

# Programmable ligand-controlled ribo regulators of eukaryotic gene expression

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# Introduction

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- importance of noncoding *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)

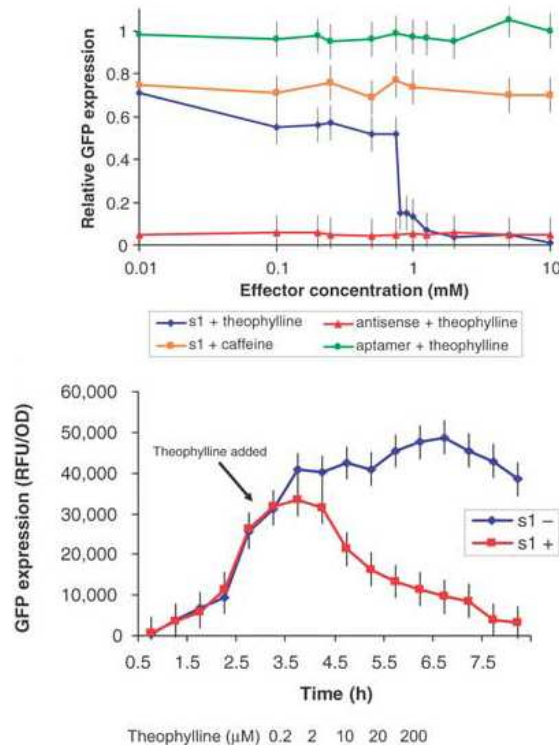
# Aptamers

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- 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 exponential enrichment)



# Results: functional activity of antiswitch regulator



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

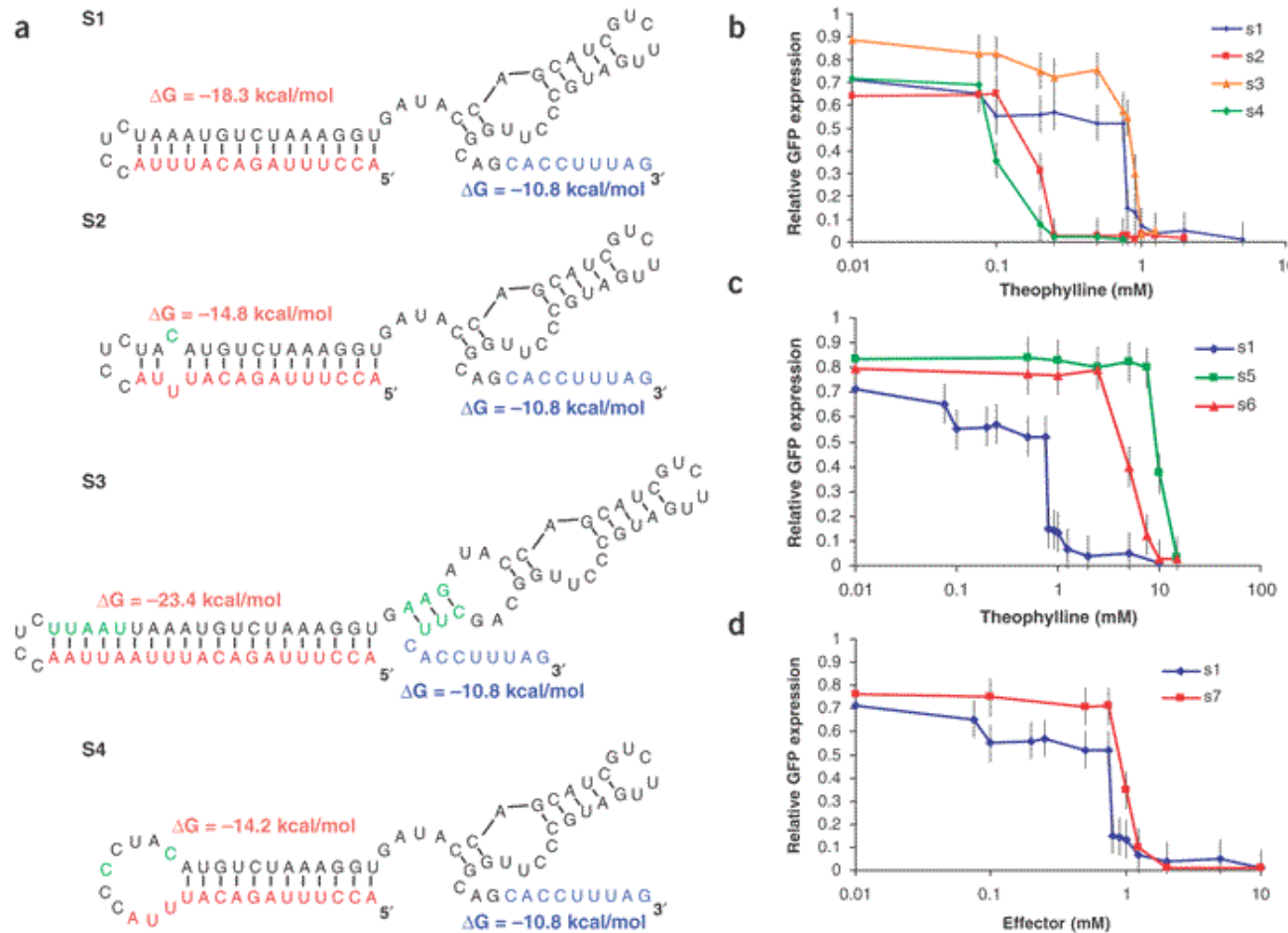
- the aptamer used in this antiswitch does not bind caffeine, which differs from theophylline by a single methyl group

- temporal response 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

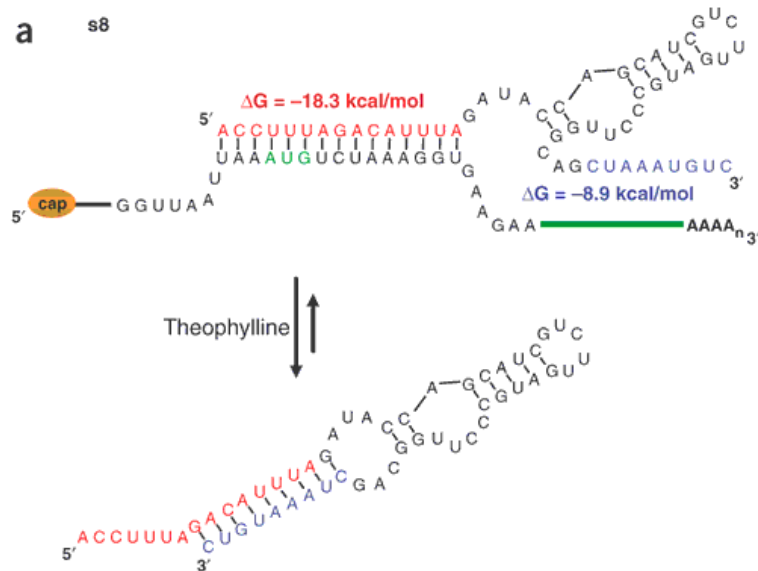
# Results: tuning and expanding the switch response of an antiswitch regulator



- Tune switching behavior by altering the thermodynamic properties of the antiswitch
- several antiswitches with varying antisense and aptamer stem stabilities
- increased antisense stem stability  $\rightarrow$  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

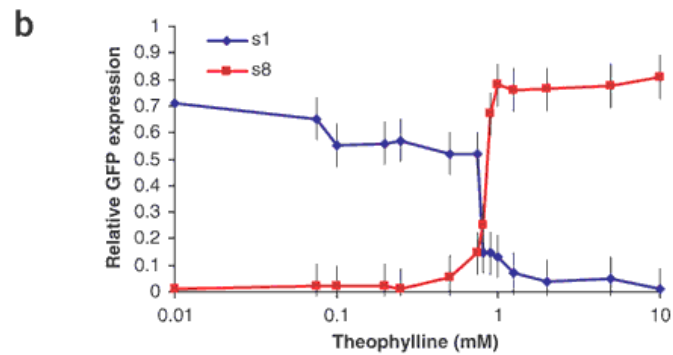
$\rightarrow$  modularity of antiswitches

# Results: redesign and characterization of an 'on' antiswitch regulator

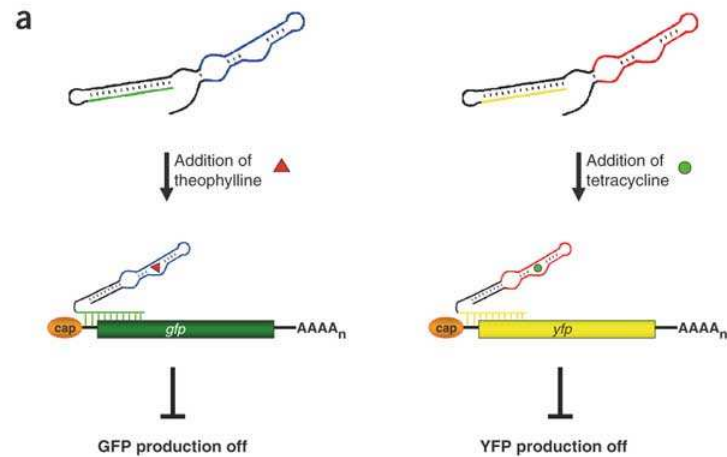


• In the absence of theophylline the antiswitch is 'on' or the antisense domain is free to bind its target

→ Flexibility of antiswitch platform and generality of design themes

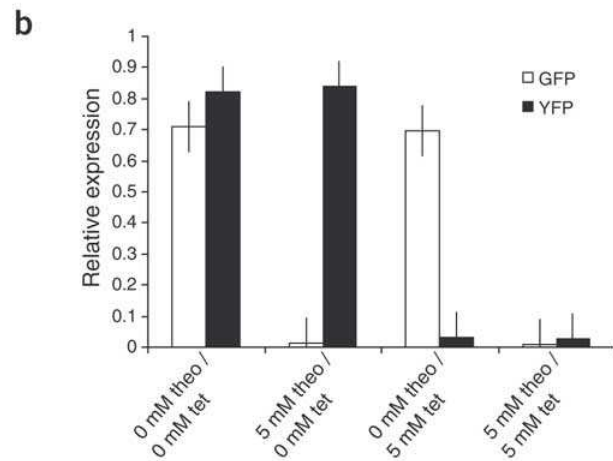


# Results: simultaneous regulation of multiple genes through multiple antiswitch regulators

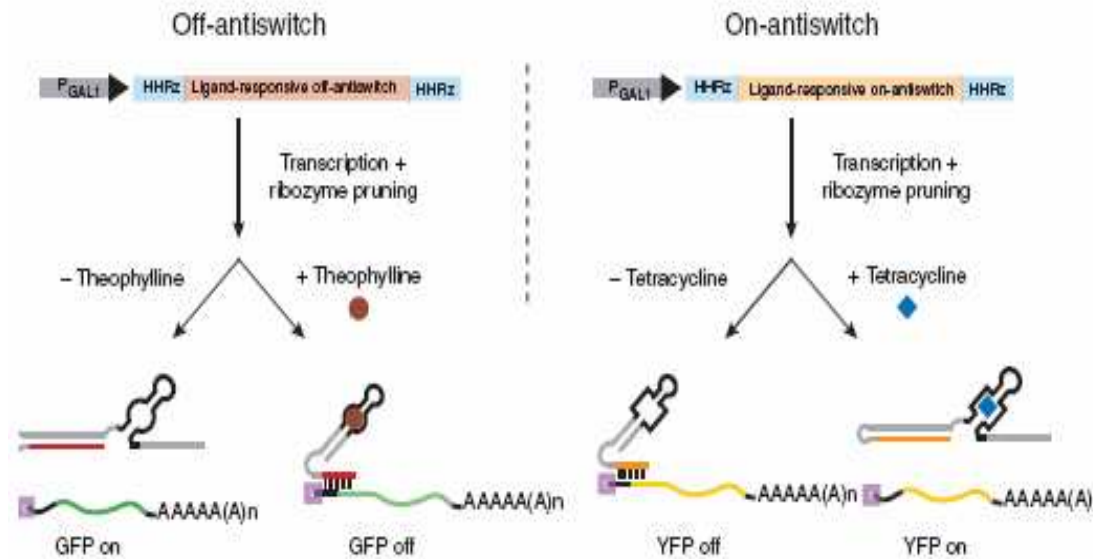


- antiswitches act independently of each other
- Modular nature of antiswitches allows combinatorial control over gene expression

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



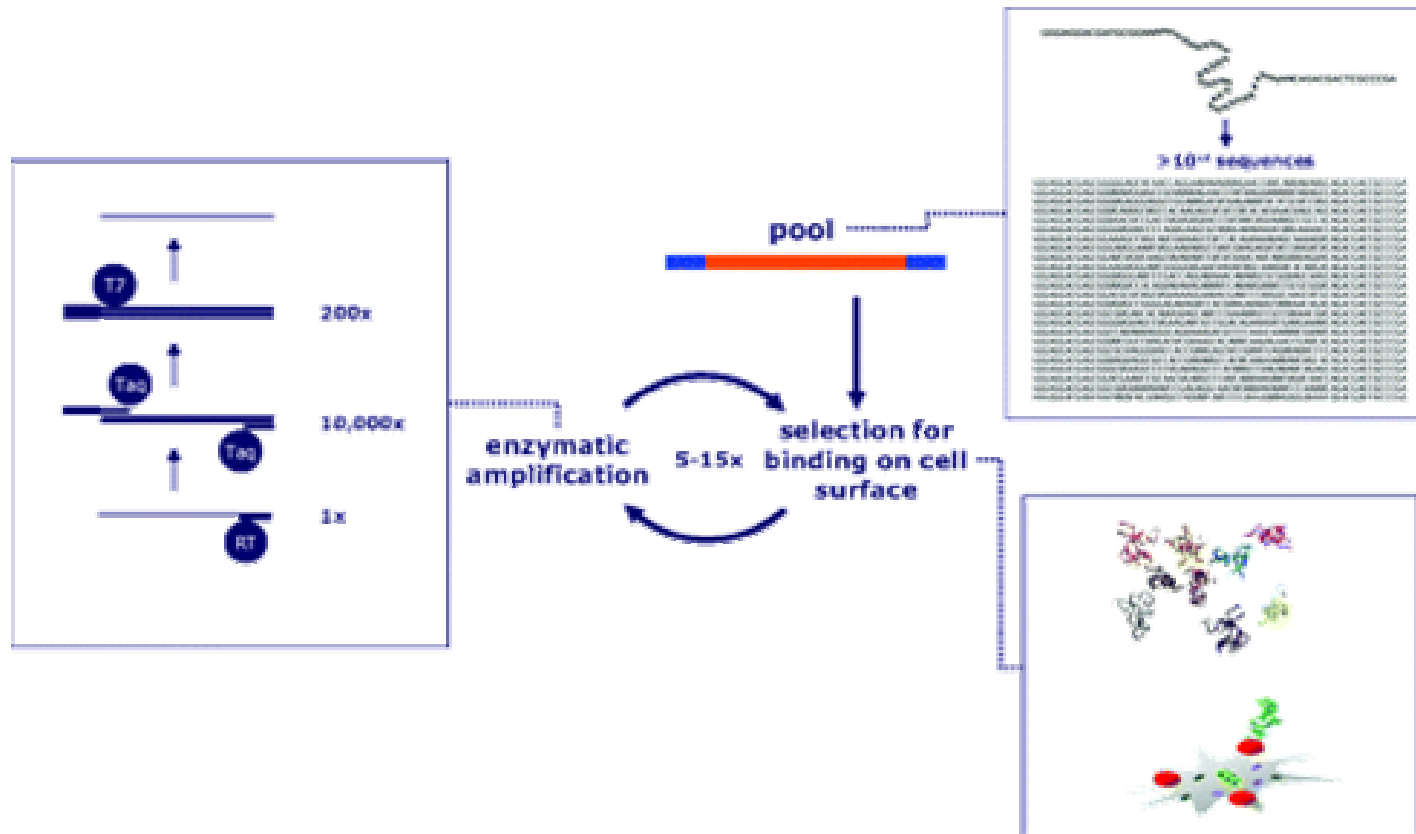
# Summary



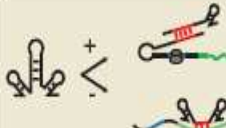
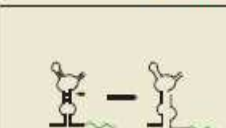







- engineered, ligand-controlled, *trans*-acting antiswitches that are allosteric regulators of gene expression
- Dual stem molecule comprised of antisense and aptamer stem
- positive and negative control possible



# SELEX



Class	Mechanism	Activity
Antisense	Prokaryotic 	Active in trans Binding represses translation
	Eukaryotic 	Active in trans Binding represses translation
Riboregulators		Active in trans Binding may repress or activate translation
Ribozymes		Active in cis or trans Activity (cleavage) in cis will repress translation Activity (cleavage) in trans may repress or activate translation
Riboswitches	Transcriptional 	Active in cis Ligand binding may repress or activate transcription
	Translational 	Ligand binding may repress or activate translation
	Metabolite-binding ribozyme 	Ligand binding may repress or activate translation
Small interfering RNA (siRNA)		Active in trans Binding represses translation
MicroRNA (miRNA)		Active in trans Binding represses translation

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