Programmable ligand-controlled riboregulators of eukaryotic gene expression

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Nature Biotechnology, March 2005

Introduction

- importance of nocoding *cis* and *trans* acting RNA elements in gene expression networks

→ regulation of complex genetic networks such as developmental timing

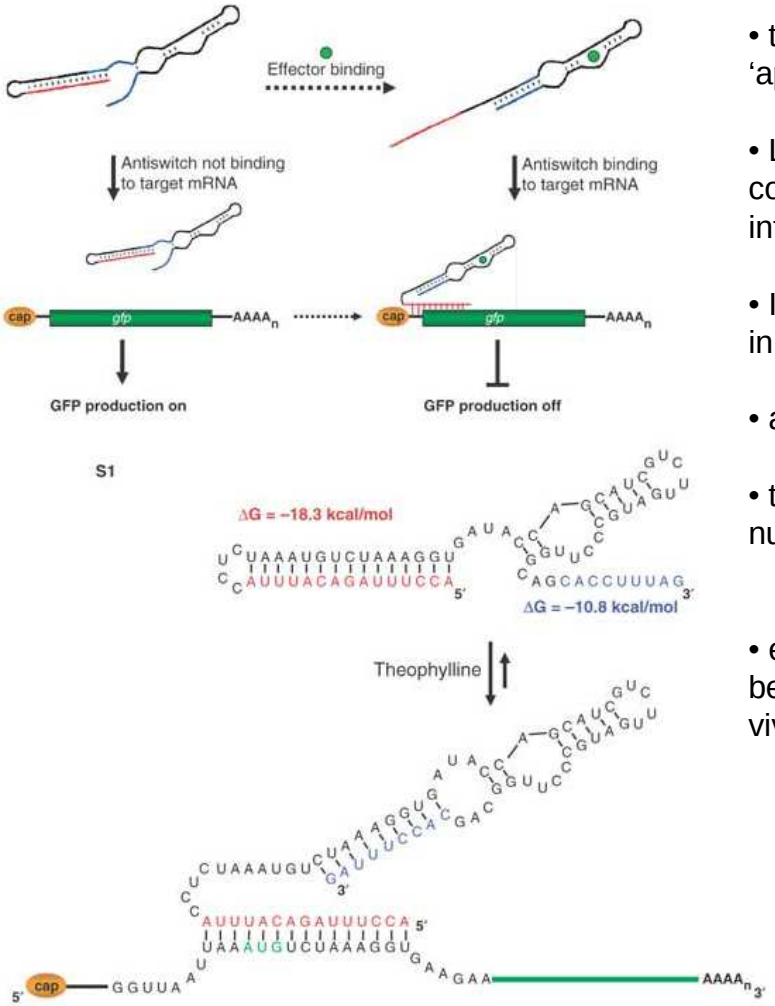
- diverse noncoding RNA elements

  (antisense RNA, taRNA, miRNA, siRNA, ribozymes, riboswitches)

Aptamers

- nucleic acid species that bind specific ligands
- impart allosteric controlled properties
- can be generated by *in vitro* selection or SELEX (systematic evolution of ligands by exponetional enrichment)

Results: designs of antiswitch regulator



• the antisense sequence is shown in red, the switching ‘aptamer stem’ in blue

• Ligand binding at the apatmer domain induces a conformational change that allows the antisense domain to interact with the target mRNA to affect translation

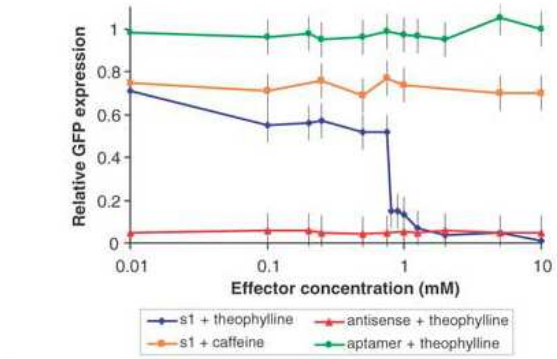
• In the absense of ligand, the antisense domain is sequestered in an ‘antisense stem’

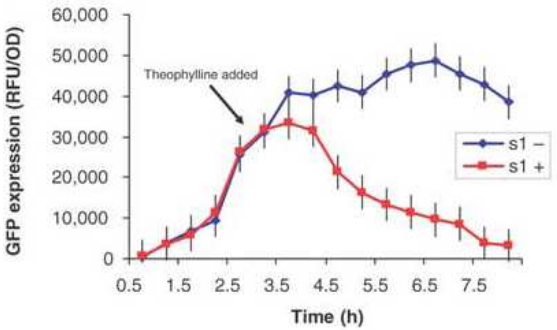
• aptamer binds theophylline with high affinity and specifity

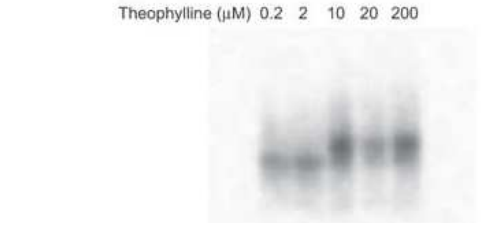
• the antisense RNA domain is designed to base pair with a 15-nucleotide region around the start codon of the target mRNA

• expression of ncRNA: the RNA to be expressed was cloned between two hammerhead ribozymes known to self-cleave in vivo

Results: functional activity of antiswitch regulator







• Protein expression assays demonstrate ligand-specific *in vivo* activity of s1

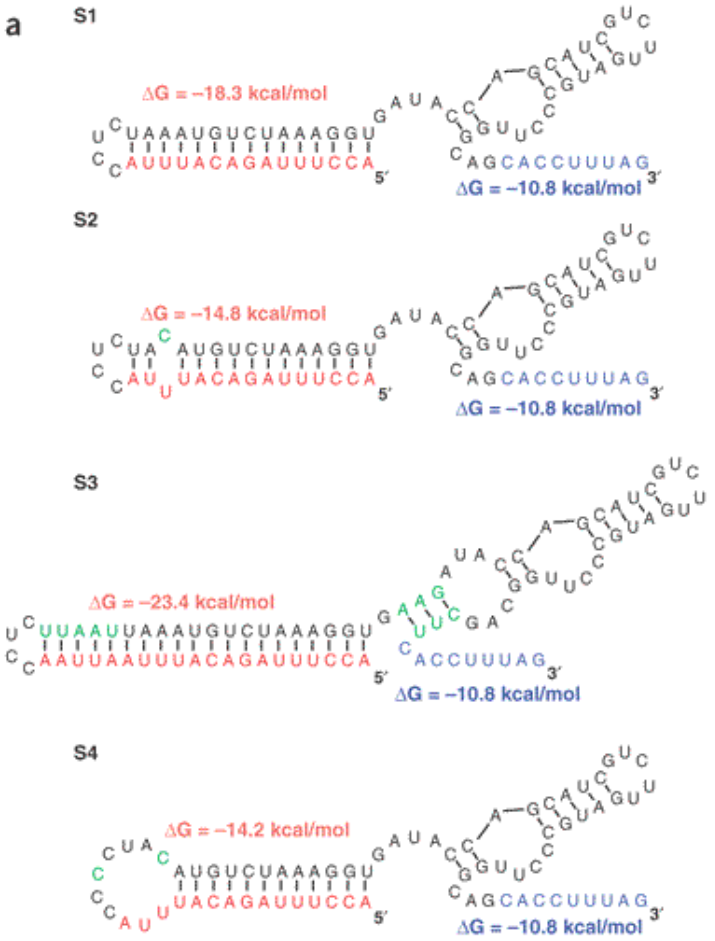
• the aptamer used in this antiswitch does not bind caffein, which differs from theophylline by a single methyl group

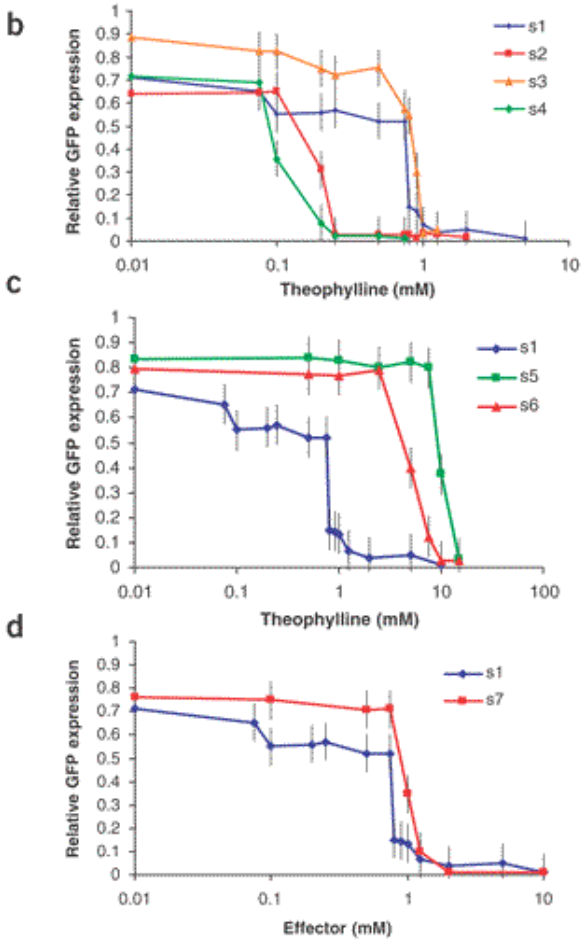
• temporal resoponse of antiswitch regulation: activation of antiswitch through addition of theophylline to cells expressing steady-state levels of GFP and with s1 in 'off' state

→ antiswitch molecules act rapidly and time required for target protein levels to decrease is determined by the protein's half-life

• gel-shift experiment to examine antiswitch ligand affinity

Results: tuning and expanding the switch response of an antiswitch regulator

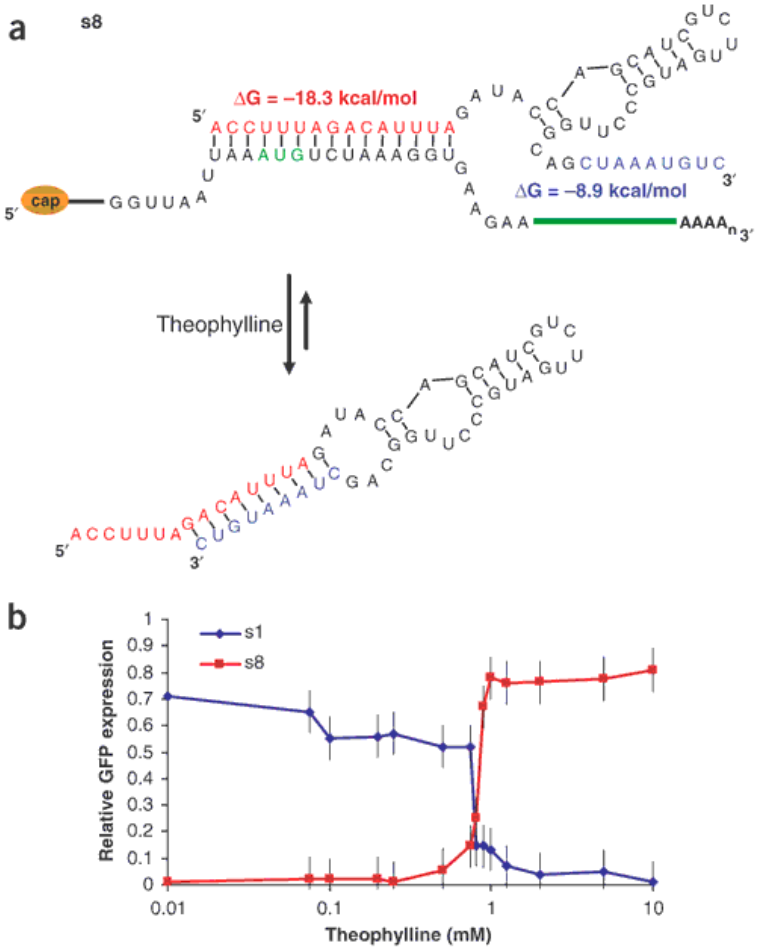




- Tune switching behavior by altering the thermodynamic properties of the antiswitch
- several antiswitches with varying antisense and aptamer stem stabilities
- increased antisense stem stability → higher concentrations of theophylline required and v.v.
- s5: antiswitch with an aptamer domain having a tenfold lower affinity to theophylline than s1
- s7: antiswitch with tetracycline aptamer based on s1

→ modularity of antiswitches

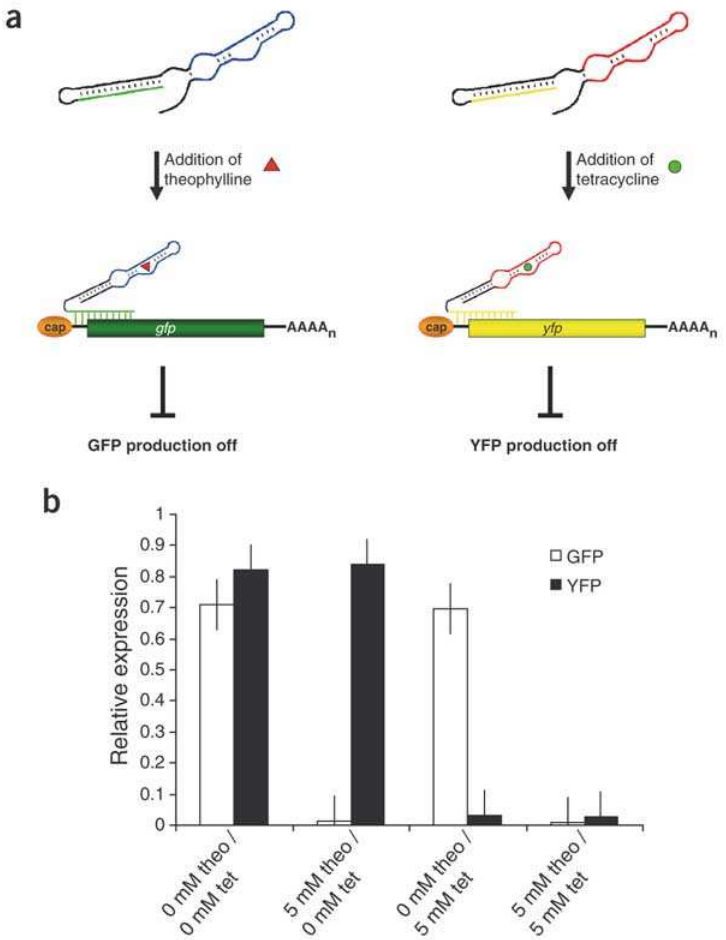
Results: redesign and characterization of an ‘on’ antiswitch regulator



•In the absence of theophylline the antiswitch is ‘on’ or the antisense domain is free to bind its target

→ Flexibility of antiswitch platform and generallity of design themes

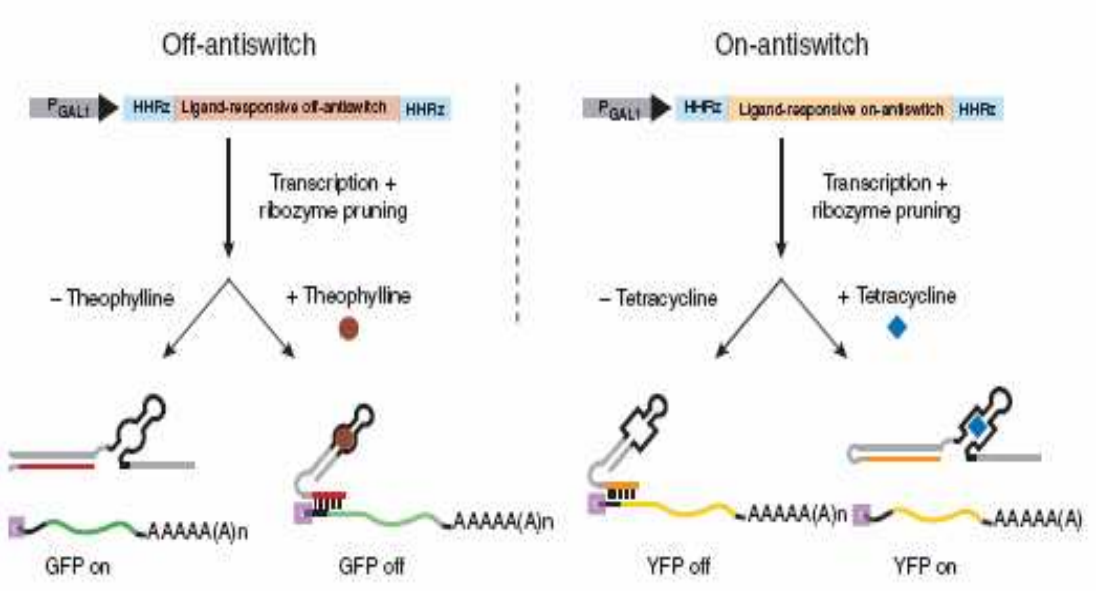
Results: simultaneous regulation of multiple genes through multiple antiswitch regulators



- antiswitches act independently of each other
- Modular nature of antiswitches allows combinatorial control over gene expression

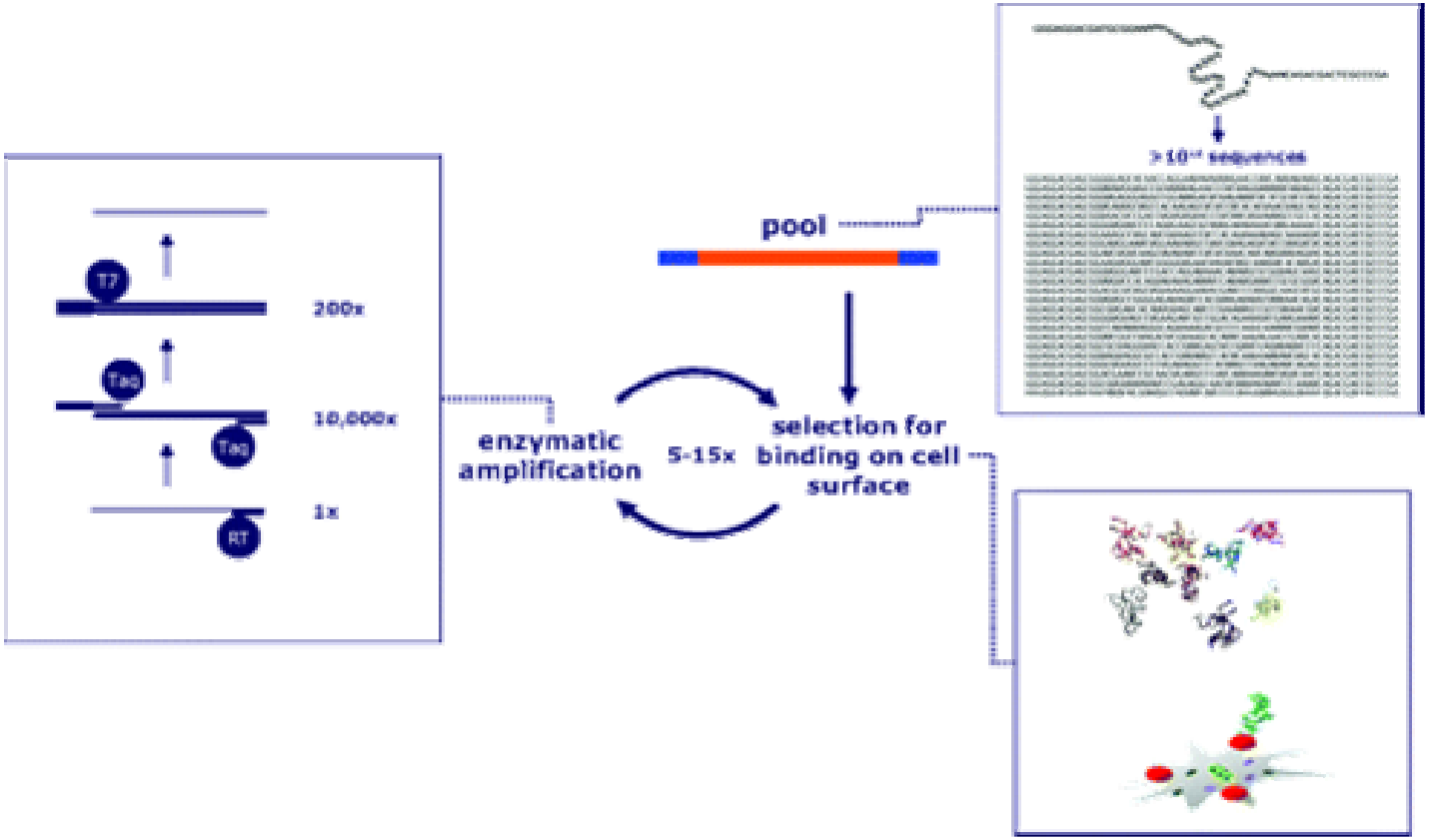
→ Illustrates the potential of building more complex genetic circuits that are precisely regulated by multiple antiswitch constructs

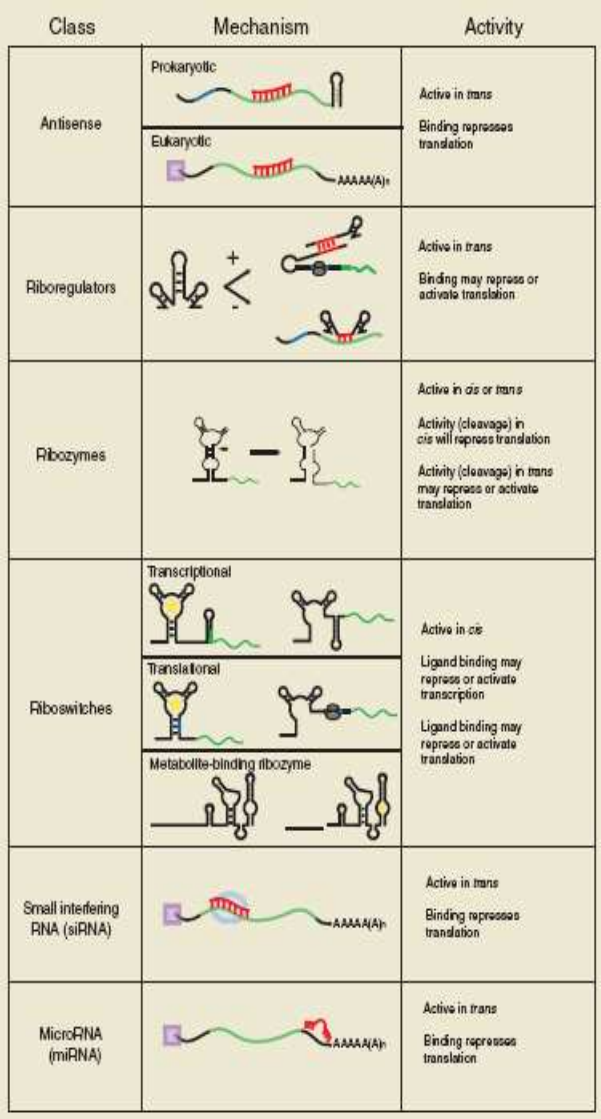
Summary



- engineered, ligand-controlled, *trans*-acting antiswitches that are allosteric regulators of gene expression
- Dual stem molecule comprised of antisense and aptamer stem
- positive and negative control possible

SELEX


T7
200x
Taq
10,000x
Taq
1x
RT
enzymatic amplification
5-15x
pool
selection for binding on cell surface



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