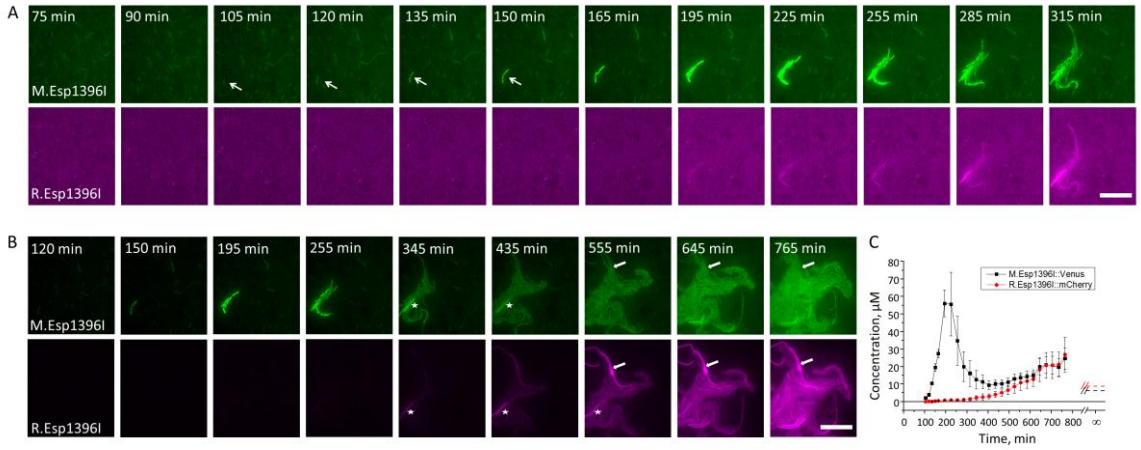
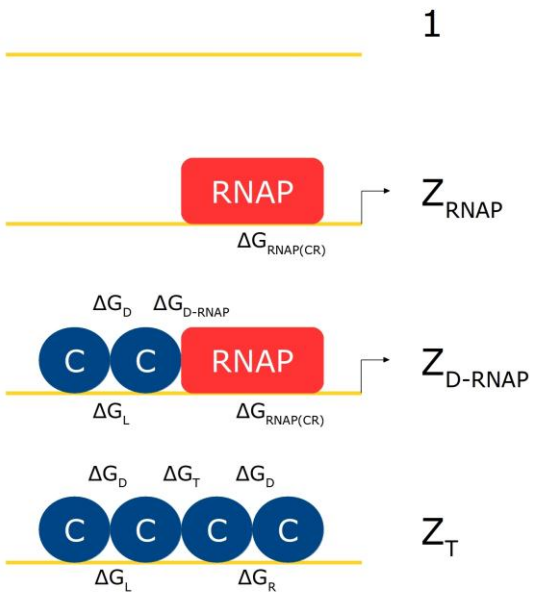


Supplementary Figure S1. Dynamics of Esp1396I enzymes accumulation in individual transformed *E. coli* cells. A and **B.** A representative kinetic series of images showing Venus (**green**) and mCherry (**magenta**) fluorescence in a microcolony growing from a single transformed cell. The initial part of the sequence is shown in **A,** in **B**, the full duration of experiment is shown. A non-linear contrast was used in **B** to promote simultaneous visualization of bright and dim signals (see Materials and Methods). Thin white arrows in **A** show transformed cell at early stages before Venus accumulation is clearly visible. In **B**, white asterisks indicate an individual cell in which mCherry fluoresecence becomes detectable. A wide white arrow in **B** shows an atypical cell (see Fig. 3C for more examples).
C. Quantification of a representative kinetic series showing changes in Venus and mCherry fluorescence intensities per individual cell in microcolony growing from a single transformed cell over time. Dashed lines at infinity show stationary R.Esp1396I::mCherry and M.Esp1396I::Venus obtained on suspension cells at exponential growth phase.

Modeling of the dynamics of the Esp1396I system

Modeling transcription activity of the CR promoter

The design of the CR and M promoter regulation model is based on experimental information on Esp1396I transcription control provided by Bogdanova et al. (1). There are 4 different allowed states of the CR promoter: 1) the empty promoter, 2) just RNA polymerase (RNAP) bound to the promoter, 3) C dimer bound to the left (distal) operator with RNAP bound to the promoter, and 4) C tetramer bound to the left and the right (proximal) operator (Figure S2). Their corresponding statistical weights are (the weight of the empty promoter is 1):



Supplementary Figure S2. A scheme representing possible CR promoter configurations. See text for details.

$$Z_{RNAP} = k\left[RNAP\right]e^{-\Delta G_{RNAP(CR)}} \quad (0.1)$$

$$Z_{D-RNAP} = k^3\left[C\right]^2\left[RNAP\right]e^{-\left(\Delta G_L+\Delta G_{RNAP(CR)}+\Delta G_D+\Delta G_{D-RNAP}\right)} \quad (0.2)$$

$$Z_T = k^4\left[C\right]^4 e^{-\left(\Delta G_L+\Delta G_R+2\Delta G_D+\Delta G_T\right)} \quad (0.3)$$

where [RNAP] and [C] denote RNA polymerase and C protein concentrations, k denotes proportionality constant for the statistical weights (with the units of concentration), while $\Delta G_{RNAP(CR)}$, ΔG_L, ΔG_R, ΔG_D, $\Delta G_{D\text{-}RNAP}$ and ΔG_T denote free energies of the respective molecular interactions (see Figure S2). Transcription activity of the CR promoter ϕ_r is proportional to its equilibrium occupancy by RNA polymerase (2):

$$\phi_r = \alpha \frac{Z_{RNAP} + Z_{D-RNAP}}{1 + Z_{RNAP} + Z_{D-RNAP} + Z_T} \quad (0.4)$$

which is transformed by introducing constants $a = k[RNAP]e^{-\Delta G_{RNAP(CR)}}$, $b = k^3[RNAP]e^{-(\Delta G_L + \Delta G_{RNAP(CR)} + \Delta G_D + \Delta G_{D-RNAP})}$ and $c = k^4 e^{-(\Delta G_L + \Delta G_R + 2\Delta G_D + \Delta G_T)}$ into:

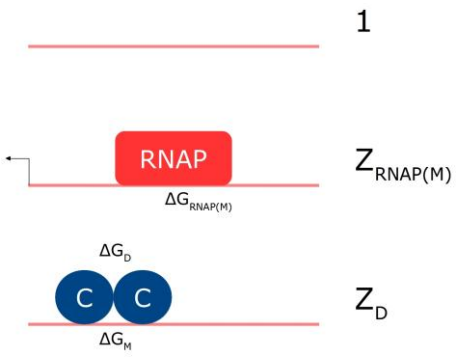
$$\phi_r(C) = \alpha \frac{a + b[C]^2}{1 + a + b[C]^2 + c[C]^4} \quad (0.5)$$

If we introduce the basal activity of the CR promoter $\phi_{baz(r)}$, which is defined as the transcriptional activity when [C]→0, the equation above takes the form which is used in further modeling:

$$\phi_r(C) = \phi_{baz(r)} \frac{1 + \frac{b}{a}[C]^2}{1 + \frac{b}{a+1}[C]^2 + \frac{c}{a+1}[C]^4} \quad (0.6)$$

Modeling transcription activity of the M promoter

We assumed 3 allowed states of the M promoter: 1) empty promoter (weight = 1), 2) promoter occupied by RNA polymerase only, and 3) C dimer bound to the operator which was experimentally proven to bind C proteins (Figure S3). The corresponding statistical weights are:



Supplementary Figure S3. A scheme representing M promoter configurations.

$$Z_{RNAP(M)} = k[RNAP]e^{-\Delta G_{RNAP(M)}} \quad (0.7)$$

$$Z_D = k^2[C]^2 e^{-(\Delta G_M + \Delta G_D)}, \quad (0.8)$$

where [RNAP] and [C] denote RNA polymerase and C protein concentrations, k denotes proportionality constant for the statistical weights, while $\Delta G_{RNAP(M)}$, ΔG_M and ΔG_D denote free energies of the molecular interactions indicated in Figure S3.

Transcription activity of M promoter ϕ_m is proportional to its equilibrium occupancy by RNA polymerase:

$$\phi_m = \beta \frac{Z_{RNAP(M)}}{1 + Z_{RNAP(M)} + Z_D} \tag{0.9}$$

If we introduce constants $f = k[RNAP]e^{-\Delta G_{RNAP(M)}}$ and $g = k^2 e^{-(\Delta G_M + \Delta G_D)}$ the above equation becomes:

$$\phi_m(C) = \beta \frac{f}{1 + f + g[C]^2} \tag{0.10}$$

If we further introduce the basal activity of M promoter $\phi_{baz(m)}$, and $K_m^2 = g/(f+1)$, the equation above becomes:

$$\phi_m(C) = \phi_{baz(m)} \frac{1}{1 + K_m^2[C]^2} \tag{0.11}$$

Modeling protein expression dynamics

The following equations describe how CR transcripts, and C and R protein change with time (notation is provided in Table 1 below):

$$\frac{dr}{dt} = \phi_r(C) - \lambda_r r \tag{0.12}$$

$$\frac{dC}{dt} = k_C r - \lambda_C C \tag{0.13}$$

$$\frac{dR}{dt} = k_R r - \lambda_R R \tag{0.14}$$

The first terms on the right-hand side of the above equations describe the generation of CR transcripts by transcription (Eq. (0.12)), and C and R proteins by translation (Eqs. (0.13) and (0.14)). The second terms on the right-hand sides of the above equations represent transcript (Eq. (0.12)) and protein decay (Eqs. (0.13) and (0.14)). To reduce the parameter number, the model uses $k_R = (2/3)\,k_C$ due to translational coupling (3) and assumes $\lambda_R = \lambda_C$, which leads to $R = Ck_R/k_C$, so that solving only two equations ((0.12) and (0.13)) is required.

Similarly, the dynamics of methyltransferase and its transcript (notation provided in Table 1), is determined by the following equations:

$$\frac{dm}{dt} = \phi_m(C) - \lambda_m m \tag{0.15}$$

$$\frac{dM}{dt} = k_M m - \lambda_M M \tag{0.16}$$

Table 1. The notation used in equations and the parameter values of the best model fit to experimental data.*

Notation	Description		Notation	Description	
Variables			Variables		
ϕ_r	Transcription activity of CR promoter		ϕ_m	Transcription activity of M promoter	
r	Concentration of CR operon transcript		m	Concentration of M gene transcript	
C	Concentration of control protein		M	Concentration of methyltransferase	
R	Concentration of restr. Endonuclease				
Constants		Best fit value	Constants		Best fit value
$\phi_{baz(r)}$	Basal transcription activity of CR promoter	0,25	$\phi_{baz(m)}$	Basal transcription activity of M promoter	36
a	Constants which absorb the relevant interaction energies and RNA polymerase concentration	0,4	K_m	Constant which absorbs the relevant interaction energies and RNA polymerase concentration	0,075
b		2,10E-07			
c		1,00E-16			
k_C	Translation rate for control protein	0,9	k_M	Translation rate for methyltransferase	0,55
k_R	Translation rate for restr. endonuclease	(=k_C*2/3)			
λ_r	Rate of CR transcript decay	0,0065	λ_m	Rate of M transcript decay	0,0025
λ_C	Rate of control protein decay	0,0025	λ_M	Rate of methyltransferase decay	0,0025
λ_R	Rate of restr. endonuclease decay	(=λ_C)			

*The left part of the Table 1 is related to the C protein and the restriction endonuclease expression dynamics modeling, while the right part is related to the methyltransferase expression dynamics modeling.

Numerically solving the equations

The differential equations provided above, which describe the dynamics of the protein synthesis and degradation, are solved numerically in MATLAB by using Runge-Kutta method. The initial conditions (the transcript and the protein concentrations) were set to zero at the time point of the first available measurement (this time being set to zero). The equation parameters were changed in the range that corresponds to biochemically realistic values (4), and the parameter combination which corresponds to the minimal R^2 (sum of the error squares) is taken to represent the best fit to the experimental data.

Determining the best fit is computationally expensive, as 12-dim parameter space (note that there are 12 independent parameters) should be searched. To reduce the dimensionality of the search, we first solve Eqs (0.15) and (0.16), as M does not regulate CR promoter, and consequently these equations can be solved independently from Eqs. (0.12)-(0.13). Once the seven parameters related to the CR part of the system were determined, the remaining five parameters related to the M part of the system were determined. To separate and simplify solving the equations on M dynamics from that on CR dynamics, the R dependence on time is presented by a quadratic term – which shows a very good fit to the experimental data - and used for describing C dynamics, as C is proportional to R (see above).

When the cell division rate becomes larger than the protein decay rate, the effective protein degradation is determined by the cell division. The cell division rate $\nu(t)$ is determined from the respective linear fits to the logarithm of the cell number. Consequently, when $\nu(t)$ is larger than the assumed rates of CR transcript λ_r and C protein λ_C degradation rates, we take that these degradation rates become equal to $\nu(t)$.

We also use the model to predict R and M expression dynamics, in the case when the

system transcription is not controlled by C protein. In this case, we solve the same set of the equations as above, with the parameters that provide the best fit to the data (see *Table 1*), but with the promoter activities that correspond to the basal transcription rates throughout the system establishment – note that in this case C protein does not influence the transcription rates of CR and M promoters. Consequently, in Eqs. (0.6) and (0.11), the respective transcription activities become $\phi_r(C) \to \phi_{baz(r)}$ and $\phi_m(C) \to \phi_{baz(m)}$, while the other equations describing the expression of the system remain unchanged.

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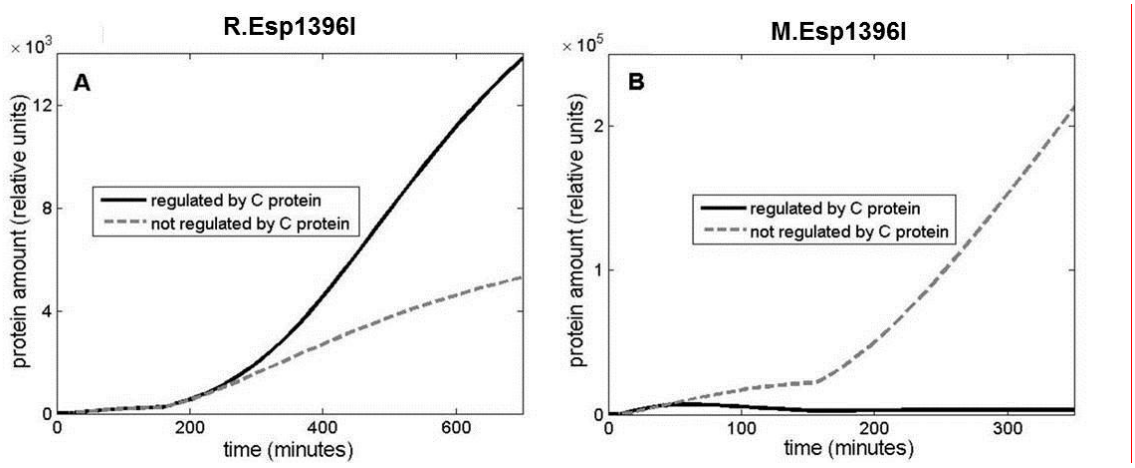
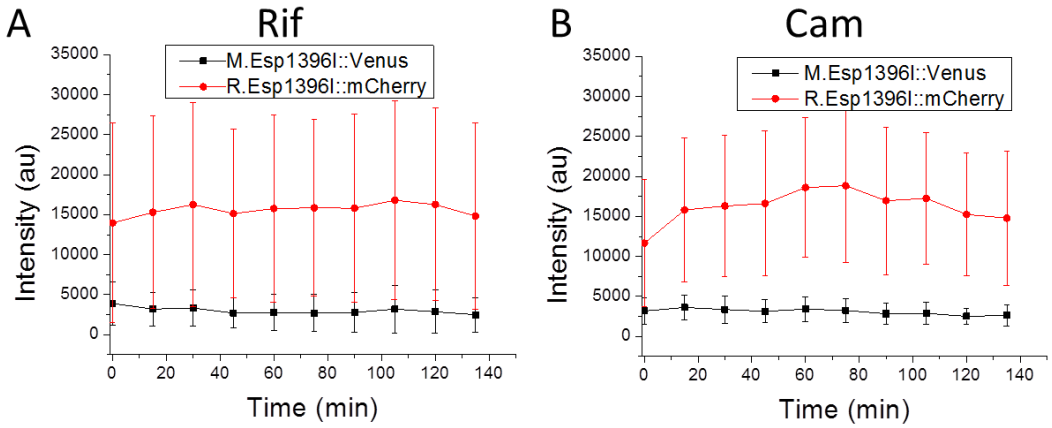


Supplementary Figure S4. Dynamic modeling of Esp1396I enzymes accumulation in the absence of control by C-protein. The same set of the equations with the same best-fit parameter values as in Figure 4 are solved with promoter activities that correspond to basal transcription rates throughout the simulated time interval. **A**. The simulated dynamics of the restriction endonuclease, in the presence and the absence of regulation by the C-protein, is

compared. **B**. As in **A** but showing methyltransferase dynamics simulation results. Note the shorter simulated time interval (up to 350 min), which is used to better compare the two curves, due to the very large levels reached by the methyltransferase when the system is not subject to negative regulated by the C-protein.



Supplementary Figure S5. Photobleaching control. Graphs of cell fluorescence intensities vs. time when cells were observed on substrate with the addition of rifampicin (**A**) or chloramphenicol (**B**). No cell division was observed at either condition.