SUGGESTED ROLE OF RESPIRATION IN BACTERIAL DNA REPLICATION

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Received August 7, 1969

SUMMARY

Uncouplers of oxidative phosphorylation stimulate DNA synthesis in intact cells of Escherichia coli. Synthesis of double-stranded DNA has been shown previously to require a functioning respiratory system even in the presence of sufficient nucleotide triphosphate pools. We suggest that the role of respiration reflects a requirement for the motion of protons across the cell membrane, away from the locus of DNA chain unwinding. This flux would be expected to be promoted by addition of uncouplers and may be required to relieve electrostatic interaction between strands of DNA. Such a vectorial feature of DNA synthesis should be considered in light of the spatial proximity of DNA replication to the bacterial cell membrane.

DNA synthesis in aerobically-grown cells of Escherichia coli appears dependent upon a functional respiratory system. Respiration is not only required for the production of deoxyribonucleoside triphosphates, but appears coupled, in some obligatory fashion, with replication of double-stranded DNA itself (1). Thus, Cairns and Denhardt have found that cyanide or carbon monoxide completely inhibits replication of E. coli DNA under conditions where deoxyribonucleoside triphosphate pools are not depleted and where synthesis of single-stranded ØX174 DNA is able to proceed (1).

The role of respiration in DNA synthesis may result from (a) sensitivity of the DNA synthetic system to the oxidation-reduction state of an electron carrier, (b) a requirement for ATP, distinct from that involved in the synthesis of deoxy-

ribonucleoside triphosphates, or (c) a requirement for some other event coupled to respiration, such as energy-linked ion transport. In considering these alternatives, we have attempted to separate the influence of respiration from other energy-linked functions by examining the effects of uncouplers of oxidative phosphorylation. Data presented in Table I indicate that, under conditions of our experiments, addition of 2,4-dinitrophenol or carbonyl cyanide *m*-chlorophenyl-hydrazone (CCCP) at concentrations sufficient to inhibit ATP formation (2) does not interfere with DNA synthesis, but, in fact, stimulates it. Addition of the antibiotic, nigericin, which was without effect itself, prevented stimulation by CCCP. Other experiments, not shown in the table, agree with the results of Cairns and Denhardt (1) and show that the addition of cyanide gives rise to nearly complete inhibition, which is not influenced by the addition of CCCP.

Failure of uncouplers of oxidative phosphorylation to prevent DNA replication indicates that the requirement for respiration is not directly associated with either ATP synthesis or with energy-linked translocation. Since, in parallel experiments under similar conditions, addition of uncoupling compounds did not influence the respiratory rate as measured with an oxygen electrode, it is unlikely that their action in increasing DNA synthesis reflects merely a stimulation of respiration or alteration in the oxidation-reduction state of an electron carrier.

On the other hand, we are impressed by the close spatial association between the locus of DNA replication and the bacterial cell membrane (3,4) where the enzymes of the respiratory chain reside (5). In this connection, a feature of bacterial

respiration which persists, even in the presence of uncouplers, is an outward movement of protons across the cell membrane (6) and, indeed, Mitchell (6,7) has shown that uncouplers act to catalyze such translocation. We believe that the dependency of DNA synthesis upon respiration may represent a requirement

TABLE I

Stimulation of DNA synthesis by uncouplers of oxidative phosphorylation

Additions	Thymidine incorporation pmoles	% Increase
Experiment I		
none	37.4	-
2,4-dinitrophenol (250 µM)	66.0	77
Experiment II		
none	49.5	-
CCCP (25 µM)	60.5	22
Nigericin (10 µg)	48.1	-
Nigericin (10 µg) + CCCP (25 µM)	45.6	-

DNA synthesis was followed as described by Cairns and Denhardt (1) by measuring the incorporation of ^{3}H-thymidine at 14°. The reaction medium contained 10 mM NaCl, 5 mM KCl, 20 mM NH_4Cl, 0.6 mM KH_2PO_4, 0.2 mM Na_2SO4, 1 mM $MgCl_2$ 0.1 mM $CaCl_2$, 10 µM $FeCl_3$, 25 mM N-Tris-hydroxymethyl-methyl-2-aminoethan sulfonic acid (pH 7.4), 25 mM Na lactate, 4 µM thymidine, 0.2 mM arginine, 0.2 mM methionone, 0.2 mM tryptophan and 70 µg dry weight of cells. The reaction medium contained about one µC of label. Cells of the TAMT strain of Escherichia coli were previously grown in the same medium, but without labeled thymidine. The reaction time was 30 sec. and the reaction was terminated by rapid dilution of cell suspension into 5 volumes of cold sucrose, immediate millipore filtration, followed by washing with 5.0 ml cold trichloroacetic acid. Radioactivity was measured by liquid scintillation.

for movement of charge in the form of protons, from the site where replication occurs, toward the cell exterior, a movement which would be prevented by cyanide and enhanced by addition of uncoupler. Since the requirement is observed only in the synthesis of double-stranded DNA, it is reasonable that such charge withdrawal be associated with strand separation prior to copying. In the simplest case, the vectorial transfer of protons (and, thus, positive charge) from the site of replication may be required to relieve electrostatic interaction between the two strands of DNA in much the same fashion that removal of cations promotes strand separation *in vitro* (see for example Reference 8). Nigericin, which acts by promoting equilibration of K^+ across biological membranes (9), probably eliminates stimulation by uncouplers by opposing the outward vectorial movement of protons with inward potassium flux.

Acknowledgments: This investigation was supported by a grant from the National Science Foundation to J.L.H. who is also recipient of a U.S. Public Health Service Research Career Development Award, and a grant from the Maine Heart Association to W.T.H. The strain of *E. coli* used in this study was obtained through the generosity of Dr. J. Cairns and Nigericin through that of Dr. R. L. Harned. The authors wish to thank Mrs. Alice Seymour and Miss Patricia Smith for skillful technical assistance.

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