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Cell Cycle-Specific Incorporation of Lipoprotein into the Outer Membrane of *Escherichia coli*

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A cell cycle-specific incorporation of free lipoprotein into the outer membrane of *Escherichia coli* was observed, with a maximal rate of incorporation occuring at the time of septation.

Approximately 30% of the lipoprotein molecules of the outer membrane of *Escherichia coli* are covalently attached to the rigid murein layer of the cell envelope, i.e., in a "bound" form (1, 2, 6). It has been suggested that assemblies of the lipoprotein molecule could act as a diffusion pore in the outer membrane of *E. coli* (5), or alternatively, that covalent linkage of the outer membrane and murein layer could be a mechanism for coordinating the synthesis of these two cell envelope layers (1, 2). We wish to describe an unusual property of the biosynthesis of the free lipoprotein which may help to distinguish these two hypotheses.

An exponential culture of *E. coli* B/r growing in M9 minimal salts-glucose medium was synchronized by sucrose gradient centrifugation. At various times after synchronization a part of the culture was pulsed with [^{35}S]methionine for 3 min to determine the rate of incorporation of the free lipoprotein into the outer membrane (7). The results as shown in Fig. 1 demonstrate a cyclical incorporation of the free lipoprotein into the outer membrane. It should be stressed that our experimental design results in the measurement of the rate of incorporation of the free lipoprotein with respect to all other outer membrane proteins, and thus reflects a specific alteration in the rate of incorporation of this protein, rather than the previously reported doubling in the rate of total membrane protein synthesis which occurs late in the cell cycle (9, 11). A similar pattern of incorporation was also observed during the second synchronous cell cycle.

Because no corresponding increase was observed in the rate of incorporation of bound lipoprotein during the cell cycle (data not shown), an appreciable decrease in the ratio of bound-to-free lipoprotein must occur shortly before septation. If the incorporation of lipoprotein is concentrated primarily at a presumptive septum site, in an analagous manner to that recently reported for the integration of the phage λ receptor of the outer membrane of *E. coli* (10), it is conceivable that the cycle-specific decrease in the ratio of bound-to-free lipoprotein is involved in the separation of outer membrane and murein observed at an early stage in septation (3, 8).

The cyclical increase in the rate of incorporation of free lipoprotein was largely abolished by

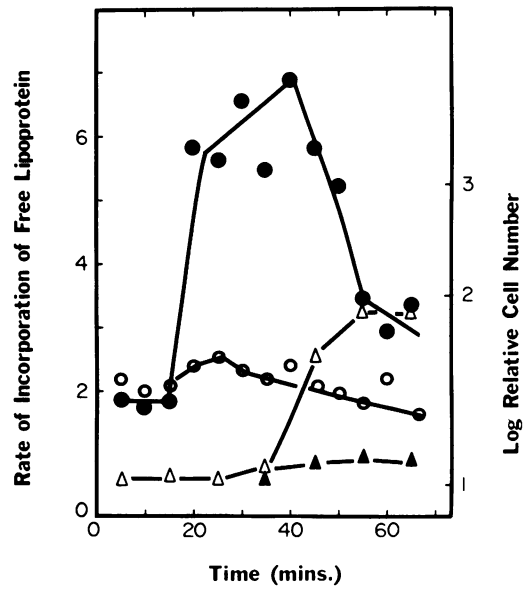

FIG. 1. *The rate of incorporation of free lipoprotein during the E. coli synchronous cell cycle. Cell number (determined by a Coulter counter) of a control culture (Δ), or after the addition of nalidixic acid (30 μg/ml) at 0 time (▲). Rate of incorporation of free lipoprotein into the outer membrane of a control culture (●), or after the addition of nalidixic acid (30 μg/ml) at 0 time (○).*

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the addition of nalidixic acid at a concentration which inhibits deoxyribonucleic acid synthesis and septation (Fig. 1). However, since thymine starvation of *E. coli* B/r *thy* or treatment of *E. coli* B/r with bleomycin, both of which inhibit deoxyribonucleic acid synthesis and cell septation, had no effect on the incorporation of the free lipoprotein (data not shown), this phenomenon cannot be a simple response to inhibition of deoxyribonucleic acid synthesis or cell division per se. The effect of nalidixic acid on the free lipoprotein is especially interesting in that the rate of incorporation of another outer membrane protein (protein G), which has very similar biosynthetic properties to that of the free lipoprotein, increased dramatically during incubation with nalidixic acid (7). It is conceivable that protein G is a precursor of the free lipoprotein, or that these two proteins compete for integration sites in the outer membrane of *E. coli*.

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