Genome Size of Cyanobacteria

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The genome sizes of 128 strains of cyanobacteria, representative of all major taxonomic groups, lie in the range 1.6×10^9 to 8.6×10^9 daltons. The majority of unicellular cyanobacteria contain genomes of 1.6×10^9 to 2.7×10^9 daltons, comparable in size to those of other bacteria, whereas most pleurocapsalean and filamentous strains possess larger genomes. The genome sizes are discontinuously distributed into four distinct groups which have means of 2.2×10^9 , 3.6×10^9 , 5.0×10^9 and 7.4×10^9 daltons. The data suggest that genome evolution in cyanobacteria occurred by a series of duplications of a small ancestral genome, and that the complex morphological organization characteristic of many cyanobacteria may have arisen as a result of this process.

INTRODUCTION

All bacteria possess genomes which are relatively uniform in size, varying from 1.0×10^9 to 3.6×10^9 daltons (see Gillis *et al.*, 1970; Wallace & Morowitz, 1973). The only exception is a group of *Mycoplasma* strains which contain small genomes of 0.4×10^9 to 0.5×10^9 daltons (Bak *et al.*, 1969). Bak *et al.* (1970) listed a number of bacterial species which appeared to contain much larger genomes, but these species were subsequently shown to fall within the normal bacterial range of genome size (Gillis *et al.*, 1970; Pemberton, 1974).

The genomes of several strains of cyanobacteria previously examined are similar in size to those of other bacteria. The DNA of *Anacystis nidulans* (classified in this communication as *Synechococcus* PCC 6301) and *Lyngbya* sp. renatured with kinetics approximately similar to those of bacterial DNA (Kung *et al.*, 1972), although quantitative estimates of genome size were not obtained. The genome sizes of *A. nidulans* (*Synechococcus* PCC 6301) and *Anabaena cylindrica* (PCC 7122) were estimated to be 2.27×10^9 and 2.47×10^9 daltons, respectively (Herdman & Carr, 1974). The DNA of these strains renatured as a single kinetic class, suggesting the absence of reiterated DNA sequences, although such sequences may not have been detected because the early stages of the renaturation reaction were not followed. Roberts *et al.* (1977) reported that the DNA of *Agmenellum quadruplicatum* (classified here as *Synechococcus* PCC 7002) had a kinetic complexity within the range 2.2×10^9 to 2.8×10^9 daltons, and that reiterated DNA sequences were absent.

This communication describes the genome sizes of 128 strains of cyanobacteria, representative of all major taxonomic groups, as measured from the kinetics of renaturation of DNA. The results are compared with values previously obtained for other bacteria, and the taxonomic and evolutionary implications are discussed.

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METHODS

Organisms and culture conditions. The strains examined are listed in Tables 1 to 5. Their generic assignments, properties, strain histories and media employed for cultivation are fully described by Rippka *et al.* (1979), with the exception of *Oscillatoria agardhii* PCC 7805 which is described by Herdman *et al.* (1979).

Extraction of DNA. DNA was extracted and purified by the hydroxyapatite method described by Herdman *et al.* (1979) and stored in $0.1 \times$ SSC buffer (SSC buffer, pH 7.0, is 0.15 M-NaCl/0.015 M-trisodium citrate) over chloroform at 4 °C.

Estimation of genome size. Genome sizes were measured from the kinetics of renaturation of thermally denatured DNA. Purified DNA samples in $0.1 \times$ SSC buffer were normally adjusted to a concentration of 50 to 65 $\mu\text{g ml}^{-1}$ and the native DNA was fragmented by passage through a French press at 145 MPa (Gillis *et al.*, 1970). DNA samples (1.2 ml) were placed in sealed 1.5 ml cuvettes (1 cm light path) in a Gilford 240 spectrophotometer fitted with an automatic cuvette programmer, thermosensor and analogue multiplexer. The samples were equilibrated to $T_m - 20$ °C (where T_m is the temperature midpoint of thermal denaturation) and then thermally denatured by raising the temperature to $T_m + 20$ °C. The change in absorbance at 260 nm was recorded. After complete denaturation, the solutions were equilibrated to the optimal temperature for renaturation (T_{OR}) in the presence of 1 M- Na^+ , and then prewarmed 5 M-NaCl was added to raise the Na^+ concentration to 1 M. Renaturation commenced immediately and the resulting change in absorbance (ΔA_{260}) was recorded. The T_m in 1 M- Na^+ was calculated from the equation

$$\% \text{ GC} = 2.44 (T_m - 81.5 - 16.6 \log M)$$

where M is the molar concentration of Na^+ (Marmur & Doty, 1962), and the T_{OR} in 1 M- Na^+ was calculated from $T_{OR} = T_m - 25$ °C (Wetmur & Davidson, 1968), using the values of mean DNA base composition determined by Herdman *et al.* (1979). Three DNA samples were examined in each experiment; the fourth cuvette contained a solution of guanine (1.2 ml, A_{260} 1.3) to enable correction to be made for solvent expansion and electronic drift.

The second-order renaturation rate constant, k_2 , was calculated from the equation

$$\frac{A_0 - A_\infty}{A - A_\infty} = \frac{k_2 P_T}{2} t + 1$$

where A_∞ and A_0 are the absorbances of native and denatured DNA, respectively, A is the absorbance of the solution at time t and P_T is the total DNA phosphate concentration (Wetmur & Davidson, 1968). The genome mol. wt was calculated by reference to an *Escherichia coli* control of known genome size (strain W1485, 2.40×10^9 daltons) using the equation $k_A/k_B = M_B/M_A$ (Gillis *et al.*, 1970) where k_A , k_B , M_A and M_B represent the second-order renaturation rate constants (k) and genome mol. wts (M) of the control (A) and test organisms (B), respectively. The genome size (N_D) of the *E. coli* control was calculated from the alkaline sedimentation velocity and k_2 using the equation (Wetmur & Davidson, 1968)

$$N_D = \frac{5.5 \times 10^8 (S_{20,w}^{pH 13})^{1.25}}{k_2}$$

The results obtained were not corrected for any possible effect of base composition on renaturation rate since it has been shown (Gillis & De Ley, 1975) that this effect is negligible.

Control of the method. DNA concentration and fragment size, ionic strength of solvent and T_{OR} were rigorously controlled throughout. The purity of the DNA samples was routinely verified as described by Herdman *et al.* (1979); no contamination with protein or RNA was detectable in any sample. The high reproducibility of the method (as shown by low standard deviations from the mean in Tables 1 to 5) was achieved by initiating the renaturation reaction by elevation of Na^+ concentration rather than by thermal change, permitting the reaction to be recorded within 30 s of initiation. This technique also permitted the detection of reiterated DNA sequences which renatured rapidly in the early stages of the reaction.

For some strains, an independent estimate of genome size was obtained by chemical measurement (Burton, 1956, as described by Mann & Carr, 1974) of the minimum cell DNA content, corresponding to a single genome. This was achieved by measuring the cell DNA content at different growth rates; the intercept of the plot of log DNA content versus growth rate represents the single genome contained by cells at zero growth rate (Mann & Carr, 1974).

RESULTS AND DISCUSSION

The reliability of the method was verified with DNA extracted from *E. coli* W1485 and *Pseudomonas aeruginosa* 6352. The genome size of *E. coli* was 2.40×10^9 daltons, as estimated from the alkaline sedimentation velocity and k_2 ; this is in agreement with previous estimates (Gillis *et al.*, 1970). Renaturation of *P. aeruginosa* DNA revealed a genome of 2.54×10^9 daltons. This value differs from that (6.96×10^9 daltons) previously obtained from renaturation kinetics (Bak *et al.*, 1970) but is in close agreement with the estimate of 2.1×10^9 daltons obtained by electron microscope observation and velocity sedimentation analysis (Pemberton, 1974) and is similar to the values obtained for *P. fluorescens* (2.72×10^9 daltons) and *P. putida* (2.53×10^9 daltons) by Gillis *et al.* (1970).

Further verification of the accuracy of the renaturation method was obtained by comparison with the genome size derived from chemical estimates of cell DNA content at different growth rates by extrapolation to zero growth rate as described by Mann & Carr (1974). The estimated genome sizes for *Synechococcus* PCC 6301 (2.08×10^9 daltons), *Anabaena* PCC 7118 (3.61×10^9 daltons) and *Nostoc* PCC 7524 (5.80×10^9 daltons) corresponded closely to the kinetic complexity values of 2.12×10^9 , 3.74×10^9 and 5.61×10^9 daltons, respectively.

The genome sizes of cyanobacteria of Sections I to V are shown in Tables 1 to 5. Within the tables, the genera have been divided into their different DNA base compositional sub-groups (Herdman *et al.*, 1979) where possible; within each genus or sub-group the strains are arranged in order of increasing genome size. Strains which appear, by other criteria, to be closely related (Rippka *et al.*, 1979) are enclosed by braces, although specific names have not been appended. Some strains, originally thought to be of independent origin, are now considered to be identical isolates (Rippka *et al.*, 1979) and in these cases the data for only one such strain are given.

Genome sizes of cyanobacteria assigned to Section I

Section I contains six genera of unicellular cyanobacteria which reproduce either by binary fission (most genera) or by budding (*Chamaesiphon*). The genome sizes are shown in Table 1.

The genus *Synechococcus* (sheathless unicellular cyanobacteria which divide by binary fission in only one plane) has been divided into three groups of different DNA base composition (Herdman *et al.*, 1979). The five strains of sub-group 1 (39 to 43 mol% GC) vary in genome size from 2.55×10^9 to 4.20×10^9 daltons, and the mean genome size (3.16×10^9 daltons) is larger than that of the other *Synechococcus* sub-groups. The wide variation in genome size of the members of this sub-group is accompanied by diversity in other phenotypic properties (Rippka *et al.*, 1979). Strains 7418 and 7424, which contain large genomes of 3.08×10^9 and 4.20×10^9 daltons, respectively, are readily distinguished from the remaining strains by their large cell size, and strain 7424 is further distinguished by its ability to fix nitrogen under anaerobic conditions (Rippka & Waterbury, 1977). Strain 7511 (3.34×10^9 daltons) is able to grow photoheterotrophically at the expense of glucose (Rippka *et al.*, 1979). The members of sub-group 2 (47 to 56 mol% GC) fall into two further groups with respect to genome size. In the first, seven strains, which show marked phenotypic diversity, possess genomes of similar sizes (1.57×10^9 to 2.12×10^9 daltons, mean 1.81×10^9 daltons); three members of this group (6311, 6301 and 6908) appear to be closely related (Rippka *et al.*, 1979) and vary in genome size from 1.57×10^9 to 2.12×10^9 daltons. Strain 6910 contains a slightly larger genome (2.52×10^9 daltons), and is the only *Synechococcus* strain in which gliding motility has been observed (Stanier *et al.*, 1971). The second group contains two strains, 7425 and 7335, which have larger genomes of 3.09×10^9 and 3.11×10^9 daltons, respectively; although these strains differ from each other in many phenotypic properties they are both distinguished from all other members of sub-group 2 by their ability to fix

Table 1. *Genome sizes of cyanobacteria assigned to Section I*

Genus	Strain number		N*	k_2 (l mol ⁻¹ s ⁻¹) ± S.D.†	10 ⁻⁹ × Genome size (daltons) ± S.D.
	PCC	ATCC			
<i>Synechococcus</i>					
Sub-group 1	7502	29172	3	3.74 ± 0.06	2.55 ± 0.04
	7202	29140	3	3.60 ± 0.08	2.65 ± 0.06
	7418	29534	3	3.09 ± 0.07	3.08 ± 0.07
	7511	29154	4	2.85 ± 0.06	3.34 ± 0.07
	7424	29155	4	2.27 ± 0.18	4.20 ± 0.33
Sub-group 2	{ 6311	27145	4	6.07 ± 0.31	1.57 ± 0.08
	{ 6908	27146	3	5.74 ± 0.10	1.66 ± 0.03
	{ 6301	27144	9	4.50 ± 0.32	2.12 ± 0.15
	{ 7002	27264	6	5.67 ± 0.41	1.68 ± 0.12
	{ 6716	27179	3	5.54 ± 0.19	1.72 ± 0.06
	{ 7003	27265	5	4.86 ± 0.27	1.96 ± 0.11
	{ 6312	27167	3	4.81 ± 0.05	1.98 ± 0.02
	{ 6910	27191	6	3.78 ± 0.38	2.52 ± 0.25
	{ 7425	29141	3	3.08 ± 0.04	3.09 ± 0.04
	{ 7335	29403	3	3.06 ± 0.07	3.11 ± 0.07
Sub-group 3	7001	27194	3	4.74 ± 0.31	2.01 ± 0.13
	{ 6603	27168	3	4.47 ± 0.06	2.13 ± 0.03
	{ 6710	27174	5	4.31 ± 0.23	2.21 ± 0.12
	{ 6307	27147	3	3.72 ± 0.39	2.56 ± 0.27
	{ 7009	29203	3	3.91 ± 0.05	2.44 ± 0.03
<i>Synechocystis</i>					
Sub-group 1	6808	27189	4	4.50 ± 0.13	2.12 ± 0.06
	6711	27175	3	4.43 ± 0.21	2.15 ± 0.10
	6804	27185	5	3.95 ± 0.13	2.41 ± 0.08
	6701	27170	9	3.81 ± 0.14	2.50 ± 0.09
Sub-group 2	{ 6803	27184	8	5.32 ± 0.15	1.79 ± 0.05
	{ 6702	27171	4	4.65 ± 0.36	2.05 ± 0.16
	{ 6905	29109	4	4.58 ± 0.29	2.08 ± 0.13
	{ 7201	29152	3	4.33 ± 0.18	2.20 ± 0.09
	{ 6806	27187	4	4.13 ± 0.16	2.31 ± 0.09
	{ 6714	27178	3	4.07 ± 0.14	2.34 ± 0.08
	{ 7005	27153	3	4.63 ± 0.16	2.06 ± 0.07
	{ 6906	27266	3	4.06 ± 0.17	2.35 ± 0.10
	7509	29235	3	2.73 ± 0.31	3.50 ± 0.23
<i>Gloeobacter</i>	7421	29082	3	3.54 ± 0.24	2.69 ± 0.18
<i>Gloeocapsa</i>	7512	29115	3	3.29 ± 0.07	2.90 ± 0.06
	73106	27928	3	3.21 ± 0.31	2.97 ± 0.29
	7501	29113	3	2.77 ± 0.16	3.44 ± 0.20
	7428	29159	3	2.75 ± 0.13	3.47 ± 0.16
<i>Gloeotheca</i>	73108	29164	3	1.90 ± 0.04	5.02 ± 0.10
	7109	29163	3	1.83 ± 0.05	5.22 ± 0.15
<i>Chamaesiphon</i>	7430	29397	3	2.62 ± 0.07	3.64 ± 0.10
	6605	27169	4	2.54 ± 0.19	3.75 ± 0.39

* Number of determinations.

† Standard deviation.

nitrogen under anaerobic conditions (Rippka & Waterbury, 1977). The five members of sub-group 3 (66 to 71 mol % GC) have genomes of similar sizes (2.01×10^9 to 2.56×10^9 daltons) with a mean of 2.27×10^9 daltons. Three of these strains (6603, 6710 and 6307) appear to be closely related (Rippka *et al.*, 1979) and vary in genome size from 2.13×10^9 to 2.56×10^9 daltons.

Although the members of the genus *Synechocystis* (sheathless unicellular cyanobacteria which divide successively in two or three different planes) fall into two groups of different base composition (Herdman *et al.*, 1979), the majority of the strains possess genomes of similar size. The members of sub-group 1 (35 to 37 mol % GC) display several phenotypes but a narrow range of genome size (2.12×10^9 to 2.50×10^9 daltons, mean 2.30×10^9 daltons). In sub-group 2 (42 to 48 mol % GC) eight of the strains occupy a similar range (1.79×10^9 to 2.35×10^9 daltons) with a mean of 2.15×10^9 daltons, six of them being closely related (Rippka *et al.*, 1979). The remaining strain of sub-group 2, 7509, has a larger genome of 3.50×10^9 daltons and is further distinguished by its ability to grow photoheterotrophically at the expense of sucrose (Rippka *et al.*, 1979).

The other genera of Section I are each represented by a limited number of isolates. The genus *Gloeobacter* (ensheathed unicellular cyanobacteria, lacking thylakoids, which divide in one plane) contains only one strain, which possesses a genome of 2.69×10^9 daltons. *Gloeocapsa* (ensheathed unicellular cyanobacteria dividing successively in three different planes) is represented by four strains which differ both in genome size (2.90×10^9 to 3.47×10^9 daltons, mean 3.20×10^9 daltons) and in other properties (Rippka *et al.*, 1979). The two strains of *Gloeotheca* (ensheathed unicellular cyanobacteria which divide in a single plane) possess the largest genomes (5.02×10^9 and 5.22×10^9 daltons) of any unicellular prokaryote so far investigated. These strains also differ from all other unicellular cyanobacteria in their capacity to fix nitrogen under aerobic conditions (Rippka & Waterbury, 1977). The two members of the genus *Chamaesiphon* are distinguished from all other unicellular cyanobacteria by their mode of reproduction (bud formation). Although these strains differ from each other in many properties (Waterbury & Stanier, 1977), their genomes are similar both in base composition (Herdman *et al.*, 1979) and size (3.64×10^9 and 3.75×10^9 daltons).

The distribution of genome sizes of all unicellular cyanobacteria of Section I is shown in Fig. 1(a). With the exception of the genus *Gloeotheca*, the overall range (1.6×10^9 to 4.2×10^9 daltons) is similar to that of other bacteria (Gillis *et al.*, 1970; Wallace & Morowitz, 1973).

Genome sizes of cyanobacteria assigned to Section II

The members of Section II (pleurocapsalean strains) reproduce by the release of small baeocytes (Waterbury & Stanier, 1978) which arise by multiple fission within a larger vegetative cell. Full descriptions of the strain properties are given by Waterbury & Stanier (1978). The genome sizes of the members of this section are shown in Table 2.

The genus *Chroococcidiopsis* is divided into two groups of different DNA base composition (Herdman *et al.*, 1979) which, from the study of a limited number of strains, also differ in genome size. Strain 6712 (40 mol % GC) contains a genome of 3.31×10^9 daltons; the remaining three strains (46 mol % GC) possess larger genomes (4.06×10^9 to 4.75×10^9 daltons, mean 4.48×10^9 daltons). The other genera of Section II (*Dermocarpa*, *Dermocarpella*, *Xenococcus*, *Myxosarcina* and the Pleurocapsa group) vary in genome size from 3.07×10^9 to 4.39×10^9 daltons; since few members of these genera have been examined the intrageneric variation in genome size remains unknown.

In spite of the wide variation in morphological and physiological properties in the members of Section II (Waterbury & Stanier, 1978), the 11 strains examined exhibit a narrow range of DNA base composition (Herdman *et al.*, 1979) and genome size (Fig. 1b); the genome sizes are clustered around the upper limit of the range of other bacteria, with a mean of 3.78×10^9 daltons.

Fig. 1. The distribution of the genome sizes of cyanobacteria assigned to Sections I to V.

Table 2. *Genome sizes of cyanobacteria assigned to Section II*

Genus	Strain number		N	k_2 ($l\ mol^{-1}\ s^{-1}$) $\pm$ S.D.	$10^{-9} \times$ Genome size (daltons)
	PCC	ATCC			$\pm$ S.D.
<i>Dermocarpa</i>	7301	29367	3	3.10 ± 0.06	3.07 ± 0.05
	7438	29372	4	2.17 ± 0.09	4.39 ± 0.19
<i>Xenococcus</i>	7307	29375	3	2.39 ± 0.06	3.99 ± 0.13
<i>Dermocarpella</i>	7326	29376	3	2.86 ± 0.06	3.33 ± 0.07
<i>Myxosarcina</i>	7312	29377	3	2.70 ± 0.08	3.53 ± 0.10
<i>Chroococcidiopsis</i>					
Sub-group 1	6712	27176	4	2.88 ± 0.10	3.31 ± 0.11
Sub-group 2	{ 7203	27900	3	2.35 ± 0.15	4.06 ± 0.26
	{ 7433	29381	3	2.01 ± 0.05	4.75 ± 0.12
	{ 7434	29382	3	2.06 ± 0.07	4.62 ± 0.15
Pleurocapsa group	7322	29391	3	3.04 ± 0.08	3.13 ± 0.08
	7320	29389	3	2.78 ± 0.07	3.43 ± 0.09

Genome sizes of cyanobacteria assigned to Section III

Section III (Table 3) contains filamentous cyanobacteria which do not produce heterocysts. The overall range of genome size (2.14×10^9 to 5.19×10^9 daltons) is similar to that of Section I, although the distribution of sizes (Fig. 1c) is different.

The single strain of the genus *Spirulina* (filamentous cyanobacteria that produce motile, helical trichomes) examined differs from the majority of the members of Section III in possessing a small genome of 2.53×10^9 daltons.

The genomes of the six strains of *Oscillatoria* (filamentous cyanobacteria that produce motile, sheathless or thinly ensheathed trichomes composed of disc-shaped cells) vary from 2.50×10^9 to 4.38×10^9 daltons (mean 3.62×10^9 daltons). The recent isolate of *Oscillatoria agardhii* (PCC 7805) is distinguished from all other *Oscillatoria* strains by the abundance and irregular distribution of gas vacuoles and contains the smallest genome (2.50×10^9 daltons) of the strains examined in this group. Three strains, which differ from one another in many properties but share the ability to fix nitrogen under anaerobic conditions (Rippka *et al.*, 1979), possess larger genomes (3.15×10^9 to 3.86×10^9 daltons). The remaining two strains, genome sizes 4.20×10^9 and 4.38×10^9 daltons, seem to be closely related in other respects; they are both obligate autotrophs, incapable of fixing nitrogen under anaerobic conditions (Rippka *et al.*, 1979).

The seven members of the genus *Pseudanabaena* (filamentous cyanobacteria that produce motile, sheathless trichomes composed of cylindrical cells which contain polar gas vacuoles) exhibit a range of genome size from 2.14×10^9 to 3.77×10^9 (mean 3.05×10^9) daltons. Although many of these strains contain genomes of similar size, they differ extensively from each other in their phenotypic properties (Rippka *et al.*, 1979). Strain 6901 contains the smallest genome of all filamentous cyanobacteria.

The LPP group is a provisional category containing a large number of strains for which generic assignments have not yet been made; the taxonomic difficulties are discussed by Rippka *et al.* (1979). The 24 strains can be divided into three distinct sub-groups of different genome size. The first contains two strains of genome size 2.58×10^9 and 2.63×10^9 daltons. The genomes of the 12 strains of the second sub-group vary from 3.16×10^9 to 4.08×10^9 daltons (mean 3.72×10^9 daltons) and the third sub-group contains 10 strains with genomes of 4.53×10^9 to 5.19×10^9 daltons (mean 4.86×10^9 daltons). The members of each of these sub-groups are highly diverse phenotypically (Rippka *et al.*, 1979) and in mean DNA base composition (Herdman *et al.*, 1979). Although five strains of the LPP group are indistinguishable by all other phenotypic characters examined (Rippka *et al.*, 1979) they fall into two different genome size groups; one contains strains 7505 and 6402 (3.86×10^9 and 3.99×10^9 daltons, respectively) and the other 6306, 7410 and 73110 (4.78×10^9 to 5.15×10^9 daltons). Two members of the latter group, 6306 and 73110, have been shown by DNA hybridization studies to share 98% homology (Stam & Venema, 1977: the strains were identified as UTEX 581 and 594 respectively).

Genome sizes of cyanobacteria assigned to Section IV

Section IV (Table 4) contains heterocystous cyanobacteria whose cells divide in a single plane to produce unbranched filaments. The genera of this section have been placed into two groups based on their ability to form hormogonia (Rippka *et al.*, 1979). Section IVa contains three genera (*Anabaena*, *Nodularia* and *Cylindrospermum*) which do not produce hormogonia; Section IVb contains the hormogonia-producing genera *Nostoc*, *Calothrix* and *Scytonema*.

The six strains of the genus *Anabaena*, which possess trichomes composed of spherical, ovoid or cylindrical vegetative cells and containing both intercalary and terminal heterocysts, vary in genome size from 3.17×10^9 to 3.89×10^9 daltons. They have been divided into

Table 3. *Genome sizes of cyanobacteria assigned to Section III*

Genus	Strain number		N	k_2	$10^{-9} \times$ Genome size
	PCC	ATCC		(l mol ⁻¹ s ⁻¹) ± s.d.	(daltons) ± s.d.
<i>Oscillatoria</i>	7805	—	3	3.81 ± 0.07	2.50 ± 0.05
	6412	29205	3	3.03 ± 0.06	3.15 ± 0.06
	7515	29209	3	2.62 ± 0.06	3.63 ± 0.08
	6602	27935	3	2.47 ± 0.07	3.86 ± 0.11
	{ 7412	29135	3	2.27 ± 0.05	4.20 ± 0.09
	{ 6304	27930	3	2.18 ± 0.09	4.38 ± 0.19
<i>Pseudanabaena</i>	6901	27263	5	4.45 ± 0.21	2.14 ± 0.10
	7402	29207	3	3.80 ± 0.15	2.51 ± 0.10
	7367	29137	3	3.23 ± 0.07	2.95 ± 0.06
	7403	29210	3	3.01 ± 0.10	3.17 ± 0.11
	7429	29536	3	2.82 ± 0.08	3.38 ± 0.10
	6802	27183	3	2.78 ± 0.22	3.43 ± 0.28
	6406	29118	3	2.53 ± 0.20	3.77 ± 0.30
<i>Spirulina</i>	6313	29542	3	3.77 ± 0.06	2.53 ± 0.04
LPP group	7407	29126	1	3.70	2.58
	7376	29410	3	3.62 ± 0.08	2.63 ± 0.06
	7123	29122	3	3.02 ± 0.17	3.16 ± 0.18
	6409	29119	1	2.85	3.34
	7105	29120	3	2.81 ± 0.05	3.39 ± 0.06
	7406	29125	3	2.69 ± 0.10	3.54 ± 0.13
	6703	27907	3	2.65 ± 0.05	3.60 ± 0.07
	7427	29129	3	2.48 ± 0.11	3.85 ± 0.17
	7505	29170	3	2.47 ± 0.07	3.86 ± 0.10
	7411	29127	3	2.43 ± 0.07	3.92 ± 0.11
	7124	29123	3	2.42 ± 0.05	3.93 ± 0.08
	7375	29409	3	2.42 ± 0.04	3.93 ± 0.06
	6402	27902	3	2.39 ± 0.07	3.99 ± 0.11
	7420	29128	4	2.34 ± 0.04	4.08 ± 0.07
	7004	27913	3	2.10 ± 0.13	4.53 ± 0.29
	7419	29346	3	2.08 ± 0.07	4.58 ± 0.16
	7409	29541	4	2.06 ± 0.12	4.63 ± 0.27
	7104	29117	3	2.05 ± 0.04	4.65 ± 0.09
	7404	29165	3	1.92 ± 0.06	4.96 ± 0.15
	{ 6306	27894	3	1.99 ± 0.08	4.78 ± 0.20
	{ 7410	29136	3	1.91 ± 0.05	4.98 ± 0.13
	{ 73110	29407	4	1.85 ± 0.04	5.15 ± 0.12
	7113	29206	1	1.87	5.10
	7408	29344	4	1.84 ± 0.03	5.19 ± 0.09

two groups (indicated by braces in Table 4) of different phenotypic properties (Rippka *et al.*, 1979). The single representative of the genus *Nodularia*, which differs from *Anabaena* primarily in cell shape (Rippka *et al.*, 1979), contains a genome of 3.34×10^9 daltons. *Cylindrospermum*, which produces only terminal heterocysts and sub-terminal akinetes, is represented by two phenotypically similar strains which contain genomes of 5.71×10^9 and 6.15×10^9 daltons. The genome sizes of *Anabaena* and *Nodularia* strains therefore fall within the normal range of bacterial genome size and are significantly smaller than those of *Cylindrospermum* and the members of Section IVb described below.

The 11 *Nostoc* strains, which differ from *Anabaena* primarily in their ability to produce hormogonia, exhibit a wide range of genome size (4.00×10^9 to 6.42×10^9 daltons, mean 5.09×10^9 daltons). Similarity of genome size, however, does not imply close relatedness since many strains of similar genome size differ markedly in other phenotypic properties (see Rippka *et al.*, 1979). The members of the genus *Calothrix* possess tapering trichomes with a terminal heterocyst at only one end; growth results both in elongation of the trichome

Table 4. *Genome sizes of cyanobacteria assigned to Section IV*

Genus	Strain number		N	k_2 (l mol ⁻¹ s ⁻¹) ± s.d.	10 ⁻⁹ × Genome size (daltons) ± s.d.
	PCC	ATCC			
<i>Anabaena</i>	7122	27899	3	3.01 ± 0.17	3.17 ± 0.18
	6309	29211	3	2.68 ± 0.17	3.56 ± 0.22
	7120	27893	3	2.55 ± 0.07	3.74 ± 0.10
	7118	27892	3	2.55 ± 0.05	3.74 ± 0.08
	7119	29151	3	2.47 ± 0.06	3.86 ± 0.09
	6411	27898	4	2.45 ± 0.14	3.89 ± 0.22
<i>Nodularia</i>	73104	29167	3	2.85 ± 0.15	3.34 ± 0.17
<i>Cylindrospermum</i>	73101	29535	3	1.67 ± 0.09	5.71 ± 0.32
	7417	29204	3	1.55 ± 0.08	6.15 ± 0.47
<i>Nostoc</i>	6719	29105	4	2.38 ± 0.09	4.00 ± 0.15
	7413	29106	3	2.14 ± 0.06	4.46 ± 0.12
	6310	27896	3	2.11 ± 0.04	4.52 ± 0.09
	7416	29107	3	1.99 ± 0.09	4.78 ± 0.22
	73102	29133	4	1.95 ± 0.16	4.89 ± 0.39
	7423	29168	3	1.94 ± 0.07	4.91 ± 0.18
	6314	27904	3	1.86 ± 0.04	5.12 ± 0.12
	6705	29131	3	1.72 ± 0.15	5.55 ± 0.48
	7524	29411	4	1.70 ± 0.11	5.61 ± 0.36
	6302	27897	3	1.67 ± 0.09	5.71 ± 0.32
	7422	29132	3	1.48 ± 0.09	6.42 ± 0.39
<i>Calothrix</i>	6303	29156	3	1.88 ± 0.07	5.07 ± 0.19
	7103	27905	3	1.82 ± 0.07	5.23 ± 0.21
	7204	29190	3	1.81 ± 0.04	5.26 ± 0.13
	7111	29199	3	1.78 ± 0.13	5.35 ± 0.40
	7415	29157	3	1.78 ± 0.10	5.35 ± 0.32
	7116	29111	3	1.76 ± 0.07	5.41 ± 0.22
	7507	29112	3	1.75 ± 0.09	5.46 ± 0.28
	7101	27914	3	1.23 ± 0.03	7.75 ± 0.18
	7426	29345	3	1.17 ± 0.07	8.15 ± 0.49
<i>Scytonema</i>	7102	27901	3	1.11 ± 0.07	8.58 ± 0.55
	7110	29171	4	1.29 ± 0.06	7.40 ± 0.36

and in increased diameter of the basal vegetative cells, and is accompanied by the differentiation of new, larger heterocysts from the sub-terminal cell. The members of this genus therefore display an organization totally different from that of any other filamentous heterocystous cyanobacteria. The 10 strains examined fall into two groups on the basis of genome size; the first contains seven strains with genomes of 5.07×10^9 to 5.46×10^9 daltons (mean 5.30×10^9 daltons), and the second contains three strains with larger genomes (7.75×10^9 to 8.58×10^9 daltons, mean 8.16×10^9 daltons). The sub-division of the genus in this way is not consistent with any other known phenotypic properties (Rippka *et al.*, 1979) of its strains. The single representative of the genus *Scytonema* resembles the *Calothrix* strains in producing hormogonia which develop a single terminal heterocyst; subsequent growth, however, produces a trichome of uniform width which, unlike *Calothrix*, contains intercalary heterocysts. The genome size of 7.40×10^9 daltons is close to those of the second sub-group of *Calothrix*.

The members of Section IV (Fig. 1*d*) therefore show a wider variation in genome size (3.17×10^9 to 8.58×10^9 daltons) than any other section. This variation is accompanied by extensive variation in morphological complexity (see Figs 33 to 69 of Rippka *et al.*, 1979) and in physiological properties. The members of the second *Calothrix* group, together with the single *Scytonema* strain, possess the largest genomes of all prokaryotes so far examined.

Table 5. *Genome sizes of cyanobacteria assigned to Section V*

Genus	Strain number		N	k_2 ($l \text{ mol}^{-1} \text{ s}^{-1}$) $\pm$ S.D.	$10^{-9} \times$ Genome size (daltons) $\pm$ S.D.
	PCC	ATCC			
<i>Chlorogloeopsis</i>	6912	27193	4	2.27 ± 0.11	4.20 ± 0.21
	6718	27181	3	1.82 ± 0.12	5.24 ± 0.35
<i>Fischerella</i>	7521	29538	3	2.63 ± 0.07	3.62 ± 0.10
	73103	29114	3	2.37 ± 0.07	4.02 ± 0.12
	7115	27929	3	2.09 ± 0.12	4.55 ± 0.26
	7414	29161	3	2.01 ± 0.08	4.75 ± 0.19

Table 6. *Rapidly renaturing fractions in cyanobacterial DNA*

Section	Genus	Strain number PCC	k_2 ($l \text{ mol}^{-1} \text{ s}^{-1}$)	$10^{-6} \times$ Kinetic complexity (daltons)	% of total genome	Copy number
I	<i>Gloeotheca</i>	73108	2464	3.9	5.9	76
I	<i>Gloeocapsa</i>	73106	3876	2.5	5.7	67
I	<i>Synechocystis</i>	6711	5904	1.6	4.8	64
		6701	1592	6.0	9.4	40
		7005	1133	8.4	10.8	26
II	<i>Xenococcus</i>	7305	385	25*	20*	33*
III	<i>Oscillatoria</i>	6412	833	11.0	11.5	32
III	LPP group	7406	287	33.2	23.3	25
		7375	273	34.9	23.6	24
IV	<i>Anabaena</i>	7120	1780	5.4	5.8	40
		7119	1321	7.2	7.4	40
		6411	1548	6.2	6.0	37
IV	<i>Nostoc</i>	6719	1183	8.1	8.8	43

* Approximate values only; the genetic complexity of strain 7305 was not accurately determined because the excessive quantity of rapidly renaturing DNA prevented the establishment of true second-order renaturation kinetics of the main genome.

Genome sizes of cyanobacteria assigned to Section V

Section V (Table 5 and Fig. 1 e) contains those heterocystous cyanobacteria which divide in more than one plane. The genera *Chlorogloeopsis* and *Fischerella* display complex morphological forms and produce hormogonia and akinetes (Rippka *et al.*, 1979). Both show a wide intragenetic diversity in genome size which is not accompanied by variation in other phenotypic properties within the members of each genus. Although *Chlorogloeopsis* and *Fischerella* differ markedly in structural respects their overall ranges of genome sizes are similar (4.20×10^9 to 5.24×10^9 daltons and 3.62×10^9 to 4.75×10^9 daltons, means 4.74×10^9 and 4.24×10^9 daltons, respectively).

Rapidly renaturing fractions in cyanobacterial DNA

Rapidly renaturing fractions were found in 13 strains of cyanobacteria. The data for these strains are shown in Table 6, and representative examples of renaturation kinetics of reiterated DNA from strains of six genera are compared with related strains not possessing reiterated sequences in Fig. 2. The kinetic complexity of such fractions varied from 1.6×10^6 to 3.5×10^7 daltons, and the number of copies varied from 24 to 76 per single copy of the major chromosomal fraction. In most strains, the rapidly renaturing fractions represented

Fig. 2. Rapidly renaturing fractions in the DNA of strains from six cyanobacterial genera. For each genus a strain of similar genome size, exhibiting normal second-order renaturation kinetics, is also shown (●). (The concentration of DNA in each test solution, expressed in $\mu\text{g ml}^{-1}$, is shown in parenthesis.) (a) *Anabaena*: ○, 7119 (46.1); ●, 7118 (44.2). (b) *Gloeocapsa*: ○, 73106 (29.7); ●, 7428 (37.3). (c) *Oscillatoria*: ○, 6412 (48.2); ●, 6602 (44.2). (d) LPP group: ○, 7375 (42.2); ●, 7420 (31.4). (e) *Synechocystis*: ○, 6701 (39.4); ●, 6808 (43.2). (f) *Xenococcus*: ○, 7305 (43.5); ●, 7307 (43.4).

between 5 and 12% of the total genome; cyanophages have not been detected in cells of these strains. In strains 7406 and 7375 (LPP group) and 7305 (*Xenococcus*) the rapidly renaturing fractions represented a significantly greater proportion (20 to 24%) of the total cell DNA; cyanophage-like structures have been observed in electron micrographs of strains 7305 (J. B. Waterbury, personal communication) and 7406 (G. Guglielmi, personal communication) and may be the source of the rapidly renaturing fractions. The nature of such fractions in the DNA isolated from the remaining 11 strains remains to be determined.

The evolution of cyanobacterial genomes

Cyanobacteria constitute the largest, most diverse and most widely distributed group of photosynthetic prokaryotes (Stanier & Cohen-Bazire, 1977). They also show a remarkable range of genetic complexity; the lower limit of cyanobacterial genome size (1.6×10^9 daltons) is similar to that of other bacteria but the upper limit (8.6×10^9 daltons) far exceeds the largest genome previously described in prokaryotes.

The results suggest that, within the cyanobacteria as a whole, there is a correlation between genome size and morphological complexity, since strains which exhibit a high degree of morphological differentiation almost invariably possess genomes which are larger

Fig. 3. Frequency distribution of cyanobacterial genome sizes.

than those of strains which display more simple morphological forms. The genome sizes of all 128 strains of cyanobacteria examined are shown in Fig. 3. The distribution is discontinuous: the genomes fall into four discrete groups which occupy the ranges ($\times 10^9$ daltons): 1.6 to 2.9, 2.9 to 4.4, 4.4 to 5.8 and 6.0 to 8.8, respectively. The first three of these groups each contain a large number of strains (34, 52 and 36, respectively) and show no internal discontinuities. The fourth contains only six strains, of markedly different genome sizes, and may possibly represent several further groups; the limited number of strains available does not permit the immediate further resolution of this group.

The mean genome sizes of the four groups are ($\times 10^9$ daltons $\pm$ standard deviation): 2.21 ± 0.31 , 3.56 ± 0.37 , 5.00 ± 0.39 and 7.40 ± 0.95 . These values correspond closely to those which would be obtained by a process of genome evolution involving a series of fusions of a smaller ancestral genome of 1.2×10^9 daltons, creating genomes containing two (2.4×10^9 daltons), three (3.6×10^9 daltons), four (4.8×10^9 daltons) and six (7.2×10^9 daltons) copies of the ancestral genome. The redundant DNA so created would be available for the development of new genetic information by mutation, permitting the subsequent evolution of a diversity of morphological forms.

This mechanism of genome evolution has been proposed previously to account for genome structure in other bacteria. Wallace & Morowitz (1973) suggested, from the frequency distribution of bacterial genome sizes, that the genome of *Acholeplasma* (mean size 1.0×10^9 daltons) arose by duplication of a *Mycoplasma* genome of 0.5×10^9 daltons, and that the more complex (larger) genomes of other bacteria arose by further duplication. This proposal was supported by the analysis of the positions of related genes on the *E. coli* genetic map which suggested that the genome had arisen by one or two duplications of a smaller ancestral genome (Zipkas & Riley, 1975). The present results support the hypothesis of genome evolution by duplication and suggest that it may have occurred in many different prokaryotic groups. The results further suggest an evolutionary mechanism by which the complex morphological organization characteristic of many cyanobacteria may have arisen.

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REFERENCES

- BAK, A. L., BLACK, F. T., CHRISTIANSEN, C. & FREUNDT, E. A. (1969). Genome size of mycoplasmal DNA. *Nature, London* **224**, 1209–1210.
- BAK, A. L., CHRISTIANSEN, C. & STENDERUP, A. (1970). Bacterial genome sizes determined from DNA renaturation studies. *Journal of General Microbiology* **64**, 377–380.
- BURTON, K. (1956). A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochemical Journal* **62**, 315–323.
- GILLIS, M. & DE LEY, J. (1975). Determination of the molecular complexity of double-stranded phage genome DNA from initial renaturation rates. The effect of DNA base composition. *Journal of Molecular Biology* **98**, 447–464.
- GILLIS, M., DE LEY, J. & DECLEENE, M. (1970). The determination of molecular weight of bacterial genome DNA from renaturation rates. *European Journal of Biochemistry* **12**, 143–153.
- HERDMAN, M. & CARR, N. G. (1974). Estimation of the genome size of blue-green algae from DNA renaturation rates. *Archives of Microbiology* **99**, 251–254.
- HERDMAN, M., JANVIER, M., WATERBURY, J. B., RIPPKA, R., STANIER, R. Y. & MANDEL, M. (1979). Deoxyribonucleic acid base composition of cyanobacteria. *Journal of General Microbiology* **111**, 63–71.
- KUNG, S. D., MOSCARELLO, M. A. & WILLIAMS, J. P. (1972). Studies with deoxyribonucleic acid from blue-green algae. *Plant Physiology* **49**, 331–334.
- MANN, N. & CARR, N. G. (1974). Control of macromolecular composition and cell division in the blue-green alga *Anacystis nidulans*. *Journal of General Microbiology* **83**, 399–405.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- PEMBERTON, J. M. (1974). Size of the chromosome of *Pseudomonas aeruginosa* PAO. *Journal of Bacteriology* **119**, 748–752.
- RIPPKA, R. & WATERBURY, J. B. (1977). The synthesis of nitrogenase by non-heterocystous cyanobacteria. *FEMS Microbiology Letters* **2**, 83–86.
- RIPPKA, R., DERUELLES, J., WATERBURY, J. B., HERDMAN, M. & STANIER, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111**, 1–61.
- ROBERTS, T. M., KLOTZ, L. C. & LOEBLICH, A. R. (1977). Characterisation of a blue-green algal genome. *Journal of Molecular Biology* **110**, 341–361.
- SIAM, W. T. & VENEMA, G. (1977). The use of DNA-DNA hybridization for determination of the relationship between some blue-green algae (Cyanophyceae). *Acta botanica neerlandica* **26**, 327–342.
- STANIER, R. Y. & COHEN-BAZIRE, G. (1977). Phototrophic prokaryotes: the cyanobacteria. *Annual Review of Microbiology* **31**, 225–274.
- STANIER, R. Y., KUNISAWA, R., MANDEL, M. & COHEN-BAZIRE, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). *Bacteriological Reviews* **35**, 171–205.
- WALLACE, D. C. & MOROWITZ, H. J. (1973). Genome size and evolution. *Chromosoma* **40**, 121–126.
- WATERBURY, J. B. & STANIER, R. Y. (1977). Two unicellular cyanobacteria which reproduce by budding. *Archives of Microbiology* **115**, 249–257.
- WATERBURY, J. B. & STANIER, R. Y. (1978). Patterns of growth and development in pleurocapsalean cyanobacteria. *Bacteriological Reviews* **42**, 2–44.
- WETMUR, J. G. & DAVIDSON, N. (1968). Kinetics of renaturation of DNA. *Journal of Molecular Biology* **31**, 349–370.
- ZIPKAS, D. & RILEY, M. (1975). Proposal concerning the evolution of the genome of *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 1354–1358.