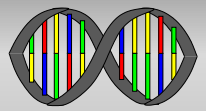
Imagine the Possibilities



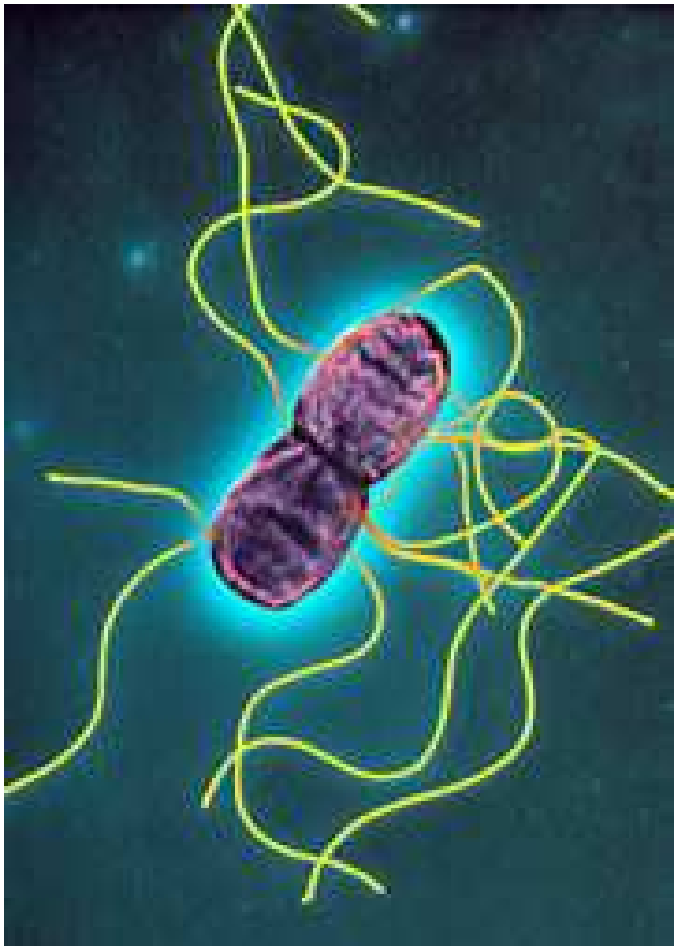
Mathematical Biology
University of Utah

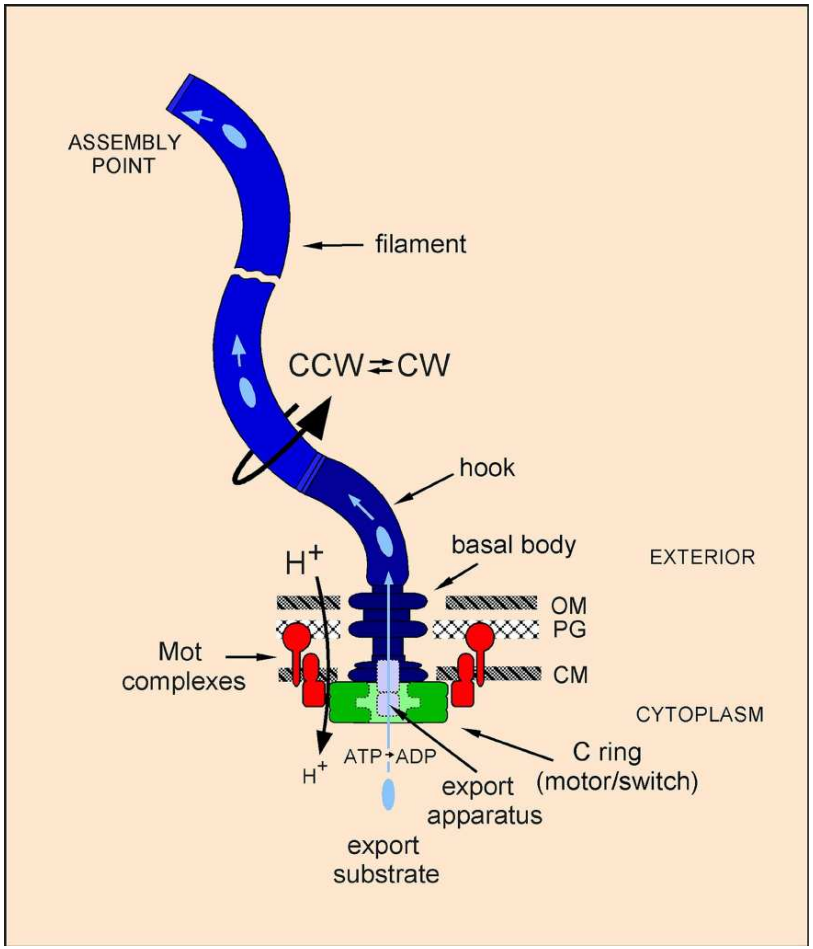
Length Regulation of Flagellar Hooks and Filaments in Salmonella

J. P. Keener

Department of Mathematics
University of Utah

Introduction

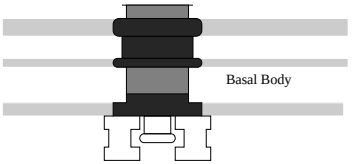




Control of Flagellar Growth

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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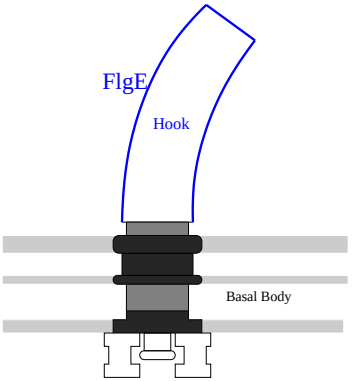

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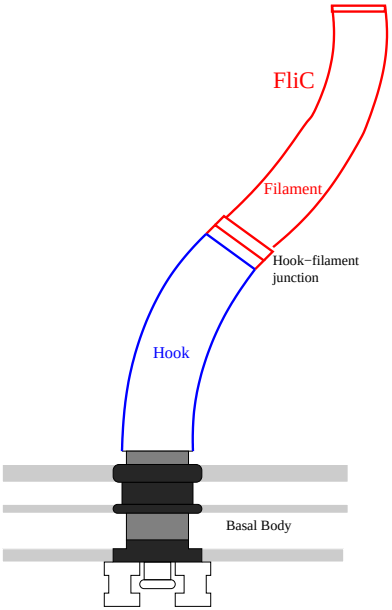

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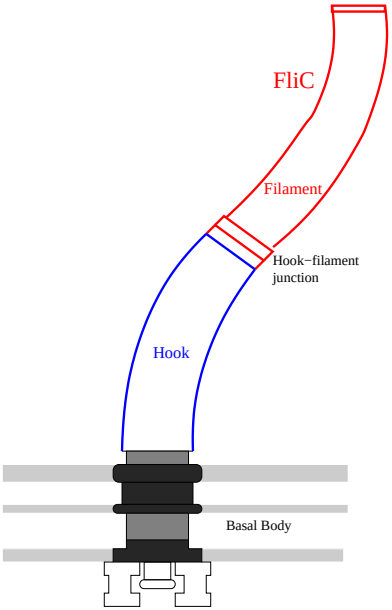
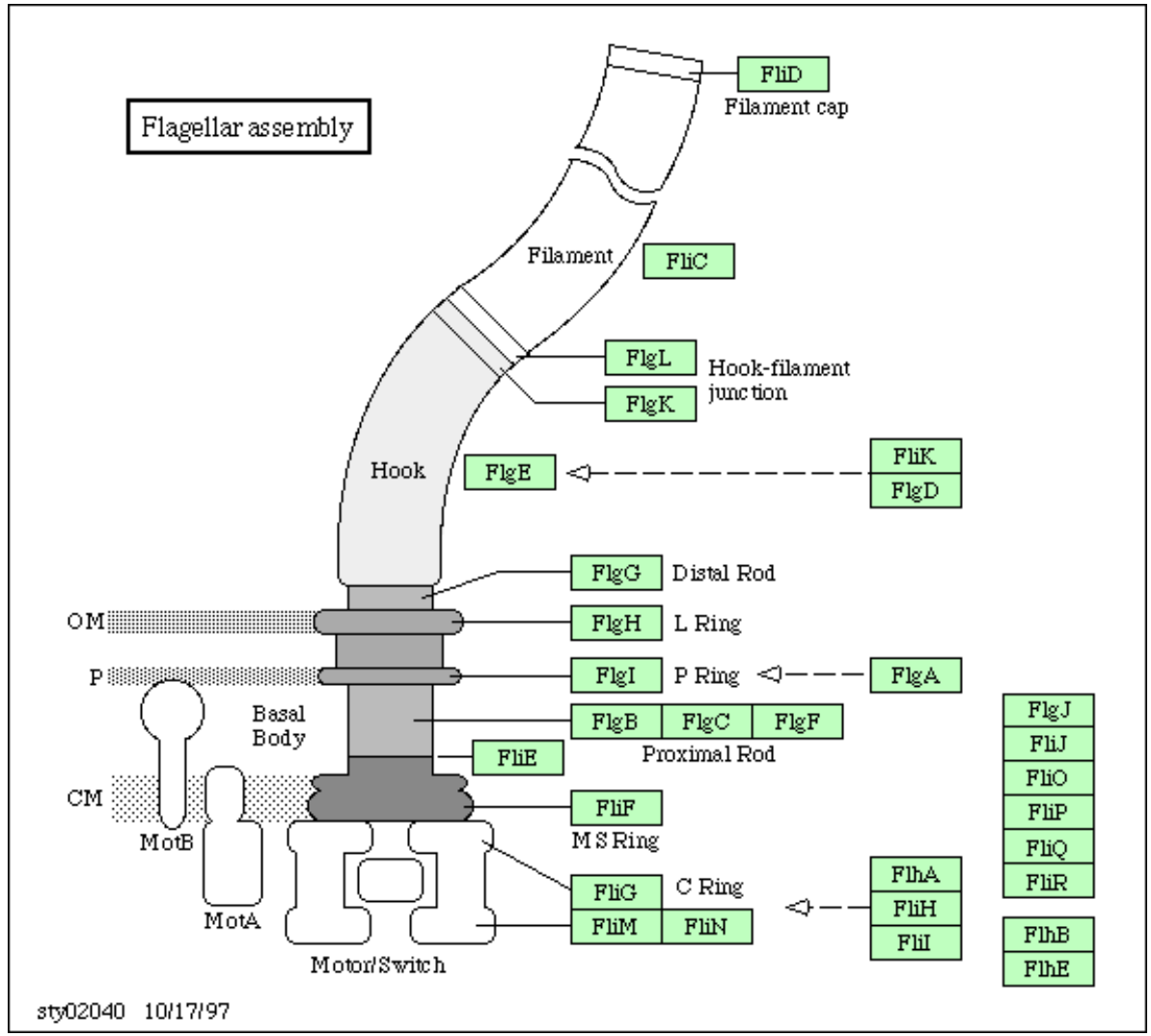


- How are the switches between steps coordinated?
- How is the hook length regulated (55 $\pm$6 nm)?
- How is the length of the filament "measured"?

Proteins of Flagellar Assembly



Hook Length Regulation

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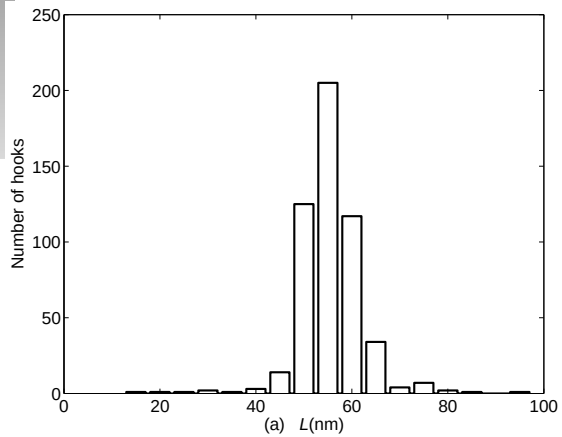
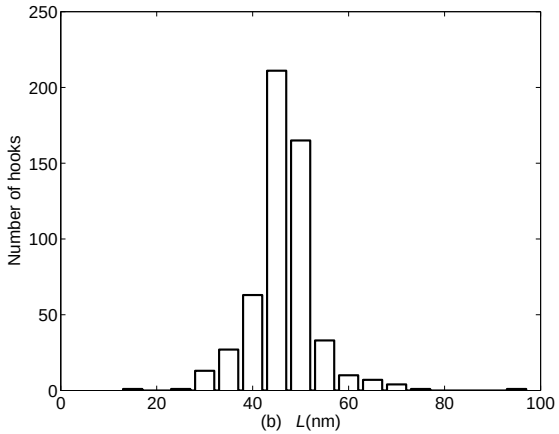
  - FliK is secreted only during hook production.
  - Mutants of FliK produce long hooks; overproduction of FliK gives shorter hooks.
  - Lengthening FliK gives longer hooks.
  - 5-10 molecules of FliK are secreted per hook (115-120 molecules of FlgE).

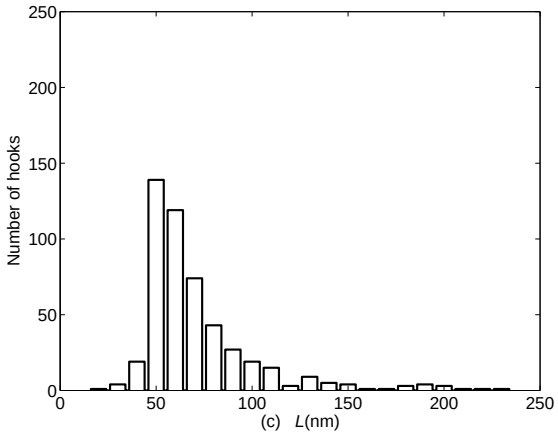
Hook Length Data



Wild type
(M = 55nm)



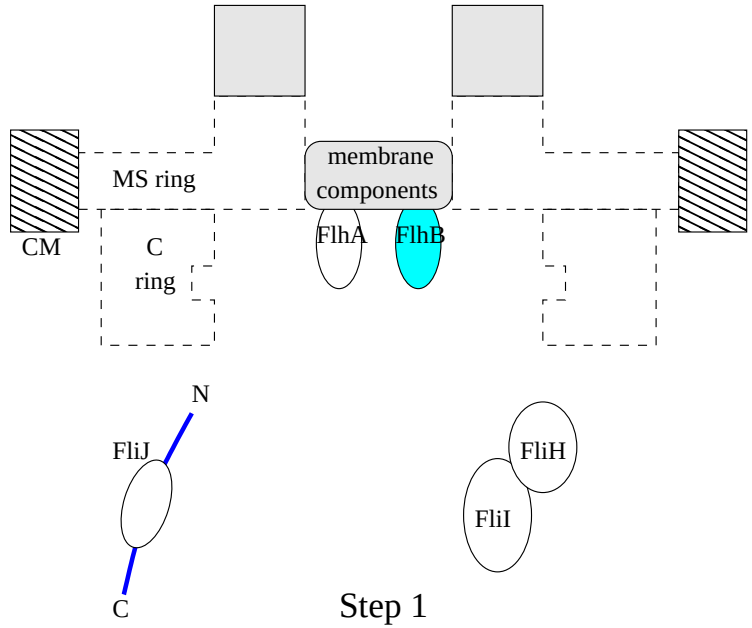
Overexpressed
(M = 47nm)



Underexpressed
(M = 76nm)

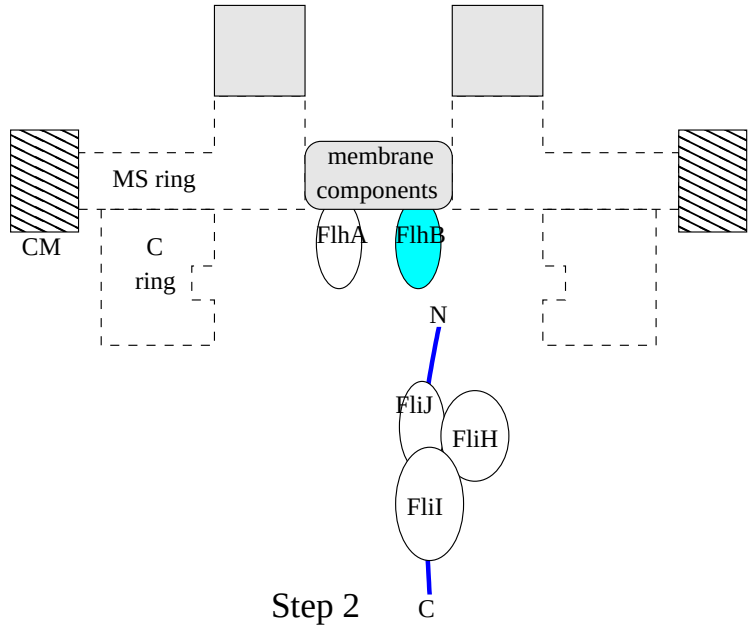
The Secretion Machinery

- Secreted molecules are chaperoned to prevent folding.



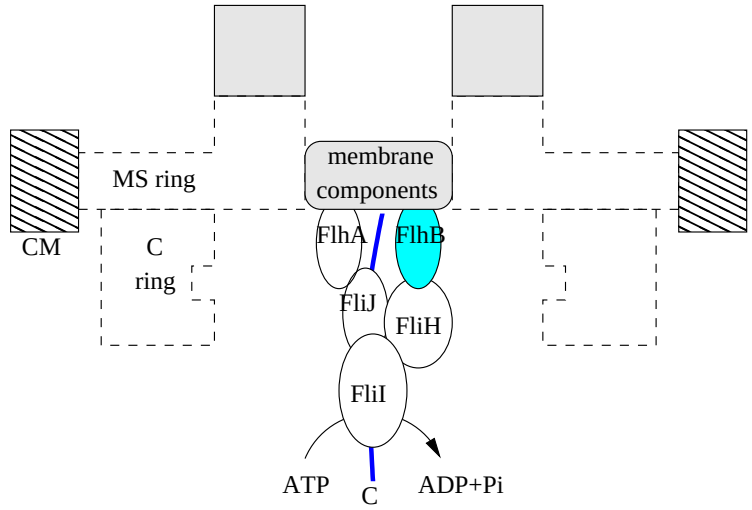
The Secretion Machinery

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



The Secretion Machinery

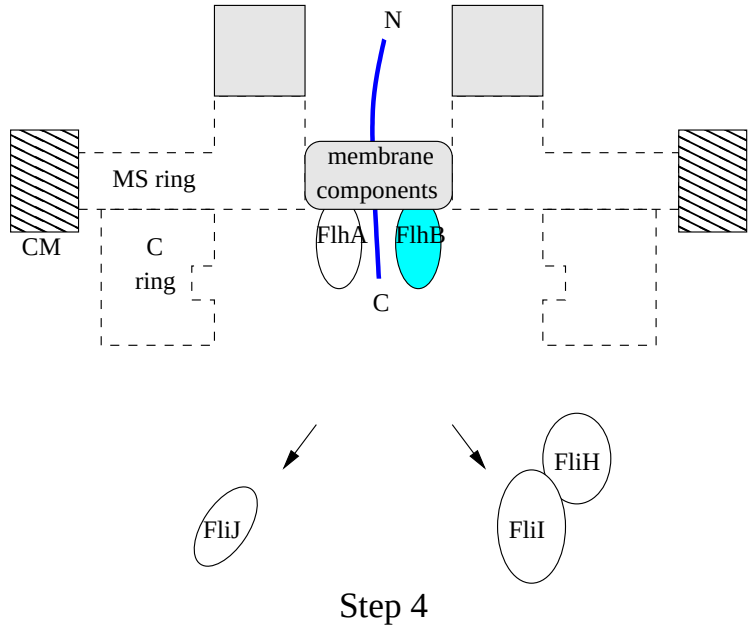
- Secreted molecules are chaperoned to prevent folding.
- FliI is an ATPase
- FlhB is the gatekeeper recognizing the N terminus of secretants.



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.



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 FlK.

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 at 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

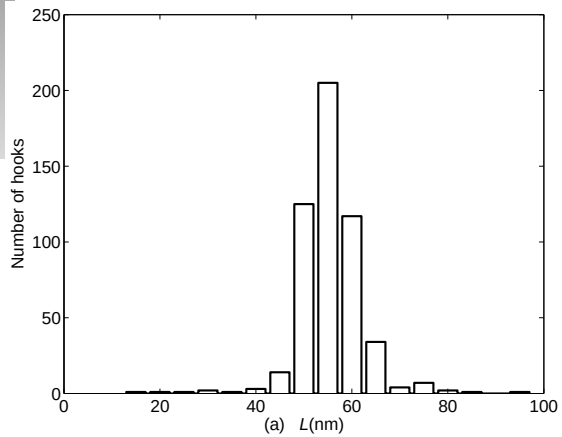
It follows that

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

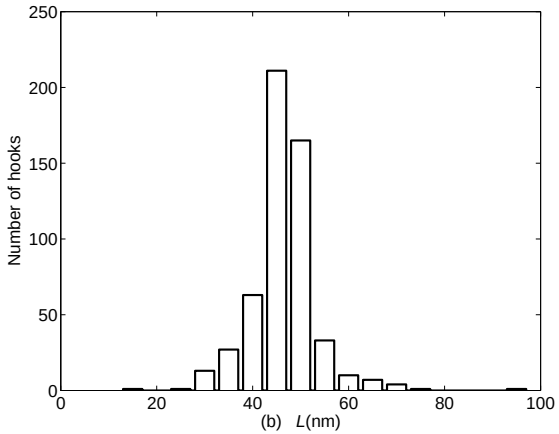
or

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

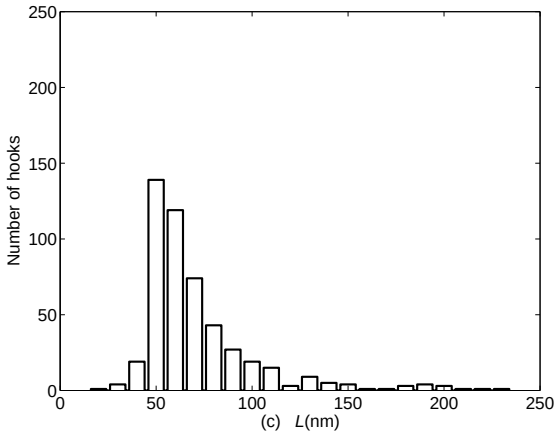
Check the Data



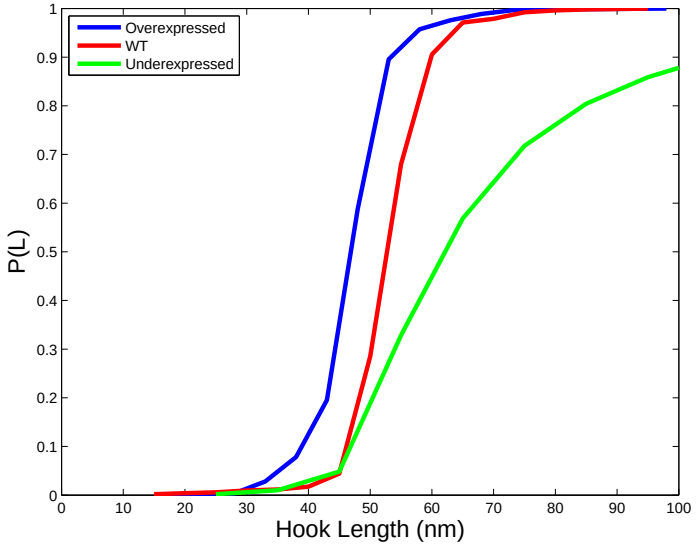
Wild type



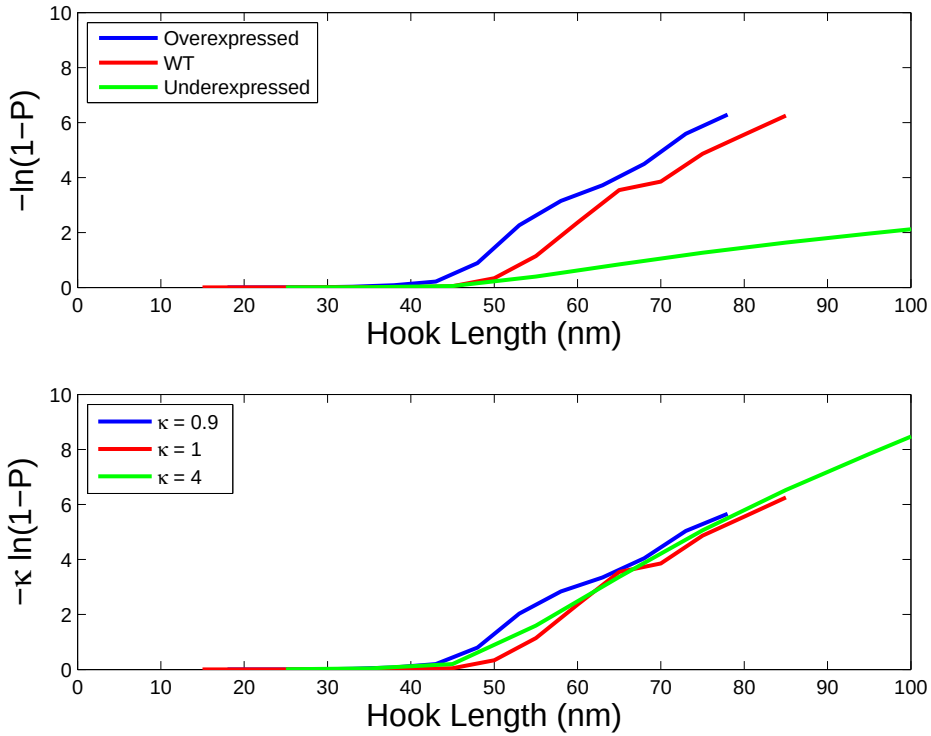
Overexpressed



Underexpressed



Check the Data



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

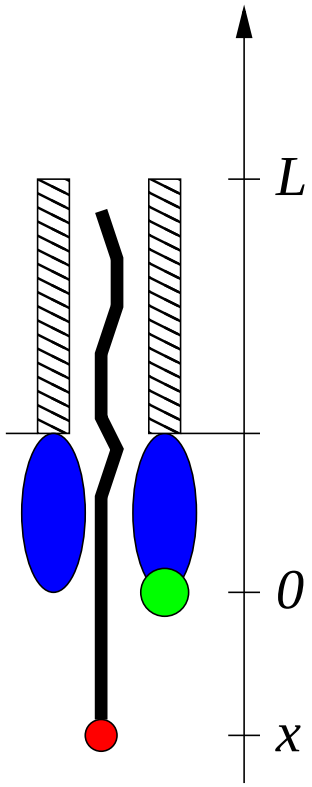
Hypothesis: How Hook Length is determined

- 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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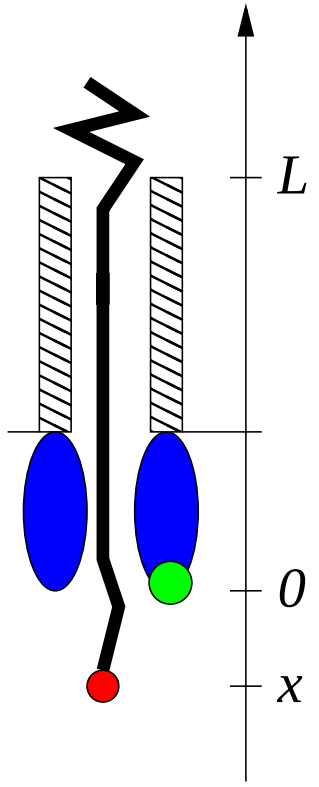

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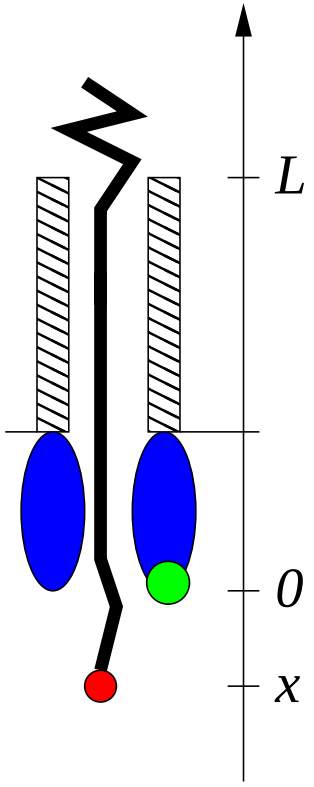

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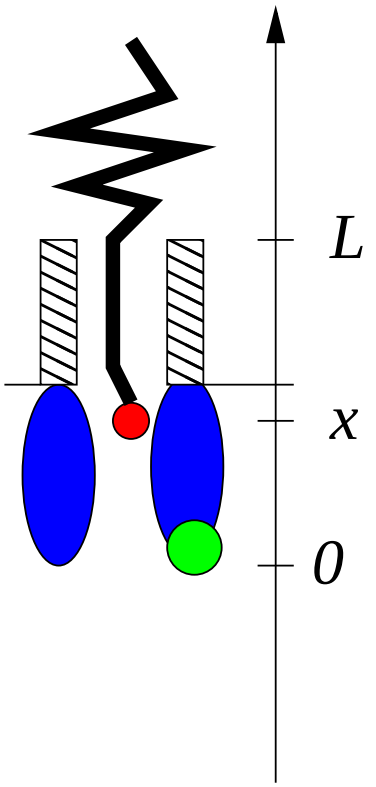

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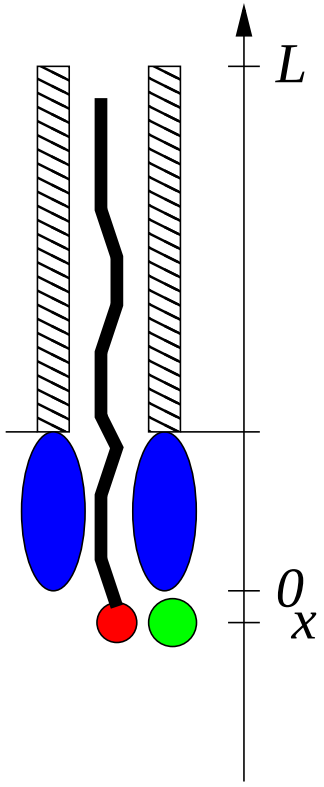
- Folding on exit prevents back diffusion, giving a brownian ratchet effect.
- For short hooks, folding prevents FlhB cleavage.
- For long hooks, movement solely by diffusion allows more time for cleavage.

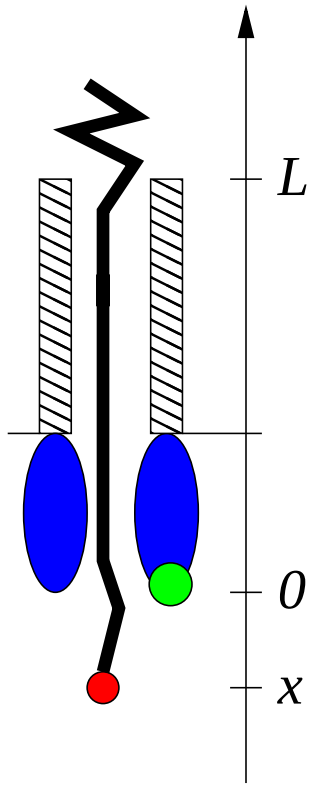


Stochastic Model

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

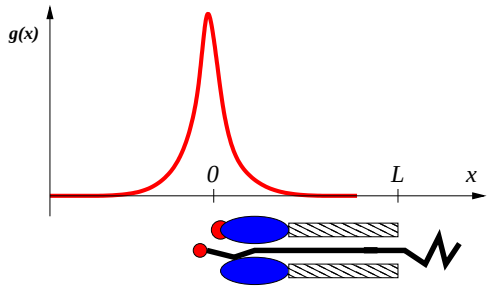
Let $P(x,t)$ be the probability density of being at position x at time t with FlhB uncleaved, and $Q(t)$ be the probability of being cleaved by time t. Then

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

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.

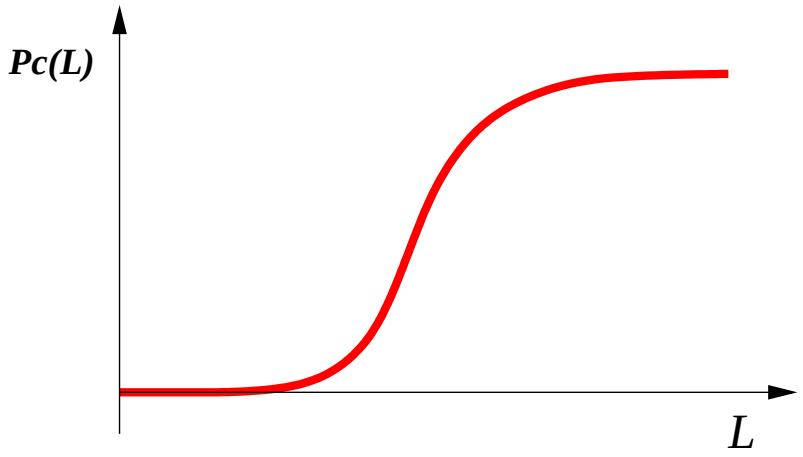


Probability of Cleavage

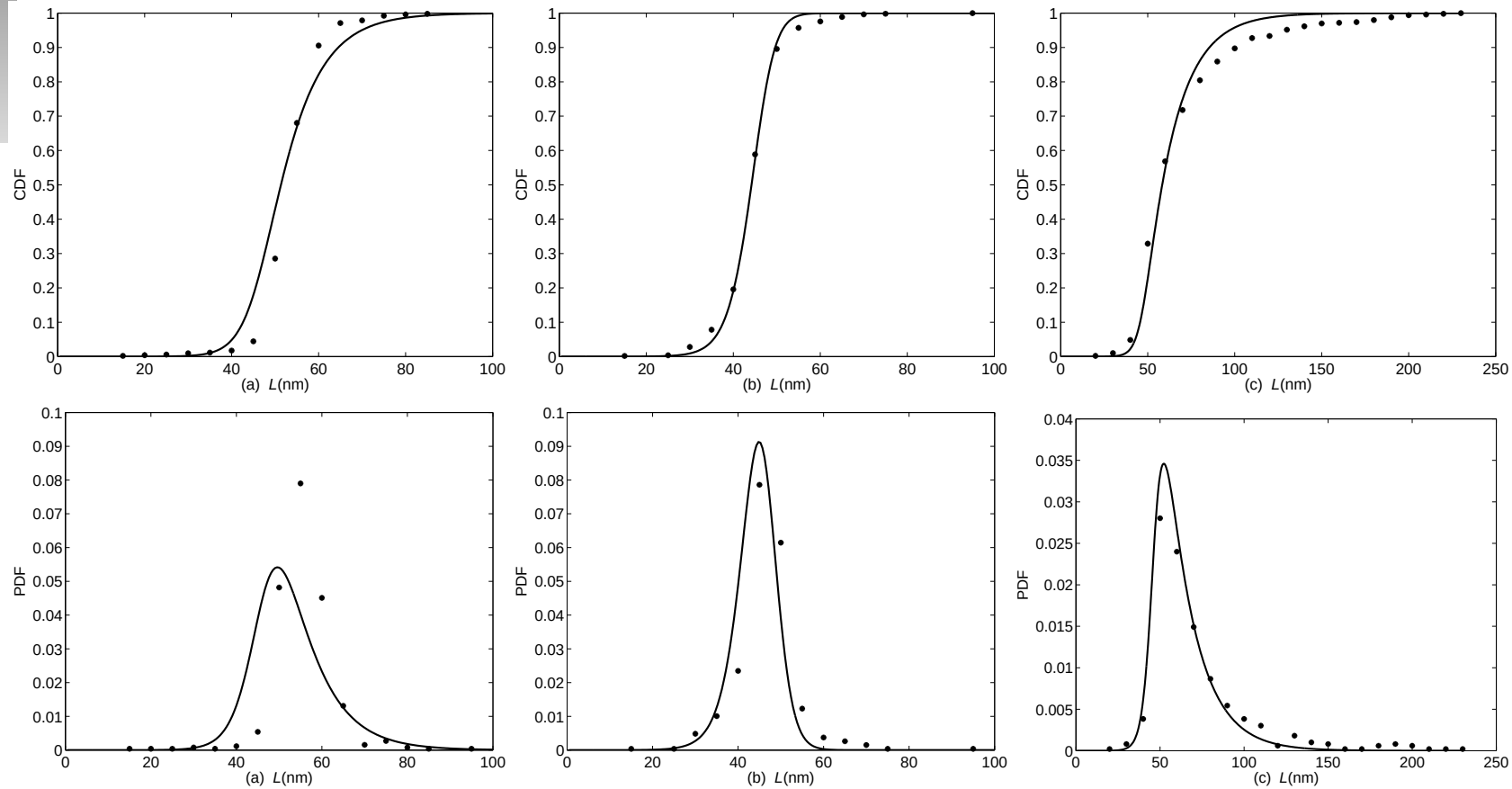
To determine the probability of cleavage $\pi_c(x)$ starting from position x, solve

$$D\frac{d^2\pi_c}{dx^2} + F(x)\frac{d\pi_c}{dx} - g(x)\pi_c = 0$$

subject to $\pi_b'(a) = 0$ and $\pi_b(b) = 1$.
Then $P_c(L) = \pi_c(a)$.



Results



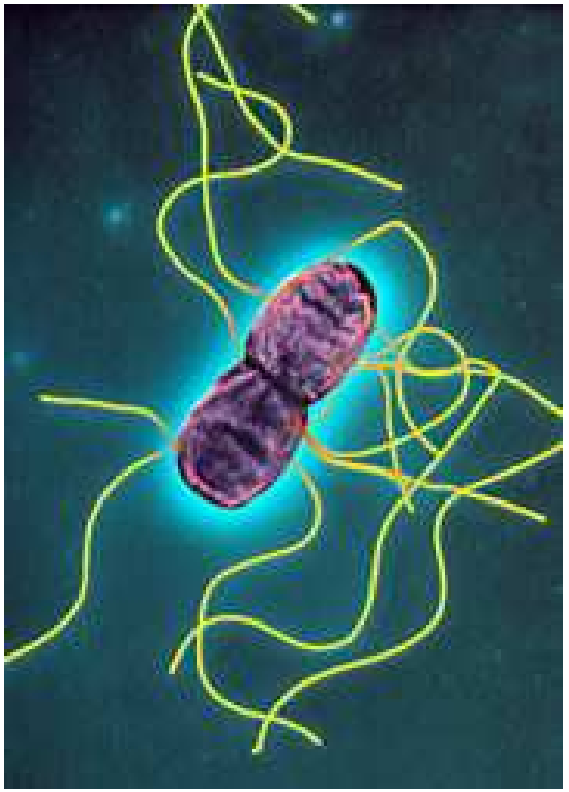
Wild type Overexpressed Underexpressed

Difficulties

- There is no direct experimental evidence either for or against this proposed length measurement mechanism.

II - Flagellar Length Detection

- Flagella grow at a velocity that decreases as they get longer.



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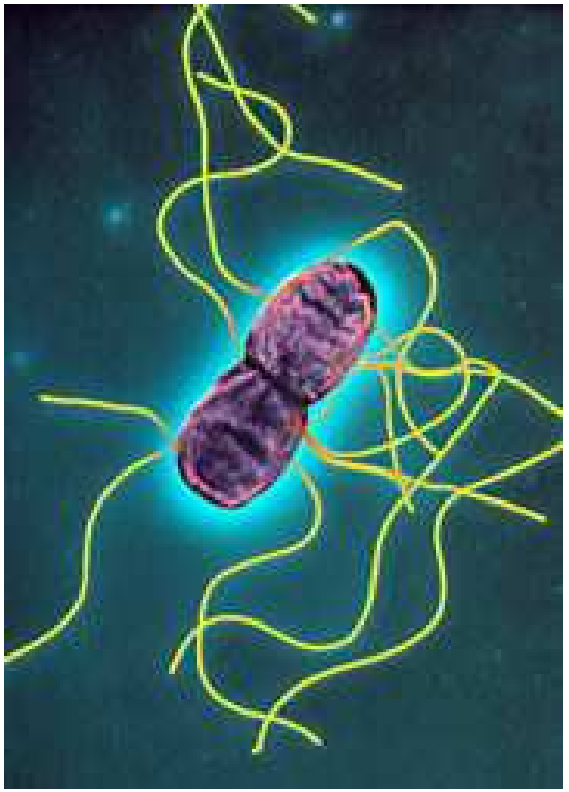
- If a flagellum is broken off, it will regrow at the same velocity as when it first grew.



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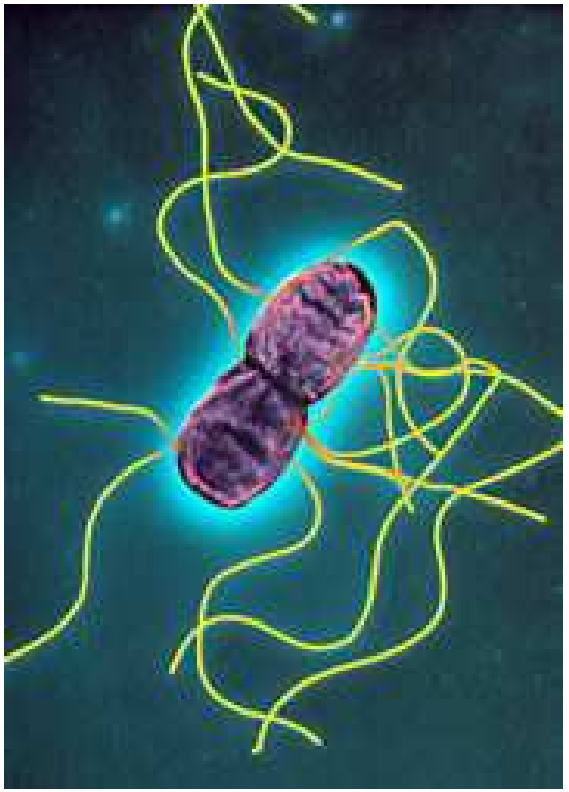
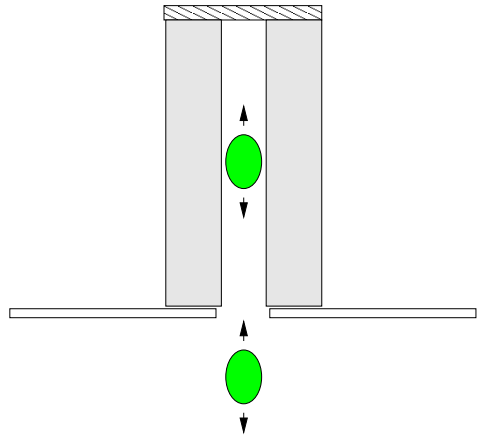


Question: How does the bacterium measure flagellar length?

How Do Flagella Grow?

- Step 1: Secretion
- Step 2: Diffusion
- Step 3: Polymerization



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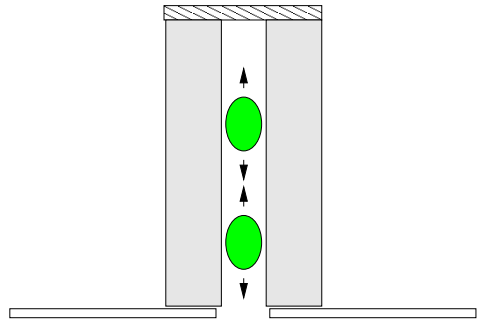

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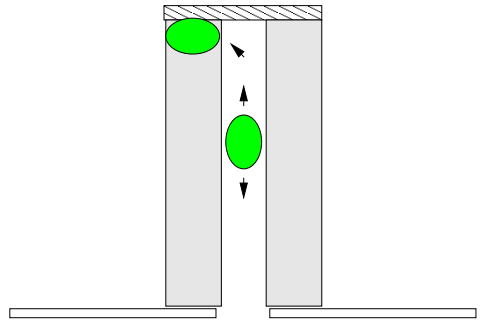


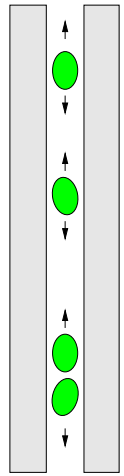
Modelling Flagellar Growth

Step 2: Diffusion

Important Fact: Filament is a narrow hollow tube, so movement (diffusion) is single file.

Let $p(x,t)$ be the probability that a molecule is at position x at time t. Then,

$$\frac{\partial p}{\partial t} + \frac{\partial J}{\partial x} = 0$$



where

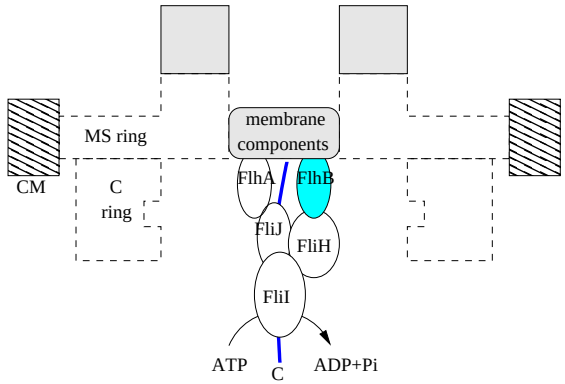
$$J = -D\frac{\partial p}{\partial x}.$$

Remark: $\frac{J}{l}$ = flux in molecules per unit time.

Rate of Secretion

Step 1: Secretion

Let $P(t)$ be the probability that ATP-ase is bound



Step 3

Rate of Secretion

Step 1: Secretion

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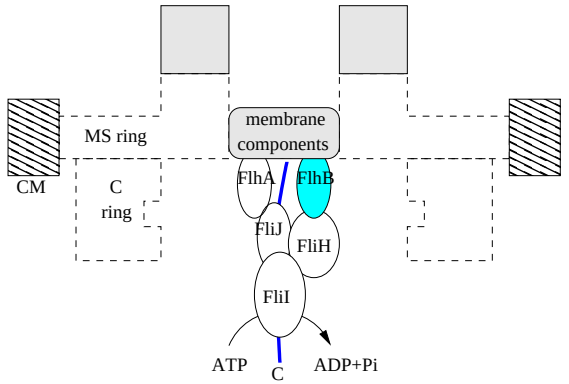

Step 3

$$\frac{dP}{dt} =$$

Rate of Secretion

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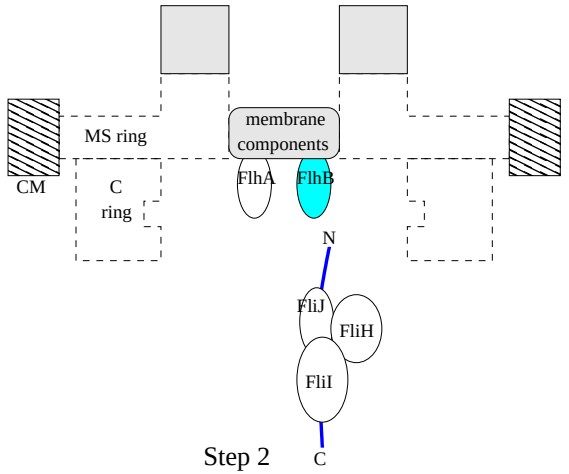

$$\frac{dP}{dt} = K_{on}(1-P)$$

on rate,

Rate of Secretion

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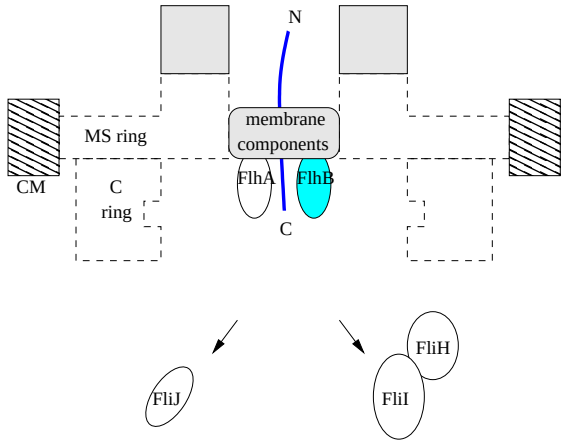


Step 4

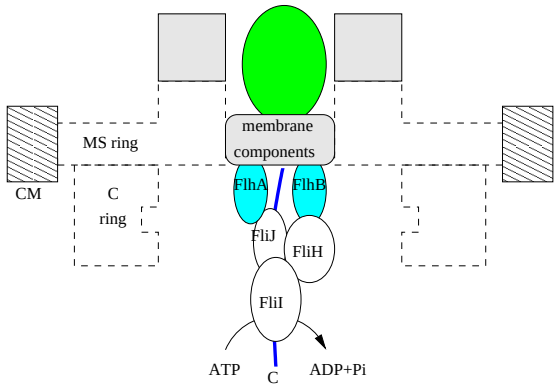
$$\frac{dP}{dt} = K_{on}(1-P) - \boxed{k_{off}P}$$

on rate, off rate,

Rate of Secretion

Step 1: Secretion

Let $P(t)$ be the probability that ATP-ase is bound



Step 4 Blocked

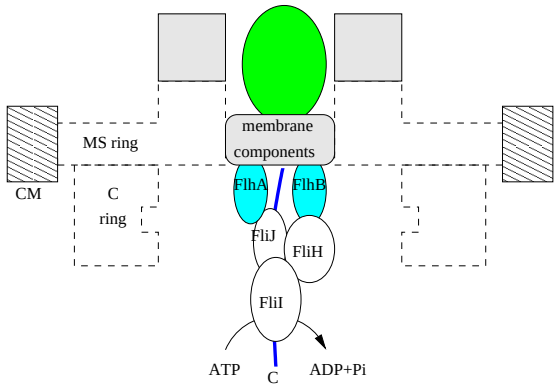
$$\frac{dP}{dt} = K_{on}(1-P) - k_{off}(1-p(0,t))P$$

on rate, off rate, restricted if blocked by another molecule in the tube.

Rate of Secretion

Step 1: Secretion

Let $P(t)$ be the probability that ATP-ase is bound



Step 4 Blocked

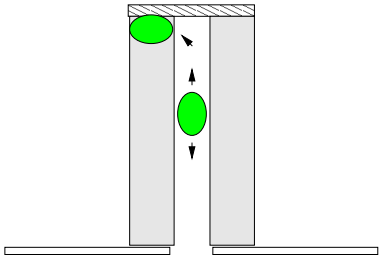
$$\frac{dP}{dt} = K_{on}(1-P) - \boxed{k_{off}(1-p(0,t))P}$$

on rate, off rate, restricted if blocked by another molecule in the tube. Thus,

$$\frac{J}{l} = \boxed{k_{off}(1-p(0,t))P} \text{ at } x = 0 \text{ (A Robin boundary condition).}$$

Rate of Polymerization

Stage 3: Polymerization



$$\frac{J}{l} = k_p p$$

at the polymerizing end $x = L$.

Then, the growth velocity is

$$\frac{dL}{dt} = \beta \frac{J}{l} \equiv V$$

where $\beta =$length of filament per monomer (0.5nm/monomer)

$\cdots$ a moving boundary problem.

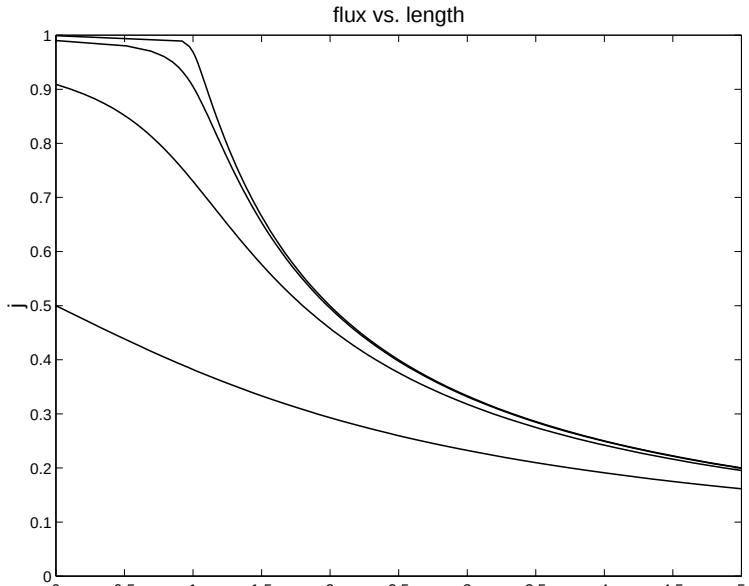
Diffusion Model

After some work, it can be shown that

$$\lambda = \frac{1}{j} - \frac{K_a}{1-j} - K_b$$

where $j = \frac{J}{lK_{on}}$, $\lambda = \frac{lLK_{on}}{D}$, $K_a = \frac{K_{on}}{k_{off}}$, $K_b = \frac{K_{on}}{k_p}$.

A good approximation $J \approx \frac{1}{K_J + \frac{L}{D}} \approx \frac{D}{L}$ for large L



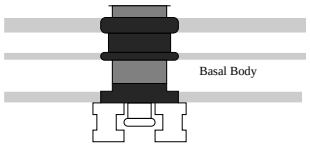
Filament Length Control

Introducing FlgM and σ^{28}:

Filament Length Control

Introducing FlgM and σ^{28}:

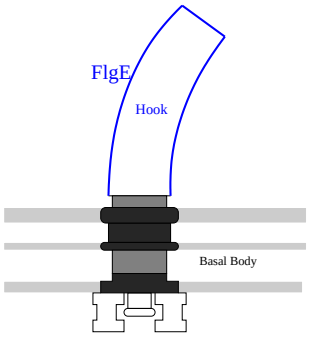
Class 1



Filament Length Control

Introducing FlgM and σ^{28}:

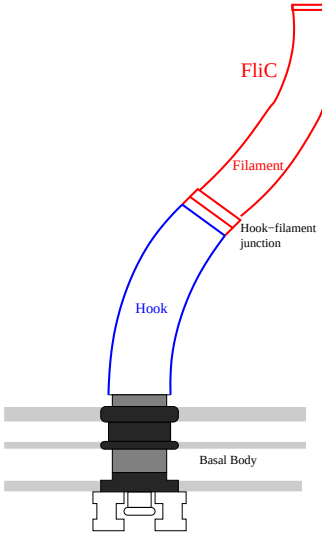
$$\text{Class 1} \rightarrow \text{Class 2} \left\{ \begin{array}{c} \sigma^{28} \\ \text{FlgE} \\ \text{FlgKL} \\ \text{FlgM} \\ \text{FliK} \end{array} \right\}$$



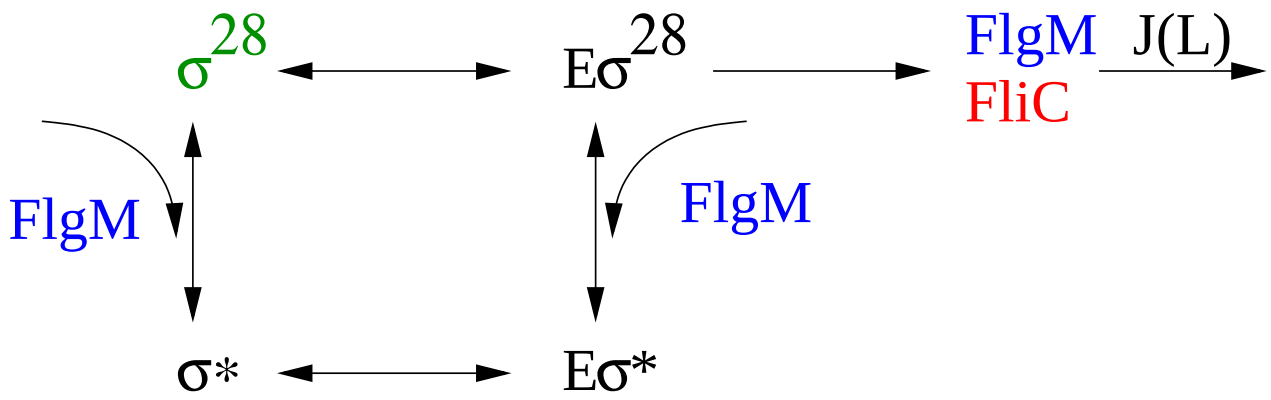
Filament Length Control

Introducing FlgM and σ^{28}:

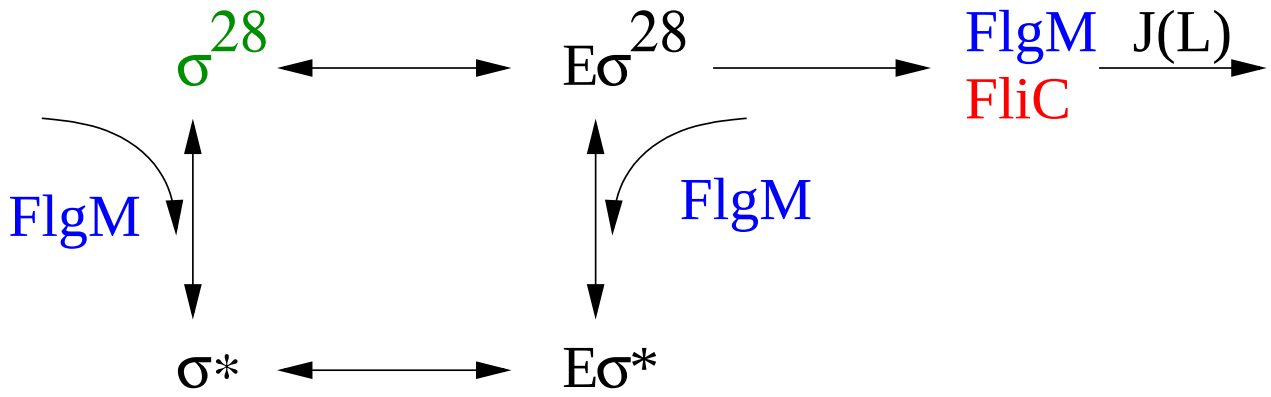
$$\text{Class 1} \rightarrow \text{Class 2} \left\{ \begin{array}{c} \sigma^{28} \\ \text{FlgE} \\ \text{FlgKL} \\ \text{FlgM} \\ \text{FliK} \end{array} \right\} \overset{E\sigma^{28}}{\rightarrow} \text{Class 3} \left\{ \begin{array}{c} \text{FliC} \\ \text{FliD} \\ \text{FlgM} \end{array} \right\}$$



FlgM-σ^{28} Chemistry

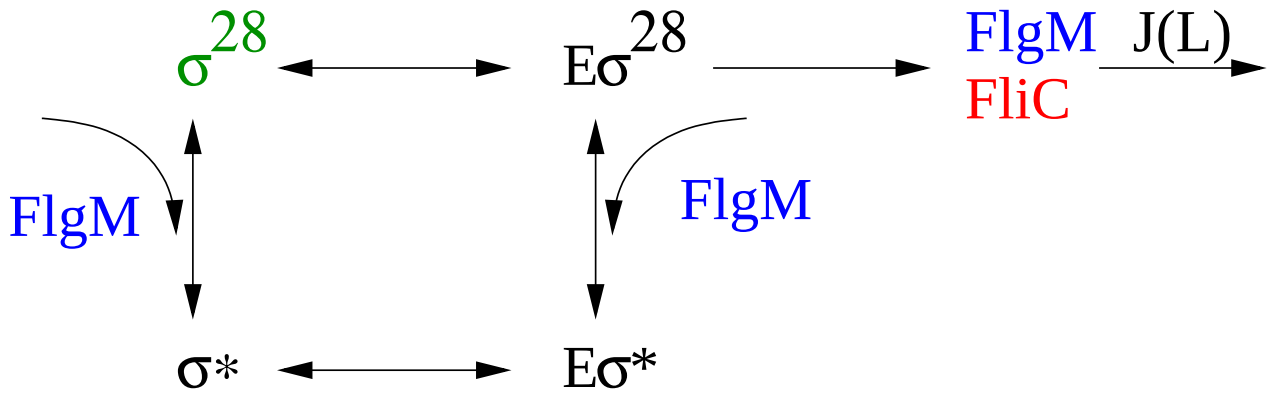


FlgM-σ^{28} Chemistry



- FlgM inhibits σ^{28} activity;

FlgM-σ^{28} Chemistry



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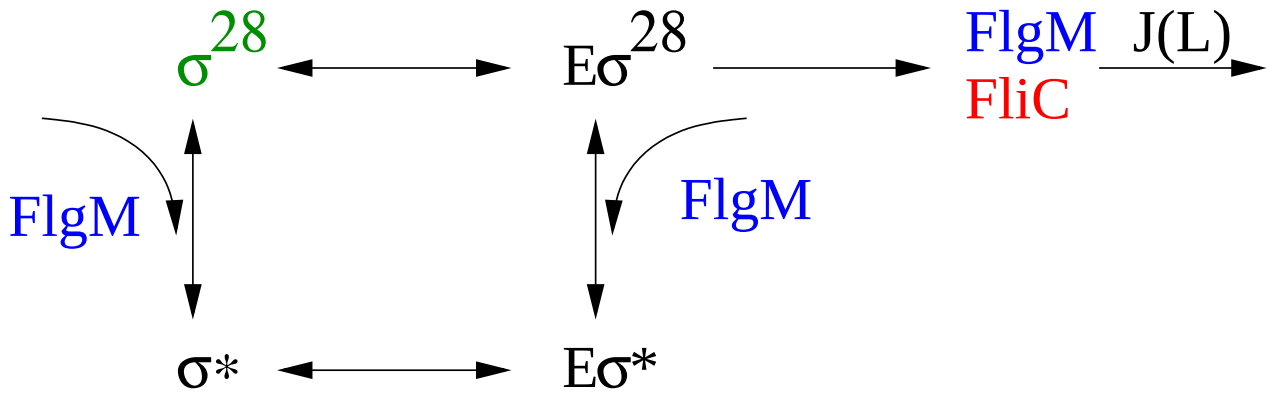
- Therefore, during stage 3, FlgM inhibits its own production (negative feedback);

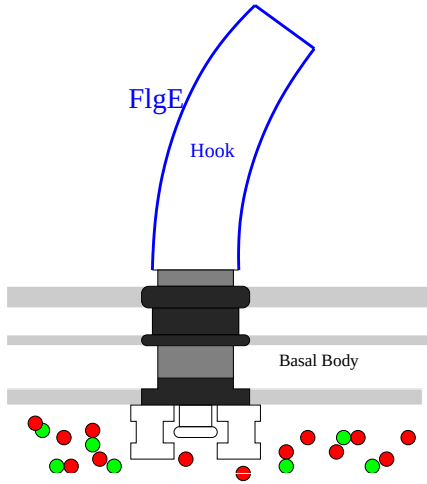
FlgM-σ^{28} Chemistry



- FlgM inhibits σ^{28} activity;
- Therefore, during stage 3, FlgM inhibits its own production (negative feedback);
- And, FlgM inhibits the production of Flagellin (FliC).

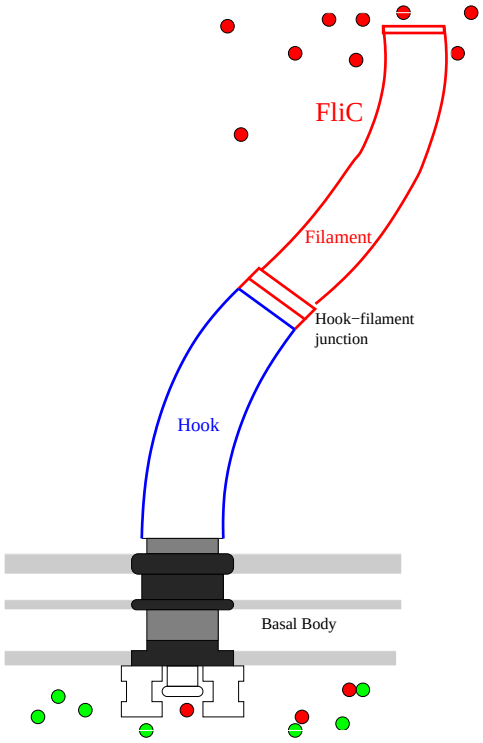
FlgM-σ^{28} Secretion Dynamics

- FlgM **is not** secreted during hook growth.



FlgM-σ^{28} Secretion Dynamics

- FlgM **is not** secreted during hook growth.
- FlgM **is** secreted during filament growth.



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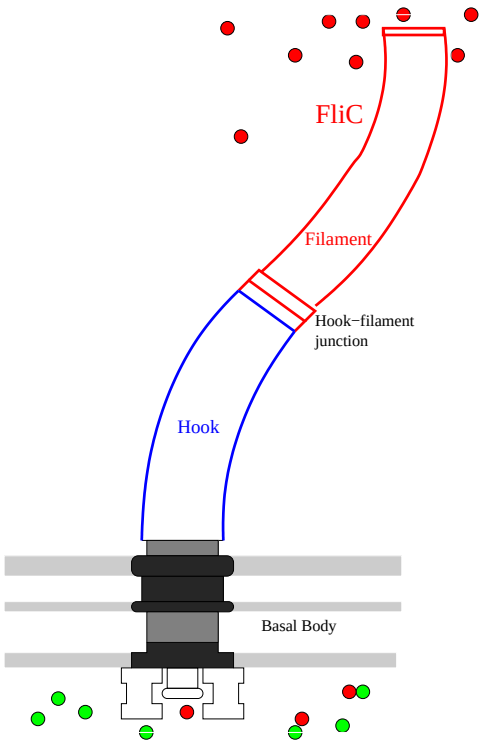


So, how fast is FlgM secreted, and why does it matter?

Tracking Concentrations

FlgM (M):

$$\frac{dM}{dt} = \text{rate of production} - \text{rate of secretion}$$

Flagellin (FliC) (F):

$$\frac{dF}{dt} = \text{rate of production} - \text{rate of secretion}$$

Filament Length (L):

$$\frac{dL}{dt} = \beta * \text{rate of FliC secretion}$$

Tracking Concentrations

FlgM (M):

$$\frac{dM}{dt} = \frac{K_*}{K_M + M} - \alpha \frac{M}{F + M} J$$

Flagellin (FliC) (F):

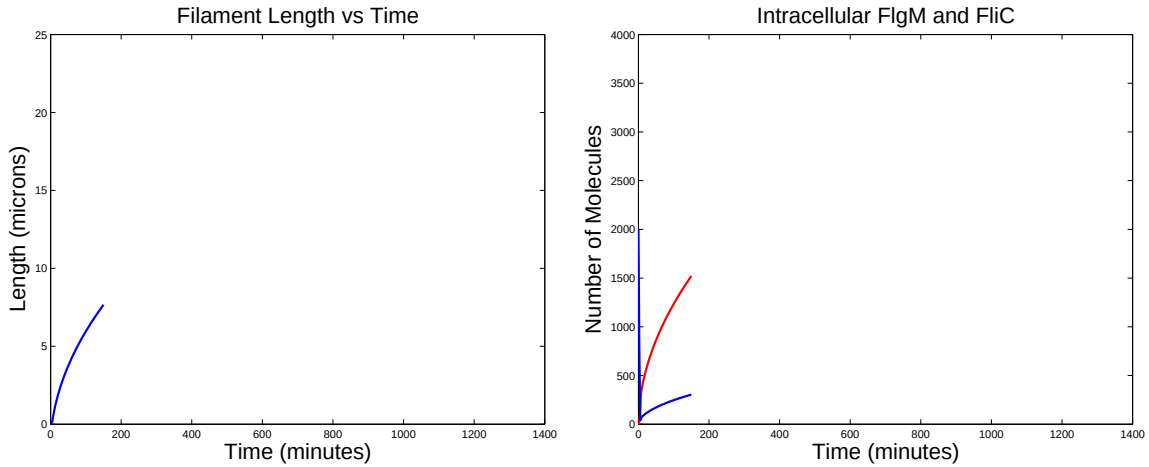
$$\frac{dF}{dt} = \frac{K_*}{K_M + M} - \alpha \frac{F}{F + M} J$$

Filament Length (L):

$$\frac{dL}{dt} = \beta \frac{F}{M + F} J$$

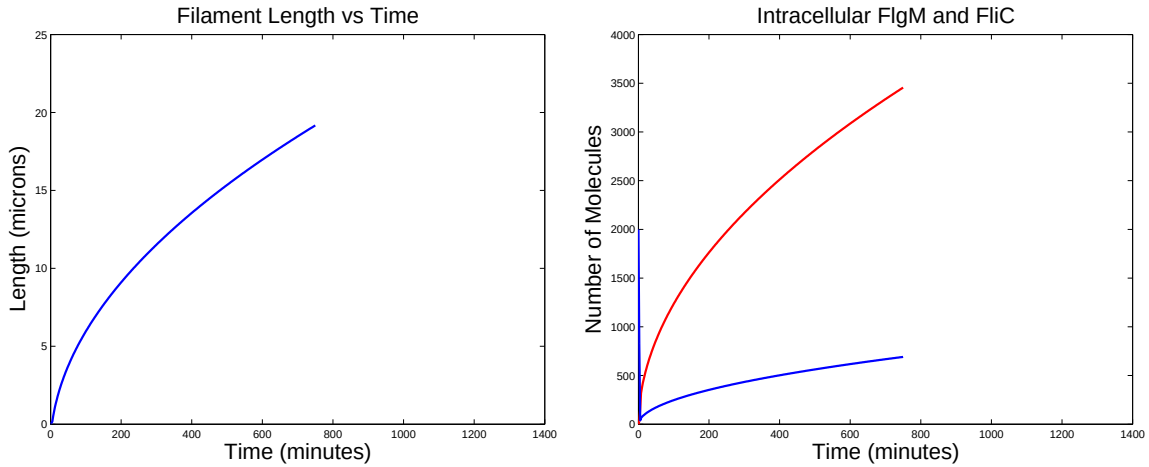
with $J = \frac{1}{K_J + \frac{L}{D}}$ (which is length dependent!) .

Filament Growth



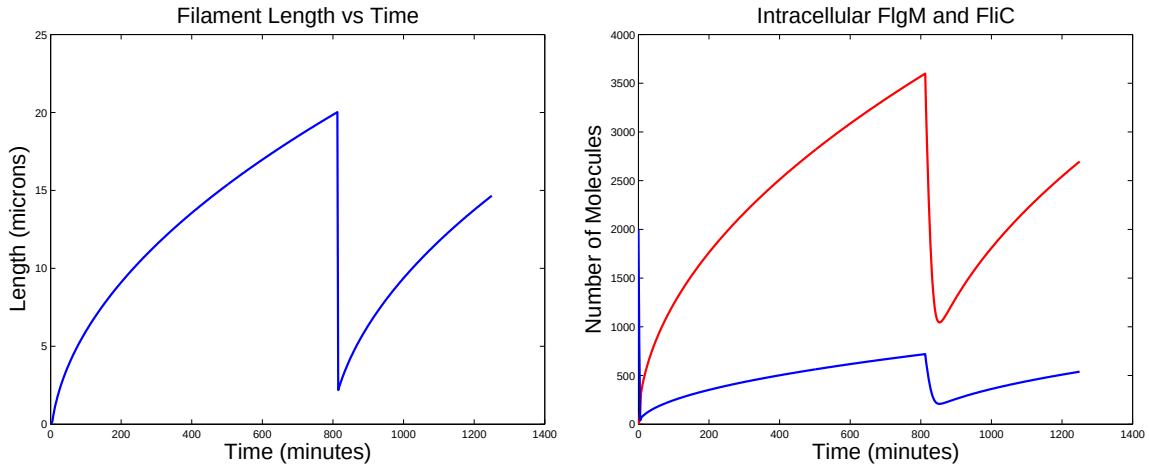
- Before secretion begins FlgM concentration is large. When secretion begins, FlgM concentration drops, producing FliC and more FlgM.

Filament Growth



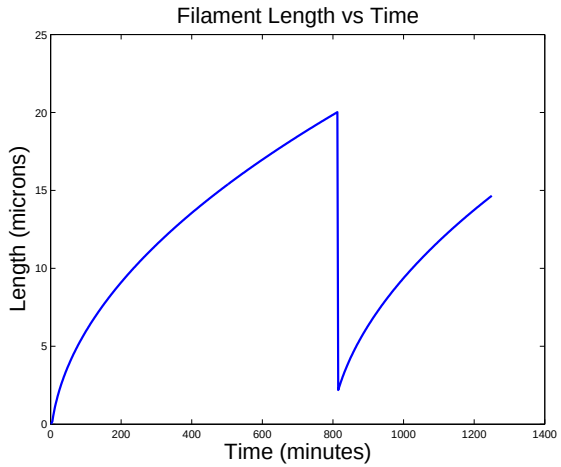
- Before secretion begins FlgM concentration is large. When secretion begins, FlgM concentration drops, producing FliC and more FlgM.
- As the filament grows, secretion slows, FlgM concentration increases, shutting off FliC and FlgM production.

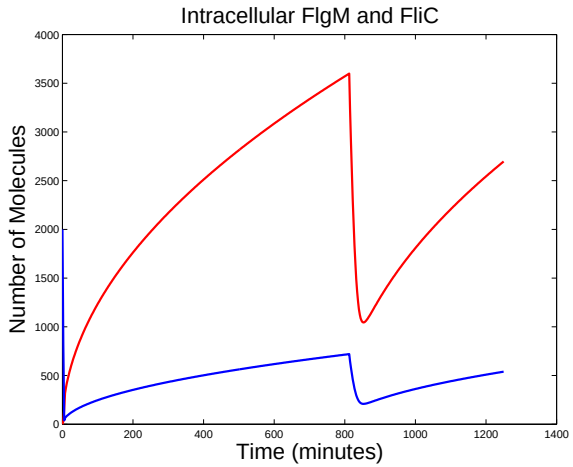
Filament Growth



- Before secretion begins FlgM concentration is large. When secretion begins, FlgM concentration drops, producing FliC and more FlgM.
- As the filament grows, secretion slows, FlgM concentration increases, shutting off FliC and FlgM production.
- If filament is suddenly shortened, secretion suddenly increases, reinitiating the growth phase.

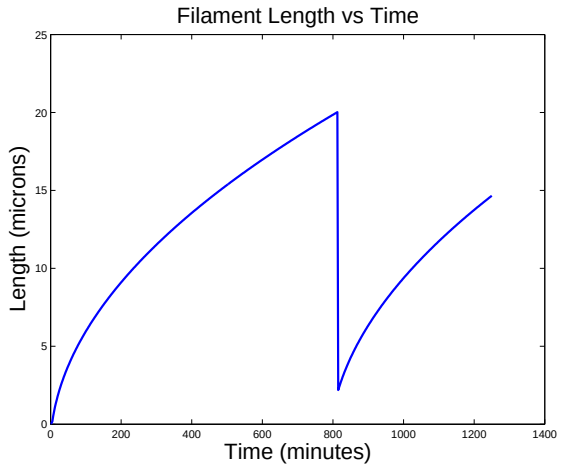
Observations

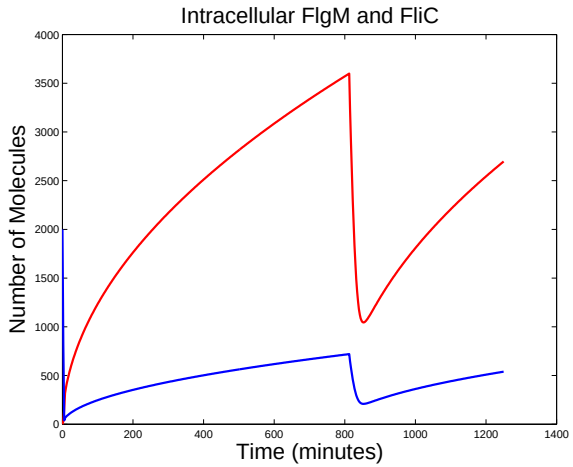




- Because the flux is inversely proportional to length, the amount of FlgM in the cell is a direct measure of the length of the filament.

Observations





- Because the flux is inversely proportional to length, the amount of FlgM in the cell is a direct measure of the length of the filament.
- Because of negative feedback, the cell "knows" to produce FliC only when it is needed.

Acknowledgments

Help came from

- Kelly Hughes, U of Washington
- Bob Guy, U of Utah
- Tom Robbins, U of Utah

No computers were harmed by Microsoft products during the production or presentation of this talk.

The End