Synthetic life
(combination of "The molecular origins of life" SoSe 2018)

WiSe 2018/19
Zbigniew Pianowski

Overview of the course

Artificial genetic polymers and oligonucleotide analogues;
unnatural base pairing – expansion of the genetic alphabet;
artificial ribozymes for efficient catalysis and recognition (SELEX, DNAzymes, foldamers);
biosynthetic incorporation of unnatural aminoacids (UAAs) into proteins;
enzyme engineering – production of enzymes with unknown or unnatural properties, ab initio protein design, directed evolution, theozymes;
Artificial lipid vesicles as models for protocell multiplication;
synthetic biological circuits – riboswitches, time-delay circuits, oscillators, optogenetics;
design of artificial organisms – minimal genome project, Synthia – fully artificial genome resulting in living bacterial species

The molecular origins of life
Life is a self-replicating chemical system capable of evolution (NASA, 2009)

Origin of the Universe – stars, planets, elements
Origin of biorelevant monomers – primordial soup
Complex chemical processes on the way to living systems
Protocells and LUCA

7 lectures (90 min. each) in English
Heidelberg: Mon. 13:30-15:00 INF 270, Seminarraum 335

1st lecture: 5th Nov. 2018 (HD)

Following lecture terms:


The most current dates, handouts – on the website:
http://www.ioc.kit.edu/pianowski/ and by Moodle

Mailing list for changes and supplementary information
What is Life? What makes it different from just matter?

Everything – living or not – is just chemicals made of atoms.

Fishes swim in water. But what makes fishes alive and not water is the way how the atoms are organized –

By the special kind of molecules: DNA – the double helix molecule that houses the genetic alphabet of
A, C, G and T, which, in different combinations, can make a flower, or a frog, or you...

Every living creature has its code, that makes it grow, reproduce, and change. DNA turns dust into life.

20 years ago, Scientists learned to read the creatures’ entire DNA sequence, from beginning to the end – the genome

Electropherograms are commonly used to sequence portions of genomes

An ABI PRISM 3100 Genetic Analyzer. Such capillary sequencers automated the early efforts of sequencing genomes.
Whole genome sequencing was initially achieved for simple organisms: bacteria, nematodes, flies and plants...

Haemophilus influenzae 1995
Caenorhabditis elegans 1998
Arabidopsis thaliana 2000

... and way up to mammals and human

Mus musculus 2002
Homo sapiens 2004
Human Genome Project (NIH)
Craig Venter – Celera Genomics (private)

With that knowledge, scientists begun to tinker...
... to take a glow from a jellyfish...
... and transfer it to a cat...
... or to a rabbit...

To make creatures do what they never did before.

As biologists got better in this, a new kind of science was born – synthetic biology

Definition: Synthetic Biology
(also known as Synbio, Synthetic Genomics, Constructive Biology or Systems Biology)

„the design and construction of new biological parts, devices and systems that do not exist in the natural world and also the redesign of existing biological systems to perform specific tasks“

Advances in nanoscale technologies – manipulation of matter at the level of atoms and molecules – are contributing to advances in synthetic biology.
What can we do with new tools of synthetic biology?

We can improve what was spelled out for the 3.5 Billion years of evolution.

We can take it beyond reading genomes or editing genomes...
...and start writing genomes.
Our own ideas of what life should be like.

Making creatures drastically different from any that have ever existed.

How could it be done?

Already a group of scientists have re-written and rebuild the entire instruction kit for yeast. And they plan to put their recipe into a cell. And if everything goes according to the plan, it will come alive and begin making baby yeast exactly like their most unusual parent. And they will be new to the world.

Synthetic biologists have been engineering chromosomes from scratch, sticking them into yeast and seeing whether the modified organisms can still function normally.

Could yeasts have evolved through alternate routes?
How much can you change a genome and still have a working organism?

Each of S. cerevisiae’s 16 chromosomes were assigned to teams of collaborators worldwide.

Each was to create a chromosome that was stable yet evolvable, and would keep yeast functioning as usual.

The teams used computer programs to design the codes of their respective chromosomes. They omitted some sequences found in naturally occurring yeast chromosomes, such as repetitive parts of the genome, in hopes of increasing the stability of the synthetic versions.

And they endowed their creations with a mechanism that mimics the random variation that drives evolution. When this scrambling system is triggered, it can shuffle, duplicate and delete genes at random.

This synthetic yeast will break the continuous chain of evolution that links every creature back to the first living cell.

It will be discontinuous in a way, a thing onto itself. And then, where do we take this new technology?

The work that the consortium has already done could help to optimize the creation of microbes to pump out alcohol, drugs, fragrances and fuel. And it serves as a guide for future research on how genomes evolve and function.
Overview of the course

Artificial genetic polymers and oligonucleotide analogues; unnatural base pairing – expansion of the genetic alphabet; artificial ribozymes for efficient catalysis and recognition (SELEX, DNAzymes, foldamers); biosynthetic incorporation of unnatural aminoacids (UAAs) into proteins; enzyme engineering – production of enzymes with unknown or unnatural properties, ab initio protein design, directed evolution, theozymes; Artificial lipid vesicles as models for protocell multiplication; synthetic biological circuits – riboswitches, time-delay circuits, oscilators, optogenetics; design of artificial organisms – minimal genome project, Synthia – fully artificial genome resulting in living bacterial species.
**Introduction**

**Basic bio-vocabulary methods of molecular biology**

Greek mythology – introduction to modern molecular biology – chimera, centaur
Because DNA can only be synthesized from the 5' to 3' direction, the two newly-synthesized strands are synthesized differently. They are called the "continuous" (on the top in the figure) and "discontinuous" (on the bottom in the figure) strand, and also the "leading" (top) and "lagging" (bottom) strand.

As the replication fork moves down the helix, you can see the leading strand synthesized continuously, while the lagging strand must initiate new sites of synthesis.
From DNA to proteins

Procaryotic DNA polymerases

<table>
<thead>
<tr>
<th>Polymerase</th>
<th>Polymerase activity</th>
<th>Exonuclease activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA polymerase I</td>
<td>Filling gap after removal of RNA primer, DNA repair, removal of RNA primers</td>
<td>5'→3' and 3'→5'</td>
</tr>
<tr>
<td>DNA polymerase II</td>
<td>DNA repair</td>
<td>3'→5'</td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td>Replication, proofreading and editing</td>
<td>3'→5'</td>
</tr>
</tbody>
</table>

*The main enzyme of replication*

Eukaryotic enzymes:

Five common DNA polymerases from mammals:

1. Polymerase α (alpha): nuclear, DNA replication, no proofreading
2. Polymerase β (beta): nuclear, DNA repair, no proofreading
3. Polymerase γ (gamma): mitochondria, DNA replication, proofreading
4. Polymerase δ (delta): nuclear, DNA replication, proofreading
5. Polymerase ε (epsilon): nuclear, DNA repair, proofreading

Polymers vary by species.
Transcription: DNA → RNA

Transcription requires the opening of the DNA helix, similar to replication.

An RNA transcript is synthesized in the 5' to 3' direction, just like a new strand of DNA.

Transcription: DNA → RNA

Here the RNA transcript continues elongation until the transcript is complete.

There is a signal in the DNA sequence that indicates a transcription start site and a transcription termination site. The transcription machinery must be able to read these signals, which implies that proteins can read the sequence of DNA.

Transcription: DNA → RNA

It is possible to transcribe the other strand in this example.

Here the RNA transcript continues elongation until the transcript is complete. Only one strand of any gene is transcribed, so the transcription initiation and termination signals must be specific to one strand.

Transcription: DNA → RNA

Because transcription start and stop signals are specific to one strand, different genes on the same chromosome can be oriented in different directions. Only one particular strand is ever transcribed for each gene.
Transcription: DNA → RNA

How do the start/stop signals look like? For procaryotes (like bacteria E. coli):

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transcription</th>
<th>Coding sequence of gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

The transcription start site does not exactly correspond to the codon that is used to initiate translation – base 1 (+1). Transcription begins upstream of this sequence. The RNA between the base 1 and the start of translation some distance downstream is called the 5' untranslated region or 5' UTR. There is also an untranslated region at the 3' end (3' UTR).

There are two conserved elements in promoter regions of E. coli: an element around -30 and an element around -35. These elements can be identified in most E. coli promoters.

Transcription: DNA → RNA

How does the transcription machinery recognize these sequences?

RNA polymerase itself does not carry out recognition of the promoter. In E. coli, there is a separate protein called sigma factor that specifically recognizes the promoter. Sigma factor and RNA polymerase form a complex that initiates transcription at the promoter. Once RNA elongation has begun, sigma factor dissociates from the complex.

Transcription: DNA → RNA

Initiation in eukaryotes is more complex.

Most eukaryotic promoters have a "TATA box" at position -30, and typically have other classes of promoter sequences that are shared by groups of genes.

Eukaryotes use a set of general transcription factors that bind to the promoter region, then recruit other protein factors including RNA polymerase.

Once the preinitiation complex is formed, RNA polymerase is phosphorylated and released from the complex to begin RNA strand elongation.

Transcription: DNA → RNA

In E. coli, there are two mechanisms for transcription termination, an intrinsic mechanism and a mechanism that depends on a specific protein called rho factor.

In the intrinsic mechanism, shown above, there is a self-complementary sequence past the end of the coding sequence that forms a hairpin loop once it is transcribed. The base-paired part of the loop is very GC-rich, so the hairpin is stable. The presence of this structure interferes with RNA elongation, and transcription terminates.
In rho-dependent transcription termination, a specific sequence at the end of the gene binds rho factor. When RNA polymerase encounters rho, the polymerase dissociates from the template, terminating transcription.

Eukaryotic transcription termination is similar to that of prokaryotes (polyT, or recruiting CPSF/CTSF).
In 1977, Phil Sharp (Nobel Prize 1993) hybridized an mRNA to its DNA template and prepared the hybrid molecule for electron microscopy by coating the nucleic acid with a basic protein, then using rotary shadowing to coat the nucleic acid-protein complex.

Eucaryotic genes are discontinuous!!!
Eukaryotic mRNAs are also modified at the 3' end by the addition of a poly-A tail.

Finally, eukaryotic mRNAs have a chemical modification of the 5' end, called a cap. The cap is added to the first base of the 5' UTR.

Alternative splicing - tropomyosin

Splicing is regulated, with many genes producing multiple isoforms of the same protein that can differ considerably in their amino acid sequence due to alternative splicing. Isoforms of the muscle protein tropomyosin derived from alternative splicing are shown below. While there are some exons common to all isoforms, some isoforms have large protein segments entirely missing from other isoforms.

Translation: RNA → proteins

The coupling of transcription and translation in bacteria
One can see the newly-synthesized peptide, and can tell which is the 5' end of the mRNA, because there are shorter protein tails on the ribosomes at that end.

**A transfer RNA has a cloverleaf structure with regions of base pairing.**

**A tRNA has the structure shown here both as a flat cloverleaf and in its folded form.**

**Two important parts of a tRNA:**
- the anticodon, which participates in base pairing with a codon in the mRNA
- the site of amino acid attachment at the 3' end of the tRNA

This shows a "charged" serine tRNA, covalently attached to the amino acid serine at its 3' end, with the anticodon paired to a serine codon.

This is a better representation of the 3D structure of a tRNA. The model is color-coded to the flat cloverleaf representation in the lower right.
Aminoacyl tRNA synthetase

A special set of enzymes "charges" tRNAs, attaching the correct amino acid to particular tRNAs.

A charged tRNA is called an aminoacyl tRNA, so the charging enzymes are more properly called aminoacyl tRNA synthetases.

There is only one aminoacyl tRNA synthetase for each amino acid, even though there can be multiple tRNAs for that amino acid. Each aminoacyl tRNA synthetase is able to recognize all of the tRNAs that need to be charged with the one amino acid that is their specialty.

Amino acids are attached to the hydroxyl (-OH) group at the 3' end of the tRNA through their carboxyl (-COOH) group.

Proteins are synthesized by ribosomes that read the sequence of mRNA and write it as protein. Translation is accomplished with the help of charged tRNAs that allow individual codons to specify the next amino acid added to the growing polypeptide. The mRNA is read from the 5' end to the 3' end, with the protein being synthesized from the amino terminus to the carboxy terminus.

Three sites are associated with tRNAs: the A (aminoacyl) site, that accepts a new aminoacyl tRNA; the P (polypeptide) site, that holds a tRNA with the growing polypeptide chain; and the E (exit) site that holds an uncharged tRNA ready to exit the ribosome.
**Translation: RNA → proteins**

**Translation elongation**

1. **Initiation**
   - Ribosome binds to mRNA at the AUG start codon.
   - tRNA carrying the first amino acid (Met) binds to the start codon.

2. **Elongation**
   - Peptidyltransferase (PTase) catalyzes the formation of a peptide bond between the two tRNAs.
   - tRNA is moved from the P site to the A site, and another tRNA enters the P site.

3. **Termination**
   - Stop codon (UAA, UAG, UGA) is encountered.
   - Release factor binds to the ribosome, releasing the peptidyl-tRNA and the completed polypeptide.
   - Polypeptide is released from the ribosome.

**Translation termination**

1. **Release factor**
   - tRNA is released from the ribosome.
   - Protein chain is released from the ribosome.

2. **Polyprotein**
   - mRNA is translated into a polypeptide chain.
   - Polypeptide is released from the ribosome.

3. **Ribosome subunits separate**
   - The ribosome subunits separate, releasing the completed polypeptide.

**Translation: RNA → proteins – the genetic code**

<table>
<thead>
<tr>
<th>Base</th>
<th>Codon</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU</td>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>UUC</td>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>UUA</td>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>UUG</td>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>CUU</td>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>CUC</td>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>CUA</td>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>CUG</td>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>AUA</td>
<td>Ile</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>AUG</td>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>GUA</td>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>GUC</td>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>GUG</td>
<td>Val</td>
<td>Valine</td>
</tr>
</tbody>
</table>

**Standard genetic code**

- Start codon: AUG (Met)
- Stop codons: UAA, UAG, UGA