Supporting Text

Variable RepA Binding to Different Iterons *in Vitro*. The present RepA mutants seem to be changed primarily in DNA binding activity. As discussed in the main text, a preferential binding defect for autorepression could increase initiator supply and thereby could increase the copy number. To test this hypothesis, DNA binding of RepA was compared between two different pairs of iterons. An iteron pair overlapping the *repA* promoter (nos. 11 and 10) (the –35 and –10 of the promoter flanks the iteron no. 11) and another furthest from the promoter (nos. 14 and 13) (Fig. 5 *A* and *B*) were used. These pairs were also most divergent from each other in their DNA sequences. A pair of iterons was used so that possible cooperative interactions could be tested, because loss of positive cooperativity in binding also could lead to poorer autorepression and increased initiator production. Single-site binding to the iterons nos. 14 and 13 was nearly identical for the three mutants and only slightly defective compared with the WT (Fig. 5*C*). The defect in the mutants became more conspicuous when side-by-side binding was compared, signaling some loss of cooperativity (Fig. 5*C*). Both single and side-by-side binding profiles for the promoter iterons differed from those of the iterons nos. 14 and 13. Compared with the WT, the defect was enhanced for mutants 120 and 123 and essentially vanished for 143 (Fig. 5*D*). The strength of binding thus could depend on the iteron and its context. The relatively weak binding of mutants 120 and 123 specifically to the promoter iterons conformed to our expectation that initiators are oversynthesized in the mutants. The basis of the mutant phenotype for 143 could be different. It appears to be only defective in side-by-side binding to the iteron-pair nos. 14 and 13. Together with the results of Fig. 3*C*, we conclude that DNA binding to origin iterons has changed both qualitatively and quantitatively in the mutants, although not identically in all three cases.

Dampening of Monomer Increase Due to Dimerization. By transcriptional autorepression, the initiator synthesis rate increases and decreases at lower and higher initiator concentrations, respectively. This mechanism makes the initiator concentration less sensitive to changes in plasmid concentration. The simplest mathematical model

depicting this phenomenon assumes that the initiator (x) represses its own gene according to the following scheme:

$$active \underset{k_{diss}}{\overset{k_{ass}[x]}{\rightleftharpoons}} inactive$$

The probability that the gene is in its inactive state is then

$$r = \frac{k_{ass}[x]}{k_{diss} + k_{ass}[x]} = \frac{[x]}{K + [x]} \qquad \textbf{[1]}$$

where $K = k_{diss}/k_{ass}$. Assuming that the gene switches rapidly between these two states, that the synthesis is constant k_1 per active gene copy, that there are n plasmid copies, and that initiator is diluted and spontaneously degraded with a rate k_2, then the rate of change of x can be described by

$$\frac{d[x]}{dt} = k_1 n \frac{K}{K + [x]} - k_2[x] \qquad \textbf{[2]}$$

Setting $d[x]/dt = 0$ results in a quadratic equation for how the steady-state concentration $[x]_{ss}$ depends on the kinetic parameters, like n. Solving the equation and calculating the sensitivity parameter S gives

$$S = \frac{\partial [x]_{ss}}{\partial n} \times \frac{n}{[x]_{ss}} = \frac{1}{1 + r} \qquad \textbf{[3]}$$

S is thus a logarithmic gain factor and measures how sensitively the steady-state initiator concentration responds to changes in plasmid copy number: if $S = ½$, then a 1% change in n eventually gives about a ½% change in $[x]_{ss}$. Eq. **3** thus shows how negative feedback reduces sensitivity, but only for large r, i.e., for strong inhibition.

Now instead assume that the initiator is made constitutively but is involved in standard monomer–dimer equilibrium. With [y] as the dimer concentration, the simplest rate equations are

$$\begin{aligned}\frac{d[x]}{dt} &= \overbrace{\tilde{k}_1 n}^{\text{synthesis}} - \overbrace{\tilde{k}_2[x]}^{\text{degradation}} - \overbrace{2k_a[x]^2 + 2k_d[y]}^{\text{monomer–dimer equilibrium}} \\ \frac{d[y]}{dt} &= \underbrace{k_a[x]^2 - k_d[y]}_{\text{monomer–dimer equilibrium}} - \underbrace{k_2[y]}_{\text{degradation}}\end{aligned} \qquad \textbf{[4]}$$

Setting these equations to zero and solving for the steady state again yields a quadratic equation that can be solved for $[x]_{ss}$. Following the same procedure as above again leads to

$$S = \frac{\partial [x]_{ss}}{\partial n} \times \frac{n}{[x]_{ss}} = \frac{1}{1+r} \text{ where } r = \frac{2[y]_{ss}}{[x]_{ss} + 2[y]_{ss}} \qquad \textbf{[5]}$$

Dimerization thus can be as efficient as autorepression in obtaining initiator homeostasis, and the mechanisms are in some senses equivalent, where the level of saturation in autorepression corresponds to the fraction of total protein present in dimer form. This equivalence is easier seen by comparing the idealized schemes

$$\frac{d[x]}{dt} = \frac{n}{[x]} - [x] \text{ and } \frac{d[x]}{dt} = n - [x]^2 \qquad \textbf{[6]}$$

where in both cases $[x]_{ss} = \sqrt{n}$, so that a 2-fold increase in plasmid copy number only gives a $\sqrt{2} = 1.4$-fold increase in initiator (Fig. 6). Accelerating dimerization thus can have the same homeostatic effect as retarding synthesis, and in general the effect depends on the difference in effective kinetic orders of elimination and synthesis (1). In terms of Eq. **6** above, the first mechanism results in 1– (–1) = 2 and the second mechanism in 2–0 = 2. In both cases, the sensitivity factor is S = ½. Both transcription and initiator multimerization schemes also can be made more sensitive by allowing cooperative

binding in the autorepression loop or by forming higher-level multimers. The schemes are also compatible with each other and result in a higher sensitivity when combined. The combined effect of the autorepression and dimerization schemes above could cause initiators to increase by $\sqrt[3]{2} = 1.3$ fold in the case of P1 and $\sqrt[4]{2} = 1.2$ fold in the case of F, when the copy number increases 2-fold (Fig. 6). The higher sensitivity in the case of F is because dimers serve as autorepressors rather than monomers as in P1.

1. Paulsson, J. (2004) *Nature* **427,** 415–418.