

Genové terapie nádorových onemocnění

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ÚOCHB AV ČR

vŠCHT PRAHA

Osnova

Pathobiochemie nádorových onemocnění

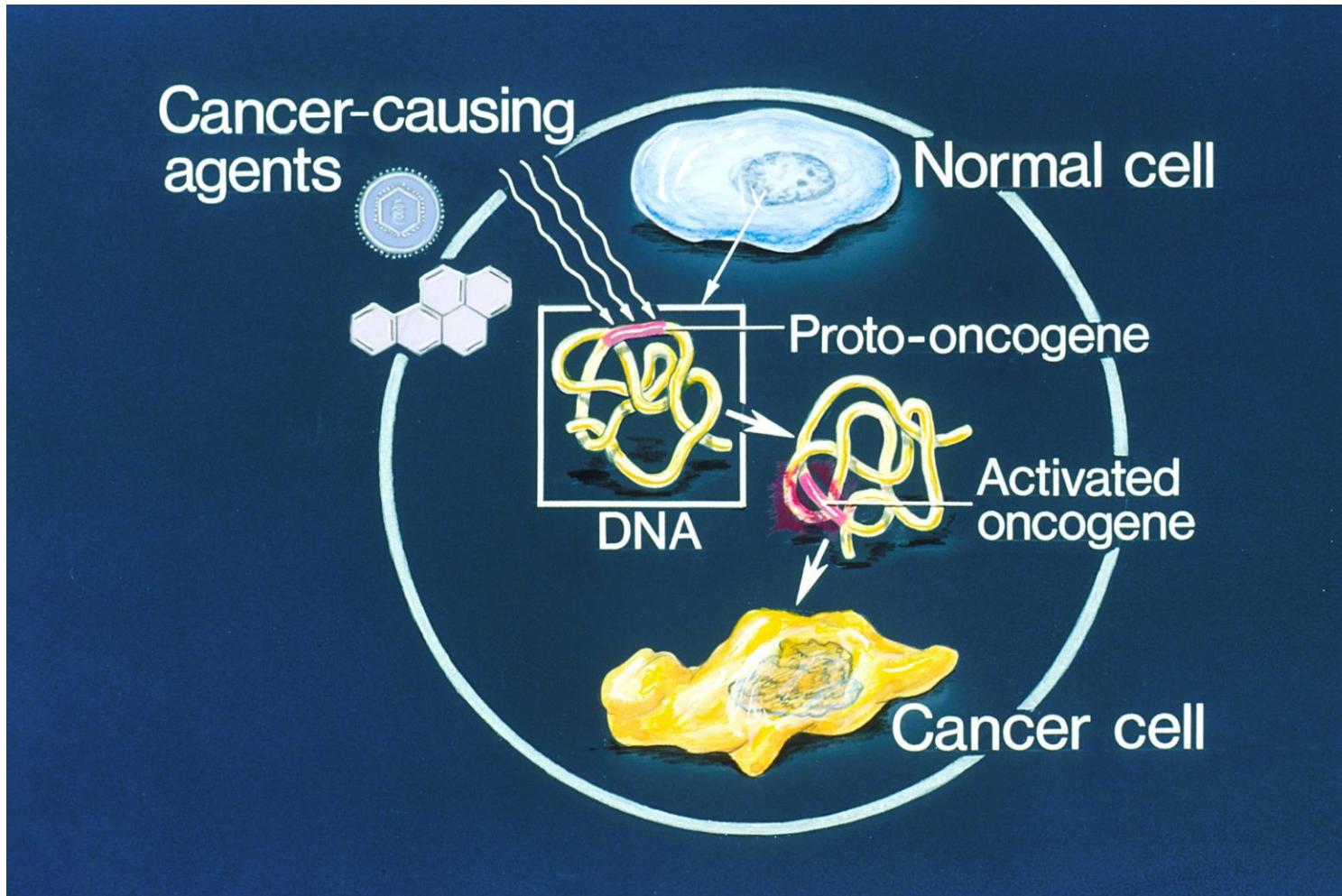
Současná terapie

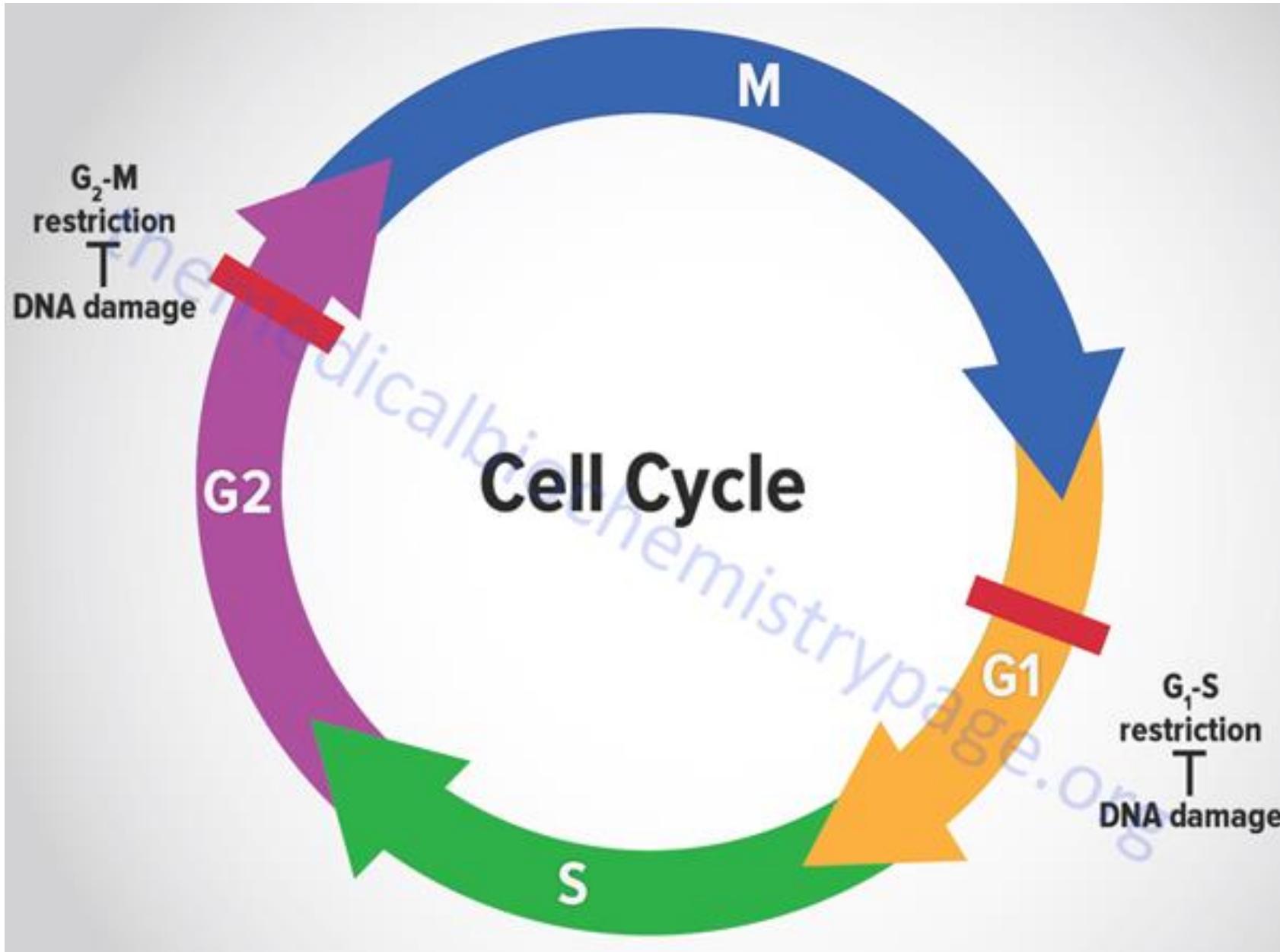
Genová terapie

Proonkogen c-Myc



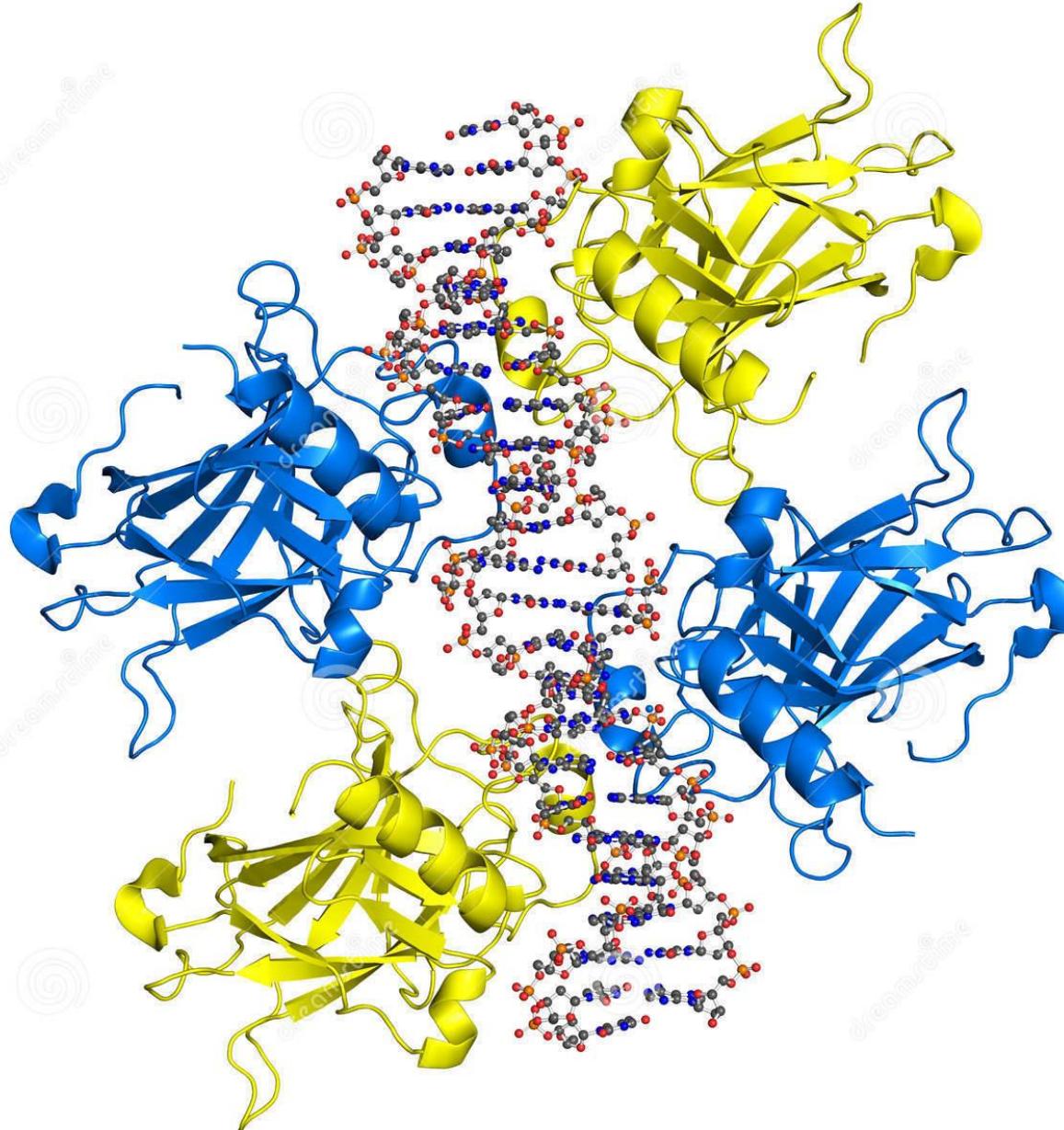
Charakteristika nádorového onemocnění





p53

Tumor supresorový gen



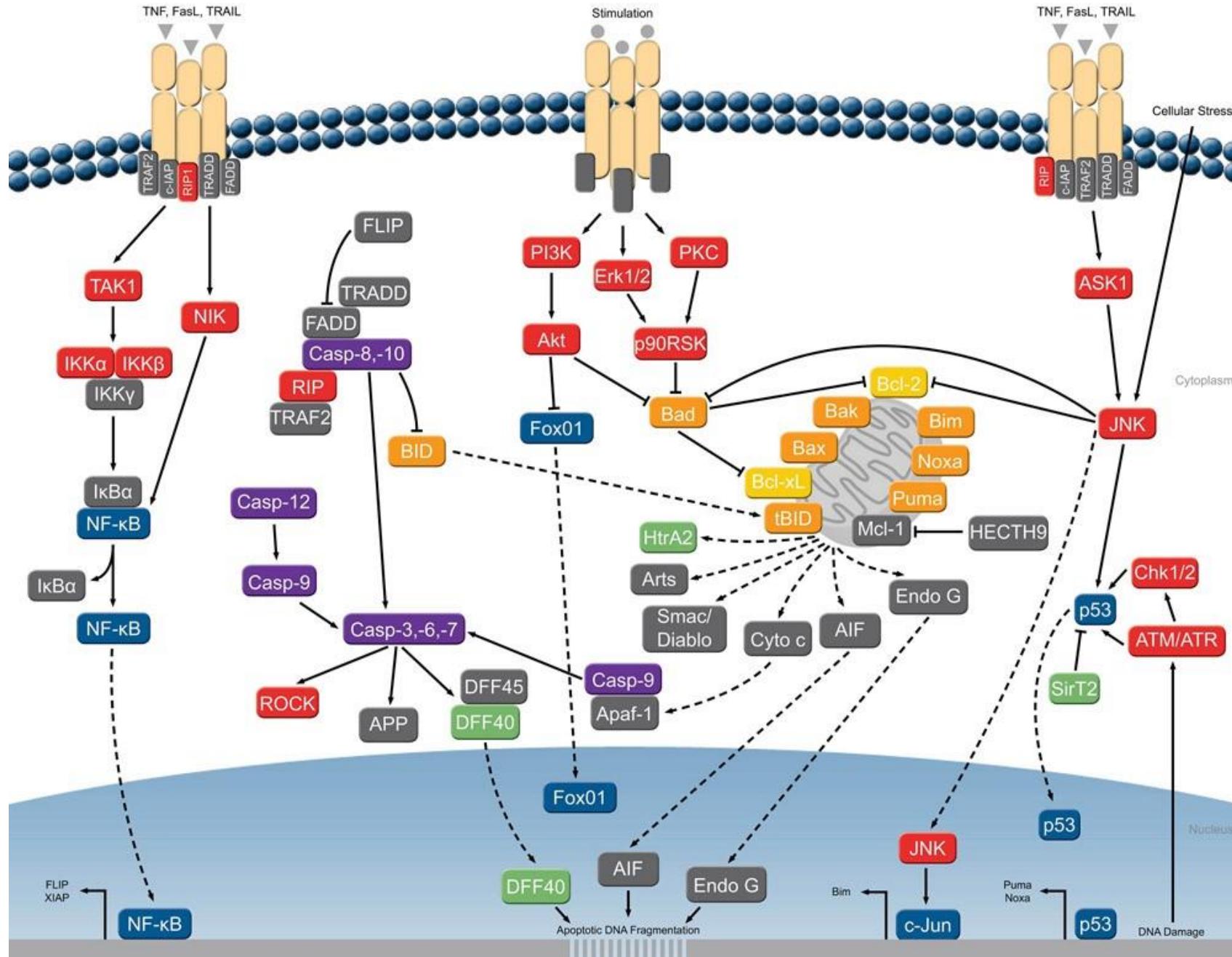
Download from
Dreamstime.com

This watermarked comp image is for previewing purposes only.

<https://thumbs.dreamstime.com/z/dna-bound-p53-tumour-suppressor-25076231.jpg>

ID 25076231

P glazar | Dreamstime.com



Současná terapie - chemoterapie

Cytostatika (mitotické jedy, prekurzory DNA, inhibitory topoizomeráz, interkalační činidla, inhibitory cyklindependentních kináz...)

Zásah do buněčného cyklu

Nejcitlivější jsou rychle se množící buňky a buňky, které mají sníženou schopnost opravovat chyby v DNA

Nespecifické => vedlejší účinky (útlum hematopoézy, alopecie, ...)

Současná terapie - ostatní

Protinádorová imunoterapie

Cytokiny (interferon α , interleukin 2)

Protinádorová hormonální léčba

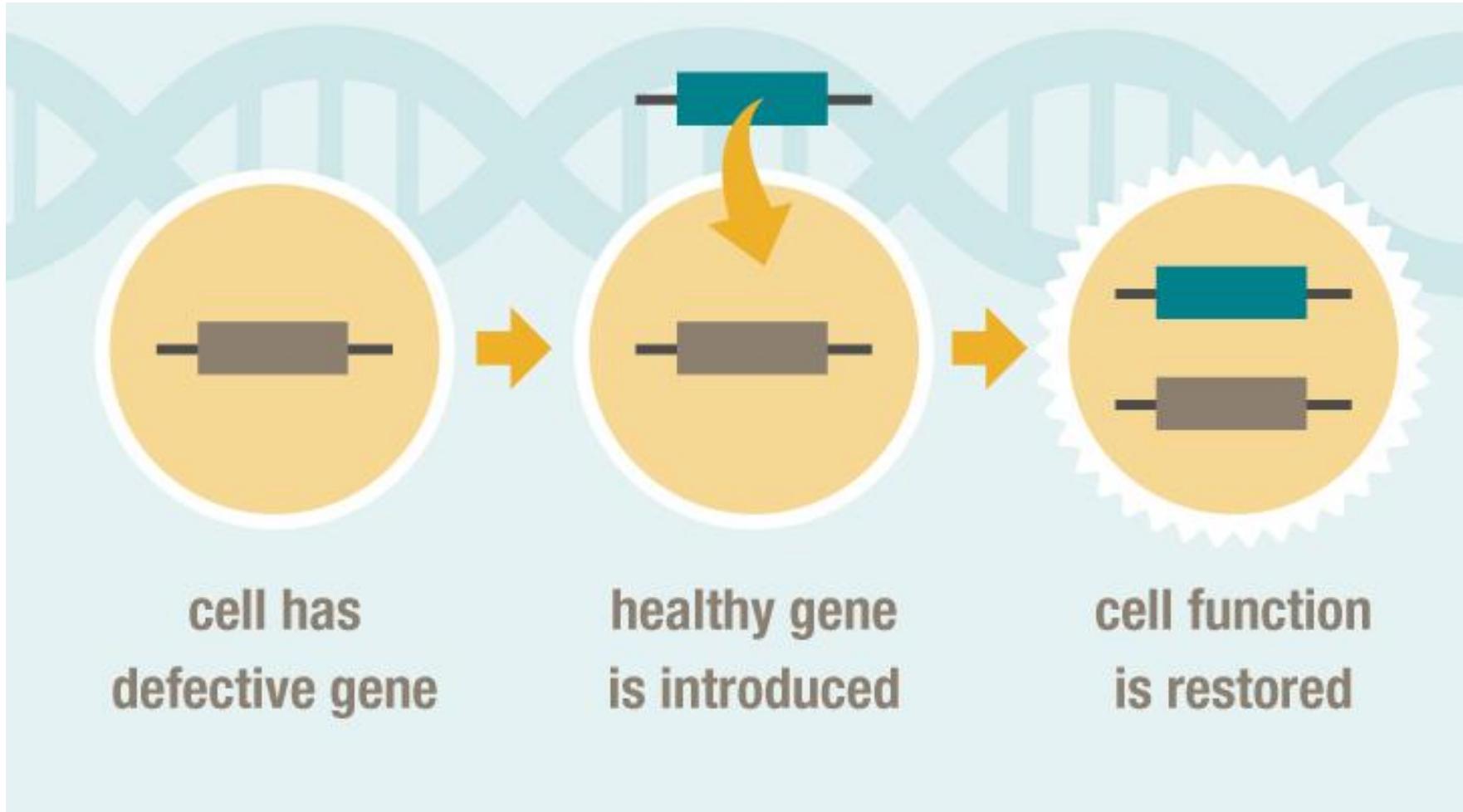
Inhibitory syntéz hormonů, antagonisté hormon. receptorů, kortikosteroidy, analogy somatostatinu...

Biologická terapie

Radioterapie

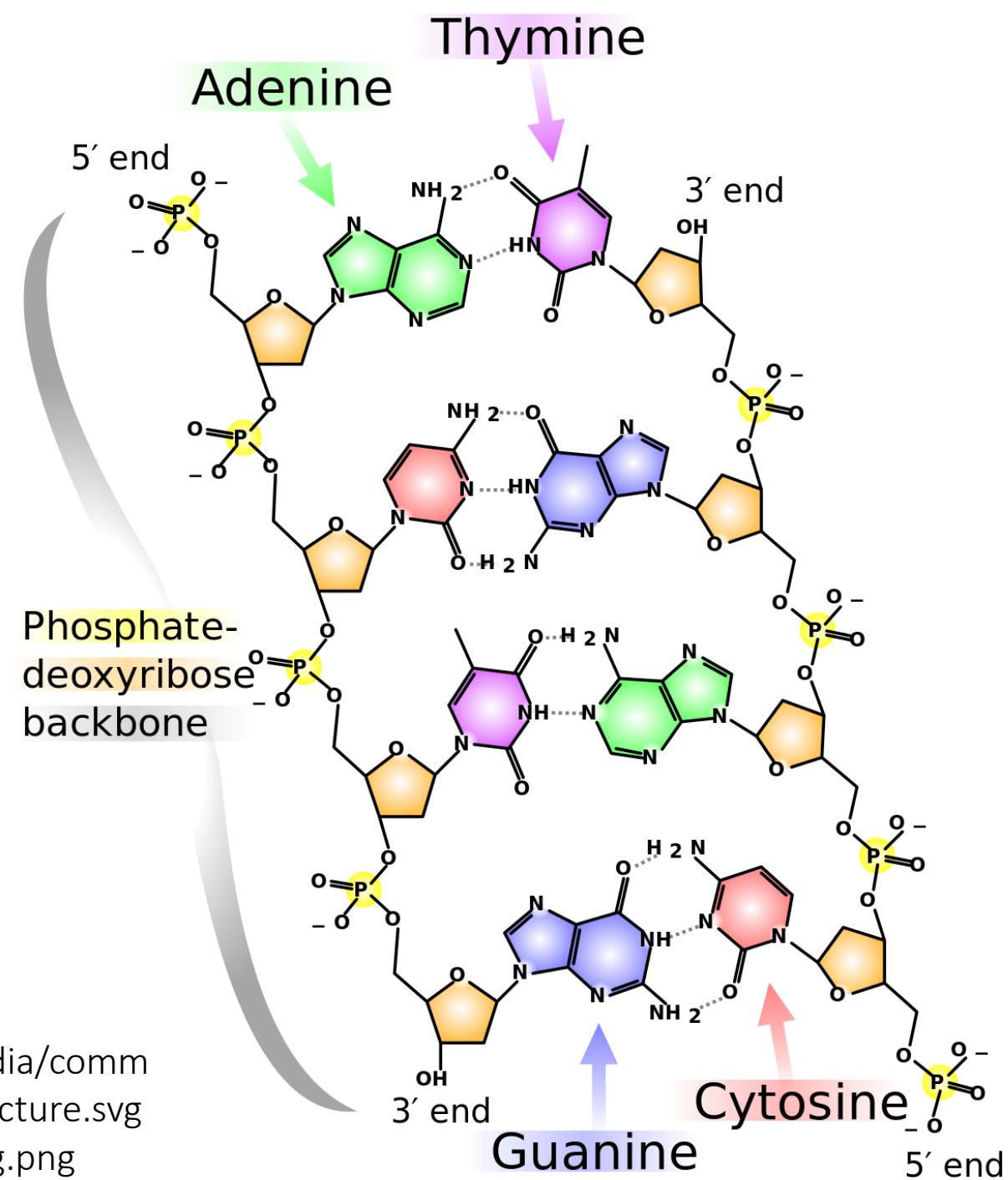
Chirurgie

Genové terapie

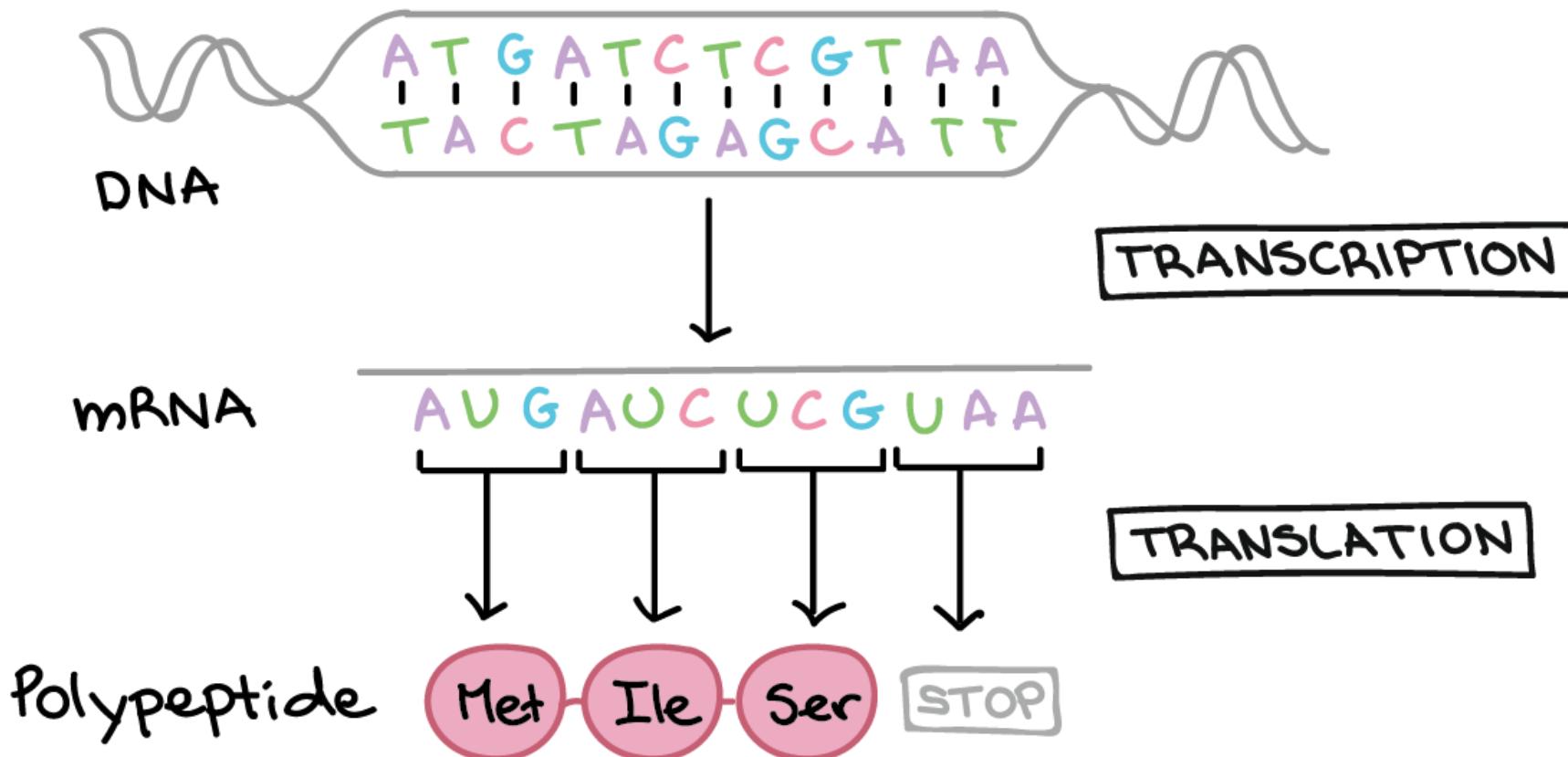




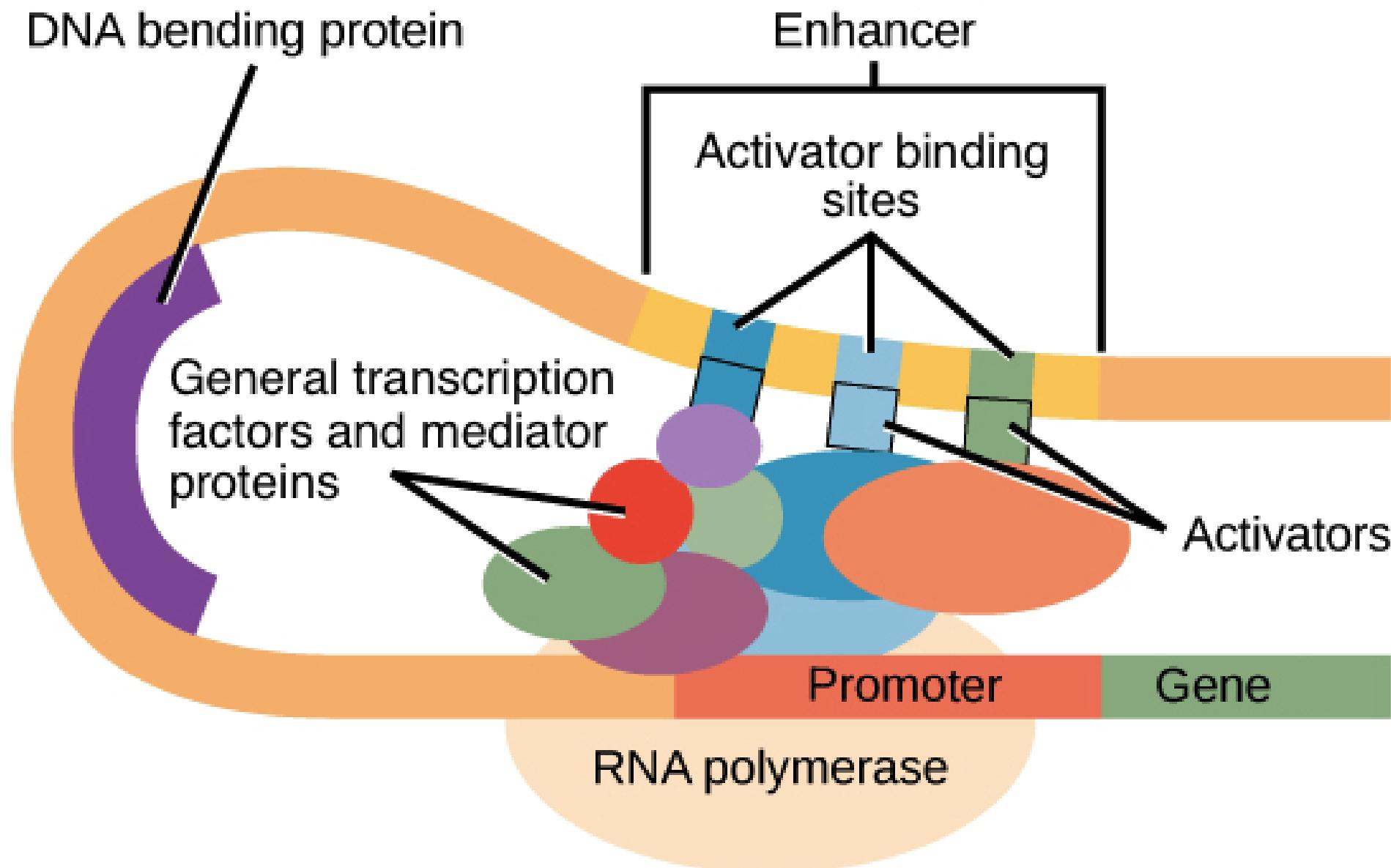
<http://ccftp.scu.edu.cn:8090/Download/uploadfile/20120812141913143.jpg>



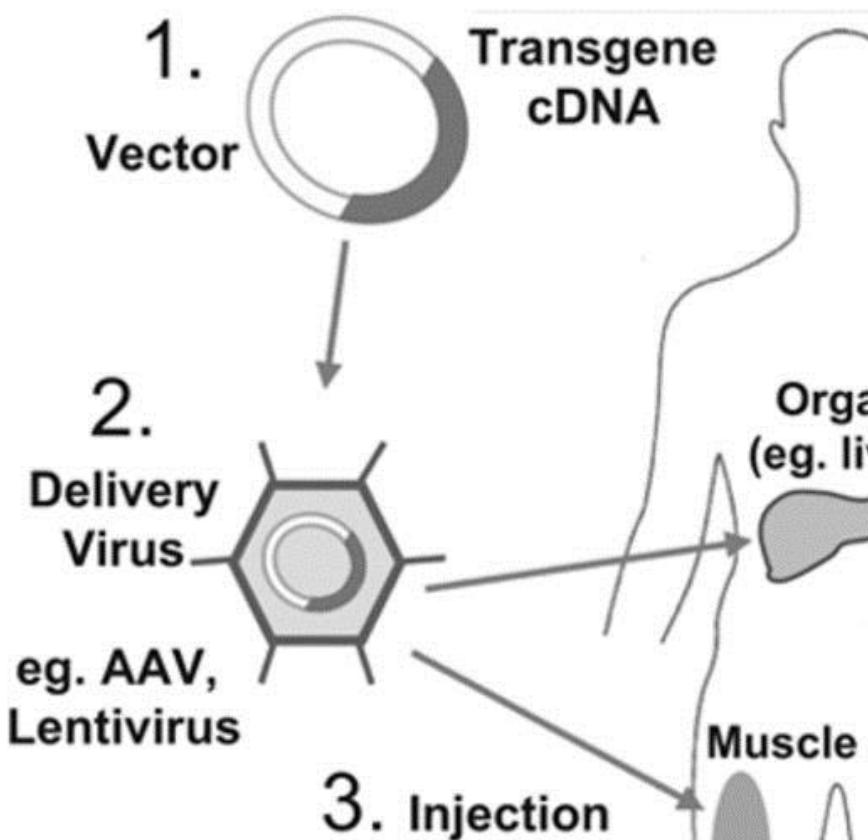
THE CENTRAL DOGMA



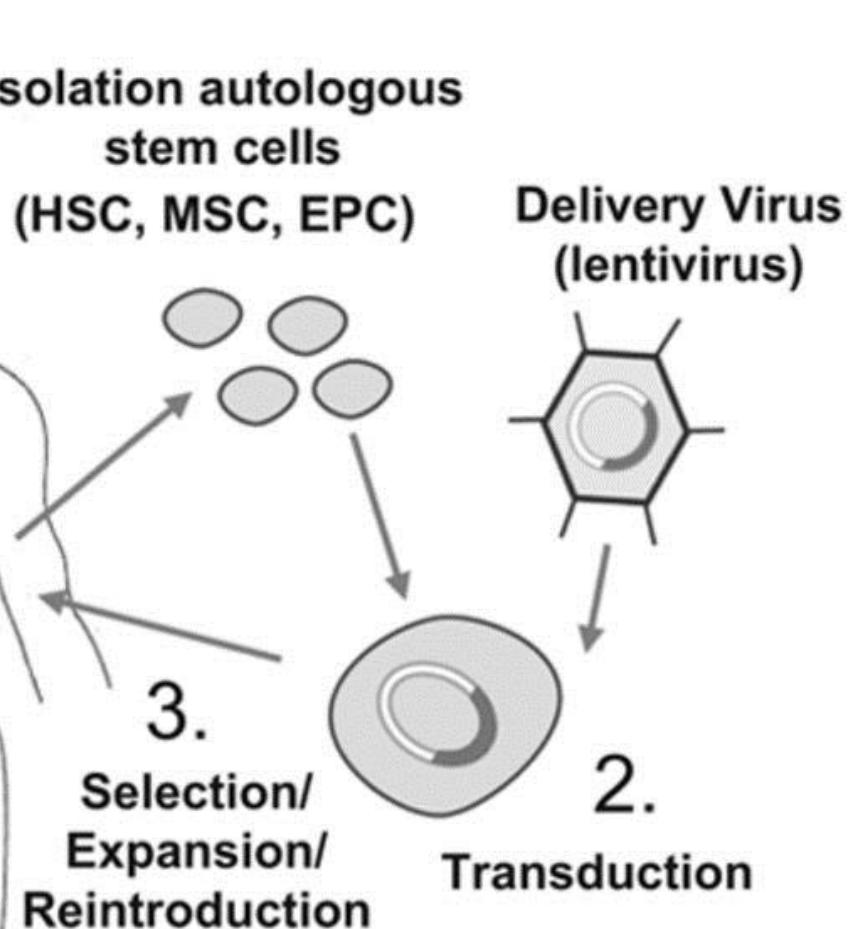
		Second base					
		U	C	A	G		
First base	U	UUU UUC UUA UUG	UCU UCC UCA UCG	UAU UAC UAA UAG	UGU UGC UGA UGG	Cysteine Serine Stop codon Tryptophan	U C A G
	C	CUU CUC CUA CUG	CCU CCC CCA CCG	CAU CAC CAA CAG	CGU CGC CGA CGG	Stop codon Leucine Proline Glutamine	U C A G
	A	AUU AUC AUA AUG	ACU ACC ACA ACG	AAU AAC AAA AAG	AGU AGC AGA AGG	Histidine Leucine Glutamine Arginine	U C A G
	G	GUU GUC GUA GUG	GCU GCC GCA GCG	GAU GAC GAA GAG	GGU GGC GGA GGG	Isoleucine Threonine Asparagine Lysine	U C A G
Third base							
Methionine start codon							
V							
A							
D							
E							
G							



A *In Vivo*
Gene Transfer



Ex Vivo
Gene Transfer



Doručení genu do tkáně

Vektory (virové, nevirové)

Lipofekce

Injekčně

Genetické dělo



<http://www.bio-rad.com/en-ch/product/helios-gene-gun-system>

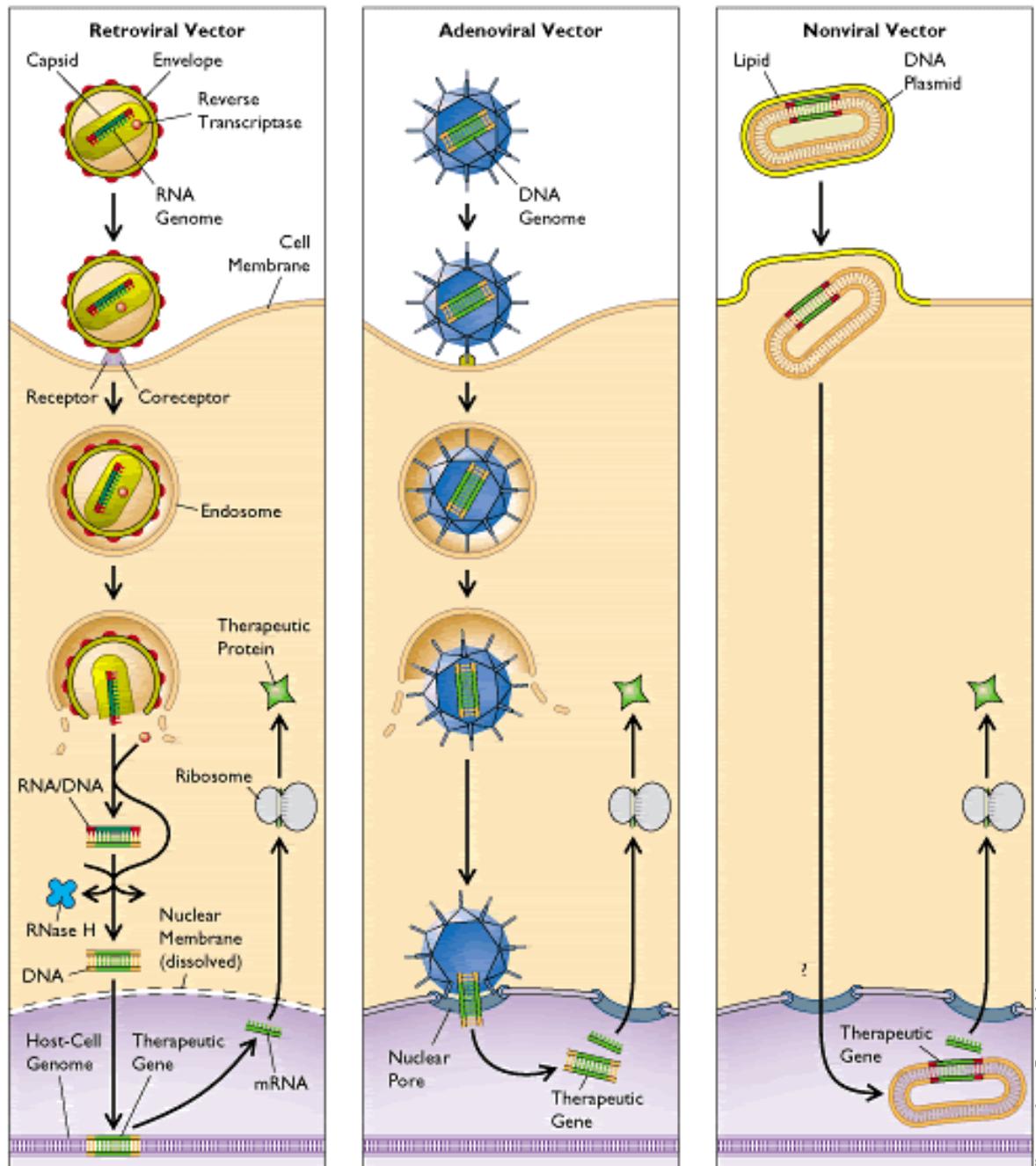
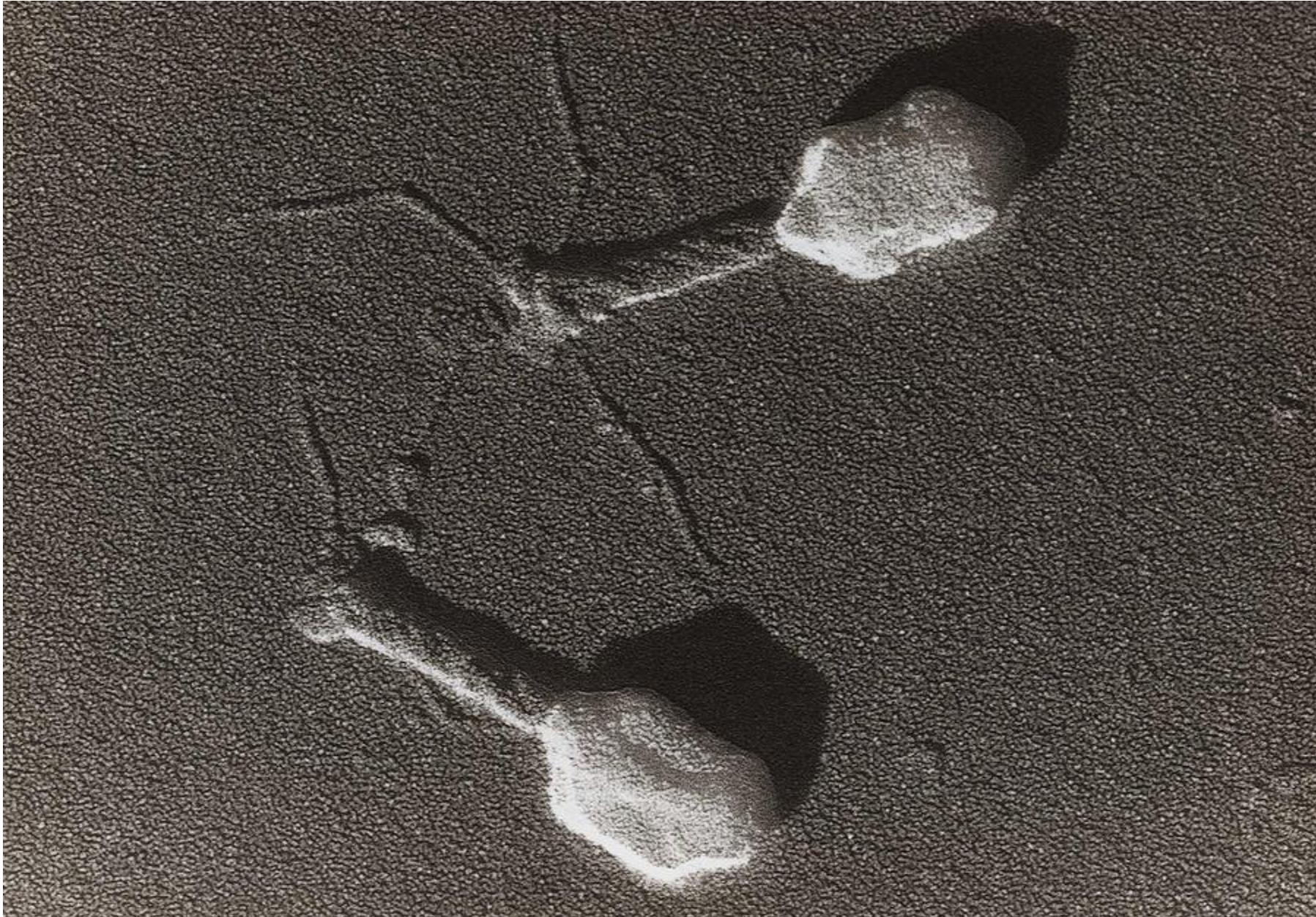
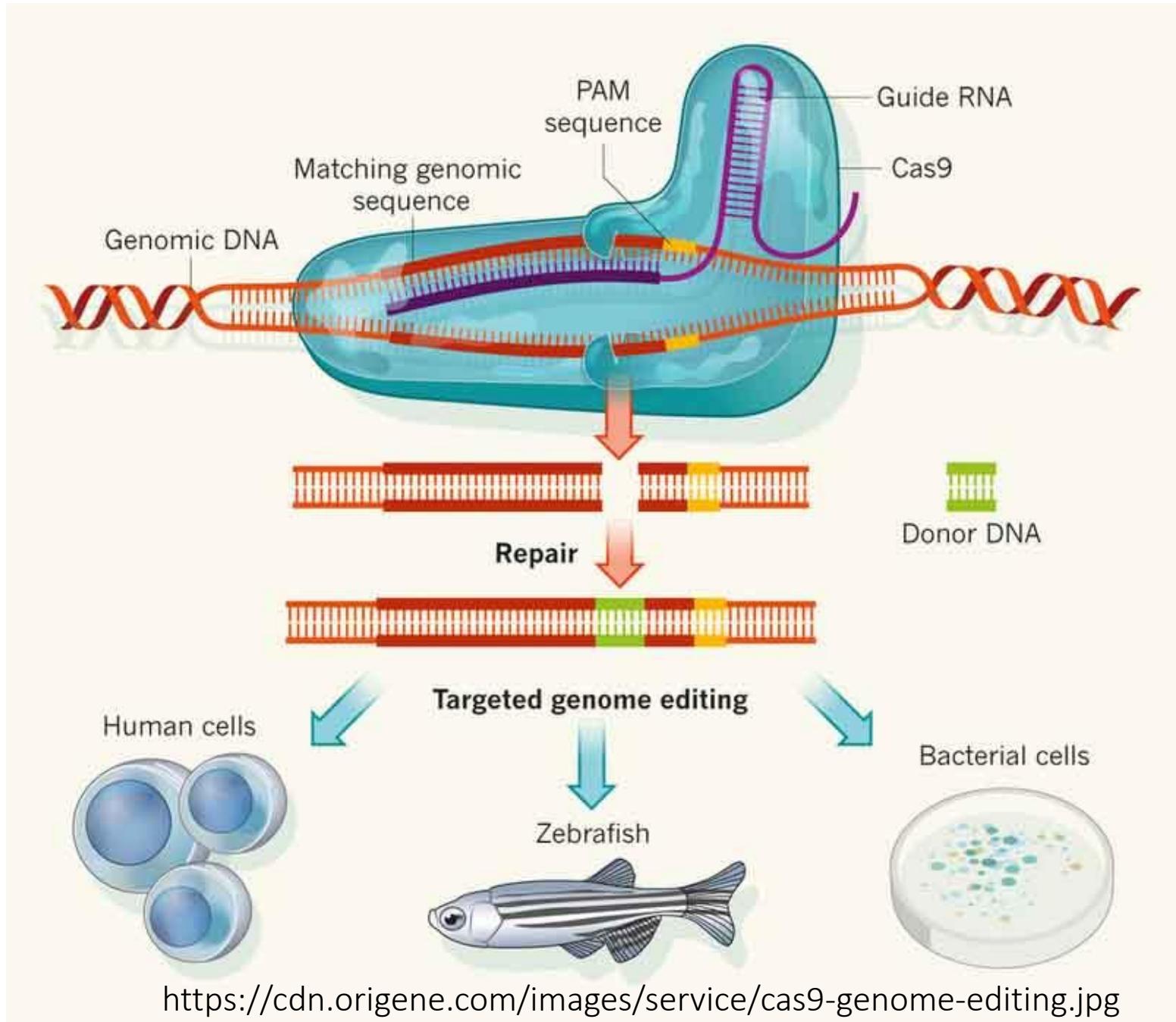


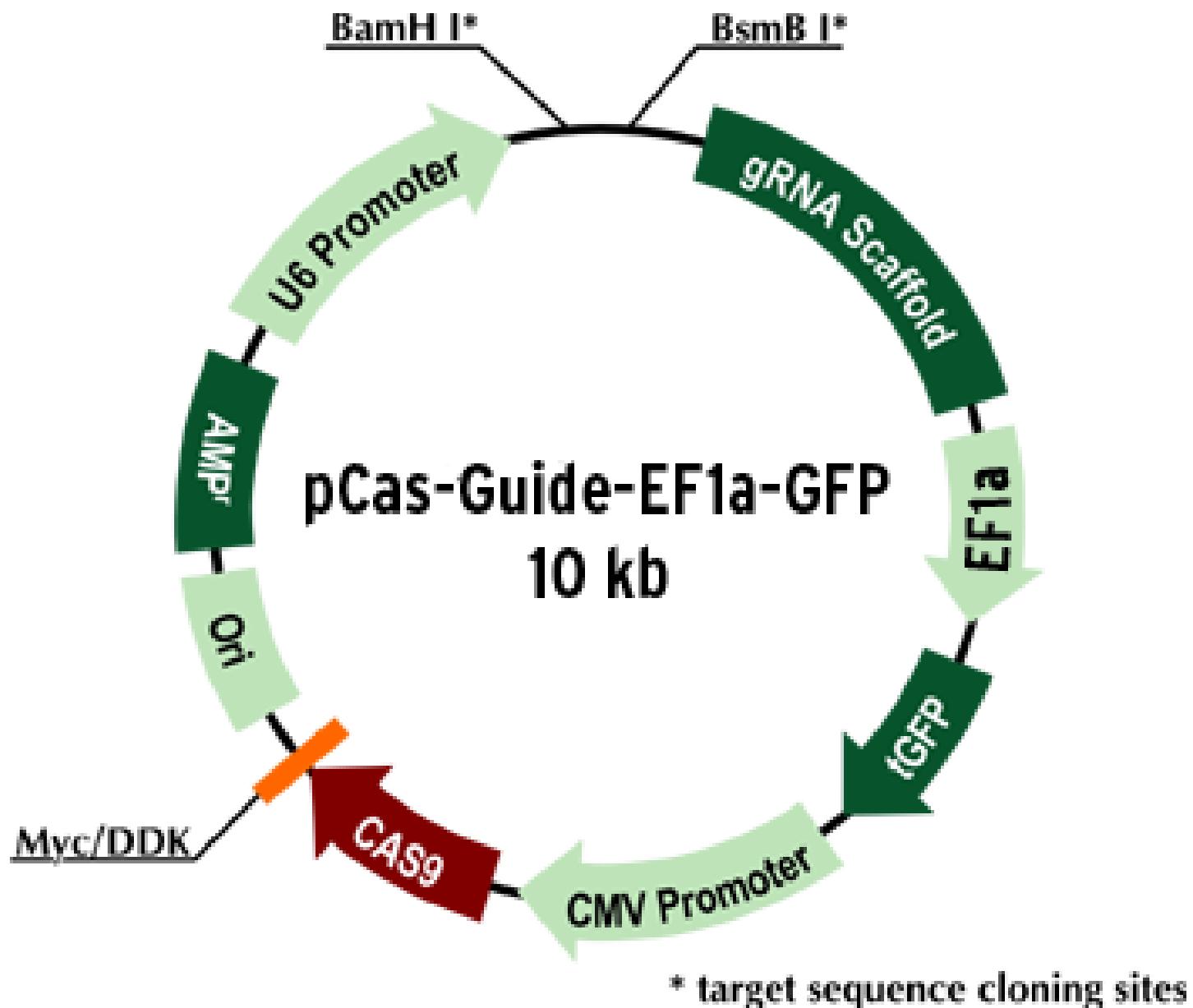
Illustration: Seward Hung



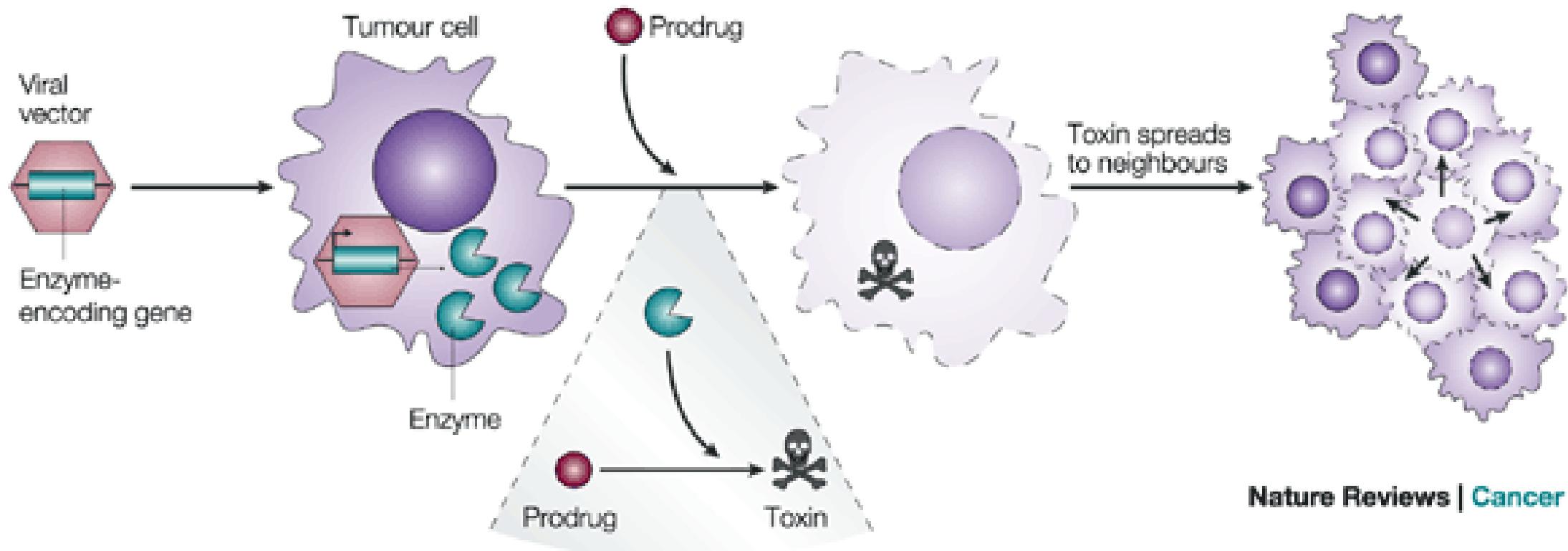
<https://images.fineartamerica.com/images-medium-large/tem-of-t4-bacteriophage-m-wurtzbiozentrum-university-of-basel-.jpg>

CRISPR/Cas9



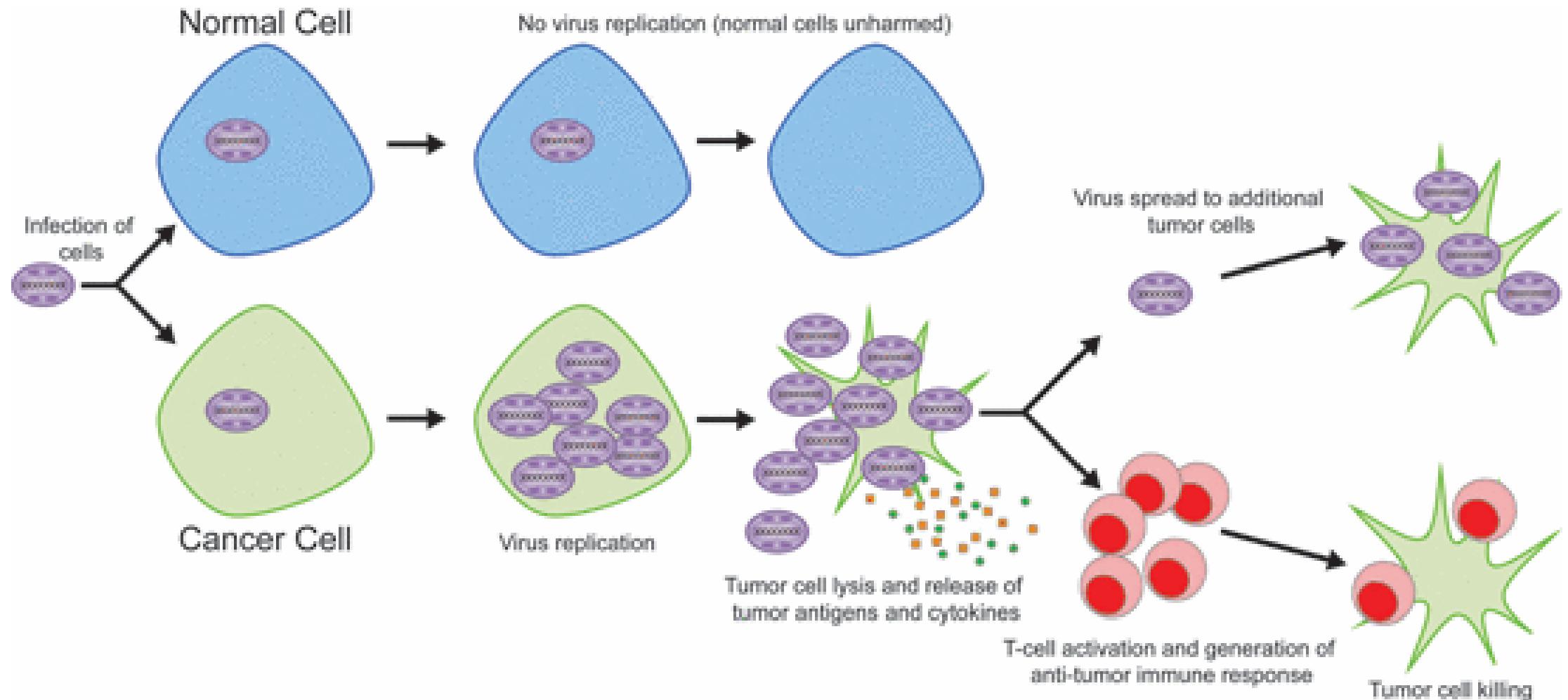


Sebevražedné geny



<https://media.nature.com/m685/nature-assets/nrc/journal/v1/n2/images/nrc1101-130a-f3.gif>

Onkolytické viry



TALE Nucleases (TALENs)



TALEN modules recognize single bases



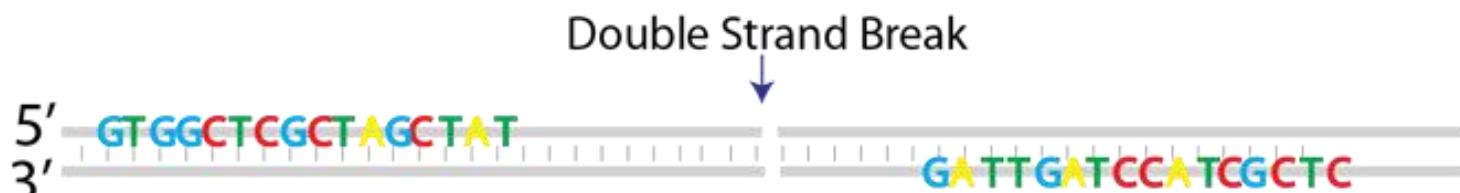
~14 bp long half-sites
separated by a ~16 bp spacer

Left TALEN



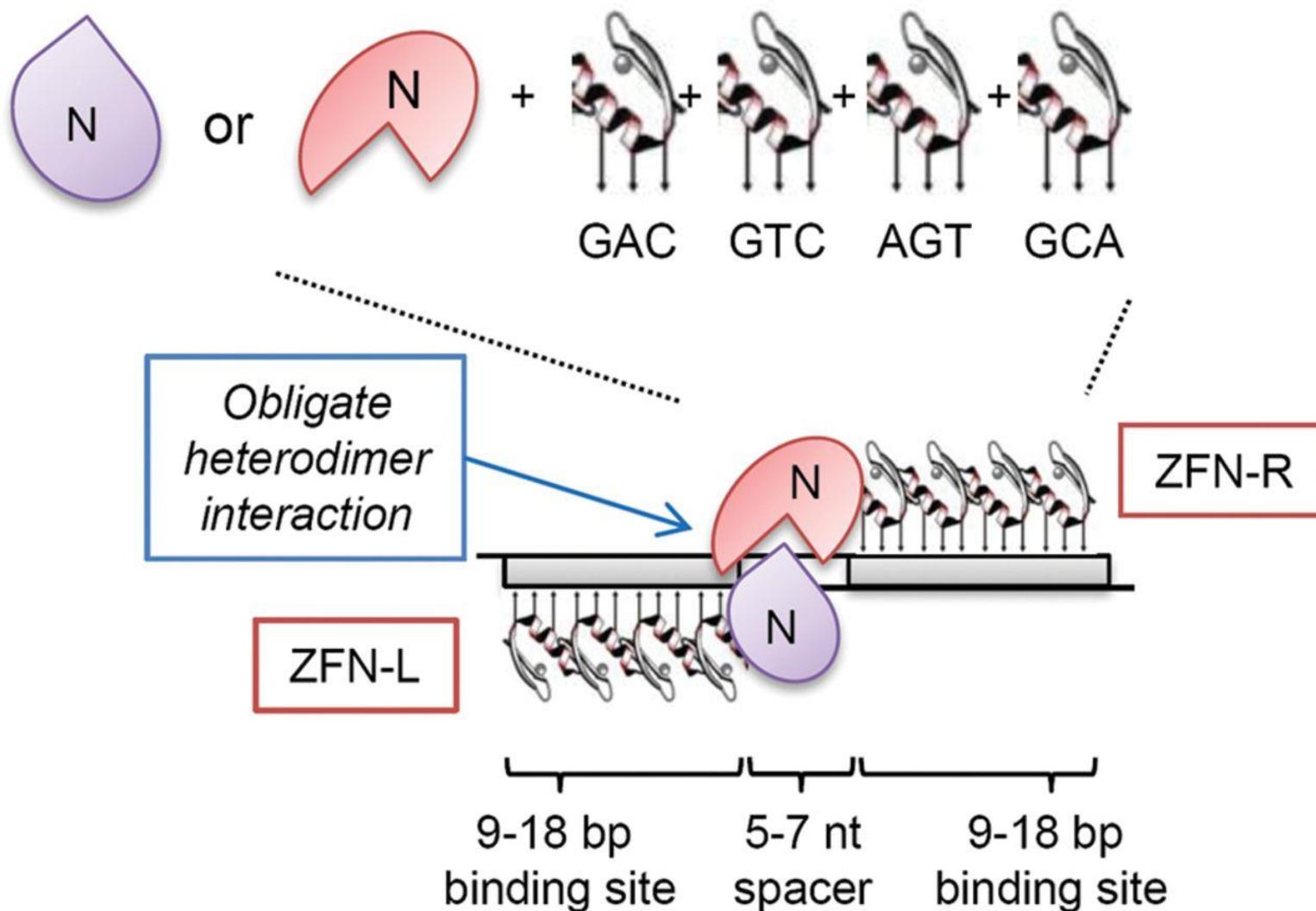
The FokI domains of pairs of TALENs dimerize

Right TALEN

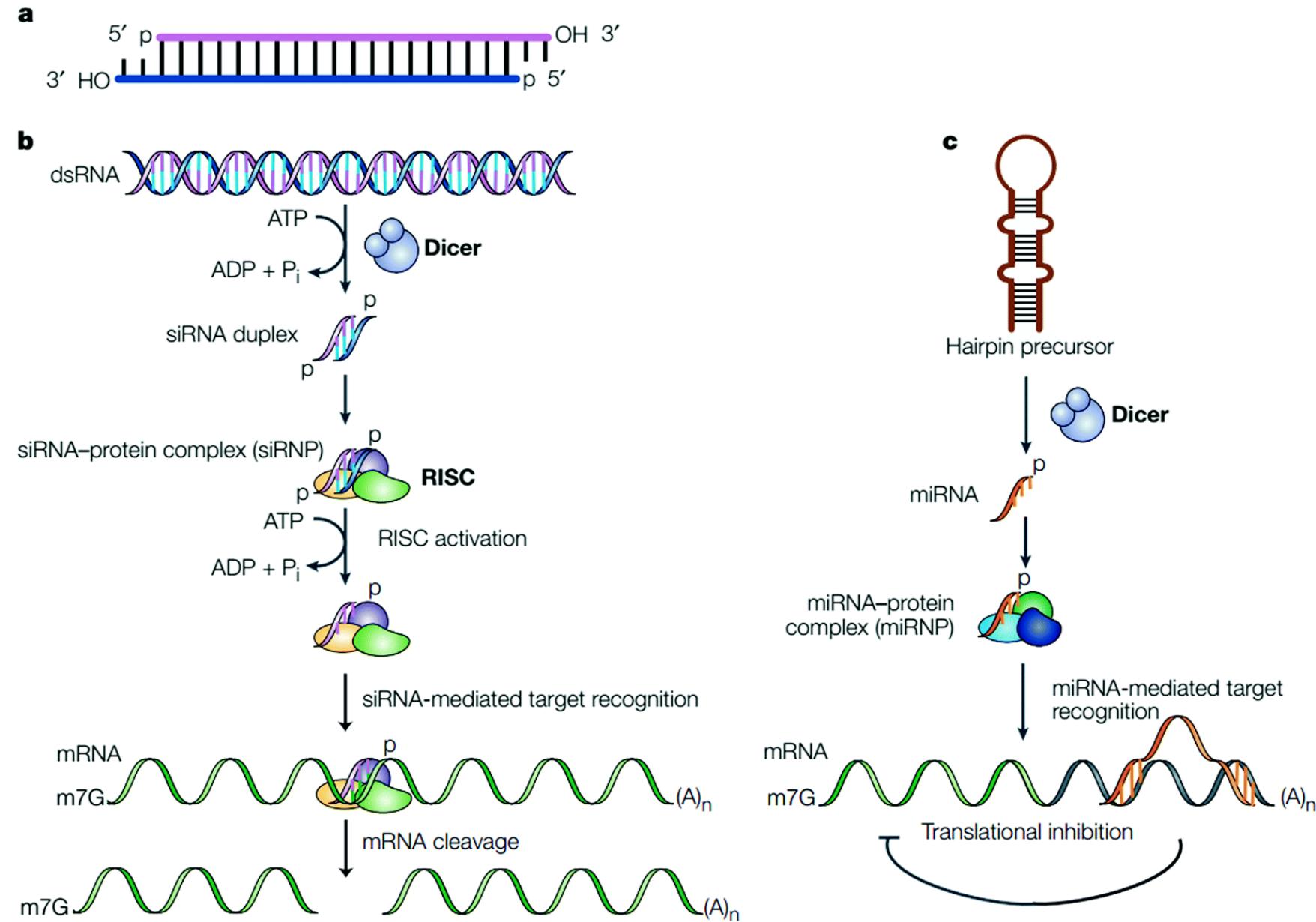


Variant FokI
Nuclease
domain

Combine zinc-finger modules



miRNA, siRNA

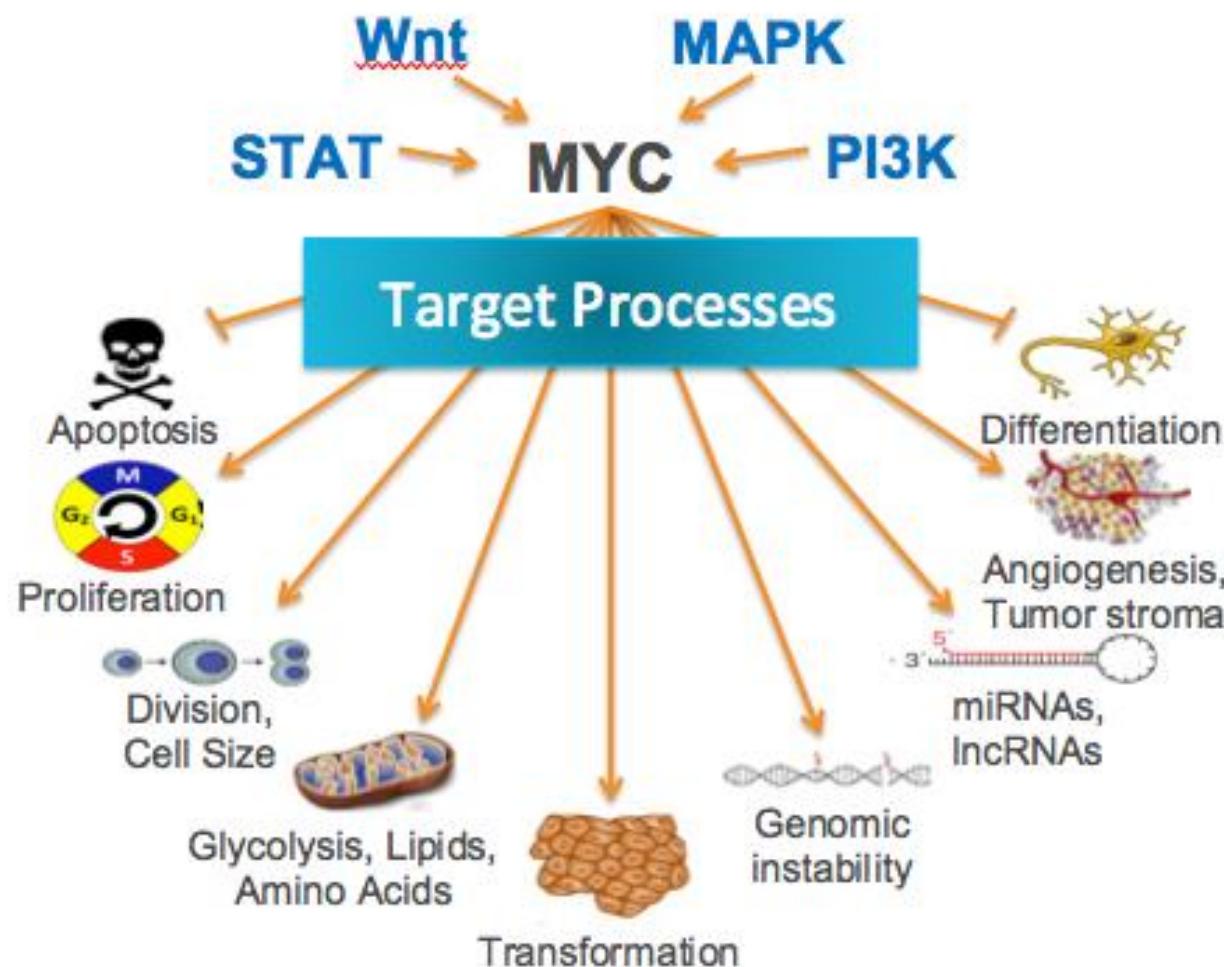


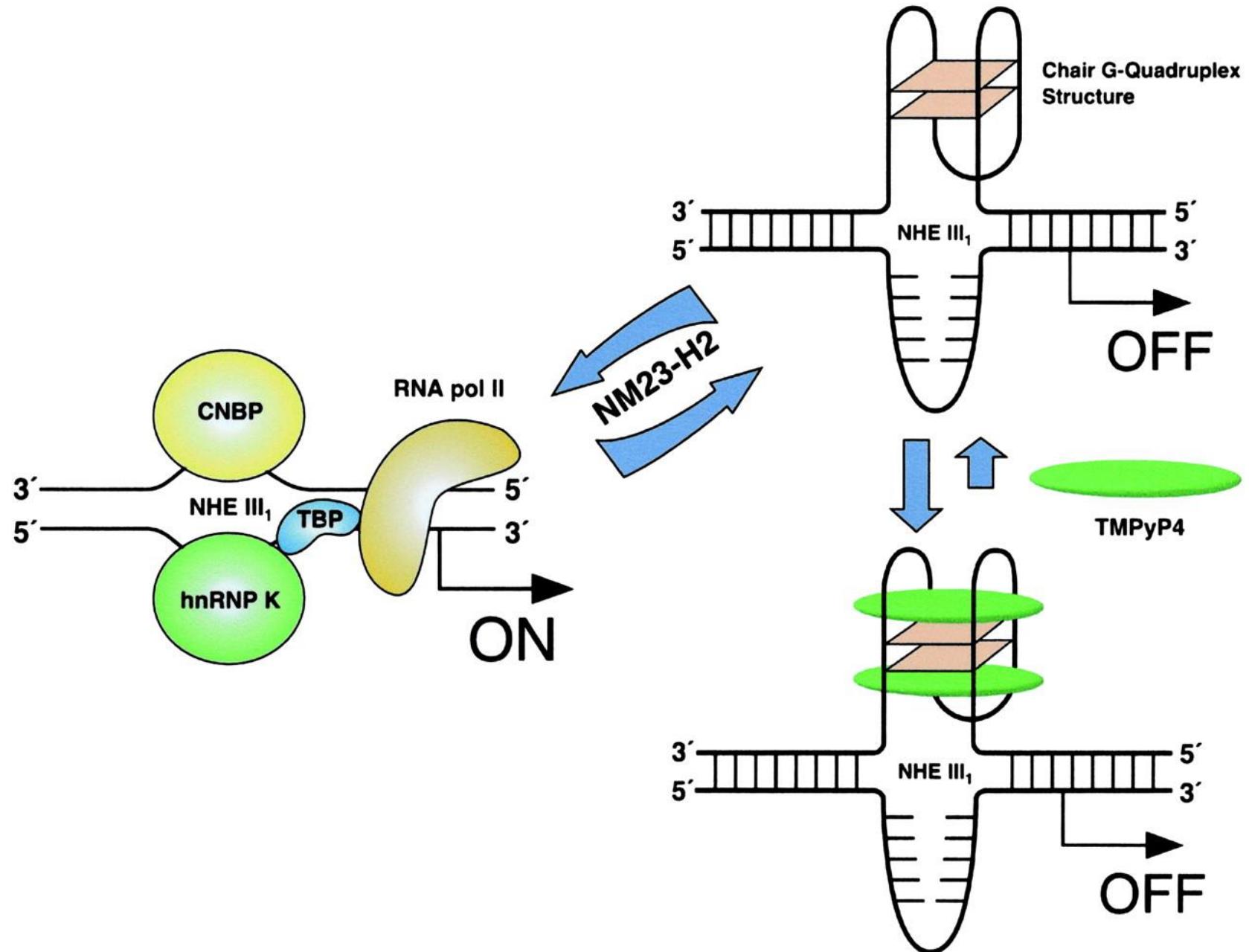
Proonkogeny

Skupina	Příklad
Růstové faktory	Sis
Tyrosinkinázové receptory	EGFR,
Tyrosinkinázy	Src
Serinové / Threoninové kinázy	Raf kinázy, CDk
GTPázy	Ras
Transkripční faktory	c - Myc

C - Myc

MYC Is a Key Inducer of Oncogenic Pathways





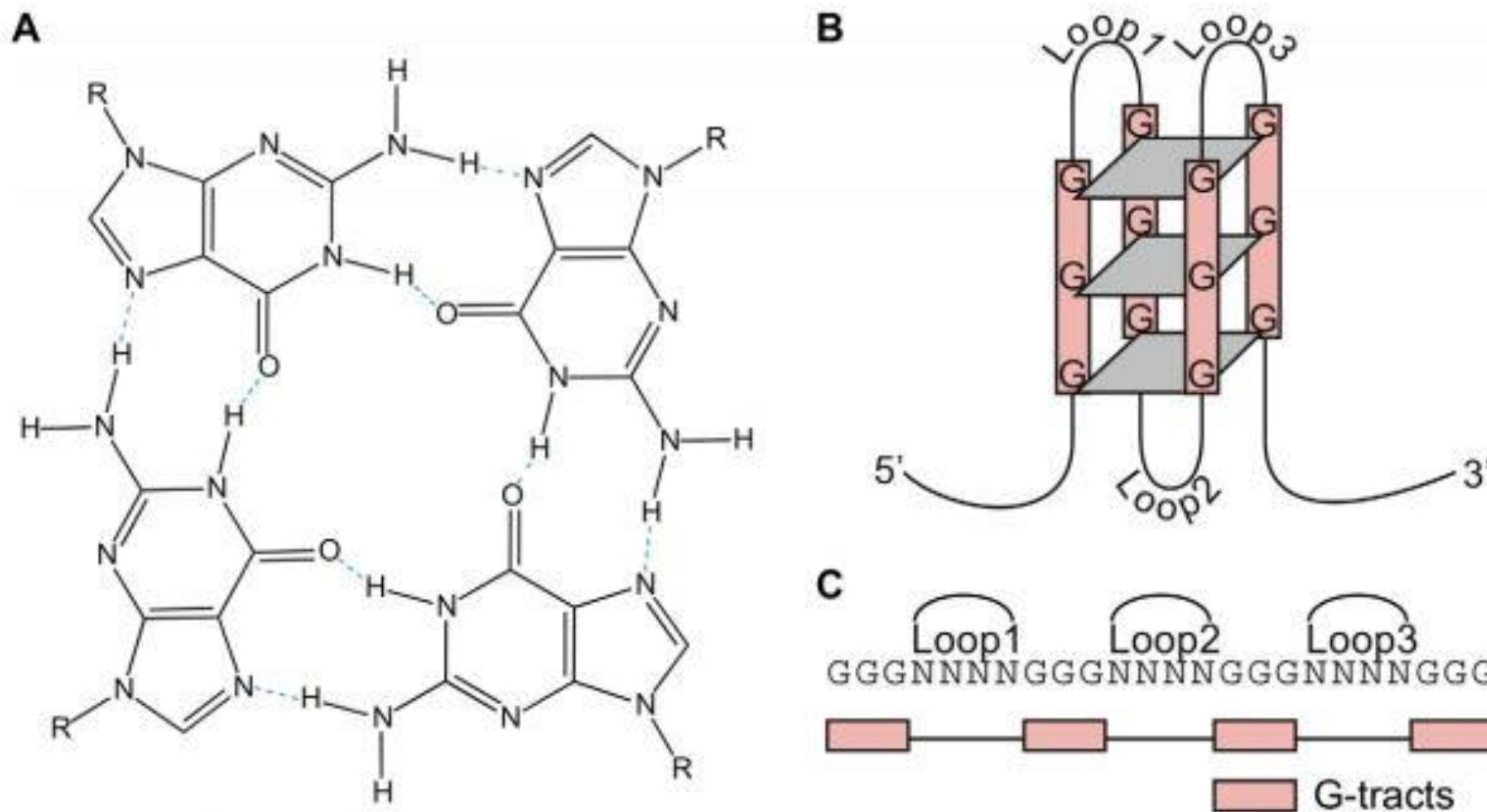
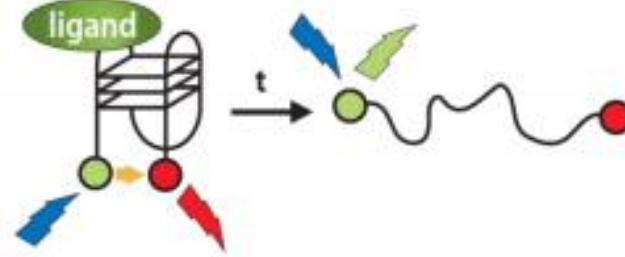


Figure 1. The G4 DNA structure and motif. (A) Structure of a G-quartet. The planar ring of four hydrogen-bonded guanines is formed by guanines from different G-tracts, which are separated by intervening loop regions in the intra-molecular G4 DNA structure. (B) Schematic of an intra-molecular G4 DNA structure consisting of three G-quartets. Inter-molecular G4 DNA structures can also form from two or four strands. (C) The G4 DNA motif sequence used in this study with four G-tracts of three guanines separated by loop regions.
doi:10.1371/journal.pcbi.1000861.g001

1. FRET assay & competition with DNA duplex



2. Luciferase reporter assay



3. Viability tests – XTT assay HUVEC and „CA-46 test”

Raji cell line

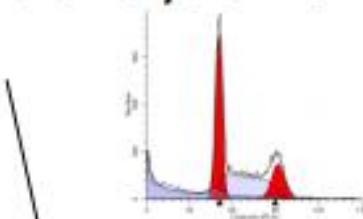


CA-46 cell line

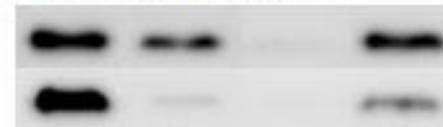


Siddiqui-Jain et al. 2002 PNAS

6. Cell Cycle analysis



5. Protein expression WB & MS



4. RT qPCR Myc mRNA expression



7. Best ligands mouse experiments