A study of the bacterial replication clock

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Molecular Biophysics at the Transition State:
From Statistical Mechanics to AI

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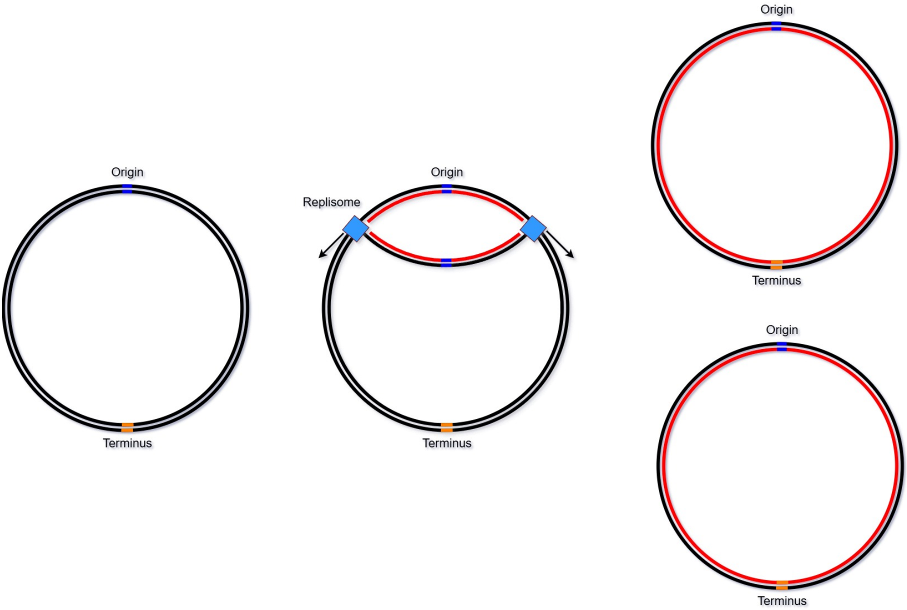
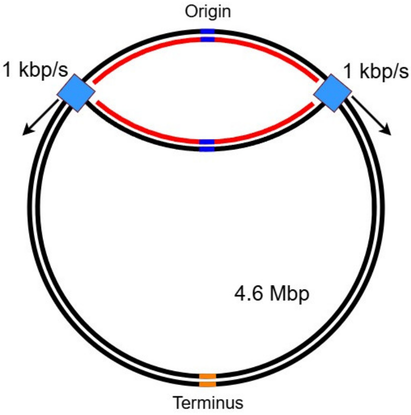
The bacterial replication clock

Replication in bacteria



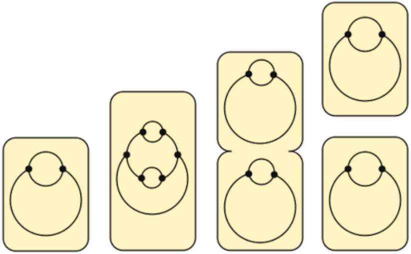
The timing puzzle



Genome size:	4.6 Mbp
Fork speed:	1 kbp/s
Replication time (C):	$\sim$40 min
Doubling time (τ): (LB, 37°C)	$\sim$20 min

$\tau < C$??

Multifork replication



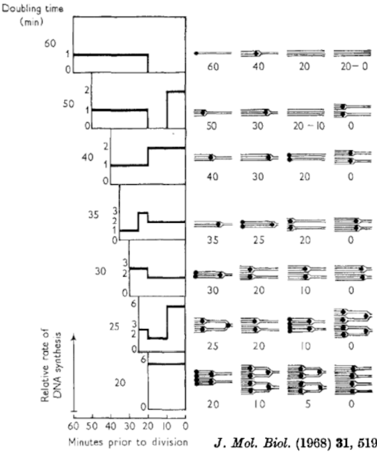
Cooper-Helmstetter model

Two constants:

Replication time C ~ 40 min

Separation time D ~ 20 min

Chromosome Replication and the Division Cycle of *Escherichia coli* B/r



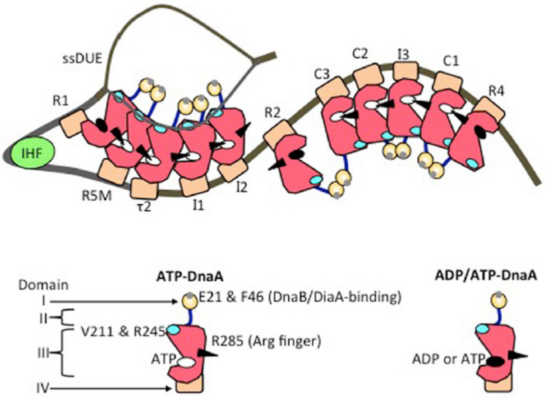
J. Mol. Biol. (1968) **31**, 519–540

Two fundamental questions

1. How does the cell "know" when to initiate replication?

2. How does it make all origins fire at the same time?

At the heart of both questions: the initiator DnaA



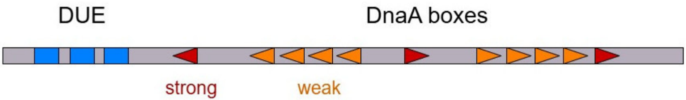
Katayama et al. Frontiers 2017

The initiator protein DnaA plays a central role in replication timing.

It binds to repeated sequences at the origin of replication and promotes the separation of the two DNA strands.

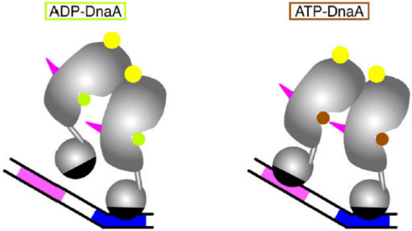
This marks the beginning of each replication cycle.

DnaA recognizes a specific DNA motif



GATCTATTTATTTAGAGATCTGTTCTATTGTGATCTCTTATTAGGATCGCACTGCCCTGTGG
ATAACAAGGATCCGGCTTTTAAGATCAACAACCTGGAAAGGATCATTAACTGTGAATGATCG
GTGATCCTGGACCGTATAAGCTGGGATCAGAATGAGGGGTTATACACAACTCAAAAACTGAA
CAACAGTTGTTCTTTGGATAACTACCGGTTGATCCAAGCTTCCTGACAGAGTTATCCACA

S. Ozaki, T. Katayama / Plasmid 62 (2009) 71–82

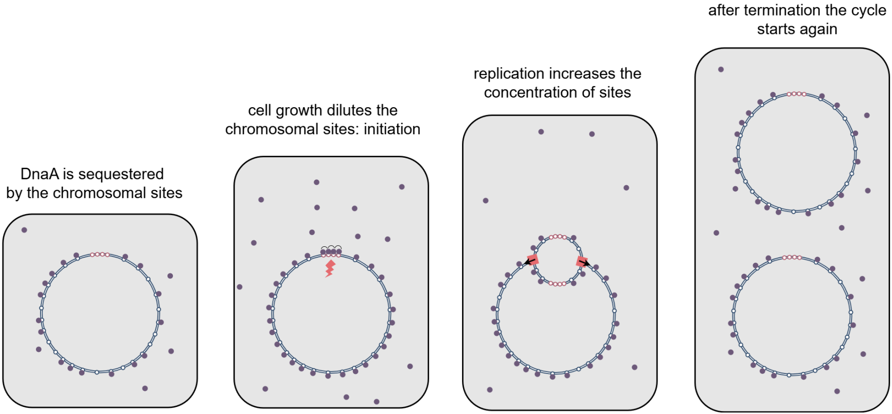


The regulatory mechanisms of DnaA

1. Titration

2. Activation/de-activation switch

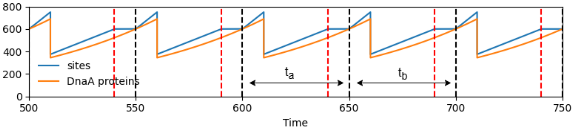
Titration

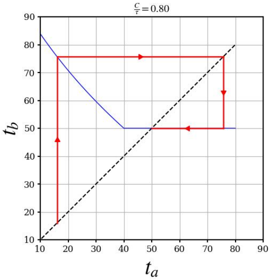


Hansen et al., Res. Microbiol. 1991.

Stability analysis: slow growth

replication time $<$ doubling time



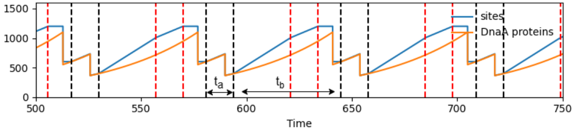


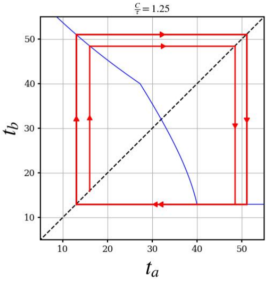
$$\left(1+\min\left(1,\frac{t_a}{C}\right)\right)\cdot e^{\lambda t_b} = 2\cdot\left(1+\min\left(1,\frac{t_b}{C}\right)\right)$$

Fu et al., PRX LIFE 2023.

Stability analysis: fast growth

replication time > doubling time

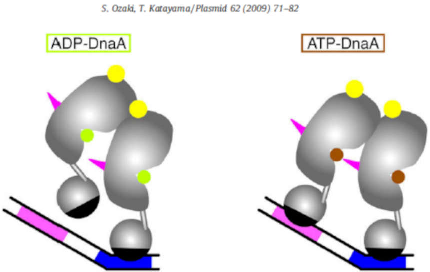


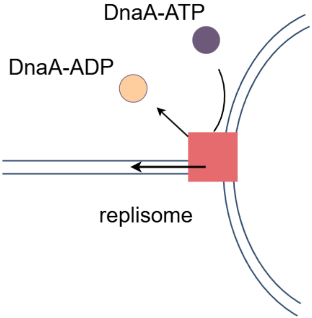


$$\left(1 + \min\left(1, \frac{t_a}{C}\right)\right) \cdot e^{\lambda t_b} = 2 \cdot \left(1 + \min\left(1, \frac{t_b}{C}\right)\right)$$

Fu et al., PRX LIFE 2023.

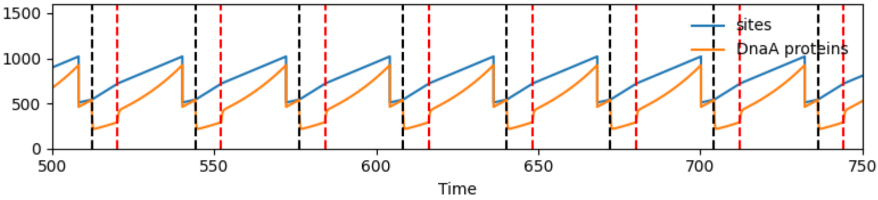
(De)-activation





Hydrolysis restores stability

$$\frac{d\,[\text{DnaA-ATP}]}{dt} = -\frac{n_{\text{forks}}}{V} k_h\,[\text{DnaA-ATP}] + (k_e + \lambda)\,[\text{DnaA-ADP}]$$
$$\frac{d\,[\text{DnaA-ADP}]}{dt} = \frac{n_{\text{forks}}}{V} k_h\,[\text{DnaA-ATP}] - (k_e + \lambda)\,[\text{DnaA-ADP}]$$



1. How does the cell "know" when to initiate replication?

2. How does it make all origins fire at the same time?

Equilibrium model for DnaA-DNA interaction

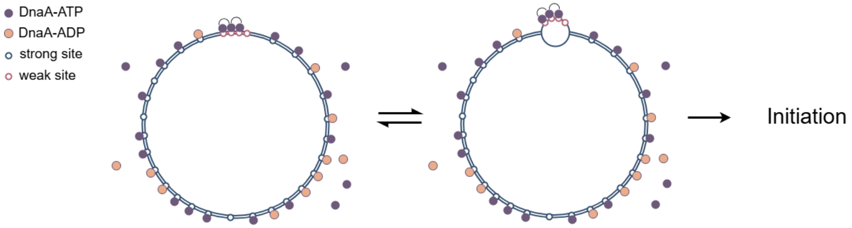
$$E^{(p)}(\{\boldsymbol{s}^{(c)}\},\{\boldsymbol{s}^{(o)}\}) = -\sum_{i=1}^{n_{\text{tot}}^{c}} \boldsymbol{\epsilon}_c \cdot \boldsymbol{s}_i^{(c)} - \sum_{i=1}^{n_{\text{tot}}^{o}} \boldsymbol{\epsilon}_o^{(p)} \cdot \boldsymbol{s}_i^{(o)} - \sum_{\langle i,j \rangle} \boldsymbol{s}_i^{(o)} \hat{J}^o \boldsymbol{s}_j^{(o)}$$

$$\boldsymbol{s}_i = \begin{cases} (1,0,0) & \text{if site } i \text{ is vacant} \\ (0,1,0) & \text{if occupied by DnaA-ATP} \\ (0,0,1) & \text{if occupied by DnaA-ADP} \end{cases}$$

The origin region can be in two conformations: $p \in \{\text{open}, \text{closed}\}$.

Only ATP-bound DnaA can bind cooperatively: $\hat{J}^o_{mm} = J_c \delta_{m2} \delta_{n2}$.

Origin activation



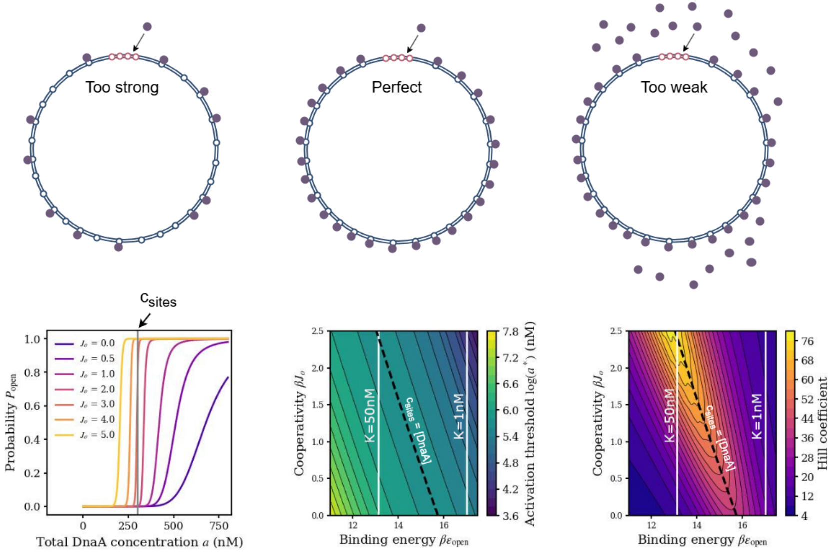
Probability of open conformation:

$$P_{\text{open}} = \frac{e^{-\beta\epsilon_{\text{cost}}} Z^{\text{open}}}{e^{-\beta\epsilon_{\text{cost}}} Z^{\text{open}} + Z^{\text{closed}}}$$

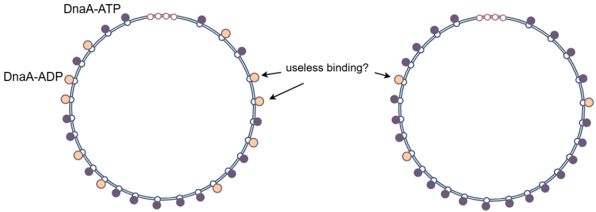
Stochastic rate of origin firing:

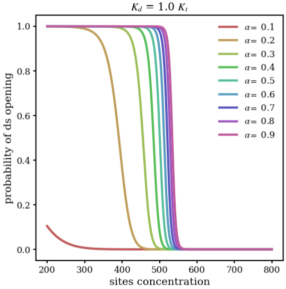
$$k = P_{\text{open}} k_{\text{max}}$$

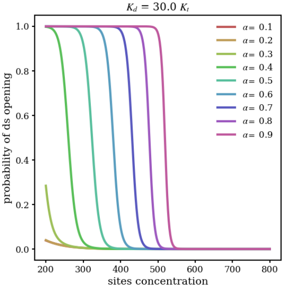
Titration leads to ultra-sensitivity



The binding of inactive DnaA reduces sensitivity







Conclusions

- Cells coordinate the replication cycle with the cell cycle via DnaA titration and (de)-activation.
- The origin design (weak, cooperative sites) is crucial to ensure a sharp response and a quick feedback.
- DnaA sequestration on the chromosome ensures ultra-sensitivity.
- A quantitative assessment of the strength of cooperative binding is needed, both at the origin and the chromosome.

Thank You!

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