Mathematical Model for the Assembly of Type III Secretion Injectisome Controlled by Timing of Substrate Switching

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Short-Abstract — The Type III Secretion System is crucial for bacterial pathogenesis. In this work, we use mathematical models to quantitatively understand how the length of the needle of this massive injectisome is controlled. This model considers both the dynamics of the relevant protein concentrations and the statistical nature of the "substrate switching" process. Length distributions predicted by this model show excellent agreement with experimental measurements using only one free parameter. This work provides quantitative evidence for the substrate switching model, as well as suggesting further experiments to understand the assembly of this massive pathogenic machinery.

I. BACKGROUND

The Type III Secretion System (TTSS) is a major virulence factor found in many gram-negative bacteria. This complex nanomachine consists of a base complex which spans the membranes and a long needle for injecting effector proteins into the host cell. Since functioning needle complexes are required for efficient pathogenesis, the assembly of this complex structure is highly regulated. One parameter of particular importance is the needle length; control of needle length is essential to the assembly of functional injectisomes [1]. Two mechanisms for the needle length control have been proposed for different bacterial systems [2,3]. For *Salmonella typhimurium*, the lining of the inner channel of the base, called inner rod, is composed of a protein PrgJ that is different from the PrgI protein, which constitutes the needle. In the proposed substrate switching mechanism [2], assembly of a *Salmonella* needle stops when the inner rod is completed.

While the substrate switching model is qualitatively consistent with experimental observations, to date there has been no attempt to quantitatively predict the effects of parameters such as the concentrations of PrgI and PrgJ on the needle-length distribution. To better understand the quantitative properties of the needle-length distribution, we have developed a mathematical model for the assembly of the needle complex using the substrate switching mechanism. In this model, the assembly of the needle complex is formulated with a combination of dynamical processes for the constituent proteins and statistical processes for the assembly of the needle and inner rod. This model has been validated with numerical simulation of the stochastic processes and the experimental data from Ref. [2].

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II. RESULTS

Using this mathematical model, we obtained the following distribution for the needle length:

$$P_{needle}(L) = \frac{(n_s + L - 1)!}{L!(n_s - 1)!} \left(\frac{\langle L\rangle}{\sigma^2}\right)^{n_s} \left(1 - \frac{\langle L\rangle}{\sigma^2}\right)^{L} \quad (1)$$

where L is the number of proteins in the outer needle and n_s is the number of PrgJ needed for substrate switching. The average of the needle length $\langle L\rangle$ and the variance σ^2 are $\langle L\rangle = n_s\beta_O O/(\beta_I I)$ and $\sigma^2 = \langle L\rangle + \langle L\rangle^2/n_s$, where O and I are the concentrations of PrgI and PrgJ, respectively, and β_O and β_I are the protein binding rates on the needle and base, respectively. Note that the value of n_s for the substrate switching can be determined with measurements of $\langle L\rangle$ and σ experimentally; this represents the only free parameter in this model. Figure 1 plots the needle-length distribution calculated from Eq. (1) for the two experimental cases in Ref. [2]. In both cases, n_s calculated from the experimental values of $\langle L\rangle$ and σ equals 6. The experimentally-measured histograms of the needle length in Ref. [2] are also plotted in Fig. 1. The excellent agreement between experimental measurements and the prediction indicate that the substrate switching model is quantitatively consistent with available data. Further experimental perturbations to PrgI and PrgJ concentration *in vivo* could be used to further test the validity of the this mechanism for *Salmonella typhimurium* and other bacteria.

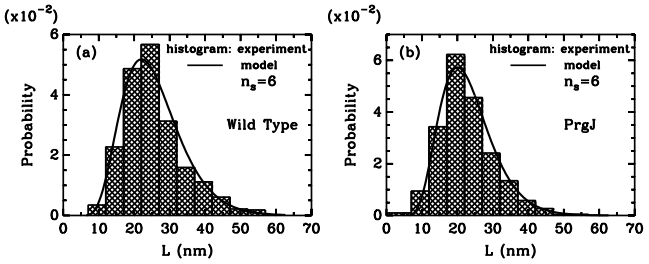

Figure 1. Needle-length distribution of (a) wild type and (b) PrgJ overexpressed Salmonella, where L is in nm. Solid line is calculated from Eq. (1) with the experimentally measured $\langle L\rangle$ and σ and the histogram is the experimental measurement from Ref. [2].

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