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Base Sequence Homology and Renaturation Studies of the Deoxyribonucleic Acid of Extremely Halophilic Bacteria

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The genetic relatedness among various strains of halophilic bacteria has been assessed by deoxyribonucleic acid-deoxyribonucleic acid (DNA-DNA) duplex formation and ribosomal ribonucleic acid (RNA) hybridization. All of the strains of extremely halophilic rods are closely related, and the extent of divergence of base sequence is similar for the major and minor DNA components. Parallel experiments with ribosomal RNA revealed a relationship between the extremely halophilic rods and cocci and a more distant relationship to moderate halophiles and to a photosynthetic extreme halophile. Renaturation studies of halophile DNA exclude the possibility that the satellite DNA represents multiple copies of a small episomal element. The kinetics of DNA renaturation show that the genome size of the extreme and moderate halophiles is similar to that of *Escherichia coli*.

The results in the preceding paper tend to exclude the possibility that the satellite deoxyribonucleic acid (DNA) of extreme halophiles results from a culture contaminant or an episome (25). The presence of two DNA components seems to be characteristic of all the non-photosynthetic strains of extreme halophiles so far examined, whether rods or cocci. The present paper deals with the genetic relatedness among the various halophiles by determining the relative homology of base sequence in both DNA components to those of other strains. Studies of the kinetics of DNA renaturation are also presented. These results are discussed in relation to the nature and origin of the satellite DNA.

MATERIALS AND METHODS

Isolation and growth of strains, preparation of DNA, and characterization of CsCl density gradients were described in the preceding paper (25).

Ribonucleic acid (RNA) preparation. ^{32}P-labeled ribosomal RNA from isolate III was prepared by growing cells in the high-salt medium with low phosphate content. Cells were grown for 10 hr in the presence of 100 μc of carrier-free ^{32}P-orthophosphate per ml. The culture was then centrifuged, and the cells were suspended in the high-salt medium with high phosphate content. After 2.5 hr of growth in this medium, the cells were again removed by centrifugation and lysed by suspending them in a small amount of medium and then rapidly adding saline-EDTA (0.1 M ethylenediaminetetracetate and 0.15 M NaCl, *p*H 8) and 0.5% sodium dodecyl sulfate. Purification of the RNA and isolation of the 23*S* ribosomal RNA component in sucrose gradients were performed as previously described (24).

^{32}P-pulse-labeled RNA from isolate III was prepared by adding 100 μc of carrier-free ^{32}P-orthophosphate per ml to cells in the exponential phase of growth in the high-salt, low-phosphate medium. After 1 hr, approximately 10% of the generation time under these conditions, the cells were harvested by centrifugation and the RNA was prepared as above. The pulse-labeled RNA was further purified by passing through a column (0.9 by 17 cm) of G-50 coarse Sephadex previously equilibrated with 2 × SSC (0.15 M NaCl and 0.005 M sodium citrate).

Preparation of labeled DNA. ^{3}H-labeled DNA from isolate III was prepared by growing the cells to early stationary phase in high-salt medium containing 10 μc of ^{3}H-adenine (New England Nuclear Corp.) per ml. The DNA was prepared as described in the previous paper (25).

DNA/RNA hybridization and DNA/DNA duplex formation. The DNA samples were heat-denatured just before use and immobilized on nitrocellulose membrane filters (B-6 coarse; Carl Schleicher and Schuell, Co., Keene, N.H.) according to the method of Gillespie and Spiegelman (13). Filters (6.5 mm in diameter) were cut from larger filters with a paper punch and incubated with various quantities of RNA or DNA overnight at 70 C in 0.2 ml of 2 × SSC (24). In all experiments involving DNA-DNA complex formation, the filters were treated with the preincuba-

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tion mixture of Denhardt (5). In the case of the RNA-DNA experiments, mismatched RNA was removed by treating the filters with 5 μg of pancreatic ribonuclease (Calbiochem, Los Angeles, California) per ml for 15 min at 37 C (13).

Fractionation of DNA on a preparative CsCl density gradient. The heavy and light components of ^{3}H-labeled DNA from isolate III were separated by CsCl density gradient centrifugation in an angle-head rotor according to the method of Flamm et al. (12).

Thermal denaturation profiles. Filters containing bound RNA-DNA were rinsed in 0.5 × SSC at 60 C, and the thermal denaturation profiles were obtained by determining the amount of RNA eluted in each 10 min of incubation in 2 ml of 0.5 × SSC at each of the specified temperatures (24).

Renaturation of DNA in solution. DNA samples at a concentration of 50 to 70 μg/ml were denatured in 0.1 × SSC in quartz cuvettes in a Beckman DU spectrophotometer with a Gilford multisample absorbance recorder, linear thermosensor, and water-bath attachment by increasing the temperature at a rate of about 30 C/hr. After denaturation, the salt concentration was increased to 1 × SSC, and the temperature was rapidly lowered to 70 C. The proportion of the DNA which had renatured at any given C_0t (concentration of nucleotides, in moles per liter, times duration of time, in seconds, in which the reaction has proceeded) value was determined by relating the decrease in the optical density at 260 mm to the maximal decrease possible (2). The maximal possible decrease was determined from the hyperchromic shift observed during denaturation, taking into consideration the collapse of denatured DNA on cooling to 70 C.

RESULTS

Genetic relatedness among various halophilic bacteria. Comparison of the overall base composition of the DNA of various bacteria may be used as a preliminary guide to their genetic relatedness (21). A more sensitive method, however, involves a comparison of the base sequences of the DNA components. The most convenient technique for accomplishing this comparison consists in measuring the extent to which RNA or the separated strands of DNA of one organism are able to reanneal with the separated strands of DNA from another organism (19). In the present study the relationships among the various bacteria used in this study were determined from the ability of radioactively labeled nucleic acids of isolate III to form heteroduplexes with the various denatured bacterial DNA components immobilized on nitrocellulose membrane filters (5, 13). The relative reactions of ^{3}H-labeled DNA or ^{32}P-pulse-labeled RNA with filters containing various DNA components are summarized in Table 1.

All the extreme halophiles of the genus *Halobacterium* which were examined show some degree of relatedness to isolate III, with DNA homology values ranging from 39% for isolate II, to 78% for *H. salinarium*. The DNA of the extremely halophilic *Halococcus morrhuae* displays no homology to that of isolate III. It will be recalled that the DNA of these two organisms exhibits nearly identical patterns when examined by analytical CsCl density gradient centrifugation (25). Little or no homology was apparent between the DNA of isolate III and those of the photosynthetic extreme halophilic SL-1, or the two moderate halophiles, isolate I and isolate V; nor does the photosynthetic bacterium, *Rhodopseudomonas spheroides* show any nucleotide sequence relationship to isolate III.

The degree of relatedness shown by reactions utilizing pulse-labeled RNA or DNA are essentially identical. The same finding was reported previously for species of *Enterobacteriaceae* (20). This suggests that the genes functioning to produce most of the messenger RNA evolve at a rate which is indistinguishable from that of the total DNA. Preliminary experiments using purified major and minor DNA components of isolate III indicated that both DNA components were capable of reacting with messenger RNA and are, therefore, both active in RNA transcription.

DNA-DNA duplex formation with the major and minor DNA components of isolate III. The question of the relative homologies of the base sequences present in the two DNA components of the various *Halobacteria* was approached by fractionating the ^{3}H-labeled DNA of isolate III into the major and minor components. This particular strain proved to be the most appropriate for this study, since the minor component represents a relatively large fraction of the total DNA. Moreover, the difference in buoyant density of the two fractions is larger than for other strains (26). After fractionation in a preparative CsCl gradient, the purity of the two separated components was checked by analytical ultracentrifugation. Samples of each DNA component were then incubated with filters containing total DNA from each of the various strains under the conditions described in Table 1. The relative amounts of heavy or light ^{3}H-DNA which reacted with the various filters is recorded in Table 2, with the values for unfractionated DNA included for comparison. The results clearly demonstrate that, when satellite DNA components are present, both DNA fractions are able to react. There is no apparent tendency for one fraction to show a higher level of cross-reaction. This would imply that the divergence of base sequence of the two DNA components between two strains is essentially the same.

The extent of mismatching of bases in these

TABLE 1. *Duplex formation between the DNA or RNA of isolate III and various bacterial DNA components*[a]

Filter DNA source	DNA			RNA	
	DNA (μg/filter)	^{3}H[b]	Reaction with DNA of isolate III	^{32}P[b]	Reaction with DNA of isolate III
			%		%
Isolate III	11.6	6.84	100	309	100
Halobacterium salinarium	10.0	5.15	78	239	77
H. cutirubrum	10.3	4.83	71	224	73
H. halobium	12.6	3.69	54	156	50
Isolate II	12.0	2.66	39	129	42
Isolate I	11.6	0.14	2	0	0
Isolate V	11.5	0.10	1	2.5	1
Halococcus morrhuae	9.9	0.10	1	1.4	1
Spirillum SL-1	5.6	0.00	0	5.9	2
Rhodopseudomonas spheroides	10.0	0.00	0	0	0

[a] ^{3}H-DNA (3,660 counts per min per μg) of isolate III was incubated in 1 ml of 2 × SSC at 70 C overnight with three DNA filters. The ratio of the ^{3}H-labeled input DNA to filter-bound DNA was 1:50. The RNA-DNA hybrids were formed by incubating 225 μg of ^{32}P-pulse-labeled RNA from isolate III (709 counts per min per μg) in 0.2 ml of 2 × SSC at 70 C overnight with a single DNA filter. After incubation, the filters were subjected to treatment with ribonuclease.

[b] Expressed as counts per minute per microgram of DNA.

heterologous duplexes was assessed by determination of their thermal dissociation profiles. This technique permits an estimate to be made of the fraction of bases mispaired in the duplex and hence the extent of base replacement (19, 22, 24). Each decrease of 1 C in the mean thermal stability from that obtained with homologous duplexes implies approximately 1.5% base mismatching (Laird et al., *submitted for publication*).

Figure 1 illustrates the thermal dissociation curves of duplexes formed by total and fractionated DNA of isolate III. The $T_{m,i}$ (mean temperature of irreversible dissociation) for the homologous complexes was 85 C for unfractionated DNA, 80 C for the light fraction, and 87 C for the heavy fraction. These values are consistent with the known base compositions determined by CsCl analytical pycnography (25). The thermal dissociation curves for heterologous complexes are displaced to lower temperatures in accordance with the extent of relatedness of the strain in question. However, except in the case of isolate I, a moderate halophile, these reductions in thermal stability are small, indicating a very high degree of base sequence homology.

Thus there appear to be base sequences closely related to both the heavy and light components of isolate III in the DNA of each of the extreme halophiles examined. Although the amount of satellite DNA varies among the various strains (25), no apparent correlation exists between this amount and the thermal stability of the heterologous duplexes formed by the light component.

TABLE 2. *Reaction of labeled DNA of isolate III with various DNA components*[a]

Filter DNA source	Counts per min of ^{3}H per μg of filter DNA		
	Unfractionated DNA	Heavy DNA	Light DNA
Isolate III	6.8	4.5	6.7
Halobacterium salinarium	5.1	6.2	5.1
H. cutirubrum	4.8	6.0	3.6
H. halobium	3.7	4.2	2.4
Isolate II	2.7	2.0	4.6
Isolate I	0.1	0.1	0.05
Isolate V	0.1	0.2	0.4

[a] The heavy and light components of H^{3}-DNA from isolate III were separated by centrifugation in a CsCl equilibrium density gradient employing an angle-head rotor. Reaction conditions and the values for unfractionated DNA are from Table 1.

Base sequence homology among the ribosomal cistrons of various halophilic bacteria. Many earlier studies have demonstrated that the base sequences of the ribosomal cistrons in bacterial DNA have been conserved during evolution relative to the rest of the genome (6, 8, 24, 31). A comparison of the base sequences of the ribosomal cistrons may therefore reveal a relationship among various bacteria, although base sequences of the majority of cistrons are too divergent to allow substantial DNA-DNA duplex formation.

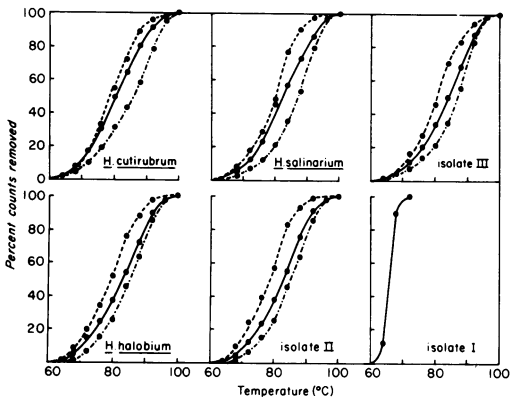

FIG. 1. *Thermal denaturation profiles of DNA-DNA duplexes formed between the DNA of isolate III and the DNA of various bacteria. ^{3}H-labeled DNA (3,660 counts per min per μg) from isolate III was fractionated into its heavy and light components. DNA from various bacteria was immobilized on nitrocellulose membrane filters and used to form duplexes with labeled, fractionated heavy DNA (dashed line), the fractionated light DNA (dotted line), and the unfractionated DNA (solid line). The duplexes were dissociated in 0.5 × SSC.*

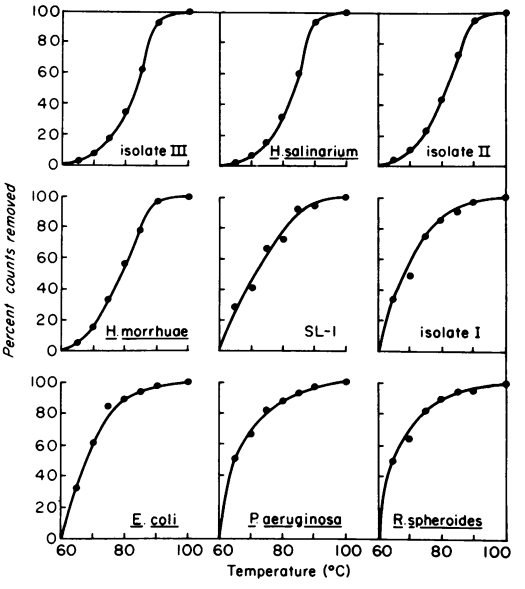

FIG. 2. *Thermal dissociation profiles of ribosomal RNA-DNA hybrids. Hybrids were formed by incubating 5 μg of ^{32}P-labeled 23S ribosomal RNA (10^6 to 5×10^6 counts per min per μg) in 0.2 ml of 2 × SSC for 15 hr at 70 C with a DNA filter. Hybridized RNA was eluted from the DNA filters in 0.5 × SSC and counted.*

This approach was employed in the present investigation as a means of elucidating more distant relationships among the halophiles. Base sequence homology of the ribosomal cistrons was determined from the thermal stabilities of the hybrids formed between the ^{32}P-labeled 23*S* ribosomal RNA of isolate III and the various bacterial DNA components. The $T_{m,i}$ values of the thermal stability profiles shown in Fig. 2 range from 83 C for the homologous complex to 65 C for the complex with the DNA of the nonhalophilic, photosynthetic *R. spheroides*. A gradation of $T_{m,i}$ values can be seen in these results, with the extreme halophiles of the genus *Halobacterium* showing the highest values, the extreme halophiles not classified in the genus *Halobacterium* showing the next highest values, and the moderate halophile and nonhalophiles having the lowest values.

The $T_{m,i}$ of the homologous complex corresponds to that expected for RNA with a base composition of approximately 54%GC. This is within the range of base compositions found in other bacterial ribosomal RNA (1, 14, 23). Thus, the grouping of bacterial types derived from these data is attributable to divergence in the base sequences of the ribosomal RNA rather than to overall differences in base composition (24), and is found to correlate well with a grouping of these bacteria based upon the NaCl concentration optimal for their growth.

Renaturation kinetics of the DNA from extreme halophiles. The renaturation of denatured DNA can be considered to be a second-order reaction with a maximum rate at a temperature of 20 to 30 C below the melting temperature (T_m) of the DNA. The rate of reaction at the optimal temperature is dependent upon the salt concentration of the solution, the molecular weight of the DNA, and its base composition (33). Furthermore, the rate of renaturation of DNA from organisms with relatively small genomes, such as bacteriophage, is more rapid than the rate of renaturation observed for DNA from organisms with very large genomes, such as vertebrates. This was convincingly demonstrated by Britten and Kohne (2) for a large number of DNA types from various organisms. By plotting semilogarithmically the fraction of DNA renatured at a given time against the concentration of nucleotides, in moles per liter times the time in seconds (C_0t), a sigmoidal curve is obtained. The $C_0t_{0.5}$ value at the midpoint of this curve (50% renaturation) is directly proportional to the size of the genome. For example, the $C_0t_{0.5}$ obtained for *E. coli* which has a genome 22.5 times larger than T4 bacteriophage is found to be 22.5 times larger than the $C_0t_{0.5}$ for T4 bacteriophage. This relationship, however, does not always hold for the complex DNA of higher organisms, owing to the presence of large numbers of partially redundant base sequences. In this case, the renaturation curve becomes biphasic

as a result of the more rapid renaturation of the redundant DNA (2). An analogous result would be expected for a bacterial DNA containing a significant proportion of episomal DNA in many copies.

Figure 3 shows the sigmoidal curve obtained from the renaturation of denatured DNA from isolate III. The curve for an idealized second-order reaction has been included and is found to be superimposable on the experimental curve. Such a result is that expected for an ideal DNA, i.e., one which contains equal quantities of unique base sequences without internal sequence redundancy (2).

The relative rates of renaturation of the minor and major DNA component were assessed by taking samples at intervals during the renaturation and examining them in an analytical ultracentrifuge. Figure 4 displays the microdensitometer tracings of ultraviolet photographs of partially renatured DNA samples subjected to analytical CsCl density gradient centrifugation. The samples were from an experiment run in parallel with the one described above. The extent of renaturation at various times was calculated from the data in Fig. 3. The first sample, which was taken at a time corresponding to approximately 5% renaturation, shows a slightly skewed peak at a density of 1.7367. This is the density expected for the single-stranded form of this DNA. The two DNA peaks were not resolvable because of the broadness of the bands obtained

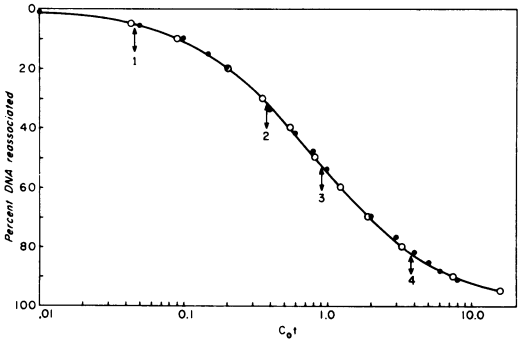

FIG. 3. *Renaturation kinetics of denatured DNA from isolate III. Denatured DNA from isolate III was allowed to renature at 70 C in 1 × SSC. Optical density at 260 nm (OD_{260}) was monitored by a Beckman DU spectrophotometer equipped with a Gilford chart recorder. Percentage of DNA renatured at a particular C_0t value was determined by the decrease in OD_{260} compared to the total decrease possible as determined by observations of the hyperchromic shift during denaturation. (○) Calculated values for an ideal second-order reaction; (●) experimental values. Arrows indicate samples taken for a parallel experiment described in Fig. 4.*

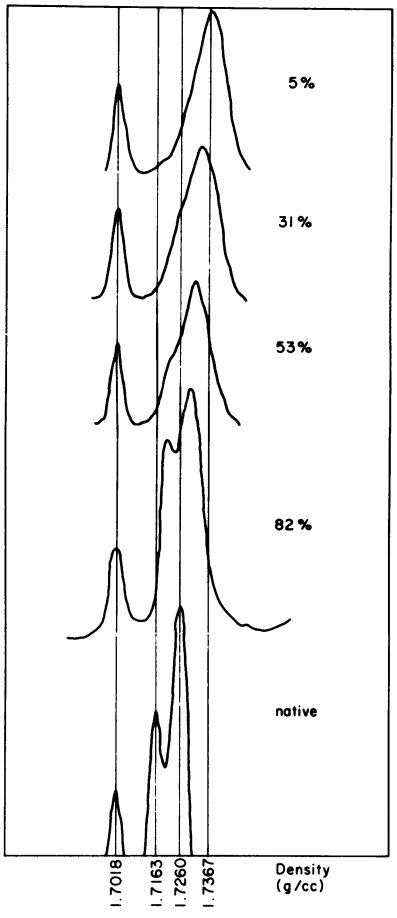

FIG. 4. *Changes in density during renaturation of DNA of isolate III. Samples taken from the experiment of Fig. 3 were analyzed by CsCl density gradient ultracentrifugation. Microdensitometer tracings of ultraviolet photographs taken at times giving 5, 31, 53, and 82% renaturation of the DNA are shown. Marker DNA is Proteus rettgeri (1.7018 g/ml).*

with single-stranded DNA. After 31% of the DNA renatured (sample 2), the band shifted approximately one-third of the way toward the lighter density of native DNA. At 53% renaturation, the DNA band moved approximately halfway between the densities of double-stranded and single-stranded DNA, and the presence of the satellite band was then easily detectable. This pattern was accentuated in the last sample taken after more than 80% renaturation. There was no apparent tendency for the denatured satellite DNA to shift toward the lighter density of fully renatured DNA at a greater rate than the major component of the DNA. One can therefore conclude that the presence of a satellite DNA is not due to a contaminating organism with a much smaller genome size, such as a bacteriophage,

although the presence of a single copy of a contaminating genome of a smaller size cannot be excluded. Nor is there any indication of the presence of multiple copies of an episomal element, since this DNA would renature more rapidly than the major DNA component.

The rate of renaturation of the DNA from four different halophilic bacteria was determined by optical density measurements as before. Since the size of DNA used influences the renaturation rate, the DNA was sheared to a uniform length of about 300,000 daltons for the single strands in a French pressure cell at 15,000 psi (20). The strains of bacteria were selected for use on the basis of the amount of satellite DNA present in their DNA. The $C_0t_{0.5}$ values for the moderate halophile with no satellite, isolate I, and for the extreme halophiles, isolate III with 36% satellite, *H. salinarium* with 19% satellite, and *H. cutirubrum* with 11% satellite, were found to be approximately 1.9 (Fig. 5).

The genome sizes of these bacteria can be determined by comparing the $C_0t_{0.5}$ values obtained to that of an organism whose genome size is known. For this purpose, the renaturation rate of *E. coli* DNA was examined under conditions identical to those used previously (Fig. 5). A $C_0t_{0.5}$ value of 2.9 was obtained. Wetmur and Davidson (33) have shown that high-GC DNA renaturates at a faster rate than that of lower GC. From their data, the rate of renaturation of *E. coli* DNA at 70 C should be 0.7 of that of the halophilic bacteria because of the difference in base composition. Taking this factor into consideration, and assuming the genome size of *E. coli* to be 4.5×10^6 nucleotide pairs (3), the genome size of the halophilic bacteria examined would be 4.1×10^6 nucleotide pairs, approximately the size of the *E. coli* genome.

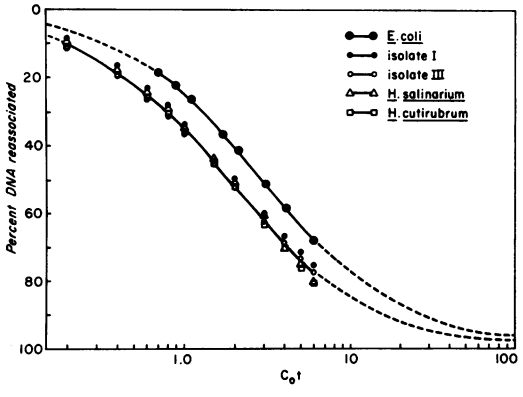

FIG. 5. *Renaturation curves of sheared, denatured DNA of halophilic bacteria. DNA was sheared at 15,000 psi in a French pressure cell and denatured by heat in 0.1 × SSC. Conditions for renaturation were the same as those described in Fig. 3. Dotted line is a theoretical function (2).*

DISCUSSION

The density distribution of the DNA from the vast majority of organisms is given by a single symmetrical peak. The base composition of individual segments in the bacterial genome is distributed in a Gaussian fashion about the mean with a range of 6%GC for molecules of 5×10^6 to 10×10^6 daltons (28). An exception to this rule exists in the crab, *Cancer borealis*, and in several mammals such as the mouse (28, 29). The only well-documented exception in bacterial DNA, however, other than cases where episomes are present, is that of the extremely halophilic bacteria. Some other exceptions may be revealed by detailed analysis of thermal denaturation curves (18).

The DNA of the temperate bacteriophage λ represents another exception to this rule. The two halves of the genome of λ differ in base composition by 10%GC (15). A similar pattern has been observed for other related lysogenic coliphages (10). The two halves of the λ molecule have also been shown to be separable from a functional as well as a physical point of view in the transcription of RNA specifying the production of the early and late enzymes (4, 26). The heterogeneity in the λ phage genome can be accounted for by supposing that the various segments have originated independently and have combined relatively recently. Whatever its origin, however, it may be supposed that each differentiated segment contains critical nucleotide sequences, each subject to different frequencies of mutation and selection (27). If one were to assume that one of the DNA components in the extremely halophilic bacteria represents a clustering of genes necessary for the unique property of halophilism, it is possible to imagine how grossly different base changes might be selected for in this group of genes eventually to give rise to a portion of the genome with large differences in base composition. At the present time, however, the assignment of the satellite DNA as a carrier of genetic determinants for halophilism is based only upon circumstantial evidence. It is simply that all extreme non-photosynthetic halophiles examined, both rods and cocci, contain this type of DNA, whereas it is absent in moderate or nonhalophiles (25).

Several lines of evidence suggest that the satellite DNA of extreme halophiles is not an episome of the usual size. Although cells containing certain episomes are often cured by acridine dyes, this could not be accomplished with the extreme halophiles (25). The sizes of most of the episomes

studied in bacteria are approximately the same as the λ DNA molecule, or 10^5 nucleotide pairs (7). Approximately 15 copies of such an episome would be required to account for the quantity of satellite DNA found in isolate III. The presence of multiple copies of identical stretches of base sequences is excluded by the kinetics of renaturation, which are those expected for a simple nonrepeating genome. The possibility that the satellite DNA represents repeated copies of segments of the chromosomal DNA originating by tandem duplication is also eliminated by the same data. In *E. coli*, repeated sequences of that part of the DNA responsible for ribosomal RNA do renature more rapidly than the bulk DNA in accordance with their higher relative concentration (D. E. Kohne, Biophys. J., *in press*). Similarly, in the DNA of *Proteus* strains containing certain R factors, the plasmid DNA renatures more rapidly and may be purified in this way (V. C. Jay, *personal communication*). However, samples taken during the renaturation of halophile DNA failed to reveal a difference in the rates of renaturation of the two components.

On the other hand, an episomal element of greater size could furnish a plausible explanation for the satellite DNA of extreme halophiles. For example, the R factor has the properties of a plasmid and can confer multiple drug resistance to individuals in all genera of the *Enterobacteriaceae* and to certain other genera (32). Available evidence shows that the different R factors contain various amounts of genetic material controlling a variable number of drug resistances, and suggests that this genetic material was accumulated from the genomes of several bacteria (11, 32). It would be possible for a plasmid of this type to account for the large amount of satellite material observed. Subsequent integration of parts of this material into the chromosome could account for the difference in the amount of satellite DNA in various strains. If this integration occurred early in the evolutionary history of the group, subsequent base changes would lead to differences in base sequence among various present-day species. This would also explain the fact that the extent of divergence of base sequence in the major and minor components among the various species is indistinguishable. The observation of almost identical buoyant density profiles for the DNA of two extreme halophiles, *H. morrhuae* and isolate III, which have completely different morphologies and show essentially no base sequence homology, is also consistent with this hypothesis. Again this mechanism would imply that the satellite DNA is functionally related to the halophilic mode of growth.

The base sequence homology studies indicate that the bacteria of the genus *Halobacterium* are closely related to one another, but not to the extremely halophilic bacteria of other genera or to the moderate or nonhalophilic bacteria. However, consistent with their relative conservatism (6, 8, 24, 31) the ribosomal cistrons show a greater degree of similarity among the various groups of halophilic bacteria. In fact, a good correlation seems to exist between a grouping of these bacteria based on their salt requirements for growth and a taxonomy based upon the stability of the ribosomal RNA-DNA heteroduplexes.

The possibility of a relationship between pigment production and the presence of satellite DNA in the extremely halophilic bacteria was suggested by the results of several investigators. Higuchi et al. (16) showed that induction of pigment synthesis in *R. spheroides* was presented by two inhibitors of DNA synthesis, mitomycin C and phleomycin, which do not inhibit the induction of amino-levulinic acid synthetase or catalase. Acriflavine was found to inhibit both pigment production and induction of these enzymes. The conclusion was drawn that pigment production requires the active synthesis of a unique extranuclear DNA. The existence of such a DNA component received support from the work of other authors who reported the presence of a satellite DNA in *R. spheroides* and *Chromatium* D (30) and in the blue-green alga, *Plectonema boryanum* (17). The presence of carotenoid pigments and satellite DNA in photosynthetic bacteria and extreme halophiles also raised the possibility of evolutionary relatedness among these two groups. Sunlight constitutes one of the dominant influences in the natural environment of both extreme halophiles and photosynthetic bacteria, and both are gram-negative, rod-shaped bacteria, often possessing polar flagella. It would, therefore, seem reasonable to speculate that the halophilic bacteria may have evolved from photosynthetic species. This could have occurred by selection following an increase in the salt concentration of the aquatic environment brought about by solar evaporation.

On the other hand, cultures of isolate III grown in the presence of mitomycin C, acriflavine, or acridine orange evidence neither a reduction in the amount of satellite DNA nor a loss of carotenoid pigments (25). Moreover, analysis of the DNA from several strains of *R. spheroides* failed to reveal the presence of a satellite DNA (25). DNA preparations from the blue-green algae examined by Edelman et al. (9) showed only a single DNA component and occasionally the presence of polysaccharides which could be misinterpreted as satellite DNA bands. Thus, there

appears to be no absolute correlation between pigment production and the presence of a satellite DNA. The results of the present study also failed to demonstrate any nucleotide sequence homology between the DNA or messenger RNA of the extreme halophile, isolate III, and DNA of the photosynthetic bacterium, *R. spheroides*. This is not necessarily unexpected, since, even if the suggestion that halophiles may have evolved from photosynthetic bacteria has validity, large changes in nucleotide sequence have certainly taken place in both DNA components from the time of the emergence of the halophilic strains. Some support for a distant relationship is offered by the homology of the conservative ribosomal RNA cistrons of isolate III with those of SL-1. A more thorough study of the relatedness between halophiles and photosynthetic bacteria is necessary, however, to draw more definite conclusions. The photosynthetic extreme halophile, SL-1, is potentially a most useful strain for this purpose, since it exhibits some of the properties of both groups.

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