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FIDELITY OF DNA REPLICATION IN VIVO

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SUMMARY

The incorporation of labeled deoxyribonucleosides into DNA during one cell cycle was measured in synchronous cultures of the mesophile, Bacillus licheniformis, and the thermophile, B. stearothermophilus, each grown at two temperatures. There were definite changes in the incorporation of adenine, guanine, and thymine for the mesophile as the temperature was raised from 37°C to 45°C. For the thermophile, only the incorporation of thymine changed as the temperature was raised from 55° to 65°C. These results may be indicative of greater fidelity of DNA replication in the thermophile. Apparent error rates of base incorporation (10^{-4} to 10^{-6}) were significantly lower than those found for in vitro systems and were within the range of theoretically calculated values.

INTRODUCTION

The fidelity of DNA replication in vivo is usually estimated indirectly either from spontaneous mutation rates of bacteria and bacteriophages (1-3), or from the fidelity of Escherichia coli DNA-dependent DNA polymerase under in vitro conditions (4). These estimates are unsatisfactory since (i) not all mutations in DNA result in mutated organisms that can be isolated and identified, and (ii) the fidelity of DNA polymerase is known to be affected by a large number of variables under in vitro conditions (5-7).

The study presented here describes an attempt to estimate directly the maximum lack of fidelity for DNA replication in vivo brought about by changes in the growth temperature. This was done by measuring the incorporation of labeled nucleosides into DNA during one cell cycle of synchronous bacterial cultures of a mesophile and a thermophile, each grown at two temperatures.

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MATERIALS AND METHODS

Synchronous cultures. The mesophile, Bacillus licheniformis (NRS 243), and the thermophile, B. stearothermophilus 10, were grown in shake flasks containing 150 ml of medium consisting of 1% Trypticase (BBL) and 0.2% yeast extract (Difco). Cells of B. licheniformis were grown at 37°C and 45°C and those of B. stearothermophilus were grown at 55°C and 65°C. Cell growth was followed either by absorbance measurements at 540 nm or by viable cell counts. Exponential growth was maintained for at least 12 generations by repeated transfers of the cells into fresh medium.

Synchronous growth was then achieved by selecting and growing the smallest cells in the population. This was done by filtering the culture under vacuum through a pad of filter paper (8-10). For synchronization, 150 ml of culture in midlog phase (A_{540} about 0.5 in all cases) were filtered at a time. Filtration was completed within about 1 minute. The filtrate was collected at the growth temperature of the cells and then reincubated.

DNA biosynthesis. The synchronous culture obtained by filtration was immediately labeled with 10 µCi of a deoxyribonucleoside and then reincubated. In the case of deoxythymidine, the labeled compound was added along with 6 µg of unlabeled deoxythymidine in order to obtain linear DNA biosynthesis. The following deoxyribonucleosides were used: {methyl-^{3}H}deoxythymidine (81.2 Ci/mmol, ICN), {8-^{3}H}deoxyadenosine (14.5 Ci/mmol, ICN), {5-^{3}H}deoxycytidine (24.7 Ci/mmol, ICN), and {8-^{3}H}deoxyguanosine (1.9 Ci/mmol, Amersham). All labeled deoxyribonucleosides were found to be more than 99% pure as determined by thin layer chromatography (11). Samples of 0.5 ml were removed from the culture at 15 to 20 minute intervals, the cells were lysed, and the labeled DNA was collected on a Millipore filter and counted (12,13).

DNA isolation. DNA was isolated from a synchronous culture according to the procedure of Sarfert and Venner (14). Aliquots of the isolated DNA were counted or hydrolyzed (15) and analyzed by thin layer chromatography (16). The DNA was determined by the diphenylamine reaction (17) and by absorbance measurements at 260 nm (18). Contamination of the DNA by RNA (<2.5%) and protein (<2%) was determined by the orcinol (17) and Lowry reaction (19), respectively.

RESULTS

Growth curves obtained for the synchronous cultures showed that synchrony could be maintained readily for the duration of one cell cycle. DNA biosynthesis was continuous and approximately linear over the same time period. Typically, synchronous cells obtained after filtration represented approximately 10% of the original number of asynchronous cells. The yield of synchronous cells at the beginning of each experiment varied to some extent, depending on the precise absorbance of the culture and the filtration rate.

For the DNA replication experiments, a labeled deoxyribonucleoside was added to the synchronous culture at time zero and the DNA was then isolated

Table I

DNA Replication as a Function of Growth Temperature

		cpm/A_{260} unit					
		B. licheniformis			B. stearothermophilus		
		37°C	45°C	$\frac{37^{\circ}C}{45^{\circ}C}$	55°C	65°C	$\frac{55^{\circ}C}{65^{\circ}C}$
A	(a)*	390	4,845	0.08	4,320	4,500	0.96
	(b)	9,912	18,052	0.55	13,636	13,075	1.04
T	(a)	3,600	4,841	0.74	1,630	500	3.26
	(b)	4,765	6,941	0.68	2,049	1,462	1.40
G	(a)	8,841	3,870	2.28	3,269	3,458	0.94
	(b)	14,210	8,073	1.76	9,288	9,629	0.96
C	(a)	9,504	9,852	0.96	3,805	4,280	0.89
	(b)	17,496	21,600	0.81	7,855	8,600	0.91

* (a) and (b) represent two separate experiments; the labeled deoxyribonucleoside added is indicated on the left.

at the end of one cell cycle. The amount of DNA (in terms of A_{260} units) and the extent of incorporation of label (in terms of cpm) was then determined. Two experiments were carried out for each system. The results are given in Table 1. In experiment (a) the yield of synchronous cells at the beginning of the experiment was significantly higher than that for experiment (b). As a result, the cpm/A_{260} unit are lower for experiment (a) compared to those for experiment (b). Some of the changes in cpm/A_{260} unit for B. licheniformis, as a function of temperature, are of such magnitude as to require specific explanation (see Discussion).

Some general trends can, however, be discerned from the data of Table 1. For B. licheniformis, there were significant differences in the incorporation of all four deoxyribonucleosides at 37°C compared to the incorporation at 45°C. On the other hand, the incorporation for B. stearothermophilus at 55°C was essentially identical to that at 65°C, except for the case of deoxythymidine. A better way of analyzing these data is given by the ratio of (cpm/A_{260} unit) values obtained for each organism at the two temperatures. These ratios (Table

1) show that there was a definite increase in the incorporation of adenine and thymine, a slight increase in the incorporation of cytosine, and a definite decrease in the incorporation of guanine for B. licheniformis as the growth temperature was increased from 37°C to 45°C. For B. stearothermophilus, on the other hand, there was a definite decrease in the incorporation of thymine but no significant changes in the incorporation of adenine, guanine, and cytosine as the growth temperature was increased from 55°C to 65°C. Analysis of the data in this fashion avoids some of the variability inherent in cpm/A_{260} unit; the ratio of cpm/A_{260} unit for two growth temperatures is, by and large, a reproducible measurement.

DISCUSSION

Taken at their face value, the data of Table 1 indicate in some instances such large changes in the incorporation of various nucleosides as a function of temperature that gross changes in the DNA base composition would result. That clearly cannot be the case. The reason for this apparent inconsistency is the fact that the experiment described here deals with a very complex metabolic system, many aspects of which could be affected by a change in temperature. Examples of such aspects include transport of deoxyribonucleosides into the cell, enzymatic modifications of nucleosides and nucleotides, sizes of the intracellular pools of nucleosides and nucleotides, and DNA repair mechanisms. We were able to rule out the involvement of some, but not all, of these aspects by the following experimental findings:

(a) no unlabeled intracellular deoxyribonucleosides could be detected by chromatographic techniques (11) so that the intracellular pool of labeled deoxyribonucleosides was a good estimate of the total pool.

(b) intracellular pool sizes of labeled deoxyribonucleosides (determined chromatographically) were constant for a given organism and deoxyribonucleoside regardless of the growth temperature so that pool size variation could not account for the observed differences in incorporation.

(c) the isolated DNA had all of its label in the base with which it had been labeled origininally (15,16) so that metabolic interconversions of nucleosides and nucleotides appeared to be negligible.

The complexity of the metabolic system studied makes a precise interpretation of the data difficult. But a definite difference between the two organisms is nevertheless apparent. The measured incorporation of deoxyribonucleosides into DNA appears to be essentially independent of temperature for the thermophile, but very temperature dependent for the mesophile.

Moreover, the data can be used to calculate an apparent error rate for DNA replication in vivo on the assumption that changes in cpm/A_{260} unit are due entirely to misincorporation. Using an extinction coefficient of 250 (1% solution, 1 cm lightpath) for the DNA, an average mol. wt. of 250 for a nucleotide, and the specific activity for each deoxyribonucleoside, an apparent error rate can be calculated. This is based on the assumption that the specific activity of the added deoxyribonucleoside does not change inside the cells and is identical to that of the intracellular deoxyribonucleoside triphosphate (the ultimate substrate of DNA polymerase). The apparent error rates thus calculated are shown in Table 2. Three conclusions can be drawn from these data. First the apparent error rates are decidedly smaller than those reported for in vitro systems (7,20,21). Low in vivo error rates are, of course, expected in order for an organism to be able to transmit genetic information from generation to generation with a high degree of fidelity. Second, the apparent error rates are in excellent agreement with theoretically calculated values based on chemical considerations of non Watson-Crick base pairs and the occurrence of alternate tautomeric forms of the bases (22). Third, generally speaking, there is greater lack of fidelity in the replication of DNA in the mesophile than in the thermophile. It is tempting to speculate that the ability to carry out DNA replication with a high degree of fidelity at elevated temperatures is one of the contributing factors to the phenomenon of thermophily.

Table 2

Apparent Error Rates of Base Incorporation*

Base	B. licheniformis ($37^{o}C \longrightarrow 45^{o}C$)		B. stearothermophilus ($55^{o}C \longrightarrow 65^{o}C$)	
	(a)**	(b)	(a)	(b)
A	$1/3.0x10^{5}$	$1/1.7x10^{5}$	$1/7.7x10^{6}$	$(1/2.5x10^{6})$
T	$1/3.1x10^{4}$	$1/1.8x10^{4}$	$(1/3.4x10^{4})$	$(1/6.6x10^{4})$
G	$(1/3.7x10^{4})$	$(1/2.9x10^{4})$	$1/1.0x10^{6}$	$1/5.3x10^{5}$
C	$1/6.6x10^{6}$	$1/5.7x10^{5}$	$1/5.0x10^{6}$	$1/3.1x10^{6}$

*error rates refer to an increase in the incoporation of a base per total number of bases incorporated as the temperature is raised as indicated; parentheses refer to a decrease in the incorporation as the temperature is raised.

**(a) and (b) represent two separate experiments.

It must be stressed again, though, that the apparent error rates, because of the complex metabolic system involved, do not necessarily represent actual misincorporation of bases into the DNA (i.e. lack of fidelity). But it is likely, since we were able to rule out the involvement of several metabolic aspects, that these apparent error rates constitute a reasonable estimate of misincorporation. As such they may serve to indicate the maximum lack of fidelity that might be expected in bacterial systems for the in vivo replication of DNA.

REFERENCES

1. Hall, Z.W. and Lehman, I. R. (1968) J. Mol. Biol. 36, 321-333
2. Kornberg, A. (1974) DNA Synthesis, Freeman, San Francisco
3. Bernardi, F. and Ninio, J. (1978) Biochemie 60, 1083-1095
4. Trautner, T. A., Swartz, M. N. and Kornberg, A. (1962) Proc. Natl. Acad. Sci. U. S. A. 48, 449-455
5. Sirover, M. A., Dube, D. K. and Loeb, L. A. (1979) J. Biol. Chem. 254, 107-111
6. Saffhill, R. (1974) Biochem. Biophys. Res. Commun. 61, 802-808
7. Stenesh, J. and McGowan, G. R. (1977) Biochim. Biophys. Acta 475, 32-41
8. Imanaka, H., Gillis, J. R. and Slepecky, R. A. (1967) J. Bacteriol. 93, 1624-1630

9. Sargent, M. (1973) J. Bacteriol. 116, 736-740
10. Helmstetter, C. A. (1969) In Methods in Microbiology (Norris, J. R. and Ribbons, D. W., eds.) vol. 1, pp. 327-363, Academic Press, New York
11. Vischer, E. and Chargaff, E. (1948) J. Biol. Chem. 176, 703-714
12. Bodmer, W. F. and Grether, S. (1965) J. Bacteriol. 89, 1011-1014
13. Stenesh, J. and Roe, B. A. (1972) Biochim. Biophys. Acta 272, 167-178
14. Sarfert, E. and Venner, H. (1965) Z. Physiol. Chem. 340, 157-173
15. Wyatt, G. R. (1951) Biochem. J. 48, 584-590
16. Holdgate, D. P. and Goodwin, T. W. (1964) Biochim. Biophys. Acta 91, 328-329
17. Ashwell, G. (1957) In Methods in Enzymology (Colowick, S. P. and Kaplan, N., eds.) vol. 3, pp. 73-105, Academic Press, New York
18. Stenesh, J., Roe, B. A. and Snyder, T. L. (1968) Biochim. Biophys. Acta 161, 442-454
19. Lowry, O., Rosebrough, N., Farr, A. and Randall, R. (1951) J. Biol. Chem. 193, 265-275
20. Shearman, C. W. and Loeb, L. A. (1979) J. Mol. Biol. 128, 197-218
21. Mizutani, S. and Temin, H. M. (1976) Biochemistry 15, 1510-1516
22. Topal, M. D. and Fresco, J. R. (1976) Nature 263, 285-289