Journal of Experimental Microbiology and Immunology (JEMI) Vol. 16: 136 – 138


# Fatty Acid *fabF* Mutants of *Escherichia coli* show limitation on Transformation Frequency

**Ada Mew and Rhena Yoo**
*Department of Microbiology & Immunology, University of British Columbia*

***Escherichia coli*** **knockouts of the *fabF* gene consequently lack the encoded protein, β-ketoacyl-acyl carrier protein (ACP) synthase II, which plays a part in fatty acid biosynthesis and by extension, in membrane fluidity. Since this enzyme is highly active at lower temperatures, we predicted that *fabF* mutants would differ from the wild-type parental strain in their membrane permeability to DNA during the cold-shock step in calcium chloride-induced transformation. Parental and mutant strains were made competent using the cold-shock transformation method, and replicates of the subsequent transformation assays with the plasmid pUC19 suggested that the *fabF* knockout had a slight negative effect on transformation frequency. It is a good candidate for further study in order to elucidate the mechanism of transformation.**

The temperature-shock transformation method is a widely used and accepted technique in molecular genetics, but even now, it is still poorly understood. Since artificial transformation appears to be a complex process consisting of many possible mechanisms working together, it is important to focus in on a part of the bigger picture. Studying the factors involved in plasmids crossing the phospholipid bilayer may aid in optimization of the transformation process. The structure and composition of the membrane is important, as it can determine the rate of permeability of genetic material. The *fabF* gene encodes for β-ketoacyl-acyl carrier protein (ACP) synthase II, an enzyme capable of changing the fatty acid profile of the cell (2). ACP synthase II produces *cis*-vaccinate most actively at low temperatures relative to overall fatty acid production. *Cis*-vaccinate is a common substrate for diunsaturated phospholipids synthesis; this keeps the plasma membrane fluid in homeostasis and at low temperatures (2).

Garwin *et al*. concluded the lack of *fabF* would cause a difference in the fatty acid composition of the plasma membrane, while revertants would have a membrane similar or identical to the wild-type (3). Knowing that *fabF* is a major contributor to the fluidity of the plasma membrane, it follows that the membrane would become more rigid and less permeable in the JW1081-4 strain. It is expected that the mutant would have a significantly lower transformation frequency than the wild-type. In this experiment, we study the possible involvement of ACP synthase II in the cold-shock transformation process.

## METHODS AND MATERIALS

**Bacterial strains.** The bacterial strains from the Keio Collection of Baba *et al.* (1) were purchased from the Coli Genetic Stock Center (CGSC) at Yale. The isogenic bacterial strains used were *E. coli* JW1081-4 and BW25113, which had the following overlapping genotype: *F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*. The difference between JW1081-4 from its parent strain is *ΔfabF759::kan*, an interruption of the *fabF* gene by a kanamycin resistance cassette. The bacterial strain DH5-α was provided from the MICB 421 Culture Collection at the Department of Microbiology & Immunology at UBC. All strains were incubated at 37°C, unless specified.

**Media.** Luria-Bertani (LB) media was originally used for bacterial propagation. 10.0 g/L peptone, 5.0 g/L NaCl (EMD, SX0420-1), and 5.0 g/L yeast extract (BD Bacto, Sparks, MD, 212750) in deionized water for broth, and 15.0g/L agar (Invitrogen, Carlsbad, CA, 30391-023, lot# 1251318) for media plates. 0.25 ug/ml kanamycin was used for the selection of JW1081-4, and 0.25 ug/ml ampicillin was used for the selection of pUC19 plasmid-containing DH5-α colonies.

**Plasmids.** DH5-α *E.coli* propagated the pUC19 plasmid that contains the $amp^R$ gene, cultures were grown to a turbidity of approximately 0.4 $OD_{600}$. The plasmid was extracted using the midi-preparation protocol described by Sambrook and Russell (4). The plasmid stock was stored at -20°C in water. Post-extraction, the plasmid stock was analyzed by 1% agarose (Invitrogen, Carlsbad, CA, 15510-027, lot# 1137923) in 1X TAE buffer (1 mM EDTA, 40 mM Tris-acetate, pH 8.5). The gel was then visualized on a MultiImage Light AlphaImager™ Cabinet (Alpha Innotech Corporation), to confirm the band size with a 1 kb DNA Ladder (Invitrogen, Carlsbad, CA, 10787018, lot# 1212311A). Plasmid stock concentration was measured in an Ultrospec 3000 (Biochrom) spectrophotometer.

**Preparation of competent cells by the calcium chloride method.** Single colonies of BW25113 and JW1081-4 strains were each inoculated into 500 ml of LB Lenox media and grown into log phase (turbidity of just under 0.5 $OD_{600}$). These cultures were made competent using the calcium chloride competency and transformation protocol described by Sambrook and Russell (4).

**TABLE 1.** Dilution Scheme for Plating

| Plating Media | Required Dilution |
|---|---|
| SOB media | 50,000X and 100,000X |
| SOB media with ampicillin | 1X and 10X |

**Transformation assay.** The transformation procedure was performed using the calcium chloride competency and transformation protocol described by Sambrook and Russell(4) using the competent strains. After the introduction of pUC19, cells were plated on media using the dilutions shown in Table 1. Growth on media with ampicillin acted as a selection method for transformants, whereas growth on non-selective media acted as a baseline for viability. The ratio between replicates showed frequency between the two strains.

**Quality assessment.** As controls, parental and mutant strains that underwent the same competency and transformation procedure, but were not given pUC19 DNA were plated concurrently as those given DNA, using the same dilution scheme as shown in Table 1.

## RESULTS

**Bacterial strains and media.** The phenotypes of the parental and mutant strains were checked by selecting growth of the mutant strain on LB plates containing 25 μg/ml kanamycin. As expected, the parental strain failed to grow.

**Quality assessment.** In the negative control, cells not exposed to DNA were plated on selective media. There was no growth on any negative control plates for all replicates. In the positive control, DNA was added to cells plated on non-selective media. There was always growth, and colony counts from these plates were used in the calculation of transformation frequency.

**Transformation assay.** For comparison, data from the five replicates were averaged and plotted in Figure 1. On average the parental strain had a transformation frequency that was two times greater than that of the mutant strain. More specifically, three of the replicates showed the expected result of lower transformation frequency in the mutant, but two of the replicates showed the opposite trend (data not shown).

## DISCUSSION

The differences in transformation frequencies between the parental and mutant strains indicate that the two strains differ in their ability to take up DNA (Figure 1). Since the only genetic distinction of the mutant from the parental is that its *fabF* gene was knocked out and replaced with a cassette for kanamycin resistance, we can assume that this difference in competence is due to the lack of ACP synthase II. Whether ACP synthase II contributes a positive or negative effect on transformation was not completely

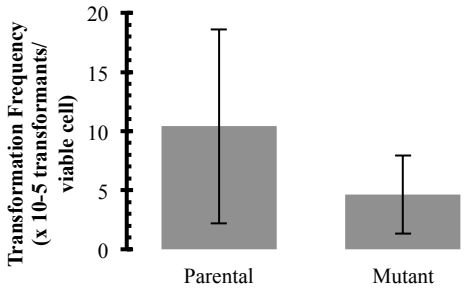


**FIG. 1.** Average transformation frequencies observed in five successive trials for the parental BW25113 and the *fabF* mutant JW1081-4 strains. Error bars represent one standard deviation.

clear due to the large variability in the control sample, but on average the parental strain had higher transformation frequencies than the mutant, so we believe that a positive effect is likely.

Since the mutants did not completely lose the ability to transform (Figure 1), knocking out *fabF* does not have an all-or-none effect on transformation. Thus ACP synthase II is not crucial for competence, but its activity in synthesizing greater quantities of *cis*-vaccinate at low temperatures would seem to play a role in maintaining membrane fluidity, and thus, by our hypothesis, in the passage of DNA through the plasma membrane. Panja *et al.* showed that temperature shocks increase transformation efficiency by triggering release of membrane proteins at low temperatures and lipids at high temperatures (5). Although there are currently no studies linking ACP synthase II activity to release of proteins from the plasma membrane, our observations on the *fabF* mutation warrant further investigation for relation to this model.

Figure 1 shows that the average transformation frequency of the mutant strain is around 5 x $10^{-5}$ transformed cells per viable cell, whereas for the parental strain, the replicates that agreed and disagreed with our expectations are gathered around 15 x $10^{-5}$ and 2 x $10^{-5}$ transformed cells per viable cell, respectively (data not shown). Since we expect the transformation frequency of a strain to be fairly constant over many replicates, and because the competent cells were not necessarily from the same batch, this pattern indicates that the two unexpected replicates may reflect errors in the transformation of the parental strains. The most likely type of error could be attributed to the highly variable initial growth of strains to make competent cells. The parental strains from these two replicates were made competent after they reached stationary phase, but they did not exhibit a greater number of

viable cells as expected. This could indicate that the late-phase cultures were adversely affected by the competency protocol, although this was not verified, as cells were plated only after both competency and transformation. Another related concern is in measuring the transformation frequency, since in three of the replicates, the mutant strain had higher viable counts than the parental strain (data not shown). Thus the lower average transformation frequency as seen in the mutants may partially be due to the viable counts.

Although evidence did not lean wholly to one side due to large variability, our experiments showed that on average, the *fabF* mutant was capable of having a two-fold lower transformation frequency when compared to the parental strain, and that overall, the knockout appeared to have a negative effect on competency. Our findings suggest that ACP synthase II could be a non-crucial, but contributory part in the transformation machinery. Also, since ACP synthase II is constitutively present but active at lower temperatures, our project also demonstrates that enzyme activity profiles should be considered alongside gene expression and protein translation profiles for elucidation of the transformation machinery.

## FUTURE DIRECTIONS

In terms of quality, the identity of the JW1081-4 and BW25113 strains received from CGSC should be confirmed by PCR or sequencing to ensure the disruption of the *fabF* gene. A membrane assay would also be performed to reconfirm the fluidity difference between mutant and wild-type. Mass spectroscopy could be used to measure the relative abundance of *cis*-vaccinate and fluorescence recovery after photobleaching (FRAP) could be used to investigate plasma membrane diffusion rates. In addition, due to variation found in the experiment, stocks of competent cells should be prepared from a single batch and large sample sets should be performed to confirm results with higher confidence. Plating cells before and after transformation would yield information on the effects of the competency protocol, storage, and transformation on cell viability. Analysis of data using transformation efficiencies (transformants/unit of DNA) would be a way to remove the effect of cell viability on measurement of competency. To ensure the presence of the kanamycin resistance cassette was not the reason for decreased transformation frequency, performing the same experiment using the revertant would provide insight.

## ACKNOWLEDGEMENTS

This work was supported by the Department of Microbiology and Immunology, University of British Columbia. Dr. W. Ramey is acknowledged for his guidance and advice.

## REFERENCES

1. **Baba, T., T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsendo, M. Tomita, B. L. Wanner, and H. Mori.** 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. **2**:1-11.
2. **Garwin, J. L., A. L. Klages, and J. E. Cronan.** 1980. β-ketoacyl-acyl carrier protein synthase II of *Escherichia coli*. J. Biol. Chem. **255**: 3263-3265.
3. **Garwin, J. L., A. L. Klages, and J. E. Cronan.** 1980. Structural, enzymatic, and genetic studies of β-ketoacyl-acyl carrier protein synthases I and II of *Escherichia coli*. J. Biol. Chem. **255**: 11949-11956.
4. **Sambrook, J. and D. W. Russell.** 2001. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Vol. 1 and 2.
5. **Panja, S., P. Aich, B. Jana, and T. Basu.** 2008. How does plasmid DNA penetrate cell membranes in artificial transformation process of *Escherichia coli*? Mol. Membr. Biol. **25**:411-422.