

## Supporting online material for Elowitz *et al.* report

### Materials and methods

**Strains and plasmids.** Plasmids expressing CFP or YFP (wild-type codons, developed by University of Washington Yeast Resource Center) from several promoters, including  $P_{LacO1}$  (*S1*) and  $\lambda P_R$  (*S2*) were constructed. The antibiotic resistance gene, promoter, ribosome-binding site, fluorescent protein gene, and transcriptional terminators from these plasmids were amplified by PCR and integrated at specific chromosomal loci by homologous recombination (*S3*), making use of primer-encoded 50 bp tails specifying the target region. For the *galK* locus, the following primers, derived from those described previously (*S3*), were used to amplify *cfp* transcriptional units (homology to the *E. coli* chromosome is indicated by uppercase letters; segments homologous to the plasmid template are in lowercase):

TTCATATTGTTTCAGCGACAGCTTGCTGTACGGCAGGCACCAGCTCTTCCG  
cacgtaaggattttgtca and

GTTTGC GCGCAGTCAGCGATATCCATTTTCGCGAATCCGGAGTGTAAGAA

cgccttgagtgagctgata. For integration into the *intC* locus, the following primers were used to amplify *yfp*:

ATAGTTGTTAAGGTCGCTCACTCCACCTTCTCATCAAGCCAGTCCGCCA  
tgaagtcagccccatacgat and

CCGTAGATTTACAGTTCGTCATGGTTCGCTTCAGATCGTTGACAGCCGCA

gagtcagtgagcgaggaagc. Chromosomal inserts were moved to other strains, as necessary, by P1 transduction (S4).

For further repression of reporter genes (such as in Fig. 3, B and C), plasmid pREP4 (Qiagen) was introduced into cells. pREP4 carries the *lacI<sup>q</sup>* promoter mutation for high-level expression of *lac* repressor.

For experiments with the Repressilator, we replaced the *amp<sup>R</sup>* gene on the original plasmid (S5) with a Spectinomycin-resistance cassette (S1).

**Statistical equivalence of reporter genes.** Our methods assume that the two reporter genes are, on average, identically regulated. In other words, we assume that the distributions of the amounts of CFP and YFP per cell are the same. To verify this assumption, single-color distributions of strains containing *lac*-repressible promoter pairs (M22 and D22, Table 1) were measured and subjected to a Kolmogorov-Smirnov test. In both cases, the individual normalized distributions for CFP and YFP were consistent with the null hypothesis, that they were both sampled from the same underlying distribution (P=0.71 and 0.68, respectively). To check for possible interactions between CFP and YFP, we constructed strains containing a constitutive promoter ( $\lambda$  P<sub>R</sub>) driving one color (CFP or YFP), and the *lac*-inducible promoter (P<sub>L</sub>lacO1) driving the other. No systematic differences were found among the measured distributions of the constitutive protein with different amounts of IPTG. With *lac*-regulated constructs, average amounts of both proteins could be varied in a co-linear fashion over two orders of magnitude.

**Measurement protocol.** Cultures were grown overnight in LB at 32°C. The next day, 2 ml of fresh LB was inoculated with 20  $\mu$ l of the overnight culture, grown at 32°C to

OD<sub>600</sub>=0.2 – 0.3, and transferred to ice. ~2 μl of cells were placed between a coverslip and a small slab of 1.5% agarose in PBS. Approximately 10 fields were acquired at 100X magnification with a Leica DMIRB/E fluorescence microscope, a cooled CCD camera (Orca II, Hamamatsu), and custom microscope control software. For each field, phase contrast and fluorescence (CFP cube = Chroma, #31044v2, and YFP cube = Chroma, #41028) images were acquired. Analysis was done off-line with custom software. Fluorescence images were corrected for inhomogeneous illumination. The program identified individual cells on phase contrast images (shapes were filtered to eliminate non-cell phase-dark areas or clumps of cells), and extracted pixel values from corresponding regions of fluorescence images.

As a negative control, cells expressing GFP were measured through CFP, YFP, and GFP (Chroma, #HQ41001) filters and processed as above. The resulting “noise”,  $\eta_{int0} \approx 0.02$ , approximates the error level of the measurement system as a whole. Intrinsic noise values may thus overestimate true values by a factor of  $\sqrt{1 + \eta_{int0}^2 / \eta_{int}^2}$  ( $\leq 12\%$  for all data).

**Definitions of noise and parameterization of data.** Intrinsic noise ( $\eta_{int}$ ), extrinsic noise ( $\eta_{ext}$ ), and total variation ( $\eta_{tot}$ ) were defined as follows:

$$\eta_{int}^2 \equiv \frac{\langle (c - y)^2 \rangle}{2\langle c \rangle \langle y \rangle}; \quad \eta_{ext}^2 \equiv \frac{\langle cy \rangle - \langle c \rangle \langle y \rangle}{\langle c \rangle \langle y \rangle} \quad \eta_{tot}^2 \equiv \frac{\langle c^2 + y^2 \rangle - 2\langle c \rangle \langle y \rangle}{2\langle c \rangle \langle y \rangle}$$

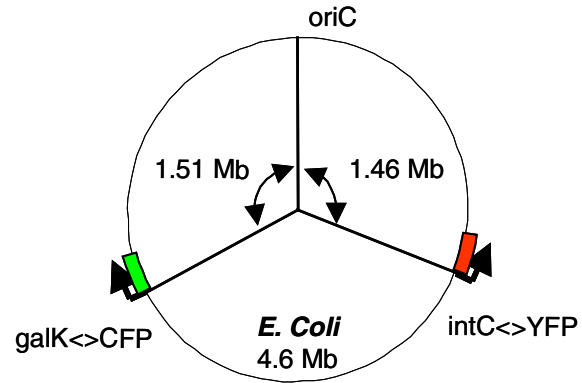
Here the  $i^{th}$  element of vectors  $c$  and  $y$  contains the average CFP or YFP intensity, respectively, of the  $i^{th}$  cell in the sample. Angled brackets denote means over the cell population. These definitions treat *cfp* and *yfp* data symmetrically, allow  $\eta_{int}$  to be

interpreted as the normalized r.m.s. distance from the line defined by CFP=YFP (Fig. 2B), and satisfy  $\eta_{int}^2 + \eta_{ext}^2 = \eta_{tot}^2$  (S6). Thus, when either source of noise decreases to 0, the other sets the total noise entirely. Error bars (see Figure 3) were calculated by the bootstrap method from the actual distributions.

In the form  $\eta_{int}^2 \approx c_1 / m + c_2$ , the  $c_1$  term represents the increase in intrinsic noise that accompanies a decrease in the rate of transcription.  $c_2$  is expected to be non-zero due to fluctuations in extrinsic variables (S6). In addition, stochastic differences in replication time may also contribute; in particular, note the increased value of  $c_2$  in  $\Delta recA$  cells (Fig. 3, B and C).

**Microcolonies.** Microcolonies were grown on low-melt Seaplaque agarose (BMA) in M9 Minimal Medium supplemented with 0.2% glycerol, 0.01% Casamino Acids, biotin (0.15  $\mu\text{g/ml}$ ), and 1.5  $\mu\text{M}$  thiamine. Cells were prepared as above, but at one twentieth the density. Custom software and ImageProPlus (Media Cybernetics) were used to control the automated microscope functions and maintain focus during timelapse.

## Supplementary Figure



**Fig. S1.** A map of the *E. coli* chromosome with the origin of replication (*oriC*), *cfp* and *yfp* loci indicated. Locations were chosen to avoid systematic, but remain sensitive to stochastic, differences in gene copy number. The <> symbol denotes replacement by homologous recombination (S3).

## Supporting References and Notes

- S1. R. Lutz, H. Bujard, *Nucleic Acids Res.* **25**, 1203 (1997).
- S2. B. J. Meyer, R. Maurer, M. Ptashne, *J. Mol. Biol.* **139**, 163 (1980).
- S3. D. Yu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 5978 (2000).
- S4. J. H. Miller, *Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia Coli and Related Bacteria* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992).
- S5. M. B. Elowitz, S. Leibler, *Nature* **403**, 335 (2000).
- S6. P. S. Swain, M. B. Elowitz, E. D. Siggia, in preparation.