Holding Biology Up to Light

single-molecule imaging of gene regulation and cell division

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Even a “simple” bacterial cell is a complex entity

Crowded molecular interactions

Heterogeneous distributions of molecules

Sensitive, high resolution single molecule methods in live cells to study cellular processes

Image credit: Xiaoli Weng
Transcription factors regulate gene expression through multiple means.

Ptashne, A genetic switch, Phage λ revisited, 2004
• How does autoregulation help minimize fluctuations in gene expression?

• How does DNA looping enhance gene regulation?
Co-translational activation by cleavage (CoTrAC) strategy to monitor TF expression dynamics

- Allow single-molecule detection
- Maintain the full functionality of TF
- Allow accurate counting of the number of TF in real time
Repressor CI utilizes both positive and negative autoregulation.

Zach Hensel
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Large Fluctuations in $\Lambda^{r3}$ CI Production

$\mu \pm \sigma$: 7.8 ± 4.4

Noise ($\sigma^2/\mu^2$): 0.32

CI production rate fluctuates ~60% around mean
wt strain $\lambda^{wt}$
(coupled negative and positive feedback)

Basal Expression strain $\lambda^b$

Repression Strain $\lambda^-$

tsr-venus-ub-cI
wt strain $\lambda^{wt}$ (coupled negative and positive feedback)

Basal Expression strain $\lambda^b$

Repression Strain $\lambda^r$

$\mu \pm \sigma$: 5.2 ± 3.2

Noise ($\sigma^2/\mu^2$): 0.40 ± 0.01

$\mu \pm \sigma$: 1.9 ± 1.8

Noise ($\sigma^2/\mu^2$): 0.96 ± 0.03

$\mu \pm \sigma$: 0.06 ± 0.3

Noise ($\sigma^2/\mu^2$): 19.4 ± 0.7
How does autoregulation of transcription factor influence fluctuations in gene expression?

• Negative autoregulation does not minimize the intrinsic fluctuations in gene expression

• It speeds up the response of the system so that fluctuations can go away quickly

• Fluctuations in protein expression levels are largely determined by heterogeneous cellular environments instead of the inherent stochasticity of gene expression

• How does autoregulation help minimize fluctuations in gene expression?

• How does DNA looping enhance gene regulation?
Transcription factors regulate gene expression through multiple means.
How to probe the mechanism and dynamics of DNA looping in live cells?

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Achieve subdiffraction-limited precision by using a minimal amount of DNA binding sites


256 binding sites, 10kb, 3 μm in length, fluorescent spot exceeds diffraction limit, unable to localize the position precisely.

3 binding sites, <100b, 30 nm in length, fluorescent spot is diffraction limited, enable the localization precision at ~15 nm.
Proof of principle using positive and negative controls

$\lambda^{\text{null}}$

Looped positive control

$\lambda^{\text{OL-}}$

Unlooped negative control

$\lambda^{\text{wt}}$
Distributions of $r^{lac/tet}$ of looped and unlooped controls are significantly different

Quantitatively measure the distance between the two sites

<table>
<thead>
<tr>
<th>Strains</th>
<th>Mean (nm)</th>
<th>Median (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda^{null}$</td>
<td>$47 \pm 1$</td>
<td>$41 \pm 1$</td>
</tr>
<tr>
<td>$\lambda^{OL}$</td>
<td>$71 \pm 1$</td>
<td>$63 \pm 2$</td>
</tr>
</tbody>
</table>

Unlooped DNA is **highly compact** in crowded cellular environment.
Wildtype and mutants strains to estimate in-vivo looping frequency

<table>
<thead>
<tr>
<th>Strains</th>
<th>$\mu_{\text{lac/tet}}$ Mean (nm)</th>
<th>Looping Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda^{\text{null}}$</td>
<td>$47 \pm 1$</td>
<td>N. A.</td>
</tr>
<tr>
<td>$\lambda^{\text{OL}-}$</td>
<td>$71 \pm 1$</td>
<td>N. A.</td>
</tr>
<tr>
<td>$\lambda^{\text{OR3}-}$</td>
<td>$58 \pm 1$</td>
<td>$53% \pm 7%$</td>
</tr>
<tr>
<td>$\lambda^{\text{OL3}-}$</td>
<td>$56 \pm 1$</td>
<td>$60% \pm 7%$</td>
</tr>
<tr>
<td>$\lambda^{\text{wt}}$</td>
<td>$52 \pm 1$</td>
<td>$79% \pm 6%$</td>
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</table>
Measuring CI expression level using single-molecule fluorescent in situ hybridization (smFISH)

<table>
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<tr>
<th>Strain</th>
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<th>CI expression level (WL)</th>
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<tbody>
<tr>
<td>λnull</td>
<td>47 ± 1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>λΔOl</td>
<td>71 ± 1</td>
<td>N/A</td>
<td>1.38 ± 0.05</td>
</tr>
<tr>
<td>λWT</td>
<td>52 ± 1</td>
<td>79 ± 6%</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td>λOr3^-</td>
<td>59 ± 1</td>
<td>53 ± 7%</td>
<td>2.50 ± 0.07</td>
</tr>
<tr>
<td>λOl3^-</td>
<td>56 ± 1</td>
<td>60 ± 7%</td>
<td>2.51 ± 0.07</td>
</tr>
</tbody>
</table>
Thermodynamic modeling using independently measured looping frequencies and expression levels

\[
P_i = \frac{d_i [C\text{Cl}_2]^n_i e^{-\frac{\Delta G_i}{RT}}}{Z}
\]

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</thead>
<tbody>
<tr>
<td>(\lambda\text{null} )</td>
<td>47 ± 1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>(\lambda\Delta O_L )</td>
<td>71 ± 1</td>
<td>N/A</td>
<td>1.38 ± 0.05</td>
</tr>
<tr>
<td>(\lambda\text{WT} )</td>
<td>52 ± 1</td>
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Looping activates transcription

Estimated transcription rates:
\[ k_{\text{unlooped}} = 1.9 \pm 0.3 \text{ nM/min} \]
\[ k_{\text{looped}} = 4.6 \pm 0.9 \text{ nM/min} \]

- Looping OR12 and OL12 is able to activate transcription
\[ (k_{\text{looped}} / k_{\text{unlooped}}) \sim 2.4 \text{ fold compared to unlooped conformation} \]
How does a bacterial cell divide?

FtsZ is a GTPase and tubulin homolog

FtsZ protein is essential for bacterial cell division

*Image credit: Xiaoli Weng*
FtsZ-ring serves as a scaffold for cell division and may generate constriction force.

Vincente and Lowe, EMBO Reports, (2003)

Scaffold for divisome assembly

Osawa and Erickson, Science, (2008)

Force generation for constriction
Fluorescence light microscopy is limited by diffraction

Diffraction-limited Spatial Resolution = \( \sim \frac{\lambda}{2} \), 200-300 nm
Diameter of an *E. coli* cell: 1 \( \mu \)m
Photoactivated Localization Microscopy (PALM) offers both specificity and resolution.

Dark

Activation (405 nm)

Bright

Excitation (561 nm)

Fluorescent

mEos2

Jackson Buss

Carla Coltharp

McKinney, et al., Nat Meth, 2009

Interferometric PALM (iPALM) to image the Z-ring in 3D

Shtengel G. et al., PNAS, (2009)

In collaboration with Harald Hess at Janelia Farm
Z-ring is heterogeneous and discontinuous
Z-ring occupies an elliptic toroidal zone

Mean: 88 ± 4 nm
Sigma: 32 ± 4 nm

Mean: 53 ± 2 nm
Sigma: 17 ± 2 nm
There are only enough FtsZ molecules to wrap around the cell three times.
Fu, et al., PLoS One, 2010
Coltharp, et al, unpublished
Z-ring consists of heterogeneous, loosely associated protofilaments overlapping in three dimensions.

**Bundle Model**

- **Width**: ~90 nm
- **Thickness**: ~50 nm
What maintains the structural integrity of the loose, heterogeneous Z-ring?

Jackson Buss
**Z-ring Associated Proteins (ZapA, ZapB)**

Localize to the Z-ring and Promote FtsZ polymerization

- **ZapA**
  - Dimer:tetramer equilibrium
  - Binds FtsZ

- **ZapB**
  - Polymerizes
  - Binds ZapA

*EM: FtsZ polymers*

- no ZapA
- +ZapA

Low and Lowe, JMB, 2004
Small et al., JMB, 2007

*EM: ZapB polymers*

Galli E & Gerdes K, J. Bact., 2012
Abnormal septa formation in the absence of ZapA or ZapB

FtsZ mislocalizes and forms smaller clusters in the absence of ZapA or ZapB

ΔzapA/FtsZ-mEos2

MatP, a physical linkage to anchor FtsZ on the chromosome?

Bacterial two hybrid shows that ZapB interacts with MatP, a DNA binding protein that has important role in chromosome segregation

Espelli, EMBO J, 2012

ΔmatP, fast growth condition

FtsZ-GFP mislocalizes in the absence of MatP

The Z-ring is maintained between the membrane and chromosome through a multi-layered protein network.

The multi-layered protein network may enable the coordination between cell wall constriction and chromosome segregation.

ZapB may form a stable, cytoplasmic raft to anchor FtsZ protofilaments through ZapA to maintain the Z-ring’s structural integrity.
Acknowledgments

Jackson Buss  Carla Coltharp

Harald Hess  Gleb Shtengel

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