Quantification of reaction rates in the *E. coli* Min system

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Abstract

Cell division of *E. coli* bacteria occurs through a remarkable pole-to-pole oscillation of the proteins MinC, MinD, and MinE. Recently, this has become a popular system for computer simulation, leading to a wide variety of models that purport to explain the dynamics. To assist in the discrimination between proposed models, and to allow the development of more accurate models, we are experimentally measuring the rates of several of the biochemical reactions that comprise this system. This is done *in vitro* using purified lipids, proteins, and other chemicals. Experiments focus primarily on the rate of ATP hydrolysis by MinD. To extract information about different rates in the system from this single observable, chemical concentrations are varied widely between different analyses. This shifts the balances of fluxes in the reaction network, thus influencing the rate of ATP hydrolysis. The resulting multi-dimensional phosphate release data will be analyzed using computer models of the reaction network to yield individual reaction rate constants.

E. coli Min system introduction

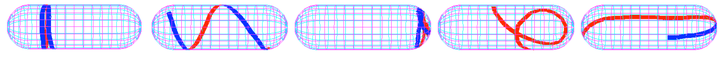
- Used by the cell to position the division plane at the cell center.
- Dynamics depend on only the proteins MinD and MinE.
- MinD and MinE oscillate from pole to pole with about a 40 second period.
- MinD forms a helical polymer on the inside of the cell membrane.
- MinD is an ATPase that is stimulated by MinE.

Figure: Dynamics of GFP-tagged MinD in an *E. coli* cell as observed by deconvolution fluorescence microscopy. Frames are 8 s apart.

Open questions with the Min system

Why is the MinD polymer mostly at a pole and helical?

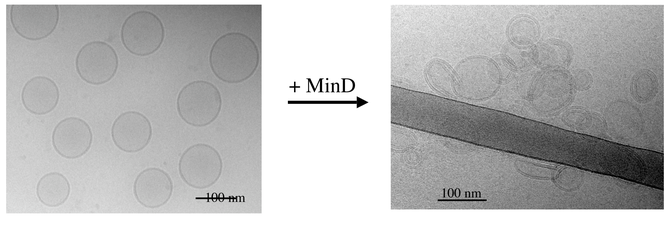
Shown in other work, the hypothesis of a co-author (SSA) is that it arises from mechanical interactions between the natural curvature of the polymer and the shape of the cell membrane. This theory leads to 5 polymer morphologies, of which MinD most closely resembles the "polar-targeted" type.



ring (FtsZ) | helix (MreB) | polar-targeted (MinD) | loop | lengthwise (TubZ)

How does MinD interact with the membrane?

Using cryo-electron microscopy, we find that MinD causes small unilamellar vesicles to deform, aggregate, and become bilamellar, even in the absence of ATP or Mg^{2+}. Similar results are found using fluorescence microscopy of giant unilamellar vesicles.

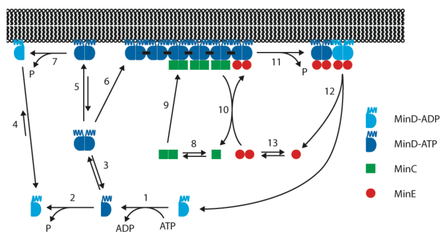


What is the Min system reaction network, including reaction rates?

This project is the topic of this poster.

The Min system reaction network

The reaction network structure is reasonably well known, but most rate constants and mechanisms are unknown.



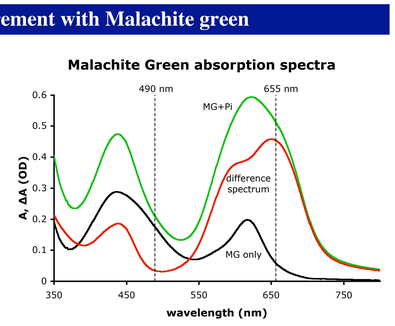
1. Transfer of nucleotide bound to MinD. This reaction is probably reversible, although the ratio of cytoplasmic ATP to ADP will strongly favor the forward reaction. 2. Hydrolysis of ATP by cytoplasmic MinD. It is likely that both monomers and dimers hydrolyze ATP, although the relative rates are not known. 3. Dimerization of MinD. 4. Interaction of MinD-ADP with membrane. This interaction is weak or non-existent, either because the amphipathic lipid-binding helix of MinD is not exposed for the ADP-binding form of MinD, or because the single binding domain of a MinD monomer does not interact strongly enough with the membrane to keep MinD from diffusion away. 5. Binding of MinD dimer to membrane with amphipathic helices of both monomers. 6. Polymerization of MinD on the membrane. While not shown, the polymer is believed to be asymmetric with plus and minus ends. 7. Hydrolysis of ATP by unactivated membrane-bound MinD. 8. Dimerization of MinC. 9. Binding of MinC to membrane-bound MinD. It is not known if only MinC monomers bind to MinD, just dimers, or both. 10. Displacement of MinC by MinE. This reaction is irreversible. 11. Hydrolysis of ATP by MinD, activated by MinE, leading to depolymerization and, possibly, retraction of the lipid-binding helices. 12. Release of MinE from MinD and MinD from the membrane. 13. Dimerization of MinE.

Every reaction affects release rate of inorganic phosphate (P_i), making this an observable that can be used to probe the reaction rates. We measure the phosphate production rate during *in vitro* reactions using purified components.

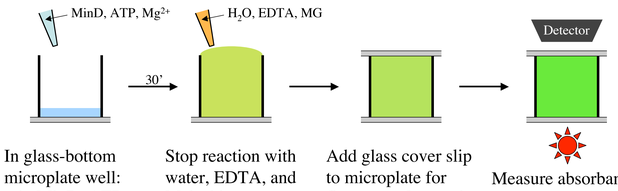
Phosphate measurement with Malachite green

Concept

Malachite green reagent (BioAssay Systems) turns green upon addition of phosphate. This is quantified with A_{655}, internally referenced to A_{490}, in a plate reader. The color change is unstable, so it is monitored over 40 minutes.



Experimental method



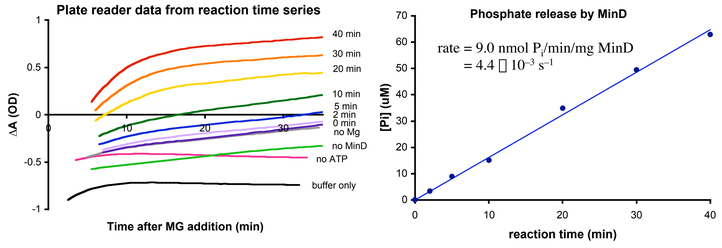
In glass-bottom microplate well: mix MinD, ATP, $MgCl_2$, buffer, etc. React ~30 minutes.

Stop reaction with water, EDTA, and Malachite green (MG). MG color starts developing.

Add glass cover slip to microplate for flat top surface and put in plate reader.

Measure absorbance difference between 655 and 490 nm for 40 minutes.

Typical data

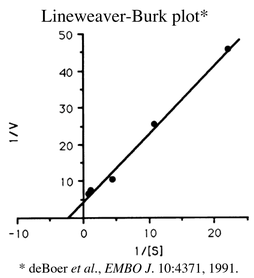


Data were analyzed by extrapolating Malachite green absorption data back to the end of the ATPase reaction, and rescaling with calibration standards. We are developing a method to fit complete kinetic traces with those from calibration standards to yield more accurate results.

Results

I. MinD reactions with ATP

Hydrolysis of ATP by MinD follows a mechanism similar to Michaelis-Menten, allowing similar analysis methods.



* deBoer *et al.*, *EMBO J.* 10:4371, 1991.

$$\text{MinD} \bullet \text{ADP} + \text{ATP} \rightleftharpoons \text{MinD} \bullet \text{ATP} + \text{ADP}$$
$$\text{MinD} \bullet \text{ATP} \rightleftharpoons \text{MinD} \bullet \text{ADP} + P_i$$

$$\frac{1}{V} = \frac{1}{k_f[\text{ATP}]} + \frac{1}{k_p} + \frac{k_r[\text{ADP}]}{k_f k_p[\text{ATP}]}$$

$$V = \frac{1}{[\text{MinD}]} \cdot \frac{d[P_i]}{dt}$$

$k_1 = 26\ \text{M}^{-1}\text{s}^{-1}$
$k_{-1} = 20\ \text{M}^{-1}\text{s}^{-1}$
$k_2 = 1.04 \times 10^{-3}\ \text{s}^{-1}$

$k_7 < 0.5 \times 10^{-4}\ \text{s}^{-1}$
$k_{11} = 0.016\ \text{s}^{-1}$

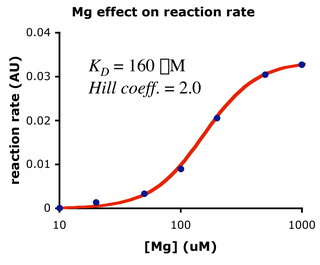
$$\text{MinE} : \text{MinD} \bullet \text{ATP} \rightleftharpoons \text{MinE} + \text{MinD} \bullet \text{ADP} + P_i$$

II. MinD activation by lipids and MinE

From literature results, lipids do not affect ATP hydrolysis (implying that k_7 is small), but the combination of lipids and MinE does. These speed up ATP hydrolysis by a factor of 10; accounting for the nucleotide transfer rates ($k_{\pm 1}$), the activated reaction rate constant is 16 times k_2:

III. Mg^{2+} binding to MinD

MinD binds ATP with a Mg^{2+} ion. We find MinD is inactive without Mg^{2+} and rates with Mg^{2+} are fit by the Hill equation. The Hill coefficient of 2.0 implies that only MinD dimers hydrolyze ATP, which is supported by other experiments in which the MinD concentration was varied.



Conclusions

Quantification of phosphate release rates is a simple and powerful method for determining reaction rates in networks that involve ATP hydrolysis. Using an improved Malachite green method, we: (*i*) determined several reaction rates in the *E. coli* Min system, and (*ii*) found that only dimeric MinD is active.

In future work, we will redraw the Min system reaction network to account for current results, quantify other reaction rates, and iterate until the *in vitro* network is fully quantified. This will require model-based parameter estimation methods.

Acknowledgements

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