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SYNTHETIC BIOLOGY

Cut up to bring together

Microorganisms with engineered genetic circuits hold promise for diverse applications, including for drug synthesis or as biological sensors. Simple engineered genetic modules, such as negative-feedback transcriptional loops that result in oscillating gene expression, are well established. However, the integration of multiple modules into controllable, more complex bionetworks is a major challenge. A new study uses competition for proteolysis to enable the rapid and customizable coupling of genetic circuits.

Prindle *et al.* used *Escherichia coli* strains engineered with a range of transcriptional gene modules that express reporter genes. They sought to temporally couple the expression of green fluorescent protein (GFP) and cyan fluorescent protein (CFP) reporter gene modules that were present in the same cells but that were driven by different inducible promoters. Previous attempts to synchronize such modules have used crosstalk at the transcriptional or translational levels, but have resulted in a substantial lag time (~30 minutes) between peaks of expression of the two reporters. Instead, the authors engineered Leu-Ala-Ala sequences into the carboxy-termini of both fluorescent proteins so that they competed for proteolytic degradation by the *E. coli* ClpXP protease. This coupling at the post-translational level resulted in tight synchronization of expression (~1-minute difference between GFP and CFP expression peaks).

The investigators then turned to more complex gene modules in which the promoter drives a negative-feedback regulator in addition to the fluorescent reporter gene, which results in oscillating gene expression. An oscillating GFP module — which had components derived from the intercellular quorum-sensing system that were also Leu-Ala-Ala tagged — successfully conferred colony-wide oscillating expression on the previously non-oscillating CFP module described above. Interestingly, this coupling could be 'tuned': engineering Thr-Ser spacers before the Leu-Ala-Ala tag in CFP increased the lag time between GFP and CFP expression peaks.

To investigate interactions between Leu-Ala-Ala-tagged oscillating gene modules with distinct properties and kinetics, Prindle *et al.* studied the quorum-sensing module and an alternative, more rapidly oscillating module based on the Lac repressor (LacI). When combined in the same cells, expression from the modules became coupled: the LacI-based module slowed so that its major expression peaks coincided with those of the quorum-sensing module, although smaller and faster oscillations of the LacI-based module still occurred during periods of low expression of the quorum-sensing module. These findings indicate that complex expression dynamics can be encoded into the coupled genetic circuits. Finally, by additionally using a redox signalling module to reduce transcriptional noise, the researchers tightened the coupling further so that it extended spatially over multiple bacterial colonies.

Such module coupling is likely to facilitate the generation of larger bionetworks for biotechnological applications.

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ORIGINAL RESEARCH PAPER Prindle, A. *et al.* Rapid and tunable post-translational coupling of genetic circuits. *Nature* **508**, 387–391 (2014)