Supporitng Text

Enhancer Mutagenesis

This method is relevant for the results shown in Fig. 6*B*. The enhancer sequence was mutagenized with Splicing by Overlap Extension (SOE) (1). The following mutagenic primers were used for the first PCR. Strong enhancer: Ss, 5'-CAAAGGTCATTGCACTAAAATGGTGCATAATGTTTCCATT GATGCACTATATTGGTGC-3', and As, 5'-CAAAGGTCATTGCACTAAAA TGGTGCATAATGTTTCCATTGATGCACTATATTGGTGC-3'. Weak enhancer: Sw, 5'-GCTTAATGTTTCCATTGATGCACCATATTAAGATAACATTCACATCGTGG TGCAGCCC-3', and Aw, 5'-GCTGCACCACGATGTGAATGTTATCTTAAT ATGGTGCATCAATGGAAACATTAAGCACC-3'. Outside primers were the following: F, 5'-GCAGCAAGCGGTCCACGCTGG-3', and R, 5'-GCATCACCTTCACCCTCTCC-3'.

PCR was carried out with (*i*) Ss/R and (*ii*) As/F for the strong enhancer and (*iii*) Sw/R and (*iv*) Aw/F for the weak enhancer. The following components were used for the PCR reactions: 200 nM concentration of each primer, 1 ng/μl template ($pGlnAp_2GFP_{mut3.1}$), 0.2 mM dNTP, and 50 milliunits/ml *Taq* polymerase. Thermal cycling parameters were 95°C for 2 min (one cycle), 95°C for 30 s, 58°C for 30 s, 72°C for 1.5 min (24 cycles), 72°C for 10 min (one cycle). The PCR products were gel-purified (Zymoclean, Zymo Research, Orange, CA), and 0.24 ng/μl Ss/R and Sw/R and 0.06 ng/μl As/F and Aw/F were used as templates for the second PCR. Concentrations were the same as in the first PCR, and thermal-cycling parameters were 95°C for 2 min (one cycle), 95°C for 30 s, 65°C for 45 s, 72°C for 1.33 min (six cycles), 72°C for 5 min (one cycle), 95°C for30 s, 53°C for 30 s, 72°C for 1.25 min (25 cycles), 72°C for 5 min (one cycle). The primers F and R were added during the incubation at 72°C for 5 min. The products of the assembly PCR were purified and used as megaprimers for Megawhop (2). PCR conditions were the following: 12 ng/μl megaprimer, 1 ng/μl template ($pGlnAp_2GFP_{mut3.1}$), 0.2 mM dNTP, 75 milliunits/ml pfu-turbo. Thermal cycling parameters were 95°C for 1.5 min (one cycle), 95°C for 30 s, 55°C for 30 s, 68°C for 9 min (24 cycles), 68°C for 30 min (one cycle). After addition of 400 units/ml *Dpn*I, the reaction solution was incubated at 37°C for 2 h. The reaction solution was used for the transformation of competent BW18793. Single colonies were picked and cultivated. Plasmids were prepared from these cultures and sequenced.

QS Experiment with a Degradable Reporter Protein

BW18793 carrying $pGlnAp_2GFP_{AAV}$ was used in a QS experiment (Fig. 7). This control experiment verifies that the behavior of the QS strain is not sensitive to the half-life of the reporter protein.

Mathematical Model

For all intracellular species, the mole balance begins with,

$$\frac{d(nVc \cdot X)}{dt} = nVc\left(r_{generation} - r_{consumption}\right), \quad \textbf{[1]}$$

where n is the number of cells, Vc is the volume of individual cells, X is the molar concentration of the species of interest, $r_{\text{generation}}$ and $r_{\text{consumption}}$ are the rates of generation and consumption of X, respectively. The left-hand side of the equation can be expanded by chain rules to give

$$\frac{d(nVc \cdot X)}{dt} = X\frac{dnVc}{dt} + nVc\frac{dX}{dt} = nVc\left(r_{generation} - r_{consumption}\right). \quad \textbf{[2]}$$

Subtracting $X\frac{dnVc}{dt}$ from both sides, then dividing both sides by nVc yields

$$\frac{dX}{dt} = r_{generation} - r_{consumption} - X\left(\frac{\mathbf{1}}{nVc}\frac{dnVc}{dt}\right), \quad \textbf{[3]}$$

where the term in parentheses is, by definition, equal to the specific growth rate of the cells,

$$\frac{\mathbf{1}}{nVc}\frac{dnVc}{dt} = \mu\,. \quad \textbf{[4]}$$

Thus, Eq. **1** becomes

$$\frac{dX}{dt} = r_{generation} - r_{consumption} - \mu X\,. \quad \textbf{[5]}$$

For extracellular species, the mole balance is

$$\frac{d([Vt - nVc]\cdot X)}{dt} = (Vt - nVc)\left(r_{generation} - r_{consumption}\right), \quad \textbf{[6]}$$

where Vt is the total culture volume. Expand by chain rules and perform algebraic rearrangements, and Eq. **6** thus becomes

$$\frac{dX}{dt} = r_{generation} - r_{consumption} + X\left(\frac{\mathbf{1}}{Vt - nVc}\frac{dnVc}{dt}\right). \qquad \textbf{[7]}$$

Multiply the term in parentheses with nVc/nVc and use of Eq. **4** yields

$$\frac{dX}{dt} = r_{generation} - r_{consumption} + X\mu\left(\frac{nVc}{Vt - nVc}\right) \qquad \textbf{[8]}$$

For species involved in diffusion across the cell membrane, the volume fraction of the cells needs to be accounted for. Hence, for the intracellular component, the mole balance is

$$\frac{d\left(nVc \cdot X_i\right)}{dt} - \left(nVc\right)\left(r_{generation} - r_{consumption}\right) = -na_s g_m \left(X_i - X_e\right) \qquad \textbf{[9]}$$

and for the extracellular counterpart, the mole balance is

$$\frac{d\left(\left[Vt - nVc\right] \cdot X_e\right)}{dt} - \left(Vt - nVc\right)\left(r_{generation} - r_{consumption}\right) = na_s g_m \left(X_i - X_e\right), \qquad \textbf{[10]}$$

where a_s is the surface area of the individual cell and g_m is the mass transfer coefficient of acetic acid through the membrane. According to the reaction scheme depicted in Fig. 8 and the derivation above, the differential equations for each species are as follows:

$$\frac{dOAc_i^-}{dt} = v_1 + v_2 - v_3 - \mu\left(OAc_i^-\right) \qquad \textbf{[11a]}$$

$$\frac{dHOAc_i}{dt} = \frac{-v_4}{Vc} + v_3 - \mu\left(HOAc_i\right) \qquad \textbf{[11b]}$$

$$\frac{dHOAc_e}{dt} = \frac{v_4}{Vt - nVc} + v_5 + \mu \frac{nVc}{Vt - nVc}\left(HOAc_e\right) \quad \textbf{[11c]}$$

$$\frac{dOAc_e^-}{dt} = -v_5 + \mu \frac{nVc}{Vt - nVc}\left(OAc_e^-\right) \quad \textbf{[11d]}$$

$$\frac{dAcP}{dt} = -v_2 - v_6 - \mu\left(AcP\right) \quad \textbf{[11e]}$$

$$\frac{dn}{dt} = \mu n \quad \textbf{[11f]}$$

$$\frac{dmgfp}{dt} = v_7 - v_8 - \mu\left(mgfp\right) \quad \textbf{[11g]}$$

$$\frac{dGfp}{dt} = v_9 - v_{10} - \mu\left(Gfp\right). \quad \textbf{[11h]}$$

Definitions each variable and velocity terms are listed in Tables 1 and 2 respectively. The definitions and values of each parameter are given in Table 3.

1. Horton, R. M. (1995) *Mol. Biotechnol.* **3,** 93-99.

2. Porter, S. C., North, A. K., Wedel, A. B. & Kustu, S. (1993) *Genes Dev.* **7,** 2258-2273.