December 22, 2020

Microbiology

The inanimate building-blocks for a living synthetic cell

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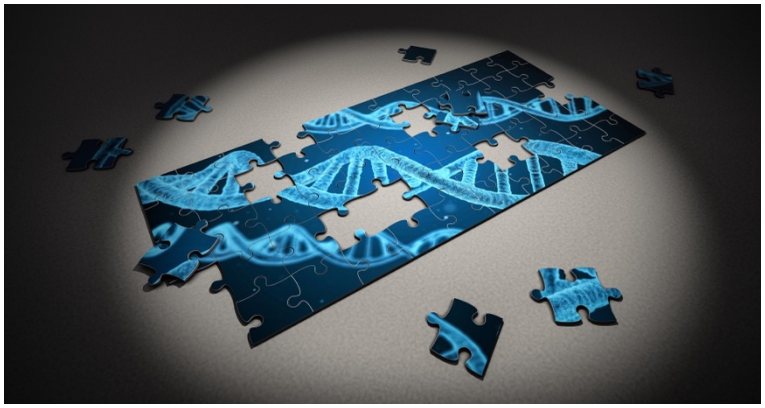
This Break was edited by Max Caine, Editor-in-chief - TheScienceBreaker

One of the most significant synthetic biology goals is the development of artificial lifelike structures that can reproduce themselves. One aspect of this is the self-replication of genomes that encode the blueprint of the whole system. We have now succeeded in reconstructing critical parts of this process in test tubes.



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In the field of "bottom-up" synthetic biology, we aim to build lifelike systems from inanimate building blocks. From this approach, we hope to gain deeper insights into the fundamental processes of life and develop new technological innovations. One such significant and possibly the most remarkable breakthrough would be creating an artificial cell that can reproduce itself. However, many steps are necessary to reach this goal. As an important milestone towards this achievement, we recently succeeded in developing a bottom-up system that can partially self-replicate.

Both DNA replication and protein synthesis are fundamental to the self-preservation and reproduction of cells. Accordingly, both often co-occur in nature. Unexpectedly, however, it is difficult to combine both processes in a test tube (in vitro). Earlier studies found that fine-tuning of some reagents enables both processes to work in parallel outside of cells. With this approach, it was possible to achieve in vitro self-replication of small DNA genomes. Nevertheless, to build a minimal cell, hundreds of genes comprising a genome of at least 113 kilobase pairs (kb) are required.

Our new system enables the replication of such large genomes. We achieved this by combining hundreds of different molecules at just the right amounts. Like in a real cell, a protein that replicates DNA (called DNA polymerase protein) is first synthesized from its encoding gene. This process is also called translation because the genetic code is translated into proteins. Upon completion, the polymerase then starts to replicate the DNA – including its own gene. Fortunately, we found that DNA replication in our system is very efficient. The length of the longest self-replicating genome exceeds the 113 kb that were predicted to be sufficient to encode all the 151 genes of a minimal cell. While our system's DNA

sequence has not yet been fully adapted to encode all of these genes, it currently contains about a third of the 151 genes, including parts of the translation machinery, enzymes for primitive energy metabolism, and the polymerases required to synthesize RNA and DNA. However, we designed the genome in a modular way such that its parts, also known as plasmids, could easily be exchanged or expanded. This modularity also helped during the analysis of the replication reaction. To prove that individual plasmids were indeed replicated in vitro, we "injected" (transformed) the replication products into living bacteria. In these bacteria, they continued to propagate (this time using the bacterial DNA replication machinery) and started to confer antibiotic resistance to their new hosts. Thus, only if the replication of the plasmids in our cell-free system worked we could subsequently use them to produce antibiotic-resistant bacteria.

At last, we could start to think about the yields of protein synthesis in our system. This is important because cell proliferation does not stop at DNA replication. When cells divide, daughter cells receive copies of the genome and the proteome, the sum of all cellular proteins. This must also happen when a minimal "bottom-up" cell divides. Therefore, the next hurdle to overcome will be the regeneration of the proteome of a minimal cell. Using a method called "mass spectrometry", we could show that some proteins are already synthesized in our current system in amounts comparable to their respective input concentrations. This is an important observation because it indicates that a self-replenishing system might be feasible.

In the future, we will have to determine whether the regenerated proteins are functional. Furthermore, synthesis levels of individual proteins need to be optimized such that they double within each generation. This will be tough with our current protein yields because in vitro translation systems are generally not very powerful. However, more sophisticated energy and recycling modules might help to achieve this goal. Indeed, our system's major limitation is its inability to get rid of its waste products, which accumulate and eventually block our chemical reactions. This cessation of reactions is also known as the thermodynamic equilibrium that all chemical systems strive for, but which means death in biological systems. To avoid their demise, all living creatures must continuously absorb energy-rich substances and emit waste products. At some point, our replicator must also acquire this ability to stay "alive" significantly longer.

Many steps like these will hopefully lead to the construction of a synthetic cell one day. Such a cell would contribute much to our understanding of the phenomenon of "life" itself. Moreover, it could be used as a chassis to produce substances such as drugs or biofuels.