Making a Synthetic Cell

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Using a computer analogy, one can think of a cell's cytoplasm as the hardware and the genome as the operating system. A synthetic cell is created by synthesizing a genome and installing it into a recipient cytoplasm. Original components of the recipient cytoplasm are replaced in early divisions and the synthetic cell takes on a phenotype determined by the synthetic genome. In the future, we expect that scientists will be able to design synthetic cells to make a variety of useful products.

We have chosen *Mycoplasma genitalium* as our model for a synthetic cell because it is the smallest known bacterium that can grow independently in the laboratory. Its genome is a single circular DNA molecule, 580kb in size. It has 485 protein coding genes and 43 RNA coding genes. Using transposon mutagenesis, we have shown that many as 100 of the protein coding genes are dispensable one at a time (1).

To make a synthetic *M. genitalium genome,* 101 DNA overlapping cassettes were made that completely spanned the natural *M. genitalium* genome. We incorporated identifying sequence tags (watermarks) in six cassettes to distinguish our synthetic chromosome. The 101 cassettes were assembled up to quarter genome size in three steps using an in vitro assembly reaction. The final assembly was accomplished in yeast using yeast recombination and the complete circular genome was propagated as a yeast centromeric plasmid (Ycp) (3, 4).

To make the synthetic cell, we must transplant the *M. genitalium* genome from yeast into a recipient mycoplasma cytoplasm so as to displace the recipient chromosome and substitute the synthetic one. However, *M. genitalium* grows very slowly and every experiment takes many weeks. Therefore, we have chosen to work out conditions for transplantation using fast growing *M. mycoides* LC cells as donor and closely related *M. capricolum* cells as recipient. We have successfully obtained transplants by isolating *M. mycoides* DNA in agarose plugs and adding it to *M. capricolum* cells in the presence of 5% PEG and calcium chloride. We typically obtain around 100 transplants per microgram of genomic donor DNA (2).

To make a synthetic cell, we must transplant DNA out of yeast. To determine conditions for this, we cloned *M. mycoides* LC DNA into yeast as a Ycp. We isolated the *M. mycoides* LC DNA from yeast in agarose plugs but were unable initially to obtain transplants into *M. capricolum*. *M. mycoides* LC and *M. capricolum* share a common restriction system. However, when *M. mycoides* LC DNA is propagated in yeast it becomes unmodified and is thus subject to cleavage by the recipient cells. We obtained transplants both by disrupting the restriction gene in *M. capricolum* or by methylating the yeast-grown donor DNA using cell extracts. We are currently working on conditions for transplanting *M. genitalium*.

1. J. Glass et al, Proc Natl Acad Sci U S A. **103**, 425-30, 2006
2. C. Lartigue et al, Science **317,** 632-38, 2007.
3. D. Gibson et al, Science **319,** 1215-20, 2008 and PNAS **109**, 20404-9, 2008.
4. D. Gibson et al, *Nat Methods.* 6, 343-5, 2009.