RESEARCH ARTICLE SUMMARY

SYNTHETIC BIOLOGY

Design and synthesis of a minimal bacterial genome

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INTRODUCTION: In 1984, the simplest cells capable of autonomous growth, the mycoplasmas, were proposed as models for understanding the basic principles of life. In 1995, we reported the first complete cellular genome sequences (*Haemophilus influenza*, 1815 genes, and *Mycoplasma genitalium*, 525 genes). Comparison of these sequences revealed a conserved core of about 250 essential genes, much smaller than either genome. In 1999, we introduced the method of global transposon mutagenesis and experimentally demonstrated that *M. genitalium* contains many genes that are nonessential for growth in the laboratory, even though it has the smallest genome known for an autonomously replicating cell found in nature. This implied that it should be possible to produce a minimal cell that is simpler than any natural one. Whole genomes can now be built from chemically synthesized oligonucleotides and brought to life by installation into a receptive cellular environment. We have applied whole-genome design and synthesis to the problem of minimizing a cellular genome.

RATIONALE: Since the first genome sequences, there has been much work in many bacterial models to identify nonessential genes and define core sets of conserved genetic functions, using the methods of comparative genomics. Often, more than one gene product can perform a particular essential function. In such cases, neither gene will be essential, and neither will necessarily be conserved. Consequently, these approaches cannot, by themselves, identify a set of genes that is sufficient to constitute a viable genome. We set out to define a minimal cellular genome experimentally by designing and building one, then testing it for viability. Our goal is a cell so simple that we can determine the molecular and biological function of every gene.

RESULTS: Whole-genome design and synthesis were used to minimize the 1079–kilobase pair (kbp) synthetic genome of *M. mycoides* JCVI-syn1.0. An initial design, based on collective knowledge of molecular biology in combination with limited transposon mutagenesis data, failed to produce a viable cell. Improved transposon mutagenesis methods revealed a class of quasi-essential genes that are needed for robust growth, explaining the failure of our initial design. Three more cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kbp, 473 genes). Its genome is smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 has a doubling time of ~180 min, produces colonies that are morphologically similar to those of JCVI-syn1.0, and appears to be polymorphic when examined microscopically.

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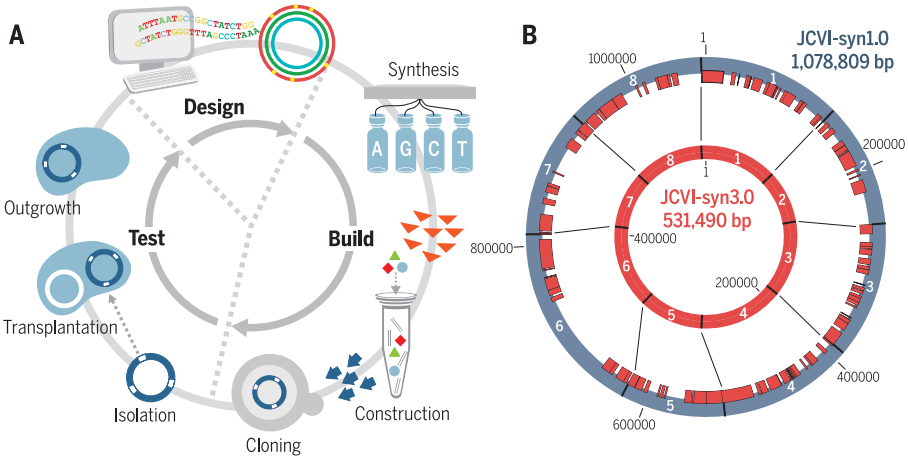
CONCLUSION: The minimal cell concept appears simple at first glance but becomes more complex upon close inspection. In addition to essential and nonessential genes, there are many quasi-essential genes, which are not absolutely critical for viability but are nevertheless required for robust growth. Consequently, during the process of genome minimization, there is a trade-off between genome size and growth rate. JCVI-syn3.0 is a working approximation of a minimal cellular genome, a compromise between small genome size and a workable growth rate for an experimental organism. It retains almost all the genes that are involved in the synthesis and processing of macromolecules. Unexpectedly, it also contains 149 genes with unknown biological functions, suggesting the presence of undiscovered functions that are essential for life. JCVI-syn3.0 is a versatile platform for investigating the core functions of life and for exploring whole-genome design. ■



Four design-build-test cycles produced JCVI-syn3.0. (**A**) The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis. (**B**) Comparison of JCVI-syn1.0 (outer blue circle) with JCVI-syn3.0 (inner red circle), showing the division of each into eight segments. The red bars inside the outer circle indicate regions that are retained in JCVI-syn3.0. (**C**) A cluster of JCVI-syn3.0 cells, showing spherical structures of varying sizes (scale bar, 200 nm).

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