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Beyond Promoters: How Genes Tweak Their Own Expression

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Abstract

The correct expression of genes is vital for cells to function. Schikora-Tamarit *et al.* show that, in addition to obeying their promoters, most genes can modulate their own expression by either buffering or amplification. This could help to avoid costly overexpression of proteins.

As the afternoon progresses, more and more cars leave the city. Highway traffic intensifies accordingly, until the lanes cannot accommodate the influx of vehicles, and beaming brakelights announce the formation of traffic jams that last until sunset. At night, traffic flow reflects again the intent of drivers to leave the city. This daily story sounds familiar to all New Yorkers – but they also know that each day is different. The time when daily congestion starts and stops depends on road construction, bridge closures, lane openings, accidents, or police on the highways.

In the same way as highway traffic adds to the economy, gene expression produces proteins – the movers and shakers of living cells that are responsible for vital tasks, from basic metabolism to sensing and responding to external stimuli. However, sometimes too much of a good thing is bad for you: the cellular energy and resource costs exceed the benefits when certain proteins are overproduced. It is therefore essential for a cell to carefully control protein amounts inside it.

Common wisdom holds that regulation (increase or decrease) of gene expression stems from the binding of transcription factor (TF) proteins to the promoter region directly in front of the gene. Thus gene expression should faithfully reflect promoter activity. However, what if other factors could play a role, decoupling expression from promoter activity similarly to afternoon traffic congestion? Schikora-Tamarit *et al.* [1] sought to answer this question by combining synthetic biology and mathematical modeling approaches. They switched the original promoters and 5' ends of 42 native genes in *S. cerevisiae* with identical inducible synthetic promoters, and fused an mCherry reporter to the native gene. They then interrogated the responses of each gene relative to a known mCherry/GFP baseline for the same promoter driving only the two fluorescent reporters. Thus, they ensured that the promoter inputs to all 42 genes and control were exactly identical, as if releasing an identical car influx onto multiple highways. Despite this, the expression of most genes deviated from

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the mCherry/GFP baseline. Specifically, promoter activity amplification (PAA) or buffering (PAB) occurred when expression rose faster or slower than expected, respectively. Overall, Promoter Activity Buffering or Amplification (PABA) seems pervasive among yeast genes, with only rare exceptions. Switching 3'-end sequences revealed that PABA could originate both in coding or non-coding regions.

Considering that feedback is well-known to reinforce or suppress input effects in natural and engineered systems, autoregulation was the logical first candidate mechanism for PABA, with PAB and PAA resulting from negative and positive feedback, respectively. Indeed, a previously constructed negative feedback system [2] showed PAB as expected. However, another negative feedback-related feature (gene dosage-dependence) was not observed for native genes, suggesting other mechanisms, such as regulation of mRNA stability by other proteins. If such regulators were scarce then they could become depleted and unable to control the rising mRNA levels. Thus, PAB could arise from depleting positive (RNA-stabilizing) regulators, acting like gas stations that stabilize highway traffic by preventing vehicles from running out of fuel – but exceedingly high car influx can still cause fuel shortages, with dire consequences for traffic flow. PAA would arise conversely, from depleting negative (RNA-destabilizing) regulators (Figure 1). Mathematical models confirmed this as a feasible possibility, in accordance with experimental results. Interestingly, although distinct from direct autoregulation, this 'regulator titration' mechanism actually corresponds to indirect autoregulation: regulators bind to mRNA, reducing the regulator pool, and the more mRNA there is, the fewer regulators are available to control mRNA levels. Beyond this regulator-based mechanism, other sources of indirect feedback with similar effects could include RNA interference or even protein level-dependent cellular growth and dilution rates.

A growing body of work suggests another intriguing but even simpler possibility without regulators. Physicists studying the Totally Asymmetric Exclusion Process (TASEP) [3] found a jamming transition to high polymerase or ribosome density when the initiation rate exceeds the threshold that elongation and termination can accommodate. In addition, slow codon variants [4] and transcriptional pausing [5] can trigger molecular traffic jams to further impede translational and transcriptional speeds, respectively, in the same way as roadworks and accidents congest traffic. Polymerases and ribosomes will then synthesize less and less mRNA or protein, failing to obey promoter input, resulting in PABA relative to other genes. Considering the pervasiveness of alternative codon usage and transcriptional pausing, PABA might be ubiquitous across biology.

Finally, the authors ask why organisms would employ such a mechanism. One possibility is to avoid excess levels of costly proteins. Indeed, removal of PAB by eliminating negative feedback caused a large fitness defect. Overexpressing five of 12 additional proteins had similar effects, indicating that expression fine-tuning is generally important. PAB would allow the cell to retain high promoter sensitivity but also reduce this cost by moderating/preventing toxicity from excessive promoter activity. These findings highlight how protein sequence-altering point mutations barely scratch the surface of the sea of molecular changes with fitness effects and evolutionary relevance, including synonymous, non-coding, and large-scale sequences alterations.

One exciting aspect to further explore is gene expression noise [6]. Noise measurements might distinguish between the multiple possible PABA mechanisms. For example, fluctuations in ribosome or polymerase density and output might peak at critical initiation rates that are specific for each gene, unlike for feedback regulation. It would be interesting to examine protein levels in single cells at increasing induction levels, and look for PABA imprints in gene expression noise, with implications for allegedly promoter-determined transcriptional bursting [7] or noise-dependent cellular phenotypes [8]. Another future direction would be to switch out sequences subject to mRNA-level regulation or slow codons so as to pinpoint sequences and mechanisms responsible for PABA. Would there be a correlation between particular combinations of base pairs and PABA, for instance?

Whatever the mechanism responsible for PABA, the Carey laboratory has characterized sources of gene expression modulation beyond promoter effects. These findings will give synthetic biologists more control over the circuits they engineer. Even more importantly, these results highlight how synthetic biology and mathematical modeling can resolve longstanding biological puzzles and give fundamental insights. Without fixing the transcriptional input of each gene by using identical promoters, the contributions of coding and 3'-end sequences would have been very difficult to unravel. After shedding light on the predictability of network evolution [9] and biological pattern formation [10], this new use of synthetic promoter constructs heralds the growing potential of synthetic gene circuits as innovative research tools to inform other fields of biology.

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Figure 1. Experimental Setup and Mechanisms of PABA (Promoter Activity Buffering or Amplification)

(A) Schikora-Tamarit *et al.* created a 42-member yeast strain library, replacing in each strain the promoter of a native gene with the same synthetic promoter and mCherry, ensuring identical 5' tagging and promoter input to all genes. A strain with the same synthetic promoter controlling mCherry alone served as control. Regulatory proteins that increase or decrease mRNA stability are shown in black and grey, respectively. (B) Expression plots (protein amounts) of three tagged genes versus mCherry alone illustrate possible mechanisms of PABA. No PABA in these plots means a simple shift or no change, whereas PAA implies a steeper response, while PAB implies a flatter response to high induction (and promoter input) relative to mCherry alone. The insets above and below the plot show two possible PABA mechanisms at induction levels 1 and 2. The top, as postulated by the

authors, consists of regulatory proteins either stabilizing or destabilizing the mRNA, resulting in higher/lower amounts of protein than the control for PAA/PAB, respectively. The bottom insets illustrate the TASEP (Totally Asymmetric Exclusion Process) mechanism, which is due to polymerases or ribosomes forming more or fewer traffic jams along the DNA/RNA strands compared to the control.