Regulation of Flagellar Motors in Salmonella

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MBI
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The Fundamental Problem

1) All living organisms make decisions (when to divide, when to differentiate, when to destroy, when to repair, when to grow, when to die)

- What information is available and how is it assessed?
- How is that information transduced into chemistry?

2) In order to operate efficiently, machines (cellular components) must be built to precise specifications.

- How are those specifications set?
- How are the decisions made to determine regarding manufacture? (When to make what and how much to make?)

Injectosomes

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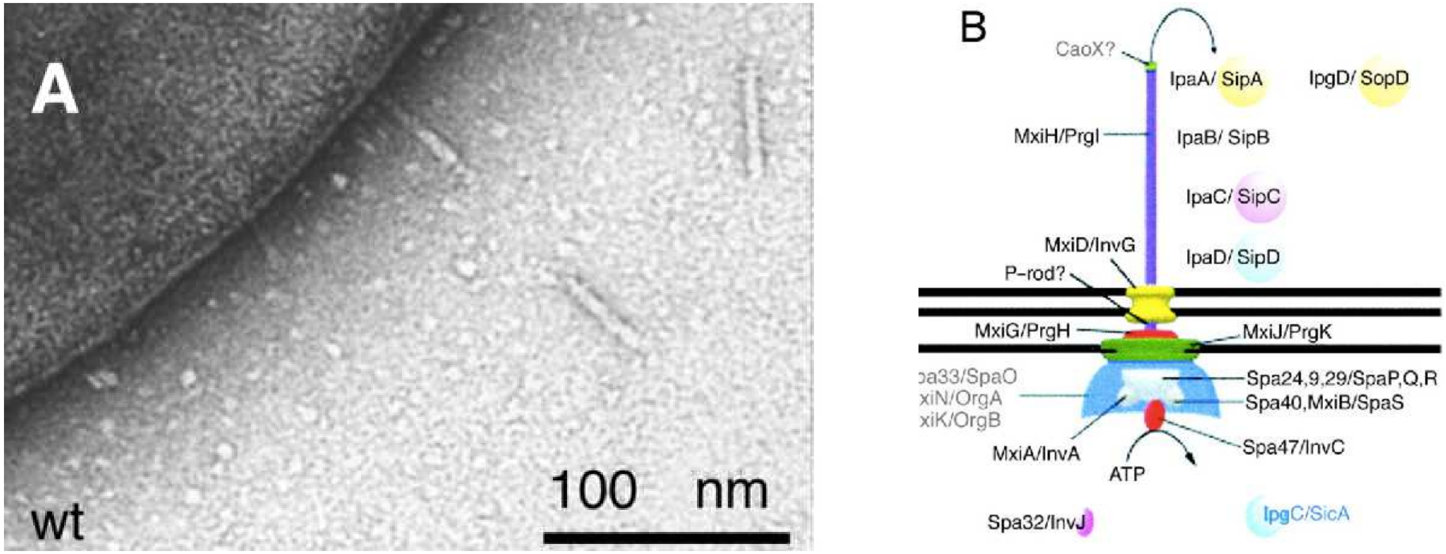
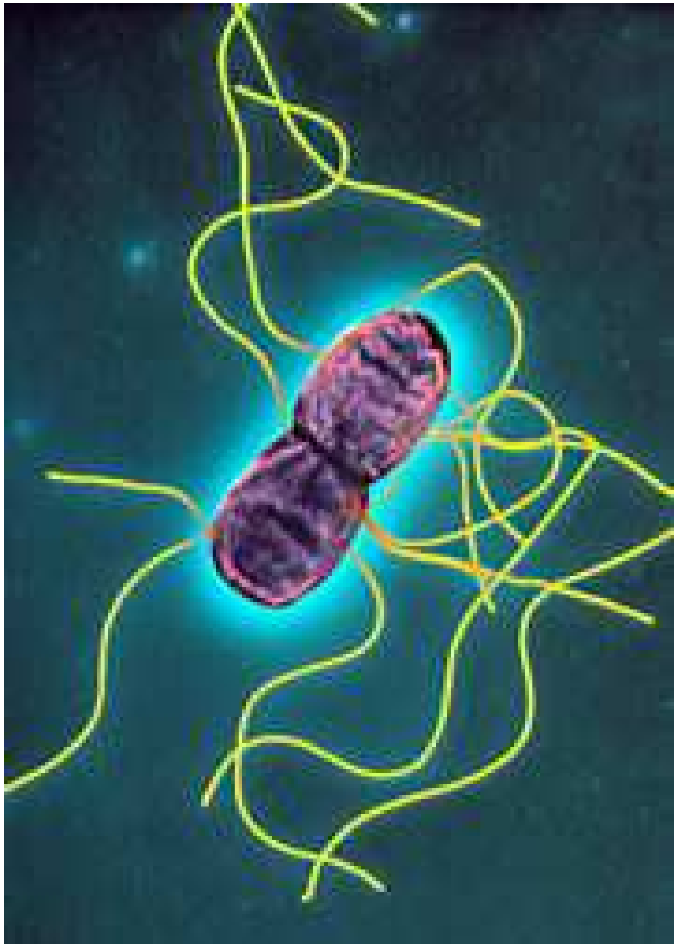
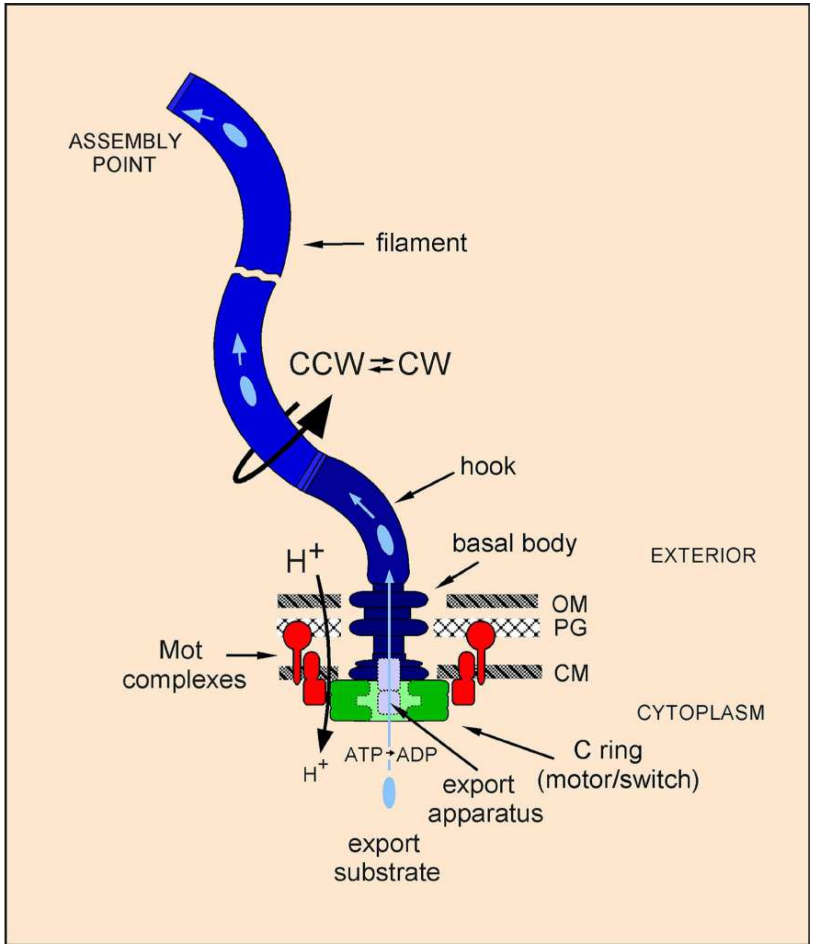


Type III Secretion System - Injectosome

Flagellar Motors

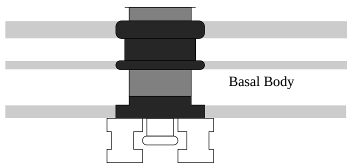




Control of Flagellar Growth

The motor is built in a precise step-by-step fashion.

- Step 1: Basal Body
- Step 2: Hook (FlgE secretion)
- Step 3: Filament (FliC secretion)



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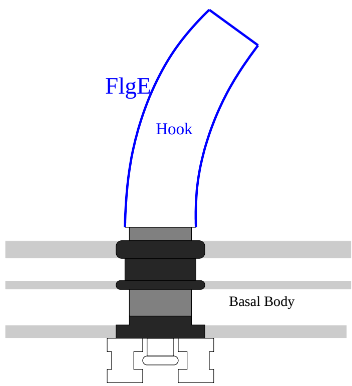

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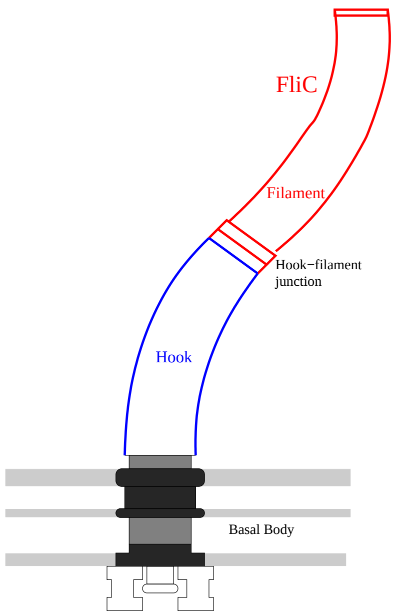

Click to see movies

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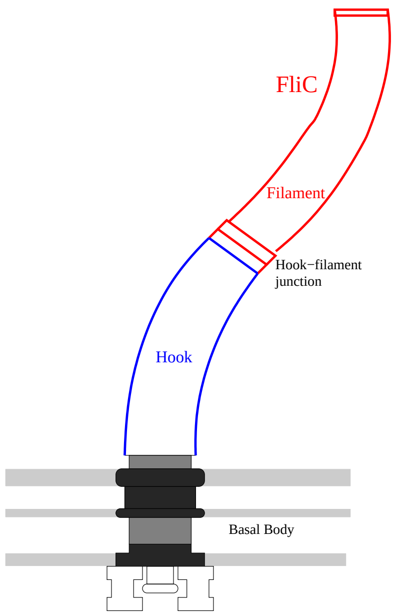


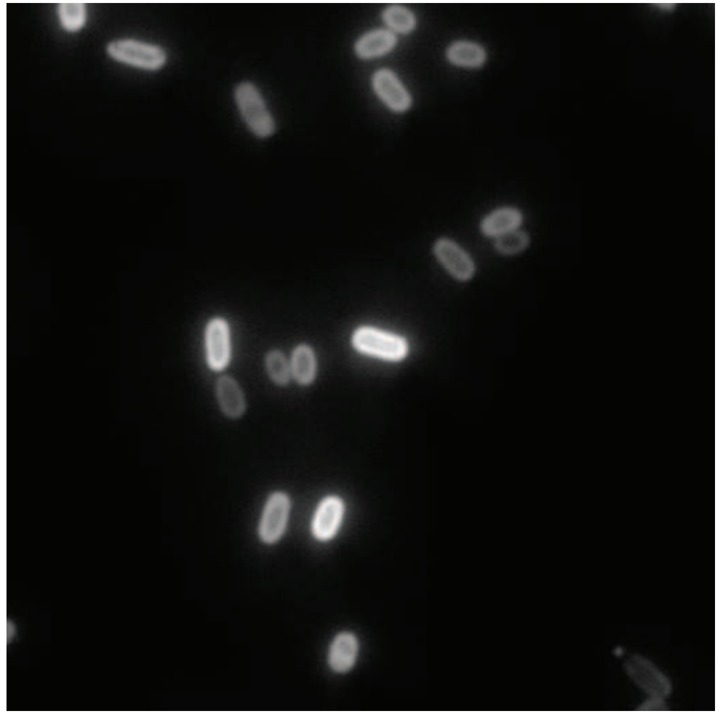
Click to see movies

Questions for this talk:

1. How is construction and number of flagella regulated?
2. How is the hook length determined (55 $\pm$6 nm)?
3. How are the switches between steps coordinated?

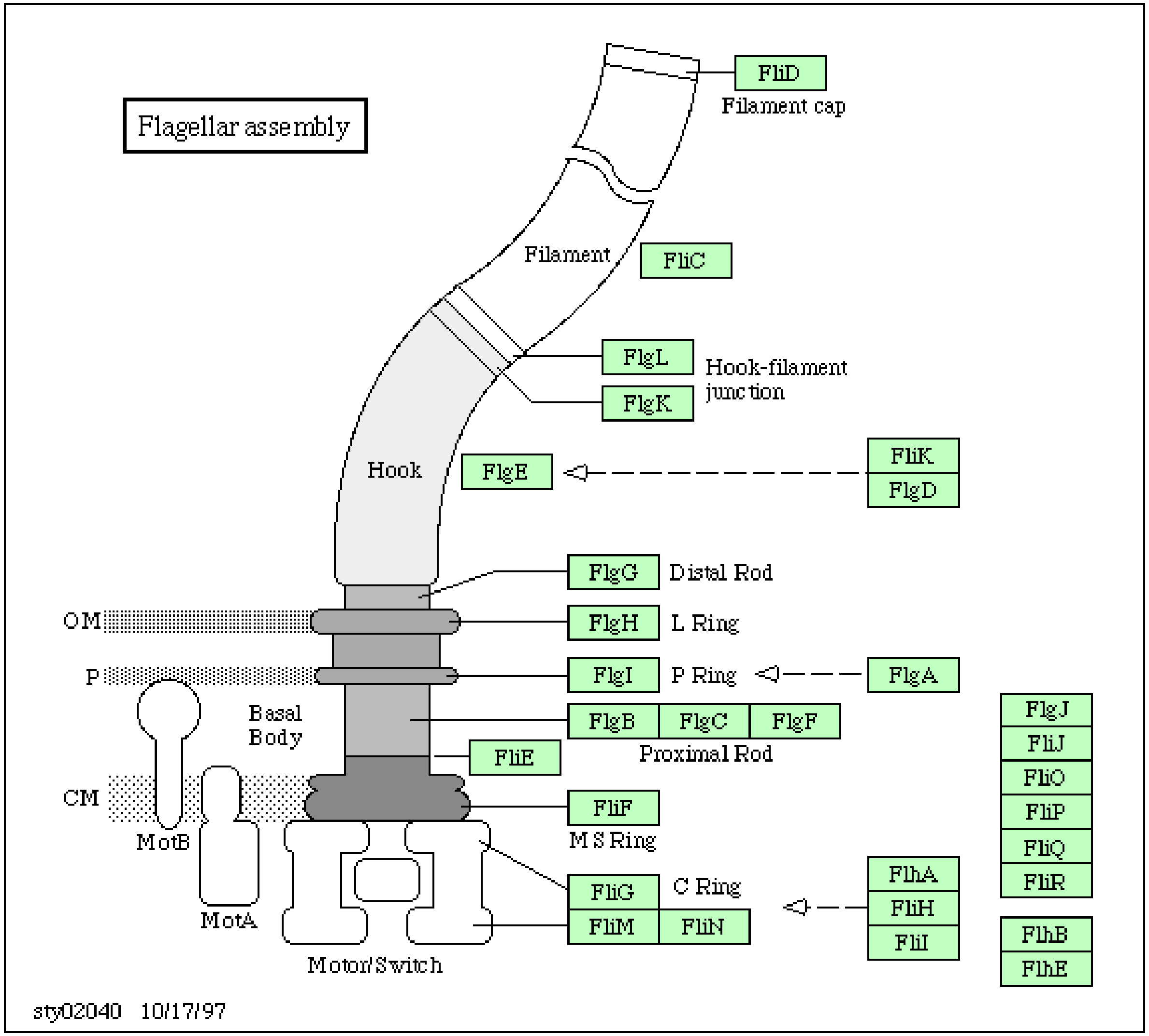
Bistability

- In a genetically identically population, some bacteria develop flagella while others do not - **bistability**
- For this image, salmonella were modified with a GFP following the *fliC* promoter. (Brighter means higher FliC flagellar protein expression level.)

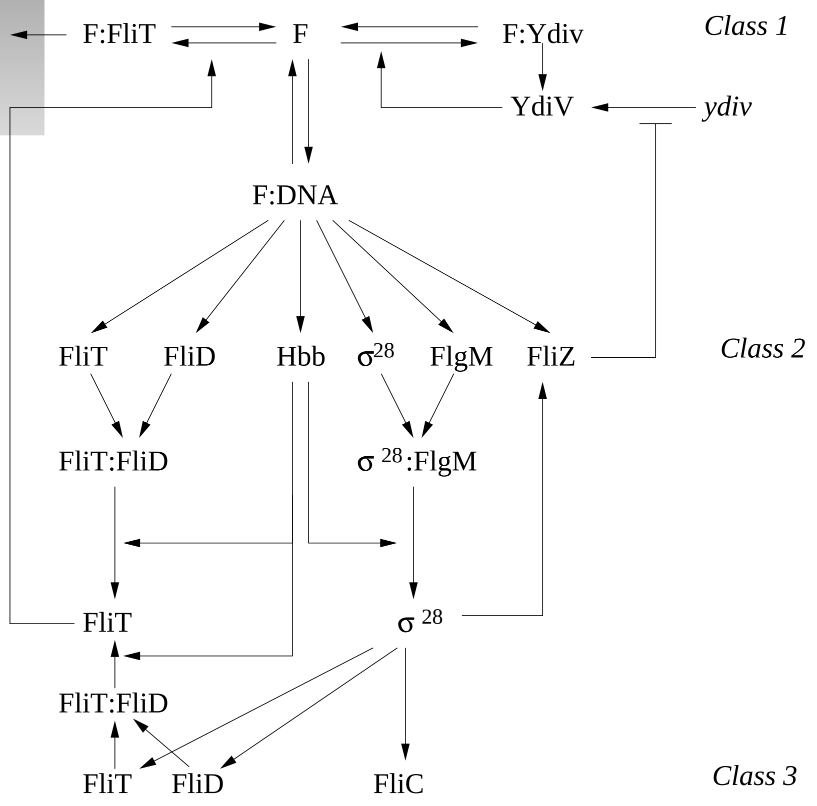


Source: Jenna Noll

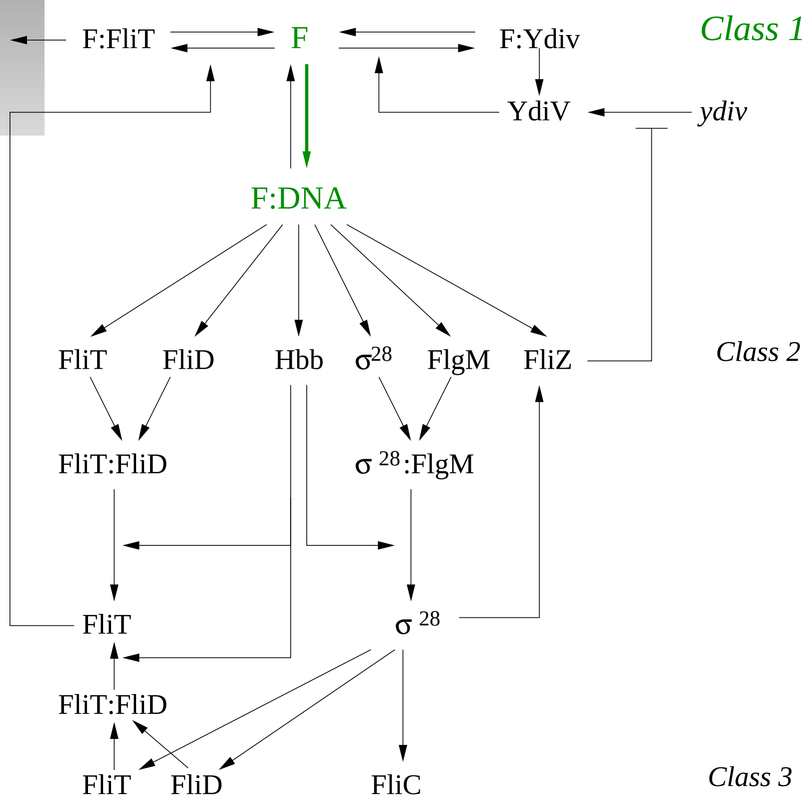
Proteins of Flagellar Assembly



Q1:Regulation of construction and Number

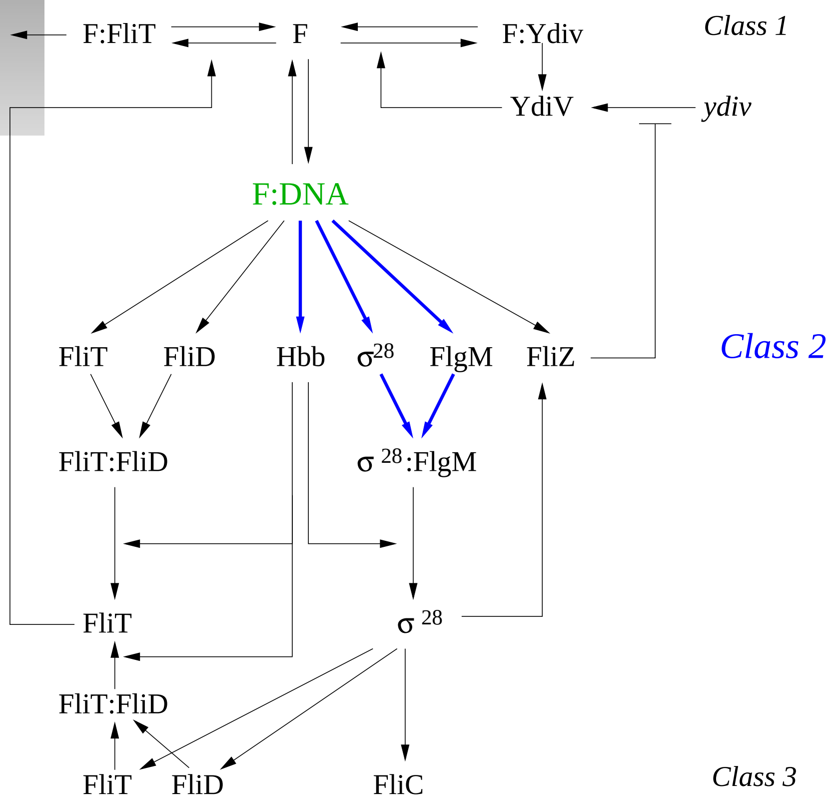


Class 1



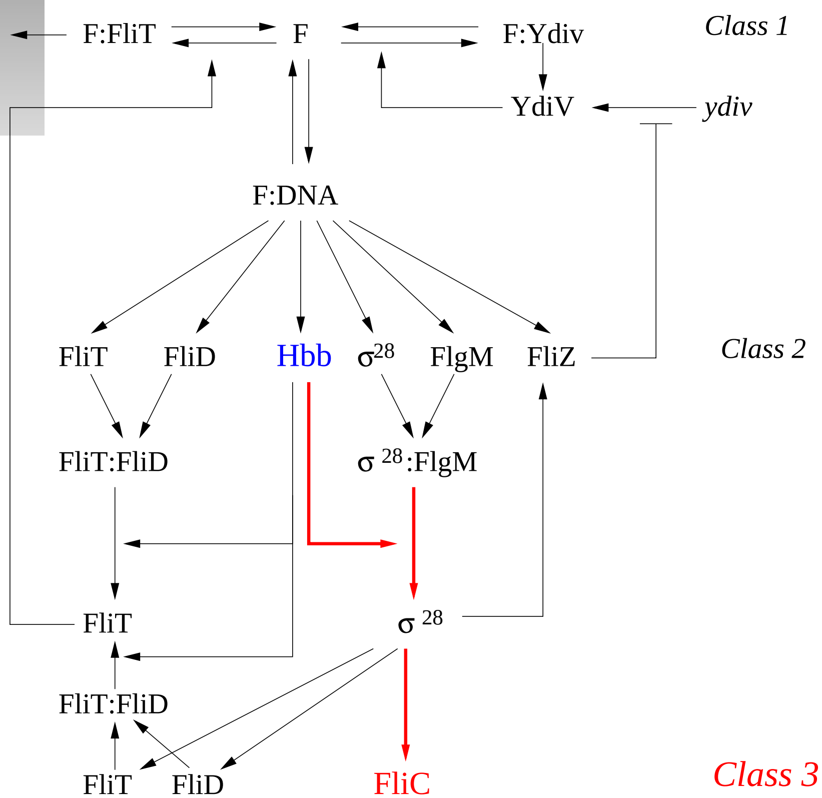
- $FlhD_4C_2$, the master operon, is made in class 1;
- $FlhD_4C_2$ is a transcription factor for class 2 production.

Class 2



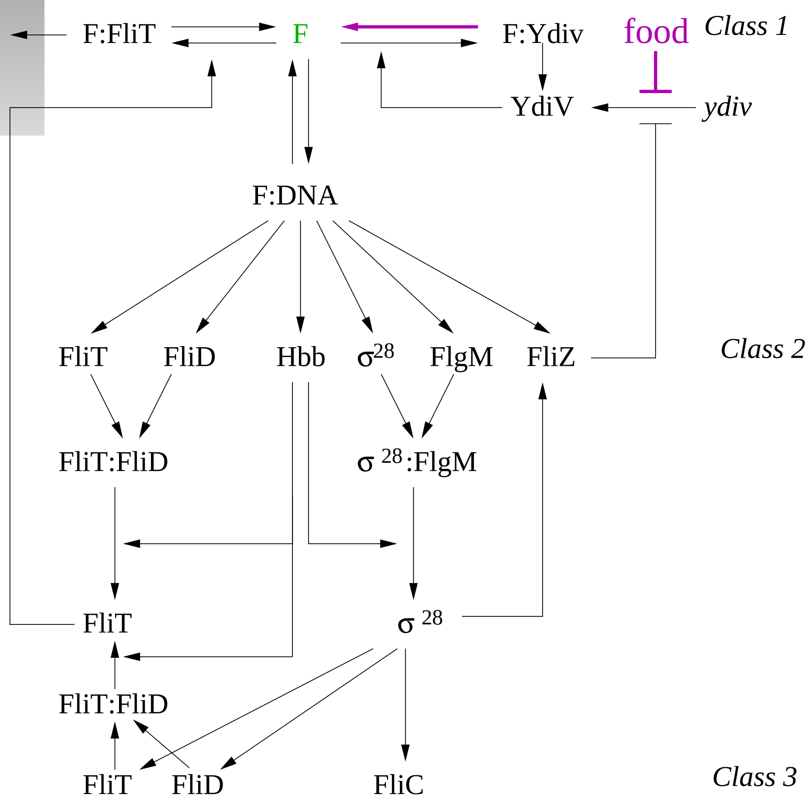
- Class 2 includes HBB and regulatory proteins.
- σ^{28} (FliA) and FlgM are produced in class 2.
- FlgM binds to σ^{28} to sequester it, keeping it inactive.

Class 3



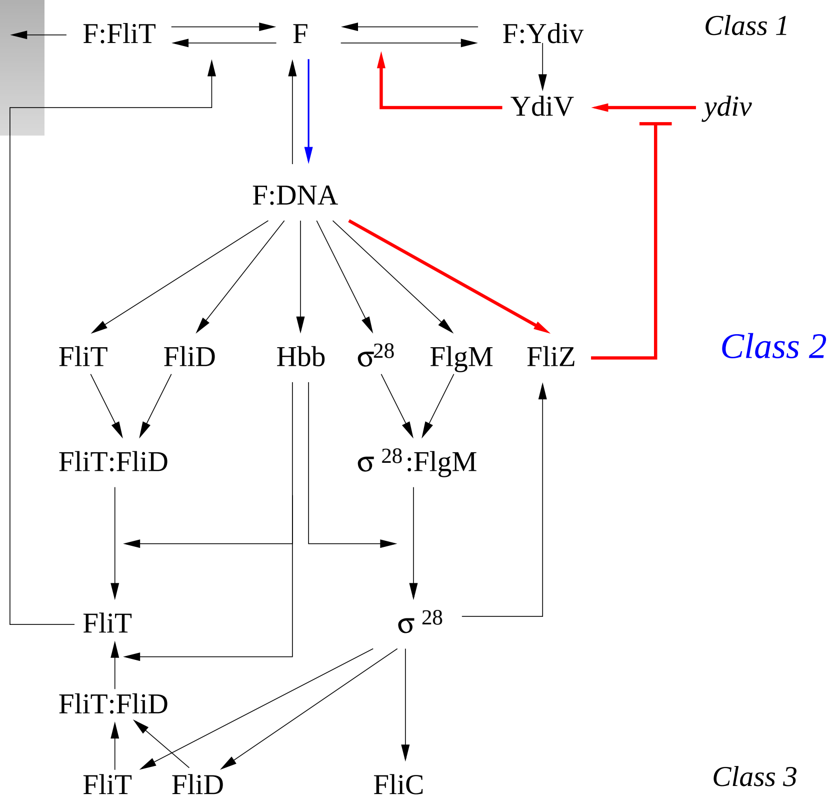
- σ^{28} is the transcription factor for class 3 production.
- FlgM is secreted from the cell when HBBs are complete.
- Flagellar (FliC) and chemosensory proteins are made in class 3.

Nutritional Response



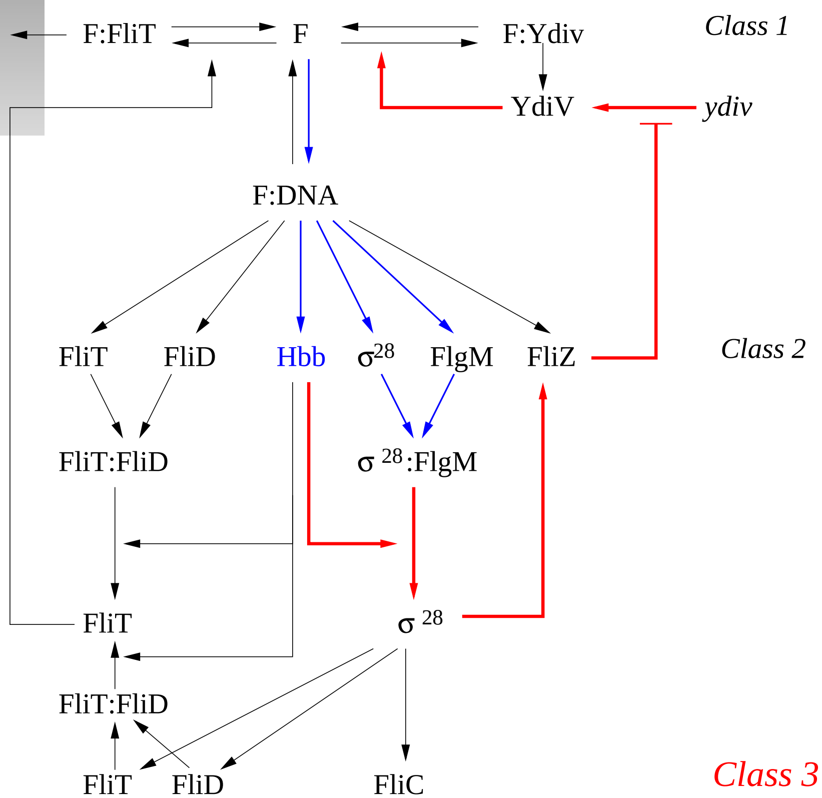
- A regulatory protein, YdiV, is increased in poor nutritional conditions, decreased in good nutritional conditions.
- YdiV binds to $FlhD_4C_2$ to sequester it, and promotes its unbinding from DNA and degradation;

Bistability - I



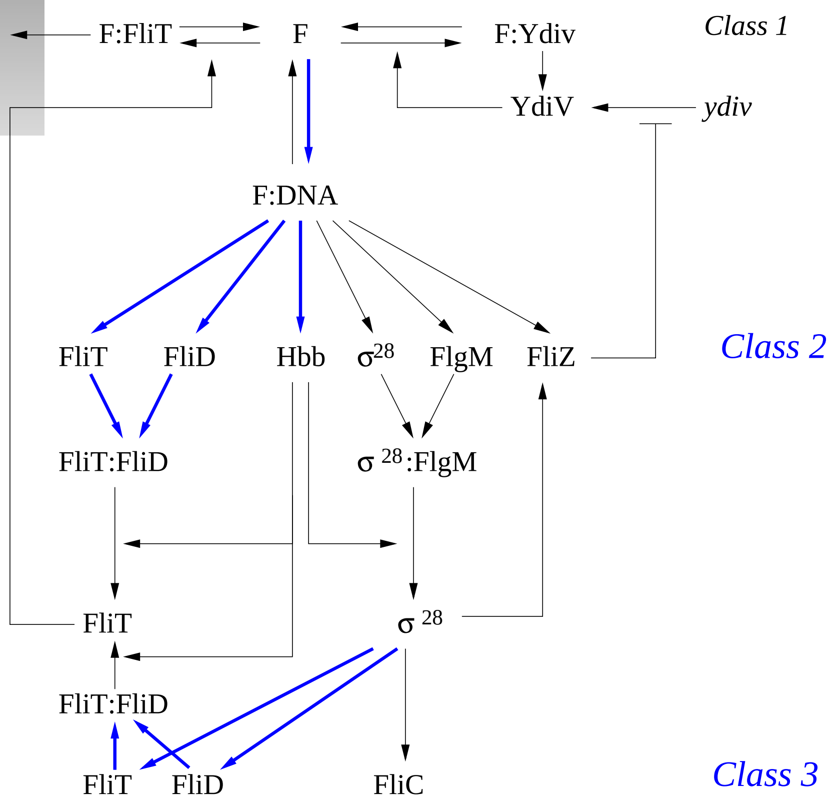
- FliZ is a class 2 and class 3 protein;
- FliZ inhibits YdiV at the transcriptional level
- giving a class 2 positive feedback loop.

Bistability - II



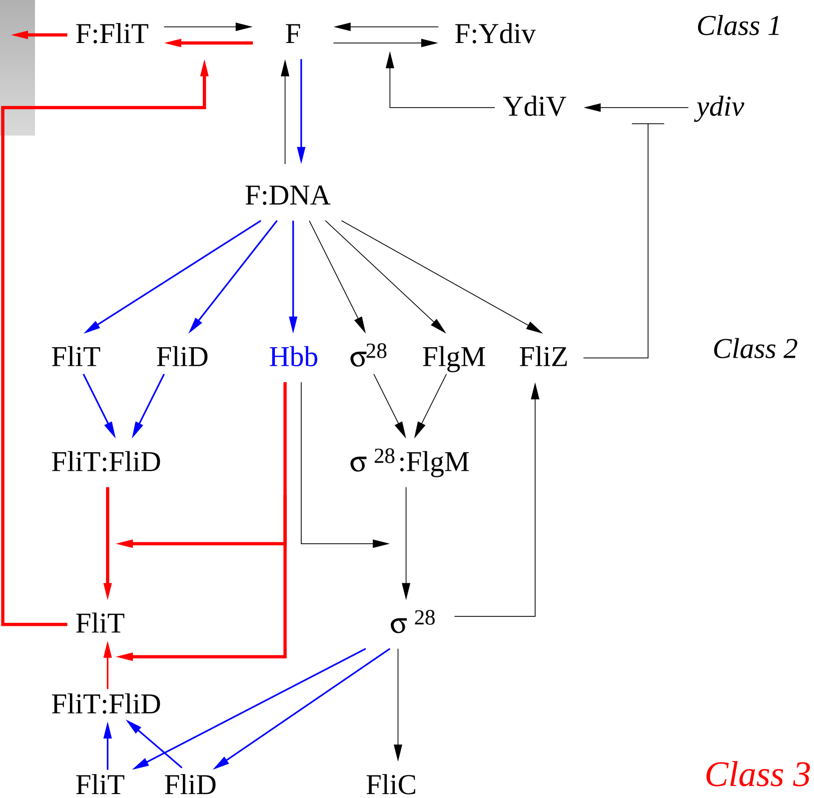
- FlgM is secreted from the cell when HBBs are complete;
- σ^{28} produces FliZ which further inhibits YdiV
- giving a class 3 positive feedback loop.

Flagellar Number Control



- FliD and FliT are both class 2 and class 3.
- FliD binds to FliT to sequester it.

Flagellar Number Control-2

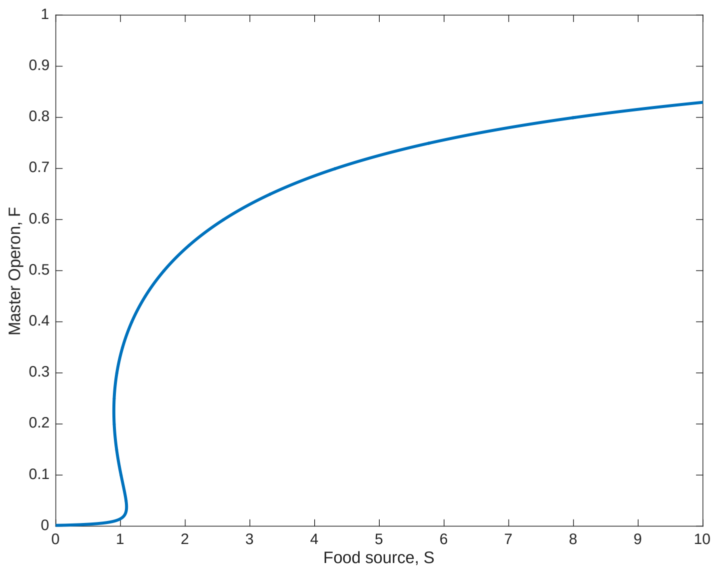


- FliD is secreted from the cell when HBBs are complete.
- FliT binds to $FlhD_4C_2$ to sequester it, and promotes its degradation.

A Mathematical Model

A mathematical model shows

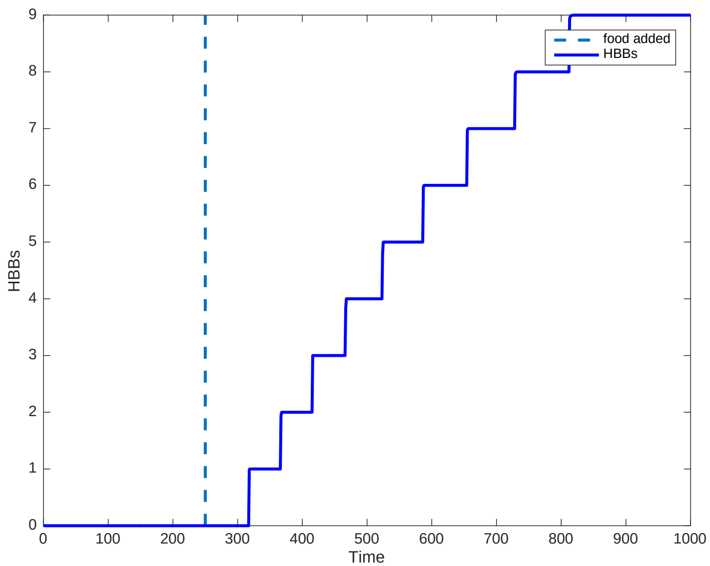
- Stochastic Switch-like behavior to turn on HBB production (bistability)

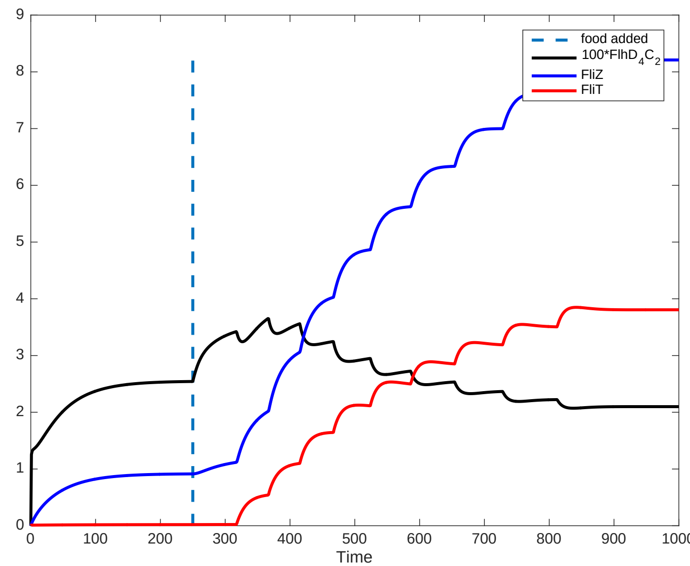


A Mathematical Model

A mathematical model shows

- Stochastic Switch-like behavior to turn on HBB production (bistability)
- Gradual buildup of FliT to turn off HBB production





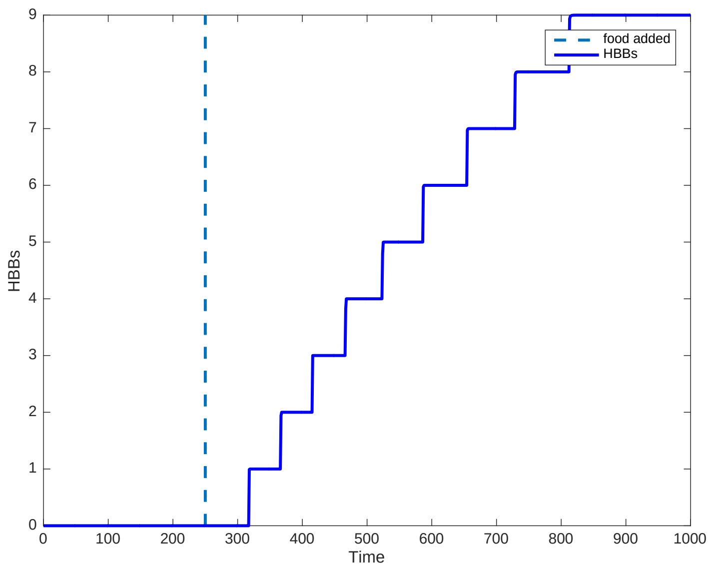
Imagine the Possibilities

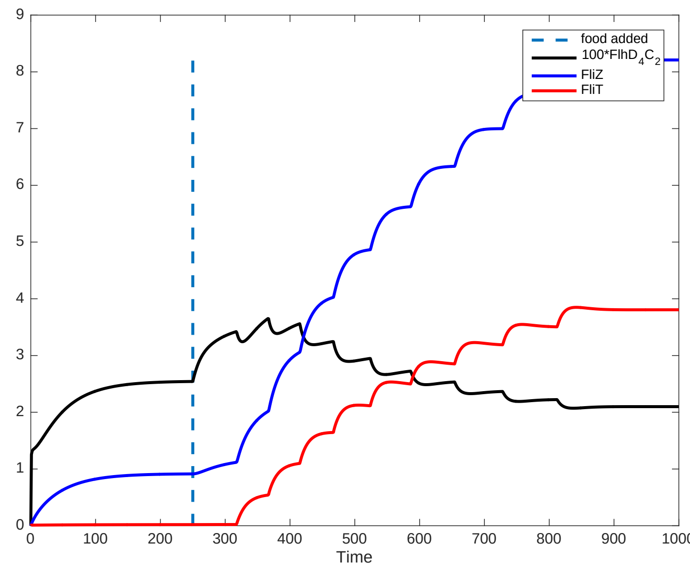
Mathematical Biology
University of Utah

A Mathematical Model

A mathematical model shows

- Stochastic Switch-like behavior to turn on HBB production (bistability)
- Gradual buildup of FliT to turn off HBB production
- Robust number of flagella = 0 or > 1





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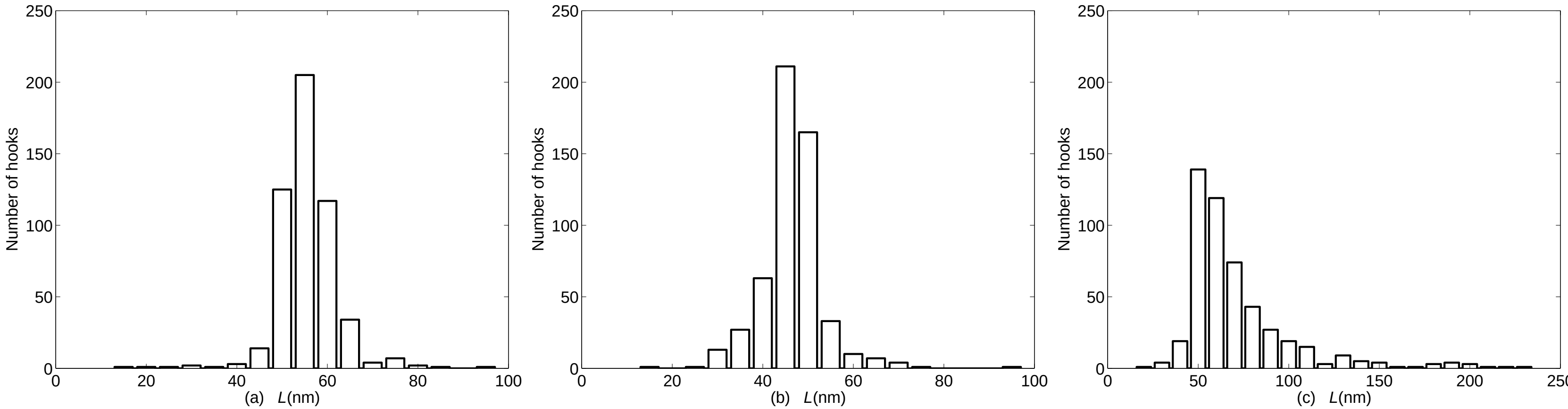
  - Lengthening FliK gives longer hooks.
  - 5-10 molecules of FliK are secreted per hook (115-120 molecules of FlgE).

Hook Length Data



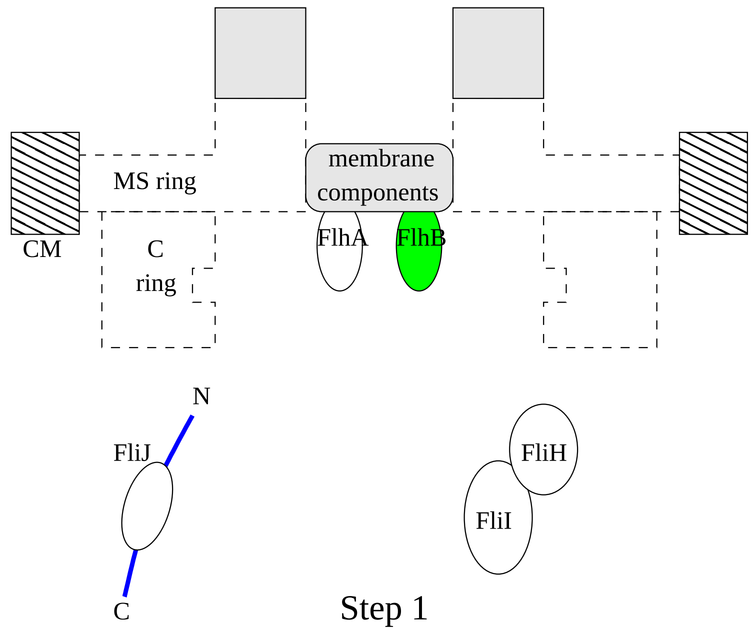
Wild type
(M = 55nm)

Overexpressed
(M = 47nm)

Underexpressed
(M = 76nm)

The Secretion Machinery

- Secreted molecules are chaperoned to prevent folding.



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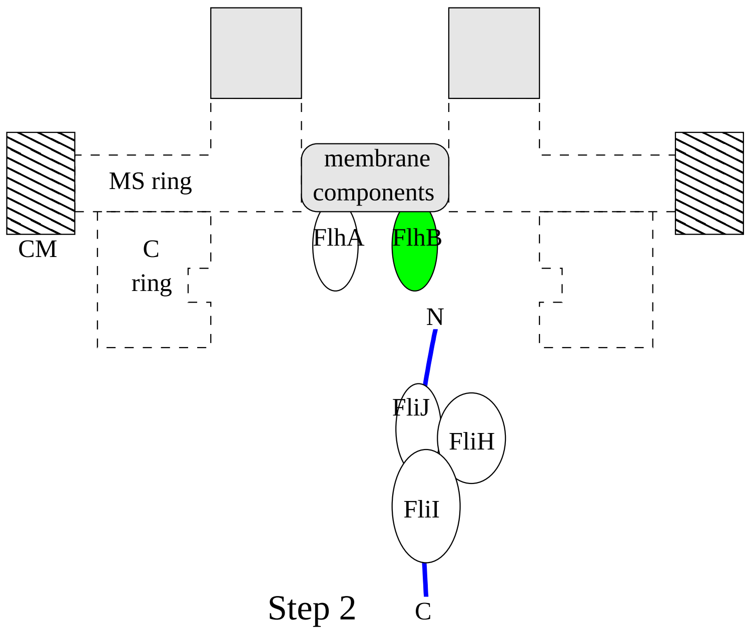
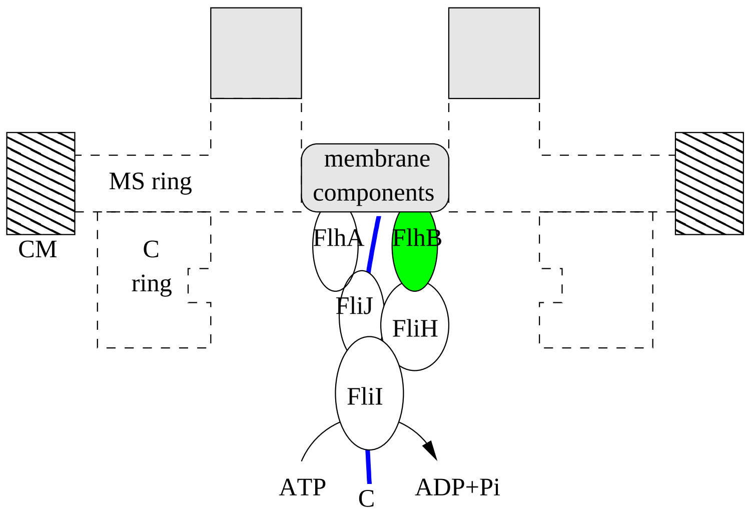
- Secreted molecules are chaperoned to prevent folding.
- FliI is an ATPase



The Secretion Machinery

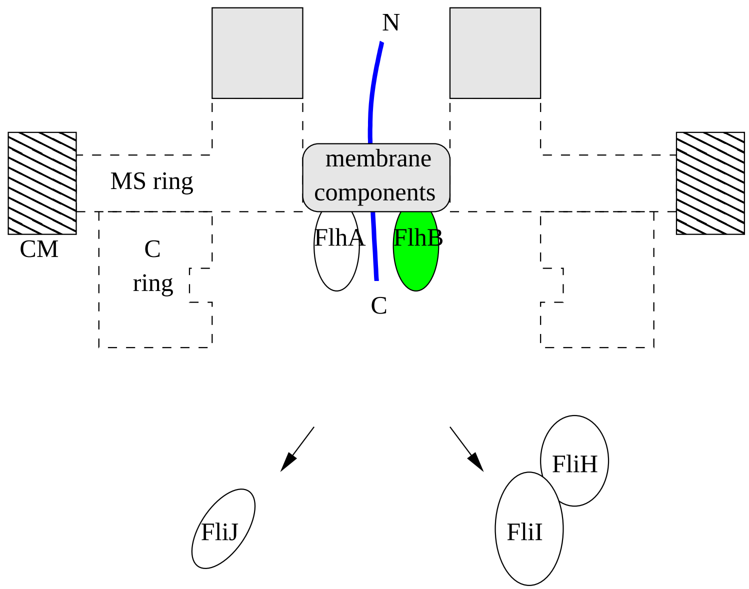
- Secreted molecules are chaperoned to prevent folding.
- FliI is an ATPase
- FlhB is the gatekeeper recognizing the N terminus of secretants.



Step 3

The Secretion Machinery

- Secreted molecules are chaperoned to prevent folding.
- FliI is an ATPase
- FlhB is the gatekeeper recognizing the N terminus of secretants.
- once inside, molecular movement is by diffusion.



Step 4

Secretion Control

Secretion is regulated by FlhB

- During hook formation, only FlgE and FliK can be secreted.
- After hook is complete, FlgE and FliK are no longer secreted, but other molecules can be secreted (those needed for filament growth.)
- The switch occurs when the C-terminus of FlhB is cleaved by FliK.

Question: Why is the switch in FlhB length dependent?

Hypothesis: How Hook Length is determined

- The Infrequent Molecular Ruler Mechanism. FliK is secreted once in a while to test the length of the hook.
- The probability of FlhB cleavage is length dependent.

Binding Probability

Suppose the probability of FlhB cleavage by FliK is a function of length $P_c(L)$. Then, the probability of cleavage on or before time t, $P(t)$, is determined by

$$\frac{dP}{dt} = \alpha r(L) P_c(L)(1 - P),$$

where $r(L)$ is the secretion rate, α is the fraction of secreted molecules that are FliK, and

$$\frac{dL}{dt} = \beta r(L)\Delta,$$

where $\beta = 1 - \alpha$ fraction of secreted FlgE molecules, Δ length increment per FlgE molecule.

Binding Probability

It follows that

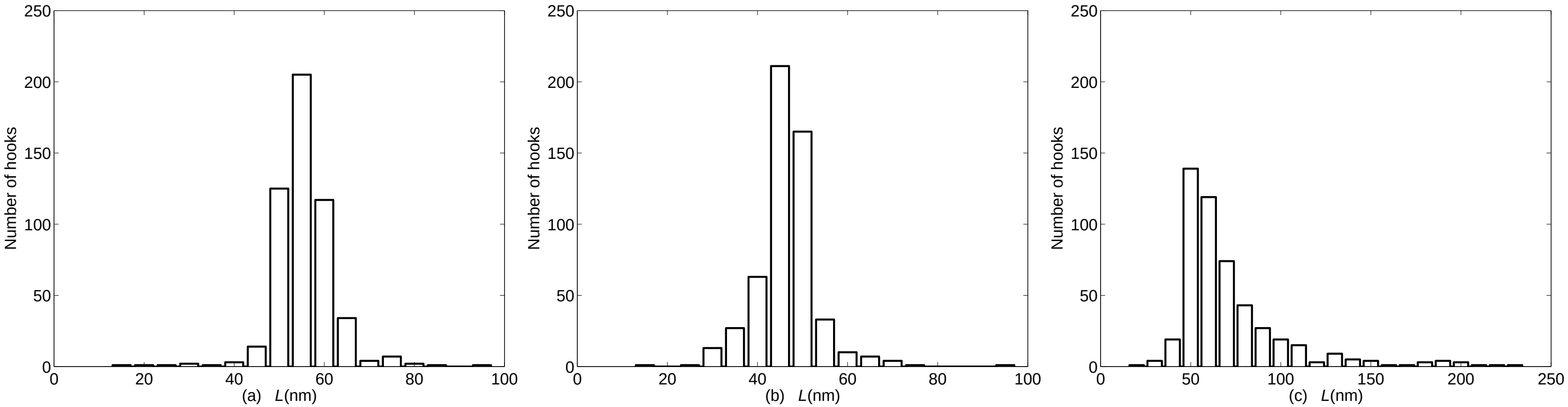
$$\frac{dP}{dL} = \frac{\alpha}{\beta\Delta} P_c(L)(1-P),$$

or

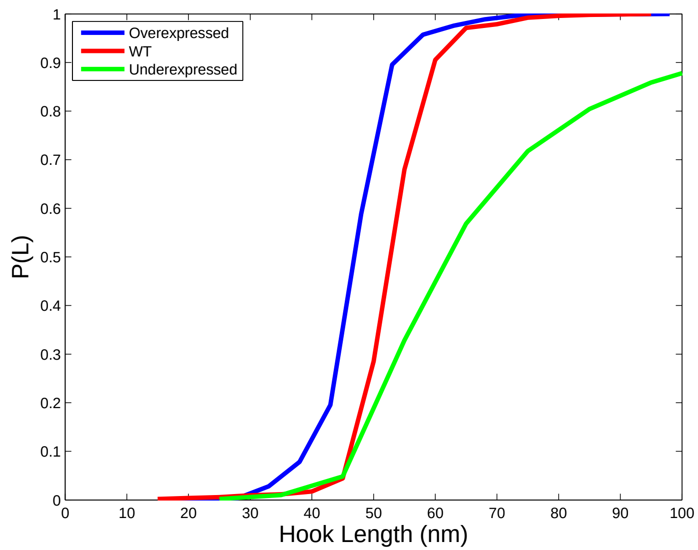
$$-\ln(1-P(L)) = \kappa \int_0^L P_c(L) dL.$$

Observation: The only difference between mutant strains should be in the parameter $\kappa = \frac{\alpha}{\beta\Delta}$.

Check the Data



Wild type Overexpressed Underexpressed



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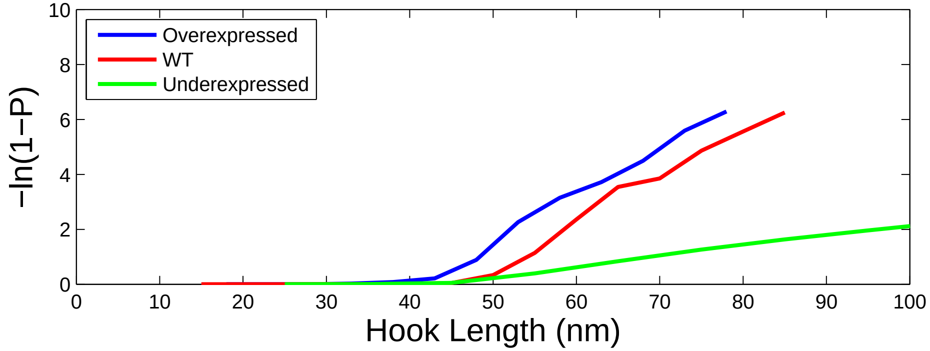
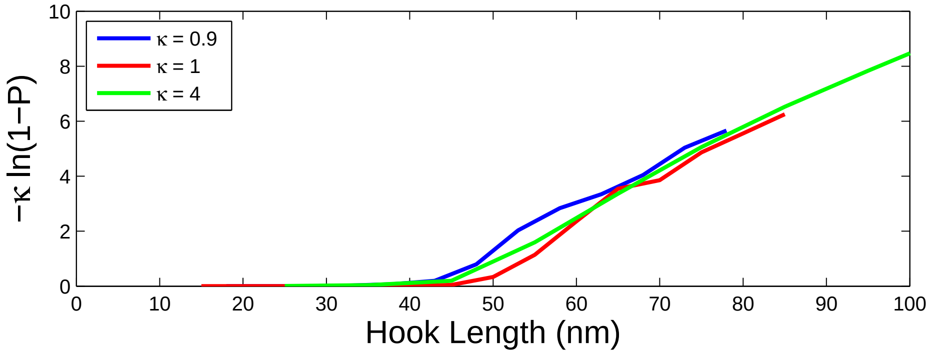


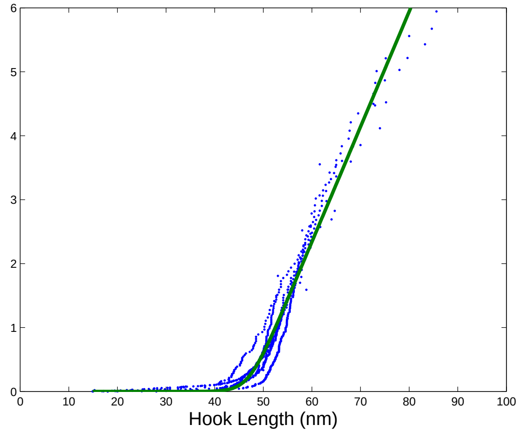


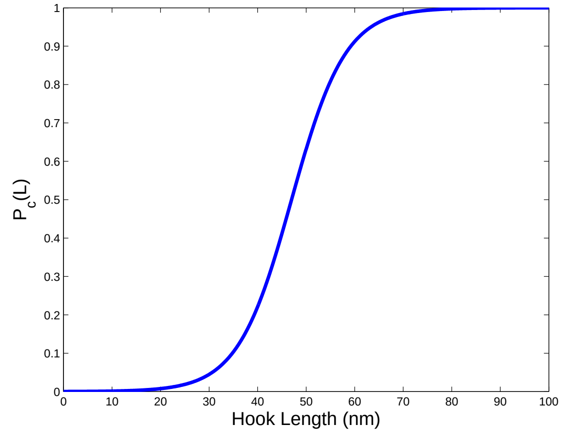
$$-\ln(1 - P(L)) = \kappa \int_0^L P_c(L) dL?$$

Use the Data

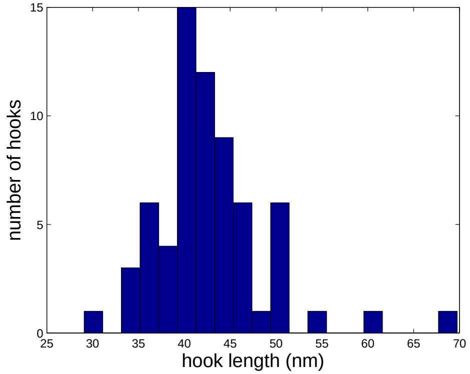
to estimate $P_c(L)$:

$$P_c(L) = \frac{1}{\kappa(1-P)}\frac{dP}{dL}$$

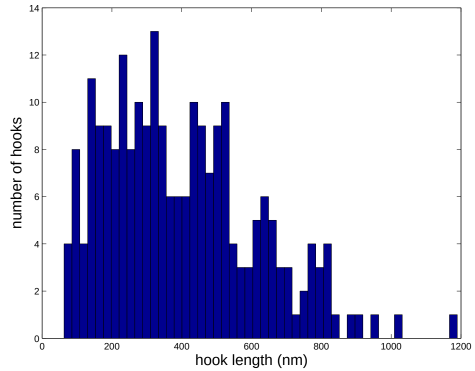




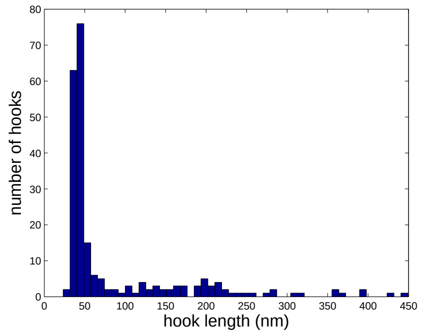
Test #2: 3 Cultures



1: WT



2: No FliK



3: FliK induced at 45 min

Question: Can 3 be predicted from 1 and 2?

Test #2

Suppose that FliK becomes available only after the hook is length L_0. How long will the completed hook be?

$$\frac{dP}{dL} = \beta P_c(L)(1 - P).$$

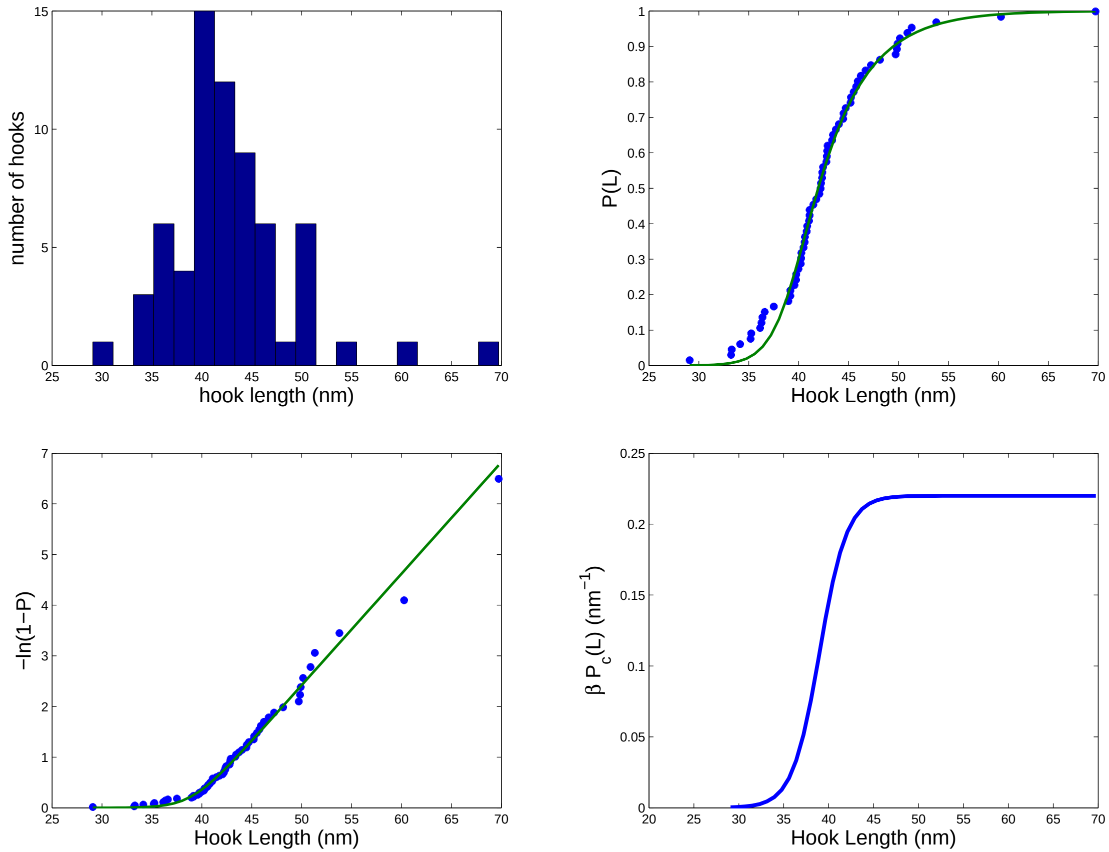
with $P(L|L_0) = 0$ so that

$$P(L|L_0) = 1 - \exp(-\kappa \int_{L_0}^{L} P_c(L) dL).$$

To see how this formula can be used:

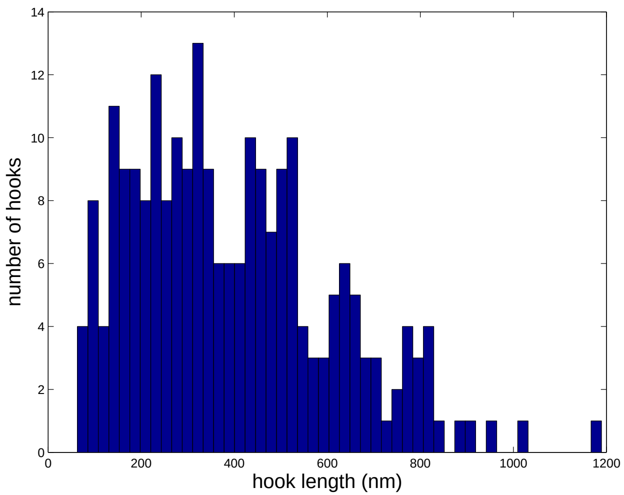
Step 1: Analysis of WT Data

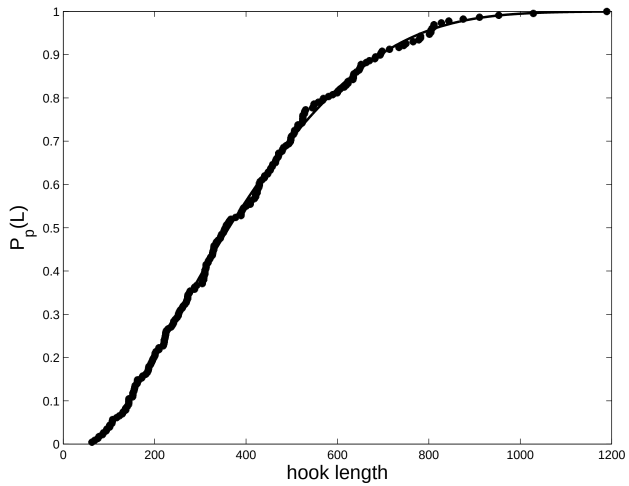
1) Determine $P_c(L)$ from WT data



Step 2: Analysis of Polyhook Data

2) Determine distribution of polyhooks $P_p(L)$ (i.e., hooks grown with no hook length control gives a measure of when hooks started growing - in a growing culture, not all hooks are initiated at the same time.)





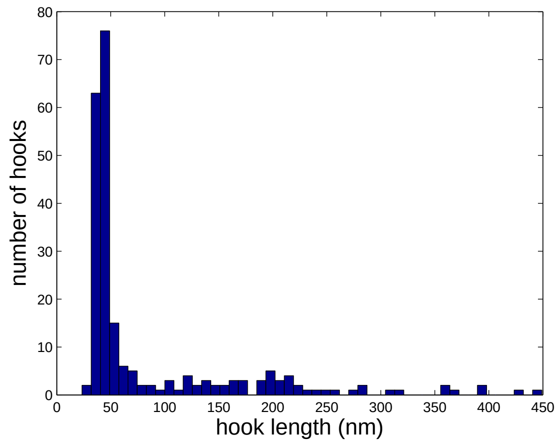
Step 3: Predict Lengths after FliK induction

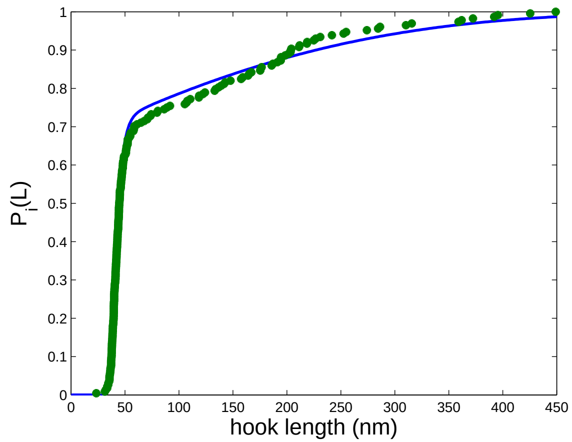
$$P_i(L) = P(L|0)\, P_p(L^*) + \int_0^L P(L|L_0)\, P'_p(L_0 + L^*)\, dL_0.$$

hooks of length $\leq L$, hooks started after FliK induction,

hooks of length L_0 at the time of FliK induction

where $P(L|L_0) = 1 - \exp(-\kappa \int_{L_0}^{L} P_c(L)dL)$.





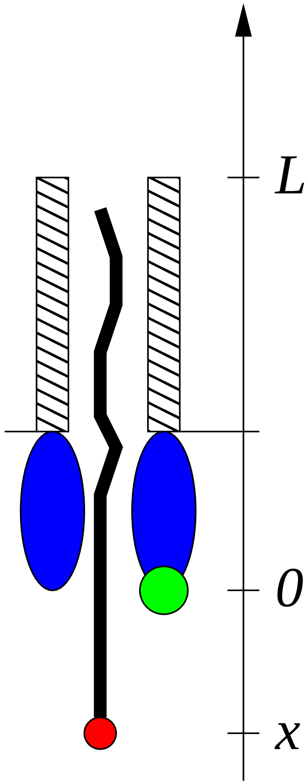
Hypothesis: How Hook Length is determined

- The Infrequent Molecular Ruler Mechanism.
- The probability of FlhB cleavage is length dependent. What is the mechanism that determines $P_c(L)$?

Secretion Model

Hypothesis: FliK binds to FlhB during translocation to cause switching of secretion target by cleaving a recognition sequence.

- FliK molecules move through the growing tube by diffusion.



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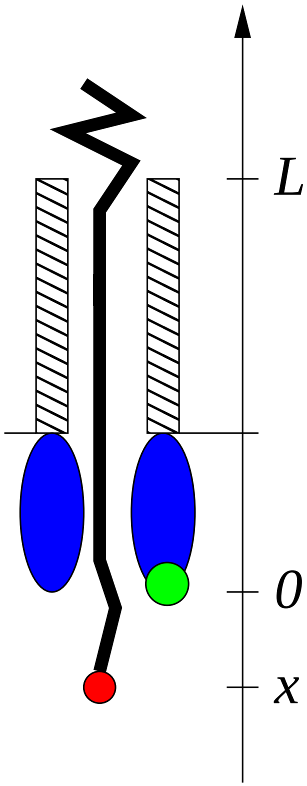
- FliK molecules move through the growing tube by diffusion.
- They remain unfolded before and during secretion, but begin to fold as they exit the tube.



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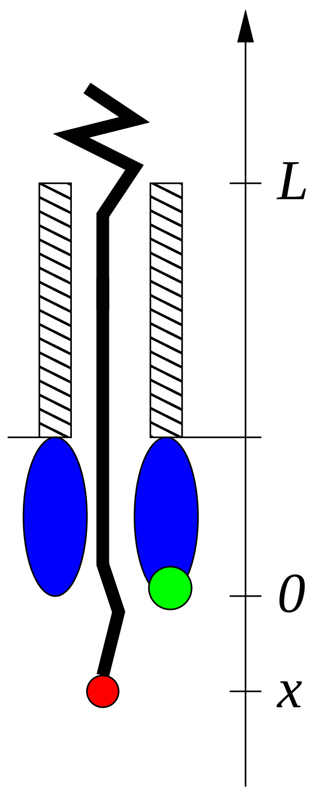
- Folding on exit prevents back diffusion, giving a brownian ratchet effect.



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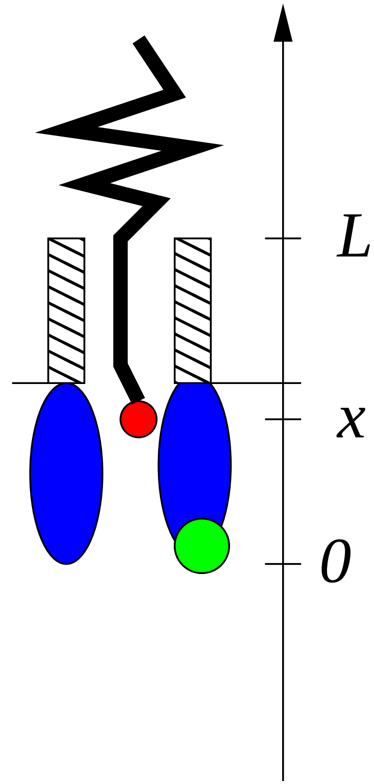
- For short hooks, folding prevents FlhB cleavage.



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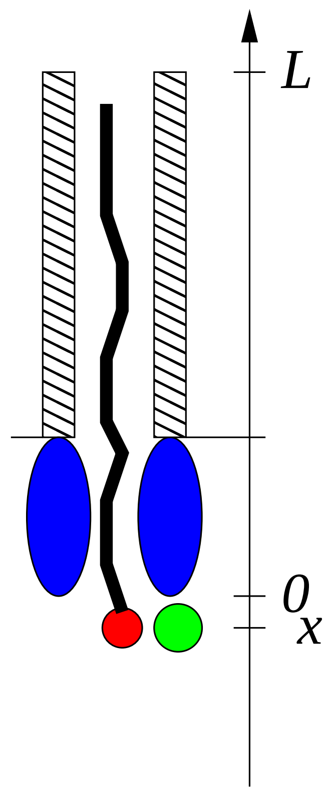
- For short hooks, folding prevents FlhB cleavage.
- For long hooks, movement solely by diffusion allows more time for cleavage.

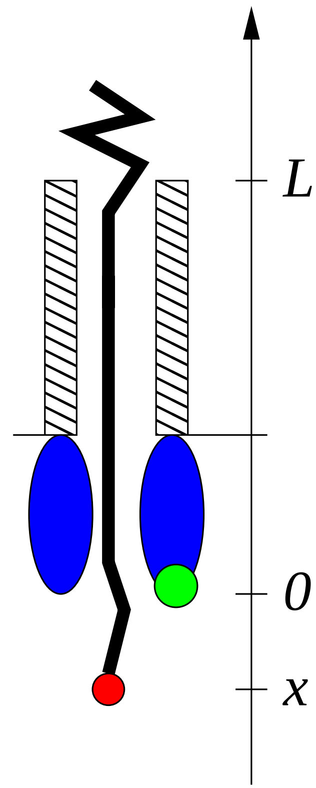


Stochastic Model

Follow the position $x(t)$ of the C-terminus using the stochastic langevin differential equation

$$\nu dx = F(x)dt + \sqrt{2k_bT\nu}dW,$$

where $F(x)$ represents the folding force acting on the unfolded FliK molecule, $W(t)$ is brownian white noise.



Fokker-Planck Equation

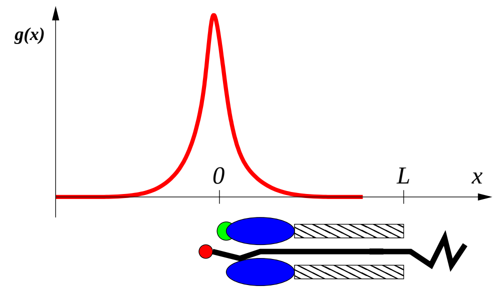
Let $P(x,t)$ be the probability density of being at position x at time t with FlhB uncleaved, and $Q(t)$ be the probability of being cleaved by time t. Then

$$\frac{\partial P}{\partial t} = -\frac{\partial}{\partial x}(F(x)P) + D\frac{\partial^2 P}{\partial x^2} - g(x)P,$$

and

$$\frac{dQ}{dt} = \int_a^b g(x)P(x,t)dx.$$

where $g(x)$ is the rate of FlhB cleavage at position x.

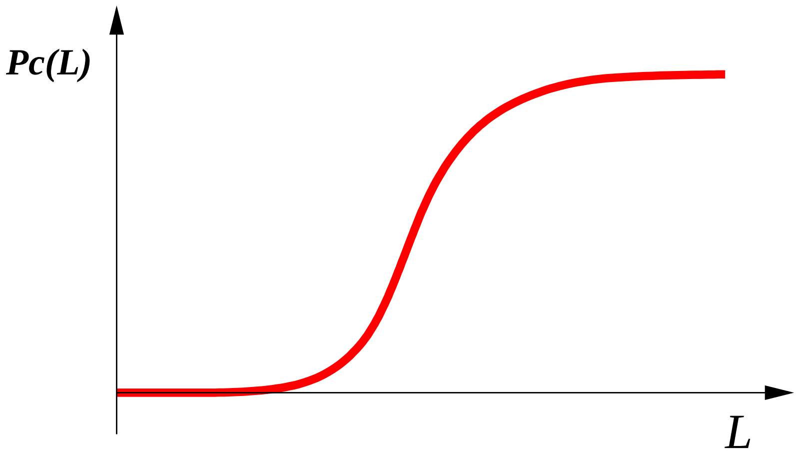


Probability of Cleavage

To determine the probability of cleavage $\pi_c(x)$ starting from position x (the splitting probability), solve

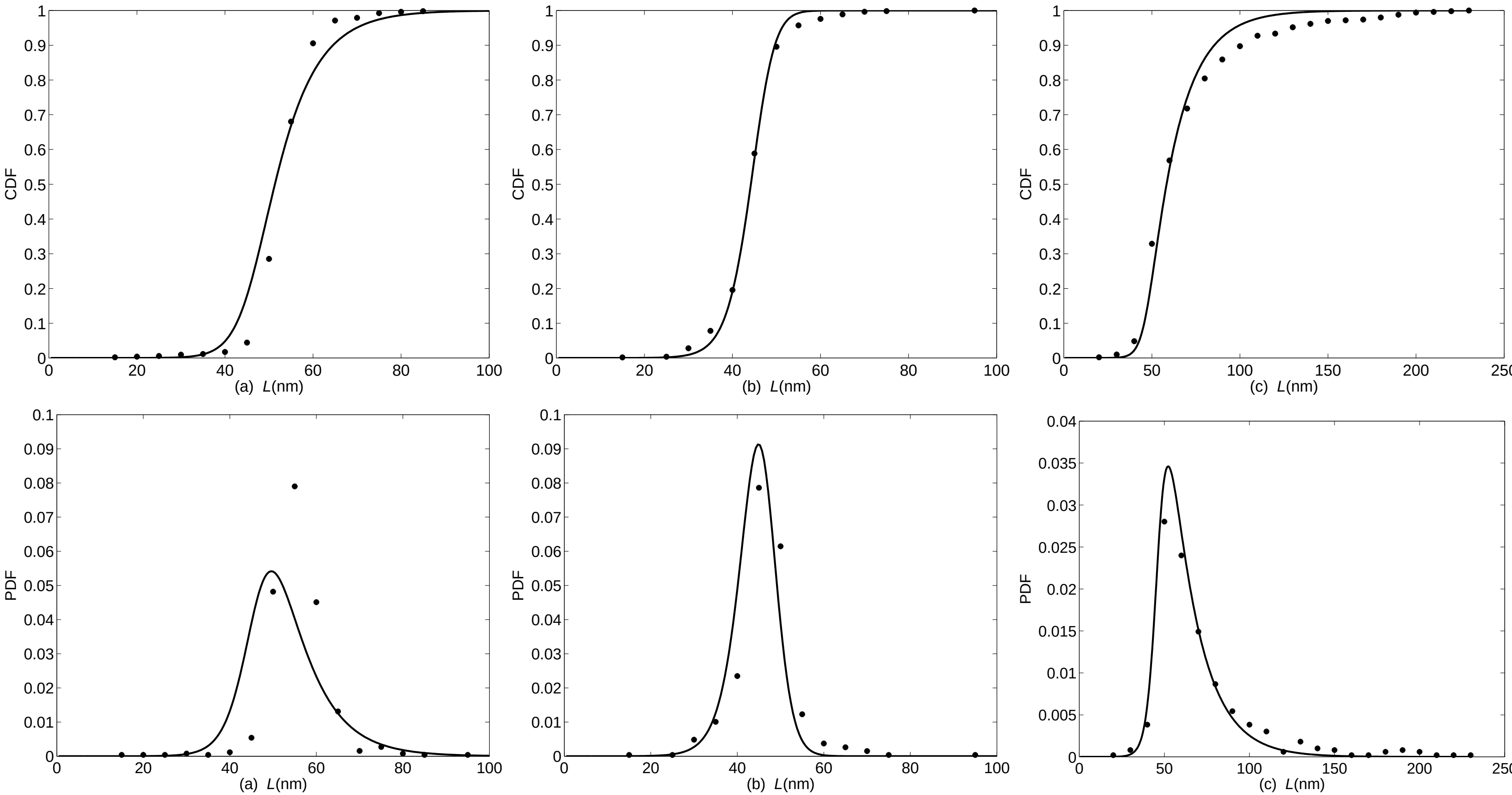
$$D\frac{d^2\pi_c}{dx^2} + F(x)\frac{d\pi_c}{dx} - g(x)\pi_c = -g(x)$$

subject to $\pi_c'(a) = 0$ and $\pi_c(b) = 0$. Then $P_c(L) = \pi_c(a)$.



gives excellent agreement with curve generated from data.

Results



Wild type | Overexpressed | Underexpressed

Overall Summary

What is the fundamental principle uncovered here?

Answer: Cells are able to count and measure using appropriate positive and negative feedback chemical reactions.

- The rate at which molecules are secreted gives information about how many secretors there are. This can be used to count and regulate the number of flagella.
- The rate at which molecules move (i.e., diffusion) contains information about their size. When appropriately coupled with chemical reactions this allows a measurement to be made leading to a decision about size.

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