Supporting Methods

Bacterial Strains and Plasmids. All cloning and expression were performed in *Escherichia coli* strain DH5α-PRO [Clontech; identical to DH5α-Z1 (1)]. This strain produces large amounts of Tet and Lac repressors, thus lowering leaky expression from the Tet and Lac promoters. All DNA, RNA, and protein manipulations were carried out according to standard molecular biology procedures (2). Bacterial techniques were as described in ref. 3. All constructs were verified by sequencing with the appropriate primers.

Construction of MS2d–GFP. The expression vector was pPROTET.E (Clontech), a ColE1-based plasmid carrying the P(LtetO-1) promoter. The selection marker was changed from chloramphenicol to kanamycin, which resulted in the plasmid K133. GFPmut3 (4) was amplified from pKEN2 (a gift of P. Wolanin, Princeton University, Princeton, NJ) and inserted into the *Not*I site of K133. MS2d (5) was amplified from p2CTdl13 (a gift of D. Peabody, University of New Mexico, Albuquerque) and inserted into the *Bam*HI site of K133. Upon induction by anhydrotetracycline (aTc), a protein product of the correct size was observed on SDS/PAGE gels. For *in vitro* assays, protein was purified by using the N-terminal 6×HN affinity tag in the pPROTET.E vector (PT3161-1) according to the manufacturer's protocol (Clontech).

RNA-Binding Assay for *in Vitro* Functionality. RNA gel mobility shifts were performed according to the method of ref. 6. MS2d–GFP was assayed by using a 6× binding site target transcribed from pBS-SK–MS2 ×6 (a gift of K. Forrest, Princeton University, Princeton).

Blue/White Test for MS2d–GFP Functionality *in Vivo*. We used the test devised by Peabody (7) to verify that our fusion protein binds to the MS2-binding site *in vivo*. The test is based on the fact that a functional coat protein represses the translation of an MS2 replicase/β-galactosidase fusion, thus preventing the formation of blue colonies on plates containing isopropyl β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal). Strain DH5α-PRO was transformed with plasmid pRZ5 encoding the hybrid replicase/β-galactosidase (a gift of D. Peabody) together with a plasmid coding for the MS2d–GFP protein, as well as various proteins serving as controls: wild-type MS2 (provided by D. Peabody), MS2 dlFG–enhanced GFP (EGFP) (provided by K. Forrest), and others. Cells were then streaked on LB plates, with antibiotic selection for both plasmids, to which X-Gal (final concentration of 60 μg/ml) and IPTG (0.2 mM) had been added.

Construction of the 96 Binding Site (96 BS) Arrays. The general scheme for constructing the tandem array of binding sites is described in the legend for Fig. 7. The following 135-base oligonucleotide, where N designates a random nucleotide, was synthesized:
CCGTCTAGAGCATGNNNNACATGAGGATCACCCATGTNNNNNNNNNNNNNNNNACATGAGGATCACCCATGTNNNNNNNNNNNNNNNNACATGAGGATCACCCATGTNNGCTAGCGTCCGAAGCTTCTACGC. This oligonucleotide mixture was

converted into double-stranded DNA and amplified by PCR using the primers GCCGTCTAGAGCATG and GCGTAGAAGCTTCGG. At this stage the ensemble consisted of $\approx 10^{13}$ molecules. The product was then cut and inserted into pBS-SK (Stratagene), and sequential doublings of the array were carried out as shown in Fig. 7. At each step, heterogeneity of the plasmid population was maintained by harvesting a few plates containing confluent colonies. The heterogeneity of the ensemble was verified by sequencing at a few doubling stages along the way. A 96 BS array was then inserted between the *Nhe*I and *Hin*dIII sites of pTRUEBLUE-BAC2 (Genomics One International, Buffalo, NY), to yield BAC2+bs96. For *in vitro* assays, RNA was transcribed from the T7 promoter in the pTRUEBLUE-BAC2 plasmid. For the RNA induction experiment, the original Plac promoter was replaced by Plac/ara-1 from plasmid pZS*24 (1) (gift of P. Wolanin).

Bacterial Growth and Induction. Cells were grown in either LB or M63 minimal medium (3), supplemented by antibiotics according to the specific plasmids. For induction of protein and RNA, cells were grown overnight from a single colony, diluted into fresh medium, and grown at 37°C to mid-logarithmic phase ($OD_{600} \approx 0.3–0.5$). The inducers IPTG (1 mM) and aTc (10 ng/ml) were then added. RNA particles were detectable from ≈45 min after induction and up to a few hours later, when background fluorescence levels became too high for further observation.

Microscopy and Imaging. A few microliters of culture was placed between a coverslip and a thin slab of 0.8% agarose containing LB. Microscopy was performed in a room maintained at 22°C. Under these conditions, cells doubled every 2 to 3 h. Microscopy was performed with a Nikon Eclipse (TE2000-U) inverted microscope equipped with a ×100 (1.3 numerical aperture) objective and epifluorescence system. Images were taken with a Cascade:512B camera (Roper Scientific, Trenton, NJ) after an additional ×6 magnification. Images and time-lapse movies were acquired with METAVIEW software (Universal Imaging, Downington, PA) and analyzed with MATLAB (Mathworks, Natick, MA). In some cases, image quality was improved by deconvolution using AUTODEBLUR software (Autoquant Imaging, Troy, NY), but intensity measurements were always performed on the original images.

1. Lutz, R. & Bujard, H. (1997) *Nucleic Acids Res.* **25,** 1203–1210.

2. Sambrook, J. & Russell, D. W. (2001) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).

3. Miller, J. H. (1992) *A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria* (Cold Spring Harbor Lab. Press, Plainview, NY).

4. Cormack, B. P., Valdivia, R. H. & Falkow, S. (1996) *Gene* **173,** 33–38.

5. Peabody, D. S. (1997) *Arch. Biochem. Biophys.* **347,** 85–92.

6. Dahanukar, A., Walker, J. A. & Wharton, R. P. (1999) *Mol. Cell* **4,** 209–218.

7. Peabody, D. S. (1989) *Nucleic Acids Res.* **17,** 6017–6027.