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
CI-YFP expressed from *tet* promoter in TetR+ background and can be induced by aTc

CI-YFP represses production of CFP from *P*<sub>R</sub> 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 $O_R2^*-\lambda$-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 $N_{\text{tot}}/2$
Calibration of Fluorescent Signal to Number of Particles

- Measured total fluorescence $Y_{\text{tot}}$ of each of the daughters, and rescaled them to units of apparent # of molecules

- $Y_{\text{tot}} = v_y \times N_{\text{tot}}$ ($v_y$ = 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 ($N_{\text{tot}}$)

- Single parameter fit of $v_y$ based on RMS error curve
Mutated $O_R2^*$-\(\lambda\)-Cascade Strain

5’-GGATAAATATCTAAACACCCTGCTCTTGTTGACTATTTTACCTCTGG
Mutated Operator Leads to Decreased Hill Coefficient and Binding Affinity

- 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 $f(R) = \frac{\beta}{1 + (R/k_d)^n}$
- Measured $k_d$ comparable to previous estimates
- Significant cooperativity possibly results from dimerization of repressor molecules

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$P_R$</th>
<th>$P_R (O_R 2^*)$</th>
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<tbody>
<tr>
<td>$n$ (degree of cooperativity in repression)</td>
<td>2.4 ± 0.3</td>
<td>1.7 ± 0.3</td>
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<tr>
<td>$k_d$ [concentration of repressor yielding half-maximal expression (nM)]</td>
<td>55 ± 10</td>
<td>120 ± 25</td>
</tr>
<tr>
<td>$\beta$ [unrepressed production rate (molecules · cell$^{-1}$ · min$^{-1}$)]</td>
<td>220 ± 15</td>
<td>255 ± 40</td>
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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.

- Descendants 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!
Cells about to divide produce CFP at about twice the rate of those newly divided.

Normalized for differences by using formula $G = M(1 + \Phi)$, where $\Phi$ = ‘phase’ of cell cycle.

Despite normalizing for these differences, standard deviation is still about 40% from mean.

Deviations from mean show log-normal distribution.
(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

- 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, $\tau_{\text{corr}}$
- Fluctuations longer than cell cycle can accumulate to produce significant effects
- Found that trajectories of single-cell lineages had $\tau_{\text{corr}} = 40 \pm 10$ min, close to the cell period
If cell produces CFP at a faster rate than mean GRF, CFP levels will accumulate to higher concentrations than predicted.

$\tau_{\text{intrinsic}} < 10$ 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
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

- 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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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 $\rightarrow r_{on}, r_{off}$

Proliferation rates $\rightarrow \gamma_{on}, \gamma_{off}$
Fast Switchers Demonstrate Greater Population Diversity

(a) Time

Environment

ON:

Fit

Unfit

OFF:

γ_{ON} r_{ON} r_{OFF}

γ_{OFF}

Phenotype

(b) Fast switchers

(r_{ON}, r_{OFF} >> 1/T_{1,2})

Slow switchers

(r_{ON}, r_{OFF} << 1/T_{1,2})

Time

Env. 1

Env. 2

Env. 1
Growth Dynamics in Fluctuating Environments

(a) Short period (●)

(b) Long period (▲)

(c)

(d)

(e)

(f)