Reprogramming Bacteria to Seek and Destroy an Herbicide

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Background

- Aptamer
- Riboswitch
- SELEX: In vitro selection method to sort through large libraries to isolate aptamers

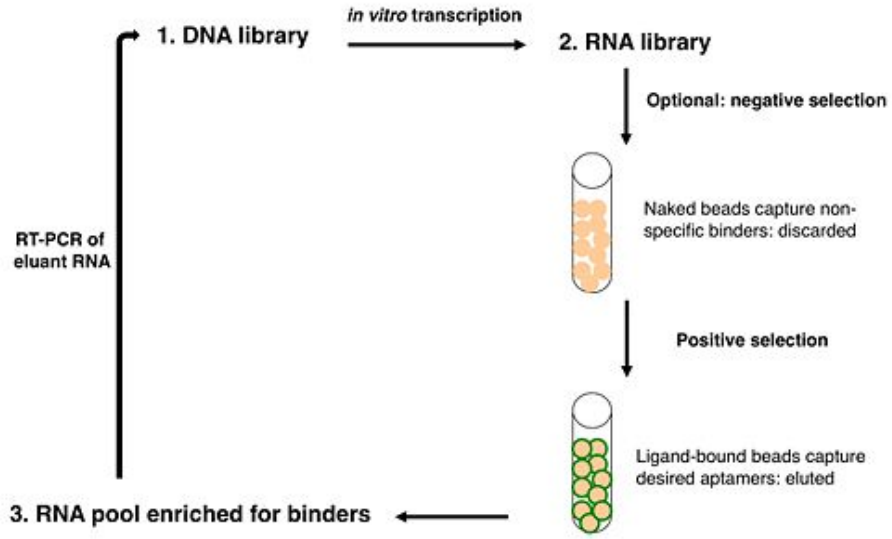


Image from: <http://openwetware.org/wiki/20.109%28S10%29:Prepare_RNA_by_IVT_%28Day3%29>

Goal and Motivation

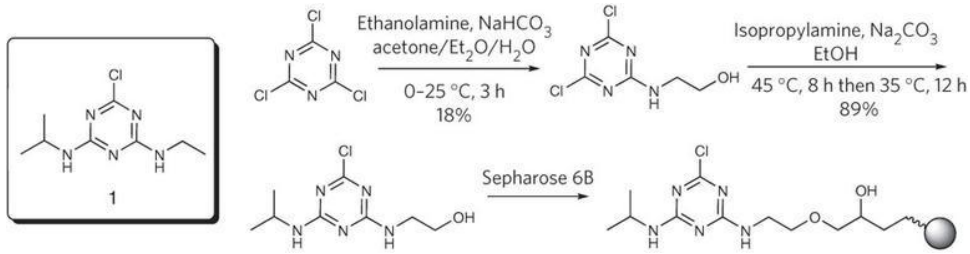
- Reprogram *E. coli* to respond to atrazine
- Environmental:
  - Heavily used herbicide
  - Persistent organic pollutant
  - Widespread groundwater contamination
- Chemical:
  - Attractive for RNA interaction due to H donors and acceptors
- Biotechnological:
  - Catabolic pathway well characterized
  - Each of the enzymes can be expressed and purified in *E. coli*

Approach

- In vitro and in vivo selection to identify riboswitch that responds to atrazine
- Riboswitch to make E. coli move in presence of atrazine (pseudotaxis)
- Add atrazine catabolism gene
- ➡ Cells seek and destroy atrazine!

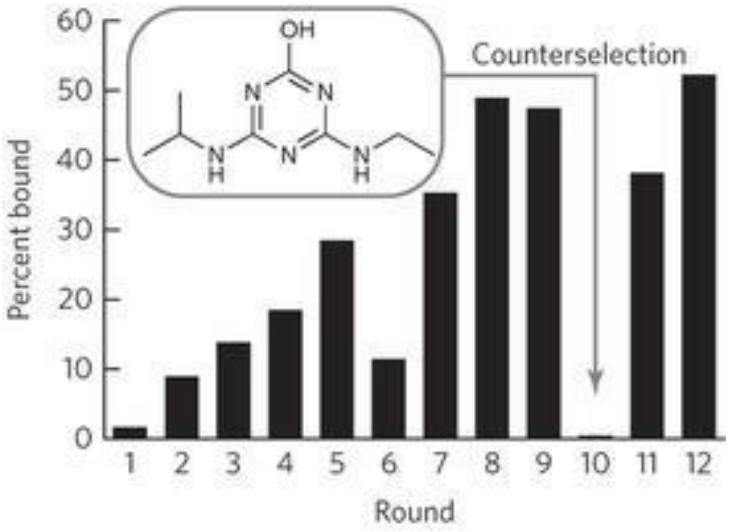
Aptamer Selection

- Atrazine derivative synthesized and coupled to a solid support



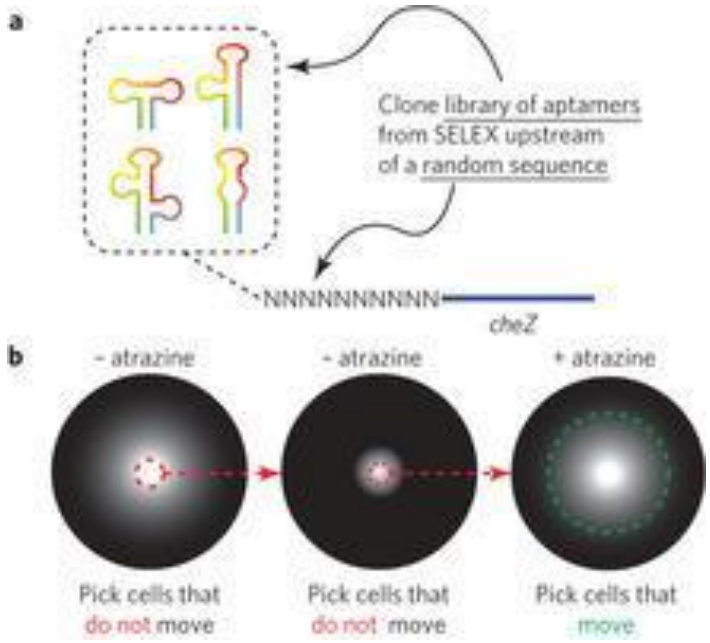
- Library of DNA sequences made

- Nine rounds of SELEX using atrazine to elute bound RNA
  - One reverse round and two more rounds of SELEX



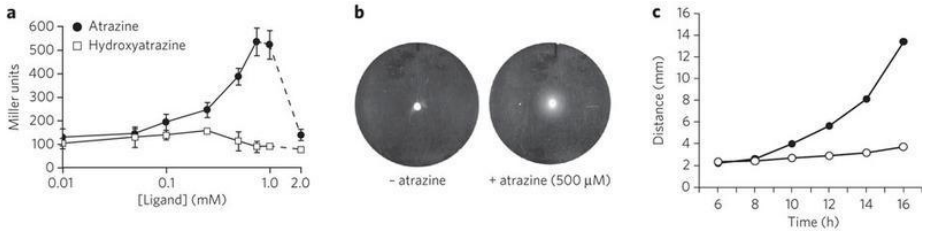
Aptamer Selection

- Problem: High affinity not sole desirable aptamer characteristic
  - "riboswitch" properties
- Extra screen for riboswitch activity
  - 12 rounds of *in vitro* selection



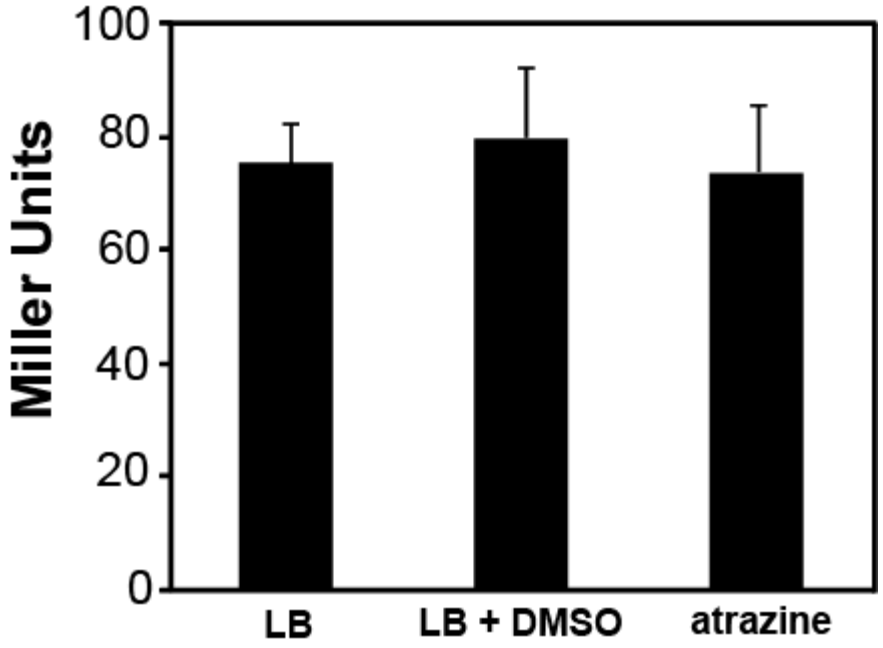
Motility Selection for Synthetic Riboswitches

- Added riboswitch to ß-galactosidase reporter gene (*lacZ*) in order to quantify gene expression



- Six of 96 clones showed fourfold increase of ß-galactosidase

- Deletion of putative aptamer sequence deleted response to atrazine



Characterization: Translational or Transcriptional Regulation?

- Created transcriptional fusion between to genes:

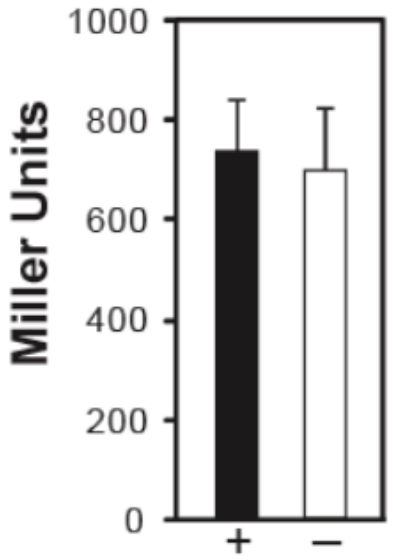
If Transcriptionally Regulated:

- Atrazine would need to be present for transcription of the whole fusion gene to be transcribed
- Expression of LacZ will be Atrazine Dependent

If Translationally Regulated:

- Transcription will occur independent of Atrazine
- Translation of LacZΔ will be atrazine activated
- Translation of LacZ will be atrazine independent because it has its own RBS

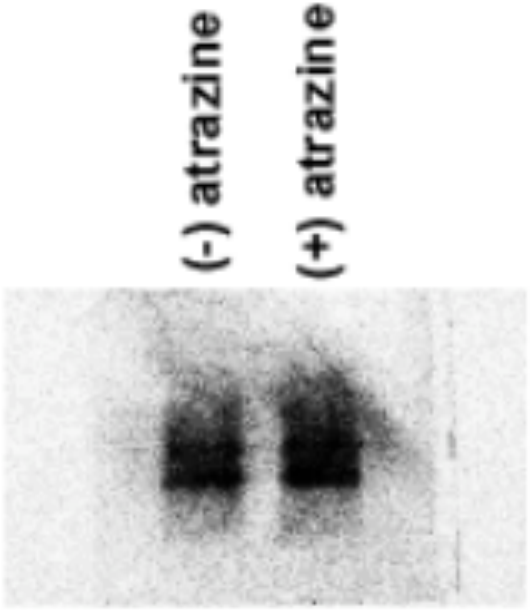
Characterization: Translational or Transcriptional Regulation?



Atrazine-dependent riboswitch acts at the translational level

Characterization: Translational or Transcriptional Regulation?

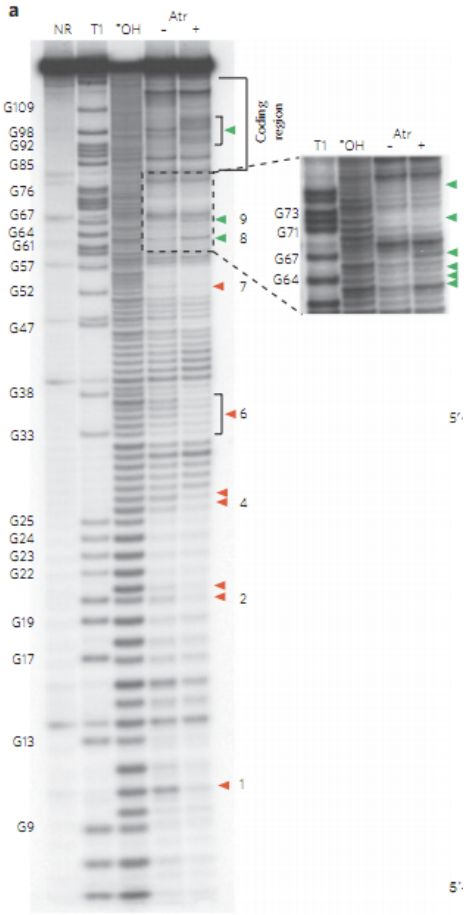
Northern Blot Analysis:



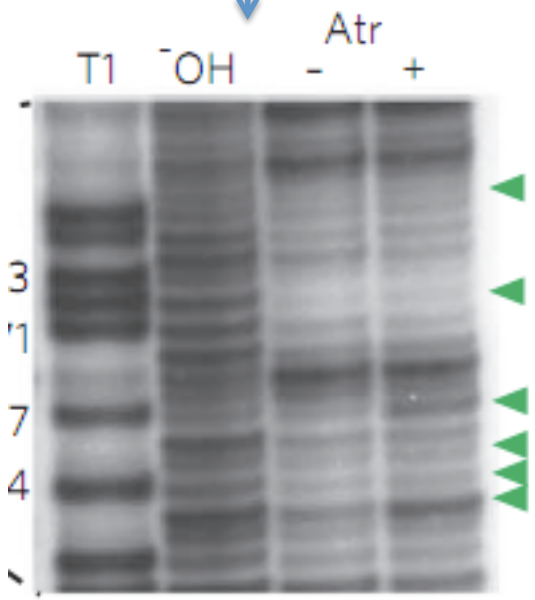
Same amount of riboswitch-encoding RNA in presence or absence of Atrazine

Characterization: Conformational Changes that Underlie Switching

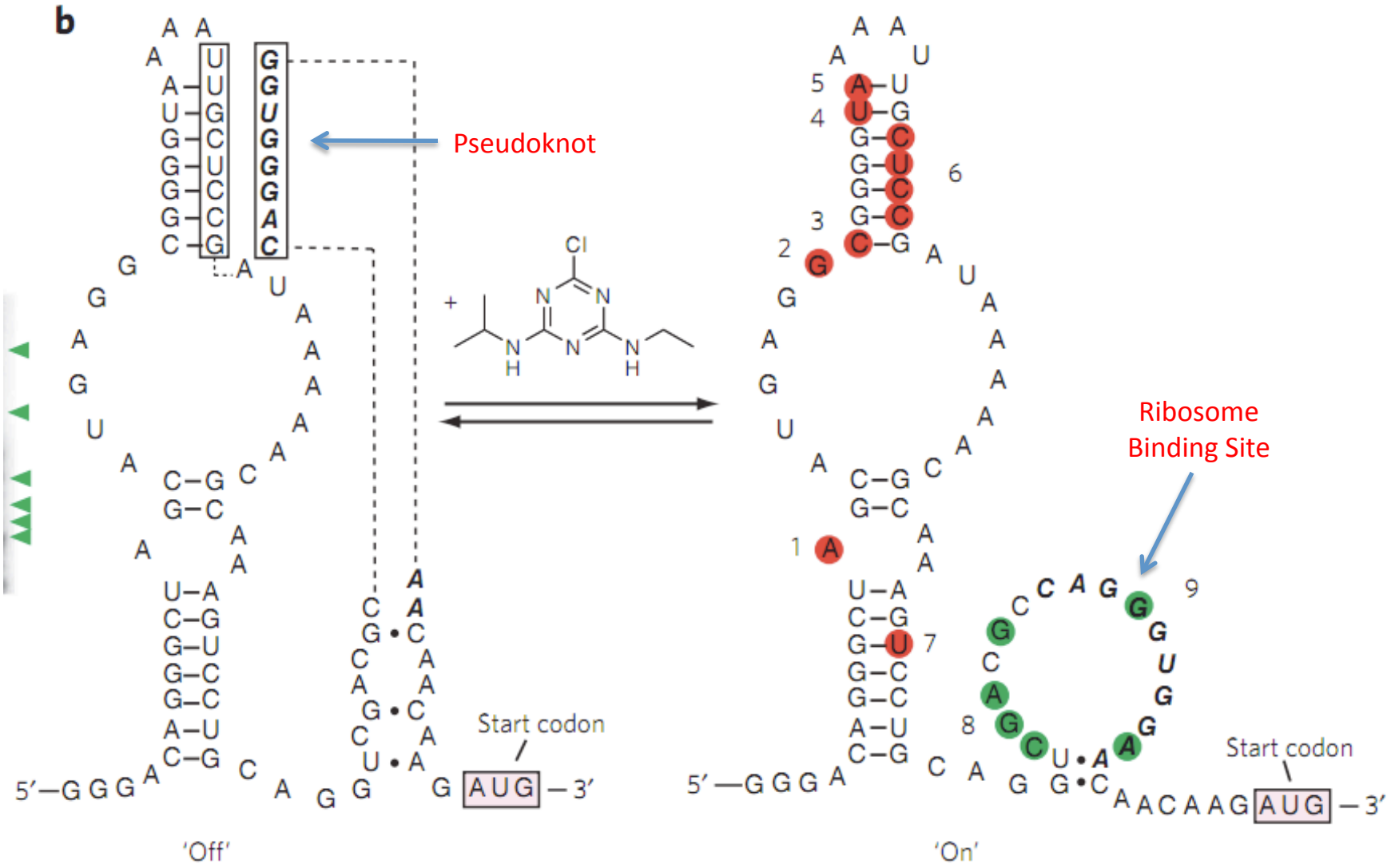
In-line probing experiment:



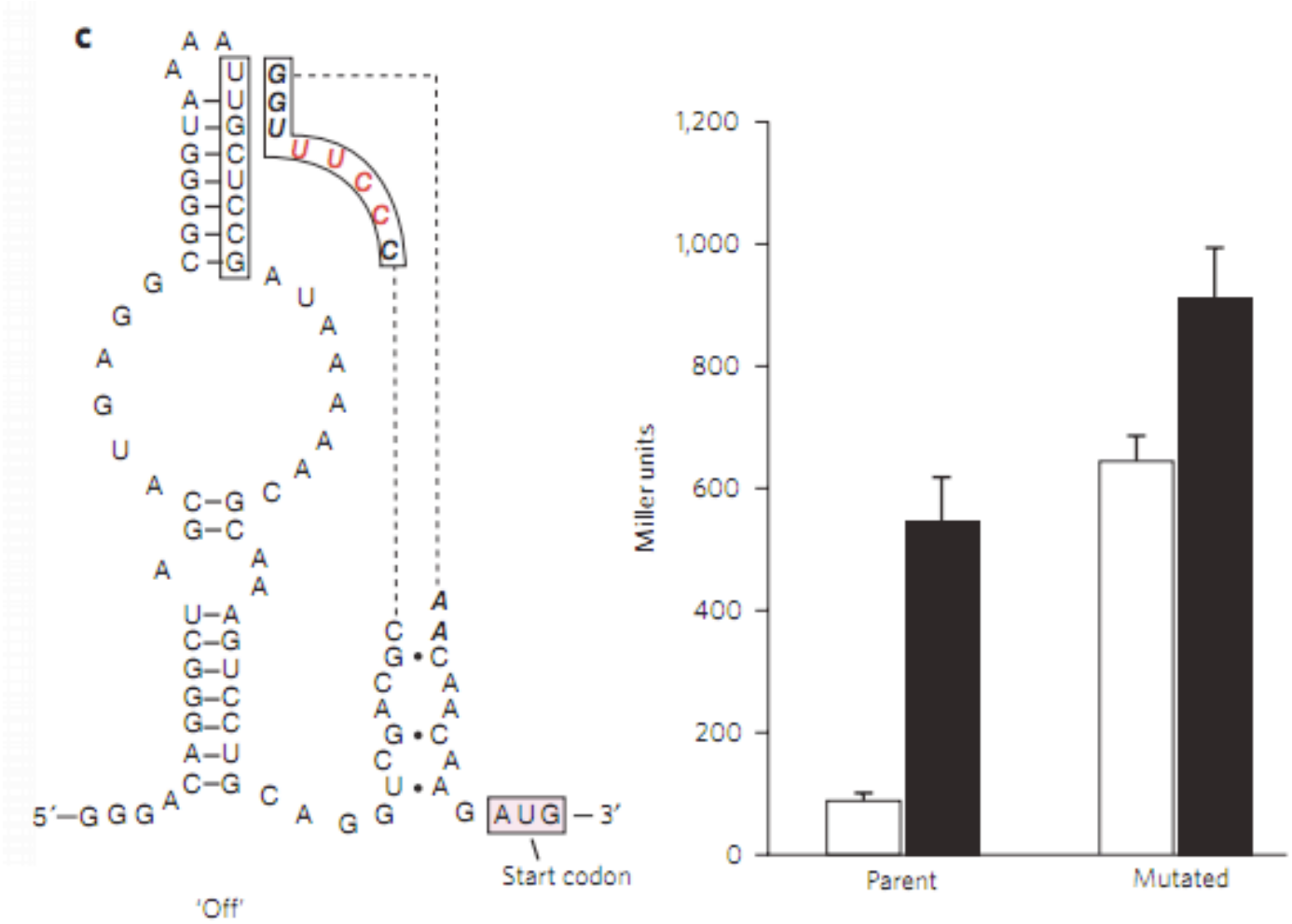
N40 Region: Proposed site of Aptamer Complex



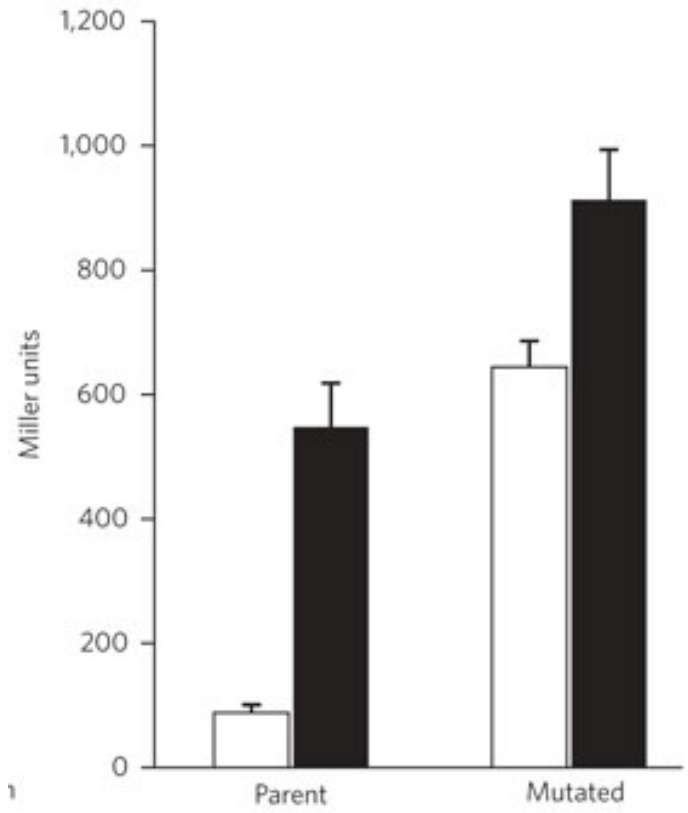
Model for Atrazine-Dependent Riboswitch



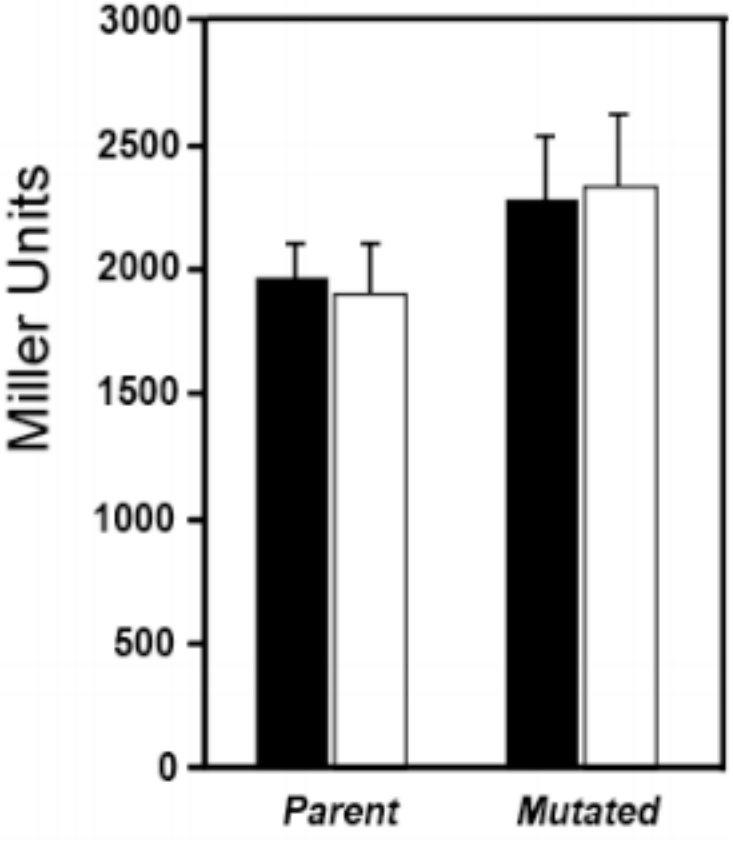
Verification of Atrazine-Dependent Riboswitch Model



Ribosome Binding Site Strength



With Aptamer and Mutated Aptamer Sequence



Without Aptamer Sequence

Destroying Atrazine

- Next: atrazine-catabolizing gene
- Existing genes
- *Pseudomonas* sp. ADP
- *AtzA*

Destroying Atrazine

- *AtzA*:

Atrazine $\xrightarrow{\text{atrazine chlorohydrolase}}$ Hydroxyatrazine

Destroying Atrazine

- Atrazine $\xrightarrow{\text{activate}}$ Riboswitch $\xrightarrow{\text{express}}$ Motility protein
- AtzA, GFPuv constitutively expressed

Destroying Atrazine

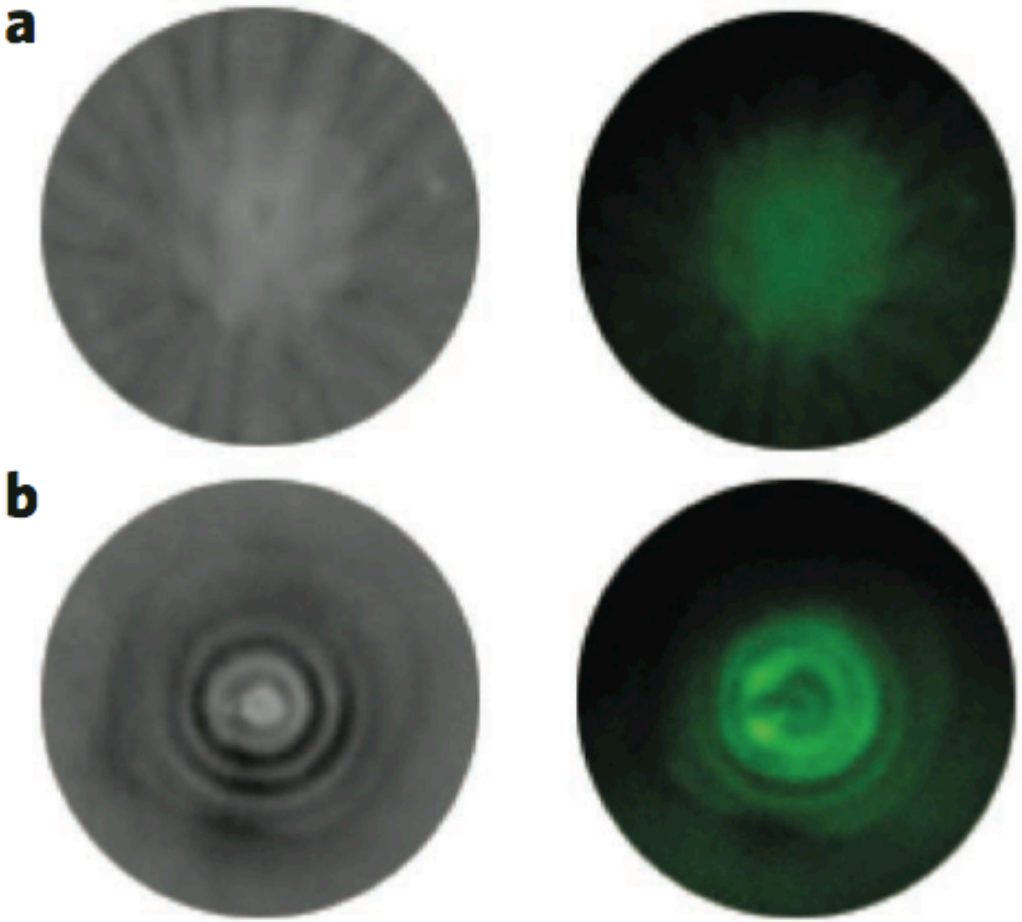


Figure 6

Outcomes

- Riboswitch activated by atrazine
  - lessons about riboswitches
- Dose-dependent motility
- *E. coli* with riboswitch degrades atrazine

Outcomes

- Riboswitch requires high concentrations in vivo
  - Delete atrazine efflux
  - Permeable chassis
- Low vs. high background expression
- Reverse switch behavior?

For Discussion

- Forward engineering vs. finding in nature
  - Find vs. design aptamer-ligand pairs
- Aptamer selection strategies
- Applications of synthetic biology