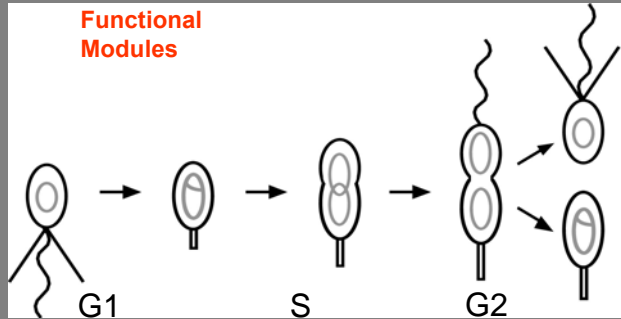


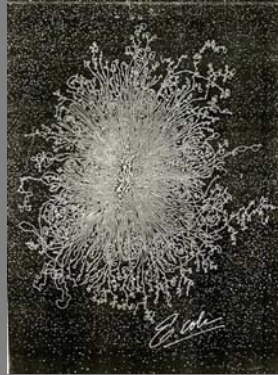
**Dynamics of bacterial chromosome organization, segregation and cytokinesis**

**Lucy Shapiro**  
**Dept. of Developmental Biology**  
**Stanford Medical School**

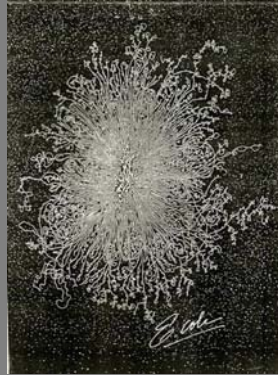
### Temporally Coordinated Events of the *Caulobacter* Cell Cycle



- Flagellar Ejection
- Cell Division
- Stalk Synthesis
- Flagellar Biogenesis
- Pili Biogenesis
- Replication Initiation
- DNA methylation



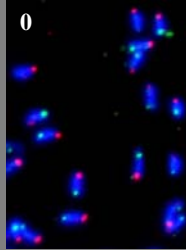
**Bacterial DNA released from the cell**



**Bacterial DNA released from the cell**

The DNA is 1000x  
longer than the cell

### Dynamic polar localization of chromosomal replication origin



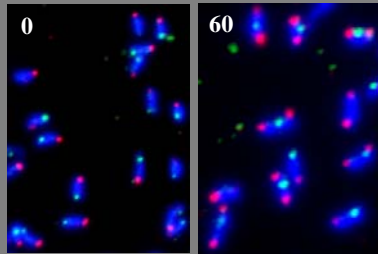
Red: Origin    Green: Terminus



Swarmer cell

- Origin
- Terminus
- McpA chemoreceptor
- Nonreplicating chromosome
- Replicating chromosome

### Dynamic polar localization of chromosomal replication origin



Red: Origin    Green: Terminus

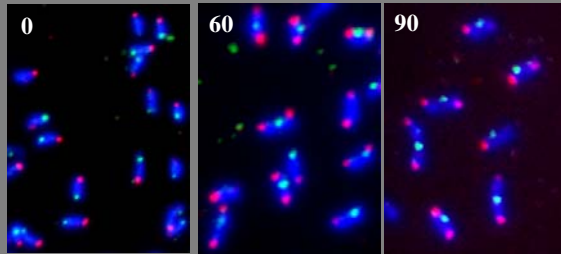


Swarmer cell

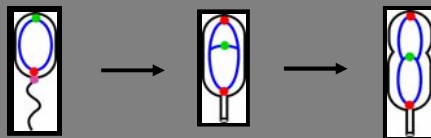


Stalked cell

### Dynamic polar localization of chromosomal replication origin



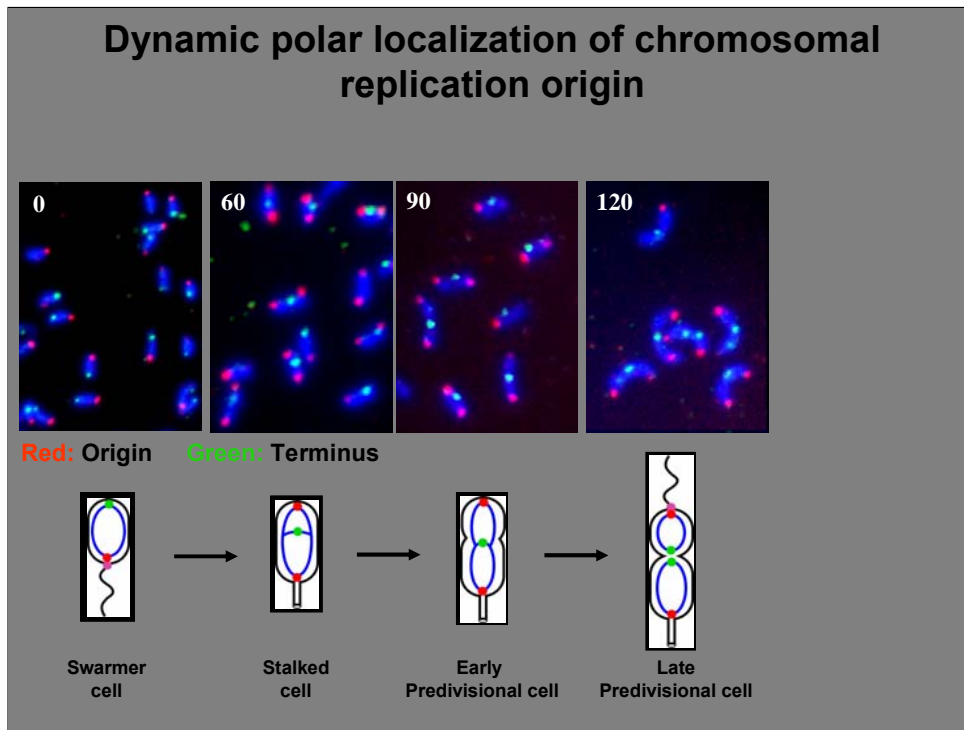
Red: Origin Green: Terminus



Swarmer cell

Stalked cell

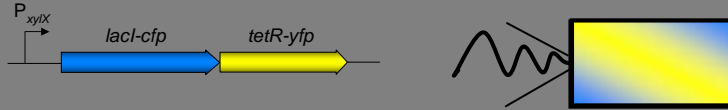
Early Predivisional cell



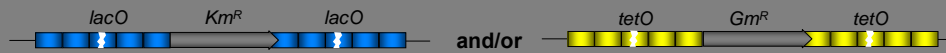


### Strategy for simultaneous visualization of two chromosomal loci in live cells

**I. Controllable expression of repressor-fluorescent protein fusion (LacI-CFP & TetR-YFP)**

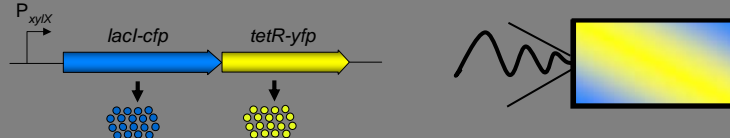


**II. Integrate tandem copies (256 ~10 kb) of repressor binding sites (*lacO* & *tetO*) into the chromosome at locus-of-interest**

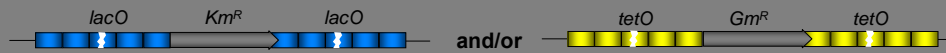


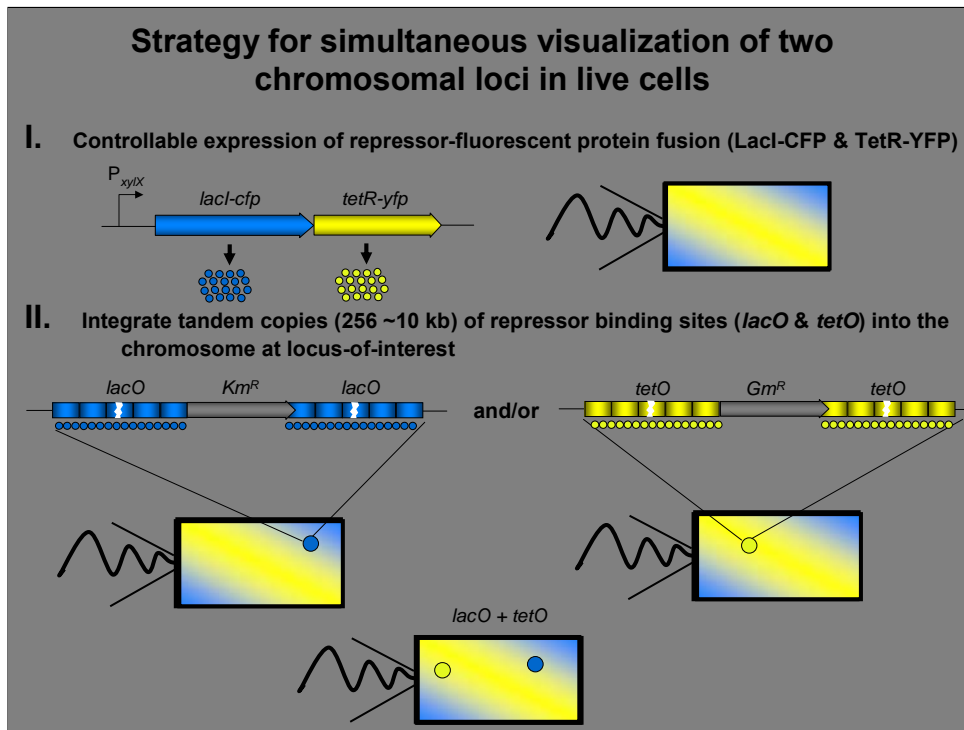
### Strategy for simultaneous visualization of two chromosomal loci in live cells

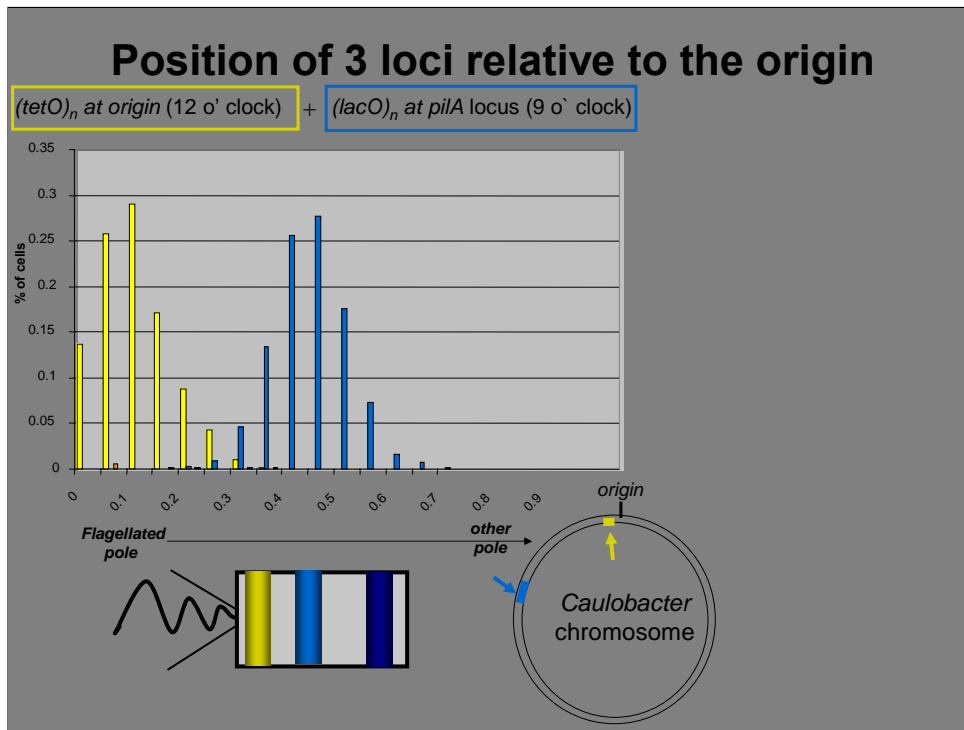
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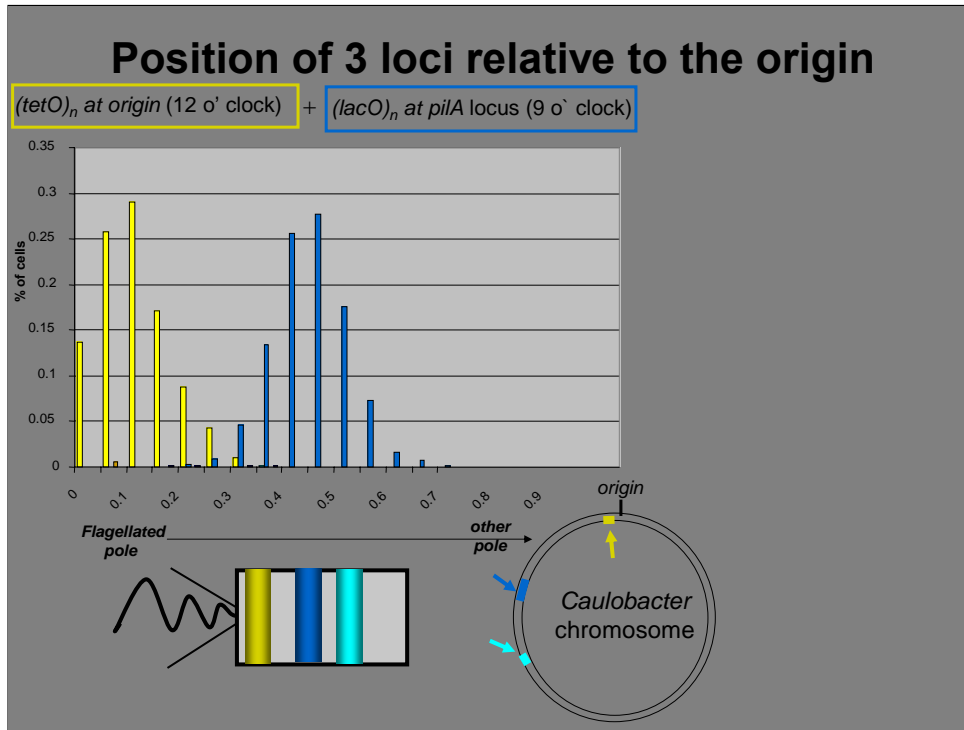


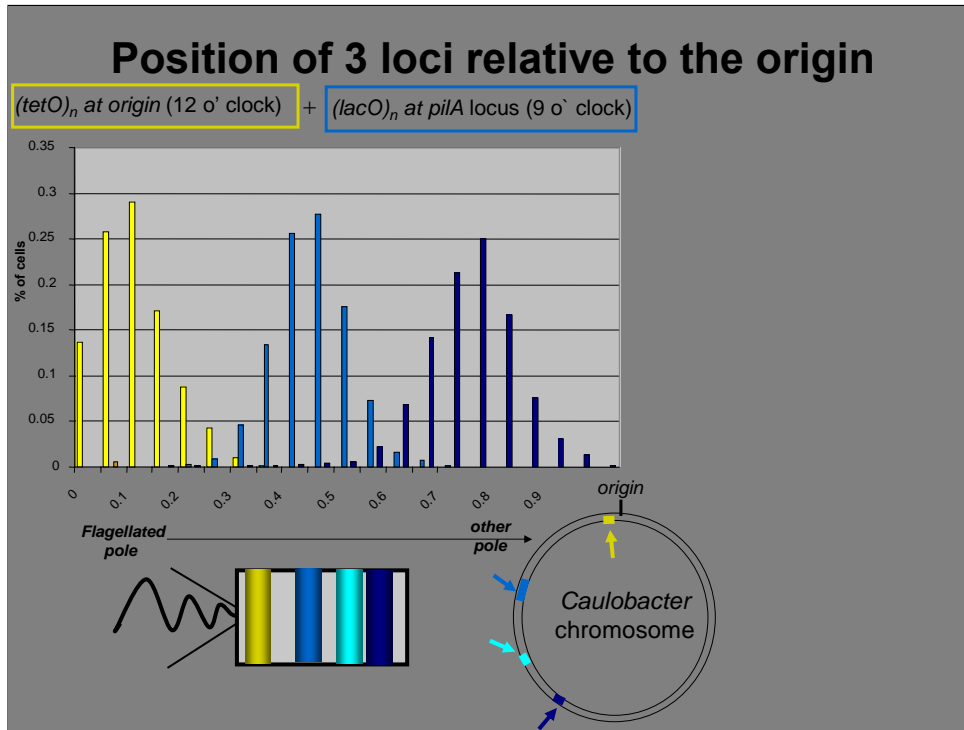
**II. Integrate tandem copies (256 ~10 kb) of repressor binding sites (*lacO* & *tetO*) into the chromosome at locus-of-interest**

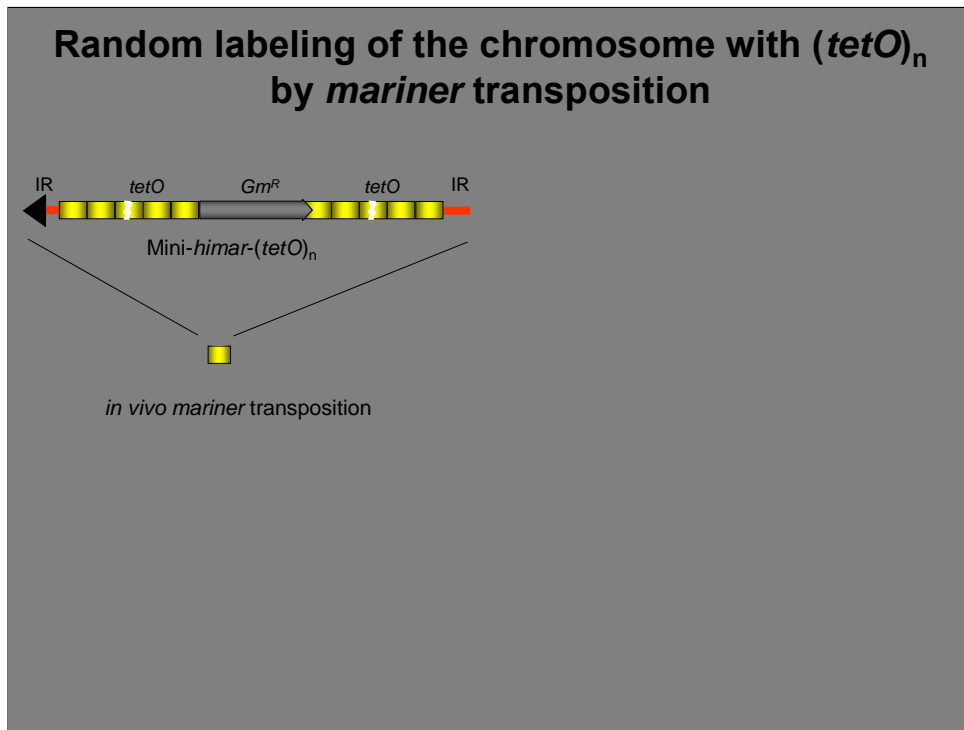




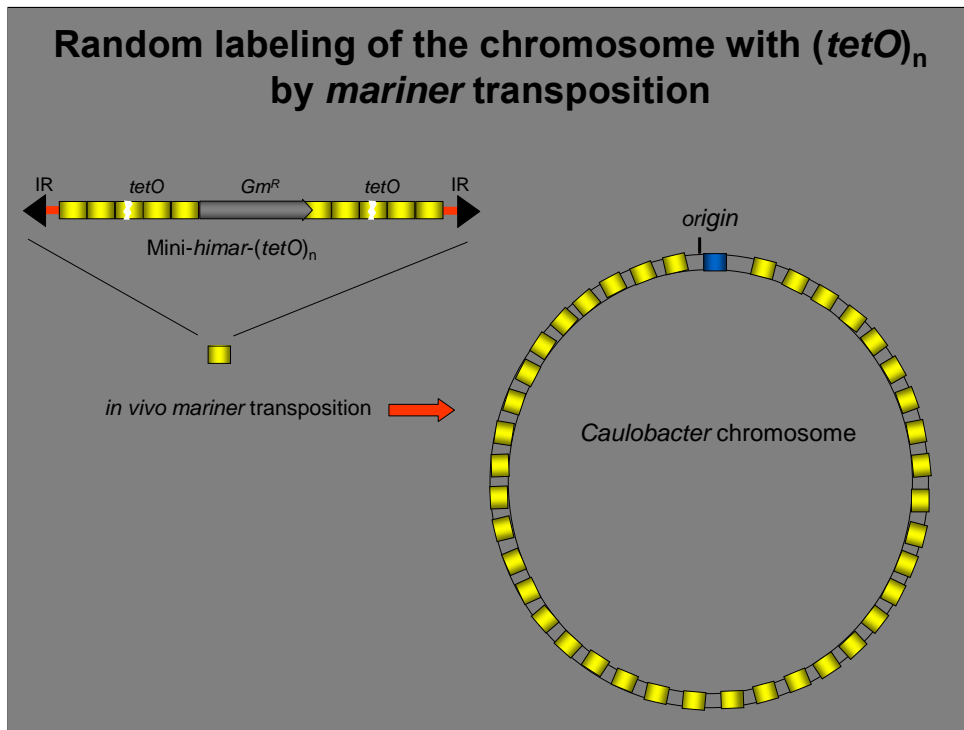








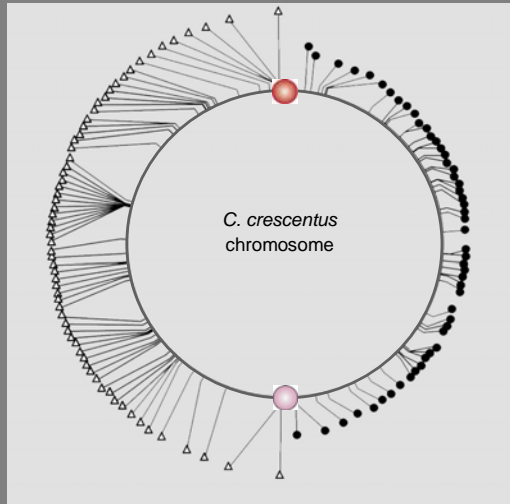
nnnn



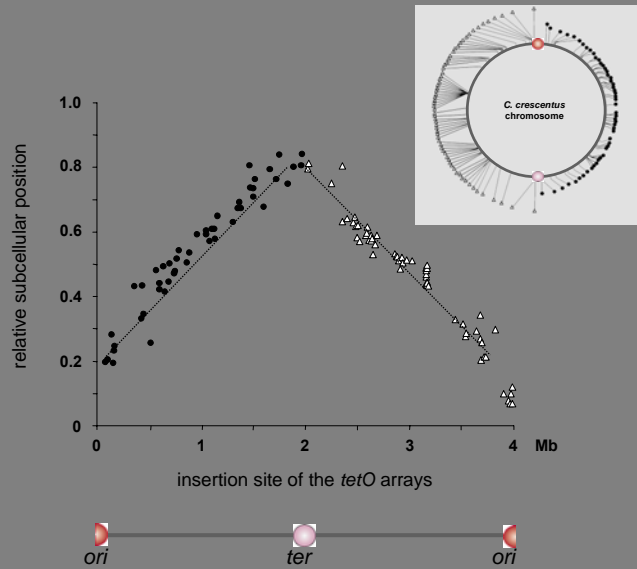
nnnn



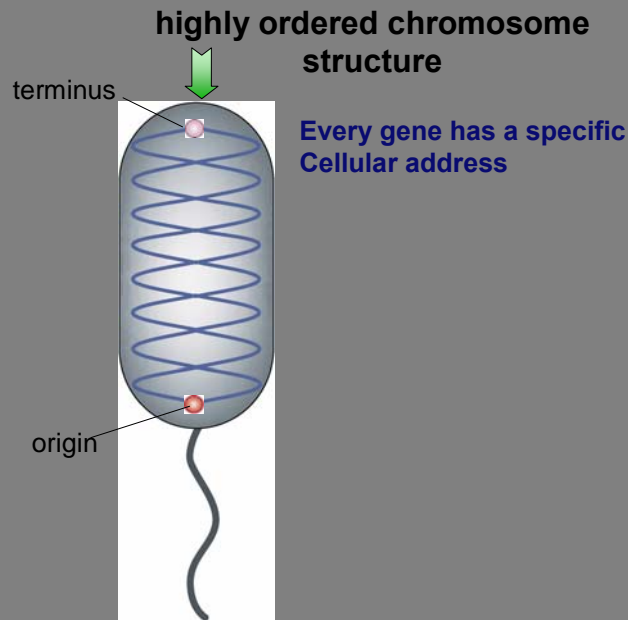
Identifying the subcellular position of chromosomal sites



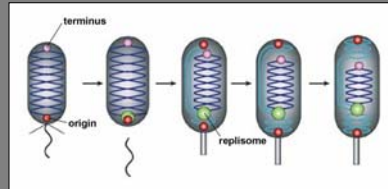
### Identifying the subcellular position of chromosomal sites



**Identifying the subcellular position of chromosomal sites**

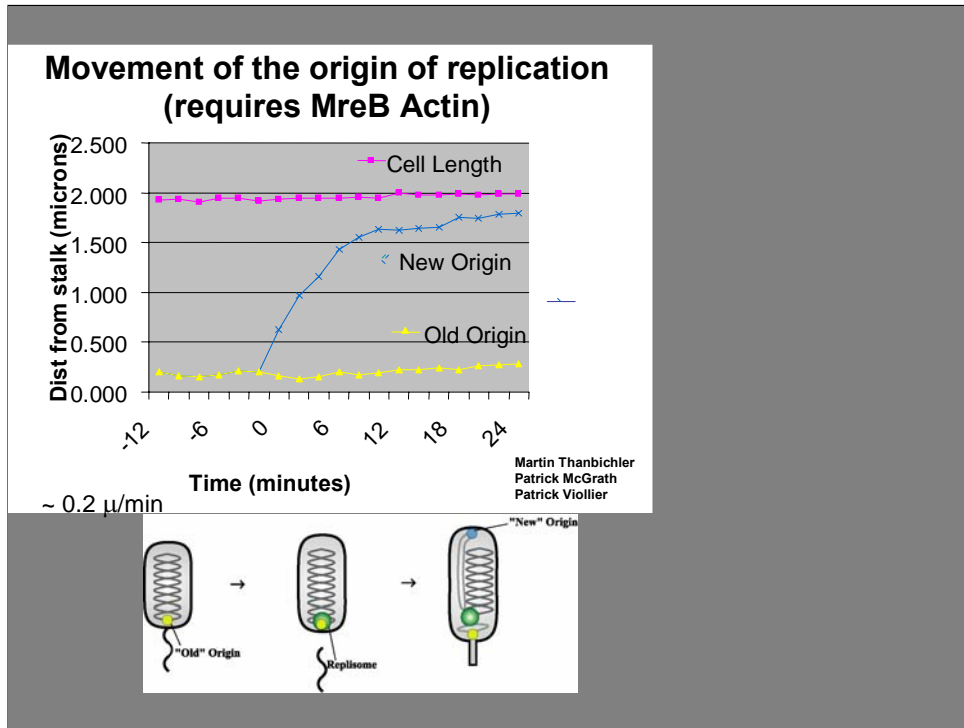


## Rapid Movement of the Origin

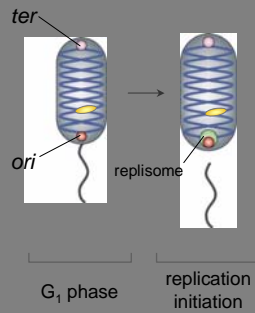


22 frames (2 min intervals)  
speed: 0.2  $\mu\text{m}/\text{min}$



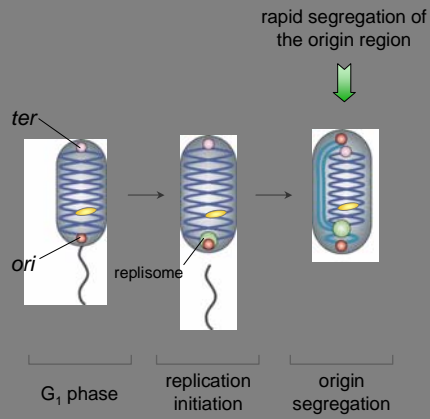


## Dynamics of bacterial chromosome segregation



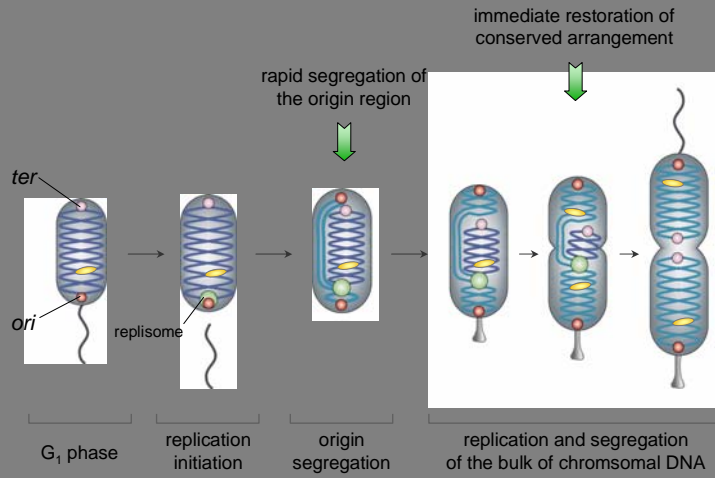
*Proc. Natl. Acad. Sci. USA* 101: 9257-9262

## Dynamics of bacterial chromosome segregation



*Proc. Natl. Acad. Sci. USA* 101: 9257-9262

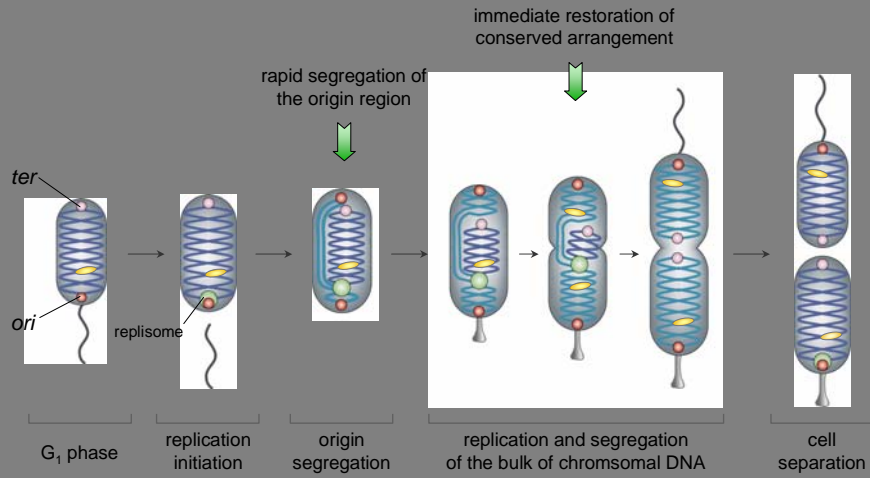
## Dynamics of bacterial chromosome segregation



*Proc. Natl. Acad. Sci. USA* 101: 9257-9262

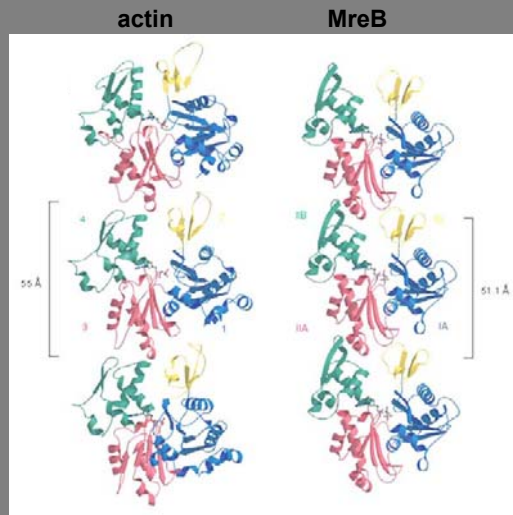


## Dynamics of bacterial chromosome segregation



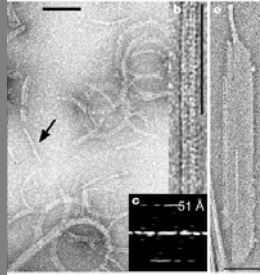
*Proc. Natl. Acad. Sci. USA* 101: 9257-9262

## MreB Actin is a candidate to mediate chromosome segregation

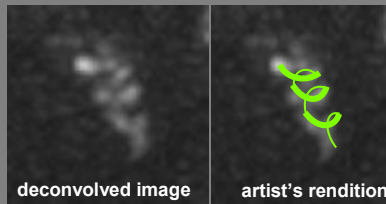


Van den Ent *et al.* Nature, 2001

## MreB Actin is a candidate to mediate chromosome segregation



*T. maritima* MreB Polymerizes *in vitro*



Forms a dynamic, lengthwise spiral in *Caulobacter*

Gitai *et al.* PNAS, 2004: MreB-GFP  
Figge *et al.* Mol Micro, 2004: MreB IF

## Dissecting MreB's cellular functions

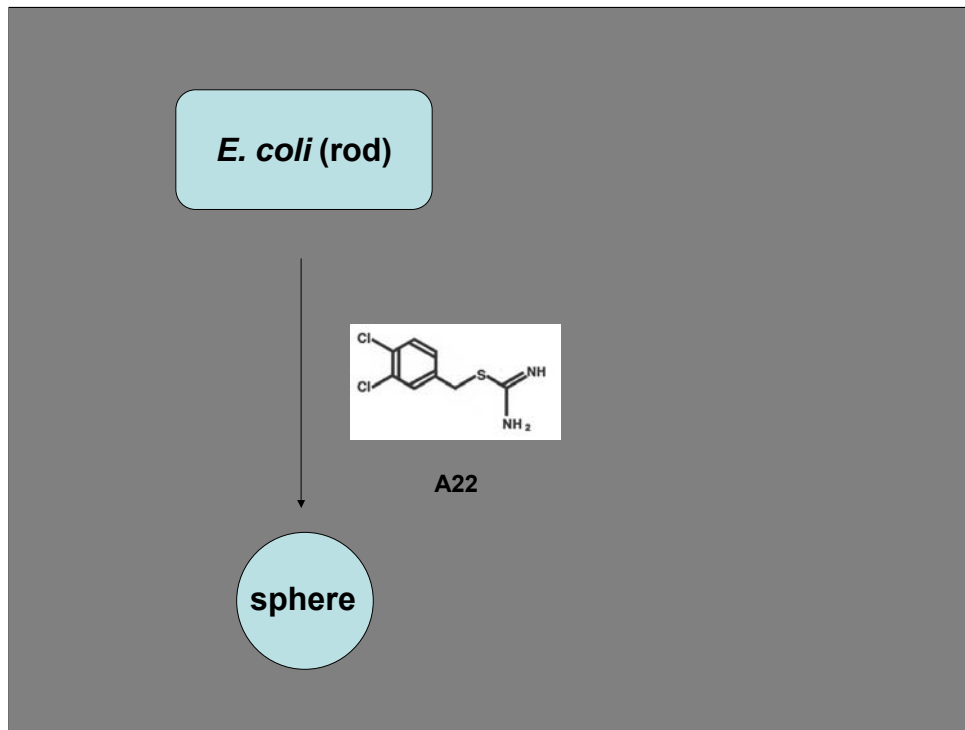
For better temporal specificity – pharmacology

Novel S-Benzylisothiourea Compound That Induces Spherical Cells in *Escherichia coli* Probably by Acting on a Rod-shape-determining Protein(s) Other Than Penicillin-binding Protein 2

Noritaka IWAI,<sup>1,\*</sup> Kazuo NAGAI,<sup>2</sup> and Masaki WACHI<sup>1†</sup>

<sup>1</sup>Department of Bioengineering, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan

<sup>2</sup>Department of Biological Chemistry, Chubu University, 1200 Matsumoto, Kasugai, Aichi 467-8501, Japan  
*Biosci. Biotech. Biochem.* 66 (12), 2658-2662, 2002

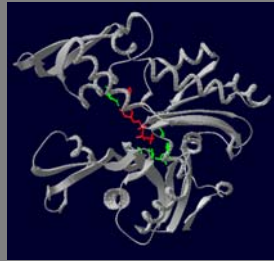


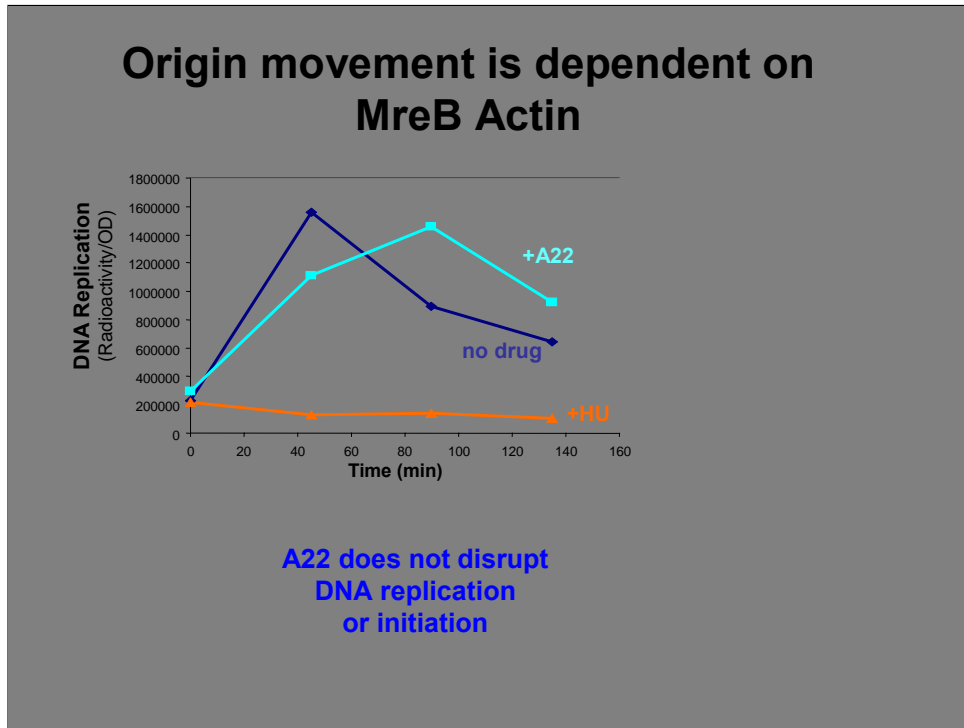
**A screen for A22 resistance yields 20 MreB alleles**

allele	mutation	# times isolated	Growth in A22	Growth in A22 when replaced by WT <i>mreB</i>	Growth in A22 in clean genetic background
Wild type	-	-	-	-	-
<i>mreB(Are1)</i>	T167A	10	+++	-	+++
<i>mreB(Are2)</i>	V324A	5	+++	-	+++
<i>mreB(Are3)</i>	D192G	1	+++	-	+++
<i>mreB(Are4)</i>	A325P	1	+++	-	+++
<i>mreB(Are5)</i>	D16G	1	+++	-	+++
<i>mreB(Are6)</i>	P112S	1	+++	-	+++
<i>mreB(Are7)</i>	L23R	1	+++	-	+++

### A screen for A22 resistance yields 20 MreB alleles

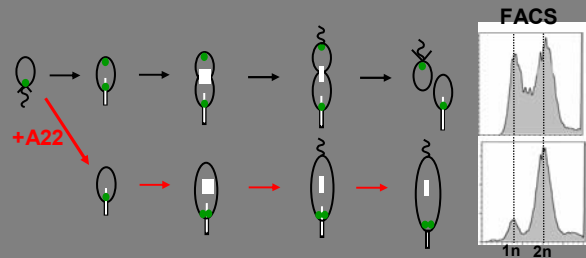
A22-resistant mutations  
mapped onto the  
*T. maritima* MreB•ATP  
structure







## Origin movement is dependent on MreB Actin



A22 disrupts movement of the origin to the pole  
MreB binds to the origin region

### Integration of Bacterial Cell Cycle Regulation

Coincident DNA  
replication and segregation

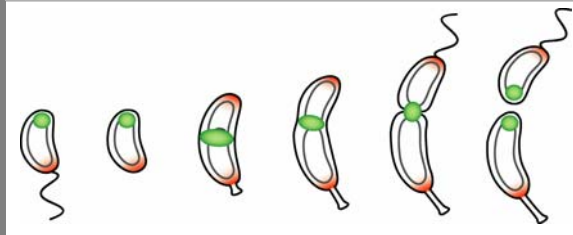


**Spatial regulation of**  
cell division site placement

**MipZ at replication origin is involved in division plane localization**

---

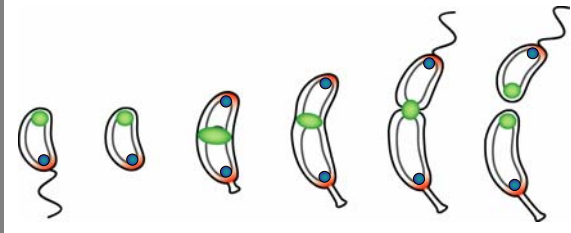
● FtsZ



**MipZ at replication origin is involved in division plane localization**

● FtsZ  
● MipZ•ori

FtsZ is a tubulin protein that forms a contacting ring for cytokinesis

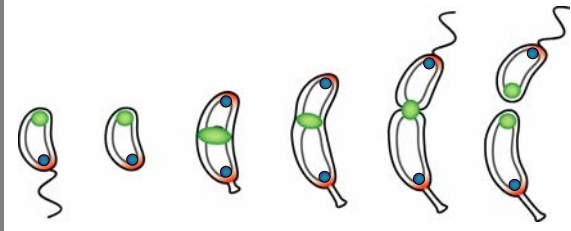


➤ FtsZ localizes to the subcellular region that is most distant from the MipZ•ori complex.

**MipZ at replication origin is involved in division plane localization**

● FtsZ  
● MipZ•ori

FtsZ is a tubulin protein that forms a ring at mid cell for cytokinesis

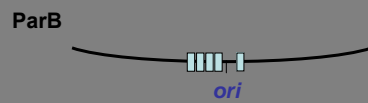


➤ FtsZ localizes to the subcellular region that is most distant from the MipZ•ori complex.

**MipZ might directly inhibit FtsZ assembly.**

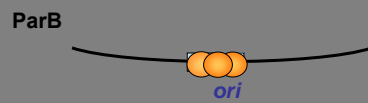
A protein complex coordinates DNA segregation and the positioning of the cell division site (dependent on MreB actin)

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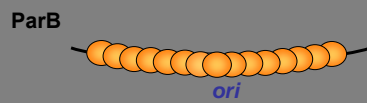
A protein complex coordinates DNA segregation and the positioning of the cell division site (dependent on MreB actin)

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A protein complex coordinates DNA segregation and the positioning of the cell division site (dependent on MreB actin)

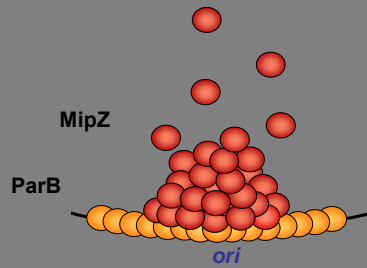
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A protein complex coordinates DNA segregation and the positioning of the cell division site (dependent on MreB actin)

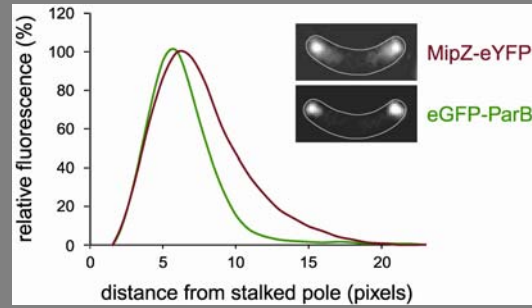
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**A protein complex coordinates DNA segregation and the positioning of the cell division site (dependent on MreB actin)**

---

**Quantitation of the fluorescent signal:**



**gradient of MipZ concentration:**

- highest at the position of the origin region(s)
- lowest at the mid-cell

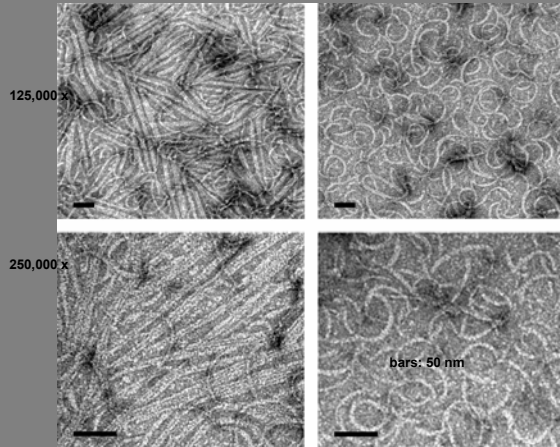
**MipZ destabilizes FtsZ polymers that form the cell division ring**

---

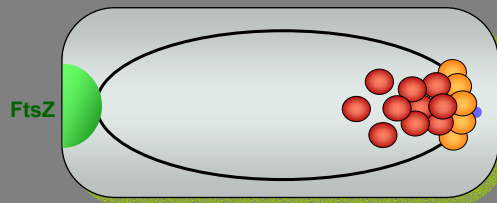
**MipZ affects FtsZ polymerization:**

**- MipZ**

**+ MipZ**

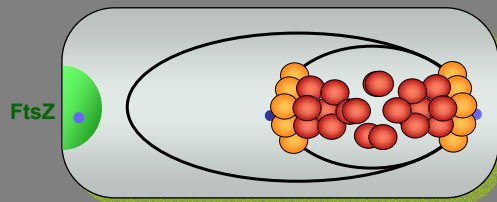


Model for placement of the Z-ring by the MipZ•ParB complex



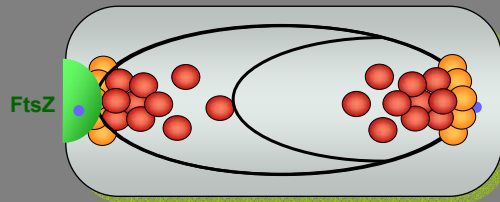
Martin Thanbichler

Model for placement of the Z-ring by the MipZ•ParB complex



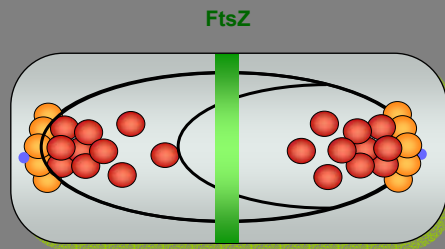
Martin Thanbichler

Model for placement of the Z-ring by the MipZ•ParB complex



Martin Thanbichler

Model for placement of the Z-ring by the MipZ•ParB complex



Martin Thanbichler

### Integration of Bacterial Cell Cycle Regulation

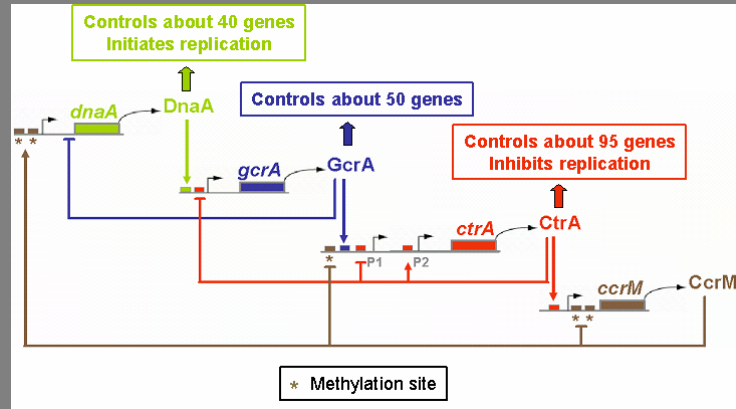
Coincident DNA  
replication and segregation

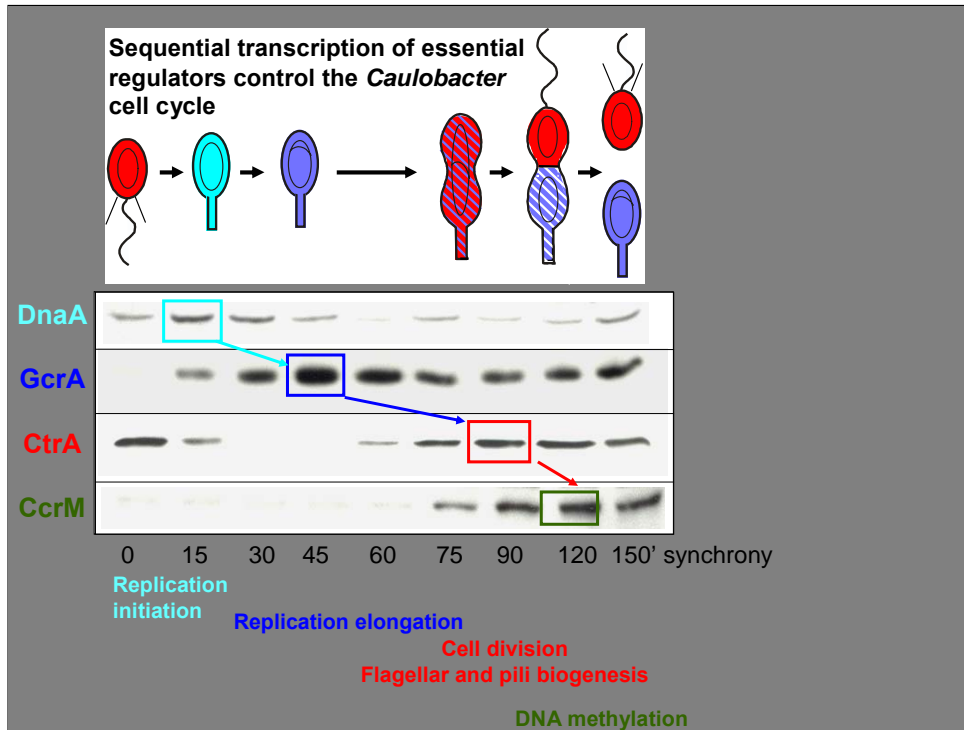


**Epigenetic control** of  
cell cycle master regulators



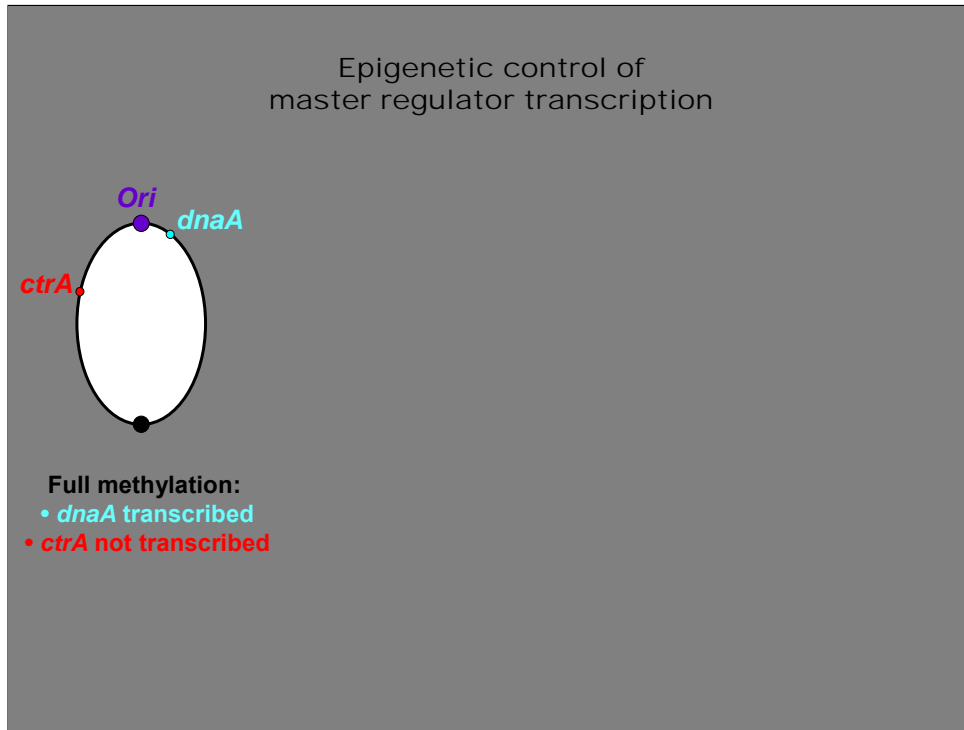
## Epigenetic control of master regulatory circuit

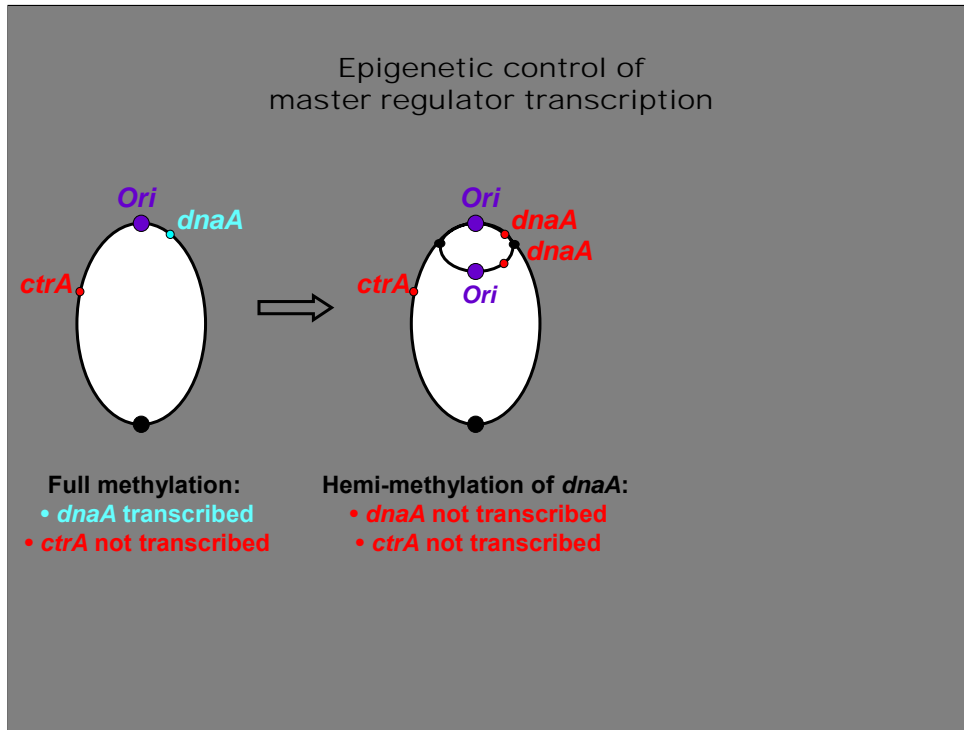


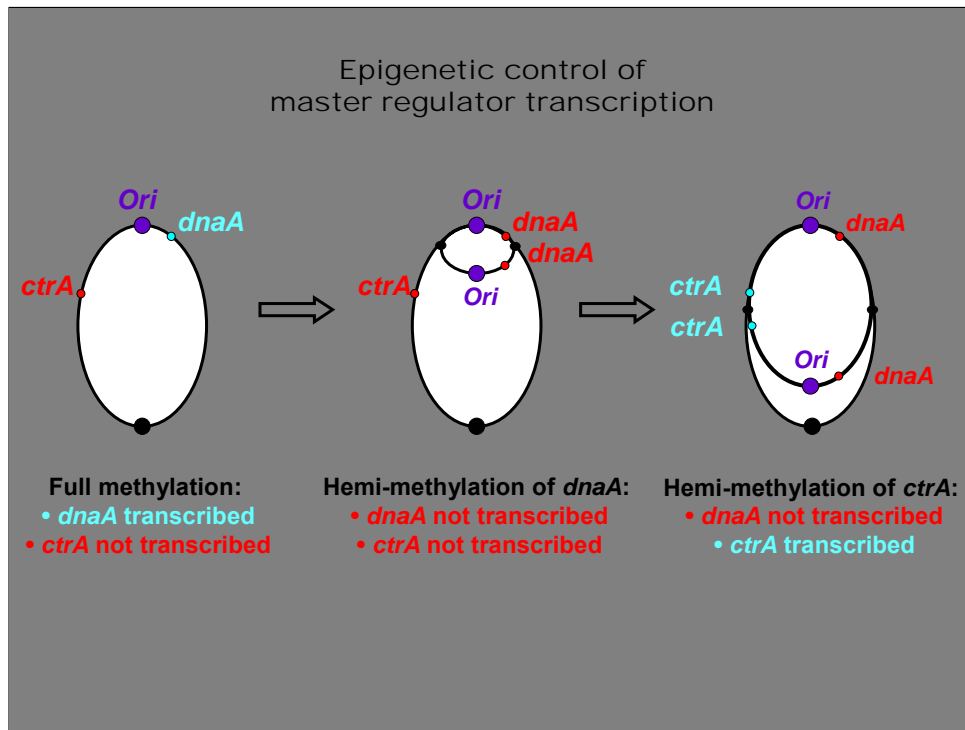


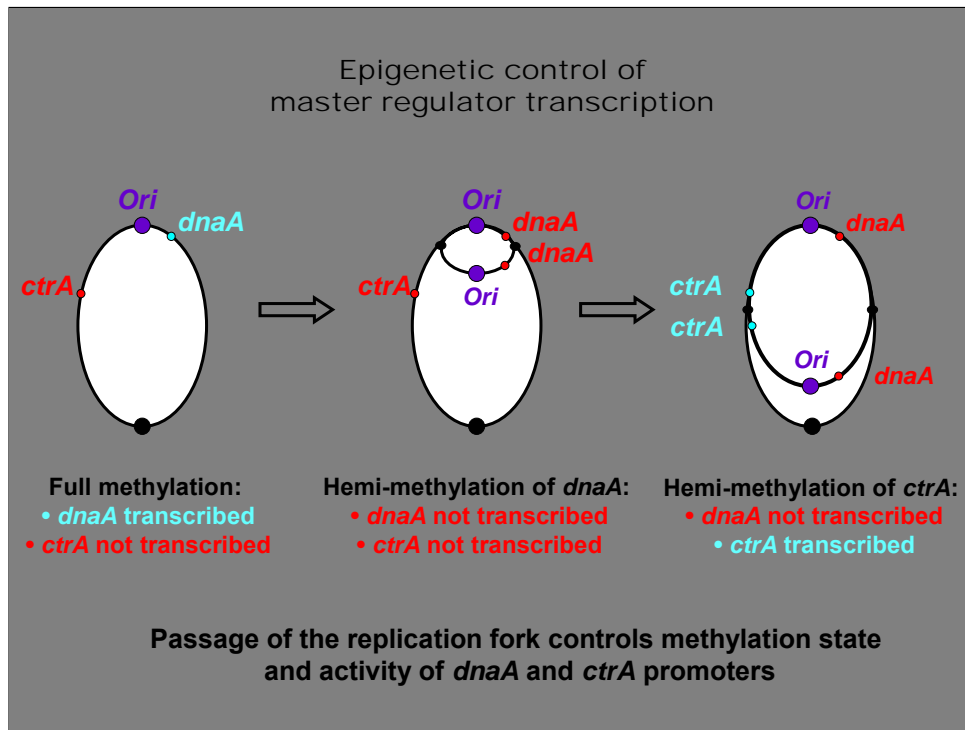


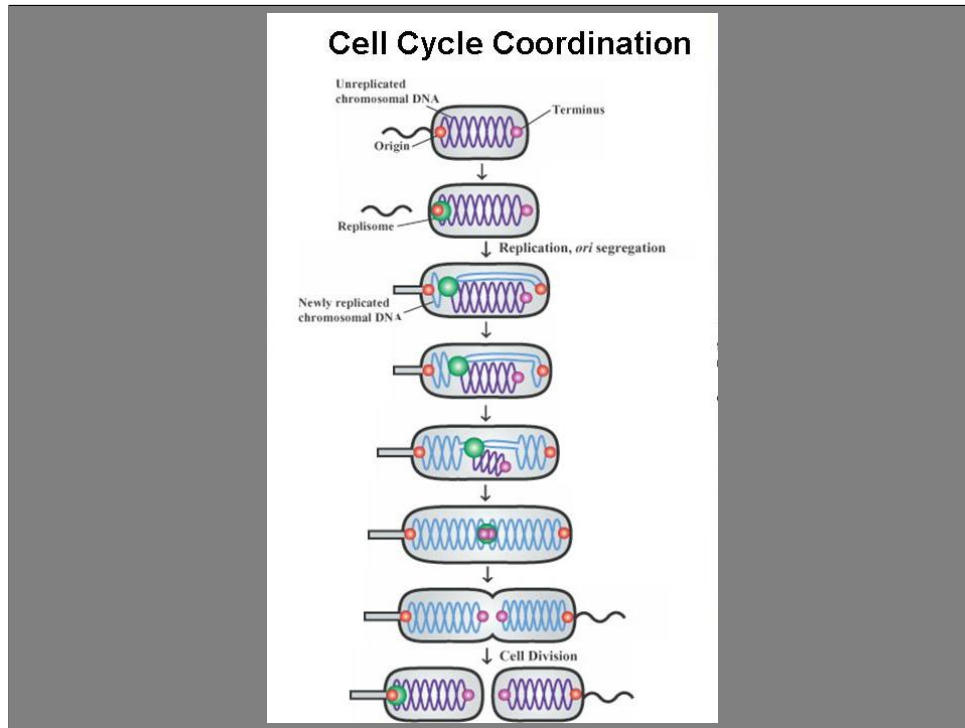
**What turns on  
this cascade?**



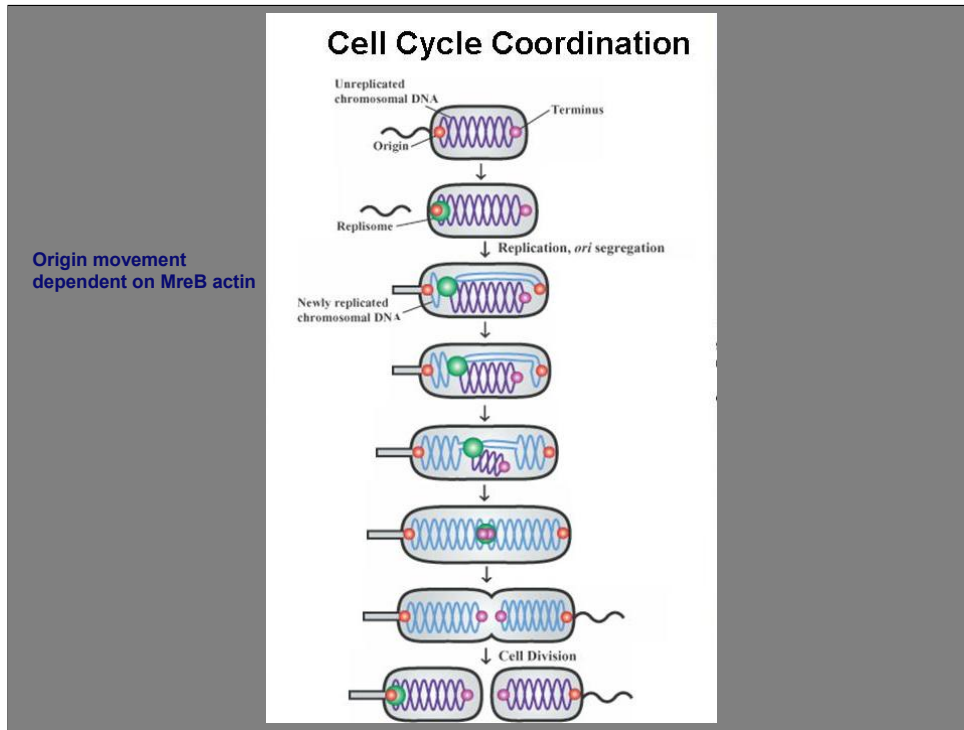


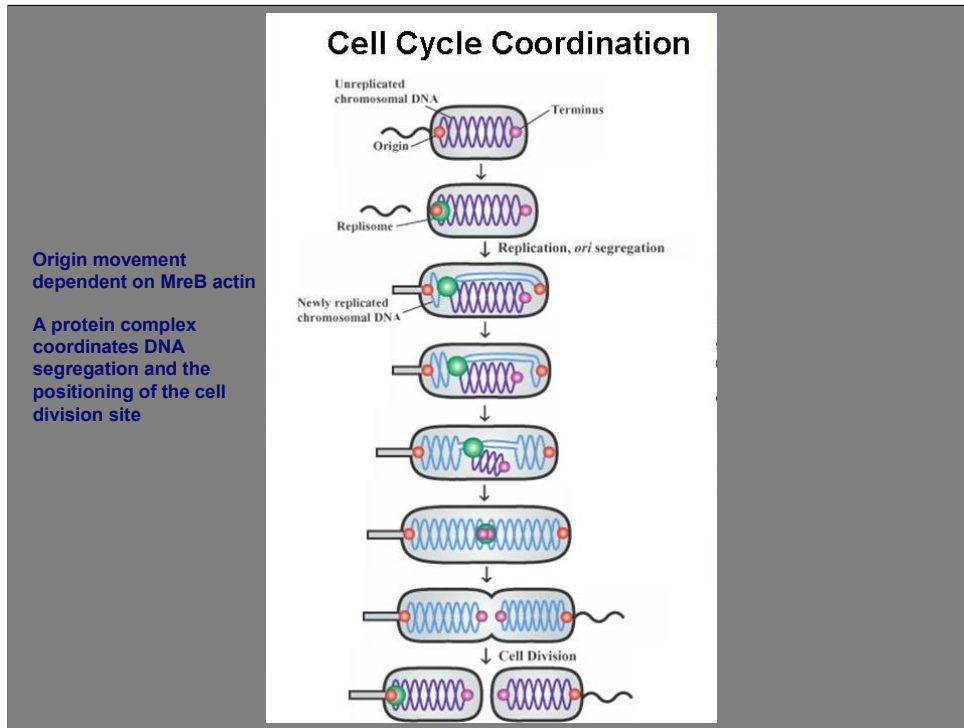


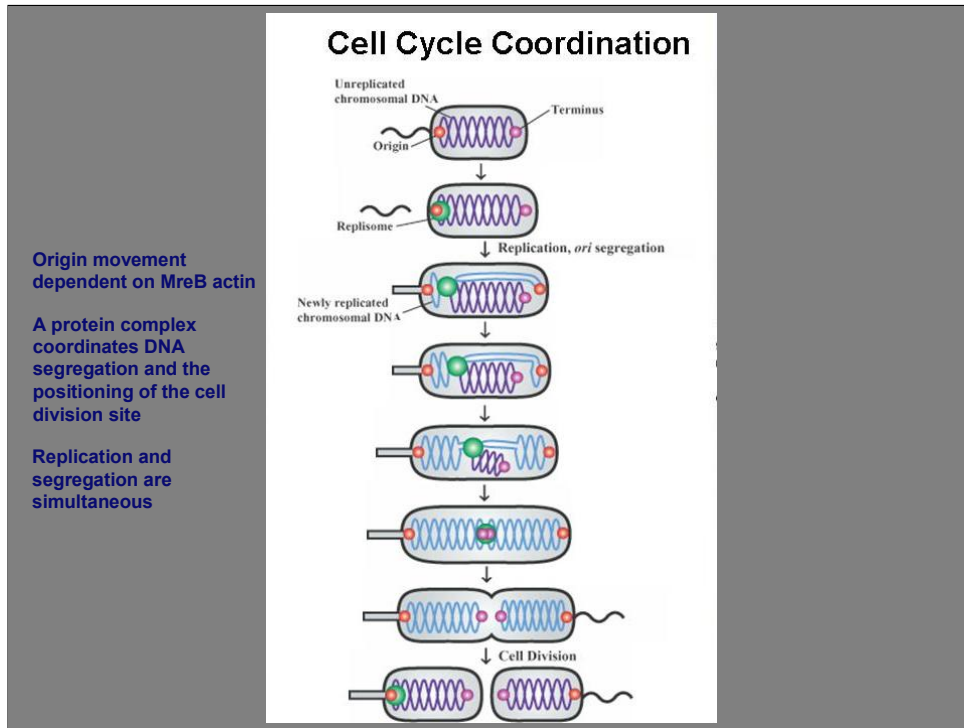


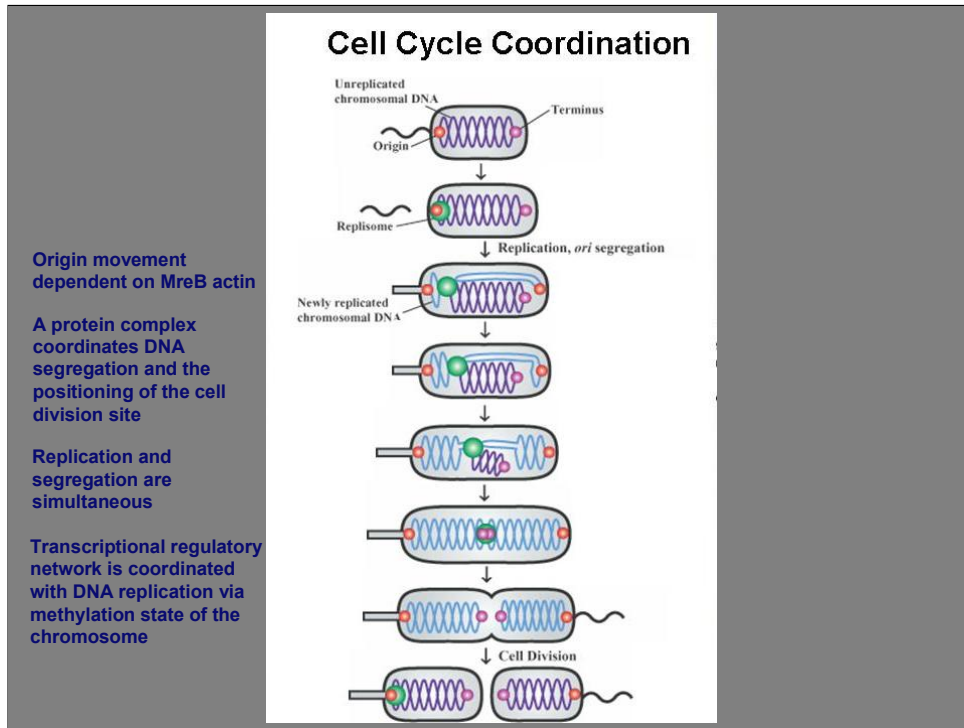












## Acknowledgments

### Lucy Shapiro Lab

- **Martin Thanbichler**  
**Justine Collier**  
**Ann Reisenauer**  
**Natalie Dye**

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- **Patrick Viollier (Case Western Reserve)**
- **Rasmus Jensen (Roskilde Univ)**

### Harley McAdams Lab

- Patrick McGrath (Physics)**  
**Alison Hottes (EE)**