A Model of Lipid A Biosynthesis in *E. coli*

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***Short Abstract* — Lipid A is a highly conserved component of lipopolysaccharide, itself a major component of the Gram-negative bacterial outer membrane. We modeled the nine enzyme-catalyzed steps its biosynthesis in *E. coli*, focusing particularly on biosynthesis regulation, which occurs through regulated degradation of the LpxC and WaaA enzymes. The model agrees with many experimental findings, including the lipid A production rate and the behaviors of several LpxA mutants. Flux control is dominated by LpxC if pathway regulation is ignored, but by LpxK if regulation is present. These results suggest that LpxK may be a useful drug target.**

I. Introduction

LIPOPOLYSACCHARIDE (LPS) is a glycolipid that forms the major component of the outer leaflet of the outer membrane of most Gram-negative bacteria, covering about 75% of the *E. coli* cell surface area. LPS helps stabilize these membranes, protects them from chemical attack, and promotes cell adhesion to surfaces. It elicits a strong immune response in humans and other animals [1].

LPS comprises lipid A, core oligosaccharide, and O-antigen, of which the lipid A component is of particular interest because it is essential for cell viability and highly conserved. These also make its biosynthetic pathway an attractive target for new antibiotics. The lipid A biosynthesis pathway has been investigated thoroughly through several decades of experimentation [1] but has received remarkably little quantitative analysis.

II. Results and Discussion

E. coli lipid A biosynthesis proceeds through nine enzyme catalyzed steps (black arrows in Figure 1). These are well established from careful experimentation, largely by the Raetz group [1]. Lipid A synthesis is regulated (red arrows in Figure 1), at least in part, through controlled degradation of LpxC and WaaA, both performed by FtsH. We assume that FtsH reversibly converts between an inactive state, an active state for degrading LpxC, and a different active state for degrading WaaA [2]. Regulation that directs FtsH to degrade LpxC appears to arise from the lipid A disaccharide concentration, based on published experimental results and on our own experiments in which we overexpressed LpxK, finding that this increased LpxC levels [2]. Regulation that directs WaaA degradation appears to arise from mature lipid A, before it has been transported to the outer membrane.

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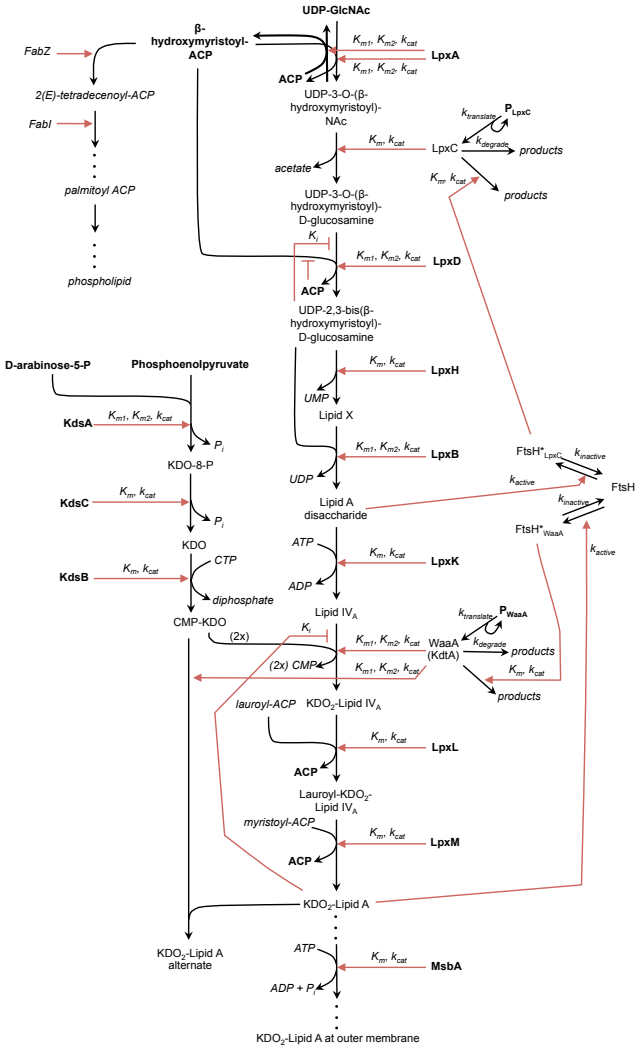

Figure 1. Model of *E. coli* lipid A biosynthesis pathway.

This model agrees with observed lipid A production rates, the behaviors of LpxA mutants, and correlations between LpxC half-lives and cell generation times. It predicts that LpxD can replace LpxA and that there may be metabolic channeling between LpxH and LpxB. It also showed that LpxC is only rate-limiting if pathway regulation is ignored, but that LpxK has the most control if not. Although LpxC has been pursued most often as a drug target, this suggests that LpxK may be a better target.

References

[1] Whitfield, C and MS Trent (2014) Biosynthesis and export of bacterial lipopolysaccharides. *Annu. Rev. Biochem.* **83**, 99-128.

[2] Emiola, A, J George, and SS Andrews (in press) A complete pathway model of lipid A biosynthesis in *E. coli*. *PLoS ONE*.