Protein-responsive ribozyme switches in eukaryotic cells

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Presented by Marianne Linz and Jennifer Thornton

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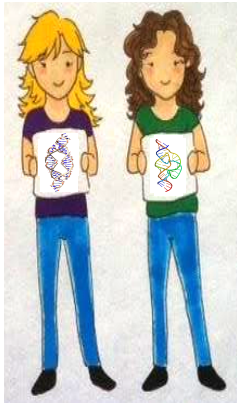
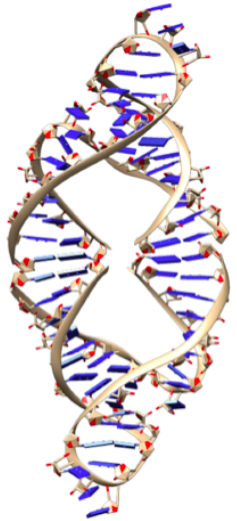


Synthetic biology would benefit from advances in protein-responsive genetic devices

- Proteins→Cell phenotype, behavior
- Protein-responsive tools for genetic control would be powerful
- Ribozymes could be these tools
  - Small molecule-responsive ribozymes had been made
  - Protein-responsive ribozymes had not



http://en.wikipedia.org/wiki/Ribozyme

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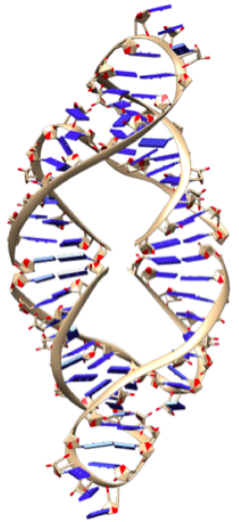

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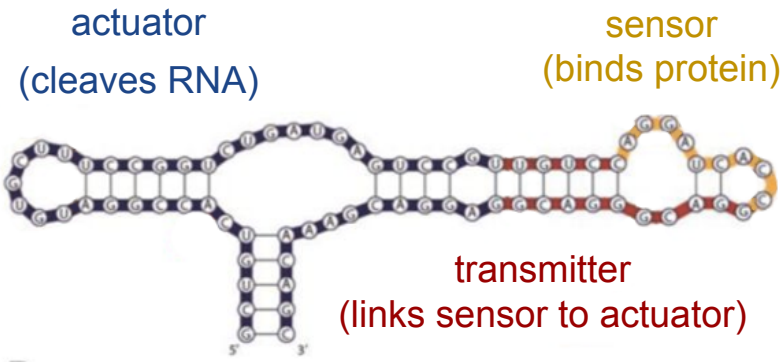
Ribozymes (**ribo**nucleic acid en**zymes**): RNA molecules that catalyze biochemical reactions, behaving like protein enzymes

Created protein-responsive ribozyme switches that can control gene expression

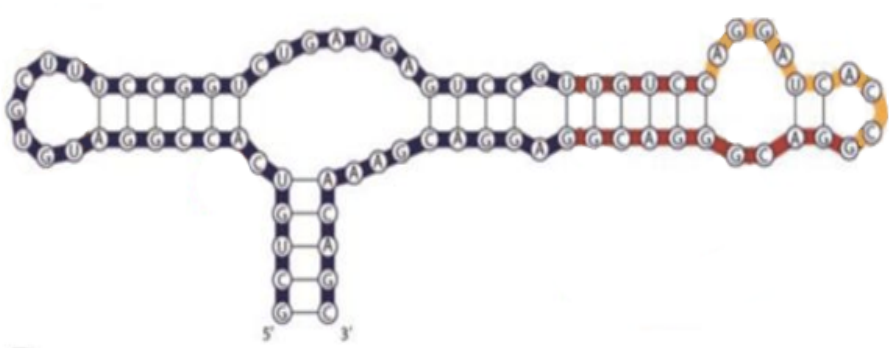
- They designed three switch components:



- Advantages:
  - Can either repress or enhance gene expression
  - Have components that can be modified
  - Work in mammalian cells, yeast, *in vitro*
  - Respond to protein binding

Multiple gene-ON and gene-OFF constructs were designed

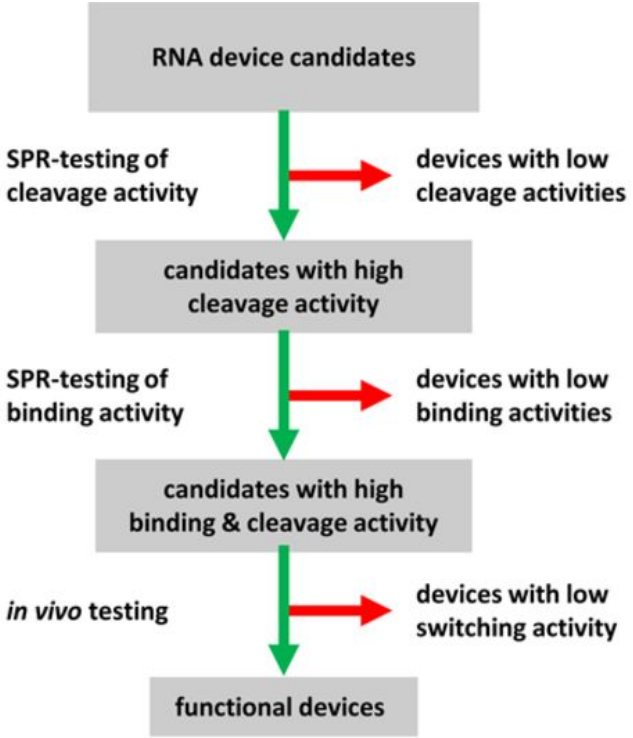
- Created ON and OFF ribozyme switches
  - ON: ligand binding ↑ gene expression
  - OFF: ligand binding ↓ gene expression
- Modified pre-existing parts
  - Sensor: MS2 coat protein aptamer (small, well-characterized)
  - Actuator: sTRSV HHRz from tobacco ringspot virus



- Built two different types of transmitter domains
  - Separate transmitter mediating secondary structure switching (gene-ON)
  - Transmitter directing displacement of HHRz loop structure (gene-ON and -OFF)

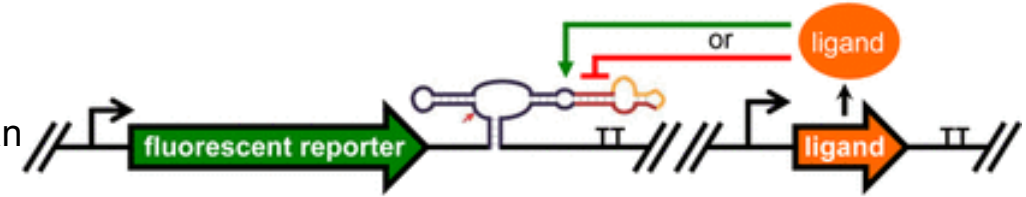
Device function was tested *in vitro* via two SPR assays

- SPR (Surface Plasmon Resonance)
  - Binding→Change in refractive index of sensor surface→Output signal
- RNA self-cleavage assay
  - Measured device cleavage when Mg^{2+} ions were added
  - RNA cleavage ↓ SPR signal
- Protein-RNA binding assay
  - Measured protein binding to devices stuck to the SPR surface
  - Protein binding ↑ SPR signal

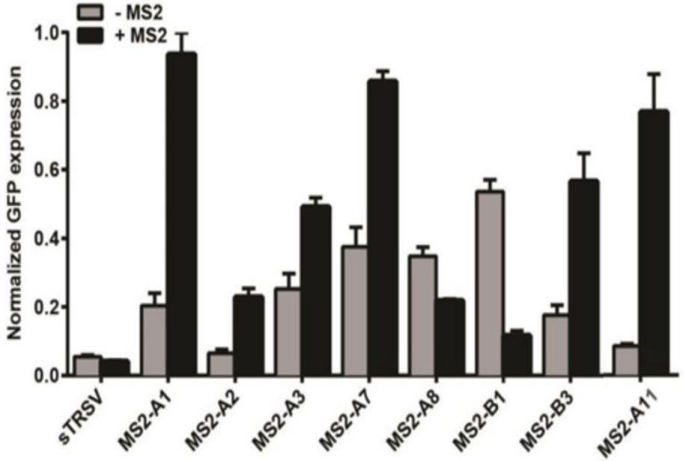


Devices that passed *in vitro* testing were tested *in vivo* with fluorescent reporters

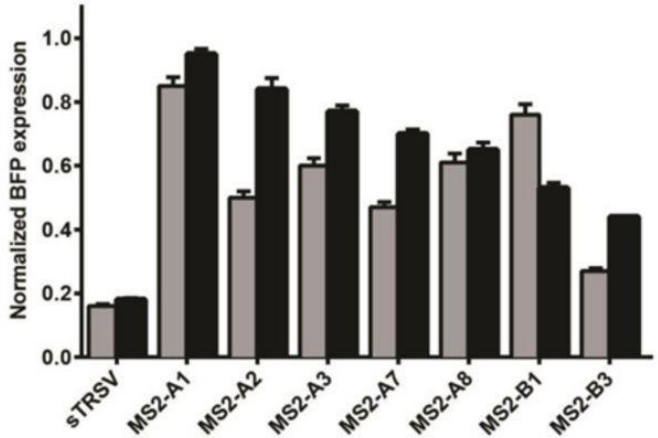
- Promising devices placed in 3’ UTR of fluorescent reporters
- Constructs placed in yeast and human embryonic kidney cells
- Cell fluorescence measured in presence/absence of MS2 ligand
- Graphs show functionality in yeast (A) and human (B) cells, variability across device activity



A

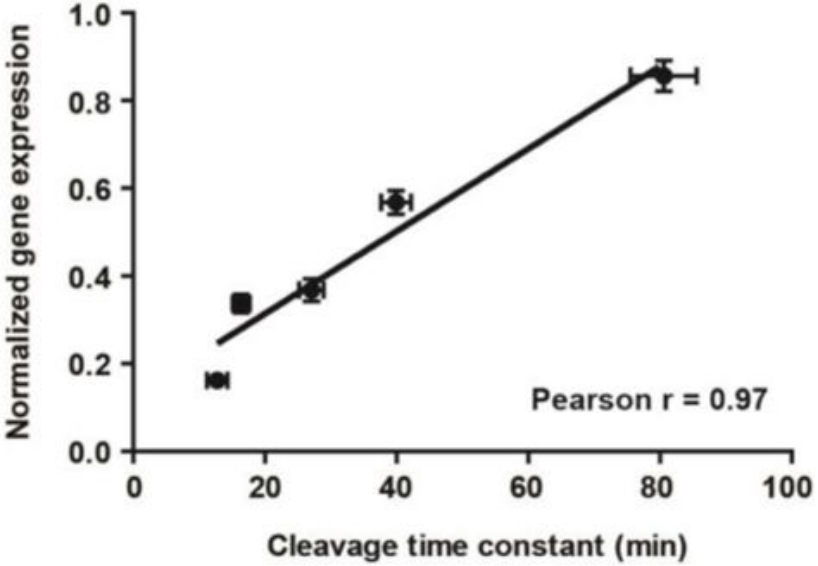


B

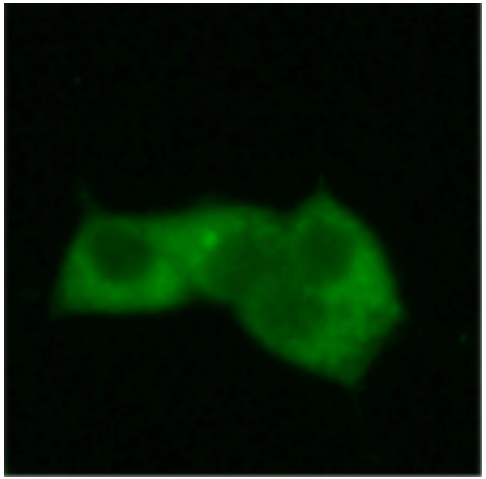


Two further experiments revealed information about how ribozyme switches function

- *In vitro* gel-based RNA cleavage kinetics correlate with *in vivo* gene expression levels (mammalian results below)



- Experiments with localized MS2 ligands show ribozyme switches respond to nuclear and cytoplasmic ligand



2MS2mut-NES localizes to the cytoplasm as expected

Assumptions and Suggestions

- The group's claims rest on a few assumptions:
  - This would work with another protein→Used only one, virus coat protein MS2
  - Sensors for different proteins can be built→MS2 sensor found in nature already
  - Enough is known about ribozymes to implement→Mechanism not well understood

- Our suggestions:
  - Build devices for more proteins
  - Condense the methods section→Move to supplementary materials

- Still, we would recommend for publishing. The work was successful and could inspire further protein-responsive switch construction.

Conclusions and Significance

- New class of ribozyme switches has many advantages:
  - Protein-responsive
  - Extendable to other proteins/genes
  - Allows for gene-ON and gene-OFF regulation
  - Can be applied to different cell types→Yeast, mammals, etc.

- No further publications yet, but future work expected to:
  - Streamline switch development
  - Elucidate ribozyme switch mechanism of action
  - Discover applications in gene therapy, diagnostic tools, cellular therapeutics, and more