TECHNIQUES IN GENETIC ENGINEERING

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CHAPTER 10

Today and Future

"There are living systems; there is no living "matter". No substance, no single molecule, extracted and isolated from a living being possess, of its own, the aforementioned paradoxical properties. They are present in living systems only; that is to say, nowhere below the level of the cell."

Jacques Monod

a. Bioinformatics and the Omics age

When Frederick Sanger developed his DNA sequencing technique (see Appendix I), the infrastructure for a genome sequencing frenzy was all set.

The Institute for Genetic Research (TIGR) has established the first genomic sequence of an organism, *Haemophilus influenza*, with the help of the new computational tools developed in this institute.

In 1989, a European consortium was set up to sequence the yeast genome, and in 1990 the Human Genome Project was announced.

The entrepreneur / scientists J. C. Venter's name should be mentioned here, since his team at TIGR, as well as his later-founded company Celera Genomics, has developed the shotgun sequencing technology which has greatly improved the speed of these genome sequencing efforts.

«OMICS» Technologies……

- **Genomics:** the study of the entire genome of an organism
- **Transcriptomics:** the study of the entire set of mRNAs that are expressed in a particular cell or tissue
- **Proteomics:** the study of the entire set of proteins that are expressed in a particular cell or tissue
- **Metabolomics:** the study of the entire set of metabolites in a particular cell or tissue
- **Lipidomics:** the study of the entire set of lipids and lipid derivatives in a particular cell or tissue

Systems biology is an interdisciplinary, or rather a *cross-disciplinary*, field that combines information obtained from all other omics approaches so as to present an integrated and interacting network of molecules, genes, proteins and pathways, studying the *system* as a whole, rather than focusing a number of molecules through a keyhole, as well as system dynamics.

The Institute for Systems Biology (ISB; http://www.systemsbiology.org) was founded in 2000, with exactly this in mind, and almost a decade and a half later this institute has made its impact with research projects on environment, brain, personalized medicine, among many others.

The web portal for systems biology researchers is also freely available at http://systems-biology.org, funded by Systems Biology Institute of Japan, also founded in 2000 (http://sbi.jp).

Synthetic biology, and Unnatural amino acids

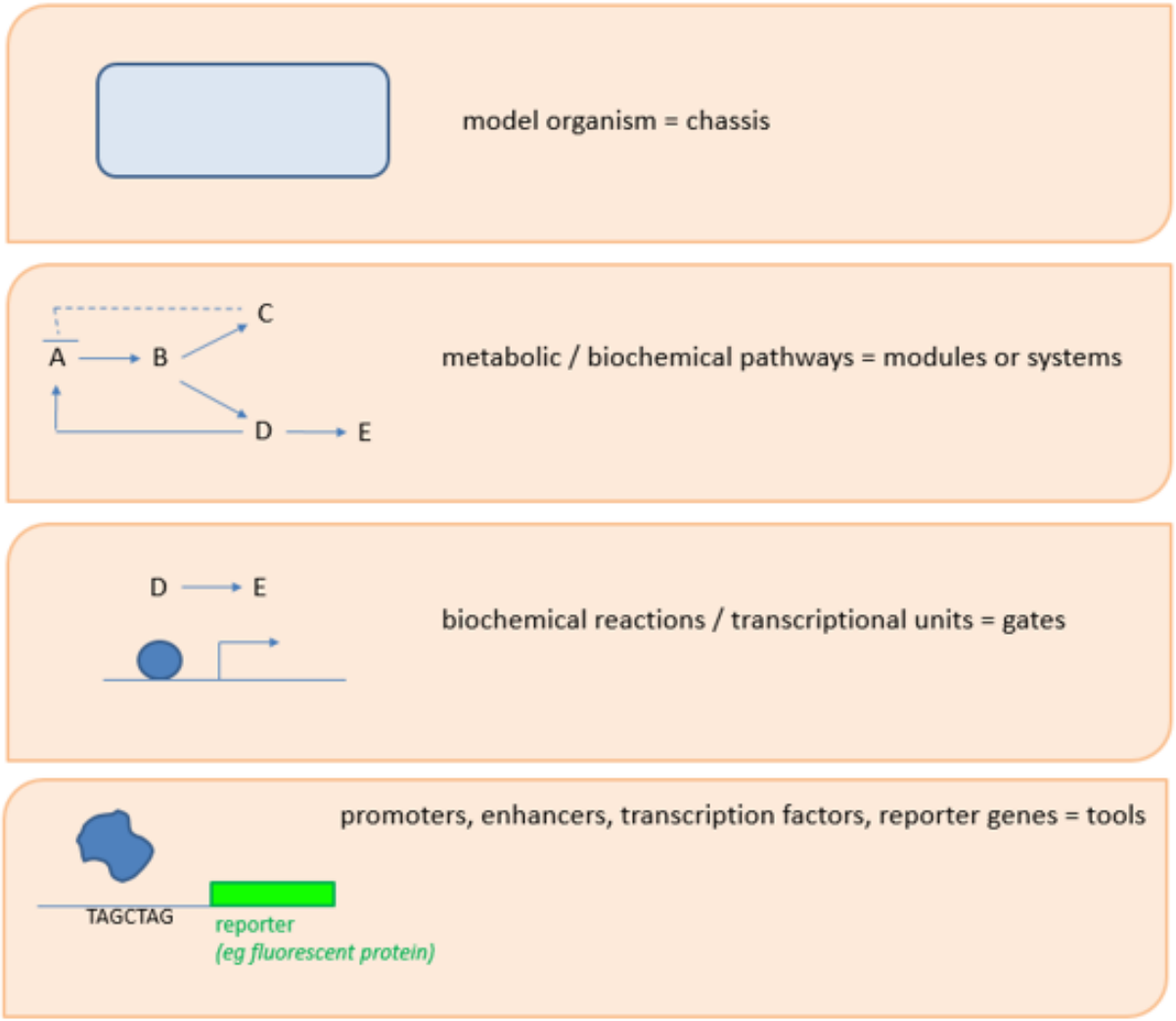
Synthetic biology is defined as

"A) the design and construction of new biological parts, devices, and systems, and

B) the re-design of existing, natural biological systems for useful purposes",

in the synthetic biology community portal (http://syntheticbiology.org).

Synthetic biology is a recently new inter-disciplinary and trans-disciplinary field, combining biology, physics, bioinformatics, engineering and many others, so as to use biological systems as, in a manner of speaking, "chassis" and molecules as "lego pieces", rearranging these pieces so as to come up with new uses that will (hopefully) benefit society.

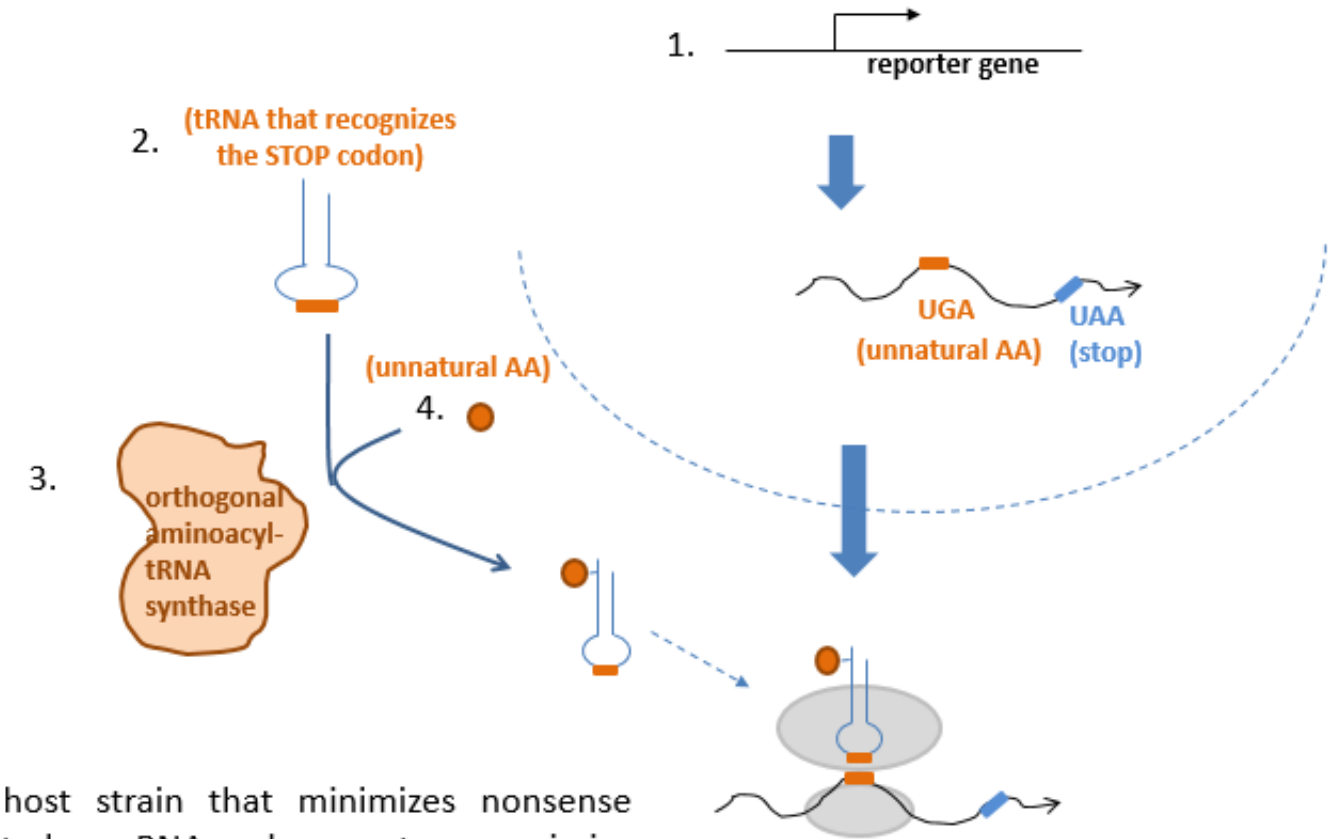


The International Genetically Engineered Machine (iGEM; http://igem.org) competition has been initiated by MIT researchers in 2003 and has since been quite "in", where new biological system designs compete every year (especially noteworthy is the iGEM high school jamboree).

The same MIT team had also introduced the BioBrick, which is a standardized platform of parts, devices and systems for easy assembly of synthetic systems (originally the "Registry of Standard Biological Parts", http://parts.igem.org/Assembly:STandard_assembly), is not also available commercially (for instance, BioBrick® Assembly Kit; http://www.neb.com/products/e0546-biobrick-assembly-kit).

It should be noted that MIT Center for Integrative Synthetic Biology has also developed the CRISPR/Cas system for genome editing, discussed previously.

Perhaps one of the most exciting developments of synthetic biology, however, was the **expansion of the genetic code with unnatural amino acids.**



Schultz laboratory has been pioneering the field, as they have engineered bacteria, yeasts and mammalian neurons that harbour an expanded genetic code.

The Standard Genetic Code contains 61 codons (leaving the 3 STOP codons) that code for 20 amino acids – however certain organisms, such as bacteria, archae or yeast, may sometimes use one of the STOP codons to code for a 21st (or sometimes a 22nd) “unnatural” amino acid, such as a Selenocysteine (Sec) or a Pyrrolysine (Pyl), using either a SECIS (Sec insertion sequence) or PYLIS (Pyl insertion sequence) elements (Zhang et al, 2005; Yuan et al, 2010). (In humans, only 25 selenoproteins are found.)

This has been the starting point of the Schultz lab, and they have re-designed the bacterial translation machinery (initially) in such a way that one of the STOP codons is re-programmed to incorporate other unnatural amino acids (Wang and Schultz, 2002).

Recently a different team has engineered a “semi-synthetic” *E. coli* that contain **unnatural genetic alphabet**, incorporating unnatural nucleotide triphosphates d5SICS and dNaM to the genome, which base pair, efficiently PCR-amplify, get transcribed, and are not removed by DNA repair machinery (Malyshev et al, 2014).

“Your theory is crazy, but it’s not crazy enough to be true.” ***Niels Bohr***

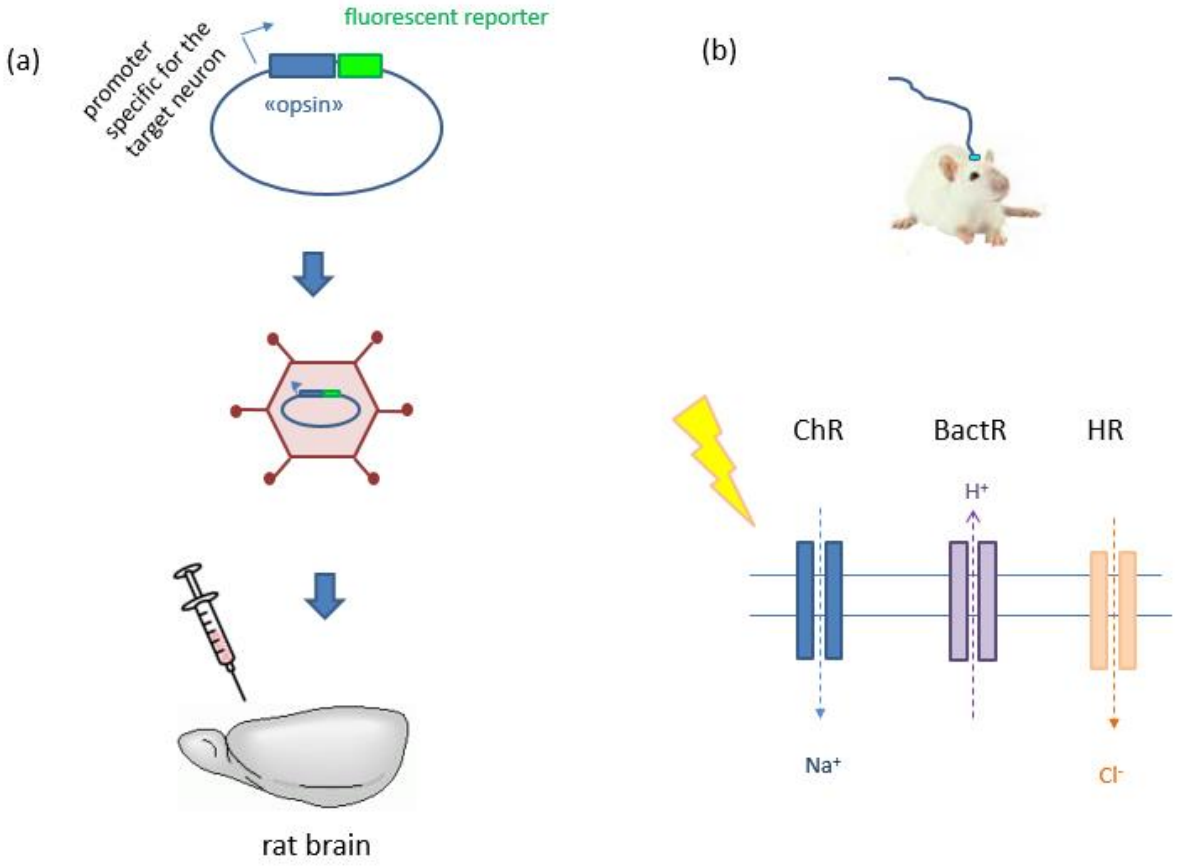
Optogenetics

Optogenetics, developed originally in Deisseroth lab, has been chosen the Method of the Year 2010 (editorial, Nature Methods, 2011; Deisseroth, 2011; http://web.stanford.edu/group/dlab/optogenetics).

The idea that light could be used to precisely control neural activity in specific cell types of the brain was originally proposed by Francis Crick since late 1970s, however it was not until 2002 onwards that rhodopsins were used to genetically target specific neurons in *Drosophila* to generate light-sensitive responses (Zemelman et al, 2002; Zemelman et al, 2003). The same group than reported the first light-induced control of wing beating, flight etc behavior in *Drosophila*, whose neurons have been genetically modified to express photosensitive channels (Lima and Miesenbock, 2005). The system was then quickly adapted to other model organisms such as *C. elegans,* and mice.

Optogenetics is principally based on light-sensitive proteins of microorganisms, mainly channelrhodopsin (ChR), halorhodopsin (HR) and archaerhodopsin (a). Bacteriorhodopsins (BactR) were known since 1970s, halorhodopsin was discoverd in late 1970s, yet it was the discovery of channelrhodopsin in early 2000s that truly opened the gate for optogenetics (Deisseroth, 2011). These light-sensitive channels can be turned on or off in response to different wavelengths, upon which channels either open or close (depends on which engineered variety is used), resulting in electrical excitation or inhibition of neuron in question.

When these light-sensitive channels are cloned downstream of a neural cell type-specific promoter and genetically knocked-in to the organism (commonly using viral vectors, as described in Chapter 8; (a)), the only other components of optogenetics required are the optical stimulation (through integrated fiberoptic and solid-state light sources for application in freely moving animals, usually directly mounted to the animal's skull; (b)) and usually another genetically knocked-in reporter (biosensor) that can provide rapid readouts, such as voltage-sensitive fluorescent protein (VSFP) (Deisseroth, 2011; Gautier et al, 2014; Knopfel et al, 2010).



What’s next ???

“Everything is theoretically impossible, until it is done”.

Robert A. Heinlein