Gene Targeting by modified Nucleobases

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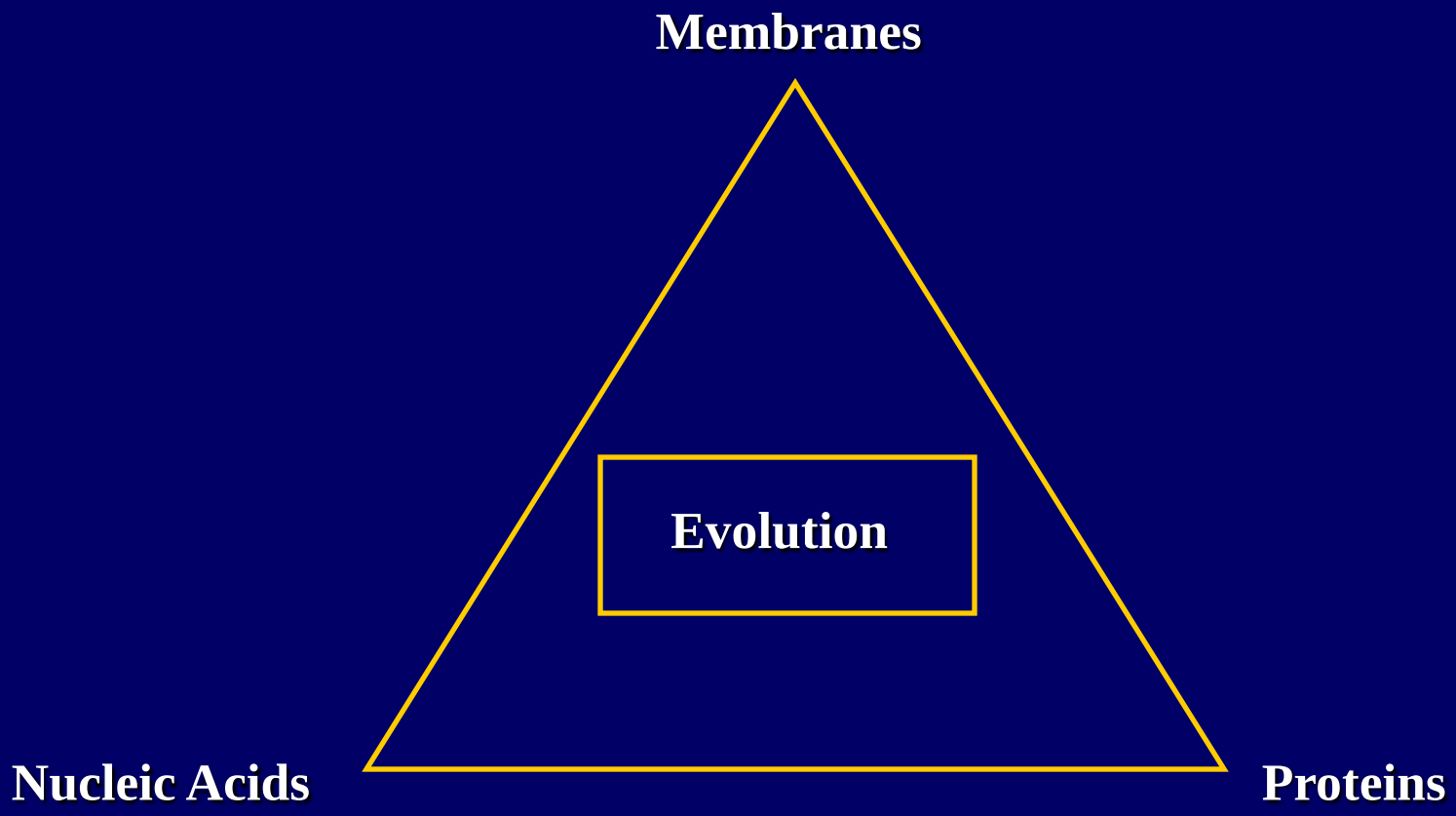
Biomesogens (1973)



S. Hoffmann, „*Molecular Matrices (I. Evolution, II. Proteins, III. Nucleic Acids, IV. Membranes*“, Akadmie-Verlag, Berlin (1978)

(LC texture of DNA between solid and isotropic state)

„Not white and black, the unlimited tones determine the world.“

Prof. Siegfried Hoffmann
(29.09.1930 – 06.09.2008)

History

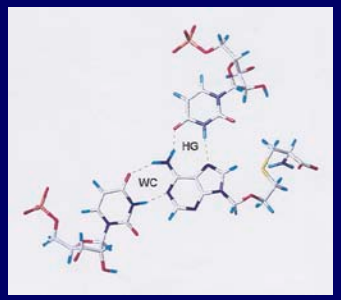
- 1868 Friedrich Miescher discovered the DNA
- 1953 Francis Crick and James Watson discovered the DNA double helix
- 1957 G. Felsenfeld and A. Rich described triple-helical nucleic acids
- 1959 K. Hoogsteen „*The structure of crystals containing a hydrogen-bonded complex of 1-methylthymine and 9-methyladenine*“ (Acta Cryst 12 (1959) 822-823),
- 1977 B.M. Paterson et al. – Single-stranded DNA inhibit translation of a complementary RNA in cell-free system
- 1986 P.B. Dervan demonstrated that short (15mers) mixed-sequence triplex-forming oligonucleotides (TFOs) form specific triple-helical complexes
- 2001 Official decoding of the human genome
- Today „*Genomic Era*“

Gene therapy strategies - Basic principles

The strategies for modulating gene expression can be directed towards blocking the process of transcription (“anti-gene” procedure), or post-transcriptional events (“anti-mRNA” procedure), including mRNA processing and translation

- Gene targeting by homologous recombination, triple-helix-forming oligodeoxynuc-leotides (TFOs),
- Targeting the mRNA by various strategies such as the use of antisense DNA, antisense RNA and RNA-decoy molecules,
- Post-transcriptional gene silencing, or RNA interference (RNAi) approach.

But the broad use of nucleic-acid molecules for treating human diseases remains uncertain up to now.



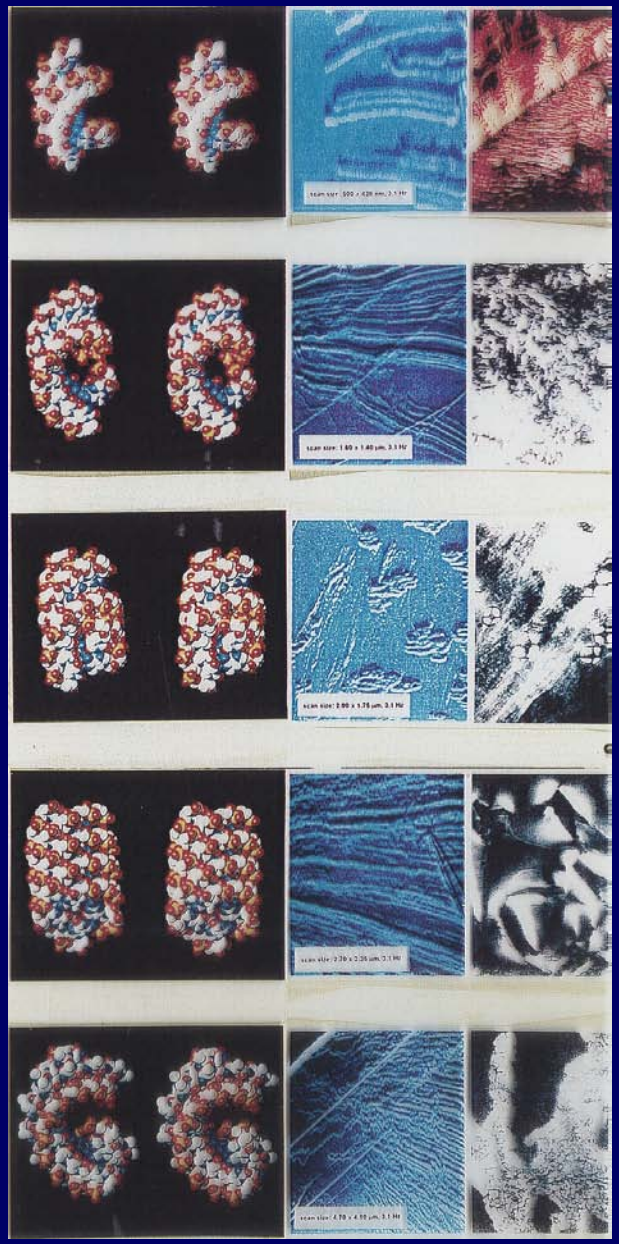


Figure 1: ***Nucleic acid geometries B-DNA duplex, A-RNA duplex, DNA/RNA triplex, G-quadruplex, DNA and RNA/$(lys)_5$ complexations (left); SFM (middle) and liquid crystal textures (right) of polydisperse chicken-DNA, $(A)_n$. $(U)_n$; $(U)_n$. $(A)_n$. $(U)_n$, $(G)_n$. $(G)_n$. $(G)_n$. (G)n and $(A)_n$. $(U)_n$ / Lsy_5 (top to bottom)***

[W.-V. Meister, Ch. Bohley, S. Lindau, U. Gromann, St. Naumann, B. Hermann, S.I. Kargov, T. Martini, J. Barthel, S. Hoffmann, *Mesophase-derived Nucleic Acid (Peptide) Self-organizations visualized by Scanning Force Microscopy*,Surface and Interface Analysis **33(2)** (2002) 126-136]

Watson-Crick and Hoogsteen base-pairing facilities

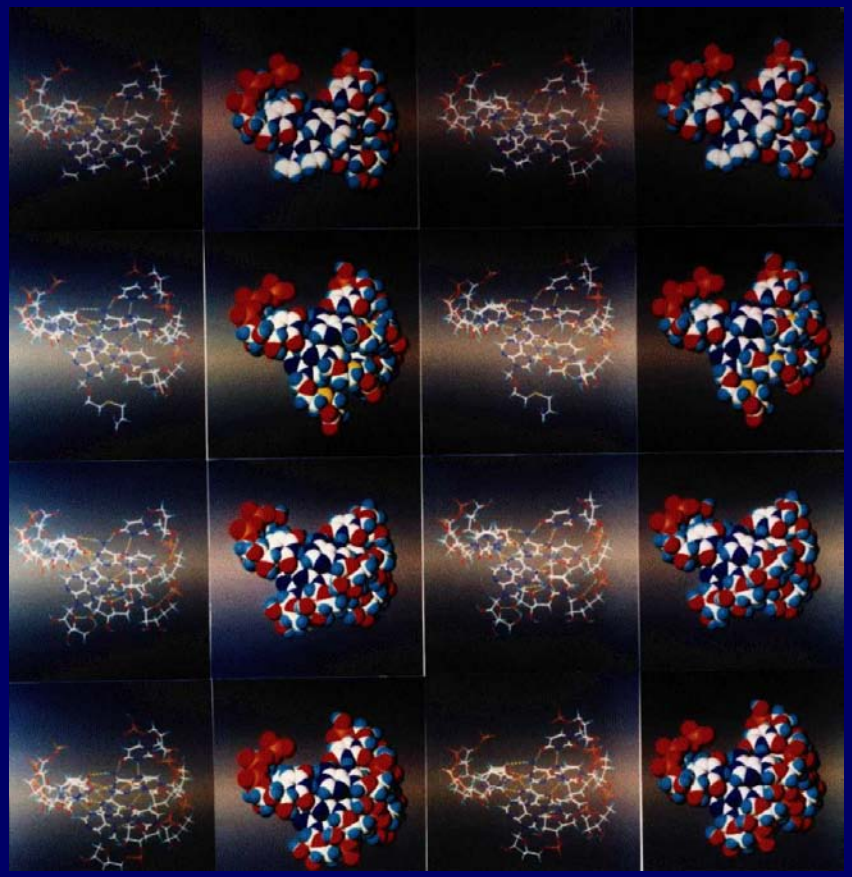


Figure 2: ***dsDNA, tsDNA and qsDNA (left) and 9-vinyladenine(vA), S-[(adenine-9-yl)methoxyethyl]-L-cysteine (cysA) and adenosine (Ado) with the native templates of polyuridylic acid, illustrated by hypothetical triplex arrangements in stick and space-fill stereo view***

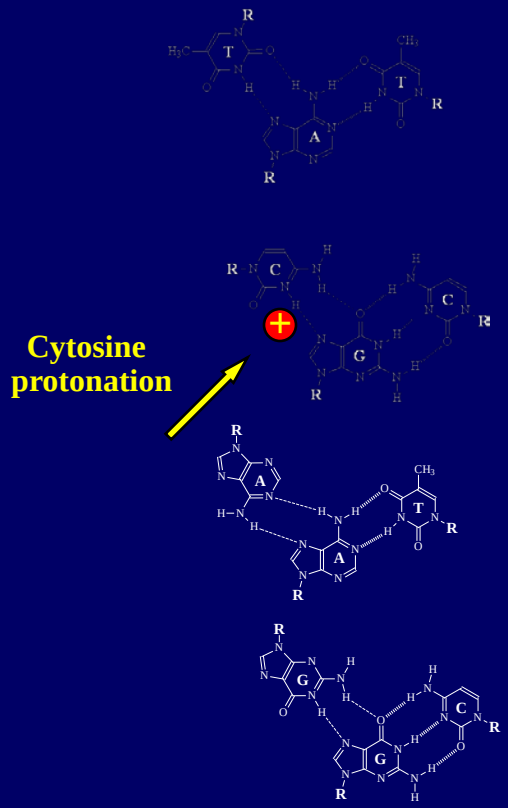


Figure 3: ***Pyrimidin (Hoogsteen) and purine (reverse Hoogsteen) motif top to bottom***

[**W.-V. Meister**, S. Lindau, Anton L. Hauser, Ch. Bohley, U. Gromann, St. Naumann, M. Madre, L. Kovalenko, G. Bischoff, R. Zhuk, S. Hoffmann *Biomesogenic Matrix Systems* Journal of Biomolecular Structure & Dynamics **18(3)** (2000) 385-392;; **W.-V. Meister**, S. Lindau, Anton L. Hauser, E. Birch-Hirschfeld, J. Reinert, K. Friese, C. Bohley, S.I. Kargov, U. Gromann, S. Hoffmann, *Biomesogenic (Pre)ordering Phenomena and Matrix Reactions of Nucleosides on Polyuridylic Acid Templates Studied by Scanning Force Microscopy* Surface and Interface Analysis **33(2)** (2002) 137-145]

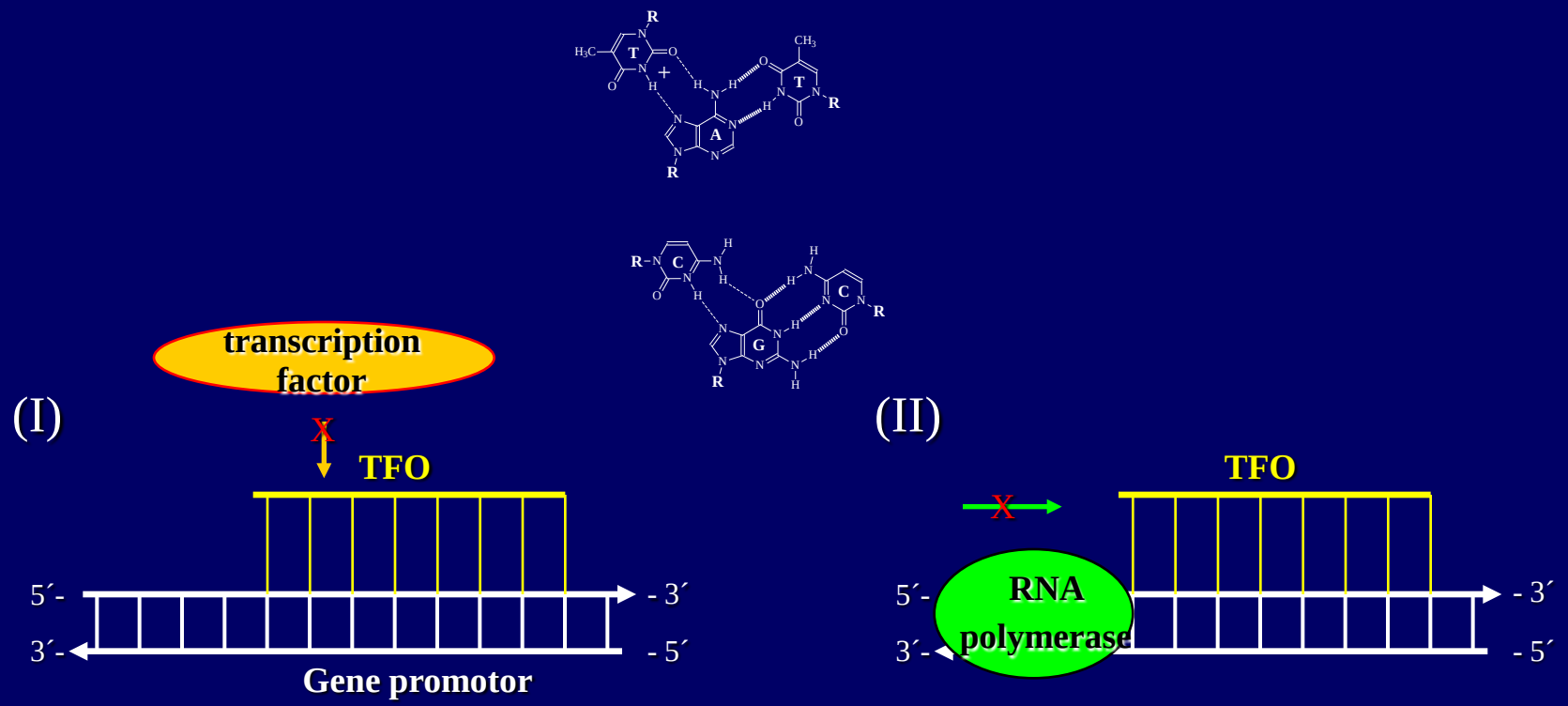


Figure 4: ***„Anti-gene“- approaches: Inhibition of transcription initiation (I) and of elongation (II)***

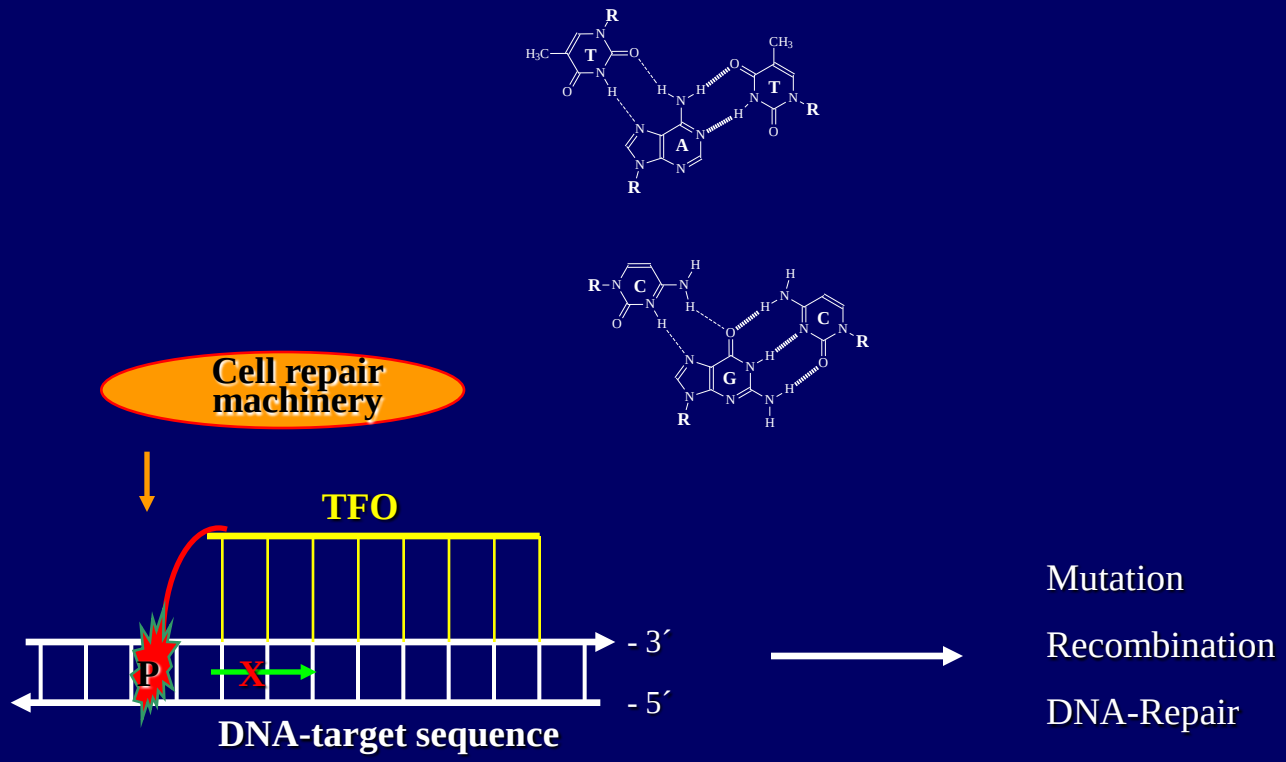


Figure 5: ***Triplex formation for directing site-spezific DNA-damage (P – photoactivatable groups such as psoralen)***

Limitations of the triplex approach to overcome:

- Weaker interaction of Hoogsteen base-pairing than the Watson-Crick base-pairing (temperature dependency),
- Low stability of triplexes under physiological conditions (repulsion between the three negatively charged strands and non-physiological levels of multivalent cations (Mg^{2+} or polyamins can lead to structure, such as G-quadruplexes or G/A-homoduplexes),
- Poor nuclease resistance of native oligonucleotides (TFO), especially RNA,
- Stable secondary structures of oligonucleotides (TFO),
- Lack optimal triplex formation and target side binding due to the intercellular pH ($G:C \cdot C^{+}$) and salt concentration,
- Use for homopurin or homopyrimidin target sequences,
- Necessary labeling of TFO for detection (diagnostics),
- TFO delivery and up-take into the cell („blood-brain barrier“)

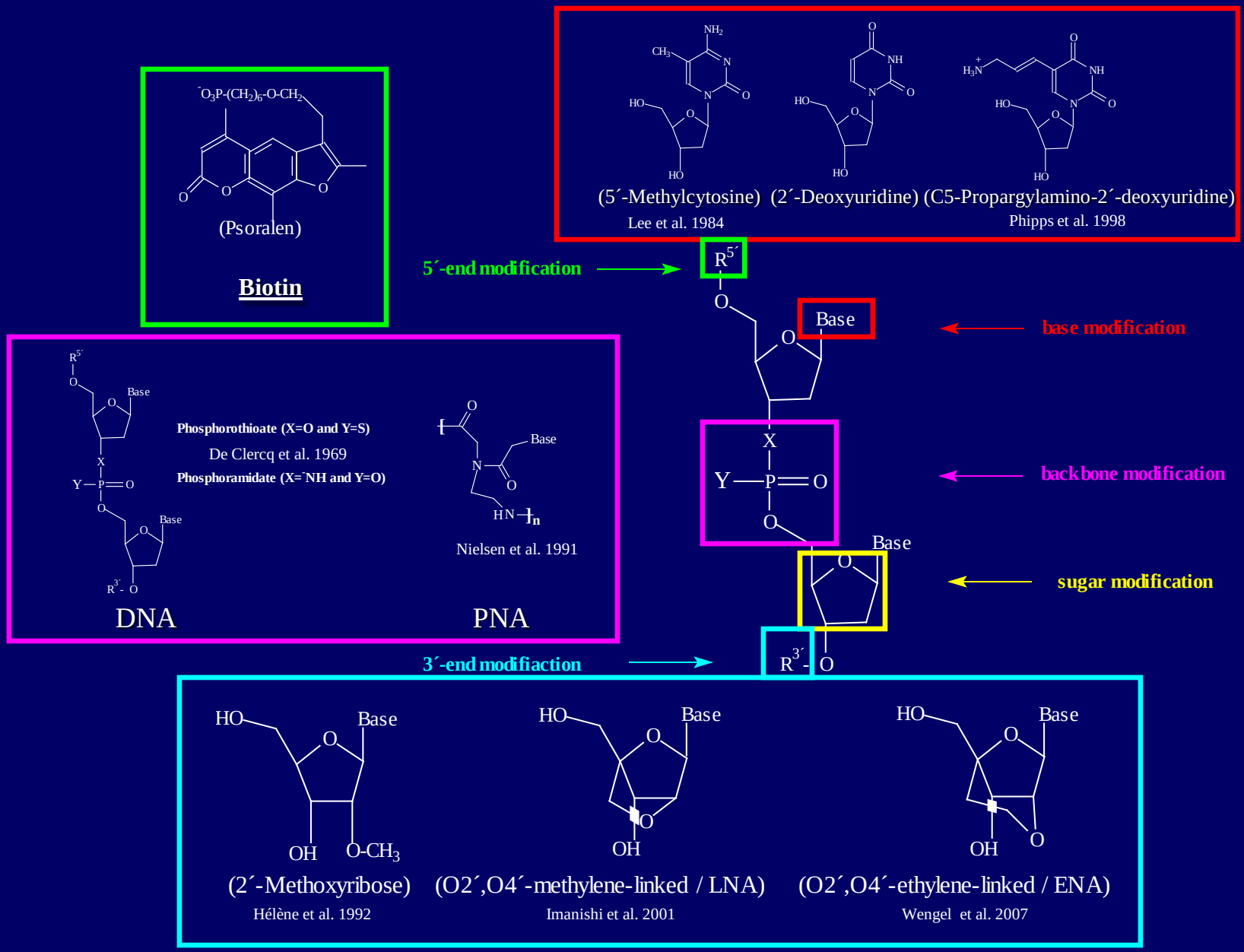


Figure 6: ***Chemical modifications of triplex-forming oligonucleotides (TFO)***

Our philosophy and plans for Fa. Biomesogen MS Technology

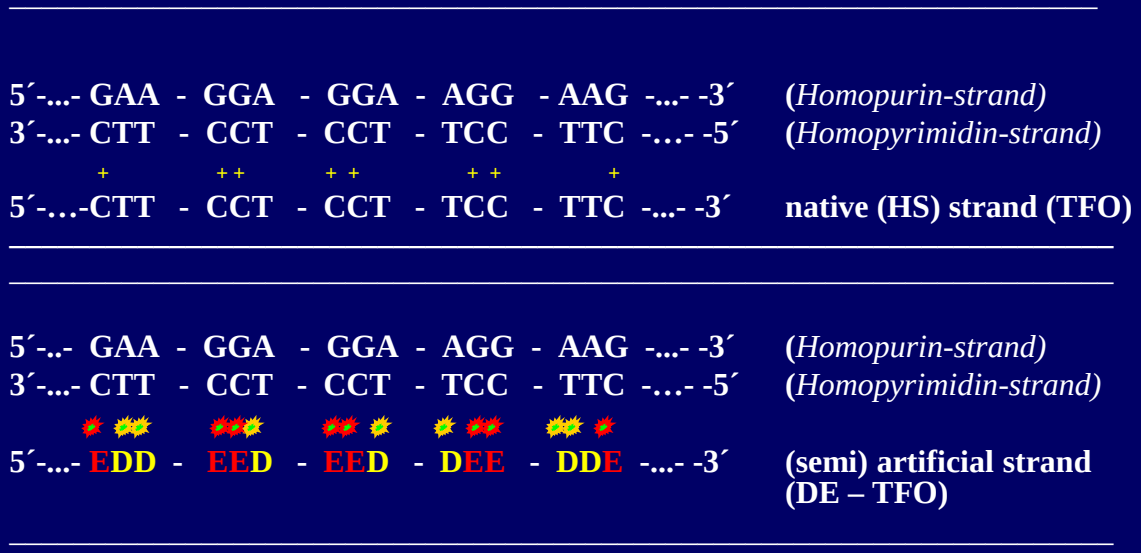


Figure 7: ***Recognition of DNA sequences by triplex-forming oligonucleotides (TFO), 2´-deoxythymidine (T) was exchanged for the new nucleobase (D) and cytosine for (E) in (DE-TFO)***

Advantages of the new D-compounds:

- Stronger interaction of base-pairing, higher stability of triplexes under physiological conditions,
- Optimal triplex formation and target side binding under physiological conditions, because no protonation of cytosine N^3 is necessary,
- Higher nuclease resistance of the new oligonucleotides (D-TFO),
- Not only use for homopurin or homopyrimidin target sequences,
- No necessary labeling of D-TFO for detection, because high fluorescence of D-molecules, slow membrane passage (single molecule analysis)

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