Synthetic life

(continuation of "The molecular origins of life" SoSe 2025)



WiSe 2025/26

Zbigniew Pianowski

7 lectures (90 min. each) in English

1st lecture: 29th Oct. 2025 (Wed.)

Zoom:

https://us02web.zoom.us/j/81927185504?pwd=bw1P5JPB3ELwcWPCaLmhshPlZT6d0d.1

Following lecture terms 14:00-15:30, Wed.:

12.11, 19.11., 26.11., 03.12., 10.12. and 17.12.2025 (backup date 07.01.2026).

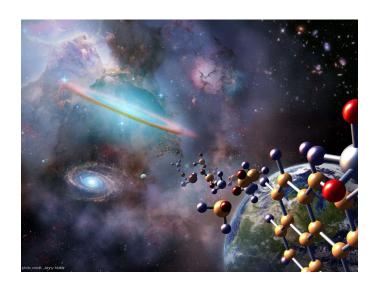
The most current dates, handouts – on the website:

https://www.ioc.kit.edu/pianowski/99_343.php

and by ILIAS (KIT)

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

Synthetic life



www.genome.gov/about-genomics/policy-issues/Synthetic-Biology

How new functions can emerge from known biological building blocks?

What is Life? What makes it different from just matter?



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

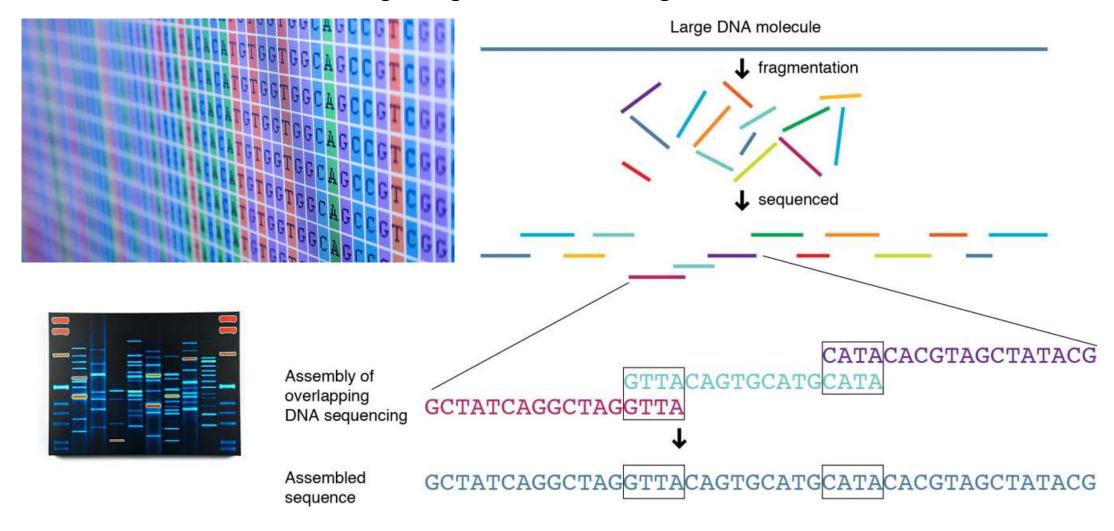
Every living creature has its code, that makes it grow, reproduce, and change.

DNA turns dust into life.

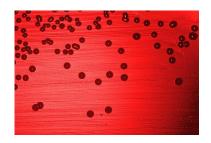


Fishes swim in water. But what makes fishes alive and not water is the way how the atoms are organized – By the special kind of molecules: DNA – the double helix molecule that houses the genetic alphabet of A, C, G and T, which, in different combinations, can make a flower, or a frog, or you...

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



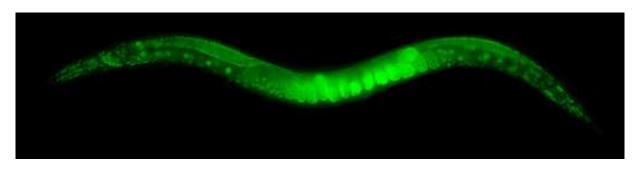
Whole genome sequencing was initially achieved for simple organisms: bacteria, nematodes, flies and plants...



Haemophilius influenzae 1995



Drosophila melanogaster 2000

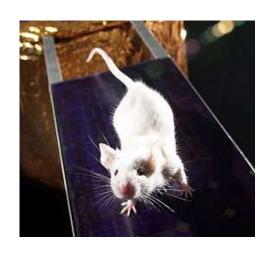


Caenorhabdis elegans 1998

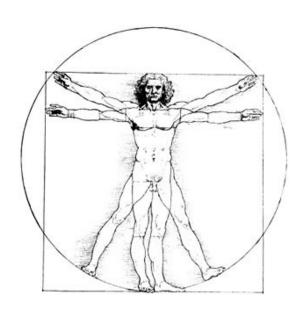


Arabidopsis thaliana 2000

... and way up to mammals and human



Mus musculus 2002



Homo sapiens
2004
Human Genome Project (NIH)
Craig Venter – Celera Genomcs (private)

With that knowledge, scientists begun to tinker...

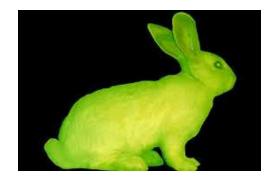
... to take a glow from a jellyfish...

... 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?

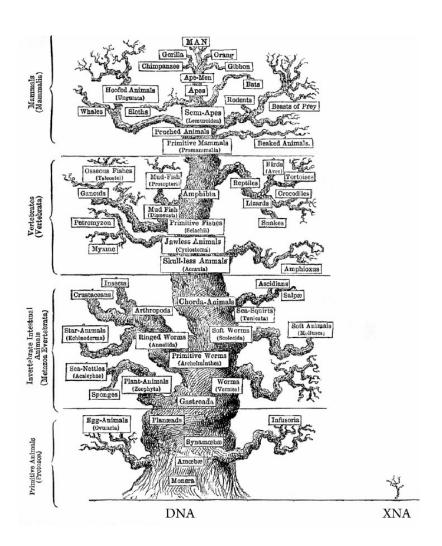
We can improve what was spelled out for the 3,5 Billion years of evolution.

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?



Overview of the course

artificial ribozymes and aptamers for efficient catalysis and recognition (SELEX, DNAzymes, foldamers);

unnatural base pairing – expansion of the genetic alphabet;

artificial genetic polymers and oligonucleotide analogues (XNA);

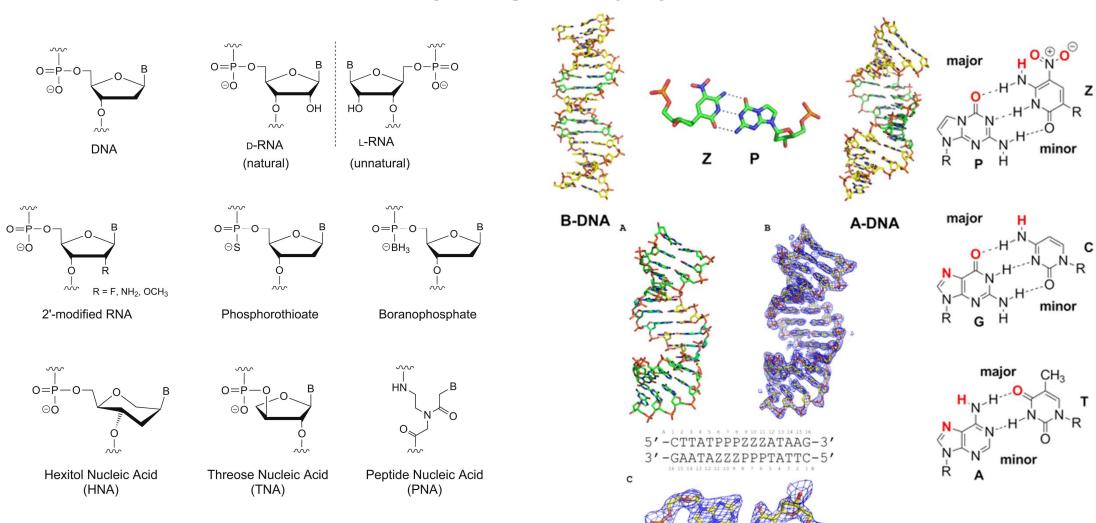
biosynthetic incorporation of unnatural aminoacids (UAAs) into proteins;

enzyme engineering – production of enzymes with unknown or unnatural properties, *ab initio* protein design, directed evolution, theozymes;

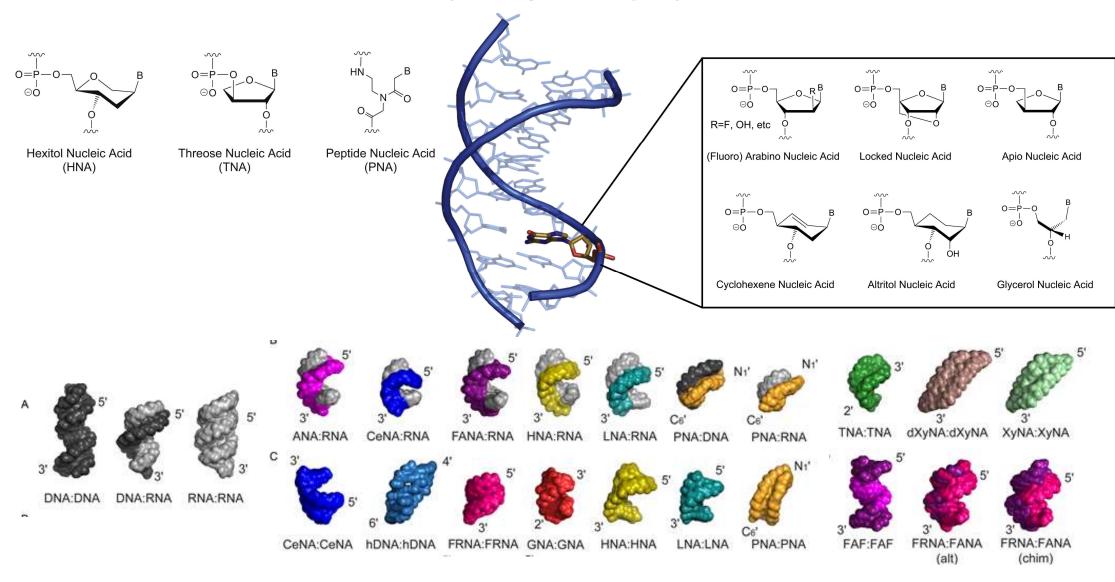
Artificial lipid vesicles as models for protocell multiplication;

design of artificial organisms

Artificial genetic polymers

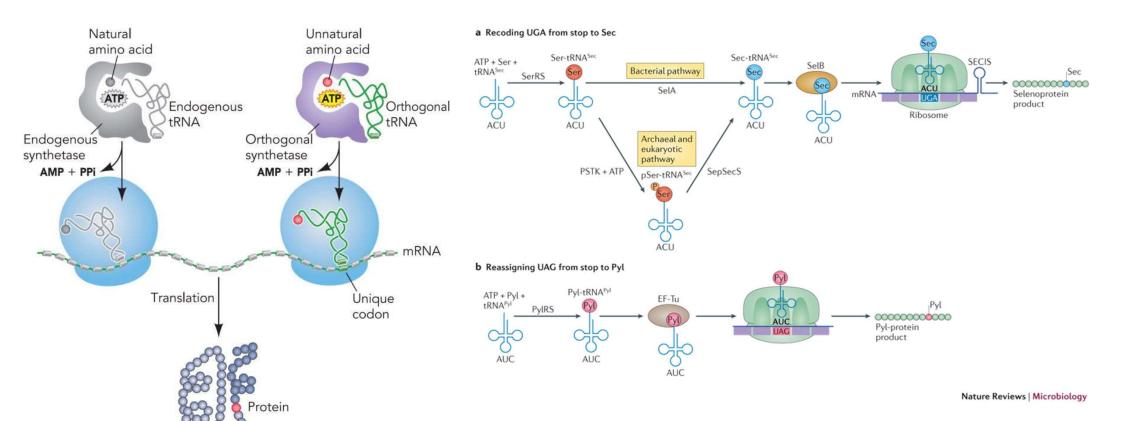


Artificial genetic polymers

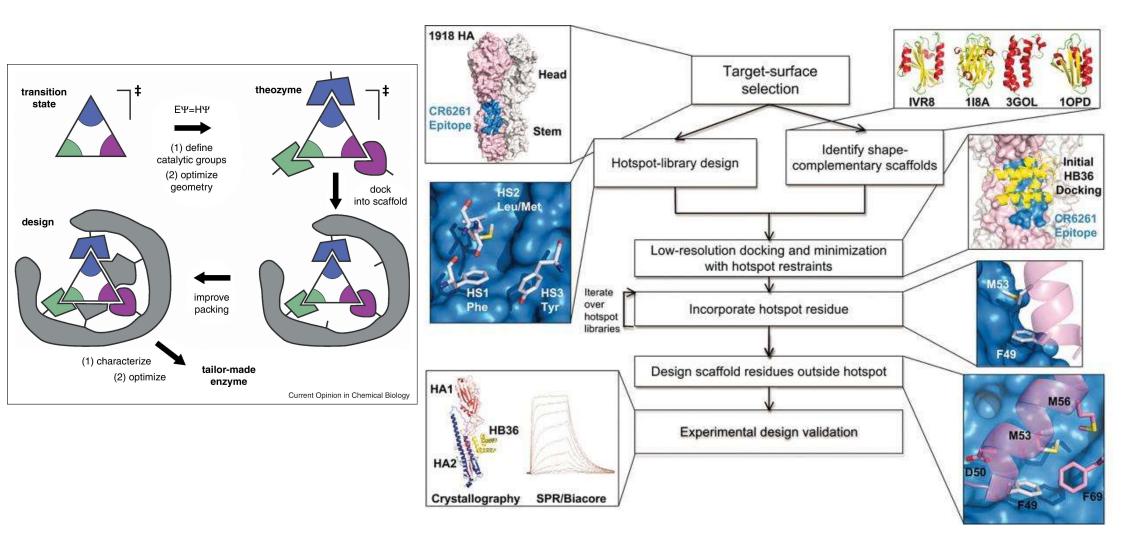


Expanded genetic code

Expanded genetic code

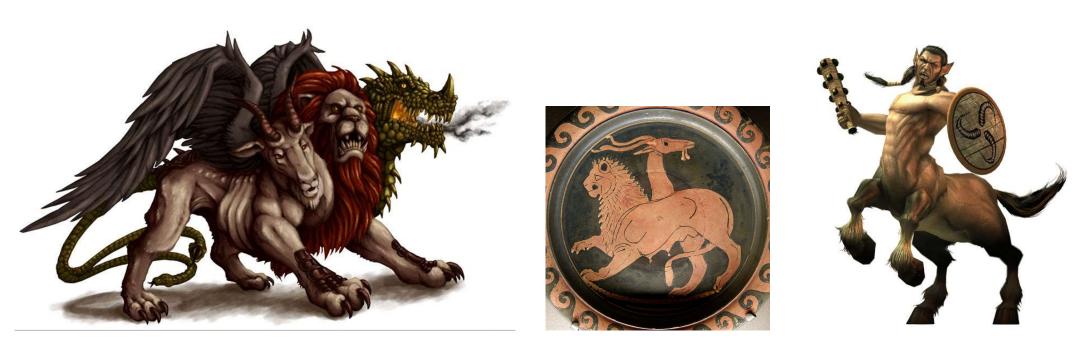


Protein engineering and de novo enzyme design



Introduction

How chemists and biologists are learning from each other?



Greek mythology – introduction to modern molecular biology – chimera, centaur

Introduction

The Central Dogma of the molecular biology – DNA → RNA → proteins

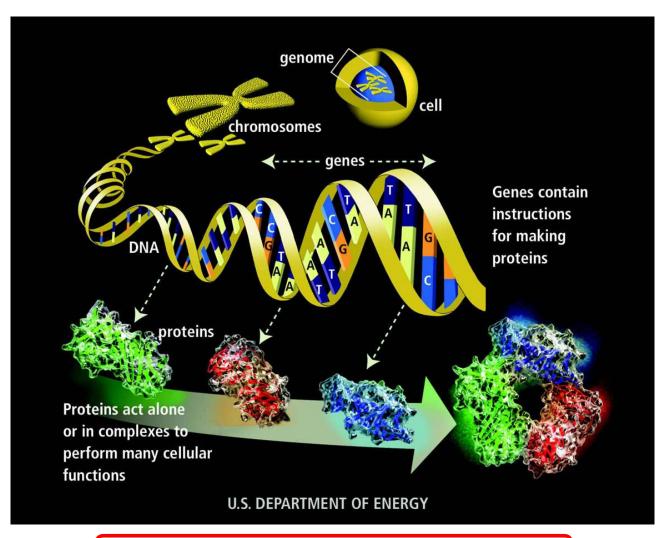
Polymerases and ribosomes - the molecular machines of life

PCR – Polymerase chain reaction – in vitro DNA amplification

Recombinant protein production – how to produce a protein in another organism

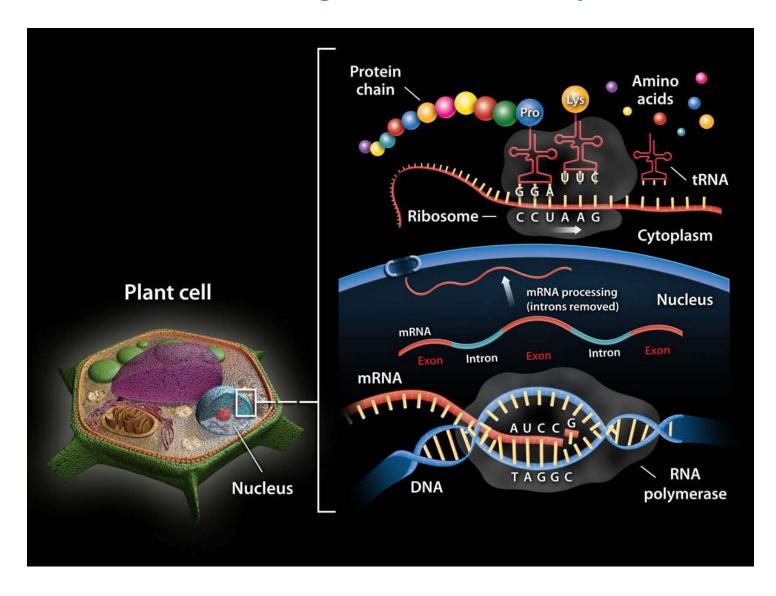
Protein engineering – how to make desired modifications in proteins

From DNA to proteins

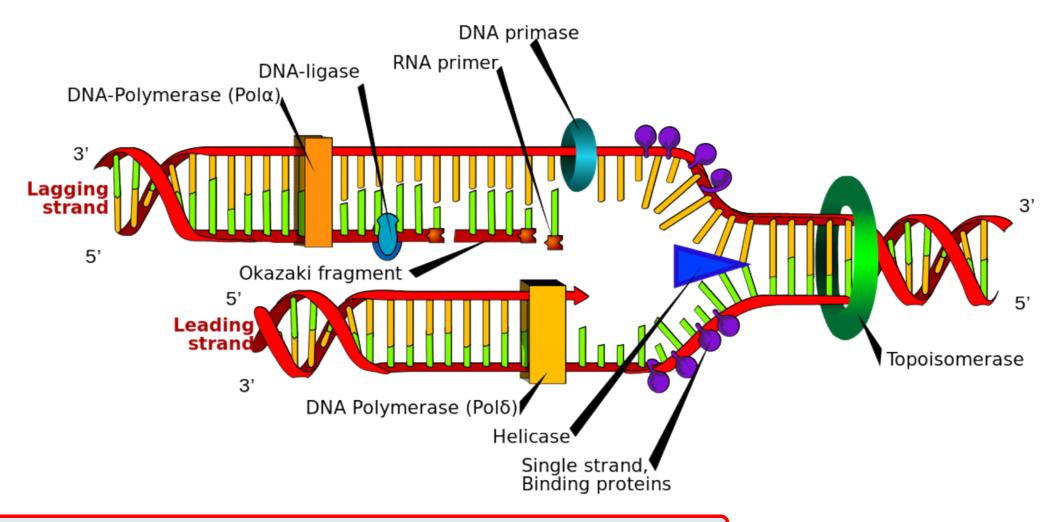


https://www.youtube.com/watch?v=gG7uCskUOrA

The Central Dogma: From DNA to proteins

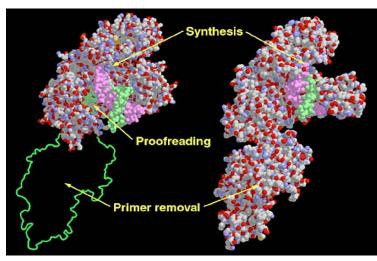


DNA replication



https://www.youtube.com/watch?v=TNKWgcFPHqw&ab_channel=yourgenome

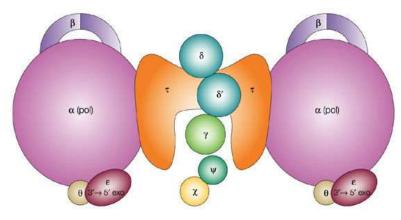
DNA polymerases



Procaryotic DNA Polymerases

Polymerase	Polymerase activity (for all enzymes 5' → 3')	Exonuclease activity
DNA polymerase I	Filling if gap after removal RNA primer, DNA repair, removal of RNA primers	$5' \rightarrow 3'$ and $3' \rightarrow 5'$
DNA polymerase II	DNA repair	3'→5'
DNA polymerase III*	Replication, proofreading and editing	3'→5'

*The main enzyme of replication



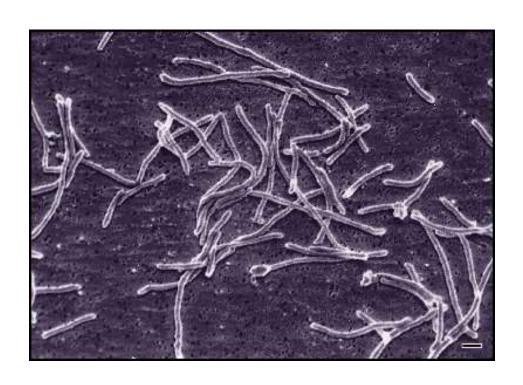
Nature Reviews | Molecular Cell Biology

Procaryotic DNA Polymerase III

SUBUNITS OF THE DNA
POLYMERASE III HOLOENZYME

Subunit	Function	Groupings
α ϵ θ	5'-3' polymerization 3'-5' exonuclease core assembly	Core enzyme: Elongates polynucleotide chain and proofreads
$\left. egin{array}{c} \gamma \ \delta \ \delta' \ \chi \ \psi \end{array} ight\}$	Loads enzyme on template (Serves as clamp loader)	γ complex
β	Sliding clamp structure (processivity factor)	
au	Dimerizes core complex	

Extremophilic organisms



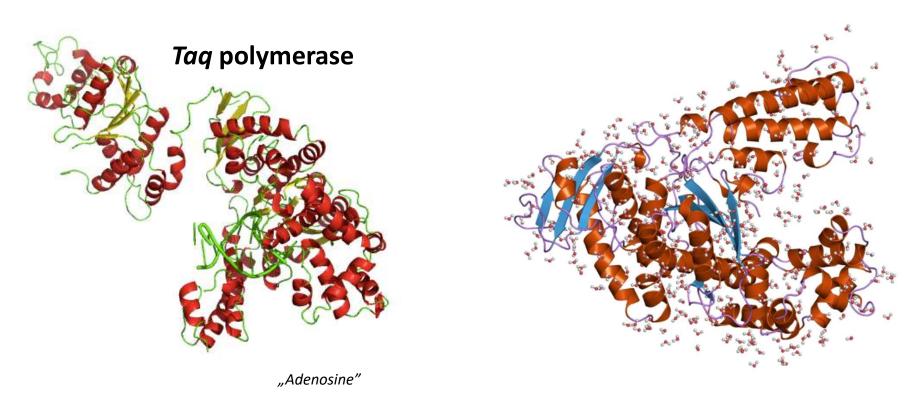


Brian W. Schaller, Yellowstone Park

Thermus aquaticus is a thermophilic bacteria from hot springs in Yellowstone Park 70°C – optimum, living range: 50-80°C

It is a source of thermostable enzymes

PCR - Polymerase Chain Reaction

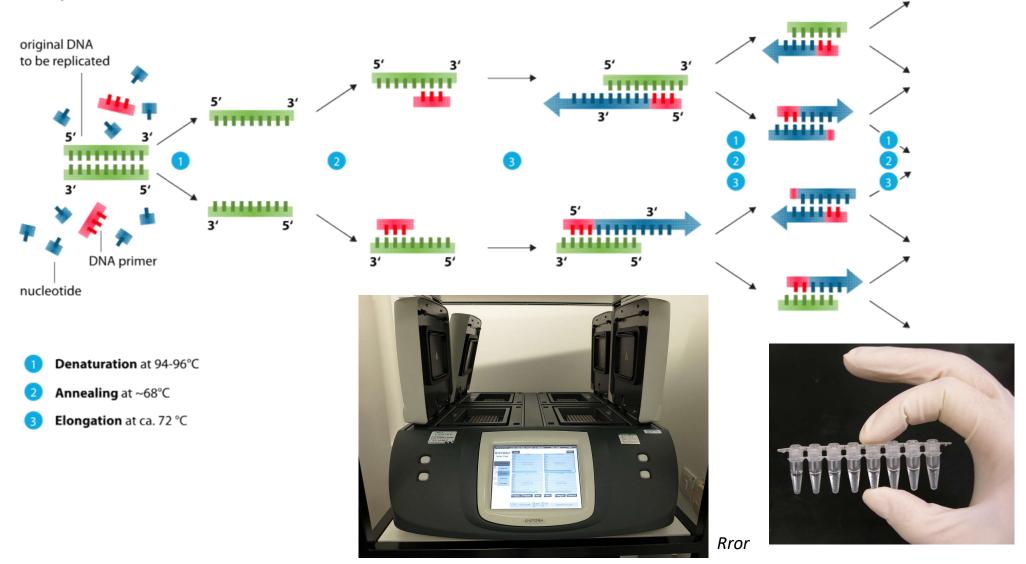


Taq polymerase withstands denaturing conditions (hot temperatures) detrimental for most enzymes. Activity optimum: 75-80 $^{\circ}$ C, half-life at 95 $^{\circ}$ C > 2.5 h

1990 – Kary Mullis optimized the PCR technique with *Taq* polymerase (1993 Nobel Prize)

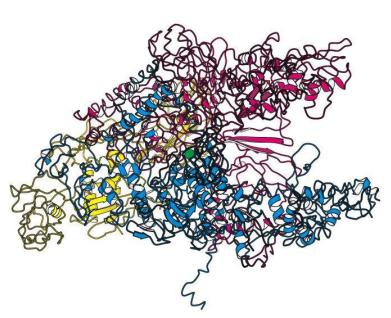
https://www.dnalc.org/view/15475-The-cycles-of-the-polymerase-chain-reaction-PCR-3D-animation.html

Polymerase chain reaction - PCR

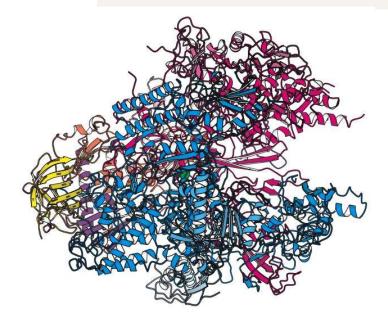


RNA POLYMERASES IN EUKARYOTES

Form	Product	Location
	rRNA	Nucleolus
	mRNA, snRNA	Nucleoplasm
	5S rRNA, tRNA	Nucleoplasm



Prokaryotic RNA polymerase



Eukaryotic RNA polymerase

Figure 29.1

Biochemistry, Seventh Edition

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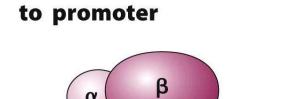
RNA Polymerase of prokaryotes

Subunit	abunit Function α,α Determine the DNA to be transcribed	
α, α		
β	Catalyze polymerization	
β′	Bind & open DNA template(unwinding)	
ω	Function is not known	
	Recognize the initiation sites called promoter	

https://www.youtube.com/watch?v=SMtWvDbfHLo

(b) Initiation

(a) RNA polymerase binding to promoter



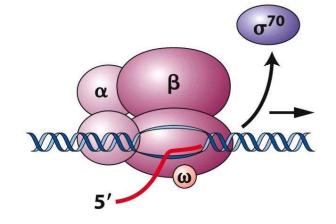
β

-35

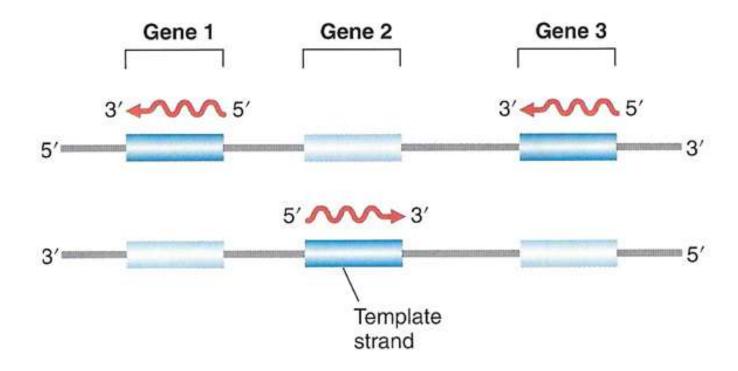
 σ^{70}

α

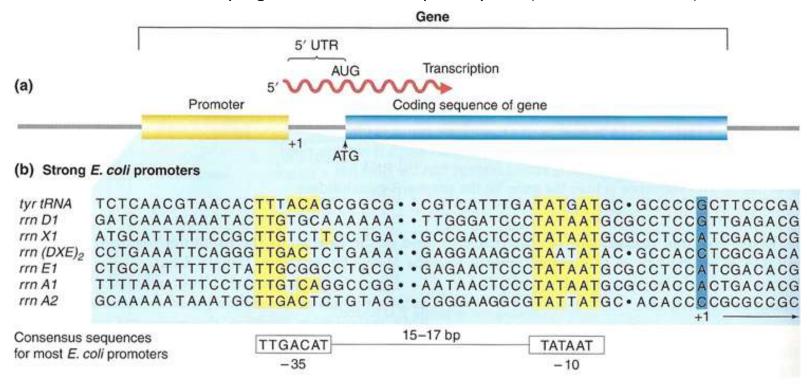
DNA



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



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

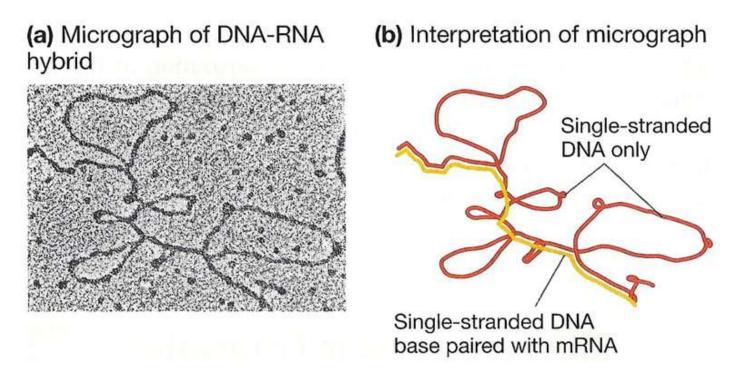


the transcription start site does not exactly correspond to the codon that is used to initiate translation – base 1 (+1). Transcription begins upstream of this sequence. The RNA between the "base 1" and the start of translation some distance downstream is called the 5' untranslated region or 5' UTR. 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 -10 and an element around -35. These elements can be identified in most *E. coli* promoters.

mRNA processing

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.



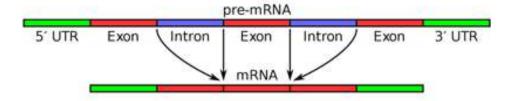
Eucaryotic genes are discontinuous!!!

mRNA processing

The transcript is discontinuous.

There are parts of the DNA template that are not represented in the mRNA.

Eukaryotic mRNA processing



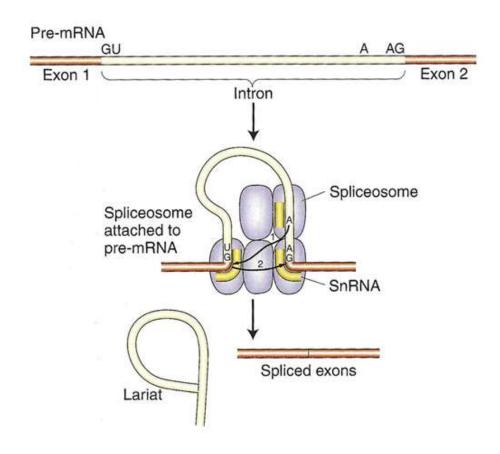
introns are removed (splicing)

When a eukaryotic gene is transcribed, the primary transcript is processed in the nucleus in several ways. The most striking modification is splicing. Parts of the primary transcript, called introns, are spliced out of the mRNA. The remaining segments of mRNA are called exons.

https://www.youtube.com/watch?v=aVgwr0QpYNE

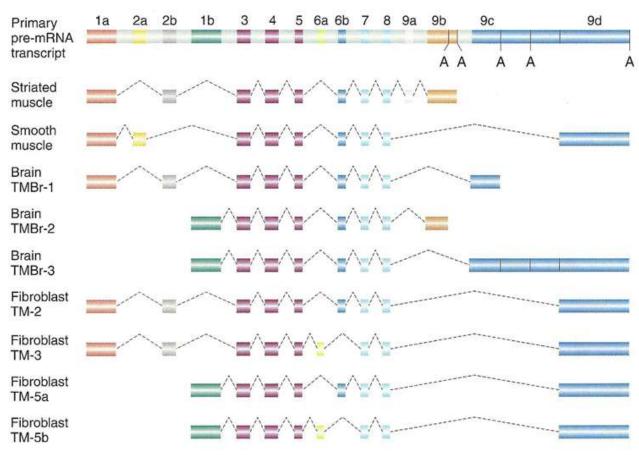
mRNA processing

Splicing is facilitated by a ribonucleoprotein complex called the spliceosome. The spliceosome carries out the removal of introns as RNA lariats, joining exons together to make a mature mRNA, as shown below.

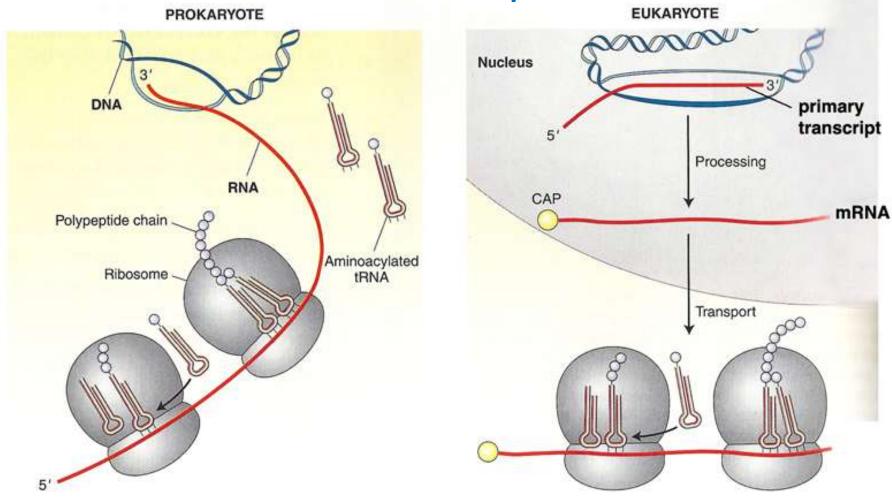


Alternative splicing - tropomyosin

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



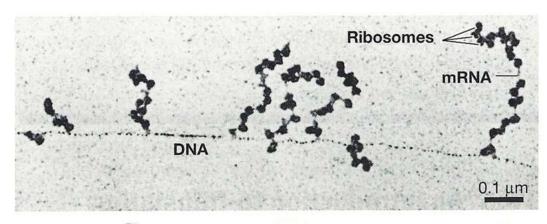
Translation: RNA → proteins

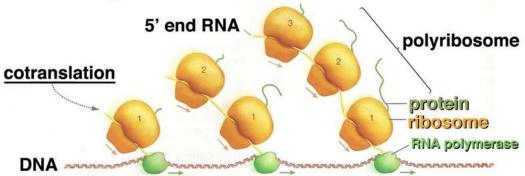


https://www.youtube.com/watch?v=TfYf_rPWUdY

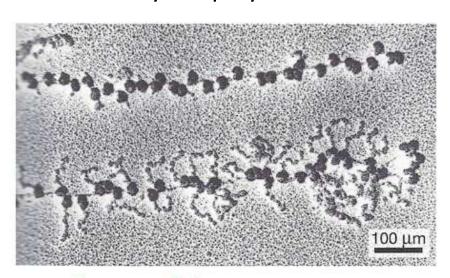
https://www.youtube.com/watch?v=kmrUzDYAmEI

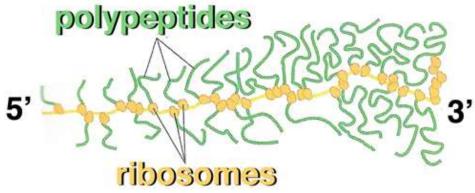
The coupling of transcription and translation in bacteria



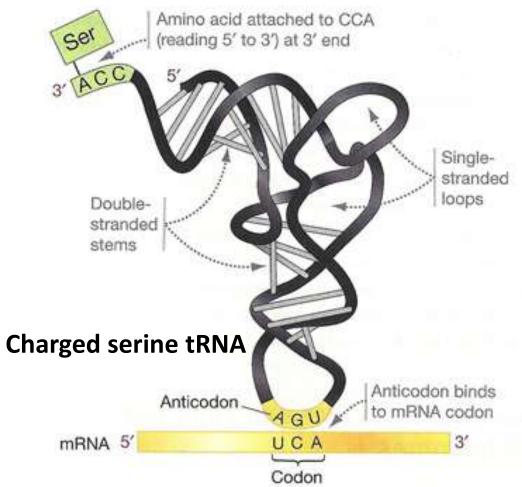


Eukaryotic polyribosomes

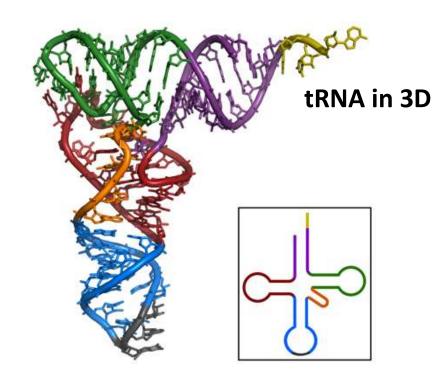




5' end of the mRNA, because there are shorter protein tails on the ribosomes at that end



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



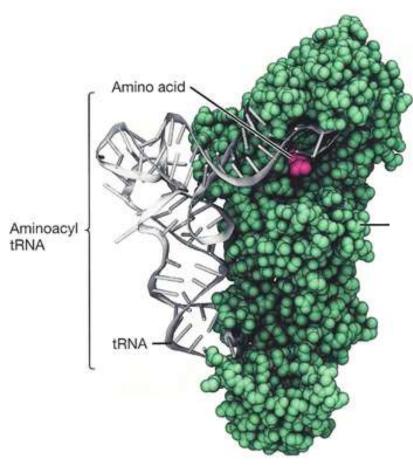
Aminoacyl tRNA synthethase

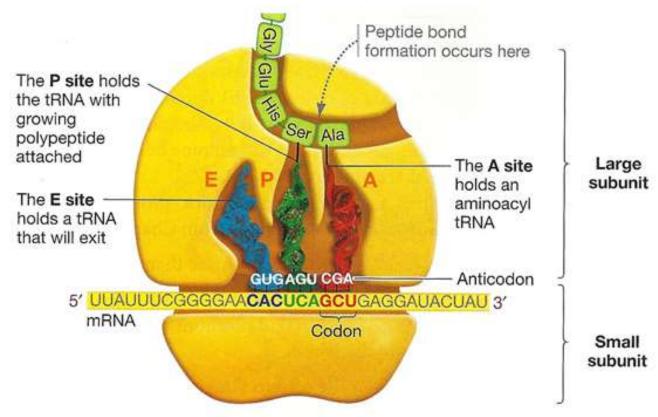
A special set of enzymes "charges" tRNAs, attaching the correct amino acid to particular tRNAs.

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.

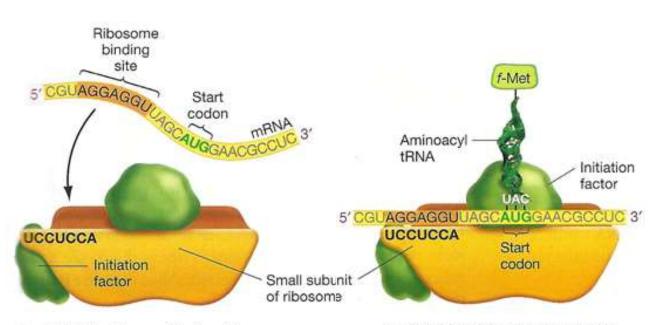
Amino acids are attached to the hydroxyl (-OH) group at the 3' end of the tRNA through their carboxyl (-COOH) group





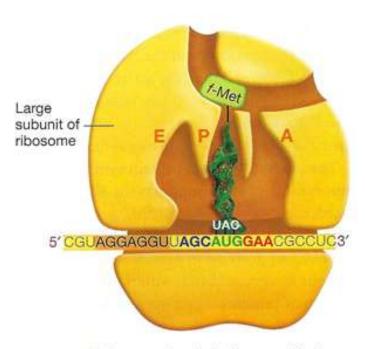
Three sites are associated with tRNAs: the A (aminocyl) site, that accepts a new aminoacyl tRNA; the P (polypeptide) site, that holds a tRNA with the growing polypeptide chain; and the E (exit) site that holds an uncharged tRNA ready to exit the ribosome

Translation initiation



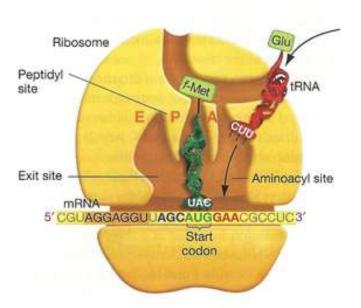
1. mRNA binds to small subunit.

Ribosome binding site sequence binds to a complementary sequence in an RNA molecule in the small subunit of the ribosome, with the help of protein initiation factors. Initiator aminoacyl tRNA binds to start codon.

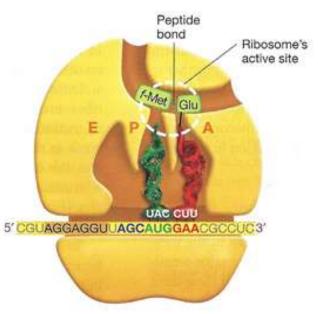


 Large subunit of ribosome binds, completing ribosome assembly.
 Translation begins.

Translation elongation



Incoming aminoacyl tRNA
 New tRNA moves into A site, where
 its anticodon base pairs with the
 mRNA codon.

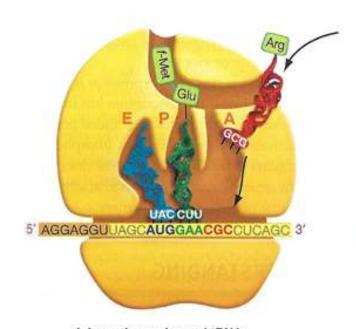


Peptide bond formation
 The amino acid attached to the tRNA in the P site is transferred to the tRNA in the A site.

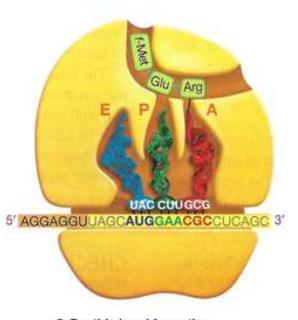


3. Translocation
mRNA is ratcheted through the ribosome
by elongation factors (not shown). The
tRNA attached to the polypeptide chain
moves into the P site. The A site is empty.

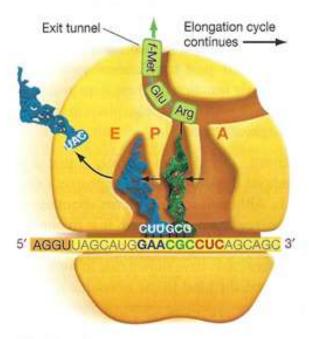
Translation elongation



Incoming aminoacyl tRNA
 New tRNA moves into A site, where its anticodon base pairs with the mRNA codon.



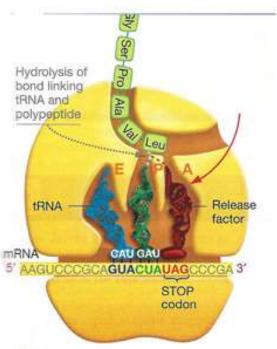
5. Peptide bond formation The polypeptide chain attached to the tRNA in the P site is transferred to the aminoacyl tRNA in the A site.



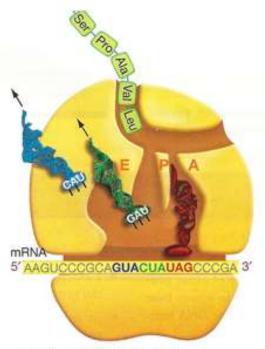
6. Translocation

mRNA is ratcheted through the ribosome again. The tRNA attached to polypeptide chain moves into P site. Empty tRNA from P site moves to E site, where tRNA is ejected. The A site is empty again.

Translation termination

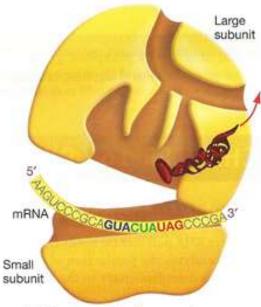


 Release factor binds to stop codon.
 When translocation exposes a stop codon, a release factor fills the A site. The release factor breaks the bond linking the tRNA in the P site to the polypeptide chain.



2. Polypeptide is released.

The hydrolysis reaction frees the polypeptide, which is released from the ribosome. The empty tRNAs are released either along with the polypeptide or...



3. Ribosome subunits separate. ...when the ribosome separates from the mRNA, and the two ribosomal subunits dissociate. The subunits are ready to attach to the start codon of another message and start translation anew.

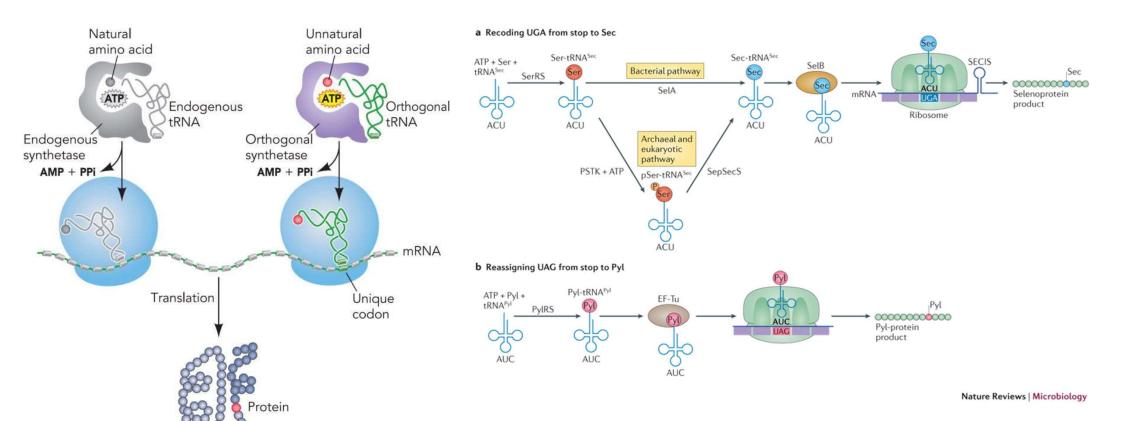
Translation: RNA → proteins – the genetic code

nonpolar polar basic acidic (stop codon)

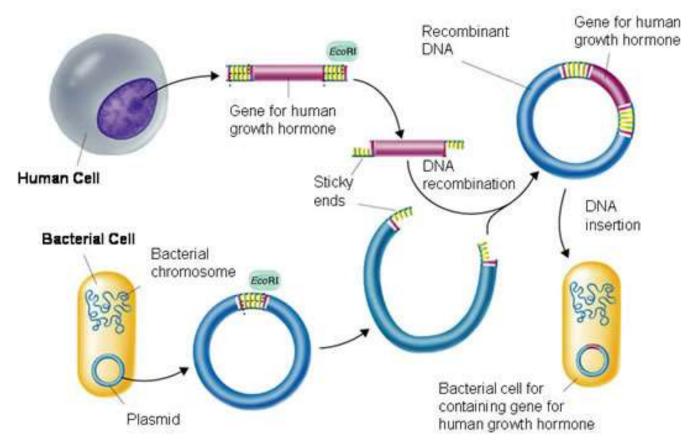
Standard genetic code

1st base	2nd base								3rd
	U		С		A		G		base
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU (Our (O) Ourtring		U
	UUC		UCC		UAC		UGC	(Cys/C) Cysteine	С
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (Ochre)	UGA	Stop (Opal)	A
	UUG		UCG		UAG	Stop (Amber)	UGG	(Trp/W) Tryptophan	G
С	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		ccc		CAC		CGC		С
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		С
	AUA		ACA		AAA	(Lvs/K) Lvsine	AGA	(Arg/R) Arginine	Α
	AUG ^[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	1	U
	GUC		GCC		GAC		GGC	(Ch/O) Chroine	С
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA	(Gly/G) Glycine	Α
	GUG		GCG		GAG		GGG		G

Expanded genetic code



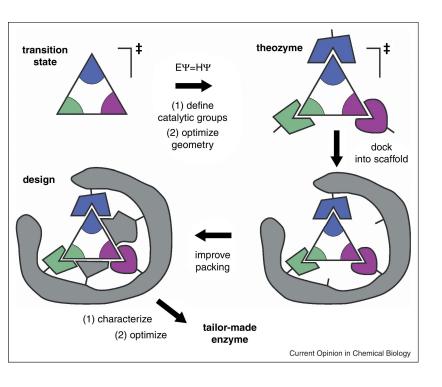
Recombinant proteins

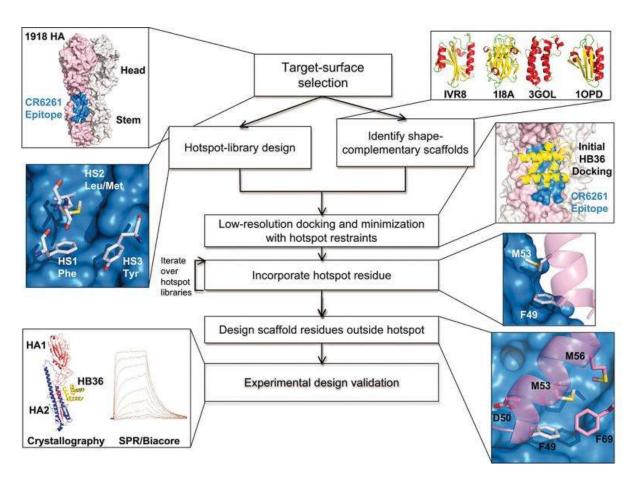


Recombinant insulin:

https://www.youtube.com/watch?v=glt8iAqK8NQ&ab_channel=ScienceForStudent

de novo enzyme design

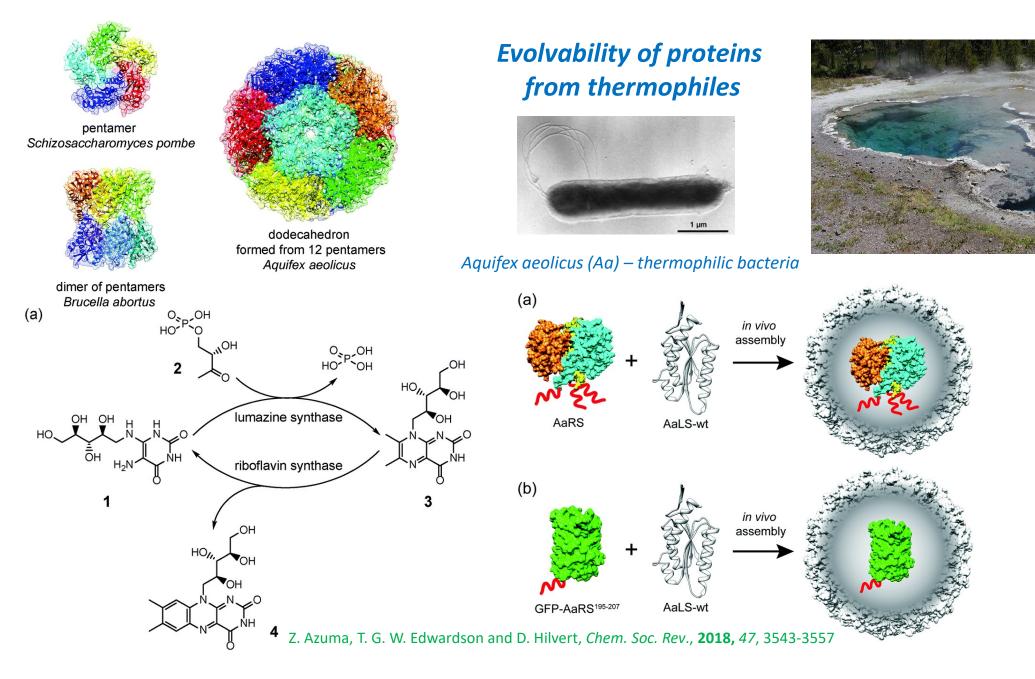


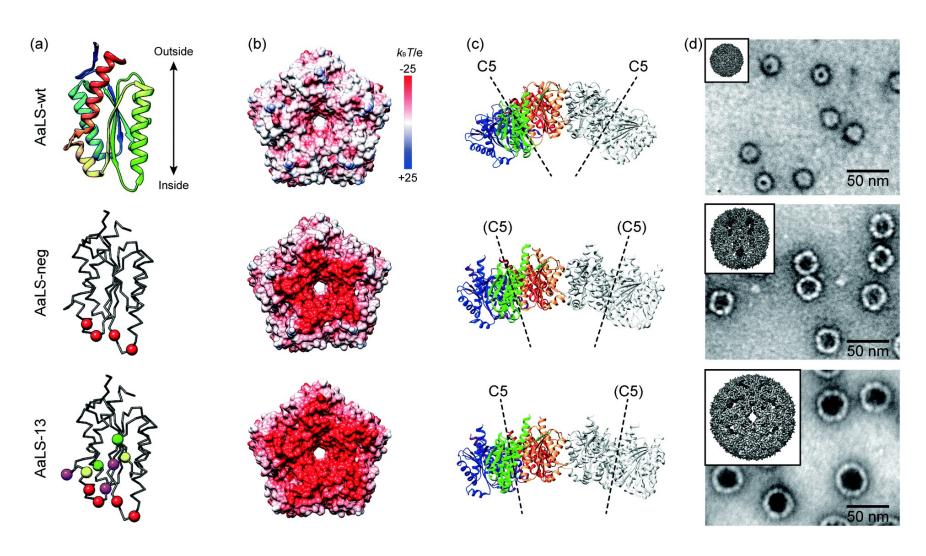


For interested in details:

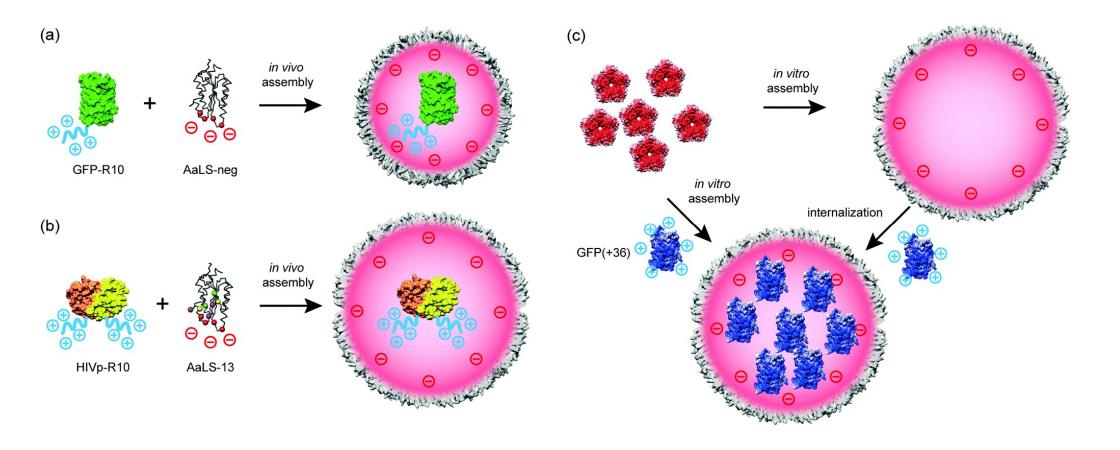
Introduction to protein design – Part1: https://www.youtube.com/watch?v=0LetJMbu7uY&ab_channel=iBiology

Introduction to protein design – Part2: https://www.youtube.com/watch?v=ZrAwWx7meTk&ab_channel=iBiology





B. Wörsdörfer, K. J. Woycechowsky and D. Hilvert, *Science*, **2011**, *331*, 589–592



Overview of the course

artificial ribozymes and aptamers for efficient catalysis and recognition (SELEX, DNAzymes, foldamers);

unnatural base pairing – expansion of the genetic alphabet;

Artificial genetic polymers and oligonucleotide analogues (XNA);

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; design of artificial organisms