Tuning Noise in Global Gene Expression

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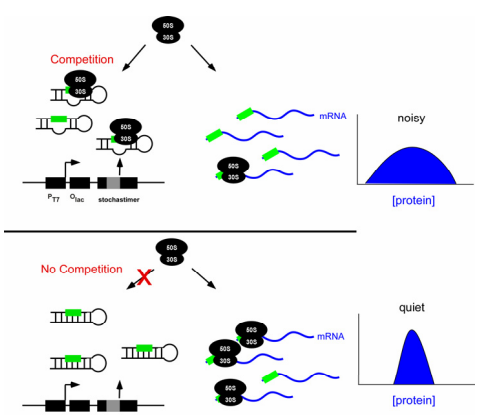
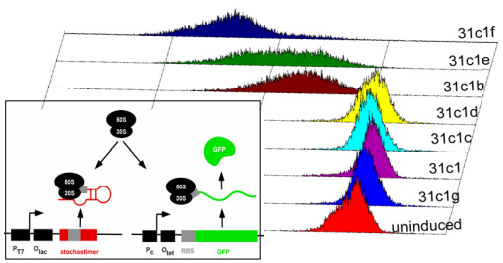
Introduction:

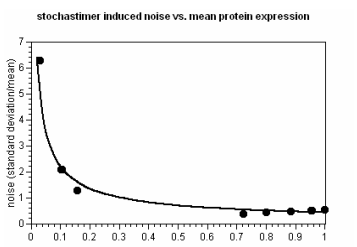
Gene expression is a probabilistic process subject to uncontrollable fluctuations which arise mostly from the small numbers of active molecules involved (1-7). This stochasticity, or noise, in gene expression has frequently been hypothesized to be dictated by mRNA fluctuations and to be affected by the concentration and state of general cellular machinery such as nucleic acid polymerases and ribosomes. Following, it has been mathematically modeled that expression levels of genes situated within operons should be more highly correlated than those of genes which are independently transcribed (6). We have engineered RNAs, which we term "stochastimers" to fine-tune global translational capacity in *Escherichia coli*. As a result, we have tuned global noise in gene expression as well as absolute levels of global protein production. We find that noise varies proportional to the inverse square root of average gene expression as translational capacity is decreased. This phenotype may be explained by finite number effects (7). We have also verified Swain's model that genes within operons are more highly correlated (6), and have further demonstrated that they are robust to large amounts of artificial noise.



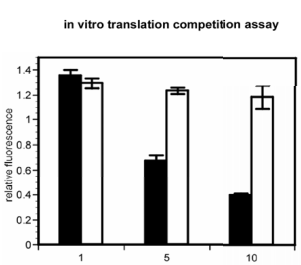
Stochastimer mechanism (above). Stochastimers are small translatable RNAs with variably accessible ribosome binding sites (RBSs). Variants with unpaired RBSs (top) readily associate with cellular ribosomes, titrating translational machinery. This reduces the probability of any given cellular mRNA being translated, broadening the distribution of expression of that gene over a population of genetically identical cells. Stochastimers engineered to have less accessible (more base-paired) RBSs (bottom) do not readily associate with ribosomes, resulting in a more wild-type or "quiet" expression profile. The availability of the RBS can be fine-tuned by simple engineering of base-pairing.

Results:





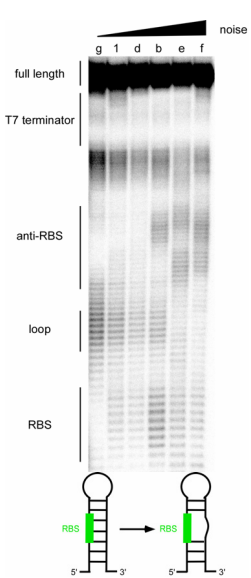
Stochastimers tune global noise and protein expression levels. (Above, top) Expression of stochastimers with increasingly available RBSs (near to far) result in increasingly noisy expression of an independently expressed cellular reporter, GFP. *E.coli* cultures were grown overnight from frozen stocks, diluted 1:100 in 100uM IPTG to induce stochastimer expression, grown to early log phase (OD ~0.1) and treated with 200ng/mL anhydrotetracycline (aTc) to induce GFP expression. 4 hours later cultures were scanned for GFP fluorescence by flow cytometry. (Above, bottom) Global protein expression and noise are inversely correlated, with noise varying as the inverse square root of protein level. The data are fit well by a negative power function, implicating finite-number effects (7), with numbers of ribosomes, translational machinery, and mRNAs potentially all becoming very small.

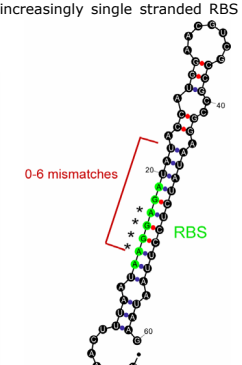


Stochastimers directly compete for translation machinery. To verify that stochastimers directly compete for translational machinery, in vitro translation reactions were performed on a GFP template with increasing amounts of an unpaired (black bars) and paired (white bars) stochastimer. The unpaired, noisy stochastimer significantly inhibits translational capacity, while the paired, quiet stochastimer has little effect on translation. Error bars represent one standard deviation, and data are normalized to a GFP only control.

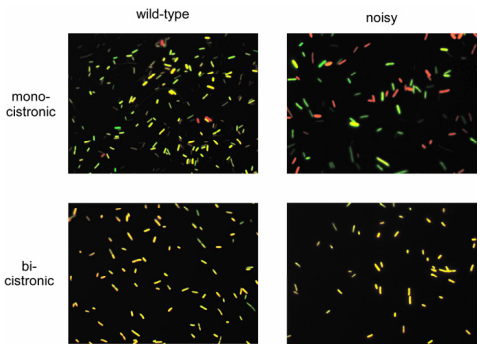
Construct	designed bulges	ΔG	normalized GFP expression	noise (SD/mean)	in vitro translation capacity
31.c1g	0	-18.8	1	0.46	1.18
31.c1	2	-13	0.96	0.49	
31.c1c	3	-9.9	0.80	0.41	
31.c1d	4	-8	0.72	0.36	
31.c1b	4	-6.1	0.16	1.26	
31.c1e	6	-4.9	0.11	2.05	
31.c1f	6 (AGGA bulged)	-8.8	0.03	6.27	0.39

Stochastimer design correlates well with structure and function. (Table, above) Stochastimer variants with increasing numbers of designed bulges in the RBS region, and decreasing overall helix stability (ΔG) reduce expression of a global reporter (GFP) and increase genetic noise. (Below) In-line probing, a structure mapping method which allows elucidates the tendency for specific nucleobases along an RNA polymer to be single or double stranded was used to visualize the structures of stochastimer constructs. In this method the RNA is transcribed in vitro, the 5'-end is radioactively labeled, and the RNA is incubated in divalent cation containing buffer allowing spontaneous degradation to occur. Nucleobases within the RNA polymer which are single stranded are free to sample an "in-line" geometry which encourages spontaneous hydrolysis at that residue. Double-stranded nucleobases are conformationally restricted, discouraging in-line geometry, and dramatically reducing the amount of spontaneous cleavage. When separated by polyacrylamide gel electrophoresis (PAGE), residues which are more single stranded show up as radioactive bands on the gel (at a position corresponding to their distance from the 5' end of the RNA), while residues which are more double stranded do not. A stochastimer which was designed to have a completely double stranded RBS (31c1g, 1st lane), shows almost no degradation in that region, or the corresponding anti-RBS region. As mispairs are designed, stochastimers show increasingly single stranded RBS and anti-RBS regions (left to right).



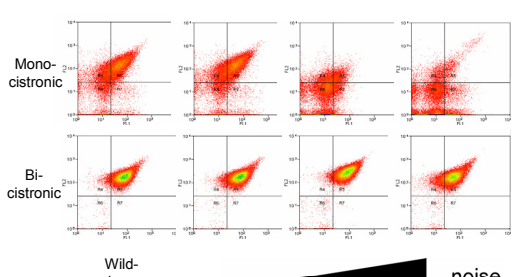


Stochastimer anatomy (above). A stochastimer variant with a completely double stranded RBS, 31.c1g (shown) was used as a template for engineering variants with increasingly unpaired RBSs. Residues marked with blue stars are most critical for ribosome binding. This RNA structure was previously selected in our lab based on the ability to tightly occlude its RBS from ribosome accessibility.



Operons are genetic noise insulators:

Bicistronic encoding, or the encoding of two genes on a single mRNA transcript, has modeled to increase the correlation (and decrease noise) in the expression of multiple genes (6). This is mostly due to a near complete reduction in relative mRNA fluctuations between the two genes. To examine this model, we are employing the use of the CFP/YFP system developed by Elowitz and coworkers (4). We have made use of the MIT Registry of Standard Biological Parts (http://parts.mit.edu) to construct a polycistronic CFP/YFP expression cassette (BBa_J13004) as well as a monocistronic version in which the two genes are independently transcribed from identical promoters in opposite directions. Our results verify Swain's model that 2 genes encoded on a single mRNA have significantly increased correlation (above, below). We have also used stochastimers to demonstrate that bicistronic genes are extremely tolerant to large amounts of artificial genetic noise (above, below).



References:

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