Synthetic life

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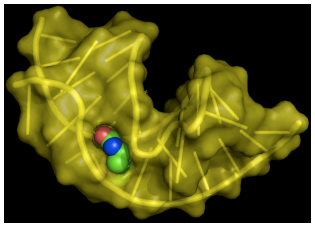
Aptamers

Aptamers (from the Latin *aptus* – fit, and Greek *meros* – part) are ***oligonucleotide*** or ***peptide*** molecules that bind to a specific target molecule.

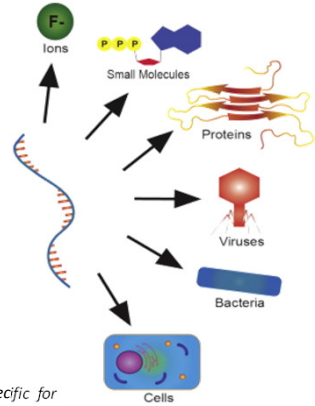
Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches.

- ***DNA or RNA or XNA aptamers*** – oligonucleotide strands (usually short)
- ***Peptide aptamers*** - one (or more) short variable peptide domains, attached at both ends to a protein scaffold.



Fdardel

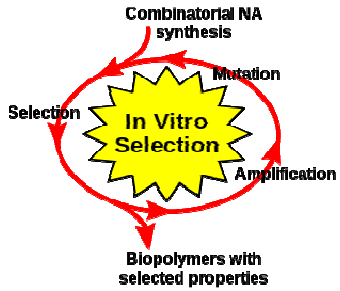
Structure of an RNA aptamer specific for biotin. The aptamer surface and backbone are shown in yellow. Biotin (spheres) fits snugly into a cavity of the RNA surface



Variety of target molecules

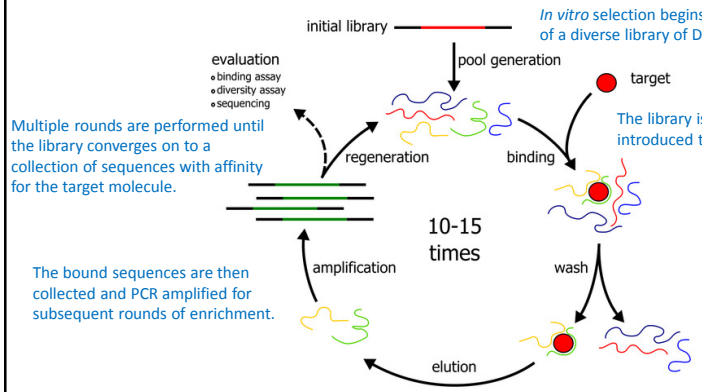
Systematic evolution of ligands by exponential enrichment - SELEX

1990 – Gold *et al.* – selection of RNA ligands against T4 DNA polymerase
1990 – J. Szostak *et al.* – selecting RNA ligands towards organic dyes



A general overview of in vitro selection protocol. NA stands for Nucleic Acids (DNA, RNA) which start as a random pool, and are enriched through the selection process

Systematic evolution of ligands by exponential enrichment - SELEX



In vitro selection begins with the generation of a diverse library of DNA or RNA molecules.

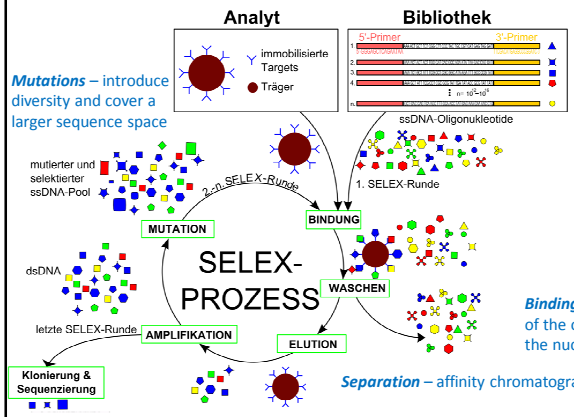
The library is then introduced to a target ligand

Multiple rounds are performed until the library converges on to a collection of sequences with affinity for the target molecule.

The bound sequences are then collected and PCR amplified for subsequent rounds of enrichment.

Sequences demonstrating affinity towards the target molecule are isolated from any unbound sequences.

Systematic evolution of ligands by exponential enrichment - SELEX



Mutations – introduce diversity and cover a larger sequence space

The initial library – usually contains sequences of 20-200 randomised nucleotides

The 5'- and 3'-termini are composed of constant primer sequences. Between them, the random sequences of *n* nucleotides result in 4^n combinations:

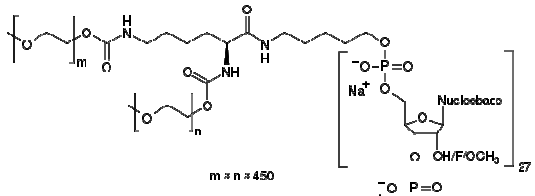
- n = 25 – a library of c.a. 10^{15} sequences,
- n = 50 – a library of c.a. 10^{30} sequences,
- n = 75 – a library of c.a. 10^{45} sequences,
- n = 100 – a library of c.a. 10^{60} sequences.

Binding strongly depends on the secondary structure of the oligonucleotides, which in turn depends on the nucleobase sequence

Separation – affinity chromatography

Amplification – reverse transcription RNA→DNA (only for RNA aptamers) + PCR (for RNA and DNA aptamers)

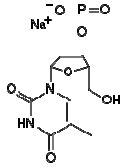
Systematic evolution of ligands by exponential enrichment - SELEX



Aptamers were evolved for a variety of target ligands:
- small molecules (ATP and adenosine)
- proteins: prions and vascular endothelial growth factor (VEGF),
- tumor cells.

Clinical uses are suggested by aptamers that bind tumor markers or GFP-related fluorophores.

A VEGF-binding aptamer trade-named ***Macugen*** has been approved by the FDA for treatment of macular degeneration.



Macugen

Additionally, SELEX has been utilized to obtain highly specific catalytic DNA or DNAzymes. Several metal-specific DNAzymes have been reported including the GR-5 DNAzyme (lead-specific), the CA1-3 DNAzymes (copper-specific), the 39E DNAzyme (uranyl-specific) and the NaA43 DNAzyme (sodium-specific).

Riboswitches

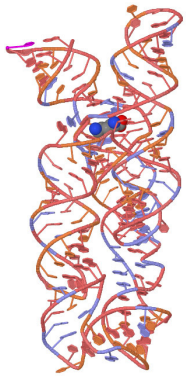
1990 - SELEX (Gold, Szostak)
2002 - the notion of aptamers in the natural world (Breaker and Nudler) – discovery of a nucleic acid-based genetic regulatory element – ***riboswitch*** - that possesses similar molecular recognition properties to the artificially made aptamers.

Riboswitches - naturally occurring regulatory segments of mRNA that bind small molecules specifically. The binding results in a change in production of the proteins encoded by the mRNA

Before discovery of ***riboswitches*** only ***proteins*** were supposed to do so in the biological context.

Most known ***riboswitches*** occur in bacteria, but functional riboswitches of one type (the TPP riboswitch) have been discovered in archaea, plants and certain fungi.

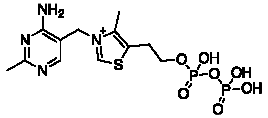
Riboswitches exist in all domains of life, and therefore are likely that they might represent ancient regulatory systems or fragments of ***RNA-world ribozymes*** whose binding domains remained conserved throughout the evolution



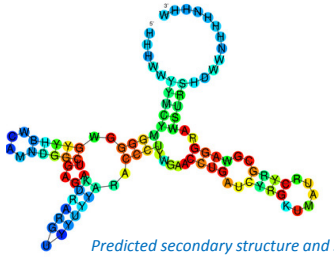
The lysine riboswitch

The TPP Riboswitch

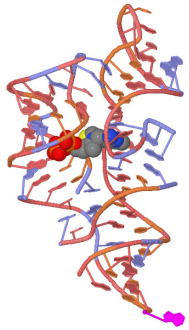
The **TPP riboswitch** (THI element and Thi-box riboswitch), is a highly conserved RNA secondary structure. It binds directly to thiamine pyrophosphate (TPP, a form of the vitamin B1, an essential coenzyme) to regulate gene expression through a variety of mechanisms in archaea, bacteria and eukaryotes.



Thiamine pyrophosphate TPP



Predicted secondary structure and sequence conservation of TPP riboswitch



The 3D structure of TPP riboswitch (by Benjamin Schuster-Böckler)

DNAzymes

Deoxyribozymes, also called ***DNA enzymes***, or catalytic DNA: DNA oligonucleotides that are capable of performing a specific chemical reaction, often but not always catalytic.

Although the working principle is similar to ***enzymes*** (and ***ribozymes***), there are no known naturally occurring ***deoxyribozymes***.

Deoxyribozymes should not be confused with ***DNA aptamers*** which are oligonucleotides that selectively bind a target ligand, but do not catalyze a subsequent chemical reaction.

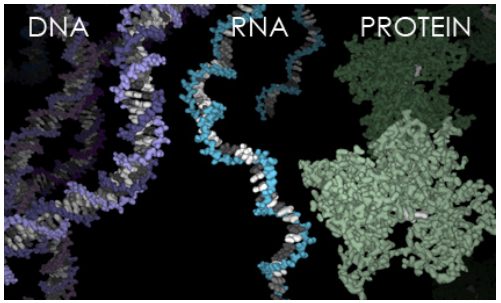
The trans-form (two separate strands) of the 17E DNAzyme. Most ***ribonuclease DNAzymes*** have a similar form, consisting of a separate enzyme strand (blue/cyan) and substrate strand (black: all-RNA or a DNA with one RNA nucleotide). Two arms of complementary bases flank the catalytic core (cyan) on the enzyme strand and the single ribonucleotide (red) on the substrate strand. The arrow shows the ribonucleotide cleavage site.

1994 – the first DNAzyme (a ribonuclease) – R. Breaker, G. Joyce – Pb^{2+} GR-5

Currently known:
- Ribonucleases
- RNA ligases
- DNA phosphorylation, adenylation, deglycosylation
- DNA cleavage

Problems: product inhibition, often single-turnover

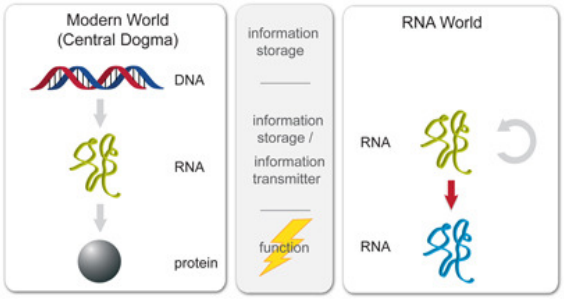
The RNA world



In modern cells, RNA (light blue, center) is made from a DNA template (purple, left) to create proteins (green, right).

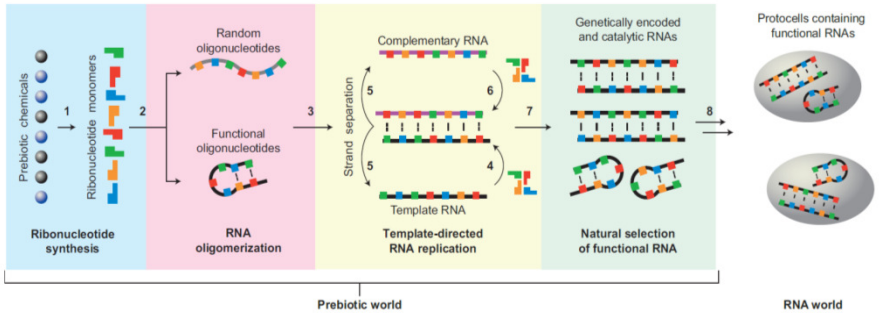
RNA folding is mediated by base-pairing interactions along different regions of a single-stranded RNA.

The RNA world

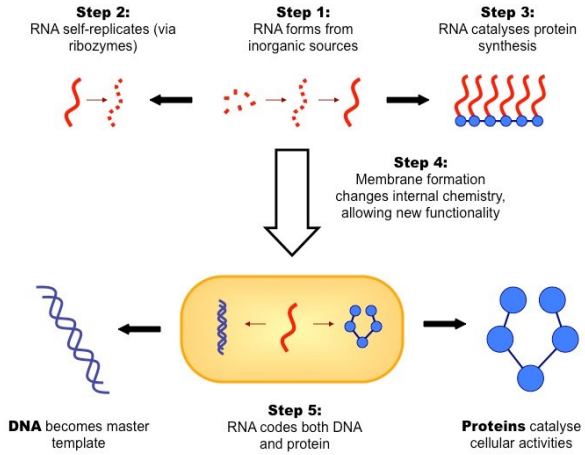


Conceptual idea that there was a period in the early history of life on Earth when RNA (or its structurally simplified analogue) carried out most of the information processing and metabolic transformations needed for biology to emerge from chemistry

The RNA world



The RNA world

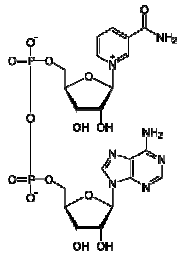


The RNA world

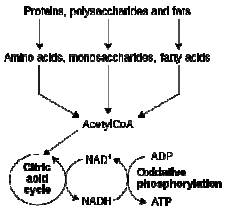
Crick, Orgel and Woese speculated in 1968 that, because RNA can form secondary structures, it has both a genotype and a phenotype and is a good candidate for the emergence of life

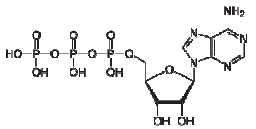
F. H. C. Crick *J. Mol. Biol.* **1968**, *38*, 367-379, L. E. Orgel *J. Mol. Biol.* **1968**, *38*, 381-393

Ribonucleotide coenzymes currently used by many proteins may be molecular „fossils" from the primoridal RNA-based metabolism



Nicotinamide adenine dinucleotide (NAD⁺)



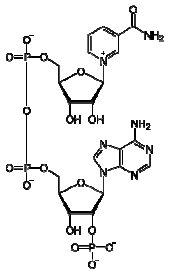


Adenosine triphosphate (ATP)

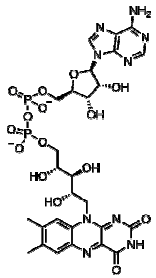
H. B. White III *J. Mol. Evol.* **1976**, *7*, 101-104

The RNA world

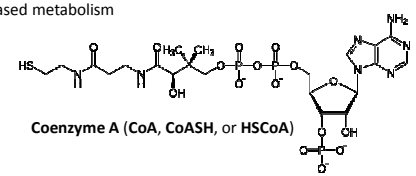
Ribonucleotide coenzymes now used by many proteins may be molecular „fossils" from the primoridal RNA-based metabolism



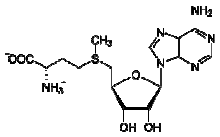
Nicotinamide adenine dinucleotide phosphate (NADP⁺)



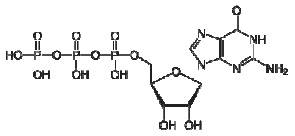
flavin adenine dinucleotide (FAD)



Coenzyme A (CoA, CoASH, or HSCoA)



***S*-Adenosyl methionine**

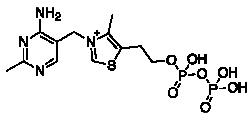


Guanosine-5'-triphosphate (GTP)

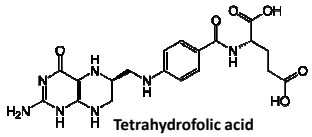
H. B. White III *J. Mol. Evol.* **1976**, *7*, 101-104

The RNA world

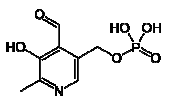
Other coenzymes contain cyclic nitrogen-containing bases that can also derive from nucleotides



Thiamine pyrophosphate (TPP or ThPP) – Vit. B_1



Tetrahydrofolic acid



Pyridoxal phosphate (PLP) – Vit. B_6

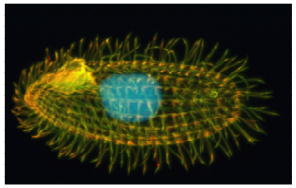
H. B. White III *J. Mol. Evol.* **1976**, *7*, 101-104

The RNA world

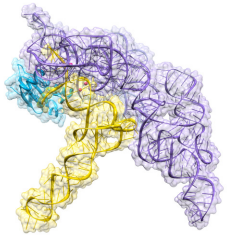
Ribozymes – Ribonucleic acid enzymes

1989 – Thomas Cech and Sidney Altman – Nobel Prize in chemistry for discovery of catalytic RNA

Thomas R. Cech was studying RNA splicing in the ciliated protozoan *Tetrahymena thermophila*
Sidney Altman and Norman Pace were studying the bacterial RNase P complex.



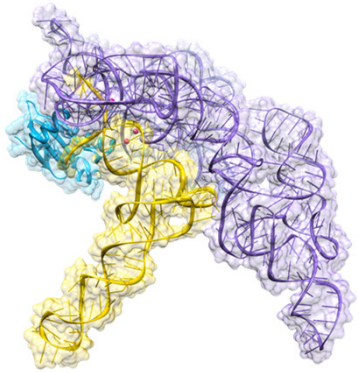
Tetrahymena thermophila



Bacterial RNAse P

The RNA world

Ribonuclease P

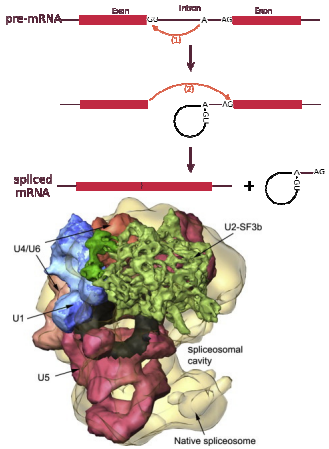


Ribonuclease P (RNase P) is a type of ribonuclease which cleaves RNA. RNase P is unique from other RNases in that it is a ribozyme – a ribonucleic acid that acts as a catalyst in the same way that a protein based enzyme would. Its function is to cleave off an extra, or precursor, sequence of RNA on tRNA molecules.

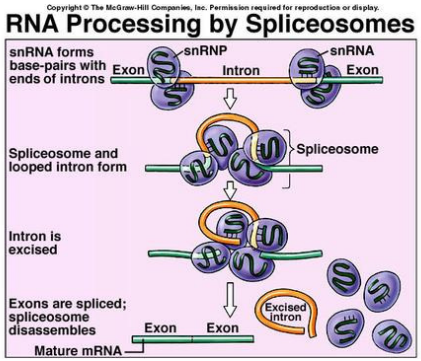
Bacterial RNase P has two components: an RNA chain, called M1 RNA, and a polypeptide chain, or protein, called C5 protein. *In vivo*, both components are necessary for the ribozyme to function properly, but *in vitro*, the M1 RNA can act alone as a catalyst. The primary role of the C5 protein is to enhance the substrate binding affinity and the catalytic rate of the M1 RNA enzyme probably by increasing the metal ion affinity in the active site.

Crystal structure of a bacterial ribonuclease P holoenzyme in complex with tRNA (yellow), showing metal ions involved in catalysis (pink)

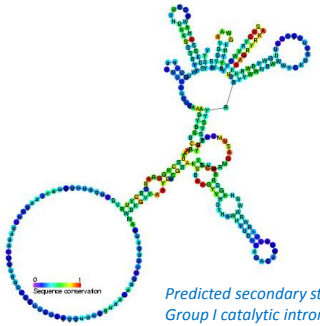
RNA splicing



Spliceosome – a complex of ribonucleoproteins



RNA splicing

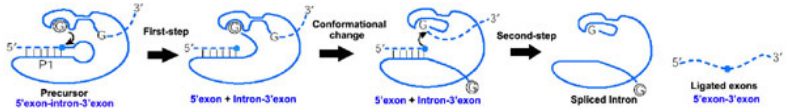


Predicted secondary structure and sequence conservation of Group I catalytic intron

Self-splicing RNA introns

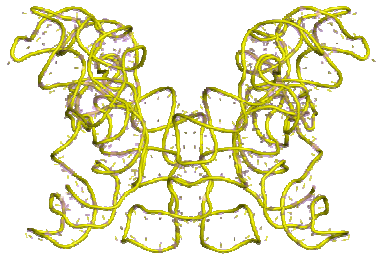
RNA splicing in *Tetrahymena* was taking place also in absence of the spliceosome - the ‚negative control' obtained after protease digestion also spliced.

In contrary to the spliceosome, the ***catalytic*** motif ***does not*** contain protein part, ***only RNA***.
First known example of a ***ribozyme*** – ribonucleic acid-composed enzyme analogue.



RNA splicing

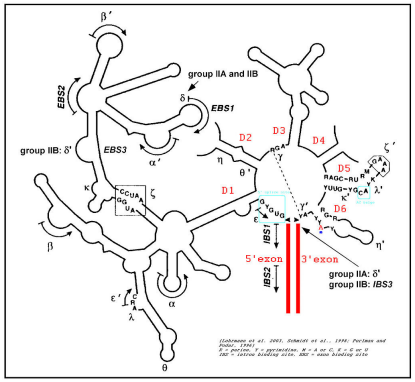
Group I catalytic introns



A 3D representation of the Group I catalytic intron.
This view shows the active site in the crystal structure of the Tetrahymena ribozyme

RNA splicing

Group II catalytic introns

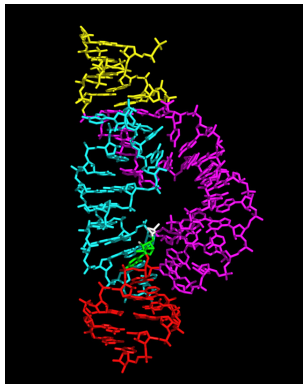


Ribozyme activity (e.g., self-splicing) can occur under high-salt conditions in vitro. However, assistance from proteins is required for in vivo splicing

It is hypothesized that pre-mRNA splicing may have evolved from group II introns, due to the similar catalytic mechanism as well as the structural similarity of the Domain V substructure to the U6/U2 extended snRNA

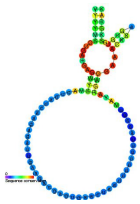
Ribozymes

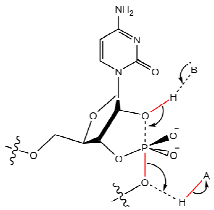
Hammerhead ribozyme



The hammerhead ribozyme is a RNA molecule motif that catalyzes reversible cleavage and joining reactions at a specific site within an RNA molecule.

- model system for research on the structure and properties of RNA,
- targeted RNA cleavage experiments,



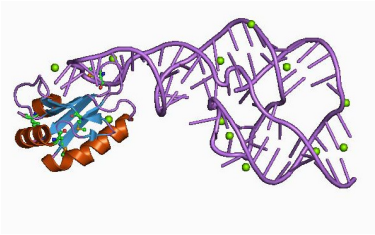


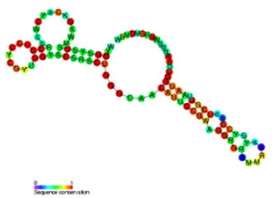
Ribozymes

HDV ribozyme

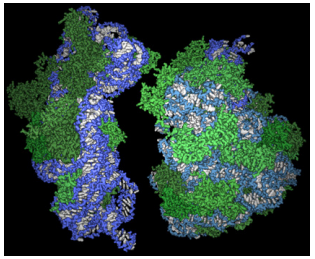
The hepatitis delta virus (HDV) ribozyme is a non-coding RNA found in the hepatitis delta virus that is necessary for viral replication and is thought to be the only catalytic RNA known to be required for viability of a human pathogen.

The ribozyme acts to process the RNA transcripts to unit lengths in a self-cleavage reaction. The ribozyme is found to be active in vivo in the absence of any protein factors and is the fastest known naturally occurring self-cleaving RNA.





Ribosome – the ‚smoking gun'



Ribosome: green - proteins, blue and white - RNA

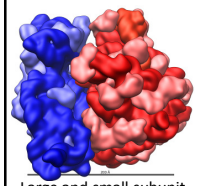
The ribosome is a simple molecular machine, found within all living cells, that serves as the site of biological protein synthesis (translation). Ribosomes link amino acids together in the order specified by messenger RNA (mRNA) molecules.

Ribosome is structurally highly conserved among all living species – most likely present in LUCA

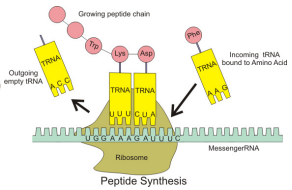
Ribosomes consist of two major components: the small ribosomal subunit, which reads the RNA, and the large subunit, which joins amino acids to form a polypeptide chain. Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and a variety of ribosomal proteins.

Ribosome – the ‚smoking gun'

Ribosome - **3**

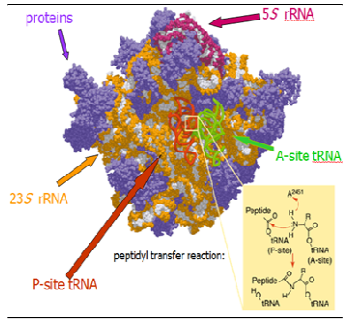


Large and small subunit



Peptide Synthesis

Ribosome is a ribozyme!



No protein within 18 Angstroms from the active site
→ proteins play a structural role, but
DO NOT CATALYZE THE ACYL TRANSFER PROCESS

T. Cech *Science*. **2000**, *289*, 878-879

Ribosome – the ‚smoking gun'

Ribosome is a ribozyme!

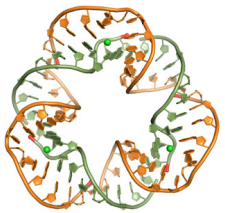
The ribosome may have first originated in an RNA world appearing as a self-replicating complex that only later evolved the ability to synthesize proteins when amino acids began to appear.

Studies suggest that ancient ribosomes constructed solely of rRNA could have developed the ability to synthesize peptide bonds.

In addition, evidence strongly points to ancient ribosomes as self-replicating complexes, where the rRNA in the ribosomes had informational, structural, and catalytic purposes because it could have coded for tRNAs and proteins needed for ribosomal self-replication.

As amino acids gradually appeared in the RNA world under prebiotic conditions, their interactions with catalytic RNA would increase both the range and efficiency of function of catalytic RNA molecules. Thus, the driving force for the evolution of the ribosome from an ancient self-replicating machine into its current form as a translational machine may have been the selective pressure to incorporate proteins into the ribosome's self-replicating mechanisms, so as to increase its capacity for self-replication

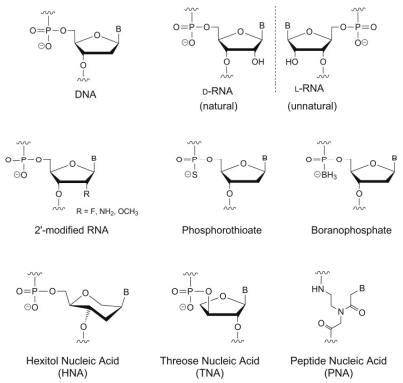
CHAPTER 1



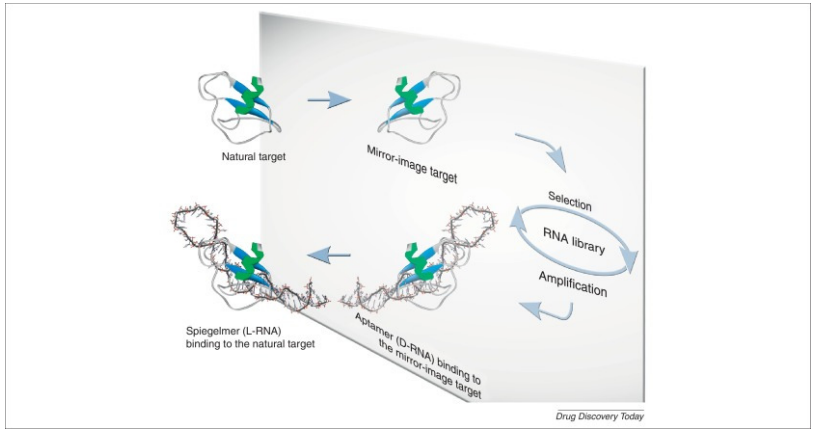
OLIGONUCLEOTIDES

Part 3 – noncanonical backbones

Artificial genetic polymers



Spiegelmers



A. Vater, S. Klussmann, *Drug Discovery Today* **2015**, *20,* 147-155

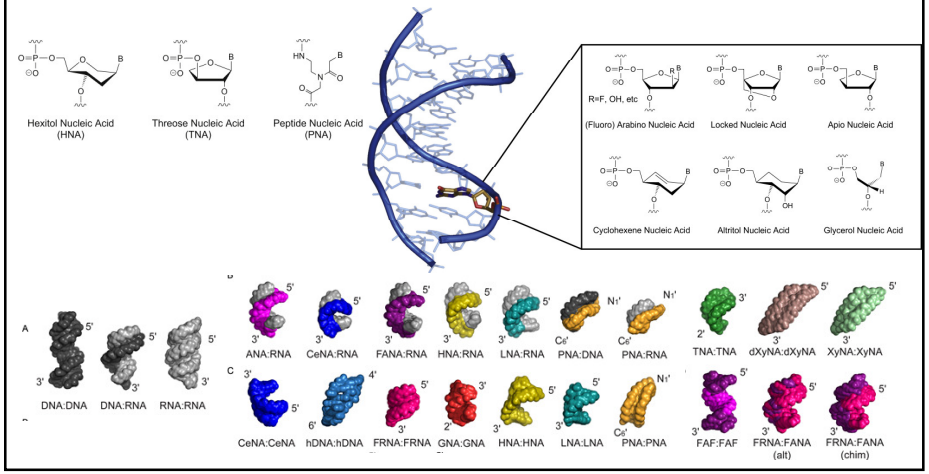
XNA – Xeno Nucleic Acids

XNA - synthetic alternative to DNA and RNA as information-storing biopolymers that differs in the sugar backbone.
- at least 6 XNAs can store and retrieve genetic information
- Ongoing research to create synthetic polymerases to transform XNA →

Xenobiology
- (XNA) as information carriers, expanded genetic code and, incorporation of non-proteinogenic amino acids into proteins
- the ***origin of life:*** *Primoridal soup → (XNA →) RNA → RNA(+DNA)+Proteins*
- development of industrial production systems with novel capabilities (pathogen resistance, biopolymer engineering)
- „genetic firewall" – excludes the risk of contaminating currently existing organisms (horizontal gene transfer)

The ***long-term goal*** - a cell that stores its genetic information on XNA, with different base pairs, using non-canonical amino acids and an altered genetic code.
So far cells have been constructed that incorporate only one or two of these features

XNA – Xeno Nucleic Acids



Synthetic genetic polymers capable of heredity and evolution

XNA are not recognized by natural polymerases.

One of the major challenges is to find or create novel types of polymerases that will be able to replicate these new-to-nature constructs. The method of polymerase evolution and design successfully led to the storage and recovery of genetic information (of less than 100bp length) from six alternative genetic polymers based on simple nucleic acid architectures not found in nature.

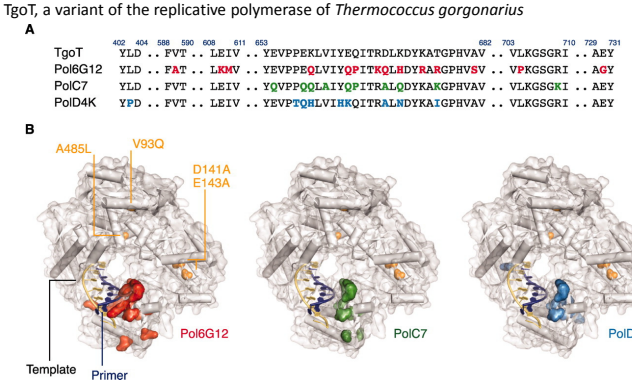
XNA aptamers, which bind their targets with high affinity and specificity, were also selected, demonstrating that beyond ***heredity***, specific XNAs have the capacity for ***Darwinian evolution*** and ***folding into defined structures***.

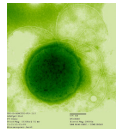
Thus, heredity and evolution, two hallmarks of life, are not limited to DNA and RNA but are likely to be emergent properties of polymers capable of information storage.

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

Engineering XNA polymerases

TgoT, a variant of the replicative polymerase of *Thermococcus gorgonarius*



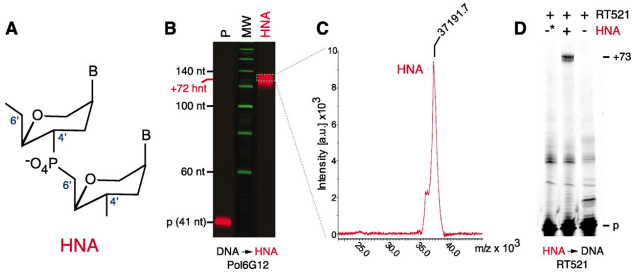


Thermococcus gorgonarius
(Angels Tapias)

(A) Sequence alignments showing mutations from wtTgo in polymerases Pol6G12 (red), PolC7 (green), and PolD4K (blue).
(B) Mutations are mapped on the structure of Pfu (PDB: 4AIL).
Yellow - template; dark blue - primer; orange - mutations present in the parent polymerase TgoT

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

HNA synthesis



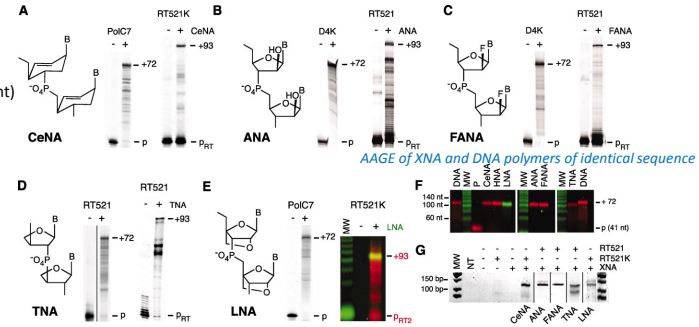
Pol6G12 extends the primer (p) incorporating 72 hNTPs against template T1 to generate a full-length hybrid molecule with a 37,215-dalton expected molecular mass.

HNA reverse transcription (DNA synthesis from an HNA template). Polymerase-synthesized HNA (from template YtHNA4) is used as template by RT521 for HNA-RT

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

XNA genetic polymers.

Structures and PAGE of synthesis (+72 xnt), and reverse transcription (+93 nt)



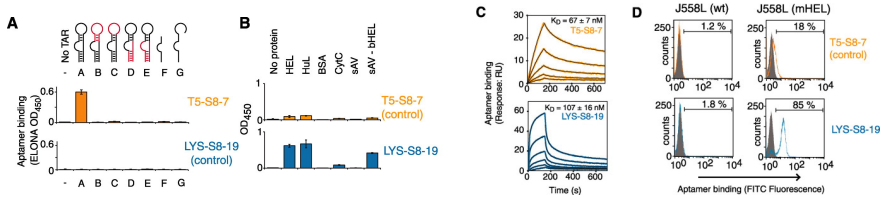
AAGE of XNA and DNA polymers of identical sequence

(E) PAGE of LNA synthesis [primer (41 nt) + 72 lnt] and LNA-RT (red). LNA synthesis (green) migrates at its expected size (113 nt) and comigrates with reverse transcribed DNA (red) synthesized from primer PRT2 (20 nt).

XNART–polymerase chain reaction. Amplification products of expected size (133 base pairs) are obtained only with both XNA forward synthesis and RT (RT521 or RT521K)

P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336*, 341-344

HNA aptamers



Characterization of HNA aptamers. Anti-TAR aptamer T5-S8-7 and anti-HEL aptamer LYS-S8-19.

(A and B) Aptamer binding specificity against TAR variants (red, sequence randomized but with base-pairing patterns maintained) and different protein antigens (human lysozyme, HuL; cytochrome C, CytC; streptavidin, sAV; biotinylated-HEL bound to streptavidin, sAV-bHEL). OD, optical density.
(C) Affinity measurements of aptamer binding by SPR. RU, response units.
(D) FACS analysis of fluorescein isothiocyanate (FITC)–labeled aptamers binding to plasmacytoma line J558L with and without expression of membrane-bound HEL (mHEL). wt, wild type.

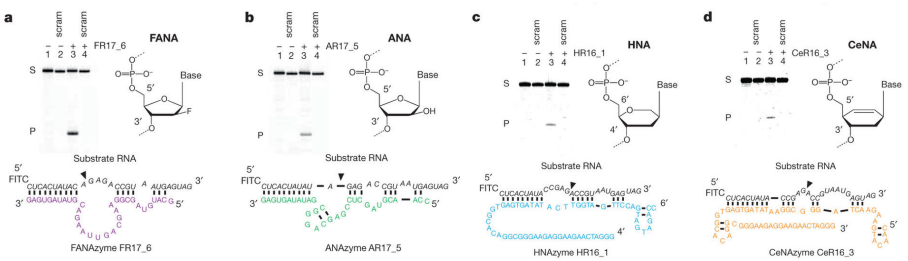
P. Herdewijn, P. Holliger, *et al. Science* **2012**, *336,* 341-344

XNA – Xeno Nucleic Acids

XNA – complementarity to DNA, also used as genetic catalysts.

FANA, HNA, CeNA and ANA - cleave RNA (***XNAzymes***).

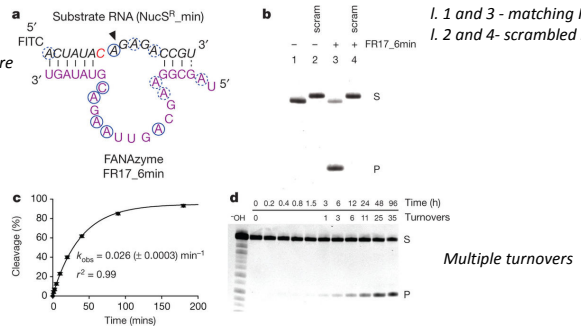
FANA XNAzymes can also ligate DNA, RNA and XNA substrates.



P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

Chemical synthesis yields an active RNA endonuclease XNAzyme

Secondary FANAzyme structure



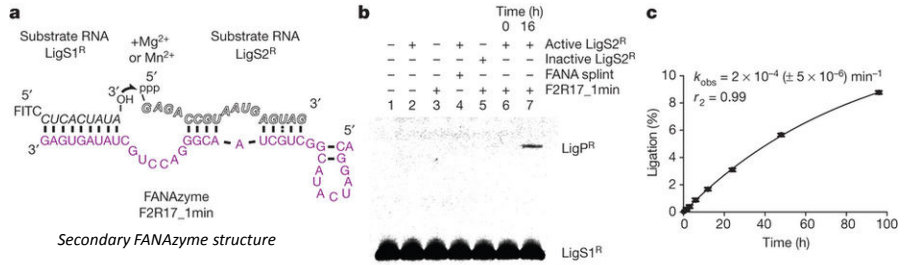
I. 1 and 3 - matching RNA
I. 2 and 4- scrambled RNA

Multiple turnovers

P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

An RNA ligase XNAzyme (FANA)

FANA XNAzymes can also ligate DNA, RNA and XNA substrates.

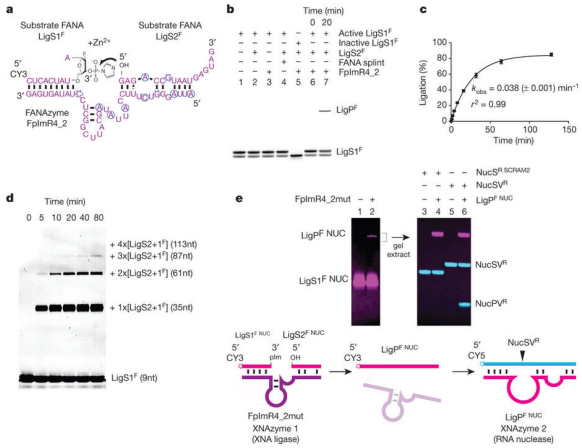


Secondary FANAzyme structure

Pre-steady state trimolecular reaction rate (k_{obs}) at 25 °C (n = 3; error bars, s.d.).

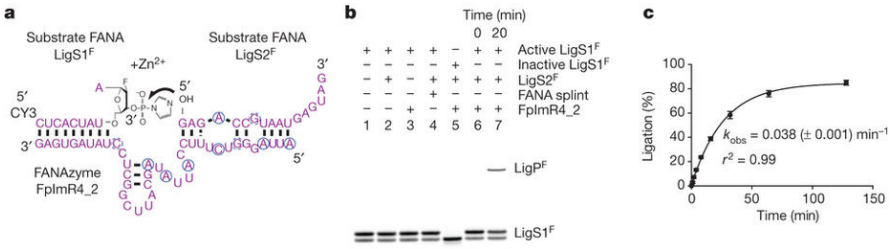
P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



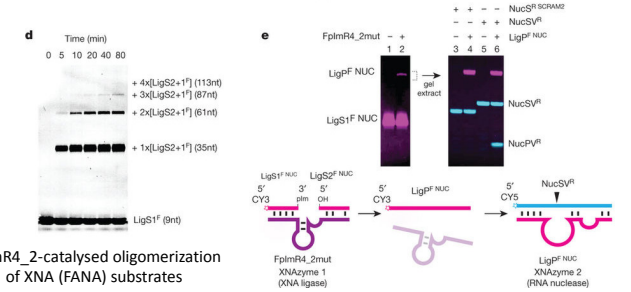
P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids



P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

XNA–XNA ligase XNAzyme (FANA): catalysis without natural nucleic acids

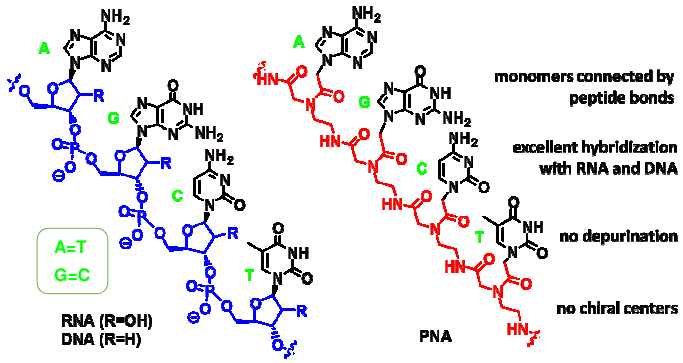


FpImR4_2-catalysed oligomerization of XNA (FANA) substrates

XNAzyme-catalysed assembly of an active XNAzyme. A variant XNA ligase (FpImR4_2mut) catalyses ligation (lane 2) of FANA substrates LigS1F NUC and LigS2F NUC. The product (LigPF NUC) is a variant of XNAzyme FR17_6 min (Fig. 2), which cleaves RNA substrate NucSVR (lanes 5 and 6), but not scrambled RNA (NucSR SCRAM2)(lanes 3 and 4).

P. Herdewijn, P. Holliger, *et al. Nature* **2015**, *518,* 427-430

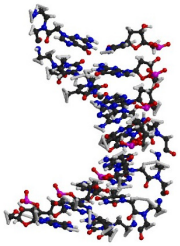
Peptidonucleic acids – functional DNA analogues



PNA – stable *ex vivo*, the backbone detected in cyanobacteria

Applications: antigene, antisense agents; fluorescent DNA probes (FISH), anticancer, antiviral, antibacterial, antiparasitic agents; diagnostics, mol. biology

Peptidonucleic acids – functional DNA analogues

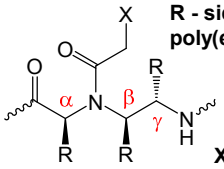


PNA-DNA duplex, NMR structure
PDB entry: 1PDT

PNA – dsDNA strand invasion due to lack of electrostatic repulsion

Problems: aggregation and low water solubility

Structural modifications of the PNA

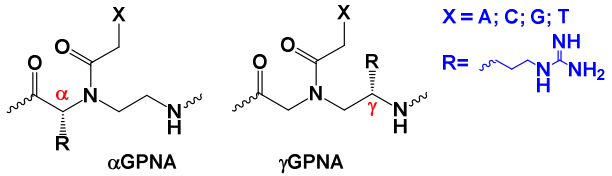


R - side chains of Ala, Lys, Glu, Arg, Ser, or poly(ethylene glycol)

X = A; C; G; T

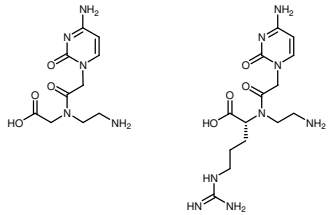
- **Polar groups → water solubility and reduced aggregation**
- **β position – detrimental for DNA/RNA recognition,**
- **α and γ - well-tolerated modifications**

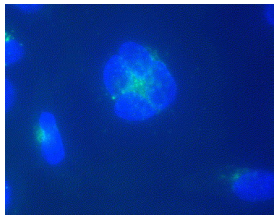
Structural modifications of the PNA - αGPNA, γGPNA



- GPNA: Alkylguanidinium residues (Arg side chains)
- enhanced water solubility
- cell permeability (analogous to oligoarginine CPPs)
- α position ← *D*-arginine
- γ position ← *L*-arginine

Cell-penetrating αGPNA

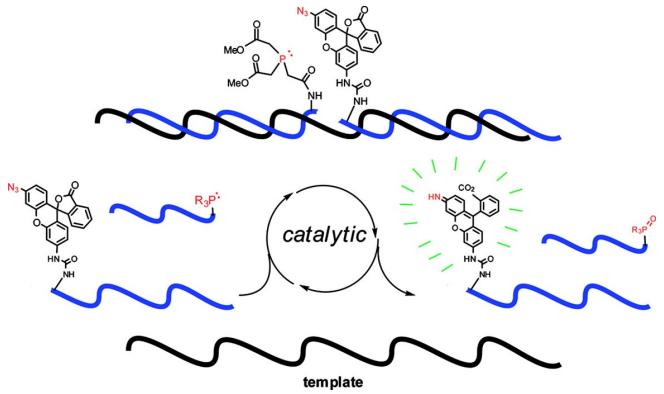




HeLa cells incubated with 1 μM GPNA (FITC-^{D}C^{D}A^{D}C^{D}T^{D}C^{D}T^{D}G^{D}C^{D}A^{D}A^{D}C^{D}G^{D}G^{D}T-NH_2) for 16 h, Fixed, stained with DAPI. Nuclei (blue), GPNA (green).

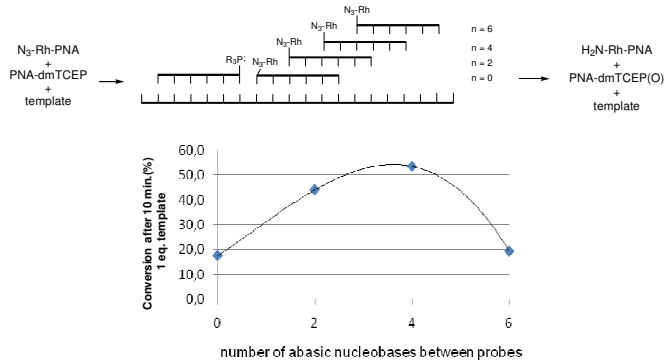
P. Zhou, A. Dragulescu-Andrasi, B. Bhattacharya, H. O'Keefe, P. Vatta, J. J. Hyldig-Nielsen and D. H. Ly *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4931
A. Dragulescu-Andrasi, S. Rapireddy, G. He, B. Bhattacharya, J. J. Hyldig-Nielsen, B. G. Zon, and D. H. Ly *J. Am. Chem. Soc.* **2006**, *128*, 16104

Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing



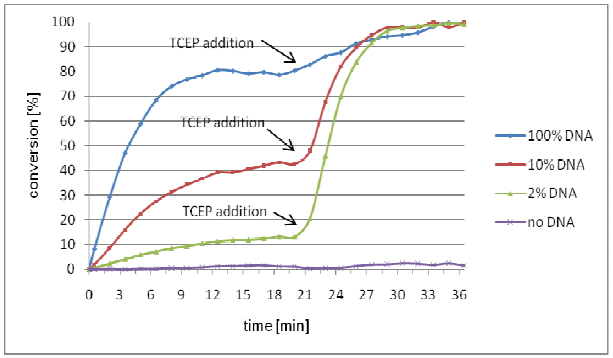
Z. Pianowski, N. Winssinger *Chem. Comm.* **2007**, *37*, 3820-3822
Z. Pianowski *et al. J. Am. Chem. Soc.* **2009**, *131*, 6492-6497

Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing



Z.Pianowski, K. Górska, L. Oswald, C. Merten, N.Winssinger *J. Am. Chem. Soc.* **2009,** *131*, 6492-6497

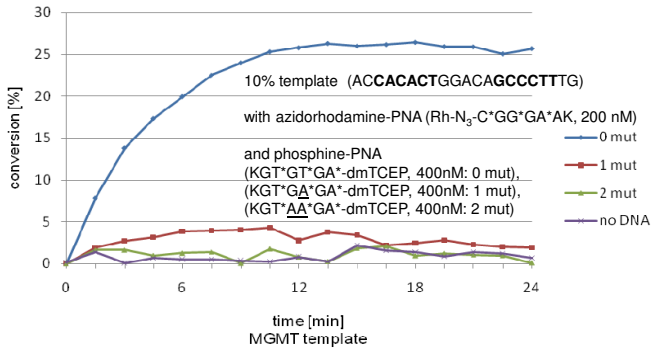
Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing



PNA-azidorhodamine (Rh-N_3-C*GG*GA*AK, 200 nM) PNA-phosphine (KGT*GT*GA*-dmTCEP, 800nM) the MGMT template (AC**CACACT**GGACA**GCCCTT**TG) (10mM PBS, pH 7.2, 154 mM NaCl, 25 mM $MgCl_2$, 0.05% Tween).
* GPNA monomer.

Z.Pianowski, K. Górska, L. Oswald, C. Merten, N.Winssinger *J. Am. Chem. Soc.* **2009,** *131*, 6492-6497

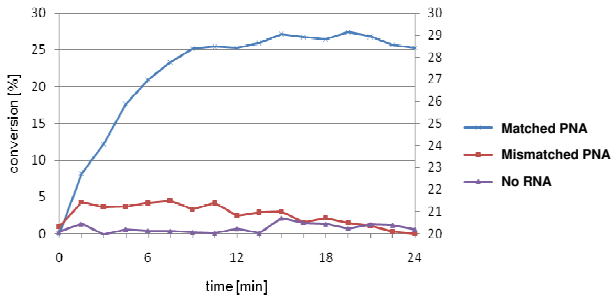
Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing



Z.Pianowski, K. Górska, L. Oswald, C. Merten, N.Winssinger *J. Am. Chem. Soc.* **2009,** *131*, 6492-6497

Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing

detection of mutations – cell extract

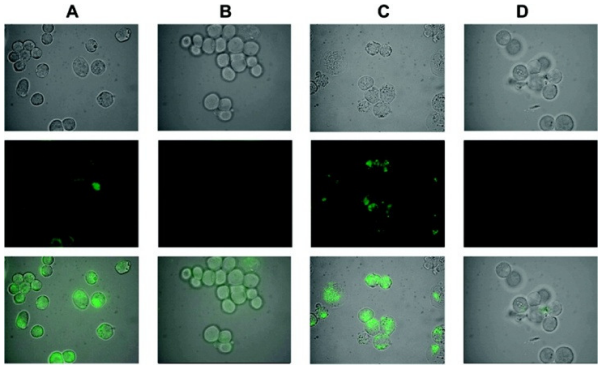


mRNA: 5′-UTR region of the O-6-metyloguanino-DNA metyltransferase (MGMT).
The expression level of MGMT correlates well with reaction of several cancers on chemotherapy using DNA-alkylating agents.
The MGMT gene has high expression level in HEK293 cells

Z.Pianowski, K. Górska, L. Oswald, C. Merten, N.Winssinger *J. Am. Chem. Soc.* **2009,** *131,* 6492-6497

Cell-penetrating αGPNA for in vivo catalytic oligonucleotide sensing

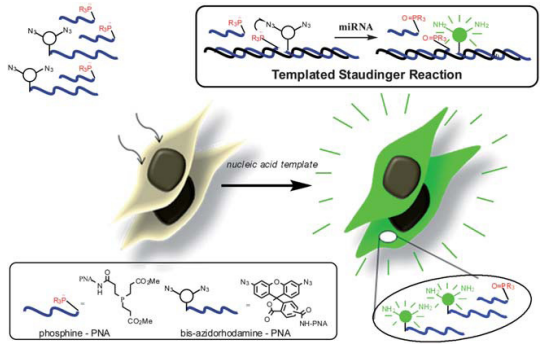
Inside living cells



A, B – controls (+/-) C – matching PNA D – mismatched PNA

Z.Pianowski, K. Górska, L. Oswald, C. Merten, N.Winssinger *J. Am. Chem. Soc.* **2009,** *131,* 6492-6497

Templated in vivo detection of miRNA



Probes targeting miR21:

Lys(N_3RhN_3) C*TG*AC*TA*C Arg

Arg AT*CG*AA*T dmTCEP – perfect match (PM)

Arg AT*GA*AA*T dmTCEP – mismatch (MM)

Probes targeting miR31:

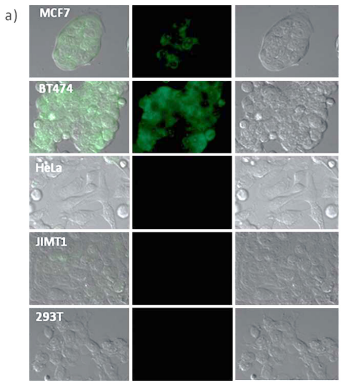
(N_3RhN_3) C*CG*TA*TC*G Arg

Arg CG*TT*CT*A dmTCEP - PM

Arg CG*TT*AT*A dmTCEP - MM

K. Gorska, I. Keklikoglou, U. Tschulena, N. Winssinger, *Chem. Sci.* **2012**, *2,* 1969-1975.

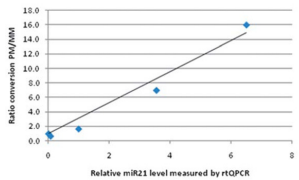
Templated in vivo detection of miRNA – miR21



b)

	MCF-7	BT-474	HeLa	JIMT-1	293T
miR21 level	6.5	3.55	1	0.08	0.015
conversion PM	16%	21%	5%	2%	0%
conversion MM	1%	3%	3%	3%	0%
ration PM/MM	16.0	7.0	1.7	0.7	1.0

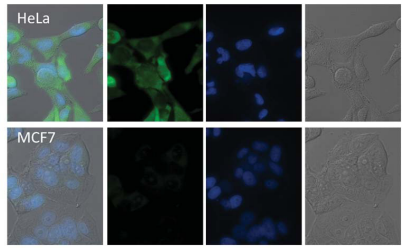
c)



K. Gorska, I. Keklikoglou, U. Tschulena, N. Winssinger, *Chem. Sci.* **2012**, *2,* 1969-1975.

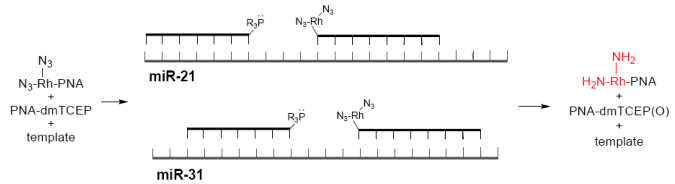
Templated in vivo detection of miRNA – miR21, miR31

a)



b)

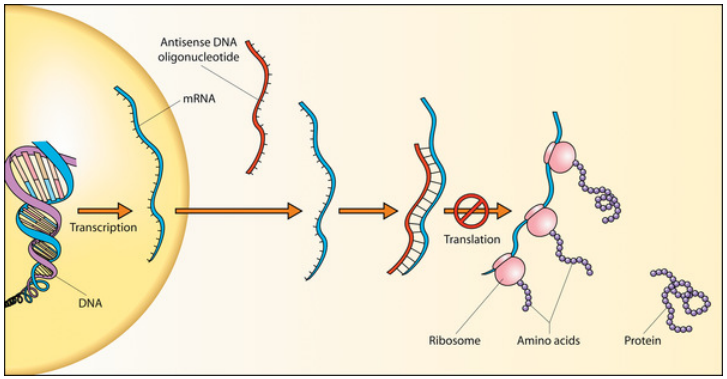
	HeLa	MCF-7
miR21 level	1	0.002
conversion PM	32%	2%
conversion MM	2%	1%
ration PM/MM	16	2



K. Gorska, I. Keklikoglou, U. Tschulena, N. Winssinger, *Chem. Sci.* **2012**, *2*, 1969-1975.

Antisense agents

- Important tools in research and molecular medicine

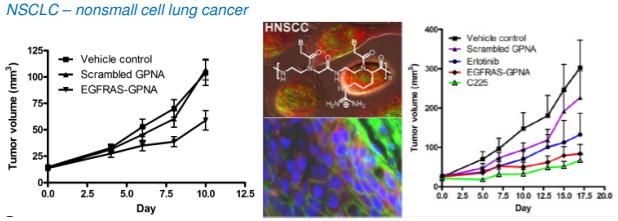


- Antisense Oligonucleotides use Watson-Crick-base pairs to form duplexes with mRNA

Antisense activity of αGPNA in vivo

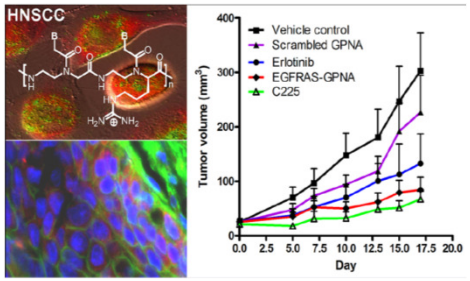
- Danith Ly et al. investigated anti-EGFR αGPNA on cells and mice
- Target: transcript of the epidermal growth factor receptor (EGFR)
- Sequence-specific inhibition (50%) of the tumor growth

HNSCC - Head and neck squamous cell carcinoma
NSCLC – nonsmall cell lung cancer



D. Ly *et. al.* ACS *Chem. Biol.* **2013**, *8*, 345-352

Antisense activity of αGPNA in vivo

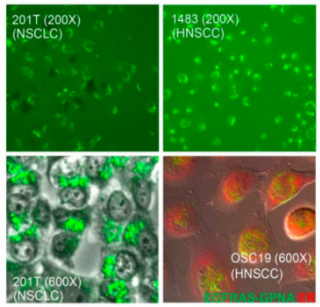


- GPNA 16-mers targeting the epidermal growth factor receptor (EGFR) in preclinical models as therapeutic modality for head and neck squamous cell carcinoma (HNSCC) and nonsmall cell lung cancer (NSCLC)
- Elicited potent antisense effects in NSCLC and HNSCC preclinical models
- When administered intraperitoneally in mice, EGFRAS-GPNA was taken-up by several tissues including the xenograft tumor
- Systemic administration of EGFRAS-GPNA induced antitumor effects in HNSCC xenografts, with similar efficacies as the FDA-approved EGFR inhibitors: cetuximab and erlotinib.

D. Ly *et. al.* ACS *Chem. Biol.* **2013**, *8*, 345-352

Antisense activity of αGPNA in vivo

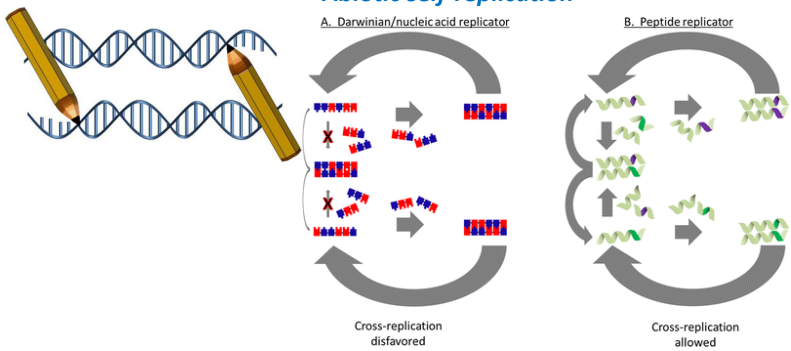
- Intracellular localization (confocal fluorescence microscope): primarily in the peri-nuclear region.
- Further co-localization with endoplasmatic reticulum (ER) (by ER staining), which is the place where mRNA molecules are translated into protein.
- Transfection efficiencies >99% with the dosage as low as 1 µM



Fluorescent images of live NSCLC and HNSCC cells following incubation with 1 µM FITC-EGFRAS-GPNA (green) in a complete medium for 24 h. Bottom right panel: an image of live cells costained with an ER dye (red), demonstrating colocalization (yellow) in the perinuclear regions. Three independent experiments were carried out showing similar results.

D. Ly *et. al.* ACS *Chem. Biol.* **2013**, *8*, 345-352

Abiotic self-replication

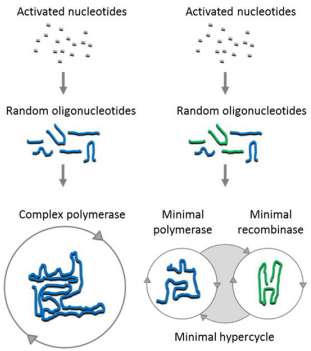


(A) For nucleic acids replicators, templating is based on base pairing, so the formation of a mutant template is rare. Once formed, the mutant replicator forms a competing replication cycle. (B) For a peptide replicator, templating is less exact, so the formation of a mutant template is common. The mutant template can catalyze formation of mutant progeny or parental progeny, and the two species form a mutualistic network.

Meyer AJ, Ellefson JW, Ellington AD. *Acc Chem Res.* **2012** *45(12)*:2097-2105.

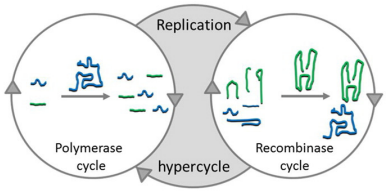
Emergence of a self-replication system through hypercycles

A. Complex emergence B. Cooperative emergence C. Cooperative-replication hypercycle



In the first scenario, a complex RNA-dependent RNA polymerase capable of full self-replication emerges from random oligonucleotides.

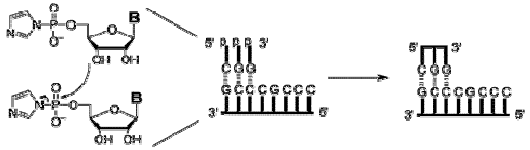
In the second scenario, a minimal polymerase and minimal recombinase emerge from random oligonucleotides. These ribozymes cooperate to perform replication.



The replication hypercycle: two intertwined polymerization and recombination cycles. In one cycle, polymerization of the short RNA fragments comprising the polymerase and recombinase occurs. In the other cycle, the reconstituted recombinase stitches the RNA fragments. Recombination is directed by internal guide sequences, forming longer, more complex ribozymes.

Meyer AJ, Ellefson JW, Ellington AD. *Acc Chem Res.* **2012** *45(12)*:2097-2105.

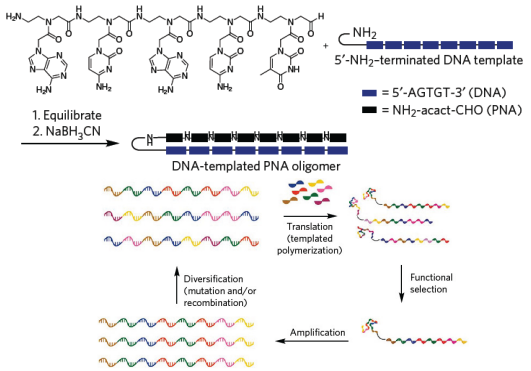
Nonenzymatic templated nucleic acid synthesis – monomer/short oligomer



Problems:
- very slow reactions
- limited range of templates (mostly C-rich)
- poor regiospecificity (2'-5' linkages, predominant in some cases)
- 3'-aminonucleotides perform better, but undergo intramolecular cyclizations as side reaction

Lohrmann, R.; Orgel, L. E. *Tetrahedron* **1978**, *34*, 853
A. Silverman, E. Kool *Chem. Rev.* **2006**, *106*, 3775

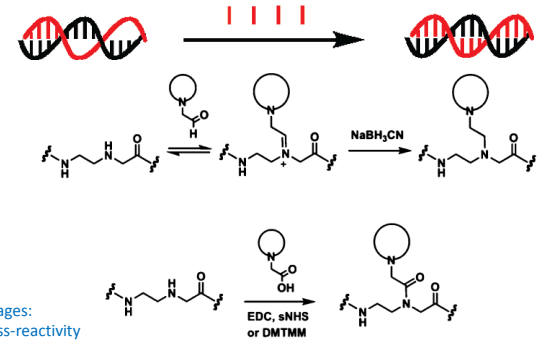
Templated nucleic acid synthesis – short oligomer coupling



Limitations:
- slightly distorted backbone (amine instead of amide backbone every 5 bases)
- only carefully designed pentamers work – limiting the diversity for functional selection

Brudno Y, Birnbaum ME, Kleiner RE, Liu DR. *Nature Chem. Biol.* **2010**, *6,* 148-155.

Templated nucleic acid synthesis – base filling

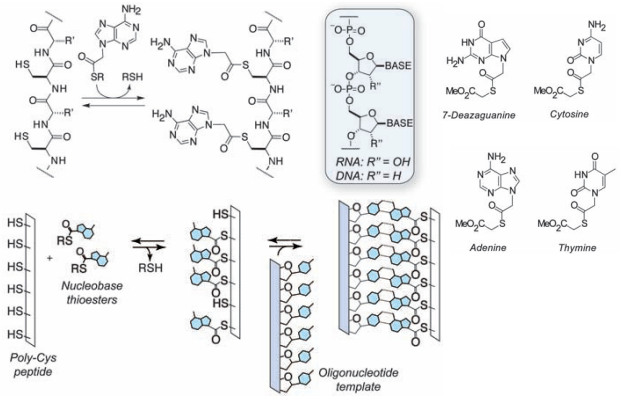


Advantages:
-no cross-reactivity
- selectivity increased by proximity of the reaction to the hybridization site

Limitations:
-single or double abasic sites (most efficient inside of the chain)
- Aldehydes give better yields and accuracy, but worse hybridization of the product

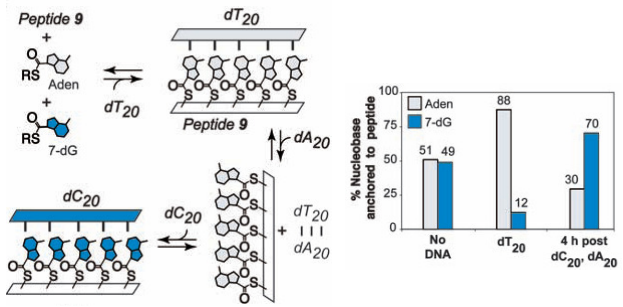
Heemstrra JM, Liu DR. *J. Am. Chem. Soc.* **2009**, *131,* 11347-11349.

A polyamide responsive to selection pressure



Ura Y, Beierle J, Leman L, Orgel LE, Ghadiri MR. *Science* **2009**, *325*, 73-77.

A polyamide responsive to selection pressure



Dynamic polymer responsive to template changes with high fidelity

Ura Y, Beierle J, Leman L, Orgel LE, Ghadiri MR. *Science* **2009**, *325*, 73-77.