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NEWS AND VIEWS

Suppressing variation in synthetic circuits

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One of the challenges in engineering biological devices is to precisely control the number of parts in each cell. Automobile engineers do not worry about control mechanisms for variation in the number of wheels on a car—if an engineer designs a car with four wheels, then all the cars roll off the assembly line with four wheels. But biology is dynamic, and biological circuits change continuously. Therefore, synthetic biologists are faced with the challenge that the number of DNA, RNA, and protein components of their devices may vary between cells, possibly by several folds. Are there simple methods for ensuring that stoichiometric changes do not affect the proper functioning of the device? In a recent *Molecular Systems Biology* article, Bleris *et al* (2011) address this question by identifying useful circuits that can make biological output insensitive to DNA dosage.

Although the network of interactions between the components of a cell is dazzlingly complex, biological networks contain smaller, recurrent subnetworks called motifs (Milo *et al*, 2002). Motifs often convey useful properties, such as altering the response time or magnitude of a propagated signal. A particular motif found in many biological contexts is a three-node motif called a Type I incoherent feed-forward loop (I1-FFL; Figure 1A; Mangan and Alon, 2003). In this motif, an 'input' node activates both an 'output' node and an 'auxiliary' node. The auxiliary node also directly regulates the output, but through an inhibitory connection.

Depending on the parameters governing the interactions of the nodes, I1-FFLs can show a wide range of behaviors. Various naturally occurring or synthetic incoherent feed-forward loops have been observed to act as pulse generators (Basu *et al*, 2004), fold-change detectors (Goentoro *et al*, 2009), or to speed up signaling responses (Mangan *et al*, 2006). Theoretical analysis (Ma *et al*, 2009) revealed that incoherent motifs can also confer adaptive properties, returning outputs to a basal level of activity following perturbation. Additionally, it was shown mathematically that the combination of positive and negative regulation on a node, such as that which occurs in an incoherent feed-forward loop, is the minimal requirement to confer invariance to fluctuations in network components (Acar *et al*, 2010). Based on these mathematical analyses, Bleris and colleagues sought to show theoretically and experimentally that I1-FFLs generate robustness with respect to changes in gene copy number in synthetic circuits.

They first identified the general qualitative behavior of three types of motifs: (1) transcriptional (t) I1-FFLs, (2) post-transcriptional (pt) I1-FFLs, and (3) transcriptional autoregulatory motifs (tAMs; Figure 1B). Computational analysis revealed that I1-FFLs are capable of adapting to changes in copy number and the extent of adaptation depends on the strength of inhibition of the output by the auxiliary node. As the inhibition weakens, the adaptability breaks down, eventually leading to a linear input–output relationship.

To experimentally validate their modeling predictions, they constructed several synthetic circuits in mammalian cells. Each individual circuit was constructed on a single plasmid, which was introduced into cells by transfection. They took advantage of the fact that transient transfection naturally generates a broad distribution of transfected plasmids for a given population of cells, which leads to a broad range of circuit input. All forms of I1-FFLs showed some degree of robustness with respect to copy number changes in comparison with the tAM (Figure 1); however, the ptI1-FFLs were the most robust circuits and also achieved the highest range of output expression. By mutating circuit components, they were also able to validate their prediction that robustness decreases as the strength of the inhibition of the output by the auxiliary node decreases. Finally, analysis of the experimental data revealed that the ptI1-FFLs were significantly less noisy than the tAM and the tI1-FFL. Although modeling provided some clues as to the possible main sources of noise and noise compensation in the circuits, more work remains to be done to validate those predictions experimentally.

This study provides much support for the idea that post-transcriptional incoherent feed-forward loops may be a powerful tool for biologists, providing a mechanism to obtain a robust output regardless of copy number variations. Can this study also teach us something new about the function of natural incoherent feed-forward loops? We believe it can. While this study focused on steady-state measurements of heterogeneous populations of cells, the results also suggest that I1-FFLs can minimize fluctuations in a particular cell over time as the cellular components change dynamically. In a dividing cell, for example, such adaptation could be used as a method to ensure that circuit output is maintained at a constant level as DNA is replicated. This may be especially important in rapidly dividing cells, such as certain bacteria that can initiate multiple DNA replication events before septation occurs (Neidhardt and Umbarger, 1996). Previous work identified an overrepresentation of incoherent feed-forward loops in bacteria (Mangan *et al*, 2006). It is intriguing

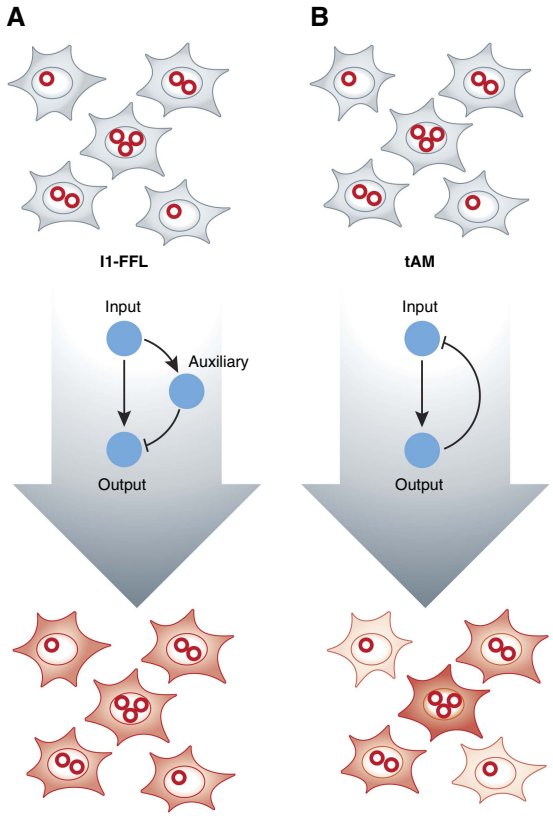

Figure 1 Type I incoherent feed-forward loops (I1-FFLs) make biological output insensitive to DNA copy number. Transient transfection generates a broad distribution of the number of transfected plasmids (red circles), causing a broad range of circuit input. (**A**) I1-FFLs suppress this variation and generate a robust output. (**B**) Transcriptional autoregulatory motifs (tAMs) generate a graded output that is proportional to the number of plasmids in each cell.

to speculate that adaptation conferred by some of these I1-FFLs may be evolutionarily advantageous. I1-FFLs may also be useful in suppressing deleterious effects arising from dramatic amplifications in cancer cells. Although Bleris and colleagues speculate that this may counteract gene amplifications, the input is not necessarily required to be the DNA itself, but could also be the concentration of a regulator (such as an overexpressed transcription factor or a hyperactive kinase) controlling the level of all parts in a particular I1-FFL. Further studies are required to test whether this is indeed the case in cancer cells. Regardless, as a result of this study, we now have a powerful tool for designing robust circuits that can adapt to, and compensate for, increased DNA amount.

Conflict of interest

The authors declare that they have no conflict of interest.

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