Cell

Synthetic Organisms Simplify Biology

You've probably heard the cliché "life is complicated." Maybe you have even picked up Marie Kondo's best-selling book *The Life-Changing Magic of Tidying Up* or clicked on a link to a self-help article promising to guide you toward the recreation of a simpler you by cutting out excess baggage—the things you probably don't really need to maintain your desired lifestyle. Perhaps some of these things are unnecessary redundancies within your home, which would only get in the way if you wanted to reconfigure the functionality of your living space.

Synthetic biologists agree that sometimes life can be unnecessarily complicated and that, for scientific applications in controlled environments, simpler single-cell organisms can be more useful than naturally occurring strains. To this end, a recent paper by Jason Chin and colleagues reports the latest milestone achievement in this exciting field of genetic minimalization, which pushes the limits of what we might think is biologically possible (Fredens et al., 2019). These scientists create a simpler, unnatural version of *E. coli* in which 3 of the 64 naturally occurring codons used across all domains of life are each replaced throughout the bacterial genome with synonymous codons from the remaining 61 (18,214 substitutions in total) to create the first ever 61-codon genome, Syn61. With the removal of these three codons (one stop codon and two for serine), three previously essential genes (for cognate tRNAs and a peptide release factor) could also now be removed. The researchers computationally designed the "compressed" genome using a refactoring and recoding approach and then completely synthesized and pieced it together about 108 kilobases at a time, to create a record-setting synthetic genome at nearly 4 million bases in size.

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Refactoring, a term borrowed from the world of software engineering, refers the process of rewriting existing code without changing its functional output. But in biology, cutting out "excess" bits of the genome is tricky business. Genomes are so densely coded with information that stretches of DNA are often multifunctional, as in the case of overlapping open reading frames (ORFs). By changing even a few bases of DNA, you could produce multiple effects. Thus, genetic refactoring was required by the Chin group to dissociate overlapping ORFs so that subsequent codon recoding would be precise. Synthetic biologists can also use refactoring to eliminate non-essential regulatory and feedback elements, to produce simplified organisms that can be more easily manipulated and controlled. Recently, a refactoring approach was used by Tom Ellis and colleagues to re-engineer the widely studied yeast mating response pathway in *S. cerevisiae* and produce a broadly applicable, cell-based G protein-coupled receptor biosensor platform, easily reprogrammable for diverse applications (Shaw et al., 2019). Both yeast and bacteria, especially *E. coli*, which has long been considered the workhorse of molecular biology, are ideal starting points for the design of customized, living factories for the manufacturing of all kinds of products. A noteworthy example is the engineering of cells capable of completely synthesizing cannabinoids and their analogs from galactose, for pharmaceutical applications (Luo et al., 2019). The Ellis group is part of a large, collaborative, global effort across more than a dozen research labs to synthesize the entire *S. cerevisiae* genome, which will yield yet another milestone for the field.

It's often hard to define what's necessary for life in a given environment. For most genomes, the functions of every base are not completely understood. Researchers have become fairly adept at identifying the protein-coding regions of genes, but many non-coding regions are also necessary for unknown reasons. Even for the protein-coding regions, context often matters since it affects the level and nature of protein expression. To determine whether the native genomic context matters for specific stretches of DNA, synthetic biologists have developed some strategies. An example recently used by Beat and Matthias Christen and colleagues in *C. crescentus* required re-introduction of synthetic essential DNA back into native cells episomally and subsequent transposon mutagenesis (Venetz et al., 2019). If the synthetic DNA, removed from its natural context, was still able to contribute its essential function to the cell, the previously essential (and previously immutable) native copy of the DNA could then be mutagenized. Further highlighting our limited understanding of the genome, refactored and recoded organisms often do not grow as robustly as their parental strains. For example, Syn61 doubles 1.6× slower than its parental strain MDS42 (Fredens et al., 2019). But as with naturally occurring organisms that have been transferred to laboratory settings, scientists can also reoptimize

unnatural strains using adaptive laboratory evolution (Choe et al., 2019).

The recovery of robust functionality is important because the end goal of synthetic biology is usually to produce a more easily manipulatable strain for downstream applications. Poor performance could render the new synthetic organism useless. A minimal organism that performs well would provide not only an easier genome to work with but also one with newly freed up space or features, where exogenous modules then could be engineered back in. The tRNAs Chin and colleagues render no longer necessary in Syn61 (Fredens et al., 2019) could potentially be repurposed to recognize an unnatural codon. The field is getting closer to creating a functional genetic code that incorporates unnatural nucleobases with another recent milestone achievement. Steven Brenner and colleagues have created a synthetic eight-letter (hachimoji) DNA molecule, where complementary unnatural bases (B, S, P, and Z) base paired with one another alongside the naturally occurring bases (A, T, G, and C), forming stable, structurally sound double helices, which could then be faithfully transcribed to eight-letter RNA (Hoshika et al., 2019). The codon sequence themselves that are freed up by the Chin group because they are no longer used for serines or the amber stop (Fredens et al., 2019) could also be repurposed. This idea of codon repurposing has been recently demonstrated by Paul Jaschke and colleagues (Vincent et al., 2019). They took a compressed *E. coli* strain also lacking amber stop codons and repurposed the UAG codon as a novel, unique initiation codon by introducing an engineered tRNA that has a CUA anticodon and recruits N-terminal methionine. This amber initiator can be used to control a synthetic protein translation system that is orthogonal to the native AUG system. Repurposed codons and engineered tRNAs could also be used to incorporate unnatural amino acids in a site-specific way.

Genetically reduced organisms can of course also be used to discover new biology. Just as the exercise of removing unnecessary items from your cluttered home will help you refocus on the things in your life that matter most to you, genetic reduction provides a simpler starting point for molecular biologists to identify essential genes and their functions. Using a genetically reduced strain of *M. mycoides capri*, the first live organism to be controlled by an entirely synthetic genome, Zaida Luthey-Schulten and colleagues developed a computer model to simulate the cell's entire metabolic network, a feat that would have been much more difficult had they started with the native organism (Breuer et al., 2019). Using available biochemical data, candidate metabolic genes were identified and associated with specific cellular reactions to construct the *in silico* network. The model nominated both essential and non-essential candidate genes for further removal, many of which were verified experimentally by transposon mutagenesis. Interestingly, the model suggests genes that could be simultaneously removed, whereas transposon mutagenesis is only able to probe the individual essentiality of genes. Discrepancies between predicted and experimentally observed essentiality provided new hypotheses concerning, for example, the existence of yet-to-be-identified genes encoding specific metabolic activities. These clues, provided by computer simulations, will direct the pursuit of even simpler lifeforms.

As synthetic biologists continue to reduce genomes and decrease the number of unknowns therein, they move us closer and closer to the day when scientists will truly be able to build an organism from the bottom up and easily reconfigure it with fewer surprises along the way. As the excess baggage is left behind, these efforts will thrust forward very practical, game-changing advances in biomanufacturing, biosensing, information storage, medicine, and beyond.

REFERENCES

Breuer, M., Earnest, T.M., Merryman, C., Wise, K.S., Sun, L., Lynott, M.R., Hutchison, C.A., Smith, H.O., Lapek, J.D., Gonzalez, D.J., et al. (2019). Essential metabolism for a minimal cell. eLife *8*, e36842.

Choe, D., Lee, J.H., Yoo, M., Hwang, S., Sung, B.H., Cho, S., Palsson, B., Kim, S.C., and Cho, B.K. (2019). Adaptive laboratory evolution of a genome-reduced Escherichia coli. Nat. Commun. *10*, 935.

Fredens, J., Wang, K., de la Torre, D., Funke, L.F.H., Robertson, W.E., Christova, Y., Chia, T., Schmied, W.H., Dunkelmann, D.L., Beránek, V., et al. (2019). Total synthesis of Escherichia coli with a recoded genome. Nature *569*, 514–518.

Hoshika, S., Leal, N.A., Kim, M.J., Kim, M.S., Karalkar, N.B., Kim, H.J., Bates, A.M., Watkins Jr., N.E., SantaLucia, H.A., Meyer, A.J., et al. (2019). Hachimoji DNA and RNA: A genetic system with eight building blocks. Science *363*, 884–887.

Luo, X., Reiter, M.A., d'Espaux, L., Wong, J., Denby, C.M., Lechner, A., Zhang, Y., Grzybowski, A.T., Harth, S., Lin, W., et al. (2019). Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. Nature *567*, 123–126.

Shaw, W.M., Yamauchi, H., Mead, J., Gowers, G.F., Bell, D.J., Öling, D., Larsson, N., Wigglesworth, M., Ladds, G., and Ellis, T. (2019). Engineering a Model Cell for Rational Tuning of GPCR Signaling. Cell *177*, 782–796.e27.

Venetz, J.E., Del Medico, L., Wölfle, A., Schächle, P., Bucher, Y., Appert, D., Tschan, F., Flores-Tinoco, C.E., van Kooten, M., Guennoun, R., et al. (2019). Chemical synthesis rewriting of a bacterial genome to achieve design flexibility and biological functionality. Proc. Natl. Acad. Sci. USA *116*, 8070–8079.

Vincent, R.M., Wright, B.W., and Jaschke, P.R. (2019). Measuring Amber Initiator tRNA Orthogonality in a Genomically Recoded Organism. ACS Synth. Biol. *8*, 675–685.

Andrew J. Rennekamp