BioE80

This is not a normal “intro/survey” class - things are changing too quickly

We are all in this together

DE and I, and your classmates, look forward to learning from you!

Keep your eyes and ears open - chances are - you will come across thing we have never heard about

Incredibly diverse, correct?

Many things, shapes sizes, and functions….

But universal (DNA) code

If you understand that code, share blueprints, and can print DNA, you can make/engineer/tinker with all those things

#1

Things are moving quickly

RESEARCH ARTICLE

Design and synthesis of a minimal bacterial genome

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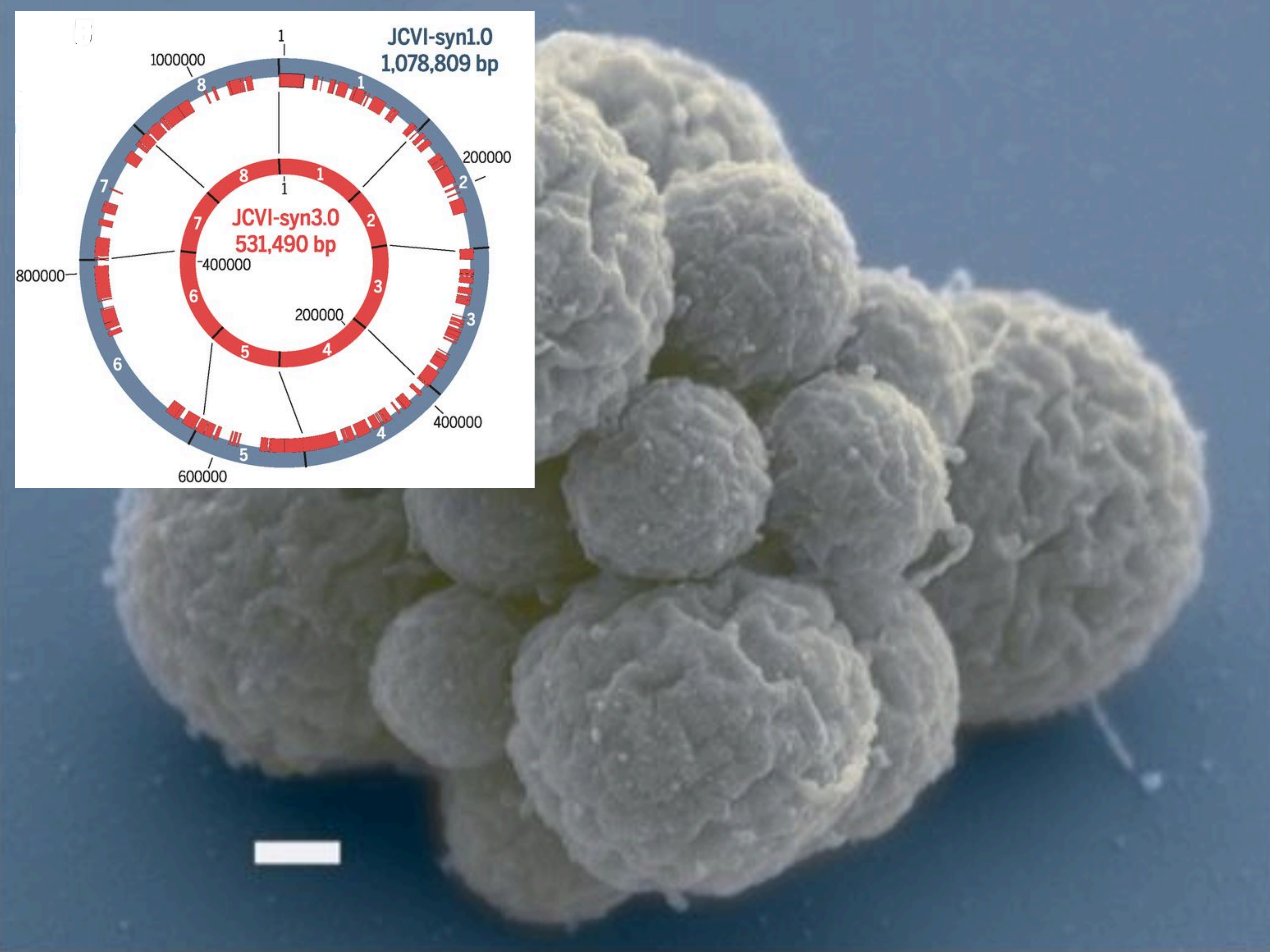
Upshot: Can make new genomes and they work!

Designing and building a minimal genome

A goal in biology is to understand the molecular and biological function of every gene in a cell. One way to approach this is to build a minimal genome that includes only the genes essential for life. In 2010, a 1079-kb genome based on the genome of *Mycoplasma mycoides* (JCV-syn1.0) was chemically synthesized and supported cell growth when transplanted into cytoplasm. Hutchison III *et al.* used a design, build, and test cycle to reduce this genome to 531 kb (473 genes). The resulting JCV-syn3.0 retains genes involved in key processes such as transcription and translation, but also contains 149 genes of unknown function.

Science, this issue p. 10.1126/science.aad6253



JCVI-syn1.0
1,078,809 bp
JCVI-syn3.0
531,490 bp
1
1000000
200000
400000
600000
800000
1
200000
400000
1
2
3
4
5
6
7
8

The Times They Are A Changin'

1990: Project was science fiction, would have cost billions and taken decades

2000: Project was technically doable but still way too expensive

2015: Project was frontier science

2017-20: More and more routine

What does this technology mean for

your kids? medicine? food?

your kitchen? art? religion? universities?

columbian drug lords? oceans?

terrorists? offshore banking?

climate?

manufacturing? your house?

language? insurance companies? law?

transportation? malaria? life expectancy?

Can make entire chromosomes

Total Synthesis of a Functional Designer Eukaryotic Chromosome

Narayana Annaluru,[1]* Héloïse Muller,[1,2,3,4]* Leslie A. Mitchell,[2] Sivaprakash Ramalingam,[1] Giovanni Stracquadanio,[2,5] Sarah M. Richardson,[5] Jessica S. Dymond,[2,6] Zheng Kuang,[2] Lisa Z. Scheifele,[2,7] Eric M. Cooper,[2] Yizhi Cai,[2,8] Karen Zeller,[2] Neta Agmon,[2] Jeffrey S. Han,[9] Michalis Hadjithomas,[10] Jennifer Tullman,[5] Katrina Caravelli,[1] Kimberly Cirelli,[1] Zheyuan Guo,[1] Viktoriya London,[1] Apurva Yeluru,[1] Sindurathy Murugan,[5] Karthikeyan Kandavelou,[1,11] Nicolas Agier,[12,13] Gilles Fischer,[12,13] Kun Yang,[2,5] J. Andrew Martin,[2] Murat Bilgel,[1] Pavlo Bohutski,[1] Kristin M. Boulier,[1] Brian J. Capaldo,[1] Joy Chang,[1] Kristie Charoen,[1] Woo Jin Choi,[1] Peter Deng,[1] James E. DiCarlo,[1] Judy Doong,[1] Jessilyn Dunn,[1] Jason I. Feinberg,[1] Christopher Fernandez,[1] Charlotte E. Floria,[1] David Gladowski,[1] Pasha Hadidi,[1] Isabel Ishizuka,[1] Javaneh Jabbari,[1] Calvin Y. L. Lau,[1] Pablo A. Lee,[1] Sean Li,[1] Denise Lin,[1] Matthias E. Linder,[1] Jonathan Ling,[1] Jaime Liu,[1] Jonathan Liu,[1] Mariya London,[1] Henry Ma,[1] Jessica Mao,[1] Jessica E. McDade,[1] Alexandra McMillan,[1] Aaron M. Moore,[1] Won Chan Oh,[1] Yu Ouyang,[1] Ruchi Patel,[1] Marina Paul,[1] Laura C. Paulsen,[1] Judy Qiu,[1] Alex Rhee,[1] Matthew G. Rubashkin,[1] Ina Y. Soh,[1] Nathaniel E. Sotuyo,[1] Venkatesh Srinivas,[1] Allison Suarez,[1] Andy Wong,[1] Remus Wong,[1] Wei Rose Xie,[1] Yijie Xu,[1] Allen T. Yu,[1] Romain Koszul,[3,4] Joel S. Bader,[2,5] Jef D. Boeke,[2,10,14]† Srinivasan Chandrasegaran[1]†

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Rapid advances in DNA synthesis techniques have made it possible to engineer viruses, biochemical pathways and assemble bacterial genomes. Here, we report the synthesis of a functional 272,871–base pair designer eukaryotic chromosome, synIII, which is based on the 316,617–base pair native *Saccharomyces cerevisiae* chromosome III. Changes to synIII include TAG/TAA stop-codon replacements, deletion of subtelomeric regions, introns, transfer RNAs, transposons, and silent mating loci as well as insertion of loxPsym sites to enable genome scrambling. SynIII is functional in *S. cerevisiae*. Scrambling of the chromosome in a heterozygous diploid reveals a large increase in a-mater derivatives resulting from loss of the *MATα* allele on synIII. The complete design and synthesis of synIII establishes *S. cerevisiae* as the basis for designer eukaryotic genome biology.

Saccharomyces cerevisiae has a genome size of ~12 megabases (MBs) distributed among 16 chromosomes. The entire genome encodes ~6000 genes of which ~5000 are individually nonessential (*1*). Which of these nonessential genes are simultaneously dispensable? While a number of studies have successfully mapped pairwise "synthetic lethal" interactions between gene knockouts, those methods do not scale well to 3 or more gene combinations because the number of combinations rises exponentially. Our approach to address this question is to produce a synthetic yeast genome with all nonessential genes flanked by loxPsym sites to enable inducible evolution and genome reduction (a process referred to as SCRaMbLEing) in vivo (*2*, *3*). The availability of a fully synthetic *S. cerevisiae* genome will allow direct testing of evolutionary questions such as "what is the maximum number of nonessential genes that can be deleted without a catastrophic loss of fitness?" and "what is the catalog of viable 3-gene, 4-gene, … n-gene deletions that survive under a given growth condition?" that are not otherwise easily approachable in a systematic unbiased fashion. Engineering and synthesis of viral and bacterial genomes have been reported in the literature (*4*–*11*). An international group of scientists, has embarked on constructing a designer eukaryotic genome, Sc2.0 (www.syntheticyeast.org), and here we report the total synthesis of the first complete designer yeast chromosome.

Yeast chromosome III, the third smallest in *S. cerevisiae* (316,617 bp), containing the *MAT* locus determining mating type, was the first chromosome sequenced (*12*). We designed synIII according to fitness, genome stability and genetic flexibility principles developed for the Sc2.0 genome (*2*). The native sequence was edited *in silico* using a series of deletion, insertion, and base substitution changes to produce the desired "designer" sequence (Figs. 1, S1, S2 and Supplementary Text). The hierarchical wet-lab workflow used to construct synIII (Fig. 2) consisted of three major steps: 1) The 750 bp "building blocks" (BBs) were produced starting from overlapping 60- to 79-mer oligonucleotides and assembled using standard PCR methods (*13*, *14*) by undergraduate students in the Build-A-Genome class at Johns Hopkins University (Fig. 2A) (*15*). The arbitrary

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REPORT

Hachimoji DNA and RNA: A genetic system with eight building blocks

Shuichi Hoshika[1,2,*], Nicole A. Leal[1,2,*], Myong-Jung Kim[1,2], Myong-Sang Kim[1], Nilesh B. Karalkar[1,2], Hyo-Joong Kim[1], Alison M. Bate...
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Most recently: Can expand alphabet

Abstract

We report DNA- and RNA-like systems built from eight nucleotide “letters” (hence the name “hachimoji”) that form four orthogonal pairs. These synthetic systems meet the structural requirements needed to support Darwinian evolution, including a polyelectrolyte backbone, predictable thermodynamic stability, and stereoregular building blocks that fit a Schrödinger aperiodic crystal. Measured thermodynamic parameters predict the stability of hachimoji duplexes, allowing hachimoji DNA to increase the information density of natural terran DNA. Three crystal structures show that the synthetic building blocks do not perturb the aperiodic crystal seen in the DNA double helix. Hachimoji DNA was then transcribed to give hachimoji RNA in the form of a functioning fluorescent hachimoji aptamer. These results expand the scope of molecular structures that might support life, including life throughout the cosmos.

Some of of you, by the time you graduate (in 2021/22), will have “fixed” and “improved” genomes of plants and animals.

Later in your careers, people in this room will design human genomes.

You will have, in your hands, unprecedented control over living things.

#2

Living matter is different

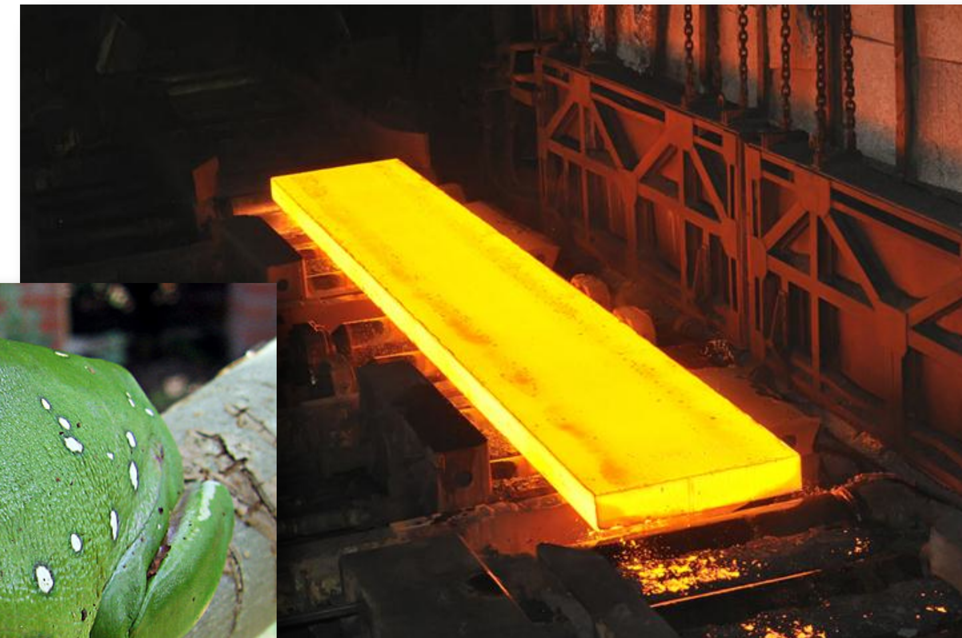
The sentence "Rose is a rose is a rose is a rose." was written by Gertrude Stein as part of the 1913 poem "Sacred Emily", which appeared in the 1922 book Geography and Plays.

"Now listen! I'm no fool. I know that in daily life we don't go around saying 'is a ... is a ... is a ...' Yes, I'm no fool; but I think that in that line the rose is red for the first time in English poetry for a hundred years."

Gertrude Stein

Living Matter

What's special about "living matter"?



What's special about "living matter"?

Lots and lots of parts;
Computation is ultra energy efficient and massively parallel

Light/air/carbon/nitrogen/water precursors

Intricately organized on multiple time and spatial scales

Self-organizing

Self-replicating

Self-healing

Each component can reproduce the whole

What's special about "living matter"?

Lots and lots of parts;
Computation is ultra energy efficient and massively parallel

Light/air/carbon/nitrogen/water precursors

Intricately organized on multiple time and spatial scales

"General facts and features"

Self-organizing

Self-replicating

Self-healing

Each component can reproduce the whole

Tasks

Build something that copies itself, perfectly, in 20 min.

Build something that can spread across the globe in 30 days.

Build something that can change the world’s climate, for free.

Build a factory that can produce the world’s most complex chemicals, with molecular fidelity, extreme purity, and chiral control, that keeps working for decades, centuries, or millennia.

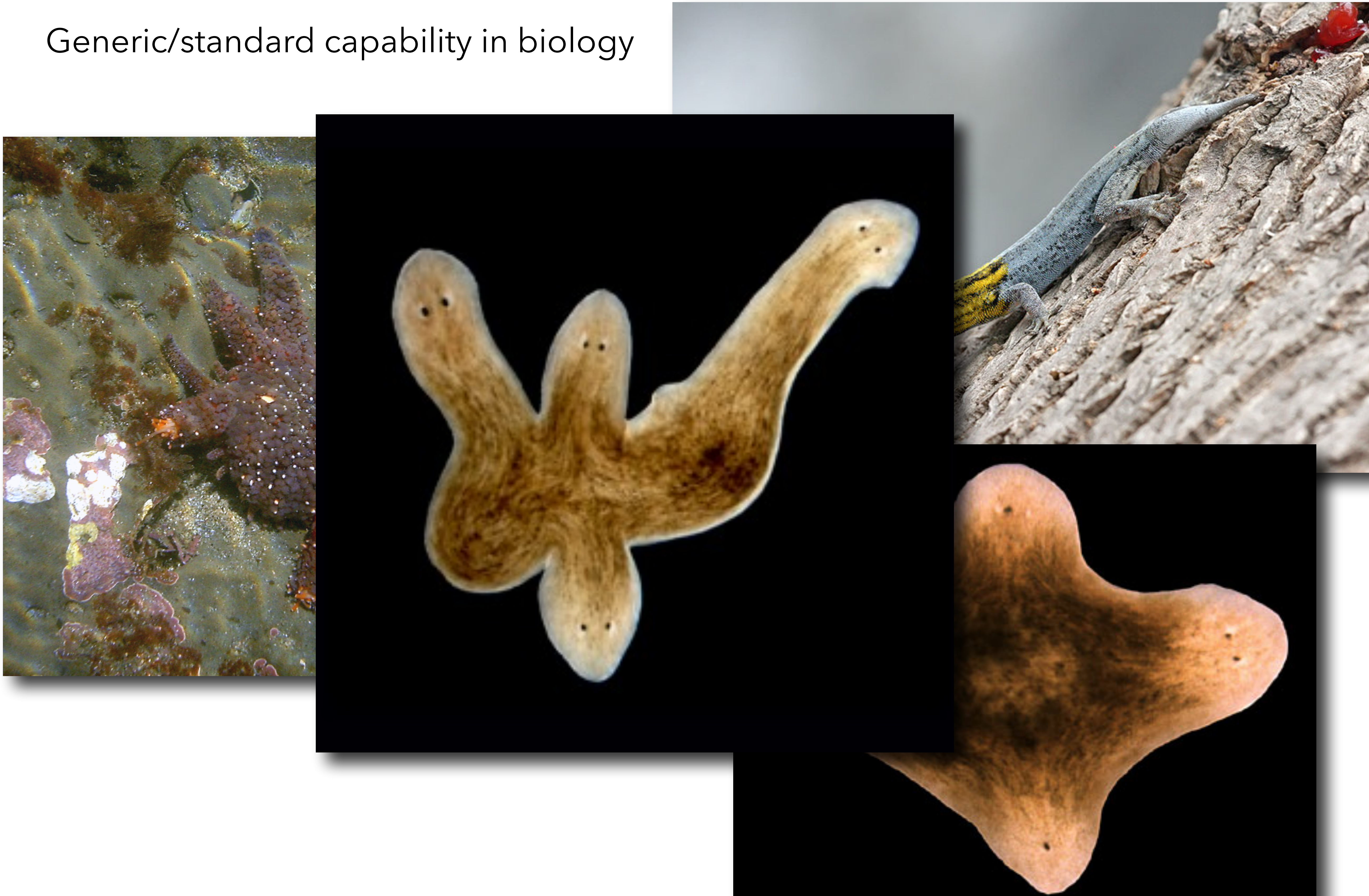
Store the library of congress on a pinhead in an indestructible manner.

These tasks, basically, sound like science fiction - but are pedestrian capabilities of living matter.

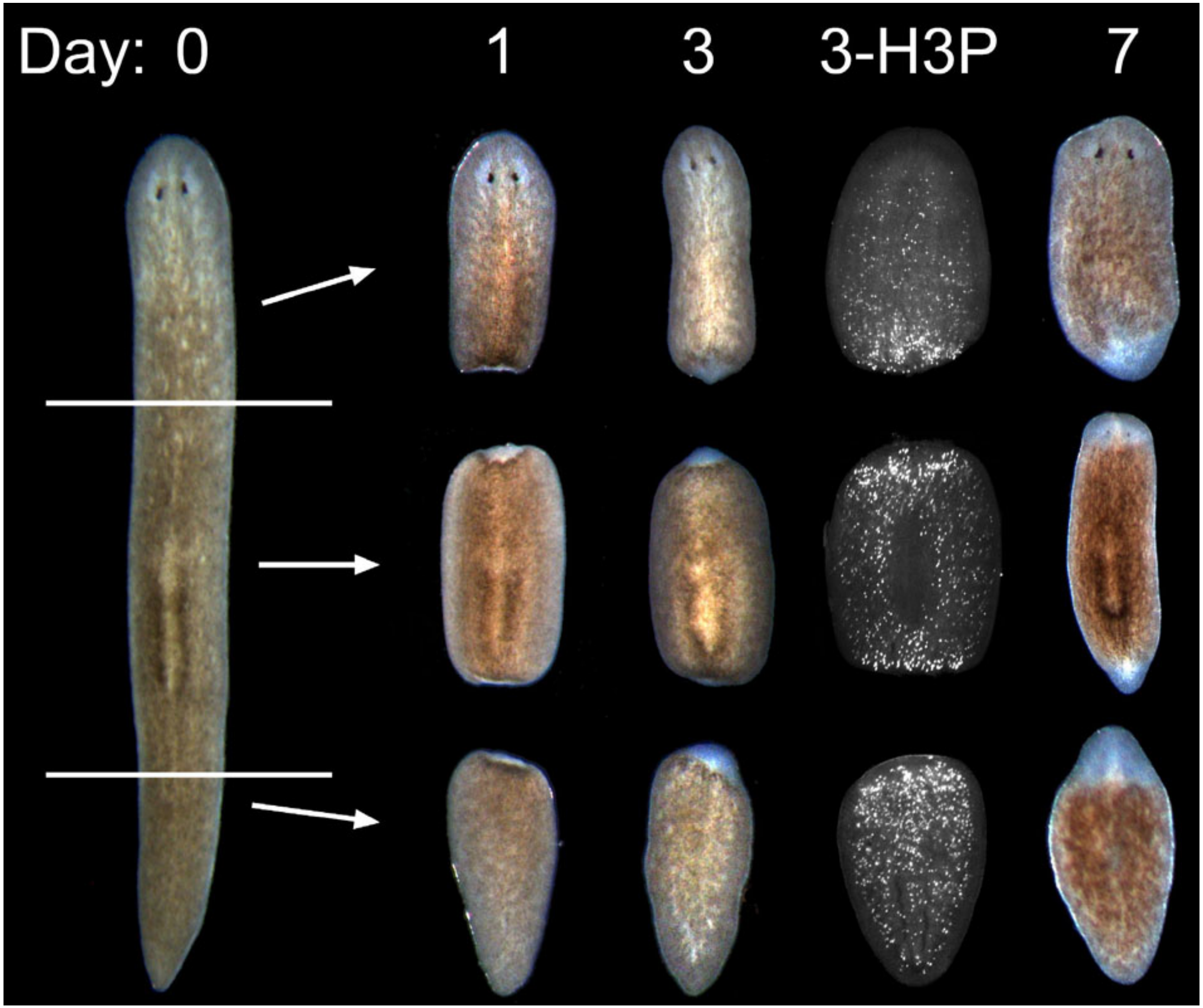
Self-replication: Bacterial cell division

Self-repair: Regeneration

Generic/standard capability in biology



Self-repair: Regeneration



http://pearsonlab.ca/images/planaria_regeneration_series.jpg

#3

Common “API”

Final thought…

Tiny number of key enabling technologies due to common blueprint:

Genome print,
CAD-life,
Encapsulation,
a trip to the grocery store…

Scared? Excited? Concerned? Surprised?

Speak up!

#4

Exercise

He Jankui told The Associated Press that he carried out his experiment to protect the twin sisters from HIV infection later in life. MARK SCHIEFELBEIN/AP PHOTO

Interesting factoid - was postdoc here at Stanford, with a BioE prof...

CRISPR bombshell: Chinese researcher claims to have created gene-edited twins

By **Dennis Normile** | Nov. 26, 2018 , 1:10 PM

HONG KONG, CHINA—On the eve of an international summit here on genome editing, a Chinese researcher has shocked many by claiming to have altered the genomes of twin baby girls born this month in a way that will pass the modification on to future generations. The alteration is intended to make the children's cells resistant to infection by HIV, says the scientist, He Jiankui of the Southern University of Science and Technology in Shenzhen, China.

The claim—yet to be reported in a scientific paper—initiated a firestorm of criticism today, with some scientists and bioethicists calling the work "premature," "ethically problematic," and even "monstrous." The Chinese Society for Cell Biology issued a statement calling the research "a serious violation of the Chinese government's laws and regulations and the consensus of the

Exercise

2019/20 - women undergoing IVF will be able to select the 'best' embryos to implant, based on a gene test. Procedure will have retail cost of $5,000 to $25,000.

2020's - women undergoing IVF will be able to fix (a small number of things) in their kid's genomes, at low risk to parent/child. May reduce disease risk. Procedure will have retail cost of $25,000 to $175,000.

You are a parent - Yes/No?

You are a world leader - Yes/No?