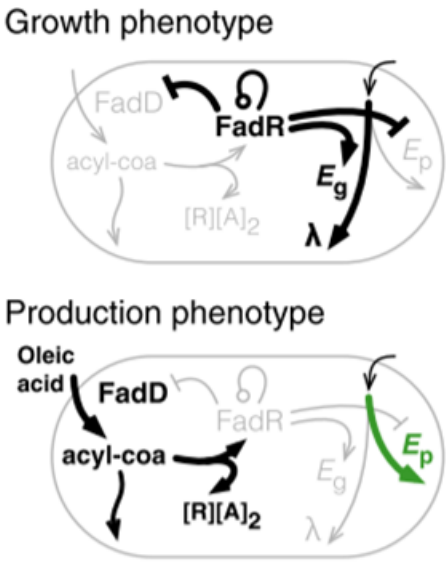
The oleic acid induction system

Mechanism

Oleic acid → acyl-CoA A → FadR protein departs from promoter PfadB → gene expresses normally, β-oxidation initiates.

Without oleic acid → FadR protein binds to promoter PfadB → gene expression is inhibited.



From the oleic acid induction mechanism, we can divide the expression of the FadR promoter into the following two scenarios:

The balance of oleic acid synthesis metabolism and decomposition metabolism in microorganisms is controlled by the transcriptional regulatory factor FadR. In the absence of oleic acid, it's a Growth phenotype with native negative autoregulation (NAR) . At this time, fatty acid synthesis metabolism is activated. FadR binds to the promoter p-fadB of the bacterial β-oxidation pathway gene fadO, inhibiting the expression of fadO, thereby inhibiting bacterial β-oxidation. In the presence of excess oleic acid, it's a Production phenotype with engineered positive autoregulation (PAR) . Fatty acid degradation metabolism is activated. After FadR couples with oleic acid, it departs from the promoter p-fadB of fadO, allowing fadO expression, and bacterial β-oxidation is completed.

Model Assumptions

1. The activation state of the oleic acid inducer (corresponding to the presence/absence of oleic acid environment) can be determined by the concentration of the regulator FadR. The expression of the product synthesis enzyme and the synthesis of the product itself depend on the control factor FadR. Therefore, we only define the state of the oleic acid inducer by the concentration of FadR.
2. FadD expression is solely controlled by FadR. In native E. coli, the expression of FadD under aerobic conditions is co-regulated by FadR and the CRP-cAMP complex (EcoCyc). However, in the presence of common carbon sources like glucose, the expression level of CRP-cAMP is very low, so its effect can be neglected.

3. The expression rate of the enzyme in two oleic acid inducer states can be modeled in the form of a Hill function. Studies have shown that the expression of gene products takes the form of a Hill function [4], either as $P_T(T) = \frac{a \cdot (K \cdot T)^n}{1 + (K \cdot T)^n}$ for T-activated expression, or as $P_T(T) = \frac{a}{1 + (K \cdot T)^n}$ for T-inhibited expression, where a is the maximum expression rate, K is the affinity with which transcription factor T binds to the operator and controls expression, and n is the Hill coefficient.
4. The growth rate of engineered bacteria linearly depends on the growth-related enzyme Eg. According to research by Usui et al. [3], the cell growth rate decreases in an approximately linear fashion. We capture this phenomenon and define the growth rate of the cell using a linear function as follows: $\lambda\left(E_g\right) = E_g \cdot \lambda_{\min} + \left(\lambda_{\max} - \lambda_{\min}\right)$. Where $\lambda_{\min}$ and $\lambda_{\max}$ represent the growth rates at zero and maximum expression of E_g, respectively.
5. Enzymes and transcription factors do not undergo active degradation. To our knowledge, proteins in the system we study are not actively degraded. Over the duration of cell doubling, protein dilution through cell growth and division is the main way proteins are lost from the system, so we model the decay of protein E as growth dilution $\lambda \cdot E$:

Model Establishment

Our endogenous system model is inspired by the model of Ahmad A. Mannan et al.[1] and the model of Hartline et al.[2], which models the expression rates of the transcription factor FadR, uptake enzyme FadD, and growth-related enzyme E_g [5] using Hill functions as $r_{\mathrm{x,R}} = P_{\mathrm{R}}(R), r_{\mathrm{x,D}} = P_{\mathrm{D}}(D), r_{\mathrm{x,E_g}} = P_{\mathrm{g}}\left(g\right)$. The reaction kinetics of FadD $\left(r_{\mathrm{D}}\right)$ and the reaction kinetics of the acyl-CoA-consuming reaction PlsB $\left(r_{\mathrm{B}}\right)$ are modeled as Michaelis-Menten equations, resulting in:

$$
\begin{aligned}
&r_{\mathrm{x,R}} = b_{\mathrm{R}} + P_{\mathrm{R}}(R), \quad r_{\mathrm{x,D}} = b_{\mathrm{D}} + P_{\mathrm{D}}(D), \quad r_{\mathrm{x},E_{\mathrm{g}}} = P_{\mathrm{g}}(g) \\
&r_{\mathrm{D}} = \frac{k_{\mathrm{cat,D}} \cdot \mathrm{OA}}{K_{\mathrm{m,D}} + \mathrm{OA}} \cdot D, \quad r_{\mathrm{B}} = \frac{k_{\mathrm{cat,B}} \cdot A}{K_{\mathrm{m,B}} + A} \cdot B.
\end{aligned}
$$

Here, the expression rate of FadR is either the native negative self-regulation (NAR) or the positive self-regulation (PAR) engineered in [2], while the expression rate of FadD remains negative self-regulation (NAR), and the E_g expression rate remains positive self-regulation (PAR):

$$
\begin{aligned}
&\mathrm{NAR}: P_{\mathrm{R}}(R) = \frac{a_{\mathrm{R}}}{1 + K_{\mathrm{R}} R}, \\
&\mathrm{PAR}: P_{\mathrm{R}}(R) = \frac{a_{\mathrm{R}} K_{\mathrm{R}} R}{1 + K_{\mathrm{R}} R} \\
&\mathrm{NAR}: P_{\mathrm{D}}(D) = \frac{a_{\mathrm{D}}}{1 + \left(K_{\mathrm{D}} R\right)^2}, \\
&\mathrm{PAR}: P_{\mathrm{g}}(g) = \frac{a_{\mathrm{g}} K_{\mathrm{g}} R}{1 + K_{\mathrm{g}} R}.
\end{aligned}
$$

Additionally, we use the mass-action kinetics model to describe the rate of formation of $[R][A]_2$ due to the sequestration of FadR by two molecules of acyl-CoA, as given by the following equation:

$$
r_{\mathrm{seq}} = r_{\mathrm{f}} - r_{\mathrm{r}} = k_{\mathrm{f}} A^2 R - k_{\mathrm{r}} C
$$

We now extend the model of Hartline and Mannan to simulate its application in oleic acid-inducible control over growth and production. The expression of product synthesis enzymes and the product itself does not provide feedback that affects the rest of the control system. Since it relies solely on FadR, we do not explicitly model production. Instead, using the same approach as

in [1], we define the native production phenotype by a low set concentration value of FadR $R \leq 0.0033\mu\mathrm{M}$. This implies that the growth phenotype of the engineered bacteria activated by the oleic acid inducer is defined as $R > 0.0033\mu\mathrm{M}$.

During the experimental process, we added the gene of the green fluorescent protein after the oleic acid inducer operator fadO. When FadR binds to the operator fadO, leading to the activation of the oleic acid inducer, the green fluorescent protein gene is expressed. Therefore, by detecting the intensity of the green fluorescent protein F in the culture medium, we can assess the activation level of the oleic acid inducer, that is:

$$F = K_p \cdot \int_0^T r_{\text{seq}}\ (t)dt + b_p$$

Now, we establish the ordinary differential equation model for the change rates of FadR(R), FadD (D), acyl-CoA (A), sequestered complex (C), growth-associated enzyme(Eg), and fluorescent protein(F):

$$\begin{aligned}
\frac{dR}{dt} &= r_{\mathrm{x,R}} - r_{\text{seq}} - \lambda\left(E_{\mathrm{g}}\right)R, \\
\frac{dD}{dt} &= r_{\mathrm{x,D}} - \lambda\left(E_{\mathrm{g}}\right)D, \\
\frac{dA}{dt} &= r_{\mathrm{D}} - r_{\mathrm{B}} - 2\cdot r_{\text{seq}} - \lambda\left(E_{\mathrm{g}}\right)A, \\
\frac{dC}{dt} &= r_{\text{seq}} - \lambda\left(E_{\mathrm{g}}\right)C, \\
\frac{dE_{\mathrm{g}}}{dt} &= r_{\mathrm{x},E_{\mathrm{g}}} - \lambda\left(E_{\mathrm{g}}\right)\cdot E_{\mathrm{g}}, \\
\frac{dF}{dt} &= r_{\text{seq}}.
\end{aligned}$$

Model expansion with additional FadO operators

References

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