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Genome Multiplication as Related to the Growth Rate in Blue-green Algae *Anacystis nidulans*

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Evidence is presented to support the hypothesis that the increase in the cellular DNA content in *Anacystis nidulans*, which occurs in association with an increase in growth rate, indicates an increase in the number of genomes in the cell.

The extent of killing and mutant reversion effected by the mutagen nitrosoguanidine was far greater in a slow-growing culture than in a fast-growing one. And, when DNA synthesis was inhibited by mitomycin C, the number of division cycles taking place before growth ceased was larger in fast-growing cultures than in slow-growing ones.

Key words: *Anacystis nidulans* — Cellular DNA — DNA synthesis — Genome — Growth rate.

The cellular organization of DNA, its quantity in the cell and its replication in relation to the growth rate have been studied extensively in heterotrophic bacteria (Cooper and Helmstetter 1968, Kubitschek and Freedman 1971). But, only a few reports have been published on this subject in the autotrophic blue-green algae. Mann and Carr (1974) investigated changes in the cell DNA content in *Anacystis nidulans* in relation to its growth rate. They found a striking difference between *Salmonella typhimurium* and the blue-green algae. In *S. typhimurium*, the DNA content per cell decreases as the growth rate decreases until the dt is equal to the C+D period. From that time on the DNA content becomes constant (Helmstetter 1974, Helmstetter et al. 1968). In *A. nidulans*, the DNA content per cell decreases continually as the growth rate decreases, even at a stage when generation time exceeds the C+D period (Herdman et al. 1970). An additional difference between heterotrophic bacteria and blue-green algae is that in the former, a great variety of auxotrophs has been isolated, whereas in the latter there has been only limited success in isolating such mutants (Delaney et al. 1976). We here propose that the differences between these organisms stem from the polyploid state of the blue-green algae, a thesis suggested by Ueda (1971) on the basis of cytochemical evidence and by Roberts et al. (1977), who reported on the DNA content per cell and on the kinetic complexity of the genome. Based on our results with NTG and the DNA-synthesis inhibitor mitomycin C, we propose that the number of genomes in the blue-green algae *Anacystis nidulans* is not fixed, but is related closely to the growth rate.

Abbreviations: dt, doubling time; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Materials and Methods

Anacystis nidulans (Strain No. 625, Culture Collection of Algae, Department of Botany, Indiana University, Bloomington, Indiana, U.S.A.) was grown in a batch culture or in a turbidostat in minimal medium as described by Allen (1968) and modified by Herdman et al. (1973). Growth flasks were aerated with an air-CO_2 mixture (97 : 3 v/v) at 38°C and illuminated by four 20-W cool white fluorescent lights. Modification of the growth rate was obtained by modifying the rate of irradiance per cell as follows: In batch cultures, the intensity of the irradiance was regulated by varying the distance between the flasks and the light source; in turbidostat cultures, the light intensity was kept constant and its availability to the culture was modified by varying the population densities.

Cell counts were made in a Neubauer counting chamber, and the increase in cell mass was measured at 650 nm with a Beckman Model 24 spectrophotometer. DNA was determined by the diphenylamine method of Burton (1956) with D-deoxyribose as the standard. Surface growth of *A. nidulans* was measured by the procedure described by Herdman et al. (1973).

Killing and mutagenesis by NTG—Cultures in the logarithmic phase of growth were washed in fresh medium and, after 1 hr of growth, NTG was added to a final concentration of 60 $\mu g \cdot ml^{-1}$. One-milliliter samples were removed at intervals and washed, then they were resuspended in fresh sterile medium and plated. During the exposure period, lights were dimmed to avoid the inactivation of the NTG.

Growth arrest by mitomycin C—Mitomycin C (Sigma) dissolved in water (500 $\mu g \cdot ml^{-1}$) was added to the culture, which then was adjusted to the desired final concentration. Mitomycin C solution was stored at −10°C for not more than one day.

Results

Growth rate and DNA content

Preliminary experiments revealed that cells of *A. nidulans* grown in a turbidostat at different rates had varied DNA contents per cell. Cells growing quickly contained a larger amount of DNA per cell than slow growing cells. The content of DNA per cell seems to be a function of the growth rate rather than of the particular light regimen under which the cells were growing, as the phenomenon of a decrease in the DNA content per cell associated a with decrease in the growth rate also was observed

Table 1 DNA content per cell[a] in *A. nidulans* as related to growth rate

NaCl (M)	Growth rate (hr^{-1})	DNA content (10^{-15} g/cell)
Control	0.139	6.3
0.1	0.118	4.5
0.2	0.101	4.0
0.3	0.058	3.0
0.4	0.046	1.4

[a] The DNA content was determined after each culture had reached a steady state of growth.

when the agent modifying the growth rate was NaCl (Table 1). In agreement with the results of Mann and Carr (1977), DNA per cell continued to decrease even after the doubling time exceeded C+D.

To check the hypothesis that the increase in the DNA content in fast-growing cells represents not only an increase in the number of replication forks, but an increase in the number of complete genomes in the cell, the response of the different cultures to a mutagen and to a DNA-synthesis inhibitor was studied. In terms of this hypothesis, slow growing cells should be more sensitive to both chemicals than fast growing cells.

Effect of NTG

NTG interferes with DNA replication and leads either to mutant formation or to the death of cells (Adelberg et al. 1965). Cultures of *A. nidulans*, in which either constant high or low growth rates were maintained by modifying the light intensity, were exposed to NTG at a concentration of 60 $\mu g \cdot ml^{-1}$ (Fig. 1). At all the exposure times, the rate of death due to NTG for cells growing at a low growth rate (dt=20 hr) was far greater than that observed in the fast growing cells (dt=5 hr). The same phenomenon was observed in cultures induced to grow at different rates by various NaCl concentrations.

In order to distinguish between the effects of light and of the DNA content on the response to NTG, a slow growing culture was exposed to a high intensity of light.

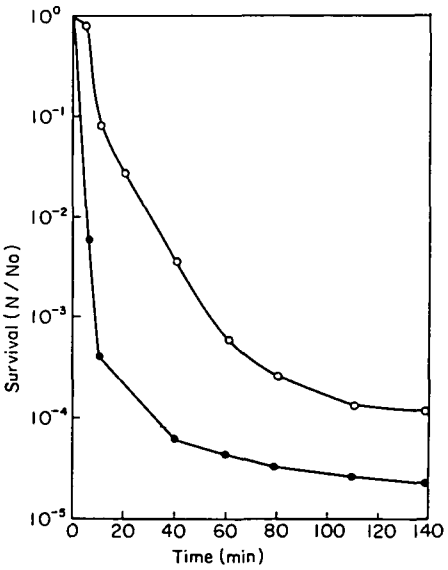

Fig. 1

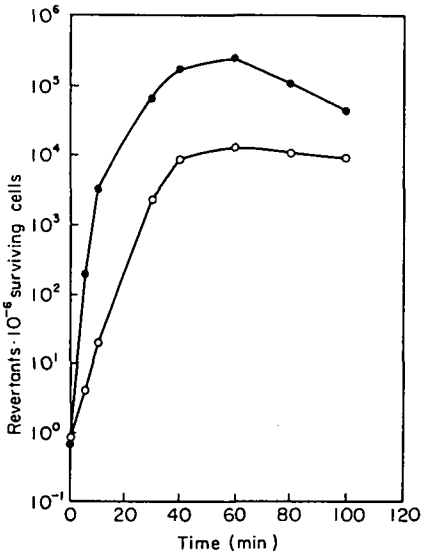

Fig. 2

Fig. 1 Killing of *A. nidulans* growing at different specific growth rates caused by NTG (60 $\mu g \cdot ml^{-1}$). -O-, dt=5 hr; -●-, dt=20 hr; N_0, Number of cells per ml at zero time; N, Number of cells per ml at any given time.

Fig. 2 Rate of reversion induced by NTG (60 $\mu g \cdot ml^{-1}$) in *A. nidulans* pyr-mutants growing at different specific growth rates. -O-, dt=5 hr; -●-, dt=20 hr.

After 2 hr of growth, during which a marked increase in the growth rate occurred with no significant change in the DNA per cell, the culture was exposed to NTG. The rate of death in this culture was the same as in the slow growing one.

By using a slow growing culture and the usual techniques for NTG-induced mutagenesis (Adelberg et al. 1965) we could isolate an auxotroph which required pyrimidine (thymine or uracil). This mutant had the same pattern of sensitivity to NTG as the wild type; the slow growing culture was more sensitive than the fast growing one. The rate of NTG-induced reversion was strikingly different in these two cultures: revertants appeared in the slow growing culture at a frequency which was some two orders of magnitude higher than in the fast growing culture (Fig. 2).

Effect of mitomycin C

Mitomycin C inhibits DNA synthesis in *A. nidulans* as soon as it is added to the culture (Mann and Carr 1977), although cell division and growth may continue for some time. Indeed, in our work, an addition of 0.1 $\mu g \cdot ml^{-1}$ mitomycin immediately inhibited [^{14}C]uracil incorporation into DNA. Cell growth and division, however, continued for some time, as indicated by the increase in the cell count (Fig. 3). The period before cell division and growth ceased (tf) lengthened as the doubling time increased due to the decreasing light intensity (Fig. 3, Table 2). A value, R, equal to tf/dt, is proposed for the comparison of the inhibitory effects of mitomycin C on cultures growing at different rates. R thus denotes the number of cell divisions taking place while DNA synthesis is inhibited. Table 2 gives the DNA content, tf and R values for cultures maintained at various doubling times. Clearly, fast growing cultures, in which the cells contained large quantities of DNA, exhibited R values greater than those for slow growing cultures, which contained smaller amounts of DNA. This fact implies that the capacity for continued cell proliferation without a net increase in cell DNA decreases as the growth rate of the culture decreases. The

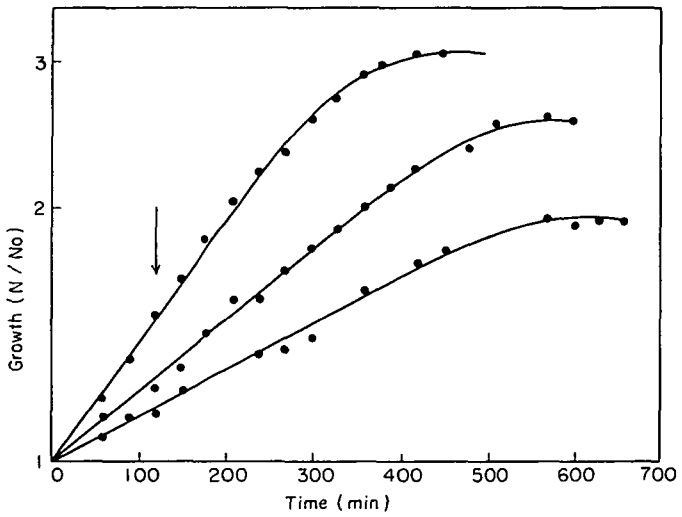

Fig. 3 Effect of mitomycin C (1 $\mu g \cdot ml^{-1}$) on the net growth of *A. nidulans* growing at different specific growth rates. The arrow designates the time mitomycin C was added.

Table 2 Effect of the growth rate (controlled by light intensity) on the response of *A. nidulans* to mitomycin C (1 $\mu g \cdot ml^{-1}$)

Exp. no.	Designation	dt[a] (min)	DNA content (fg/cell)	tf[b] (min)	R (tf/dt)	Δtf[c] (min)
1	a	195	11.0	280	1.44	
2	b	236	8.4	305	1.24	
3	c	276	7.0	320	1.16	
4	A	400	4.7	390	0.98	110
5	B	480	4.2	420	0.88	115
6	C	539	3.8	440	0.82	120
7		603	3.5	480	0.74	

[a] $dt = \frac{\mu}{\ln 2}$

[b] tf: The time elapsing between the introduction of the inhibitor and complete cessation of net growth.

[c] Δtf: Difference in tf between cultures A and a, B and b, C and c. a, A, b, B and c, C are designations relating cultures which differ in doubling time by a factor of two. Thus, the dt of a, b and c is about one half that of A, B and C, respectively.

same response of *A. nidulans* to mitomycin C was obtained for cultures whose growth rates had been modified by changing the osmoticum of the growth medium (Table 3).

It is notable that when the tf of a culture with a short dt is subtracted from the tf of a culture with a dt twice as large, a constant value of 110–120 min is obtained (Table 2). This period is approximately equivalent to the duration of phase D of the cell cycle, during which no DNA synthesis takes place (Herdman et al. 1970).

Discussion

We here provide evidence for polyploidy in the blue-green algae *A. nidulans* and show that the cellular DNA content in this organism is directly related to the growth rate of the culture. We postulate that the increase in the DNA content per cell as the growth rate increases represents not only an added number of DNA-replication forks, but an increase in the number of complete copies of the genome as well. According to this premise, cells maintained at a relatively low growth rate would

Table 3 Effect of the growth rate (controlled by the NaCl concentration) on the response of *A. nidulans* to mitomycin C (1 $\mu g \cdot ml^{-1}$)[a]

NaCl (M)	dt (min)	tf	R
Control	300	360	1.20
0.1	355	390	1.10
0.2	410	418	1.00
0.3	720	360	0.50
0.4	1,050	115	0.11

[a] Abbreviations, see Table 2.

have fewer genome duplicates than fast growing cells and would, therefore, be more sensitive to DNA-specific agents, such as mutagens and inhibitors of DNA-synthesis.

In keeping with this expectation, both the killing rate and mutagenicity of NTG were far greater (more than one order of magnitude) in slow growing cultures than in fast growing ones. These results could not be explained by assuming a different penetrability to NTG of cells growing at different rates. If this were the case, killing in the fast growing cultures should have progressed with the time of exposure; it would not have leveled off at 110 min. Neither can these results be explained by the higher activity of a repairing system in cells growing under a high light intensity, since cells of a slow growing culture transfered to a high intensity of light for 2 hr manifested their original sensitivity to NTG rather than that of a fast growing culture. Moreover, the same results were obtained when the factor controlling the growth rate was concentration of salt, not the amount of light.

Additional support for our thesis has been obtained from experiments with the DNA-synthesis inhibitor mitomycin C. Upon application of this inhibitor, cell division without further DNA synthesis will take place only if several copies of DNA exist in the cell. Thus, the number of division cycles taking place without DNA synthesis should be directly related to the number of DNA copies. In agreement with the results of experiments with NTG, the number of division cycles taking place place before growth ceases (R) was larger in fast growing cells than in slow growing ones.

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