Gene Regulation at the Single-Cell Level

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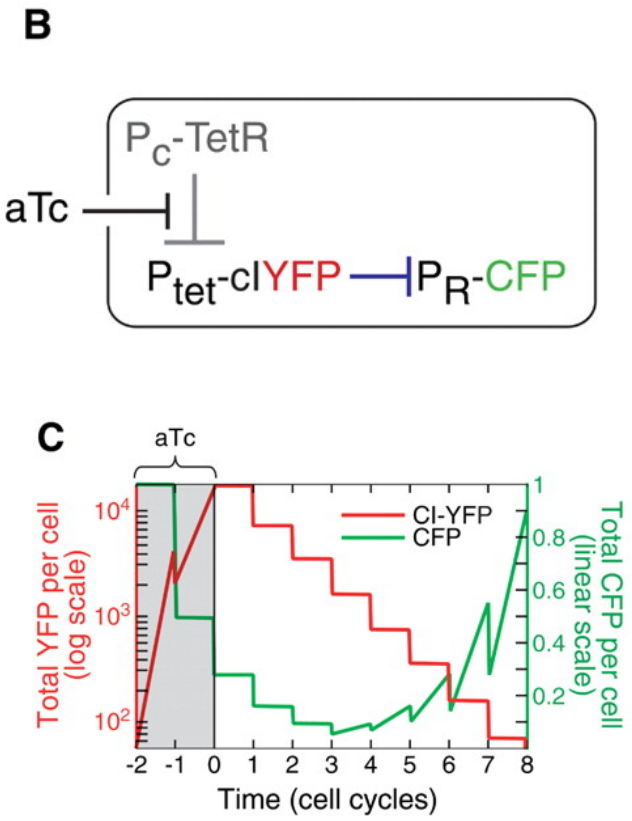
Gene Regulation at Single Promoter Level

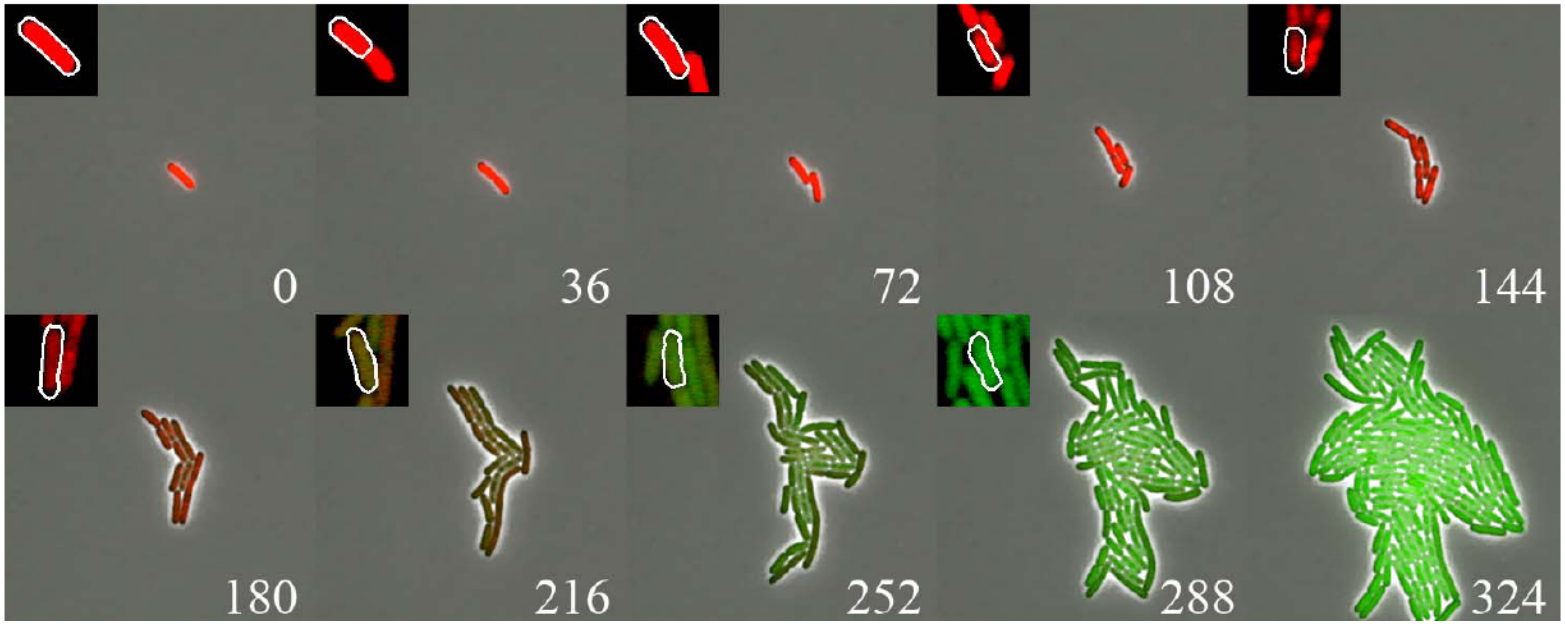
- Gene Relation Function (GRF) is relationship between concentration of active transcription factor & production of downstream gene products
- Shape and sharpness of GRF determines key features of cellular behavior
- Three fundamental aspects of GRF that specify the behavior of transcriptional circuits
  - 1) mean shape
  - 2) deviation from the mean
  - 3) time scale of fluctuations
- Must observe gene regulation in individual cells over time

λ-Cascade in *E. coli*



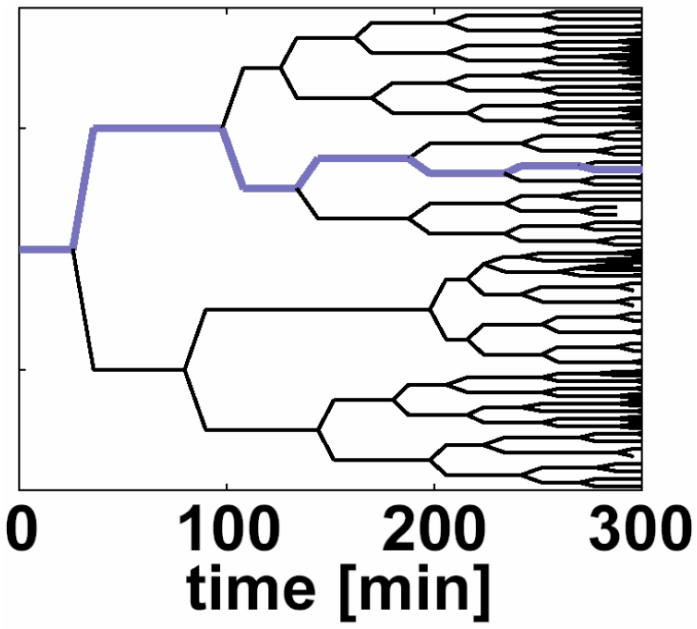
- Cl-YFP expressed from *tet* promoter in TetR+ background and can be induced by aTc
- Cl-YFP represses production of CFP from P_R promoter
- Repressor production switched off in growing cell so that concentration decreases exponentially by dilution as cell divides (schematic shown in C)

Fluorescence Time-Lapse Microscopy Used to Reconstruct Lineage Tree



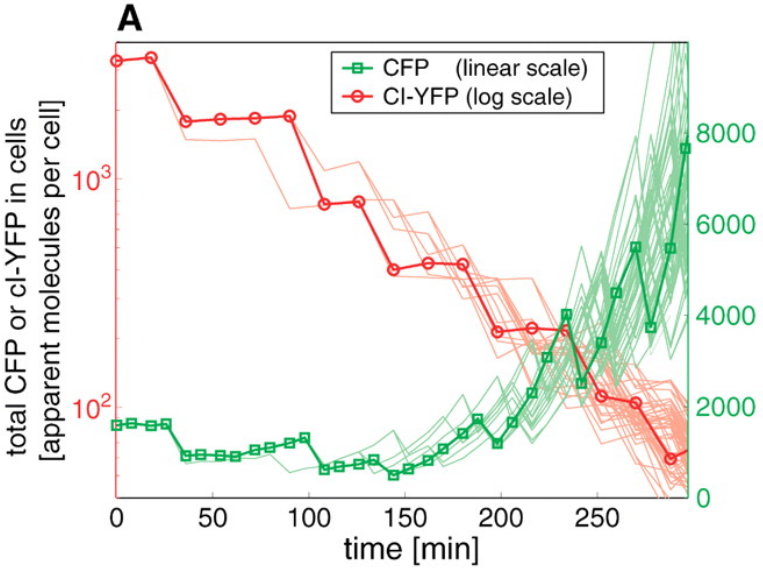
- Snapshots of "regulator dilution" experiment using O_R2^*-λ-cascade strain
- CI-YFP shown in red
- CFP shown in green

Lineage Tree Tracks Heritage of Microcolony



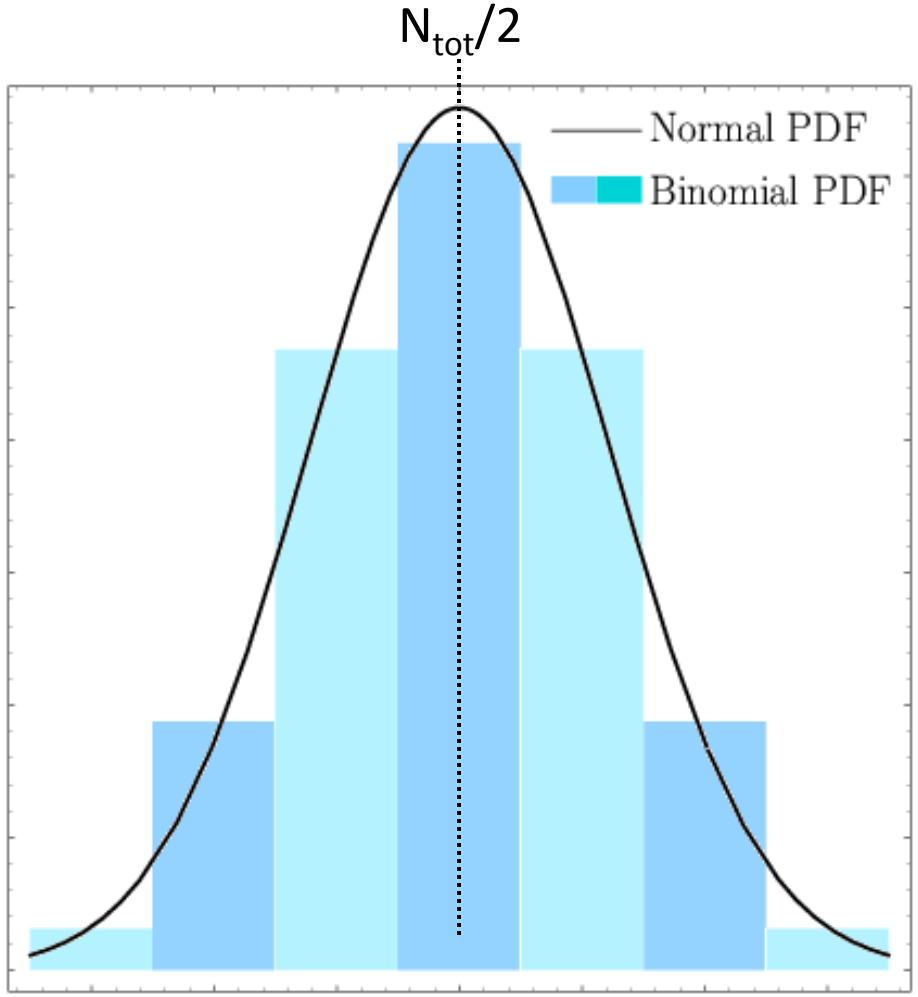
- Lineage tree determined from fluorescence time-lapse microscopy
- Each splitting point in lineage tree corresponds to one division event
- Highlighted lineage is the one outlined in other figures

CFP Production Rate Increases as CI-YFP Levels Decrease



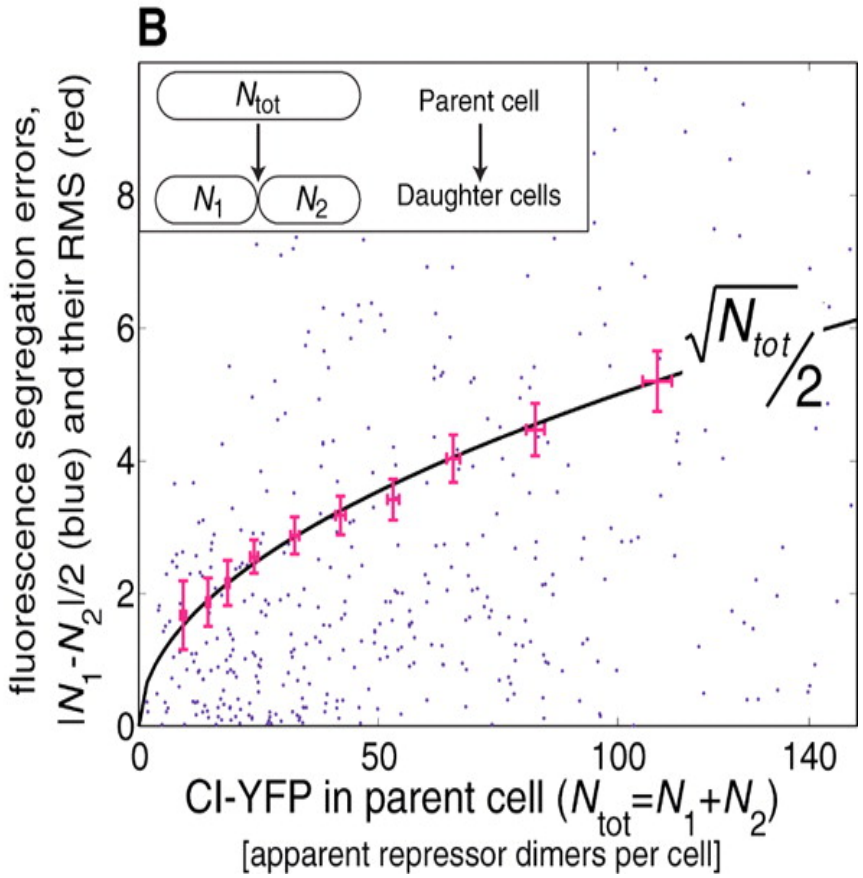
- Fluorescence intensities of CI-YFP and CFP in individual cells plotted over time
- Red = CI-YFP, plotted on log axis to highlight exponential dilution
- Green = CFP, plotted on linear axis to show increasing slope (increasing CFP production rate)

Fluorescence Partitioning During Cell Division is Binomial



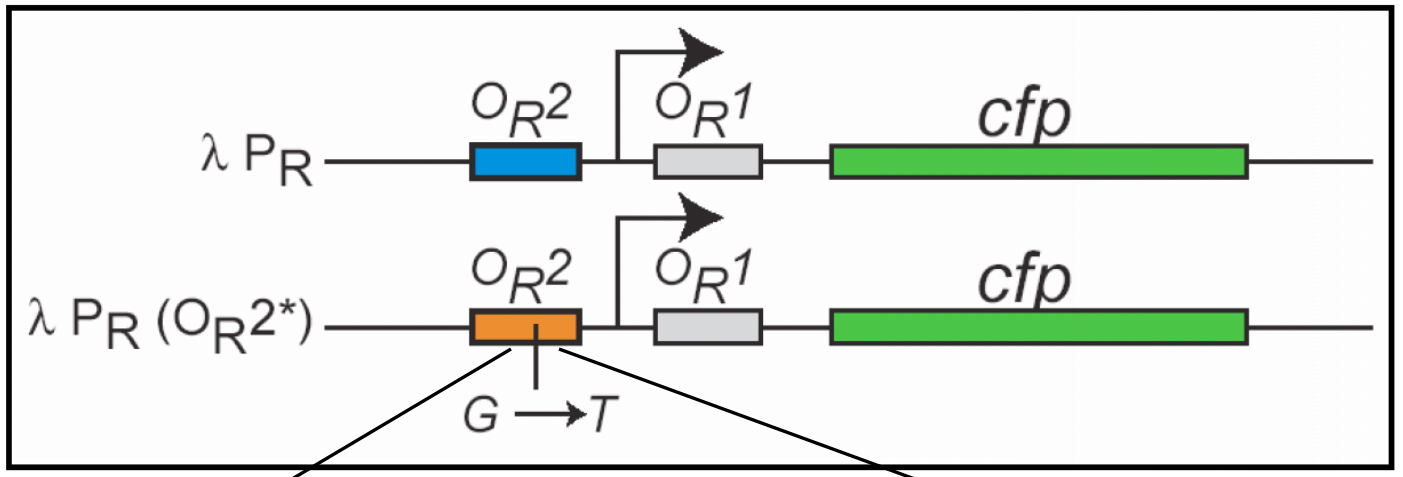
- Partitioning of CI-YFP fluorescence to daughter cells obeyed binomial distribution
- Compared differences between (real) daughter cells and a "virtual" randomly generated daughter set
- Kolmogorov-Smirnov test (80% significance level) showed that daughter distribution is consistent with the virtual set
- Average number of particles received by daughter cell is $N_{tot}/2$

Calibration of Fluorescent Signal to Number of Particles



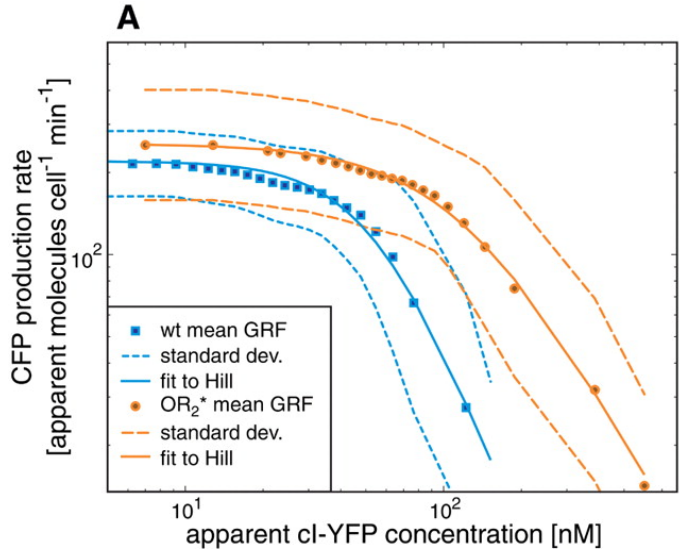
- Measured total fluorescence Y_{tot} of each of the daughters, and rescaled them to units of apparent # of molecules
- $Y_{tot} = v_y * N_{tot}$ (v_y = fluorescence reading given by one CI-YFP dimer)
- RMS error in CI-YFP partitioning between daughters increases as square root of parent cell CI-YFP (N_{tot})
- Single parameter fit of v_y based on RMS error curve

Mutated O_R2^*-λ-Cascade Strain



5′-GGATAAATATCTAACACCGTGC**T**TGTTGACTATTTTACCTCTGG

Mutated Operator Leads to Decreased Hill Coefficent and Binding Affinity



Parameter	P_R	P_R (O_R2*)
n (degree of cooperativity in repression)	2.4 ± 0.3	1.7 ± 0.3
k_d [concentration of repressor yielding half-maximal expression (nM)]	55 ± 10	120 ± 25
β [unrepressed production rate (molecules · cell^{-1} · min^{-1})]	220 ± 15	255 ± 40

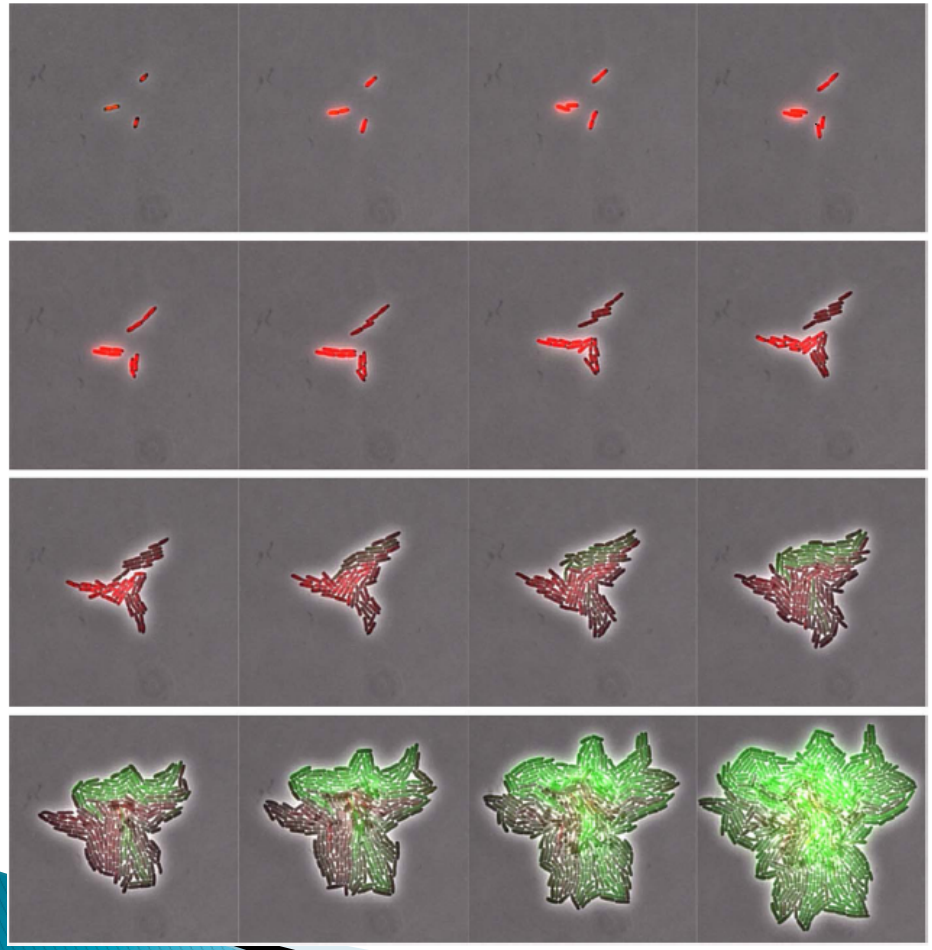
- CFP Production Rate found by determining slope of total CFP vs. time curve for a given time interval (8-9 min)
- Hill function in the form $f(R) = \beta/[1 + (R/k_d)^n]$
- Measured k_d comparable to previous estimates
- Significant cooperativity possibly results from dimerization of repressor molecules

What are the factors causing deviations from the mean GRF?

- At a given repressor concentration, standard deviation of production rates is ~55% of mean value

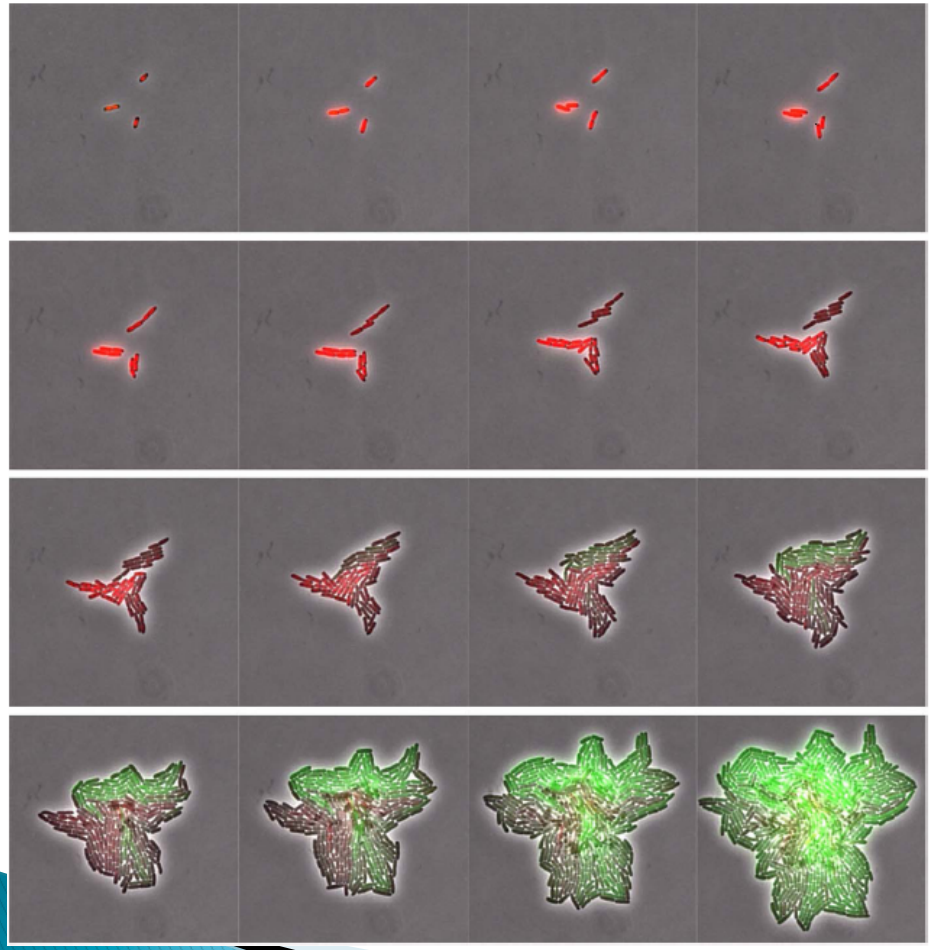
- Possible causes
  - Micro-environmental differences
  - Cell cycle-dependent changes in gene copy number
  - Intrinsic noise
  - Extrinsic noise

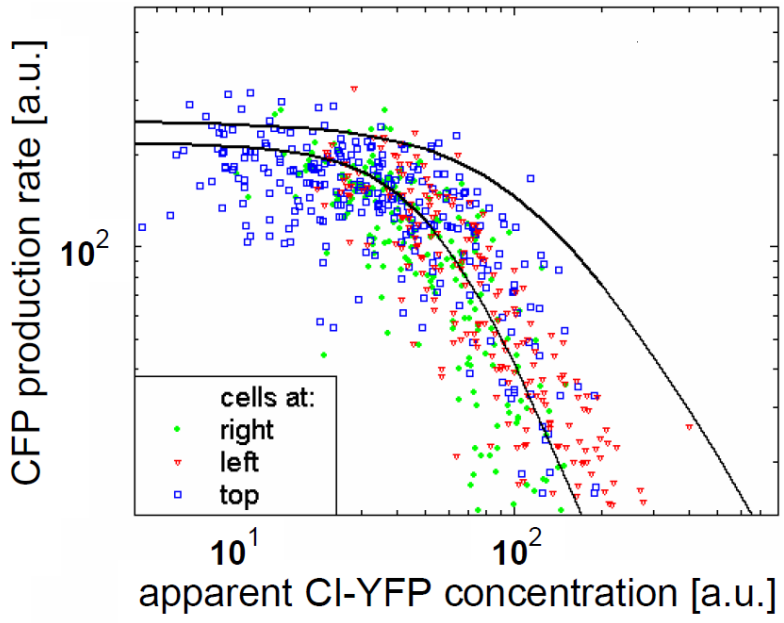
Does Local Micro-Environment Cause Deviations in GRF Value?



- Three cells (top, right, left) containing different initial amounts of repressor were grown simultaneously
- Descendents of initial cells increased CFP expression at different times
- GRFs obtained from descendants of each initial cell could be superimposed

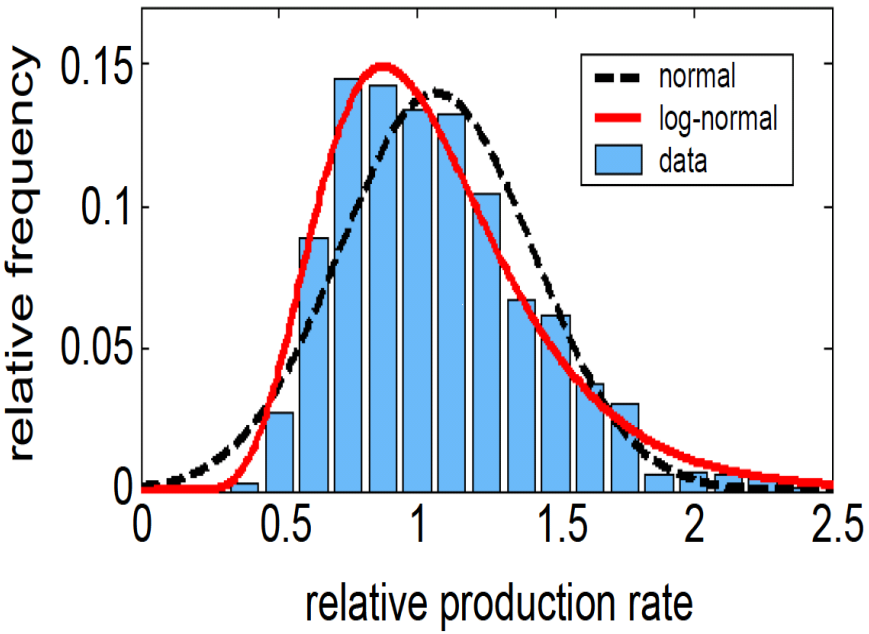
Local Micro-Environment has Little Detectable Effect on GRF





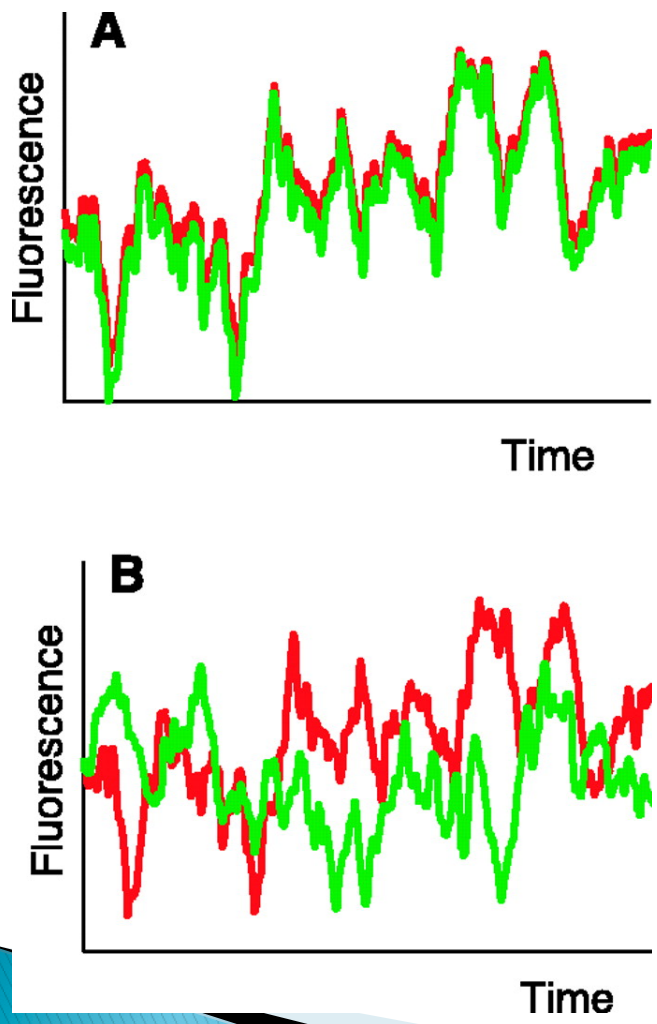
- Measured GRF is robust to differences among growth environments!

CFP Production Rate is Correlated Strongly with Cell-Cycle Phase



- Cells about to divide produce produced CFP at about twice the rate of those newly divided
- Normalized for differences by using formula G = M(1+Φ), where Φ = 'phase' of cell cycle
- Despite normalizing for these differences, standard deviation is still about 40% from mean
- Deviations from mean show log-normal distribution

Intrinsic vs. Extrinsic Noise



- (A) Extrinsic noise – caused by variations in cellular components, such as RNA Pol or ribosomes (has global effect)
- If there is only extrinsic noise, the level of expression of two proteins expressed from the same promoter will fluctuate in a correlated fashion
- (B) Intrinsic Noise – caused by stochasticity inherent in the biochemical process of gene expression
- Expression of two proteins maybe become uncorrelated because of intrinsic noise

Extrinsic Component of Noise is Dominant over Intrinsic Component

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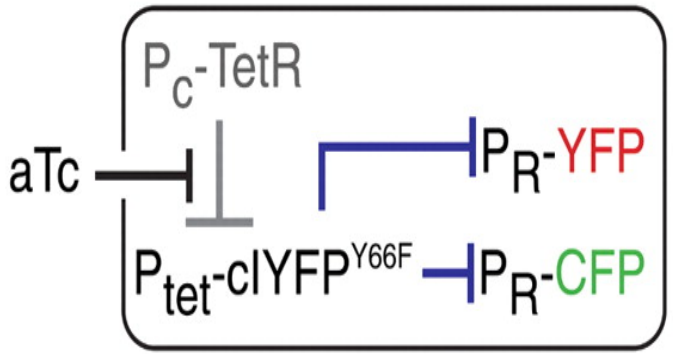
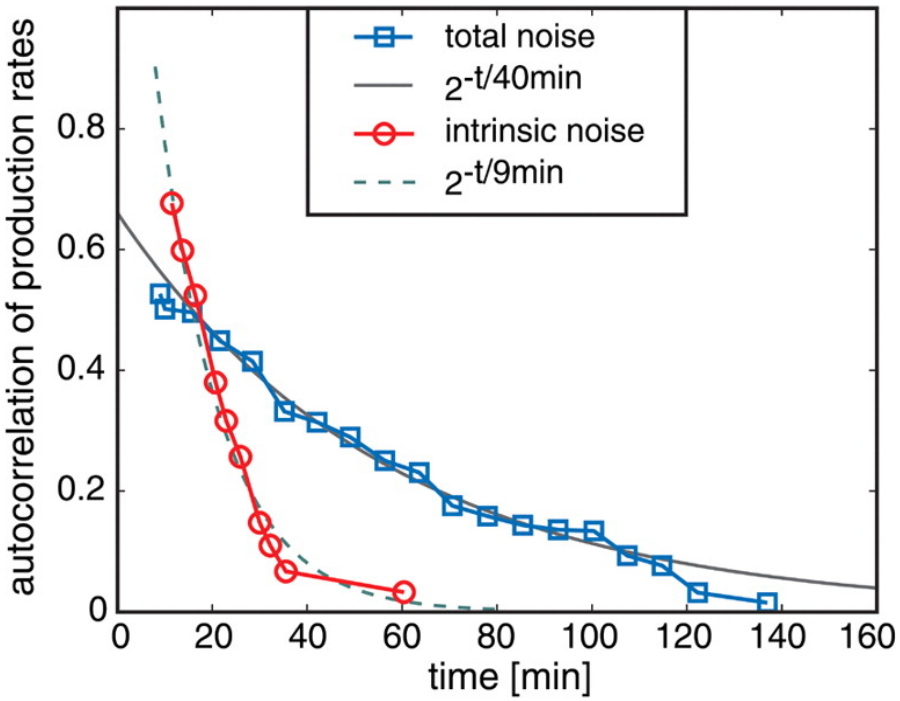


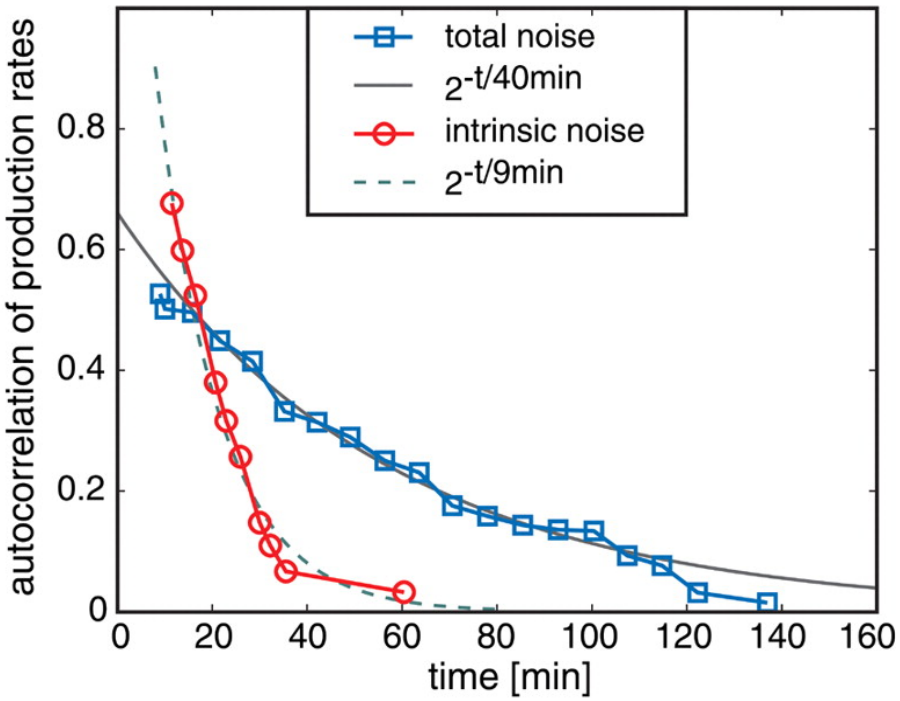
- Used symmetric branch strain that produces CFP and YFP from identical pair of P_R promoters
- Difference in CFP and YFP production rates indicated ~20% intrinsic noise
- Since the total deviation is ~55%, ~35% of the deviation is due to extrinsic noise

Cellular Autocorrelation Time is Approximately Equal to One Cell Cycle Period



- Fluctuations can be characterized by autocorrelation time, τ_{corr}
- Fluctuations longer than cell cycle can accumulate to produce significant effects
- Found that trajectories of single-cell lineages had τ_{corr} = 40 ± 10 min, close to the cell period

Cellular Autocorrelation Time is Approximately Equal to One Cell Cycle Period



- If cell produces CFP at a faster rate than mean GRF, CFP levels will accumulate to higher concentrations than predicted
- $\tau_{intrinsic}$ < 10 minutes, decays rapidly
- Therefore, observed fluctuations represent noise extrinsic to CFP expression

Conclusions

- Protein production rates fluctuate over a time scale of about one cell cycle
- Single-cell GRF cannot be represented by single-valued function
  - Biochemical parameters, noise, and slowly varying cellular states determine the effective GRF
- Slow extrinsic fluctuations limit the accuracy with which transcriptional genetic circuits can transfer signals

Significance

- Results form a basis for quantitative modeling of natural gene circuits and design of synthetic circuits
- Data provides an integrated, quantitative characterization of biochemical parameters along with amplitude and time scale of fluctuations
- Methods used here can be generalized to more complex genetic networks

Future Work

- Tuning and controlling gene expression noise in synthetic gene networks. K. F. Murphy, R. M. Adams, X. Wang, G. Balazsi, and J. J. Collins (2010), *Nucleic Acids Res.*
- Using noise to probe and characterize gene circuits. C. D. Cox, J. M. McCollum, M. S. Allen, R. D. Dar, and M. L. Simpson (2008), *PNAS* **105**, 10809-10814
- Transcriptional control of noise in gene expression. A. Sanchez and J. Kondev (2008), *PNAS* **105**, 5081-5086

Stochastic Switching as a Survival Strategy in Fluctuating Environments

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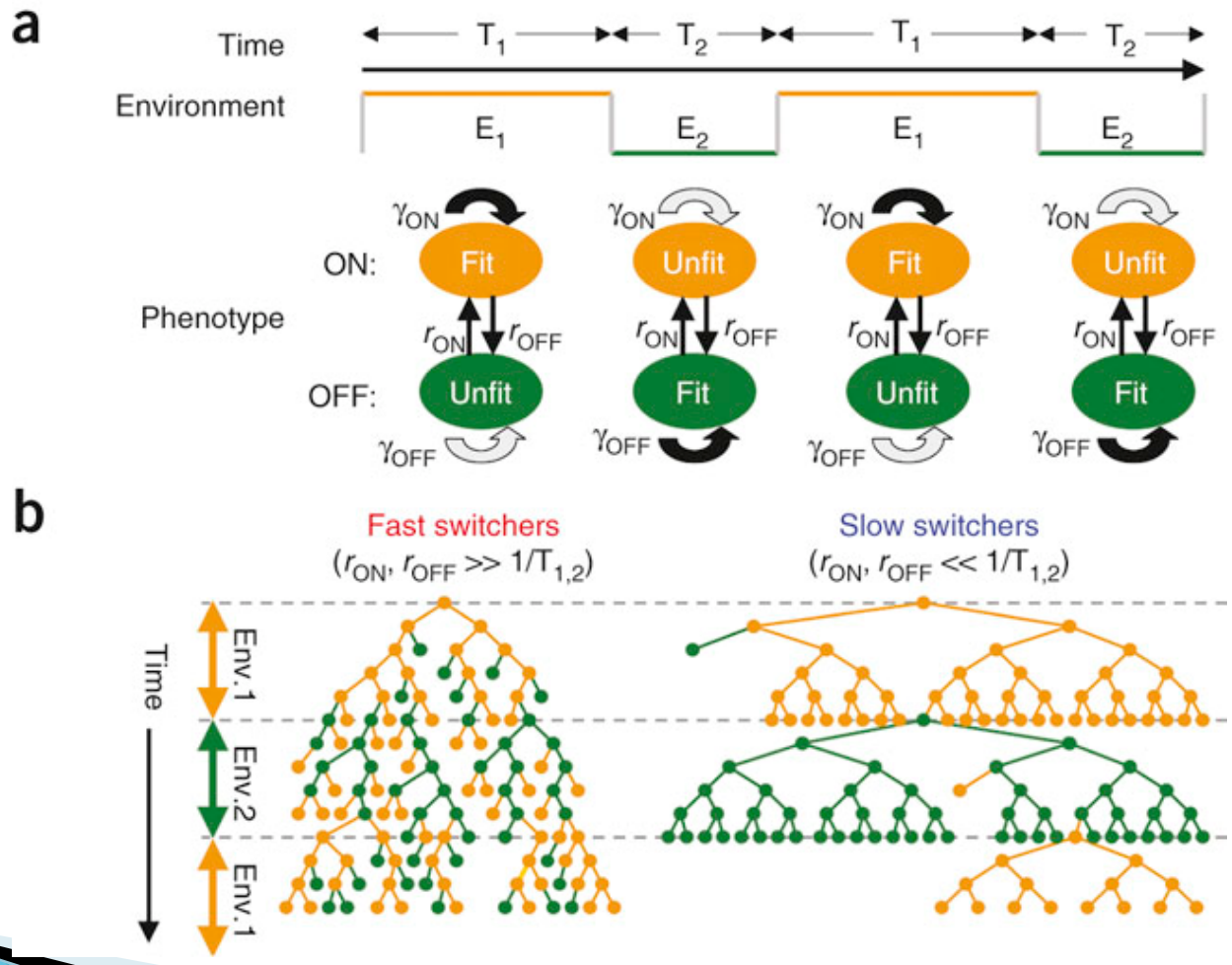
Background & Variables

- Cells may improve fitness by randomly transitioning between multiple phenotypes
- ON = URA3 expressed (GAL1 promoter activated)
- OFF = URA3 not expressed (GAL1 promoter not activated)
- E1 – lacks uracil
- E2 – contains uracil and 5-FOA
- Switching rates -> r_{on}, r_{off}
- Proliferation rates -> γ_{on}, γ_{off}

Fast Switchers Demonstrate Greater Population Diversity



Growth Dynamics in Fluctuating Environments