UNIVERSIDAD PERUANA
CAYETANO HEREDIA

BIOLOGÍA SINTÉTICA

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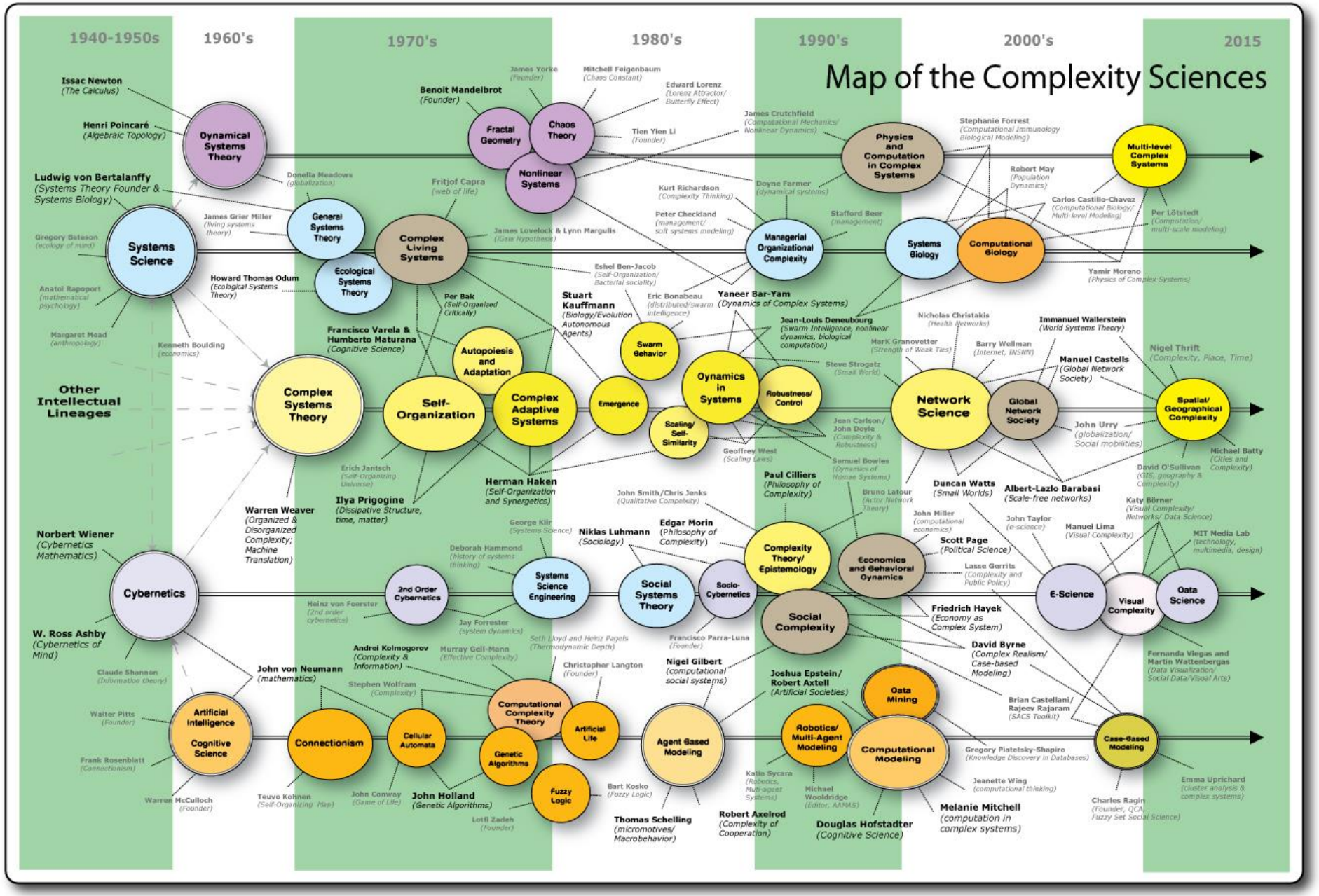
Facultad de Ciencias y Filosofía
Universidad Peruana Cayetano Heredia

- Desde hace tres décadas la Biología está experimentando una transición acelerada: Está dejando de ser una ciencia **predominantemente descriptiva**, a ser una **disciplina cuantitativa y explicativa**.


Map of the Complexity Sciences
1940-1950s
1960's
1970's
1980's
1990's
2000's
2015
Issac Newton (The Calculus)
Henri Poincaré (Algebraic Topology)
Dynamical Systems Theory
Ludwig von Bertalanffy (Systems Theory Founder & Systems Biology)
Systems Science
Gregory Bateson (ecology of mind)
Anatol Rapoport (mathematical psychology)
Margaret Mead (anthropology)
Kenneth Boulding (economics)
Other Intellectual Lineages
Donella Meadows (globalization)
James Grier Miller (living systems theory)
General Systems Theory
Howard Thomas Odum (Ecological Systems Theory)
Ecological Systems Theory
Benoit Mandelbrot (Founder)
James Yorke (Founder)
Mitchell Feigenbaum (Chaos Constant)
Edward Lorenz (Lorenz Attractor/ Butterfly Effect)
Fractal Geometry
Chaos Theory
Nonlinear Systems
Tien Yien Li (Founder)
Fritjof Capra (web of life)
Complex Living Systems
James Lovelock & Lynn Margulis (Gaia Hypothesis)
Per Bak (Self-Organized Criticality)
Francisco Varela & Humberto Maturana (Cognitive Science)
Autopoiesis and Adaptation
Complex Systems Theory
Self-Organization
Complex Adaptive Systems
Erich Jantsch (Self-Organizing Universe)
Warren Weaver (Organized & Disorganized Complexity; Machine Translation)
Ilya Prigogine (Dissipative Structure, time, matter)
Herman Haken (Self-Organization and Synergetics)
Eshel Ben-Jacob (Self-Organization/ Bacterial sociality)
Stuart Kauffmann (Biology/Evolution Autonomous Agents)
Eric Bonabeau (distributed/swarm intelligence)
Swarm Behavior
Emergence
Scaling/ Self-Similarity
Geoffrey West (Scaling Laws)
Yaneer Bar-Yam (Dynamics of Complex Systems)
Jean-Louis Deneubourg (Swarm Intelligence, nonlinear dynamics, biological computation)
Oynamics in Systems
Robustness/ Control
Jean Carlson / John Doyle (Complexity & Robustness)
Samuel Bowles (Dynamics of Human Systems)
Paul Cilliers (Philosophy of Complexity)
Bruno Latour (Actor Network Theory)
John Smith/Chris Jenks (Qualitative Complexity)
Niklas Luhmann (Sociology)
Edgar Morin (Philosophy of Complexity)
Complexity Theory/ Epistemology
Social Systems Theory
Socio-Cybernetics
Social Complexity
Economics and Behavioral Dynamics
John Miller (computational economics)
Scott Page (Political Science)
Lasse Gerrits (Complexity and Public Policy)
Friedrich Hayek (Economy as Complex System)
David Byrne (Complex Realism/ Case-based Modeling)
Brian Castellani / Rajeev Rajaram (SACS Toolkit)
Joshua Epstein / Robert Axtell (Artificial Societies)
Nigel Gilbert (computational social systems)
Agent Based Modeling
Robotics/ Multi-Agent Modeling
Computational Modeling
Data Mining
Case-Based Modeling
Gregory Piatetsky-Shapiro (Knowledge Discovery in Databases)
Jeanette Wing (computational thinking)
Melanie Mitchell (computation in complex systems)
Douglas Hofstadter (Cognitive Science)
Michael Wooldridge (Editor, AAMAS)
Katia Sycara (Robotics, Multi-agent Systems)
Robert Axelrod (Complexity of Cooperation)
Thomas Schelling (micromotives/ Macrobehavior)
Bart Kosko (Fuzzy Logic)
Fuzzy Logic
Lotfi Zadeh (Founder)
John Holland (Genetic Algorithms)
Genetic Algorithms
Artificial Life
Christopher Langton (Founder)
Computational Complexity Theory
Andrei Kolmogorov (Complexity & Information)
Murray Gell-Mann (Effective Complexity)
Seth Lloyd and Heinz Pagels (Thermodynamic Depth)
Stephen Wolfram (Complexity)
John von Neumann (mathematics)
Claude Shannon (Information theory)
Cybernetics
Norbert Wiener (Cybernetics Mathematics)
W. Ross Ashby (Cybernetics of Mind)
Artificial Intelligence
Cognitive Science
Walter Pitts (Founder)
Frank Rosenblatt (Connectionism)
Warren McCulloch (Founder)
Connectionism
Teuvo Kohnen (Self-Organizing Map)
Cellular Automata
John Conway (Game of Life)
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Heinz von Foerster (2nd order cybernetics)
Jay Forrester (system dynamics)
Deborah Hammond (history of systems thinking)
George Klir (Systems Science)
Systems Science Engineering
Francisco Parra-Luna (Founder)
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James Crutchfield (Computational Mechanics/ Nonlinear Dynamics)
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Managerial Organizational Complexity
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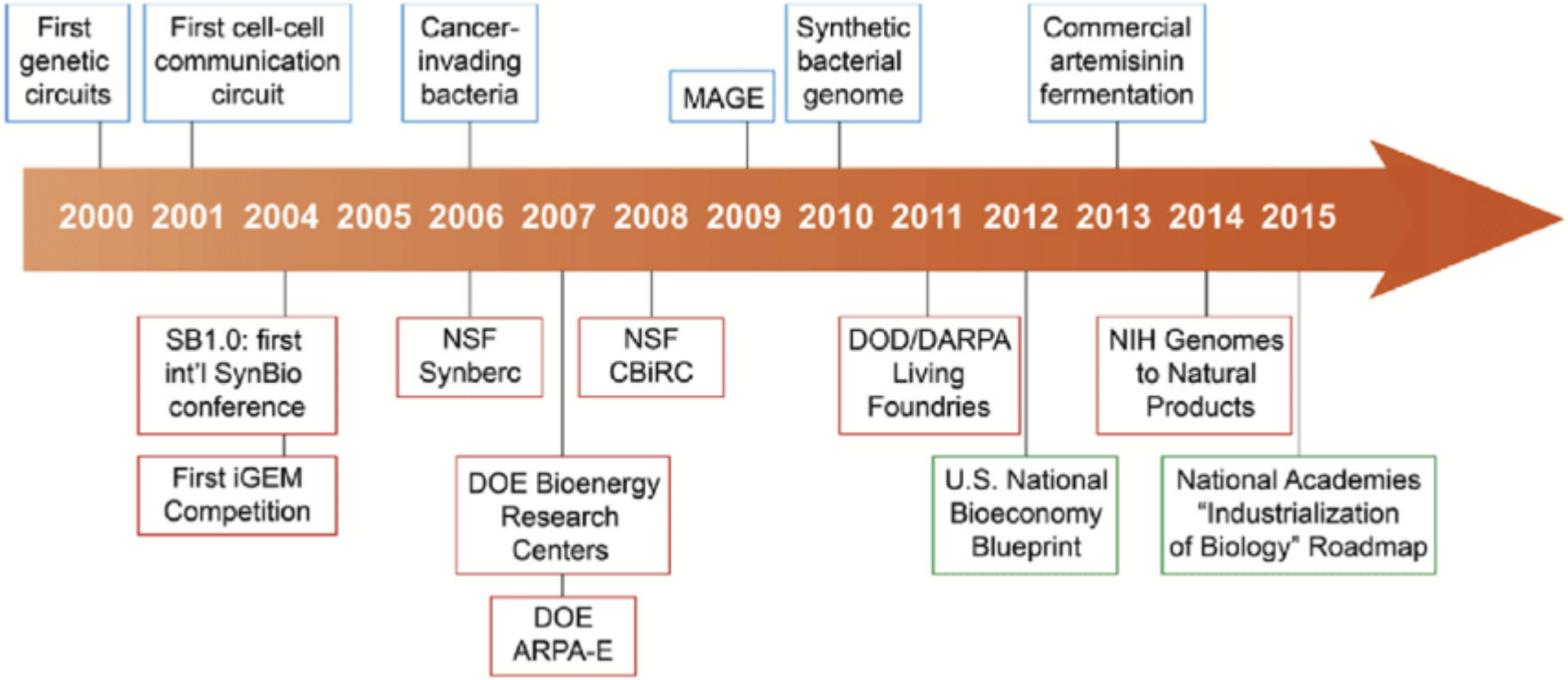
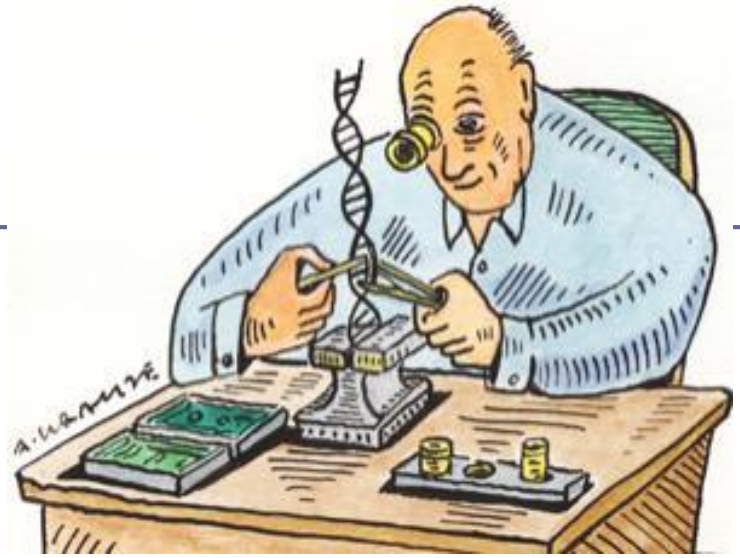
Stephanie Forrest (Computational Immunology Biological Modeling)
Robert May (Population Dynamics)
Systems Biology
Computational Biology
Carlos Castillo-Chavez (Computational Biology/ Multi-level Modeling)
Per Lötstedt (Computation/ multi-scale modeling)
Yamir Moreno (Physics of Complex Systems)
Multi-level Complex Systems
Network Science
Nicholas Christakis (Health Networks)
Mark Granovetter (Strength of Weak Ties)
Barry Wellman (Internet, INSNW)
Steve Strogatz (Small World)
Duncan Watts (Small Worlds)
Albert-Lazlo Barabasi (Scale-free networks)
Immanuel Wallerstein (World Systems Theory)
Manuel Castells (Global Network Society)
Global Network Society
John Urry (globalization/ Social mobilities)
Nigel Thrift (Complexity, Place, Time)
Spatial/ Geographical Complexity
David O'Sullivan (GIS, geography & Complexity)
Michael Batty (Cities and Complexity)
Katy Börner (Visual Complexity/ Networks/ Data Science)
John Taylor (e-science)
Manuel Lima (Visual Complexity)
MIT Media Lab (technology, multimedia, design)
E-Science
Visual Complexity
Data Science
Fernanda Viegas and Martin Wattenbergas (Data Visualization/ Social Data/Visual Arts)
Emma Uprichard (Cluster analysis & complex systems)
Charles Ragin (Founder, QCA, Fuzzy Set Social Science)


First genetic circuits
First cell-cell communication circuit
Cancer-invading bacteria
MAGE
Synthetic bacterial genome
Commercial artemisinin fermentation
2000 2001 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
SB1.0: first int'l SynBio conference
First iGEM Competition
NSF Synberc
DOE Bioenergy Research Centers
DOE ARPA-E
NSF CBiRC
DOD/DARPA Living Foundries
U.S. National Bioeconomy Blueprint
NIH Genomes to Natural Products
National Academies "Industrialization of Biology" Roadmap

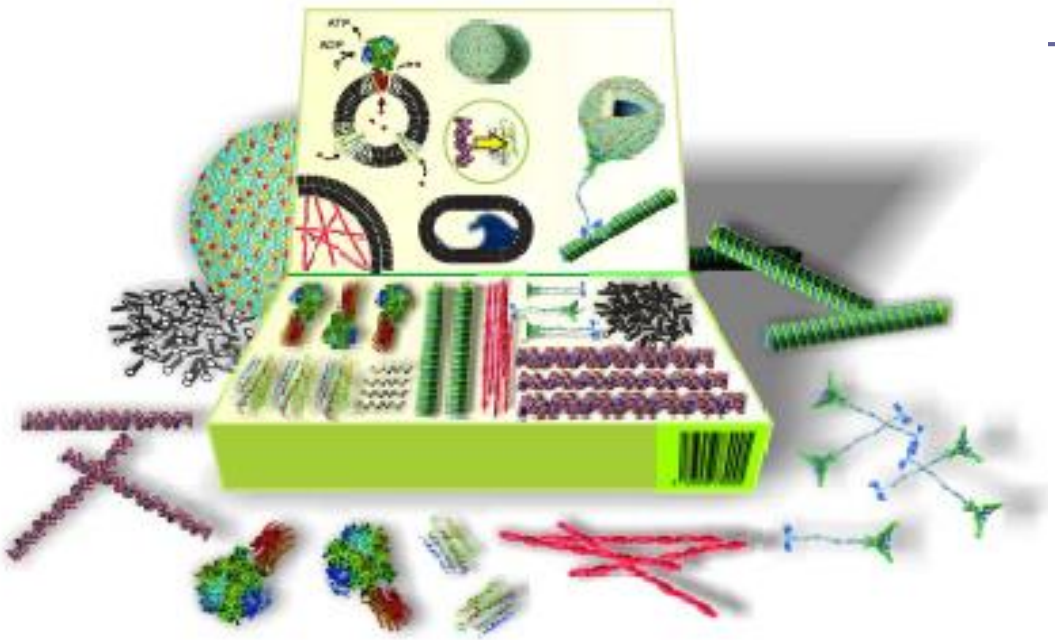


Hacia una Nueva Biología

- La **Biología Sintética** se pudo desarrollar a partir de la **Biología de Sistemas**, la computación, la biología molecular, la ingeniería genética y la genómica.
- La **Biología sintética** se define como la síntesis de biomoléculas o ingeniería de sistemas biológicos con funciones nuevas que no se encuentran en la naturaleza. Se trata de una disciplina que posee como objetivo el diseño de sistemas biológicos que no existen en la naturaleza, que contribuiría a mejorar nuestra calidad de vida y remediar los problemas medioambientales que han sido generados.

¿Qué significa "BIOLOGÍA SINTÉTICA"?

- Leyes Físicas + Propiedades Materiales= Puentes que no colapsan. Motores que convierten energía en movimiento mecánico.
- La **bacteria más simple**: Despliega una complejidad no equiparable por nada hecho por los humanos...
- *Objetivo Central de la Biología Sintética*: Transformar lo biológico en un sistema que pueda ser **diseñado** al igual que diseñamos puentes y sistemas mecánicos.
- Este enfoque proporcionará una mejor comprensión del mundo viviente.
- Y nos permitirá aprovechar el repertorio diverso biológico para aplicaciones útiles y fascinantes.

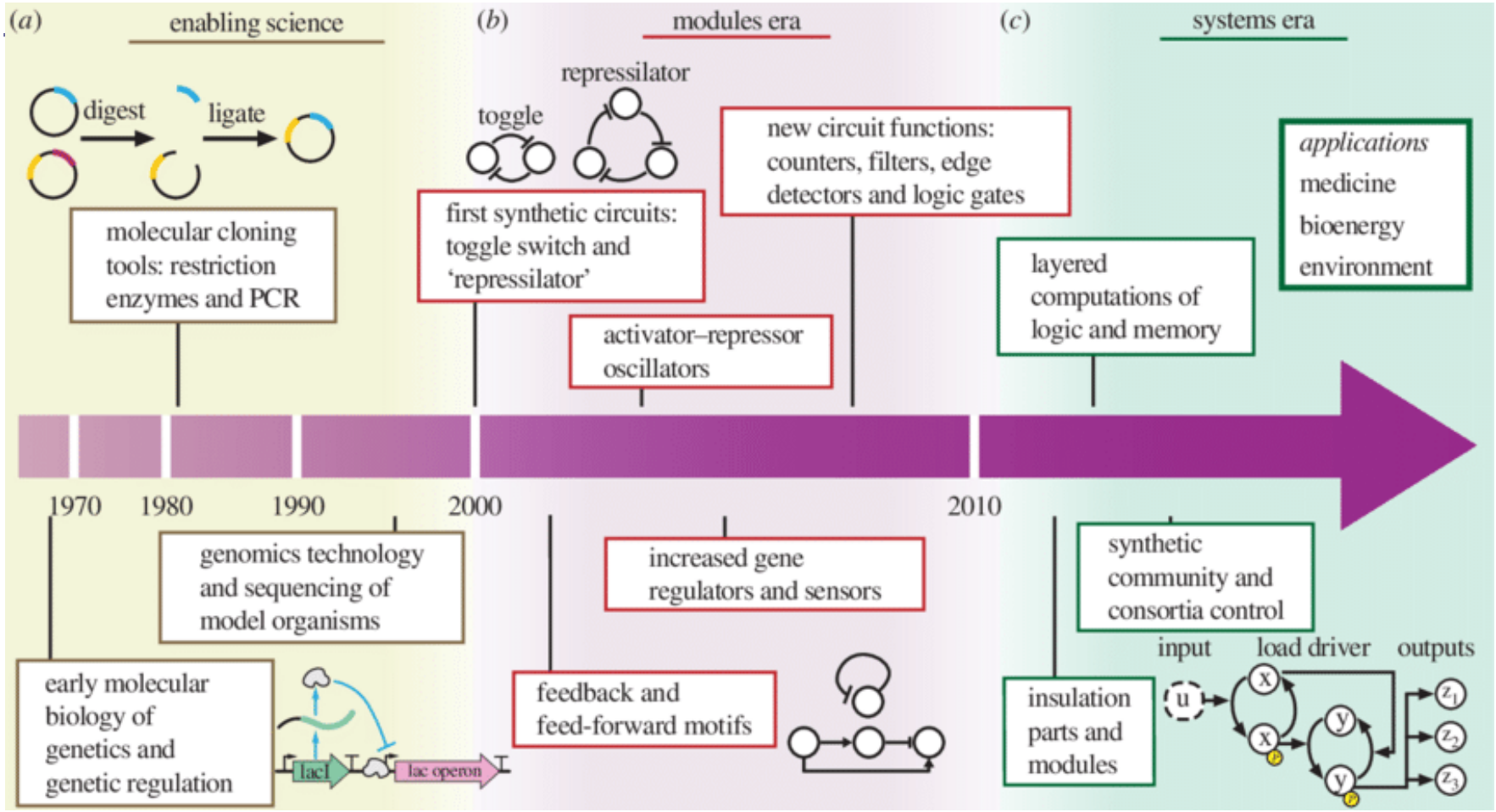


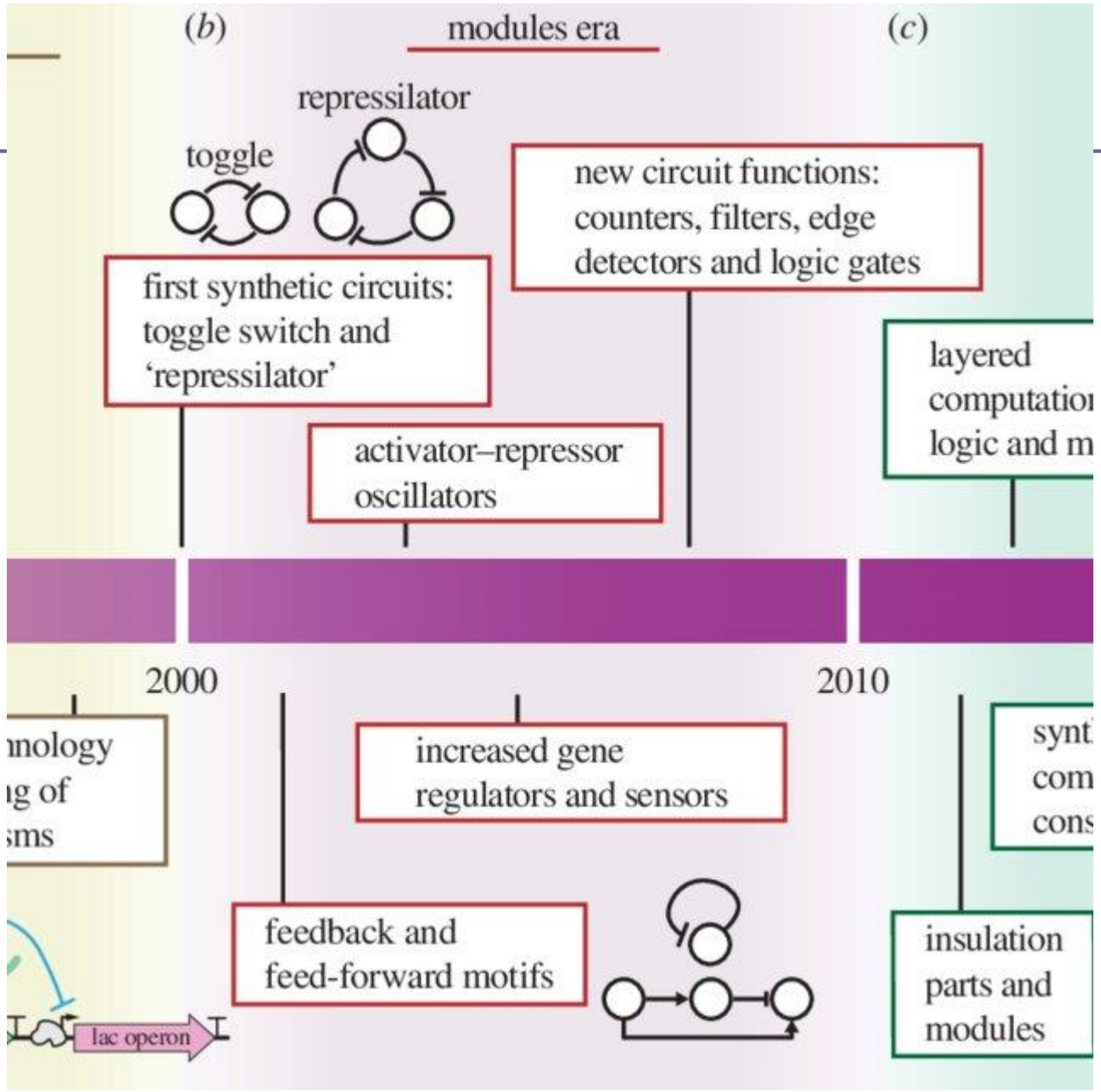
Visión de la Biología Sintética

El enfoque de la biología sintética sobre los sistemas vivos:

Kit de partes biológicas funcionales, con posibilidad de ser ensambladas en circuitos...¡Que nosotros diseñamos...!

DOS ERAS EN BIOLOGÍA SINTÉTICA




(b)
modules era
(c)
repressilator
toggle
new circuit functions:
counters, filters, edge
detectors and logic gates
first synthetic circuits:
toggle switch and
'repressilator'
layered
computation
logic and m
activator–repressor
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Construction of a genetic toggle switch in *Escherichia coli*

Timothy S. Gardner*†, Charles R. Cantor‡ & James J. Collins*†

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It has been proposed[1] that gene-regulatory circuits with virtually any desired property can be constructed from networks of simple regulatory elements. These properties, which include multistability and oscillations, have been found in specialized gene circuits such as the bacteriophage λ switch[2] and the Cyanobacteria circadian oscillator[3]. However, these behaviours have not been demonstrated in networks of non-specialized regulatory components. Here we present the construction of a genetic toggle switch—a synthetic, bistable gene-regulatory network—in *Escherichia coli* and provide a simple theory that predicts the conditions necessary for bistability. The toggle is constructed from any two repressible promoters arranged in a mutually inhibitory network. It is flipped between stable states using transient chemical or thermal induction and exhibits a nearly ideal switching threshold. As a practical device, the toggle switch forms a synthetic, addressable cellular memory unit and has implications for biotechnology, biocomputing and gene therapy.

The design and construction of synthetic gene-regulatory networks would be greatly facilitated by a theory with predictive capability. Previous work using a reconstituted enzyme system[4] showed that nonlinear mathematics can predict qualitative behaviours of biochemical reaction networks, including multistability and hysteresis. However, a practical theory of gene-regulatory networks has lagged behind that of enzyme regulatory networks. A variety of physical and mathematical approaches, including logical (discrete)[5–10], piecewise linear[11], nonlinear[12–14], statistical–mechanical[15,16] and stochastic[17,18] formulations of the underlying biochemical dynamics, have been used in the past. Owing to the difficulty of testing their predictions, these theories have not, in general, been verified experimentally. Here we have integrated theory and experiment by constructing and testing a synthetic, bistable gene circuit based on the predictions of a simple mathematical model.

The toggle switch is composed of two repressors and two constitutive promoters (Fig. 1). Each promoter is inhibited by the repressor that is transcribed by the opposing promoter. We selected this design for the toggle switch because it requires the fewest genes and *cis*-regulatory elements to achieve robust bistable behaviour. By robust, we mean that the toggle exhibits bistability over a wide range of parameter values and that the two states are tolerant of the fluctuations inherent in gene expression (the toggle switch will not flip randomly between states). Although bistability is theoretically possible with a single, autocatalytic promoter, it would be less robust and more difficult to tune experimentally. In addition, the chosen toggle design does not require any specialized promoters, such as the P_R/P_{RM} promoter of bacteriophage λ. Bistability is possible with any set of promoters and repressors as long as they fulfil the minimum set of conditions described in Box 1 and Fig. 2.

The bistability of the toggle arises from the mutually inhibitory arrangement of the repressor genes. In the absence of inducers, two stable states are possible: one in which promoter 1 transcribes repressor 2, and one in which promoter 2 transcribes repressor 1. Switching is accomplished by transiently introducing an inducer of the currently active repressor. The inducer permits the opposing repressor to be maximally transcribed until it stably represses the originally active promoter.

All toggle switches are implemented on *E. coli* plasmids conferring ampicillin resistance and containing the pBR322 ColE1 replication origin. The toggle switch genes are arranged as a type IV plasmid, as shown in Fig. 3. Although all genes and promoters are

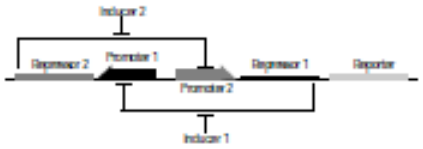

Figure 1 Toggle switch design. Repressor 1 inhibits transcription from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is induced by Inducer 2.

Box 1

The toggle model

The behaviour of the toggle switch and the conditions for bistability can be understood using the following dimensionless model for the network:

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^\beta} - u \tag{1a}$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^\gamma} - v \tag{1b}$$

where u is the concentration of repressor 1, v is the concentration of repressor 2, α_1 is the effective rate of synthesis of repressor 1, α_2 is the effective rate of synthesis of repressor 2, β is the cooperativity of repression of promoter 2 and γ is the cooperativity of repression of promoter 1. The above model is derived from a biochemical rate equation formulation of gene expression[26,27]. The final form of the toggle equations preserves the two most fundamental aspects of the network: cooperative repression of constitutively transcribed promoters (the first term in each equation), and degradation/dilution of the repressors (the second term in each equation).

The parameters α_1 and α_2 are lumped parameters that describe the net effect of RNA polymerase binding, open-complex formation, transcript elongation, transcript termination, repressor binding, ribosome binding and polypeptide elongation. The cooperativity described by β and γ can arise from the multimerization of the repressor proteins and the cooperative binding of repressor multimers to multiple operator sites in the promoter. An additional modification to equation (1) is needed to describe induction of the repressors (Fig. 5).

The geometric structure of equation (1), illustrated in Fig. 2a and b, reveals the origin of the bistability: the nullclines ($du/dt = 0$ and $dv/dt = 0$ in Fig. 2) intersect at three points, producing one unstable and two stable steady states. From Fig. 2a and b, three key features of the system become apparent. First, the nullclines intersect three times because of their sigmoidal shape, which arises for $\beta, \gamma > 1$. Thus, the bistability of the system depends on the cooperative repression of transcription. Second, the rates of synthesis of the two repressors must be balanced. If the rates are not balanced, the nullclines will intersect only once, producing a single stable steady state. This situation arises in plasmid pKE105. Third, the structure of the toggle network creates two basins of attraction. Thus, a toggle with an initial condition anywhere above the separatrix will ultimately settle to state 1, whereas a toggle starting below the separatrix will settle to state 2.

The conditions for a bistable toggle network are illustrated in Fig. 2c and d. As the rates of repressor synthesis are increased, the size of the bistable region increases. Furthermore, the slopes of the bifurcation lines, for large α_1 and α_2, are determined by β and γ. Thus, to obtain bistability, at least one of the inhibitors must repress expression with cooperativity greater than one. Moreover, higher order cooperativity will increase the robustness of the system, allowing weaker promoters to achieve bistability and producing a broader bistable region.

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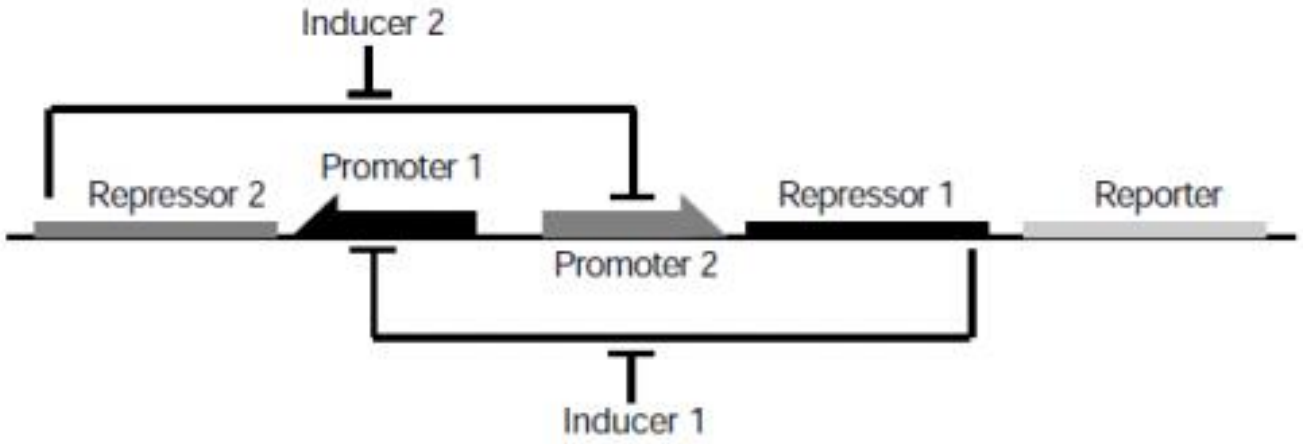
Construction of a genetic toggle switch in *Escherichia coli*

Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

Se propone que se pueden construir circuitos de regulación genética con prácticamente cualquier propiedad deseada a partir de redes de elementos reguladores simples. Estas propiedades, que incluyen la multiestabilidad y las oscilaciones, se han encontrado en circuitos de genes especializados como el “interruptor” del bacteriófago lambda y el oscilador circadiano de las cianobacterias. Sin embargo, estos comportamientos no se han demostrado en redes de componentes regulatorios no especializados. Aquí presentamos la construcción de un interruptor de palanca genético, una red reguladora de genes biestable sintética, en *Escherichia coli* y proporcionamos una teoría simple que predice las condiciones necesarias para la biestabilidad. La palanca se construye a partir de dos promotores reprimibles cualesquiera dispuestos en una red mutuamente inhibitoria. Se cambia entre estados estables mediante inducción química o térmica transitoria y exhibe un umbral de conmutación casi ideal. Como dispositivo práctico, el interruptor de palanca forma una unidad de memoria celular direccionable sintética y tiene implicaciones para la biotecnología, la biocomputación y la terapia génica.

¿Cómo funciona el **INTERRUPTOR GENÉTICO**?



La biestabilidad de la palanca surge de la disposición mutuamente inhibitoria de los genes represores. En ausencia de inductores, son posibles dos estados estables:

El promotor P1 transcribe el represor R2 y

El promotor P2 transcribe el represor R1.

El cambio se logra mediante la introducción transitoria de un inductor del represor actualmente activo.

Si R2 activo, y se **adiciona I2**, entonces, P2 deja de ser reprimido, y se transcribe R1 (lo que provoca que R2 ya no se transcriba): **R1 Activo**

¡SE HA PRODUCIDO LA CONMUTACIÓN!

APLICACIONES DEL GENETIC TOGGLE SWITCH

- El Genetic Toggle Switch se ha utilizado para una serie de aplicaciones.

Por ejemplo:

- La construcción de un Reloj Genético Sintético
  [*Cell* 2003; 113(5):597–607],
- La Regulación de la Expresión Génica en Mamíferos
  [*Nat Biotechnol*. 2004; 22(7):867–70; *Cell*. 2007; 130(2):363–72],
- El desarrollo de un Temporizador Genético Predecible
  [*Nat Biotechnol*. 2009; 27(5):465–71.],
- La formación de Biopelículas en Respuesta a Estímulos Diseñados
  [*Proc Nat Acad Sci USA.* 2004; 101(22):8414–419.].

Western blotting

Ribosomal complexes assembled and purified as described above were TCA-precipitated. Proteins were resolved on 12% polyacrylamide gel, transferred to nitrocellulose membrane and probed for eIF1 and eIF2B using T7-tag antibodies (Novagen) and for eIF1a and eIF3 (p170) using specific antibodies.

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1. Merrick, W. C. Mechanism and regulation of eukaryotic protein synthesis. *Microbiol. Rev.* **56**, 291–315 (1992).
2. Pestova, T. V., Hellen, C. U. & Shatsky, I. A. Canonical eukaryotic initiation factors determine viral internal ribosomal entry site activity. *Mol. Cell. Biol.* **16**, 6859–6869 (1996).
3. Pestova, T. V., Shatsky, I. N., Fletcher, S. P., Jackson, R. J. & Hellen, C. U. A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. *Genes Dev.* **12**, 67–83 (1998).

Acknowledgements

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A synthetic oscillatory network of transcriptional regulators

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Networks of interacting biomolecules carry out many essential functions in living cells[1], but the 'design principles' underlying the functioning of such intracellular networks remain poorly understood, despite intensive efforts including quantitative analysis of relatively simple systems[2]. Here we present a complementary approach to this problem: the design and construction of a synthetic network to implement a particular function. We used three transcriptional repressor systems that are not part of any natural biological clock[3–5] to build an oscillating network, termed the repressilator, in *Escherichia coli*. The network periodically induces the synthesis of green fluorescent protein as a readout of its state in individual cells. The resulting oscillations, with typical periods of hours, are slower than the cell-division cycle, so the state of the oscillator has to be transmitted from generation to generation. This artificial clock displays noisy behaviour, possibly because of stochastic fluctuations of its components. Such 'rational network design' may lead both to the engineering of new cellular behaviours and to an improved understanding of naturally occurring networks.

In the network shown in Fig. 1a, the first repressor protein, LacI from *E. coli*, inhibits the transcription of the second repressor gene, tetR from the tetracycline-resistance transposon Tn*10*, whose protein product in turn inhibits the expression of a third gene, *cI* from λ phage. Finally, CI inhibits *lacI* expression, completing the cycle. That such a negative feedback loop can lead to temporal oscillations in the concentrations of each of its components can be seen from a simple model of transcriptional regulation, which we used to design the repressilator and study its possible behaviours (Box 1). In this model, the action of the network depends on several factors, including the dependence of transcription rate on repressor concentration, the translation rate, and the decay rates of the protein and messenger RNA. Depending on the values of these parameters, at least two types of solutions are possible: the system may converge toward a stable steady state, or the steady state may become unstable, leading to sustained limit-cycle oscillations (Fig. 1b, c).

We found that oscillations are favoured by strong promoters coupled to efficient ribosome-binding sites, tight transcriptional repression (low 'leakiness'), cooperative repression characteristics, and comparable protein and mRNA decay rates (Box 1, Fig. 1b). A general obstacle to the design of biochemical networks is uncertainty about the values of parameters that characterize the interactions between different components. In our network, estimates of the order of magnitude of the relevant parameters seem to be compatible with the possibility of oscillations. Nevertheless, to increase the chances that the artificial network would function in the oscillatory regime, we made two alterations to natural components. First, to address transcriptional strength and tightness, we used strong, yet tightly repressible hybrid promoters, developed previously, which combine the λ P_L promoter with lac and tet operator sequences[6]. Second, to bring the effective repressor protein lifetimes closer to that of mRNA (about 2 min, on average, in *E. coli*[7]), we inserted a carboxy-terminal tag, based on the *ssrA* RNA sequence[8], at the 3′ end of each repressor gene. Proteases in *E. coli* recognize this tag and target the attached protein for destruction[9,10]. Such tags have been shown to reduce the half-life of the λ repressor DNA-binding domain from more than 60 min to around 4 min (ref. 8) and diminish the half-life of green fluorescent protein (GFP) to about 30–40 min (ref. 11).

With these considerations in mind, we used standard molecular biology techniques to construct a low-copy plasmid encoding the repressilator and a compatible, higher-copy reporter plasmid containing the tet-repressible promoter $P_R$tetO1 (ref. 6) fused to an intermediate stability variant of *gfp*[11] (Fig. 1a). Because the inducer IPTG interferes with repression by LacI, we expected that a transient pulse of IPTG might be capable of synchronizing a population of repressilator-containing cells. A culture of *E. coli* MC4100 containing the two plasmids and grown in media containing IPTG displayed what appeared to be a single damped oscillation of GFP fluorescence per cell after transfer to media lacking IPTG (results not shown). Because individual cells have no apparent means of maintaining synchronization, we studied the repressilator by isolating single cells under the microscope and monitoring their fluorescence intensity as they grew into small two-dimensional microcolonies consisting of hundreds of progeny cells. In these experiments, total observation time was limited by the colony entering a stationary phase after about 10 hours of growth at

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A synthetic oscillatory network of transcriptional regulators

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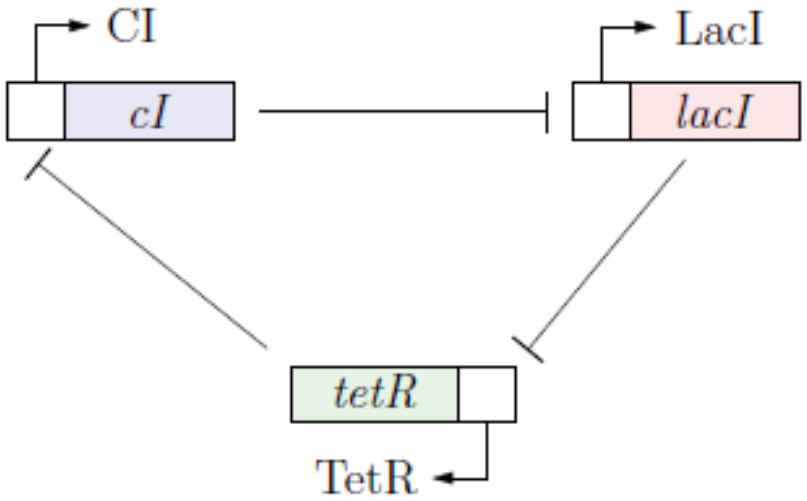
Las redes de biomoléculas que interactúan llevan a cabo muchas funciones esenciales en las células vivas, pero los "principios de diseño" subyacentes al funcionamiento de tales redes intracelulares siguen siendo poco conocidos, a pesar de los intensos esfuerzos que incluyen el análisis cuantitativo de sistemas relativamente simples. Aquí presentamos un enfoque complementario a este problema: el diseño y construcción de una red sintética para implementar una función particular. Usamos tres sistemas represores transcripcionales que no forman parte de ningún reloj biológico natural para construir una red oscilante, denominada **“repressilator”**, en Escherichia coli. La red induce periódicamente la síntesis de proteína verde fluorescente como una lectura de su estado en células individuales. Las oscilaciones resultantes, con periodos típicos de horas, son más lentas que el ciclo de división celular, por lo que el estado del oscilador debe transmitirse de generación en generación. Este reloj artificial muestra un comportamiento ruidoso, posiblemente debido a las fluctuaciones estocásticas de sus componentes. Tal "diseño de red racional" puede conducir tanto a la ingeniería de nuevos comportamientos celulares como a una mejor comprensión de las redes naturales.

EL REPRESSILATOR

- Otro circuito molecular, el repressilator, es un hito y una de las publicaciones más importantes en Biología Sintética. Esta red oscilatoria mostró que era posible diseñar una red sintética que pudiera realizar una función.
- En la red se muestra la primera proteína represora, **LacI** de E. coli, inhibe la transcripción del segundo gen represor, *tetR* del transposón Tn10 resistente a tetraciclina, cuyo producto proteico a su vez inhibe la expresión de un tercer gen, *cI* del fago lambda. Finalmente, la proteína **CI** inhibe la expresión de *lacI*, completando el ciclo.
- Básicamente consiste en una red de retroalimentación negativa de tres represores, que cuando se combinan con el gen de la proteína fluorescente verde (GFP), hace que las células de la E. coli produzcan destellos de modo periódico demostrando que las oscilaciones se pueden programar genéticamente.
- Consta de tres genes conectados en un bucle de realimentación, de manera que cada gen reprime al siguiente gen en el bucle, y es reprimido por el gen anterior. Además, la proteína fluorescente verde se utiliza como un reportero, y la red funciona de la siguiente manera:

CIRCUITO SINTÉTICO GENÉTICO OSCILANTE

- Funciona de la siguiente manera cíclica:
- El gen *lacI* de E. coli expresa la proteína **LacI** que inhibe la transcripción del gen *tetR*.
- El gen *tetR* expresa la proteína **TetR** que inhibe la transcripción del gen *cI*.
- El gen *cI* expresa la proteína **CI** que inhibe la transcripción del gen *lacI*.



- **IMPORTANTE**:

Un ciclo de retroalimentación negativa de este tipo, puede conducir a oscilaciones temporales en las concentraciones de cada uno de sus componentes.

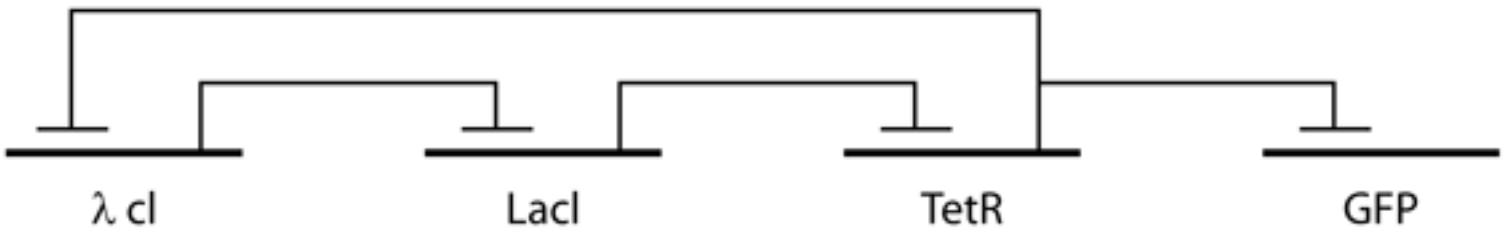


FIG: La red reguladora genética **repressilator**, en la que cada proteína traducida por un gen reprime a la siguiente en el ciclo.

Aplicaciones

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- Perez-Carrasco R, Barnes CP, Schaerli Y, Isalan M, Briscoe J, Page KM. **Combining a Toggle Switch and a Repressilator within the AC-DC Circuit Generates Distinct Dynamical Behaviors**. *Cell Syst*. 2018 Apr 25;6(4):521-530.e3.
- Garcia-Ojalvo J, Elowitz MB, Strogatz SH. **Modeling a synthetic multicellular clock: repressilators coupled by quorum sensing**. *Proc Natl Acad Sci U S A*. 2004 Jul 27;101(30):10955-60.

EDICIÓN GENÓMICA

- **Nucleasas Efectoras Tipo Activador de Transcripción (TALEN)**
- **Efectores Tipo Activadores de Transcripción (TALE)**
- **TECNOLOGÍA CRISPR/CAS**

¿Qué es la tecnología del CRISPR?

- CRISPR se origina como un mecanismo inmunológico que utilizan las bacterias para defenderse contra la infección por virus. Lo fascinante de CRISPR, es que las bacterias se llevan un trozo de ADN del virus y lo almacenan dentro de su genoma.

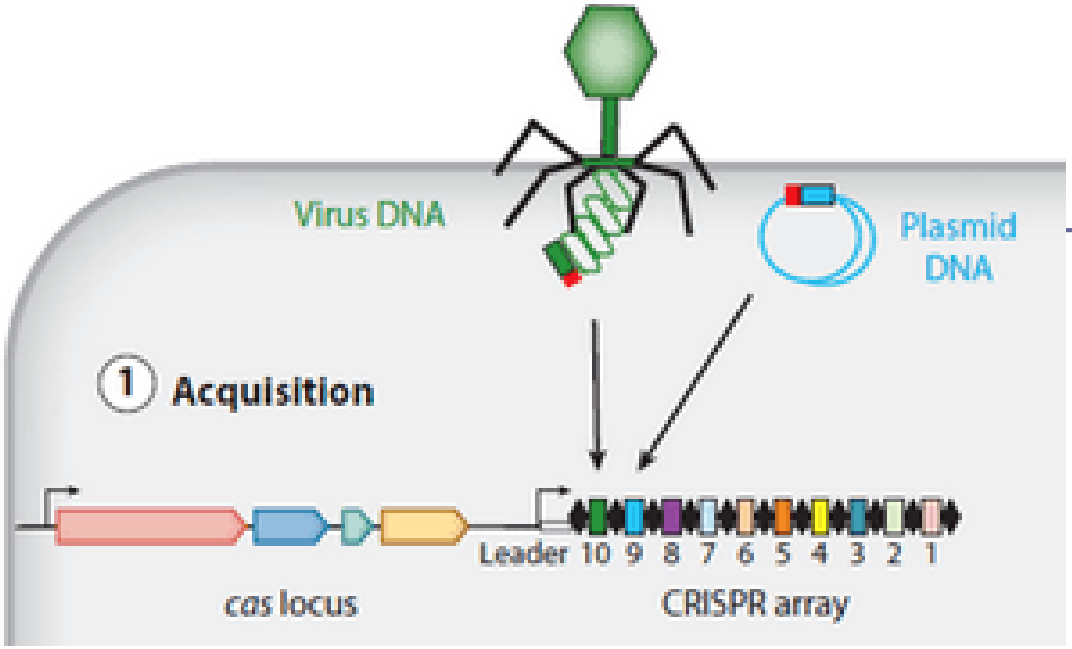
- Entonces, cuando el virus infecta las bacterias de nuevo, la bacteria es capaz de utilizar el trozo de ADN que robó como una táctica de vigilancia. Una vez que coincide con el ADN del virus, se desencadena una cascada de edición del ADN del virus para deshacerse de la infección.

Antecedentes

- Las secuencias repetidas (que luego se conocerían como CRISPR), fueron identificadas por Francisco J. M. Mojica de la Universidad de Alicante a principios de los años 90 en una arquea Haloferax mediterranei ("The Heroes of CRISPR," by Eric S. Lander, January 14, 2016 edition of *Cell*.)
- Este hallazgo lo llevó a plantear la hipótesis –correcta-, de que CRISPR es un sistema inmunológico adaptativo.
- Con frecuencia se hallan asociados con los genes cas, que codifican para proteínas nucleasas relacionadas con los CRISPR.
- El sistema CRISPR/Cas es un sistema inmune procariótico que confiere resistencia a agentes externos como plásmidos y fagos y provee una forma de *inmunidad adquirida*.
- Los espaciadores de los CRISPR reconocen secuencias específicas y guían a las *nucleasas cas* para cortar y degradar esos elementos génicos exógenos de una manera análoga al *ARNi* en sistemas eucarióticos.

Acción de los CRISPR

- Jennifer Doudna y Emmanuelle Charpentier habían estado explorando de manera independiente a las proteínas asociadas a CRISPR para aprender cómo las bacterias utilizan a los espaciadores en sus sistemas inmunes.
- Estos sistemas de defensa se basan en pequeños ARN para la detección y el silenciamiento de secuencias específicas de los ácidos nucleicos extraños.
- Los sistemas CRISPR / Cas están compuestos de genes cas organizados en *operones* y arrays CRISPR que consisten en secuencias del genoma blancos (llamados espaciadores) intercalados con repeticiones idénticas.



Observamos el genoma de las bacterias en la parte inferior. El locus CRISPR (etiquetado como un array de CRISPR) se identifica por las repeticiones de ADN (diamantes negros), con el ADN del virus específico (cajas multicolores) espaciados entre estas repeticiones. A la derecha de este locus CRISPR es el locus Cas, que consiste en enzimas importantes y las proteínas que se ensamblan y facilitan la escisión.

Biología Sintética y Diseño

- Concebir una función biológica deseada,
- Diseñar un sistema biológico para realizar dicha función,
- Construir el sistema, que se desempeña como se predijo.
- Sin embargo, la ingeniería biológica aún requiere de mucho ensayo y error porque carecemos de métodos eficaces para conectar "partes básicas" al interior de las redes de orden superior (para que se comporten como se ha previsto).
- Para mejorar el rendimiento y sofisticación de nuestros diseños contamos con dos perspectivas generales: “top-down” y “bottom-up”

“Top-Down”

- Se distingue, principalmente, de la biotecnología tradicional, por el grado de complejidad de las manipulaciones genéticas.
- En lugar de transferir genes simples, son **redes de genes enteros** los que se manipulan y provocan la *"reprogramación"* de las células.
- Constante aumento del conocimiento, sobre los nuevos módulos y funciones genéticas -y la disponibilidad de métodos y tecnologías cada vez más sofisticados-, para analizar y manipular las vías celulares .
- Reducción de la ***“Complejidad Molecular”***.

Craig Venter

- Es conocido por liderar el primer borrador de secuencia del genoma humano y reunió al primer equipo para transfectar una célula con un cromosoma sintético. Venter fundó Celera Genomics, el Instituto de Investigación Genómica (TIGR) y el Instituto J. Craig Venter (JCVI), donde actualmente se desempeña como CEO.
- El empresario de genómica Craig Venter ha creado una célula sintética que contiene el genoma más pequeño de cualquier organismo independiente conocido. Funcionando con 473 genes, la célula es un hito en la búsqueda de 20 años de su equipo para reducir la vida a sus elementos esenciales y, por extensión, para diseñar la vida desde cero.

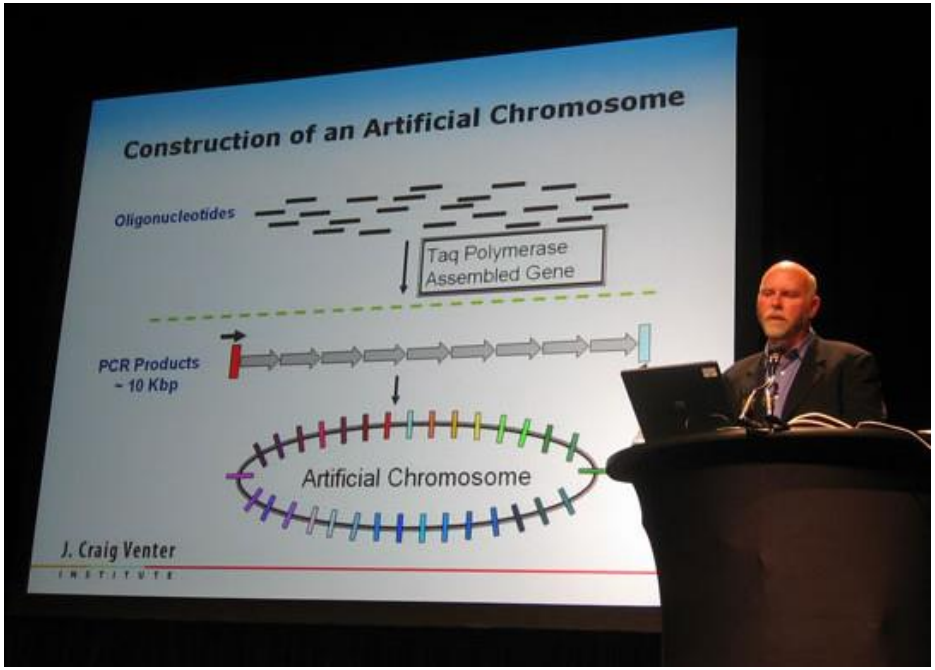
RESEARCH ARTICLE

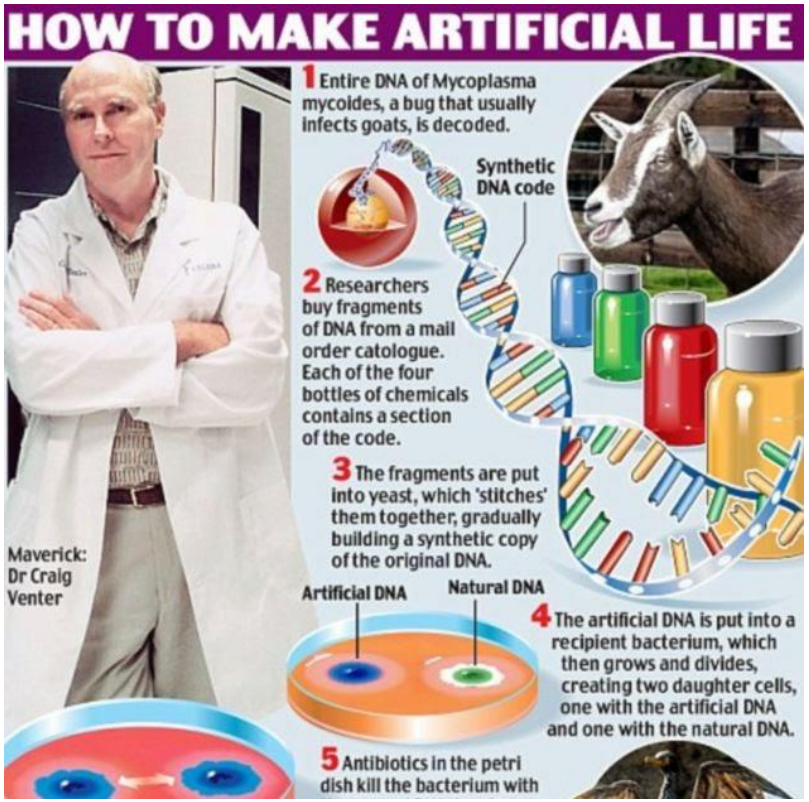
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

By Daniel G. Gibson, John I. Glass, Carole Lartigue, Vladimir N. Noskov, Ray-Yuan Chuang, Mikkel A. Algire, Gwynedd A. Benders, Michael G. Montague, Li Ma, Monzia M. Moodie, Chuck Merryman, Sanjay Vashee, Radha Krishnakumar, Nacyra Assad-Garcia, Cynthia Andrews-Pfannkoch, Evgeniya A. Denisova, Lei Young, Zhi-Qing Qi, Thomas H. Segall-Shapiro, Christopher H. Calvey, Prashanth P. Parmar, Clyde A. Hutchison III, Hamilton O. Smith, J. Craig Venter

Science | 02 Jul 2010 : 52-56 |

A synthetic *Mycoplasma mycoides* genome transplanted into *M. capricolum* was able to control the host cell.





El ensamblaje de Gibson es un método de clonación molecular que permite la unión de múltiples fragmentos de ADN en una única reacción isotérmica.

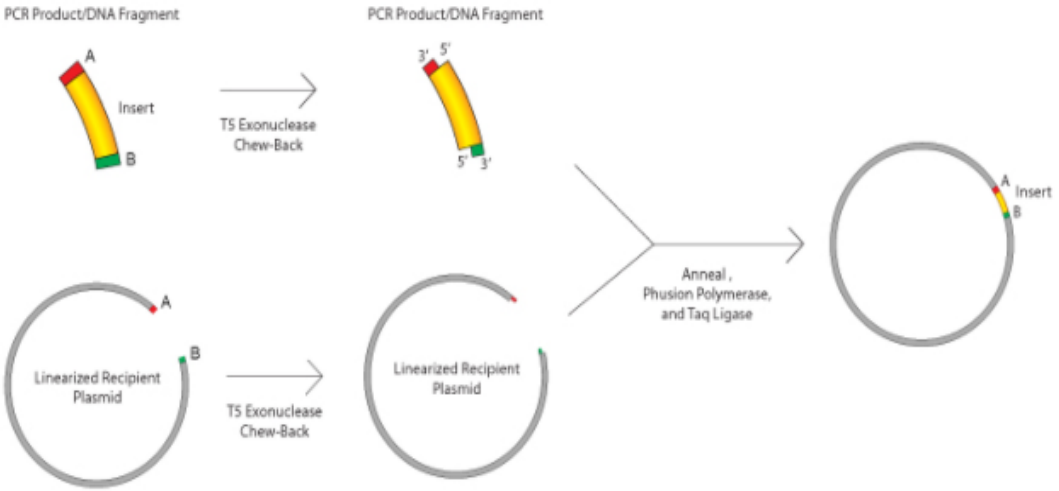
Gibson Assembly Cloning

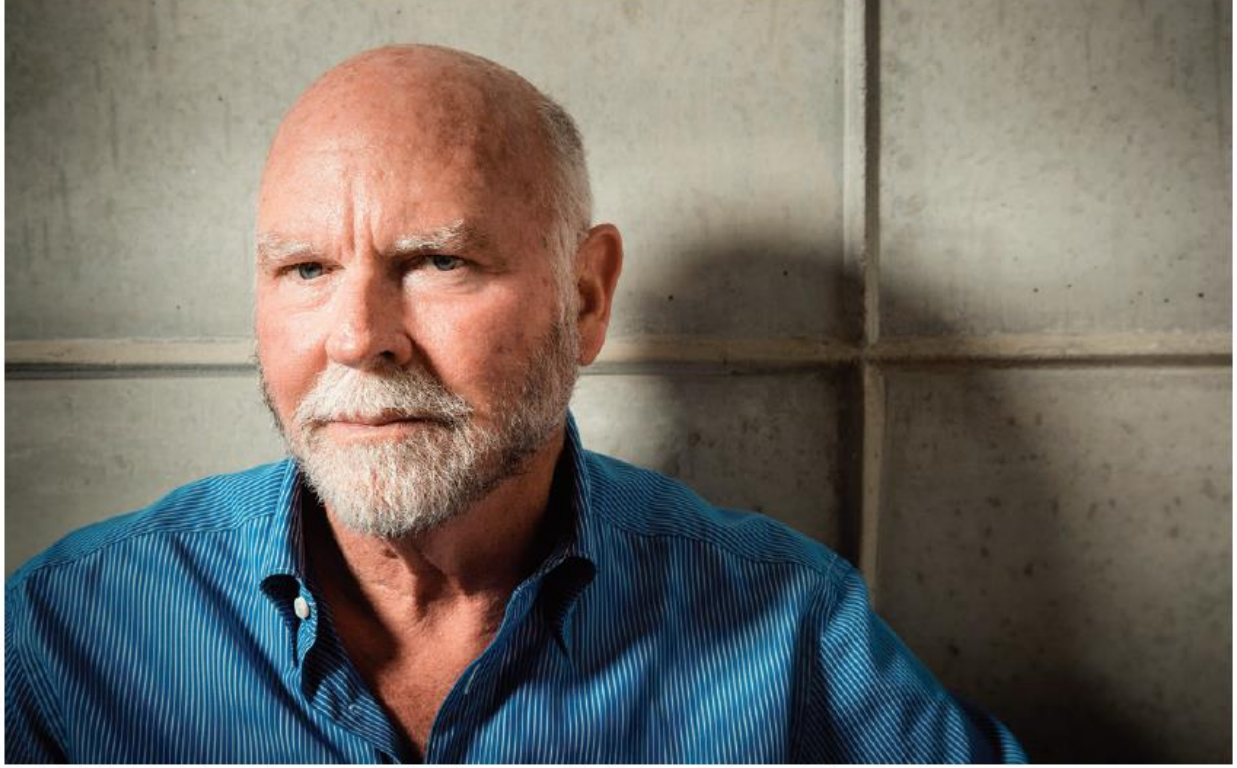
Summary

In 2009 Dr. Daniel Gibson and colleagues at the J. Craig Venter Institute developed a novel method for the easy assembly of multiple linear DNA fragments (Nat Methods 2009;6(5):343-5). Regardless of fragment length or end compatibility, multiple overlapping DNA fragments can be joined in a single isothermal reaction. With the activities of three different enzymes, the product of a Gibson Assembly is a fully ligated double-stranded DNA molecule. This has proven to be an efficient and effective method for the assembly of plasmids, and molecular biologists now use this method extensively.

Why Gibson Cloning?

- No need for specific restriction sites. Join almost any 2 fragments regardless of sequence.
- No scar between joined fragments.
- Fewer steps. One tube reaction.
- Can combine many DNA fragments at once.





Synthetic biologist Craig Venter, whose team embarked on its quest to whittle life down to its bare essentials two decades ago.

BIOTECHNOLOGY

Race to design life heats up

Craig Venter's minimal-cell triumph comes as the CRISPR gene-editing method provides alternative ways to tinker with life's building blocks.

BY EWEN CALLAWAY

Genomics entrepreneur Craig Venter has created a synthetic cell that contains the smallest genome of any known, independent organism. Functioning with 473 genes, the cell is a milestone in his team's 20-year quest to reduce life to its bare essentials and, by extension, to design life from scratch.

Venter, who has co-founded a company that seeks to harness synthetic cells for making industrial products, says that the feat heralds the creation of customized cells to make drugs, fuels and other products. But an explosion in powerful 'gene-editing' techniques, which enable relatively easy and selective tinkering with genomes, raises a niggling question: why go to the trouble of making new life forms when you can simply tweak it?

Unlike the first synthetic cells made in 2010 (ref. 1), in which Venter's team at the J. Craig Venter Institute in La Jolla, California, copied an existing bacterial genome and transplanted it into another cell, the genome of the minimal cells is like nothing in nature. Venter says that the cell, which is described in a paper released on 24 March in *Science*[2], constitutes a brand-new, artificial species.

"The idea of building whole genomes is one of the dreams and promises of synthetic biology," says Paul Freemont, a synthetic biologist at Imperial College London who is not involved in the work.

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RESEARCH ARTICLE SUMMARY

SYNTHETIC BIOLOGY

Design and synthesis of a minimal bacterial genome

Clyde A. Hutchison III,*† Ray-Yuan Chuang,† Vladimir N. Noskov, Nacyra Assad-Garcia, Thomas J. Deerinck, Mark H. Ellisman, John Gill, Krishna Kannan, Bogumil J. Karas, Li Ma, James F. Pelletier, Zhi-Qing Qi, R. Alexander Richter, Elizabeth A. Strychalski, Lijie Sun, Yo Suzuki, Billyana Tsvetanova, Kim S. Wise, Hamilton O. Smith, John I. Glass, Chuck Merryman, Daniel G. Gibson, J. Craig Venter*

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.

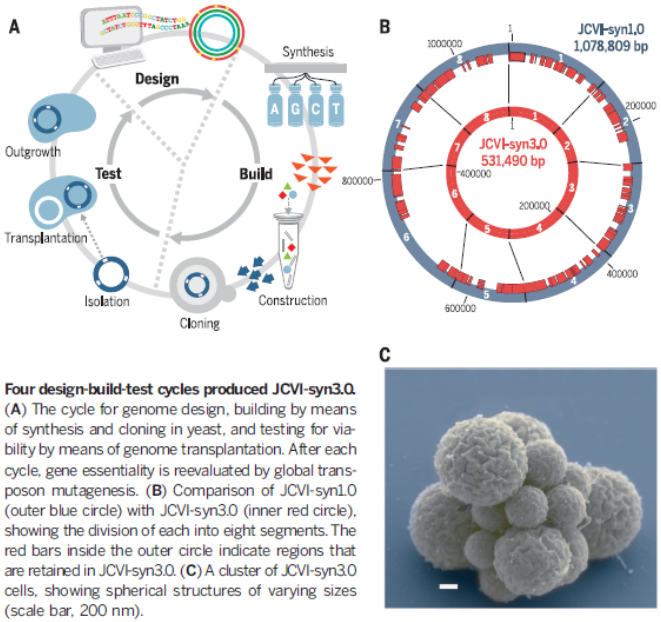
RATIONALE: Since the first genome sequences, there has been much work in many bacterial models to identify nonessential genes and define core sets of conserved genetic functions, using the methods of comparative genomics. Often, more than one gene product can perform a particular essential function. 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.

ON OUR WEB SITE

Read the full article at http://dx.doi.org/10.1126/science.aad6253

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).

The list of author affiliations is available in the full article online.
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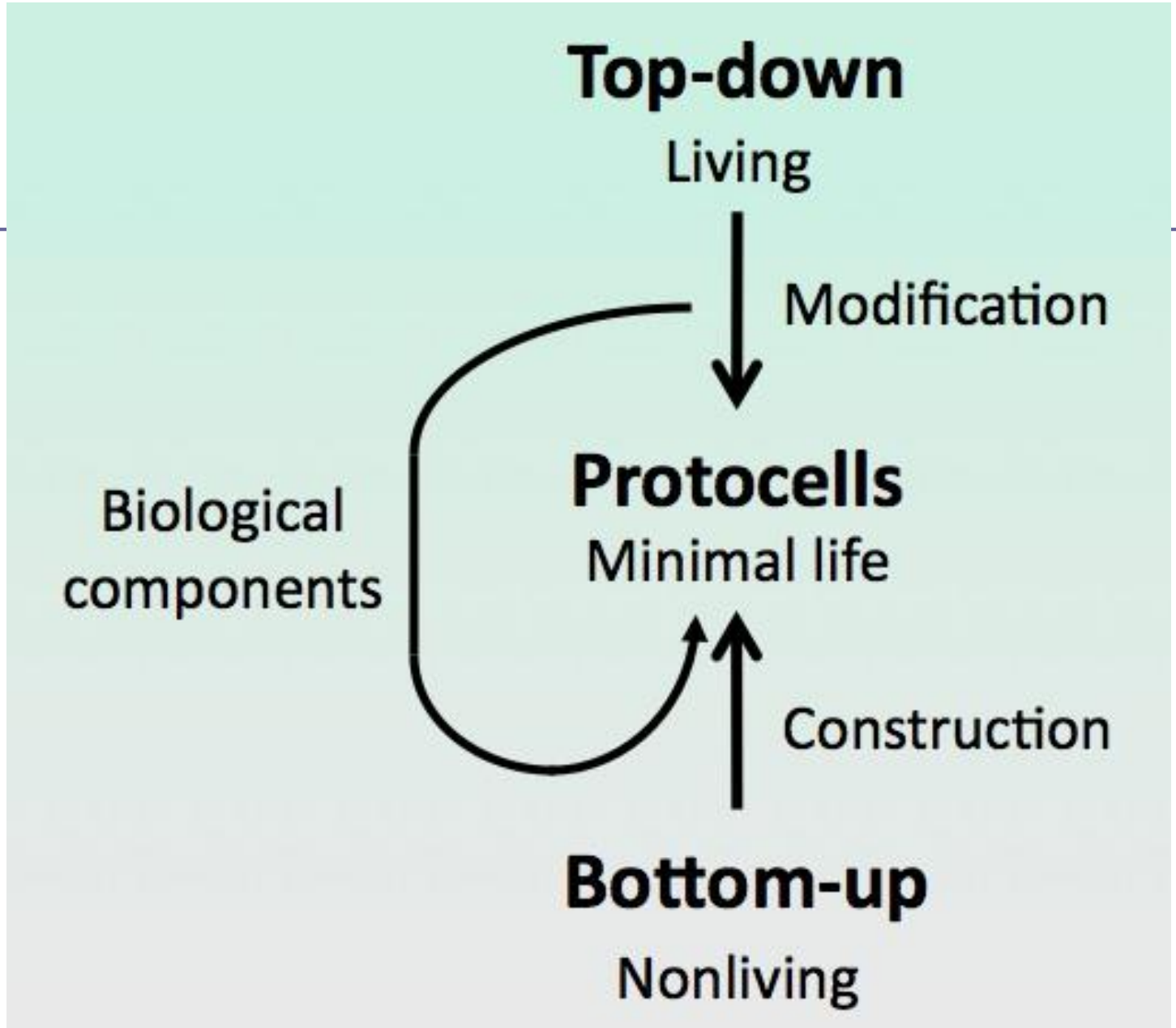
†These authors contributed equally to this work.
Cite this article as C. A. Hutchison III *et al.*, *Science* 351, aad6253 (2016). DOI: 10.1126/science.aad6253

“Bottom-Up”

- Desde esta perspectiva, la biología sintética no se pregunta cómo un sistema biológico funciona realmente.
- Más bien, se pregunta por la forma en que podría, en principio, funcionar con un conjunto mínimo de elementos.
- La comprensión de las características bioquímicas de subsistemas limitados -requisito previo importante para la interpretación cuantitativa de la célula-, no es suficiente para **(1)** abordar a la complejidad celular, **y menos (2)** La organización y funcionamiento de los organismos en su totalidad.
- ¿Conjunto mínimo de elementos funcionales suficientes para reconstituir las propiedades de la vida?


Top-down
Living
Modification
Protocells
Minimal life
Biological components
Construction
Bottom-up
Nonliving

Aplicaciones Posibles

- Próxima generación de biocombustibles,
- Productos químicos y materias primas industriales renovables (“verdes”),
- Terapias y medios de diagnóstico baratos para promover la salud mundial,
- Tecnologías que permiten la remediación ambiental,
- Materiales con nuevas propiedades.

Una mirada al Futuro

- En cada escala de organización biológica, este enfoque orienta tanto el diseño y el análisis de sistemas novedosos.
- Así como, nuestras investigaciones sobre los mecanismos fundamentales que influyen en el rendimiento de los sistemas biológicos que son rediseñados.
- La biología sintética está cambiando la forma de pensar la producción de moléculas útiles, la ingeniería de nuestra propia biología y la ampliación de la química de la vida.
- Avanzar en estos y otros frentes, requerirá enfoques interdisciplinarios y nuevas estrategias pedagógicas para la formación de jóvenes **“whole-brain thinkers”**, que comandarán las investigaciones de **“la biología por diseño”**.

synbiobeta

Asistir Leer Reloj Escuchar Sobre nosotros

***SynBioBeta*: lo ayudamos a conectarse, comprender mejor y hacer negocios con aquellos que usan la biología para hacer del planeta un lugar mejor.**

synbiobeta

Asistir Leer Reloj Escuchar Sobre nosotros

SynBioBeta es la principal red de innovación para ingenieros biológicos, innovadores, empresarios e inversores que comparten la pasión por utilizar la biología para construir un planeta mejor y más sostenible. Brindamos a los miembros de nuestra comunidad apoyo para el desarrollo personal y profesional, así como valiosas oportunidades para establecer contactos, asociaciones, colaboración y educación.

Todos los años organizamos **la Cumbre Global de Biología Sintética,** que muestra los desarrollos de vanguardia en biología que están transformando la forma en que alimentamos, curamos y alimentamos al mundo. Además, organizamos una variedad de otras conferencias, retiros de líderes de opinión y reuniones locales durante todo el año, tanto en los Estados Unidos como en todo el mundo. Para obtener más información sobre cómo puede unirse a nosotros, haga clic **aquí** .

SynBioBeta Digest **: su fuente de noticias de la industria, contenido original perspicaz y actualizaciones sobre todo lo relacionado con la biotecnología.**

synbiobeta digest

Hola ,

Siempre es bueno dar la bienvenida a nuevos miembros a la comunidad de biología sintética. Esta semana, los asistentes se inscribieron para unirse a SynBioBeta 2023 de Corteva Agriscience, Seventure Partners, el Centro de Investigación Técnica VTT de Finlandia, así como los directores ejecutivos de Bloom Science, AKT Therapeutics y Colabra. También quiero agradecer a nuestra nueva patrocinadora Antheia, que está elaborando la próxima generación de medicamentos inspirados en plantas. Espero que te unas a ellos y más en SynBioBeta 2023 .

Noticias de Bioeconomía

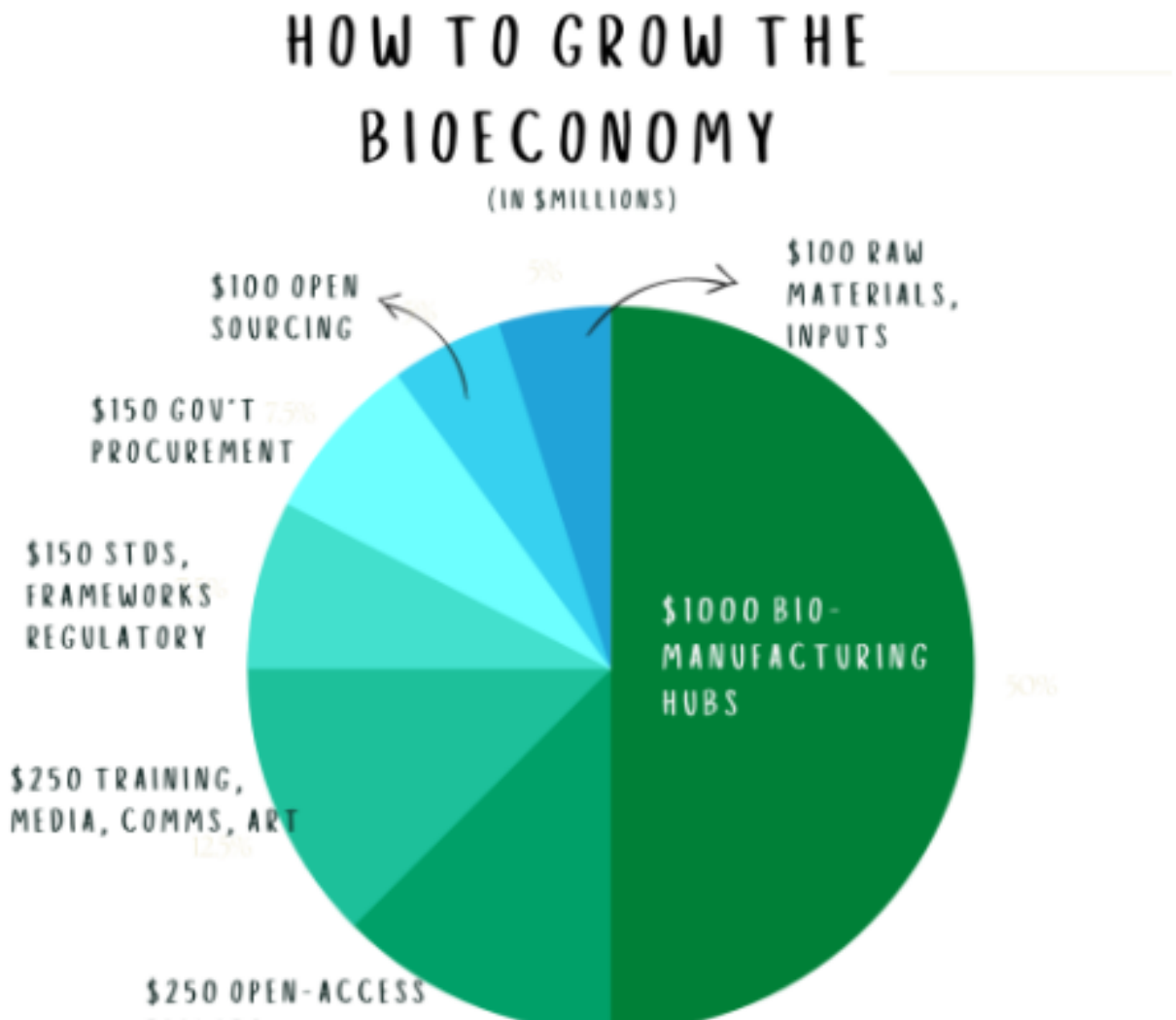
- Grandes noticias para Twist Bioscience en el cuarto aniversario de su salida a bolsa: el fabricante de ADN promueve a Paddy Finn, quien ha ayudado a aumentar los ingresos de la compañía de $2 millones a lo que se proyecta será más de $200 millones para fin de año. El nuevo cargo de Paddy será el de presidente y director de operaciones a medida que Twist ingresa al mercado de terapias con anticuerpos de $186 mil millones.

Y ahora nuestro resumen semanal de las últimas noticias sobre bioeconomía:

Noticias de financiación

- Solugen recauda $ 200 millones para fabricar productos químicos más ecológicos con biología sintética : la compañía tiene la misión de descarbonizar la industria química y está obteniendo un capital significativo en el camino. Ha recaudado $ 635,2 millones hasta la fecha, además de obtener $ 100 millones en ganancias este año, y planea expandirse aún más.
- Recursion anuncia una colocación privada de 150 millones de dólares: la empresa de diseño de fármacos impulsada por IA está cambiando su enfoque a su canal de oncología como parte de un replanteamiento estratégico impulsado por la inversión de la empresa sueca Kinnevik.
- Indee Labs se adjudicó un contrato de 2 millones de dólares para ampliar y ampliar Hydropore para terapia celular : Indee Labs anuncia un contrato de 2 millones de dólares con el Instituto Nacional del Cáncer para ampliar y ampliar la plataforma Hydropore para terapia celular con Eyquem Lab en UCSF.
- Time BioVentures lanza un nuevo fondo de biotecnología no convencional : Respaldado por una lista A de luminarias de biotecnología, como la ex estrella de rock DA Wallach y el desarrollador de fármacos en serie Tim Wright, la firma de capital de riesgo ha cerrado su primer fondo con $ 100 millones en inversiones para financiar "juegos". -innovaciones cambiantes en el cuidado de la salud".

- Cómo gastar $ 2 mil millones: la reciente Orden Ejecutiva inyectará $ 2 mil millones en la bioeconomía de los EE. UU. al reforzar la capacidad de los EE. UU. en bienes de alto valor y alta tecnología hechos de recursos biológicos, como proteínas alternativas, insumos agrícolas sostenibles y biotecnología farmacéutica. Karl Schmieder le pide a su red que haga una lluvia de ideas sobre cómo gastarían el dinero para lograr el mayor impacto.



¡MUCHAS GRACIAS!