. U. S. 60*, 915.

Karkas, J. D., Rudner, R., and Chargaff, E. (1970), *Proc. Nat. Acad. Sci. U. S. 65*, 1049.

Mandell, J. D., and Hershey, A. D. (1960), *Anal. Biochem. 1*, 66.

Margulies, L., Remeza, V., and Rudner, R. (1970), *J. Bacteriol. 103*, 560.

Margulies, L., Remeza, V., and Rudner, R. (1971), *J. Bacteriol. 107*, 610.

Mushynski, W. E., and Spencer, J. H. (1970a), *J. Mol. Biol. 52*, 91.

Mushynski, W. E., and Spencer, J. H. (1970b), *J. Mol. Biol. 52*, 107.

Petersen, G. B., and Reeves, J. M. (1966), *Biochim. Biophys. Acta 129*, 438.

Rudner, R., Karkas, J. D., and Chargaff, E. (1968a), *Proc. Nat. Acad. Sci. U. S. 60*, 630.

Rudner, R., Karkas, J. D., and Chargaff, E. (1968b), *Proc. Nat. Acad. Sci. U. S. 60*, 921.

Rudner, R., Karkas, J. D., and Chargaff, E. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 152.

Rudner, R., LeDoux, M., and Mazelis, A. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 2745.

Rudner, R., and Remeza, V. (1973a), *J. Bacteriol. 113*, 739.

Rudner, R., and Remeza, V. (1973b), *J. Bacteriol. 113*, 754.

Rudner, R., Shapiro, H. S., and Chargaff, E. (1966), *Biochim. Biophys. Acta 129*, 85.

Spencer, J. H., Cape, R. E., Marks, A., and Mushynski, W. E. (1969), *Can. J. Biochem. 47*, 331.

Spencer, J. H., Cape, R. E., Marks, A., and Mushynski, W. E. (1969), *Can. J. Biochem. 47*, 331.

Spencer, J. H., and Chargaff, E. (1963), *Biochim. Biophys. Acta 68*, 9.

Summers, W. C. (1969), *Virology 39*, 175.

Szybalski, W., Kubinski, H., and Sheldrick, P. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 129.

Taylor, K., Hradecna, Z., and Szybalski, W. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1618.

Thomas, C. A., Jr., Berns, K. I., and Kelly, T. J., Jr. (1966), *Proc. Nucleic Acid Res. 1*, 535.

Udvardy, A., and Venetianer, P. (1971), *Eur. J. Biochem. 20*, 513.

Udvardy, A., and Venetianer, P. (1973), *Biochim. Biophys. Acta 308*, 234.