Length Regulation of Flagellar Hooks and Filaments in Salmonella

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Introduction
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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Control of Flagellar Growth

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Control of Flagellar Growth

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

- Step 1: Basal Body
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- How are the switches between steps coordinated?
- How is the hook length regulated (55 ± 6 nm)?
- How is the length of the filament "measured"?
Proteins of Flagellar Assembly

Flagellar assembly

FlhD - Filament cap
FlhC
FlhL
FlhK

Hook-filament junction

FlgE

Distal Rod
FlgG
FlgH

L Ring

P Ring
FlgI
FlgB
FlgC
FlgF

Proximal Rod

FlgA

C Ring
FlhA
FlhI
FlhN

MotorSwitch

Basal Body

MotA
MotB

CM

P

Length Regulation of Flagellar Hooks – p.4/36
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  - 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)
• Secreted molecules are chaperoned to prevent folding.
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• FlihB is the gatekeeper recognizing the N terminus of secretants.
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 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 FIK.

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.
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, $\alpha$ 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, $\Delta$ length increment per FlgE molecule.
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


\[ -\ln(1 - P(L)) = \kappa \int_0^L P_c(L) dL? \]
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 $P_c(L)$?
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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.
- 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.
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.
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 $x$. 
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 $P_c(L) = \pi_c(a)$. 

$$P_c(L)$$

$L$
Results

Wild type  Overexpressed  Underexpressed

Length Regulation of Flagellar Hooks – p.19/36
• There is no direct experimental evidence either for or against this proposed length measurement mechanism.
• Flagella grow at a velocity that decreases as they get longer.
II - Flagellar Length Detection

- Flagella grow at a velocity that decreases as they get longer.
- 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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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: $\frac{J}{t} = \text{flux in molecules per unit time.}$
Step 1: **Secretion**

Let $P(t)$ be the probability that ATP-ase is bound
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$$\frac{dP}{dt} = \text{rate of secretion}$$
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Let \( P(t) \) be the probability that ATP-ase is bound

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

on rate,
Rate of Secretion

Step 1: Secretion
Let $P(t)$ be the probability that ATP-ase is bound

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

on rate,  off rate,
Step 1: **Secretion**

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

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

- **on rate**
- **off rate**
- **restricted** if blocked by another molecule in the tube.
Step 1: **Secretion**

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

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

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

\[
\frac{J}{l} = k_{off}(1 - p(0, t))P
\]

at $x = 0$ (A Robin boundary condition).
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 \( \beta = \text{length of filament per monomer (0.5nm/monomer)} \)

\( \cdots \) a moving boundary problem.
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 $L$.
Introducing FlgM and $\sigma^{28}$:
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Class 1
Introducing FlgM and $\sigma^{28}$:

$$
\begin{align*}
\sigma^{28} \\
\text{FlgE} \\
\text{FlgKL} \\
\text{FlgM} \\
\text{FliK}
\end{align*}
$$

Class 1 $\rightarrow$ Class 2

Length Regulation of Flagellar Hooks – p.27/36
Introducing FlgM and $\sigma^{28}$:

Class 1 $\rightarrow$ Class 2

- $\sigma^{28}$
- FlgE
- FlgKL
- FlgM
- FliK

$E\sigma^{28}$ $\rightarrow$ Class 3

- FliC
- FliD
- FlgM

Length Regulation of Flagellar Hooks – p.27/36
**FlgM-\(\sigma^{28}\) Chemistry**

![Chemistry Diagram](image-url)
**FlgM-σ^{28} Chemistry**

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

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

So, how fast is FlgM secreted, and why does it matter?
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 ($L$):

\[ \frac{dL}{dt} = \beta \times \text{rate of FliC secretion} \]
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!) .
• Before secretion begins FlgM concentration is large. When secretion begins, FlgM concentration drops, producing FliC and more FlgM.
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• As the filament grows, secretion slows, FlgM concentration increases, shutting off FliC and FlgM production.
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• 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.
• 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.
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• Because of negative feedback, the cell "knows" to produce FliC only when it is needed.
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The End