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Phospholipid Turnover During the Division Cycle of *Escherichia coli*

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The turnover of phospholipids in *Escherichia coli* B/r was analyzed in synchronously growing populations. The turnover of presynthesized phosphatidylglycerol and cardiolipin continued at a constant exponential rate throughout the division cycle.

The membrane compositions of bacterial cells can vary in response to changes in growth conditions, e.g., during growth at different temperatures (8), during inhibition of chromosome replication (3), and during inhibition of phospholipid synthesis (9, 10). In addition, membrane phospholipids turnover to an extensive degree even during steady-state growth (2), and membrane proteins might migrate after their synthesis into the cytoplasm (12). There are a few reports that synthesis and turnover of membrane components vary during the cell division cycle. For example, phosphatidylethanolamine, phospholipids, and membrane proteins appear to increase their rate of synthesis at either initiation (O. Pierucci, unpublished data) or completion of chromosome replication (4, 5). The rate of phospholipid turnover has been reported to increase around the time of cell division (11).

To determine whether the synthesis and turnover of the unstable phospholipid components phosphatidylglycerol and cardiolipin vary during the cell division cycle of *Escherichia coli* B/r, we analyzed the composition of radioactively labeled phospholipid at various times after the exposure of synchronously growing populations to radioactive glycerol.

For studies of phospholipid synthesis, multiple samples of newborn cells were selected from membrane-bound, exponential-phase populations of *E. coli* B/r A and F (6), and the samples were exposed to 2.9 μCi of [2-^{3}H]glycerol per ml (7.8 Ci/mmol; New England Nuclear Corp.) for 2/10 of a generation at various times during synchronous growth. The phospholipids were extracted and separated by chromatography (as indicated in detail in legend to Fig. 1), and the radioactivity associated with phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin was measured. The distribution of the radioactivity among the three components was found to be independent of the age of the cells at the time of exposure to the radioactive precursor. The composition of the newly synthesized phospholipids was also the same in cells growing at 37°C at various growth rates (between 1.3 and 2.4 doublings per h). For *E. coli* B/r A, the composition was 68% phosphatidylethanolamine, 29% phosphatidylglycerol, and 3% cardiolipin; for *E. coli* B/r F, it was 66, 26, and 8%, respectively.

For studies on phospholipid turnover, multiple samples of newborn cells growing in glucose minimal medium (1.3 doublings per h) were exposed to 2.9 μCi of [2-^{3}H]glycerol per ml for 8 min (equivalent to ~2/10 of a generation) at either the time of collection (age 0) or after 20 min of synchronous growth (age 0.4). In cultures growing synchronously in glucose minimal medium, cells of age 0.4 to 0.6 have either one completely replicated chromosome or two chromosomes that have just initiated a round of chromosome replication. At about this time in the cycle, the septum starts to be formed. Cells of age 0 to 0.2 have one partially replicated chromosome (7). Turnover of the phospholipid synthesized at these ages was measured by analyzing the radioactivity that remained associated with phosphatidyglycerol, cardiolipin, and phosphatidylethanolamine during subsequent synchronous growth. The ratio of the radioactivity associated with phosphatidylglycerol to the stable phosphatidylethanolamine is shown in Fig. 1. This ratio decreased exponentially in both *E. coli* B/r A and F, independent of the time at which the phospholipids were synthesized. A similar decrease was also observed when the ratio of the radioactivity associated with cardiolipin to phosphatidylethanolamine was determined (data not shown). Thus, the rate of phospholipid turnover did not change during the cell cycle. The rate of turnover was also independent of the age at which the phospholipids were synthesized. Ohki (11) reported that the turnover of phosphatidylglycerol in cells labeled with either

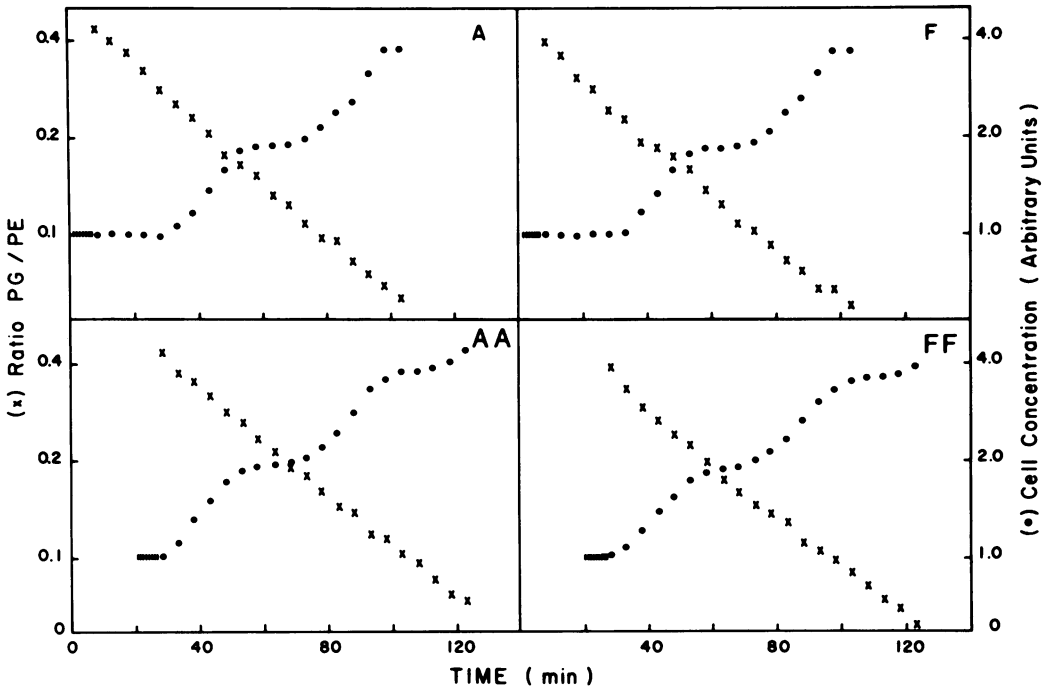

FIG. 1. *Phospholipid turnover during synchronous growth of E. coli B/r A and F. Samples (3.4 ml) of 1 × 10^7 to 2 × 10^7 newborn cells per ml were collected consecutively during 1-min intervals from membrane-bound cultures of E. coli B/r A (A and AA) and F (F and FF) growing in glucose minimal medium (μ, 1.3 doublings per h). Samples were exposed to 2.9 μCi of [2-^{3}H]glycerol per ml (7.8 Ci/mmol) for 8 min either immediately after collection (A and F) or 20 min after synchronous growth (AA and FF). The pulse was stopped by the addition of a large excess of nonradioactive glycerol (10 mg/ml), and synchronous growth continued for various periods of time. At the end of synchronous growth, cells were collected by centrifugation and washed with a medium containing nonradioactive glycerol (100 μg/ml). About 10^{10} nonradioactive carrier cells were added to the cell pellets. Pellets were then extracted at 55°C for 20 min with methanol (2 ml) in tightly stoppered test tubes. After cooling at room temperature, 4 ml of chloroform were added, and the mixtures were spun in a Vortex mixer and centrifuged. The supernatant was evaporated at room temperature, and the resulting lipid film was dissolved into 0.2 ml of methanol and 0.4 ml of chloroform. Lipids were analyzed by thin-layer chromatography on Eastman chromagram sheets–silica gel with fluorescent indicator (Eastman Kodak Co., Rochester, N.Y.)—with the chloroform-methanol (3:1 [vol/vol]) ascending technique. Three spots of radioactivity were detected, corresponding to the locations of E. coli phosphatidylethanolamine, E. coli phosphatidylglycerol, and beef heart cardiolipin (General Biochemicals Div. [Mogul Corp.], Chagrin Falls, Ohio). Symbols: x, Ratio between the radioactivities associated with phosphatidylglycerol (PG) and phosphatidylethanolamine (PE); ●, ratio between the cell concentrations at the end of synchronous growth and at the time of collection; IIIIII, length of the pulse. For E. coli B/r A, the radioactivity per 10^6 cells at the end of the pulse was 217 cells exposed immediately after collection and 207 for those exposed 20 min after synchronous growth, respectively; for E. coli B/r F, the radioactivity was 160 (exposed immediately) and 210 (exposed 20 min after growth).*

$^{32}P_i$ or [^{14}C]acetate increased near the time of cell division in synchronized cultures of *E. coli* K-12. Under the conditions used in our experiments, stepwise turnover of acylated phosphatidylglycerol could have been obscured by the continuous turnover of nonacylated phosphatidylglycerol, which is preferentially labeled during short exposure to [2-^{3}H]glycerol (1). Alternatively, the preferential turnover of phosphatidylglycerol around the time of cell division reported by Ohki could be a consequence of the temperature shift used to obtain synchronously dividing populations and not a characteristic of the cell division cycle.

In conclusion, our results suggest that the major phospholipid components in *E. coli* B/r—phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin—are synthesized coordinately during the division cycle. Furthermore, no evidence was found of enhanced turnover of presynthesized phospholipids at specific times during the cell cycle.

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LITERATURE CITED

1. **Ballesta, J. P. G., C. L. de Garcia, and M. Schaechter.** 1973. Turnover of phosphatidylglycerol in *Escherichia coli*. J. Bacteriol. **116**:210–214.
2. **Cronan, J. E., and P. R. Vagelos.** 1972. Metabolism and function of the membrane phospholipids of *Escherichia coli*. Biochim. Biophys. Acta **265**:25–60.
3. **Gudas, L. J., and A. B. Pardee.** 1976. DNA synthesis inhibition and the induction of protein X in *Escherichia coli*. J. Mol. Biol. **101**:459–477.
4. **Hakenbeck, R., and W. Messer.** 1977. Oscillations in the synthesis of cell wall components in synchronized cultures of *Escherichia coli*. J. Bacteriol. **129**: 1234–1238.
5. **Hakenbeck, R., and W. Messer.** 1977. Activity of murein hydrolases in synchronized cultures of *Escherichia coli*. J. Bacteriol. **129**:1239–1244.
6. **Helmstetter, C. E.** 1967. Rate of DNA synthesis during the division cycle of *Escherichia coli* B/r. J. Mol. Biol. **24**:417–427.
7. **Helmstetter, C. E., and O. Pierucci.** 1976. DNA synthesis during the division cycle of three substrains of *Escherichia coli* B/r. J. Mol. Biol. **102**:477–486.
8. **Marr, A. G., and J. L. Ingraham.** 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. **84**:1260–1267.
9. **Mindich, L.** 1970. Membrane synthesis in *Bacillus subtilis*. I. Isolation and properties of strains bearing mutations in glycerol metabolism. J. Mol. Biol. **49**:415–432.
10. **Mindich, L.** 1970. Membrane synthesis in *Bacillus subtilis*. II. Integration of membrane proteins in the absence of lipid synthesis. J. Mol. Biol. **49**:433–439.
11. **Ohki, M.** 1972. Correlation between metabolism of phosphatidylglycerol and membrane synthesis in *Escherichia coli*. J. Mol. Biol. **68**:249–264.
12. **Sargent, M. G.** 1973. Membrane synthesis in synchronous cultures of *Bacillus subtilis* 168. J. Bacteriol. **116**:397–409.