



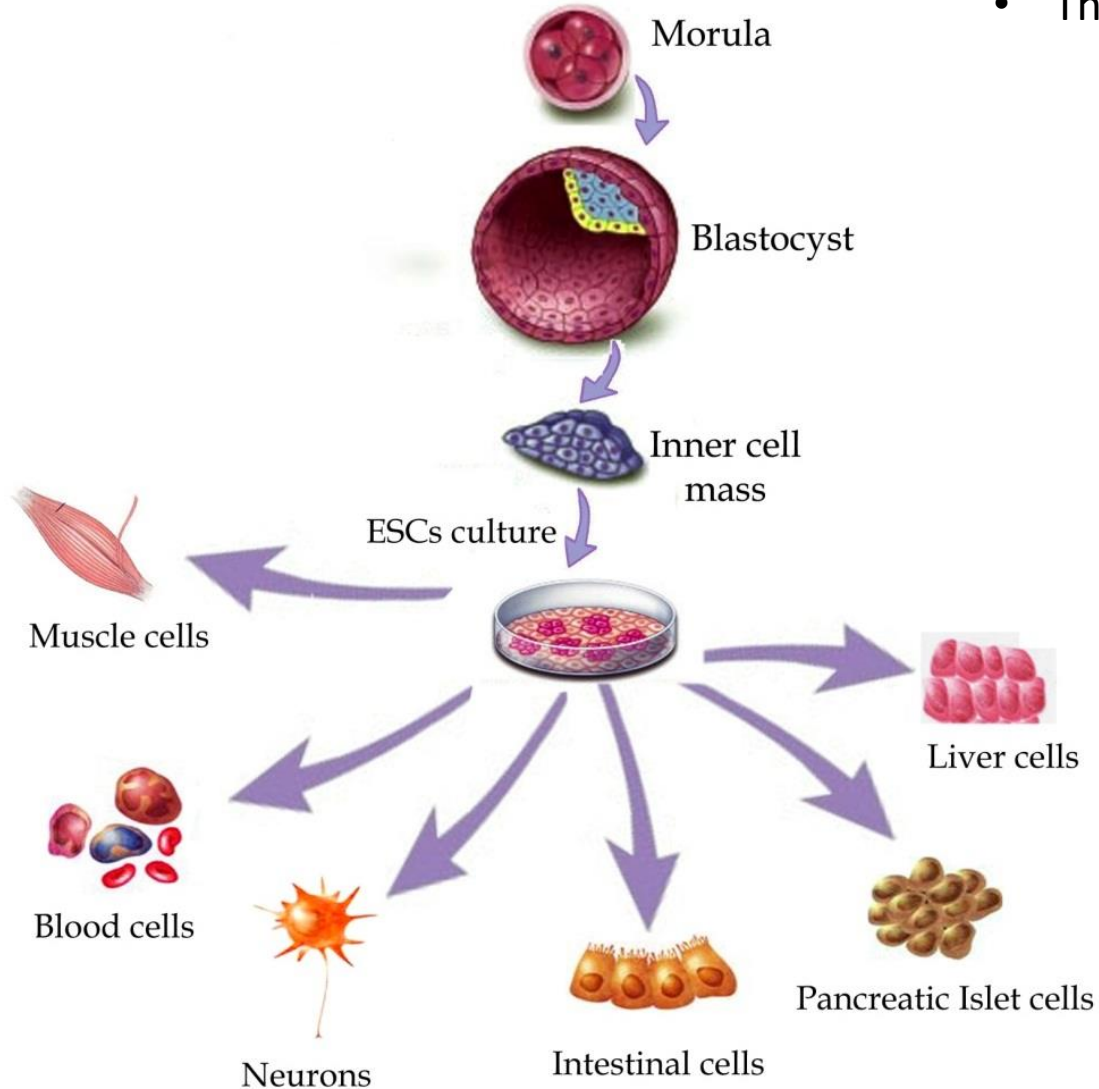
Molecular biology of cancer cell

Petr Müller, RECAMO, Masaryk Memorial Cancer Institute

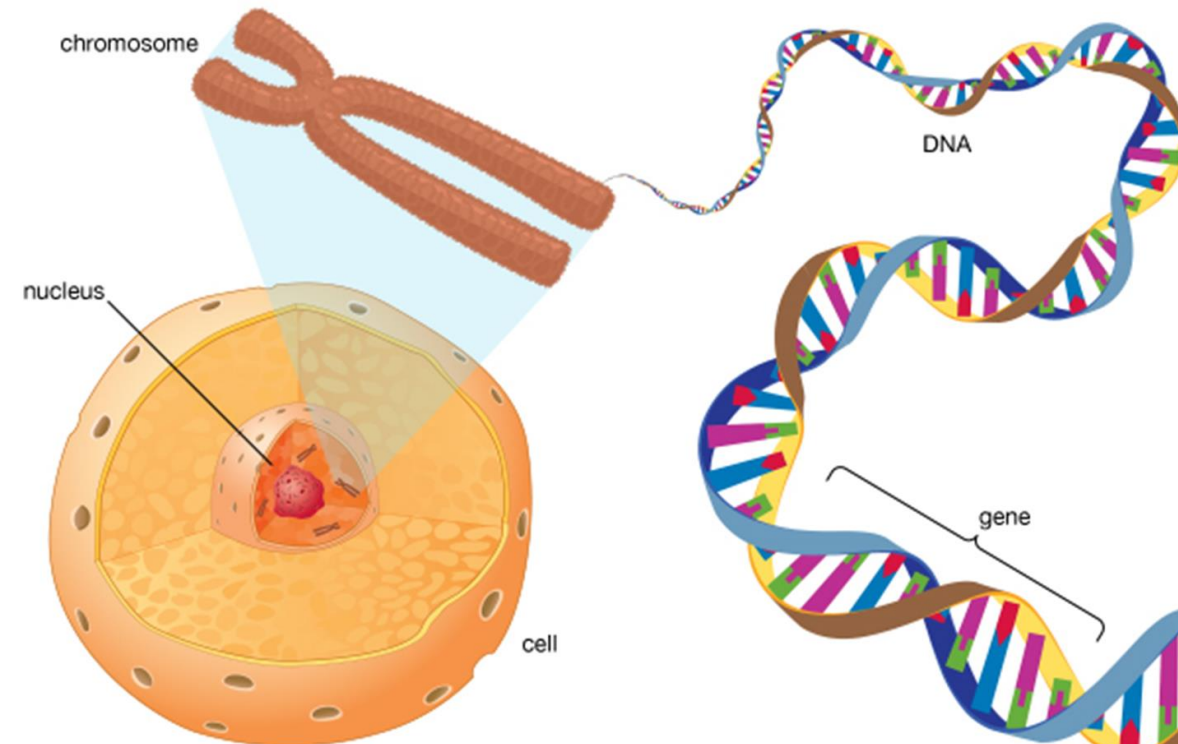
What is the difference between a normal and a tumor cell?

Human body is composed of differentiated cells

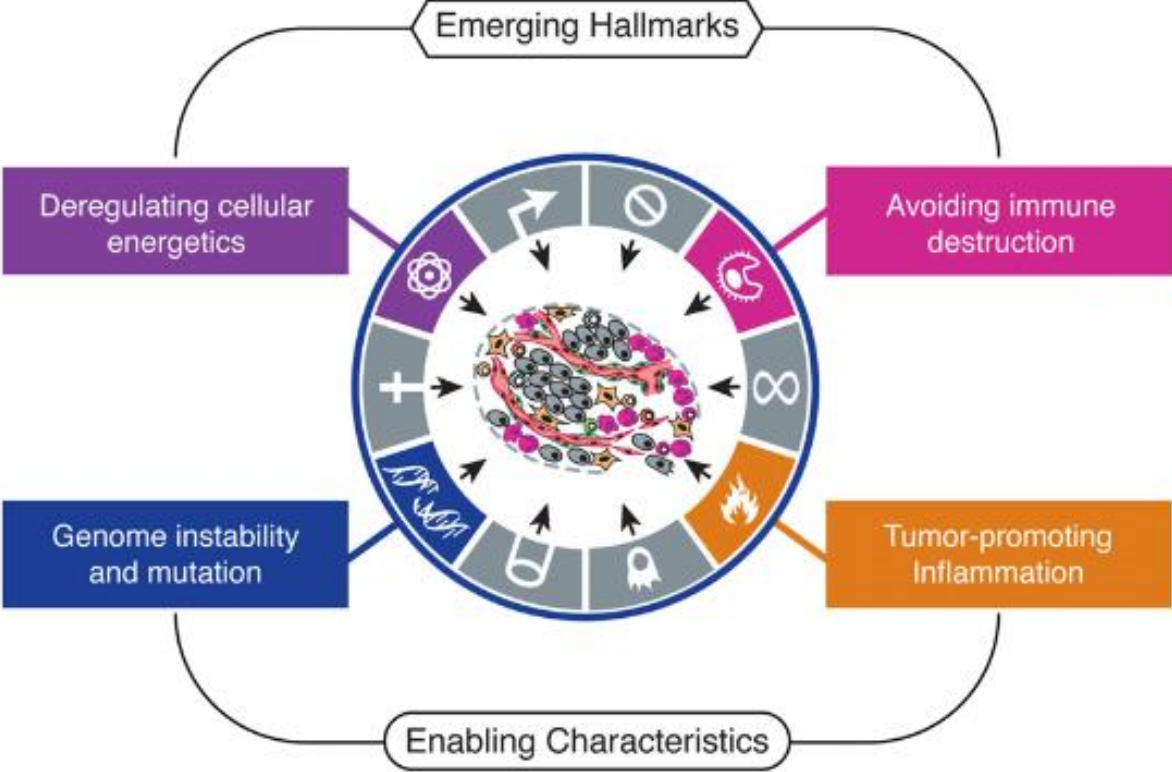
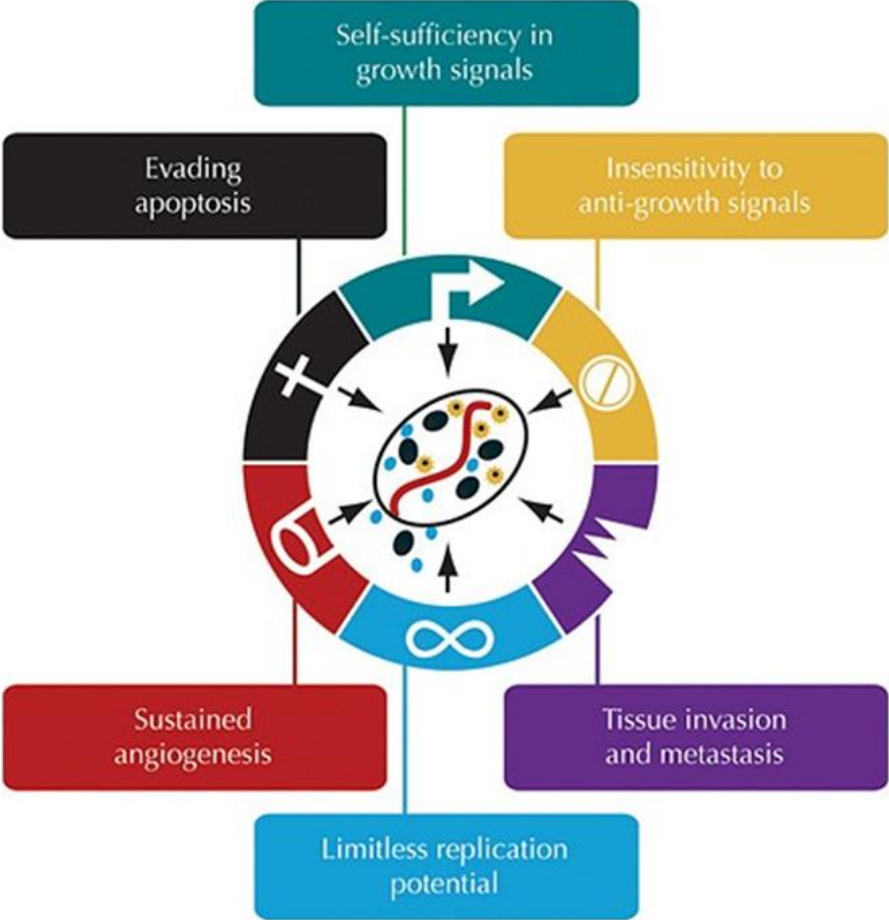
- each cell contains complete genetic information
- The cells are programmed to serve whole organism



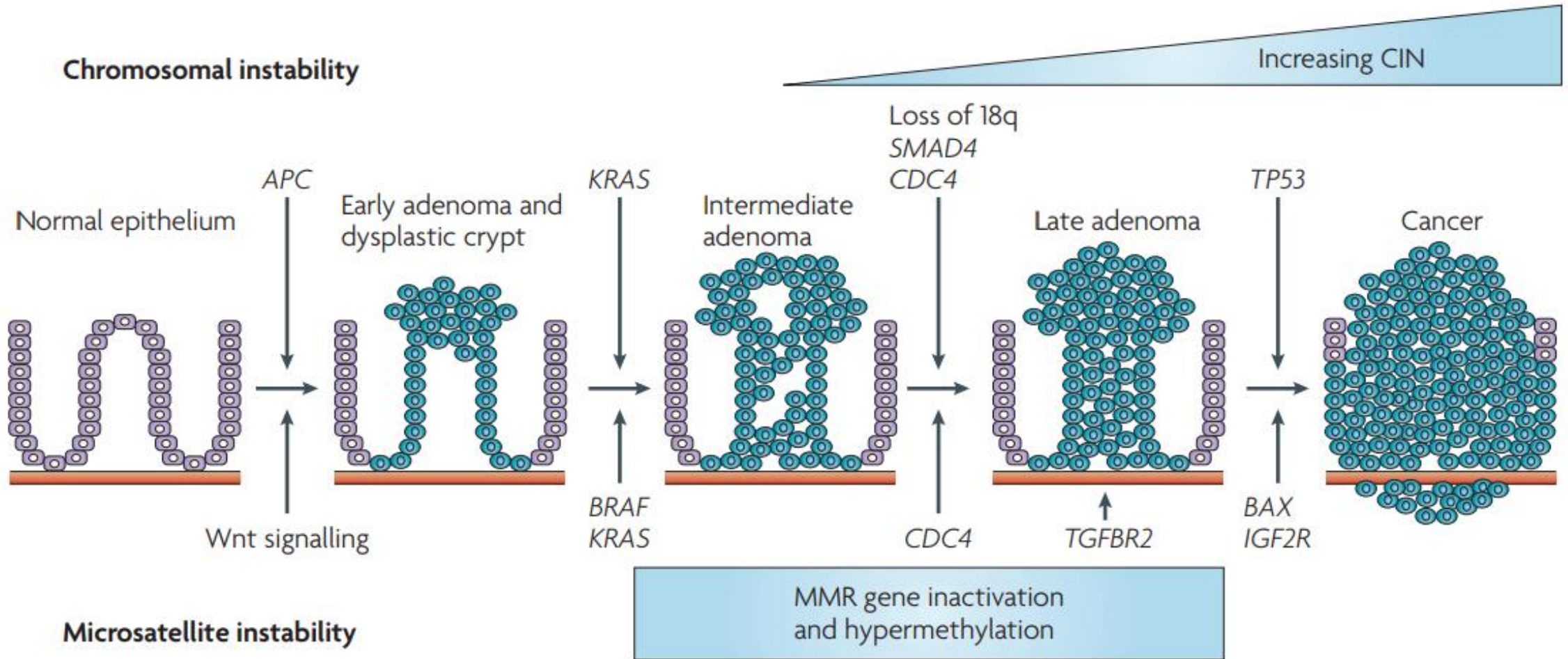
Every cell in the body contains complete genetic information

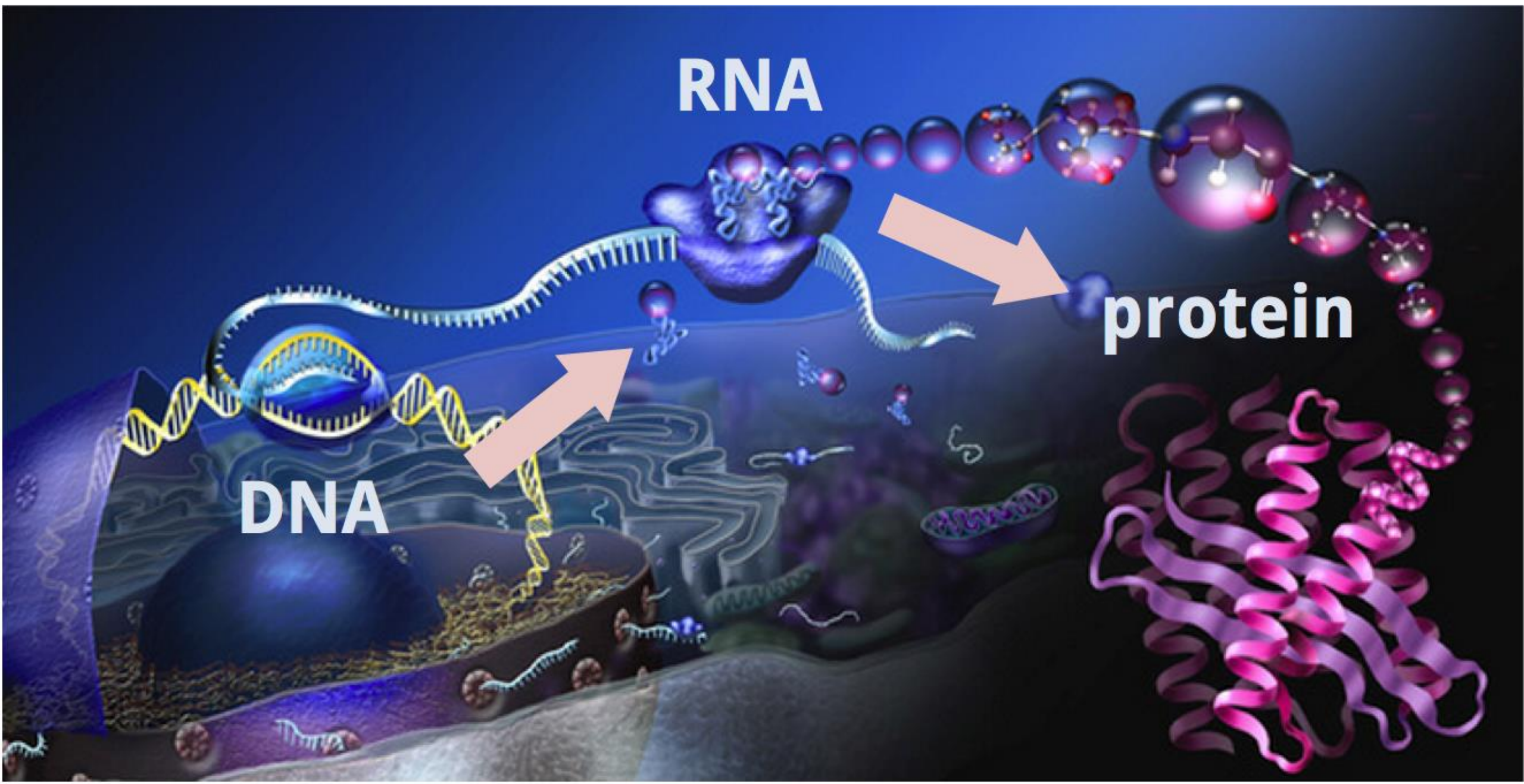


Hallmarks of cancer



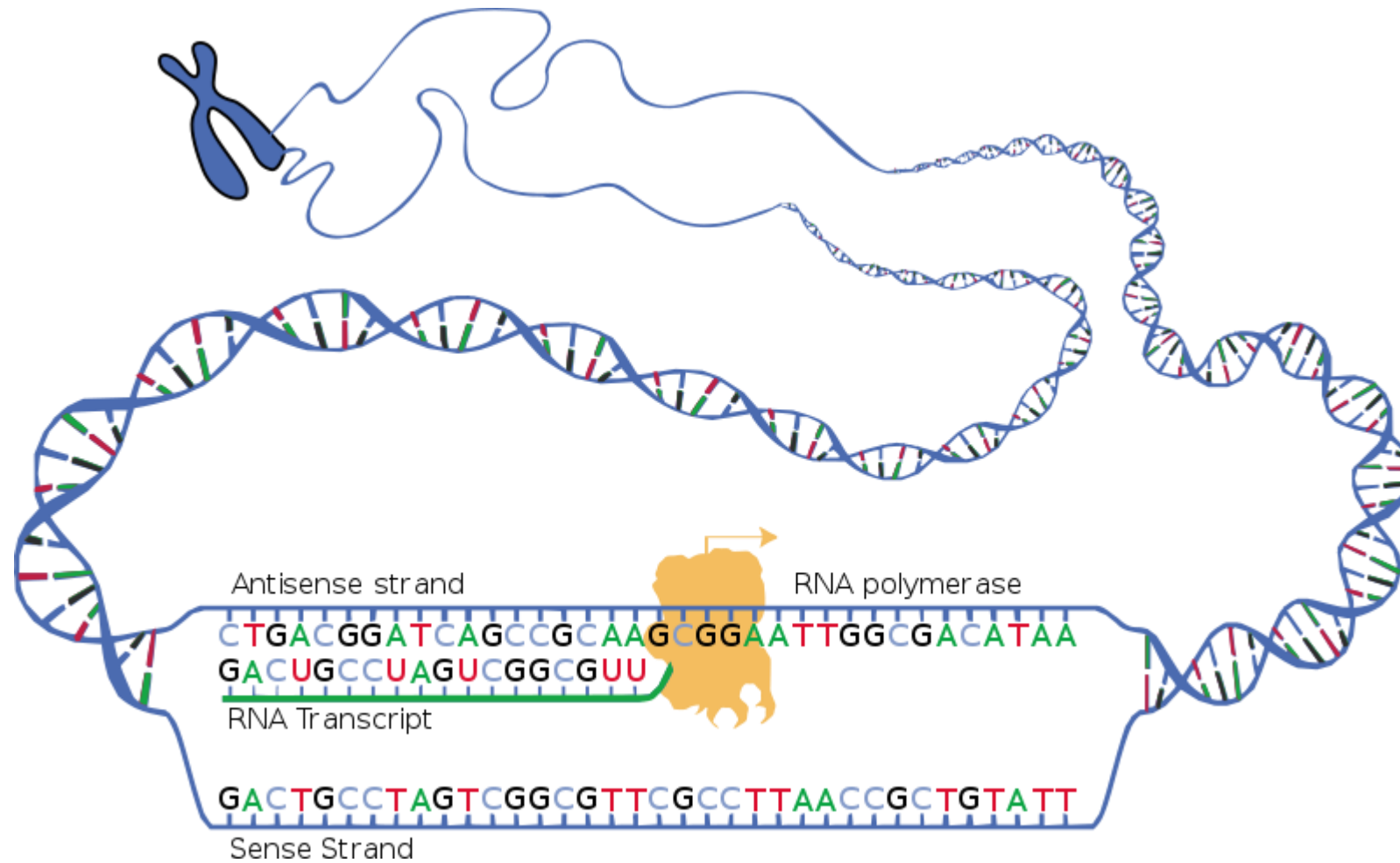
Cancerogenesis is an evolutionary process driven by genetic changes





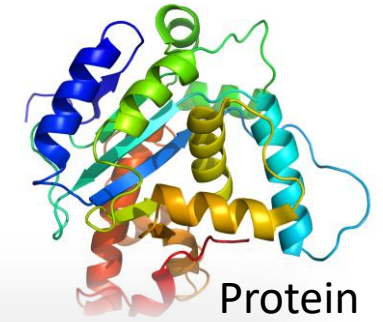
Transkripce

Transkripce je přepis genetické informace z DNA do molekuly RNA.

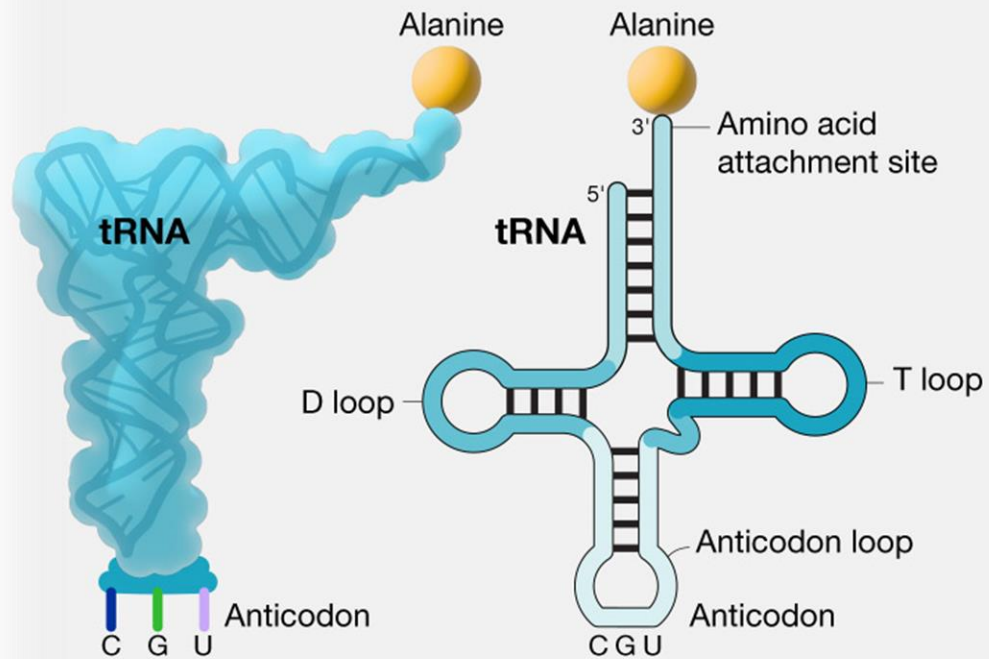


Translation

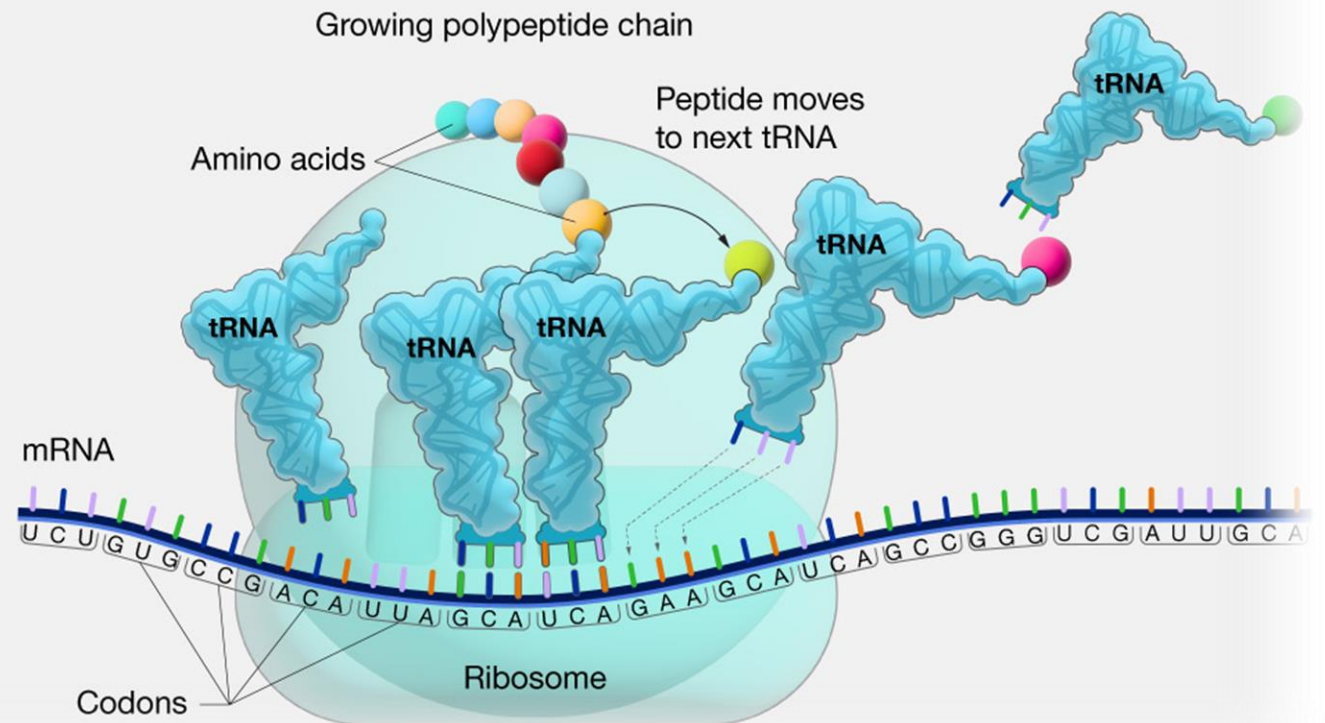
- How the protein is made
- The process in which proteins are synthesized on the ribosome according to mRNA
- Amino acids are ordered based on the genetic code



Common ways of depicting transfer RNA (tRNA)

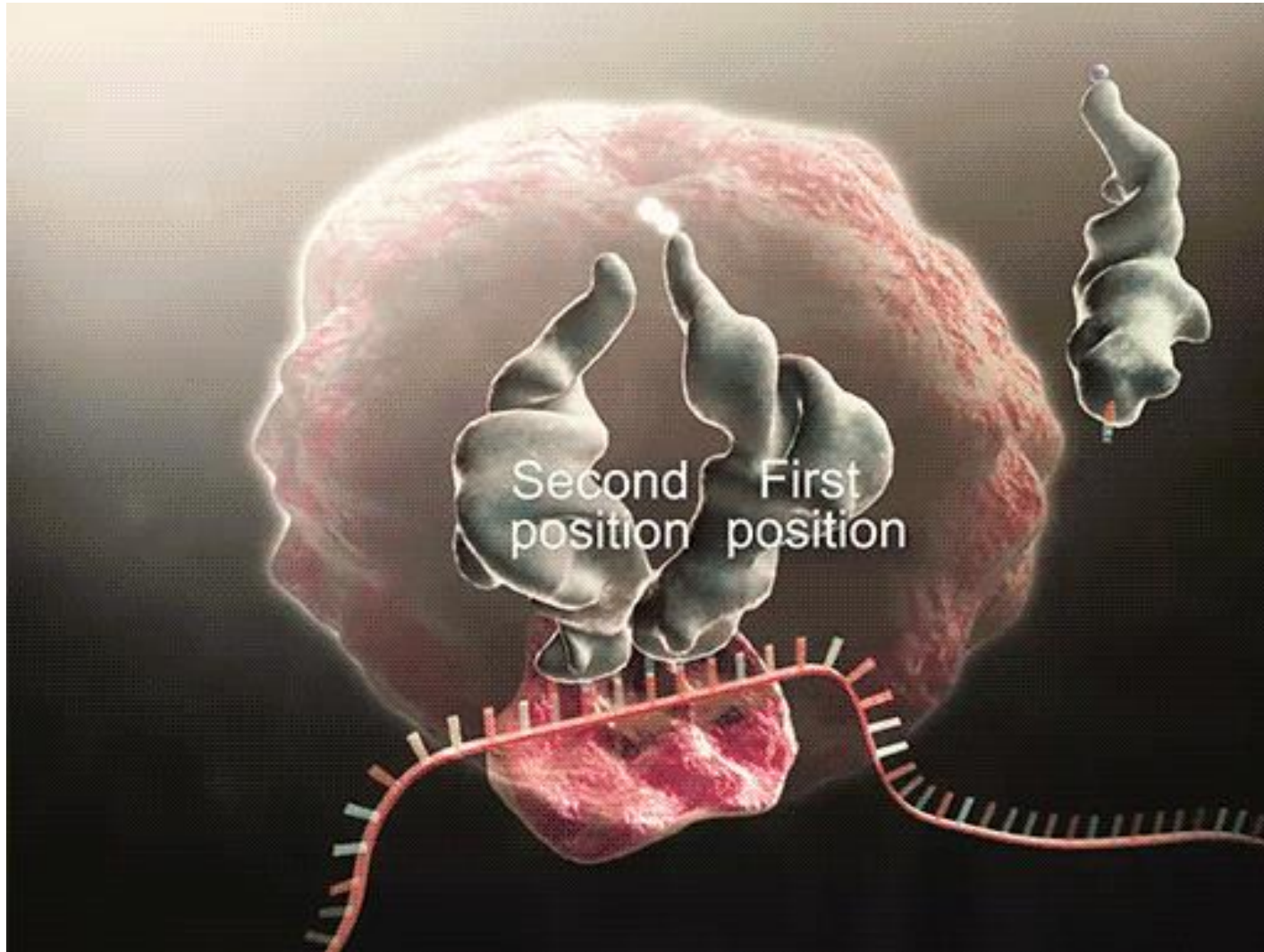


During translation



Translation

- The process in which proteins are synthesized on the ribosome according to mRNA
- Amino acids are ordered based on the genetic code

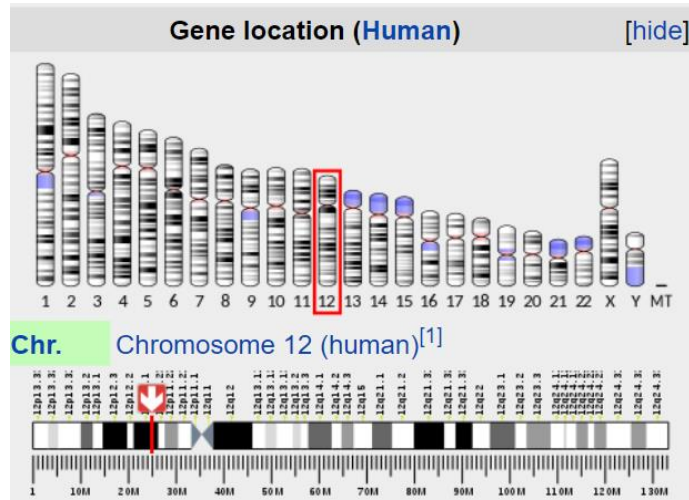


		second base in codon				
		T	C	A	G	
T	first base in codon	TTT Phe	TCT Ser	TAT Tyr	TGT Cys	T
		TTC Phe	TCC Ser	TAC Tyr	TGC Cys	C
		TTA Leu	TCA Ser	TAA stop	TGA stop	A
		TTG Leu	TCG Ser	TAG stop	TGG Trp	G
C	first base in codon	CTT Leu	CCT Pro	CAT His	CGT Arg	T
		CTC Leu	CCC Pro	CAC His	CGC Arg	C
		CTA Leu	CCA Pro	CAA Gln	CGA Arg	A
		CTG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	first base in codon	ATT Ile	ACT Thr	AAT Asn	AGT Ser	T
		ATC Ile	ACC Thr	AAC Asn	AGC Ser	C
		ATA Ile	ACA Thr	AAA Lys	AGA Arg	A
		ATG Met	ACG Thr	AAG Lys	AGG Arg	G
G	first base in codon	GTT Val	GCT Ala	GAT Asp	GGT Gly	T
		GTC Val	GCC Ala	GAC Asp	GGC Gly	C
		GTA Val	GCA Ala	GAA Glu	GGA Gly	A
		GTG Val	GCG Ala	GAG Glu	GGG Gly	G
						third base in codon

Genetic code

- 64 codon combination
- 20 amino acids

An example of how a change in the DNA sequence affects the function of a protein in tumors. How we can use this change for targeted treatment of tumors.



TGT

		second base in codon					
		T	C	A	G		
first base in codon	T	TTT Phe	TCT Ser	TAT Tyr	TGT Cys	T	C
	TTC Phe	TCC Ser	TAC Tyr	TGC Cys	A	C	
	TTA Leu	TCA Ser	TAA stop	TGA stop	A	G	
	TTG Leu	TCG Ser	TAG stop	TGG Trp	G	C	
C	CTT Leu	CCT Pro	CAT His	CGT Arg	T	C	
	CTC Leu	CCC Pro	CAC His	CGC Arg	C	C	
	CTA Leu	CCA Pro	CAA Gln	CGA Arg	A	C	
	CTG Leu	CCG Pro	CAG Gln	CGG Arg	G	C	
A	ATT Ile	ACT Thr	AAT Asn	AGT Ser	T	C	
	ATC Ile	ACC Thr	AAC Asn	AGC Ser	C	C	
	ATA Ile	ACA Thr	AAA Lys	AGA Arg	A	C	
	ATG Met	ACG Thr	AAG Lys	AGG Arg	G	C	
G	GTT Val	GCT Ala	GAT Asp	GGT Gly	T	C	
	GTC Val	GCC Ala	GAC Asp	GGC Gly	C	C	
	GTA Val	GCA Ala	GAA Glu	GGA Gly	A	C	
	GTG Val	GCG Ala	GAG Glu	GGG Gly	G	C	

Mutation description:

KRAS
c.34G>T
p.G12C

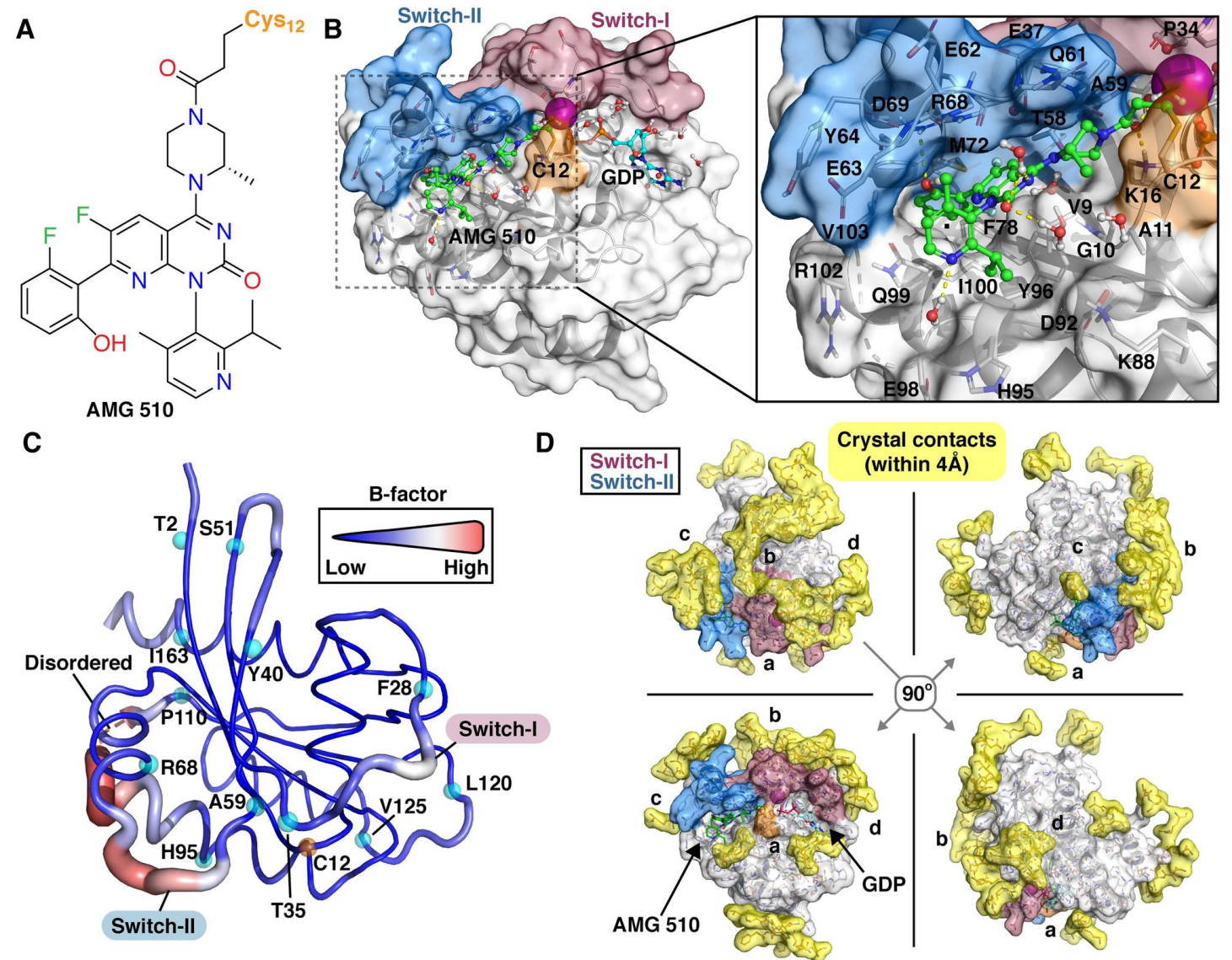
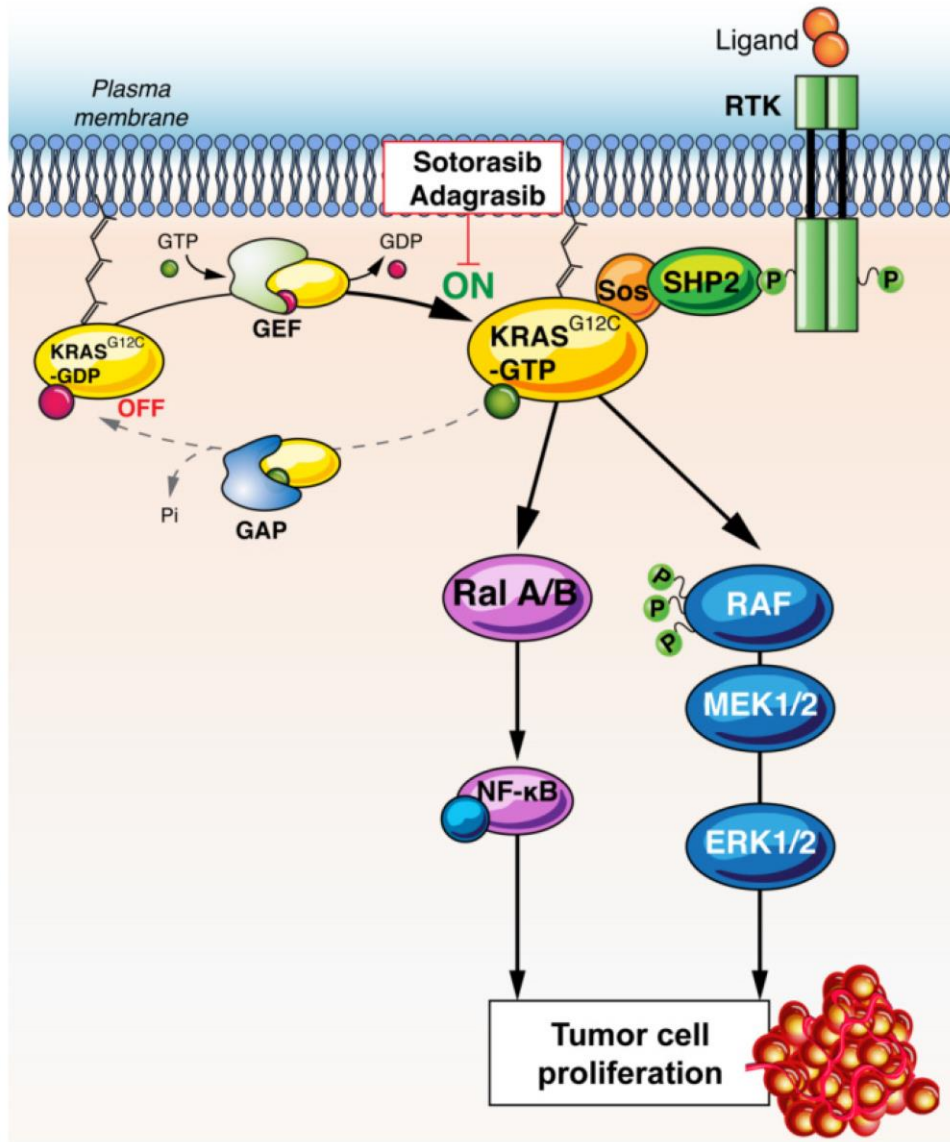
ATGACTGAATATAA ACTTGTGGT AGTTGGAGCTGGTGGCGTAGGCAAGAGT
GCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAA
CAATAGAGGATTCTACAGGAAGCAAGTAGTAATTGATGGAGAAACCTGTCT
CTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTACAGTGCAATGAGGGA
CCAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGTATTTGCCATAAATAAT
ACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATTAAGAGTTAA
GGACTCTGAAGATGTACCTATGGTCCTAGTAGGAAATAAATGTGATTTGCCT
TCTAGAACAGTAGACACAAAACAGGCTCAGGACTTAGCAAGAAGTTATGGA
ATTCCTTTTATTGAAACATCAGCAAAGACAAGACAGGGTGTGATGATGCCT
TCTATACATTAGTTTCGAGAAATTCGAAAACATAAAGAAAAGATGAGCAAAGA
TGGTAAAAGAAGAAAAGAAGTCAAAGACAAAGTGTGTAATTATGTAA

Translation

MTEYKLVVV**G**AGG**C**VGKSALTI
QLIQNHFVDEYDPTIEDSYRKQ
VWIDGETCLLDILDITAGQEEYS
AMRDQYMRTGEGFLCVFAIN
NTKSFEDIHHYREQIKRVKDSE
DVPMLVGNKCDLPSRTVDTK
QAQDLARSYGIPFIETSAKTRQ
GVDDAFYTLVREIRKHKEKMSK
DGKKKKKKSKTKCVIM

Coding sequence - mRNA

KRAS mutation G12C and targeted treatment



Mutations

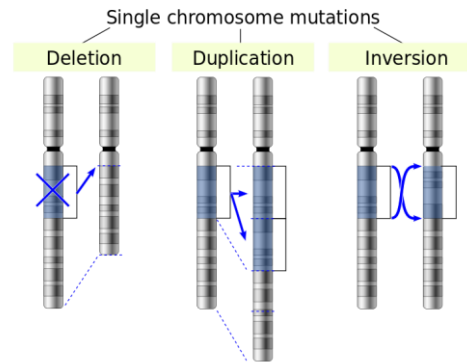
mutation is an alteration in the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA

By effect on structure

Large scale mutations (Chromosomal abnormalities)

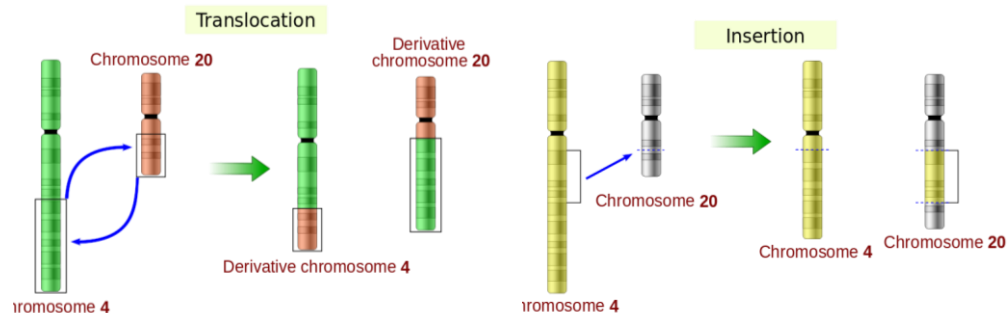
- Deletion
- Duplication, amplification
- Inversion
- Translocation
- Insertion

- Loss of heterogisosity
- Aneuploidity



Small-scale mutations

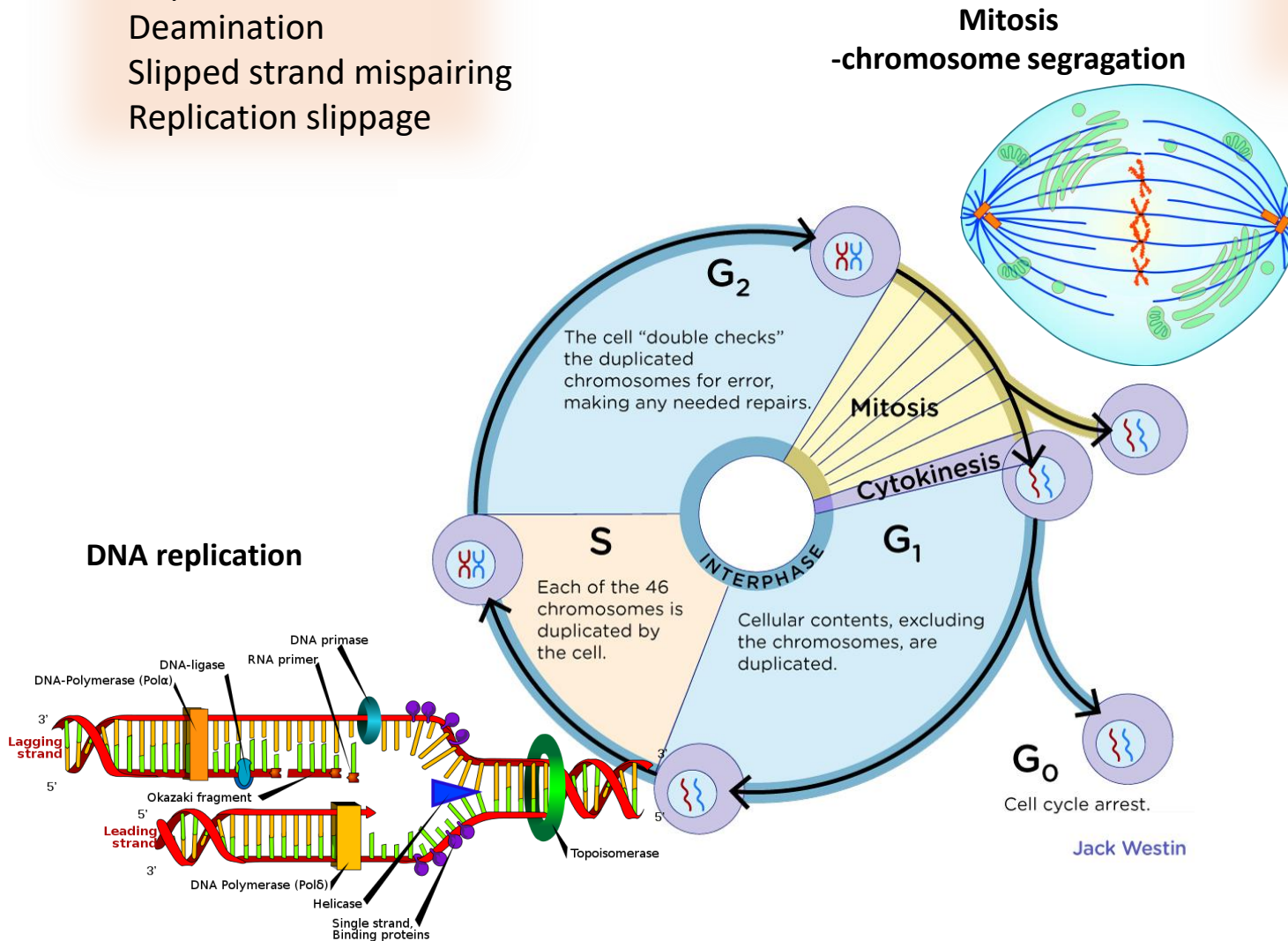
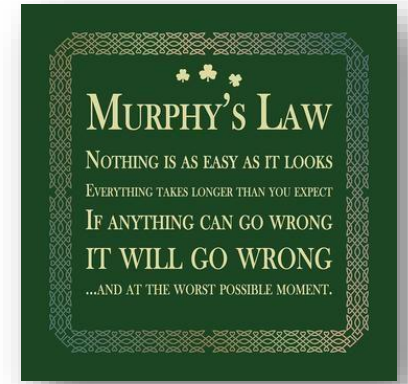
- Insertions
- Deletions
- Substitution mutations / point mutations
 - Missense
 - Nonsense
 - Silent



Spontaneous vs induced mutations

Tautomerism
 Depurination
 Deamination
 Slipped strand mispairing
 Replication slippage

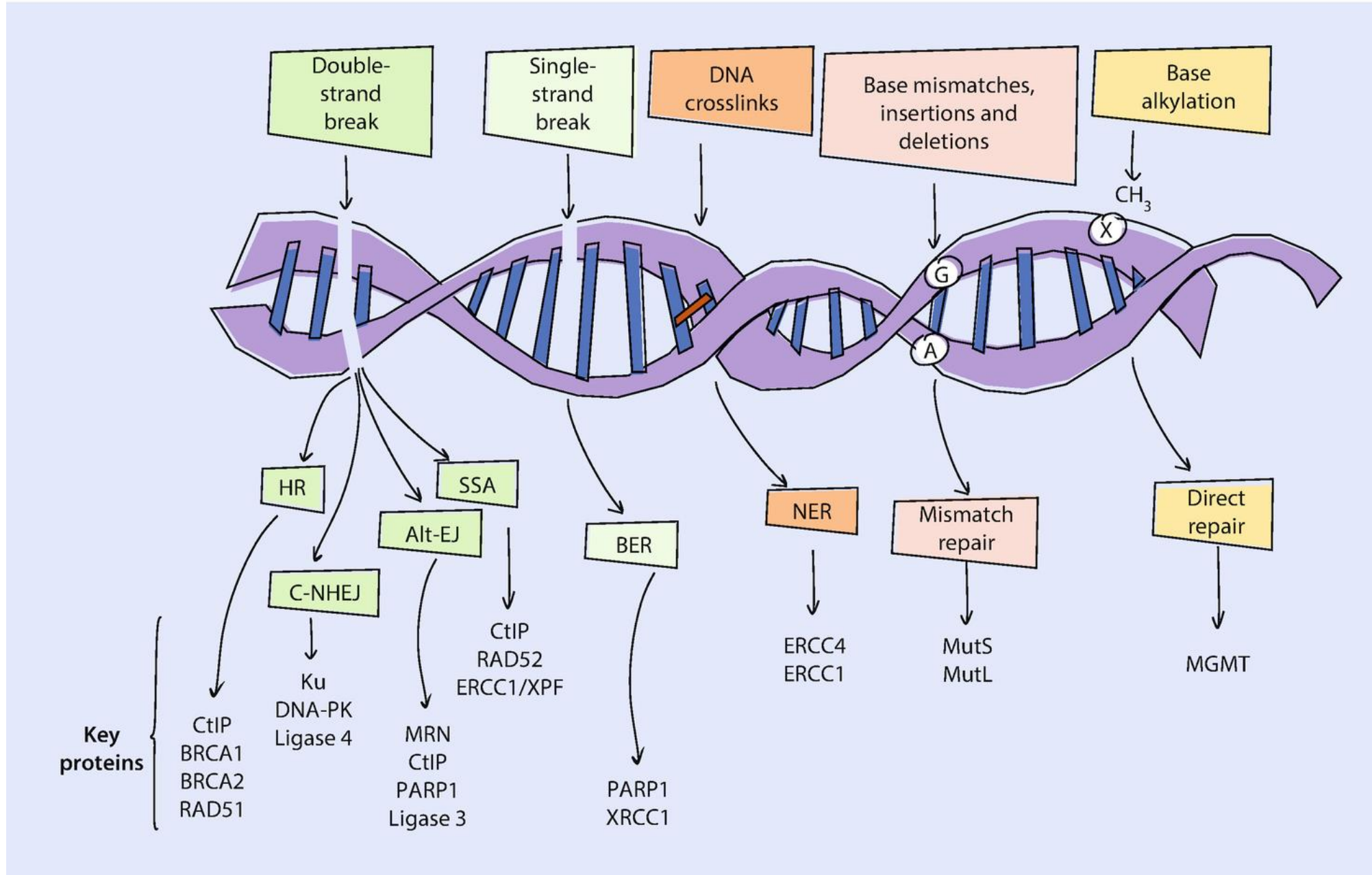
Chromosome abnormality
 Amplification
 Translocation
 Aneuploidy



DNA repair -> genomic instability

- Double strand break repair
 - BRCA1, BRCA2
 - CHK2
 - ATM
- Mismatch repair (MMR)
 - MLH1, MLH2, MLH3, PMS1, and PMS2
- Nucleotide excision repair (NER)
 - Xeroderma pigmentosum (XPC,..)
- DNA polymerase
 - POLD, POLE

Genomic instability and cancer



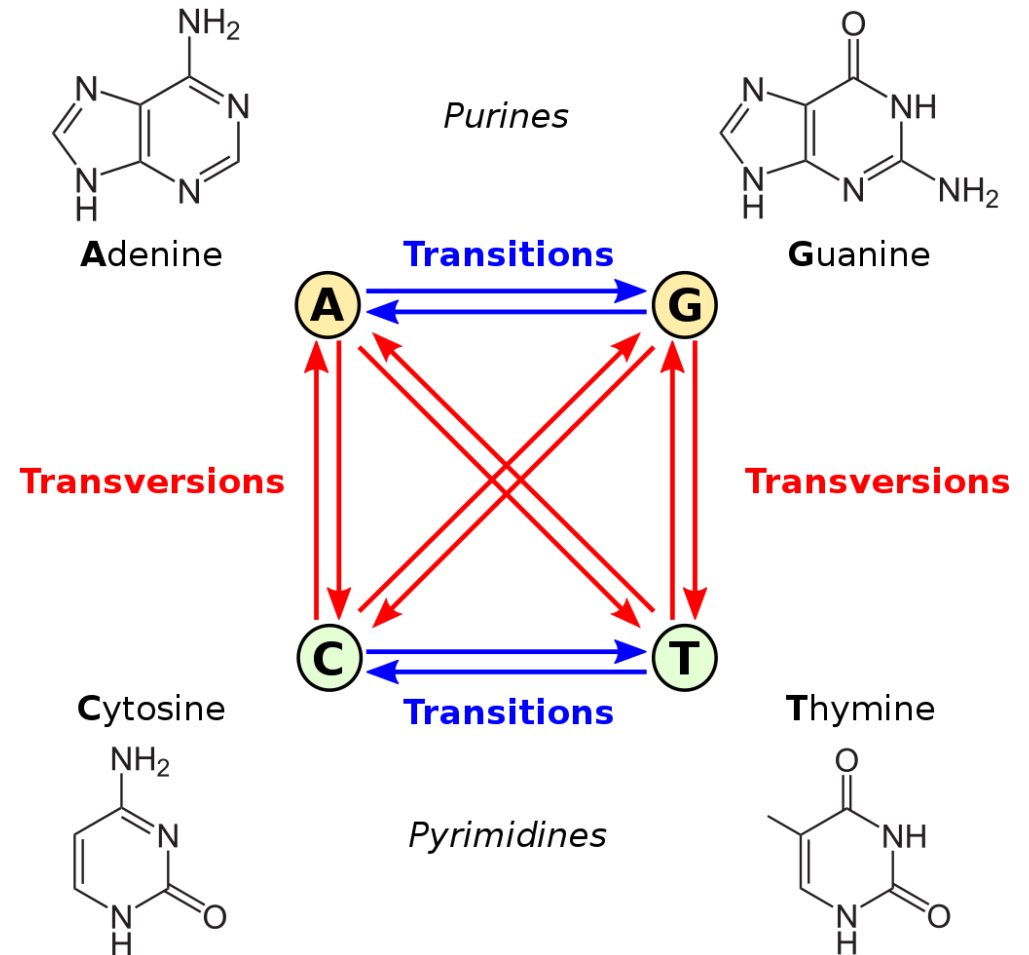
Point mutations

Transition

- mutation that changes a purine nucleotide to another purine (A ↔ G), or a pyrimidine nucleotide to another pyrimidine (C ↔ T).
- Approximately two out of three single nucleotide polymorphisms (SNPs) are transitions.
- Transitions can be caused by oxidative deamination and tautomerization.

Transversion

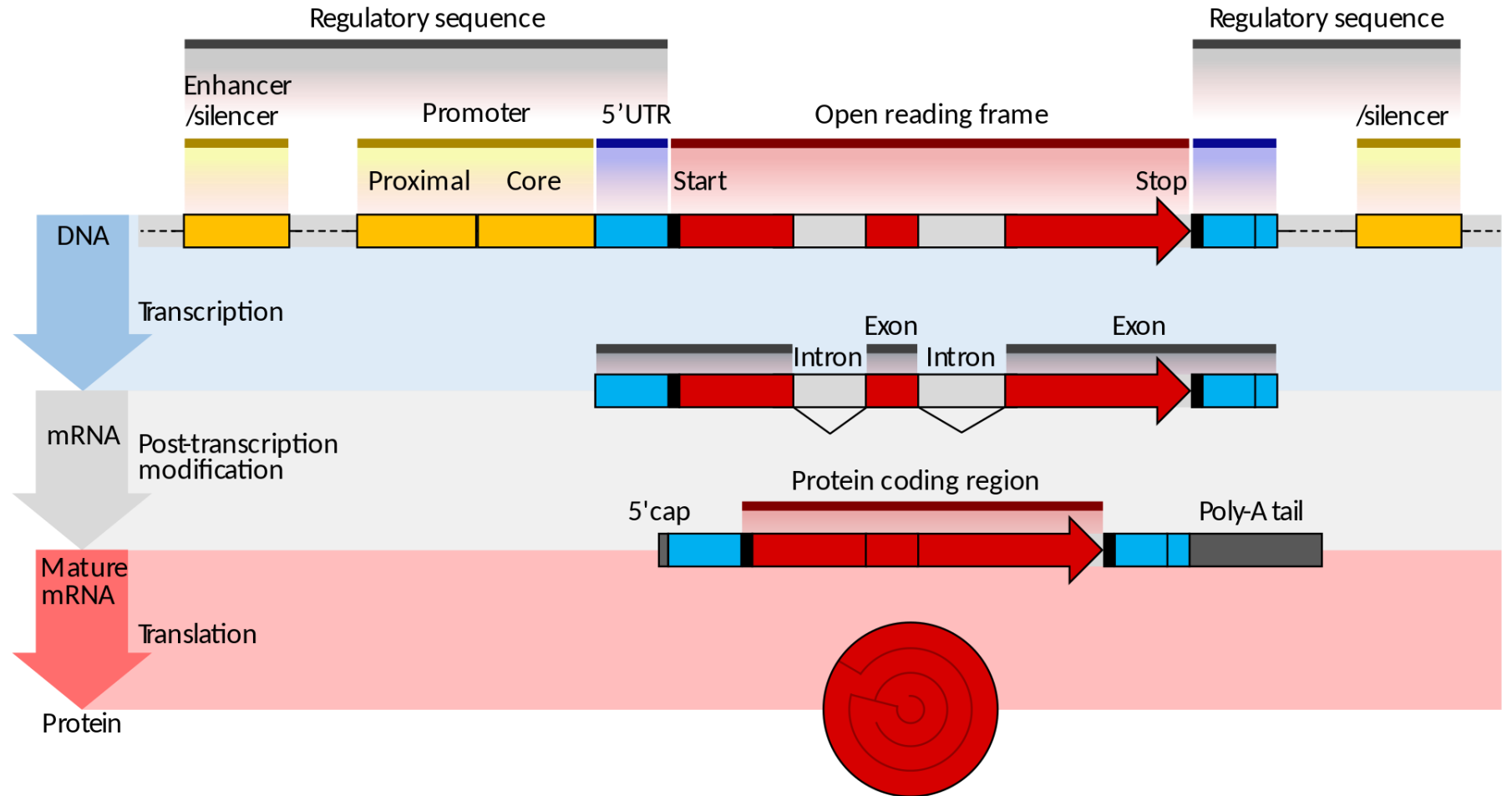
- mutation in DNA in which a single (two ring) purine (A or G) is changed for a (one ring) pyrimidine (T or C), or vice versa.
- A transversion can be spontaneous, or it can be caused by ionizing radiation or alkylating agents.



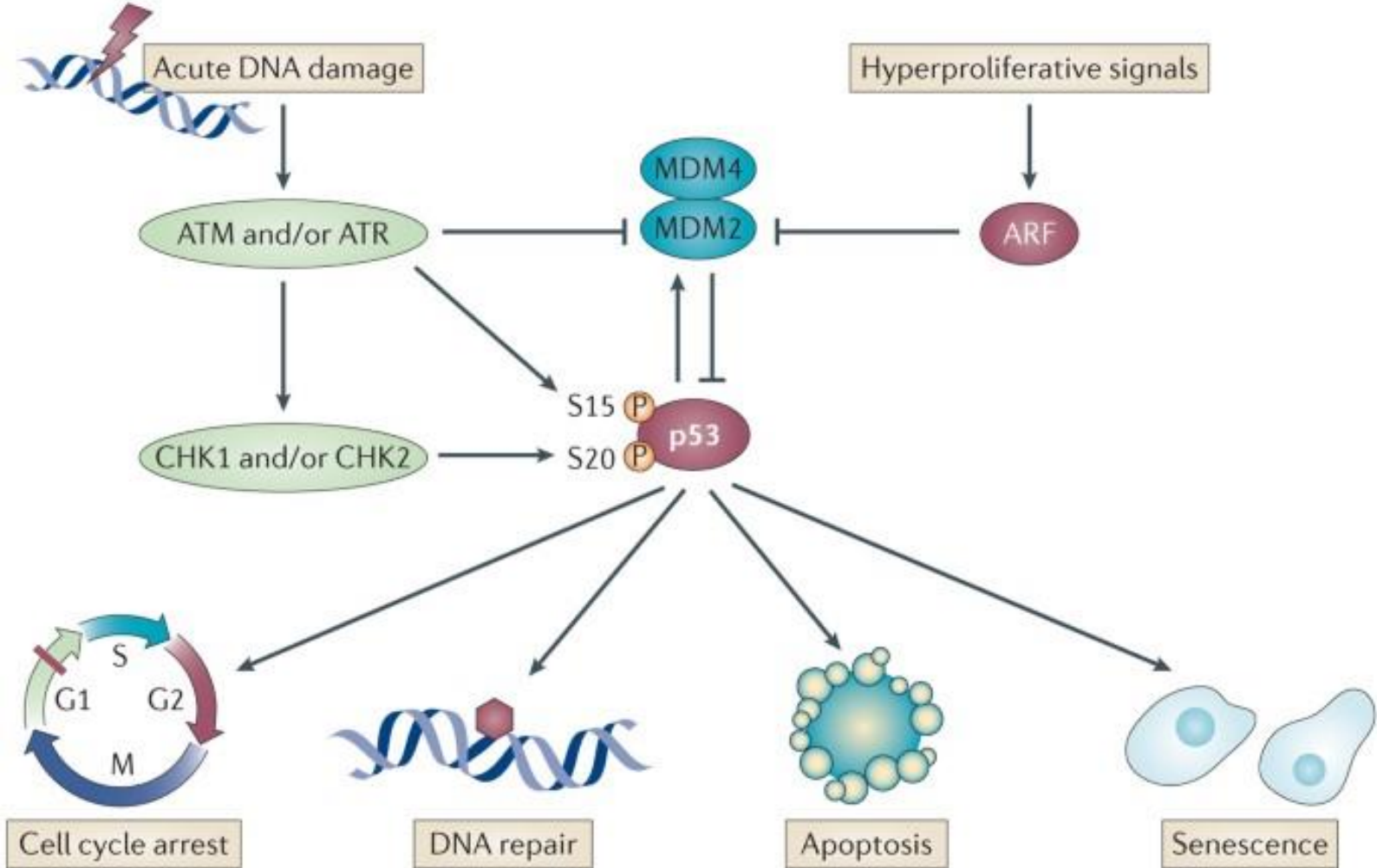
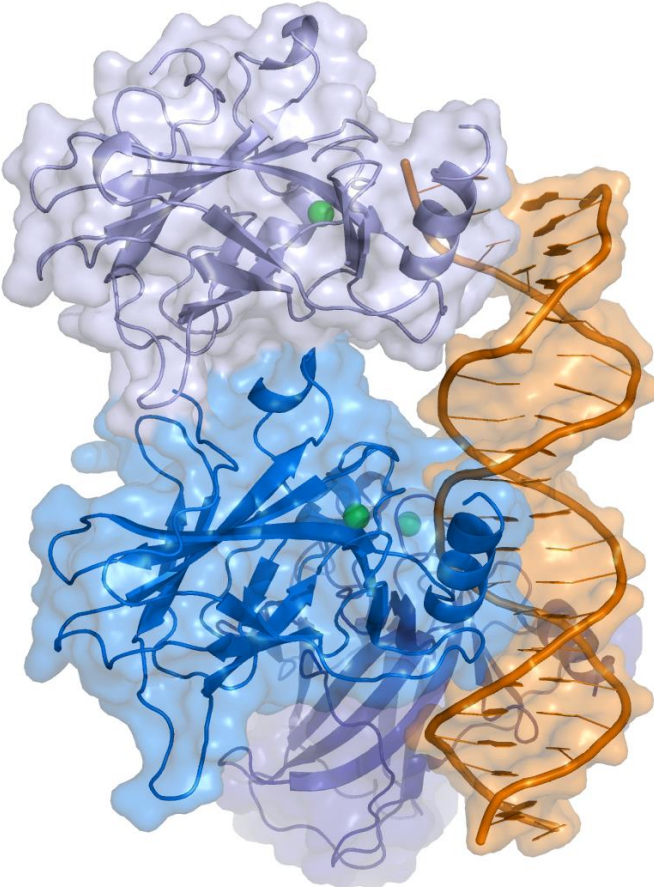
Mutations by impact on protein sequence

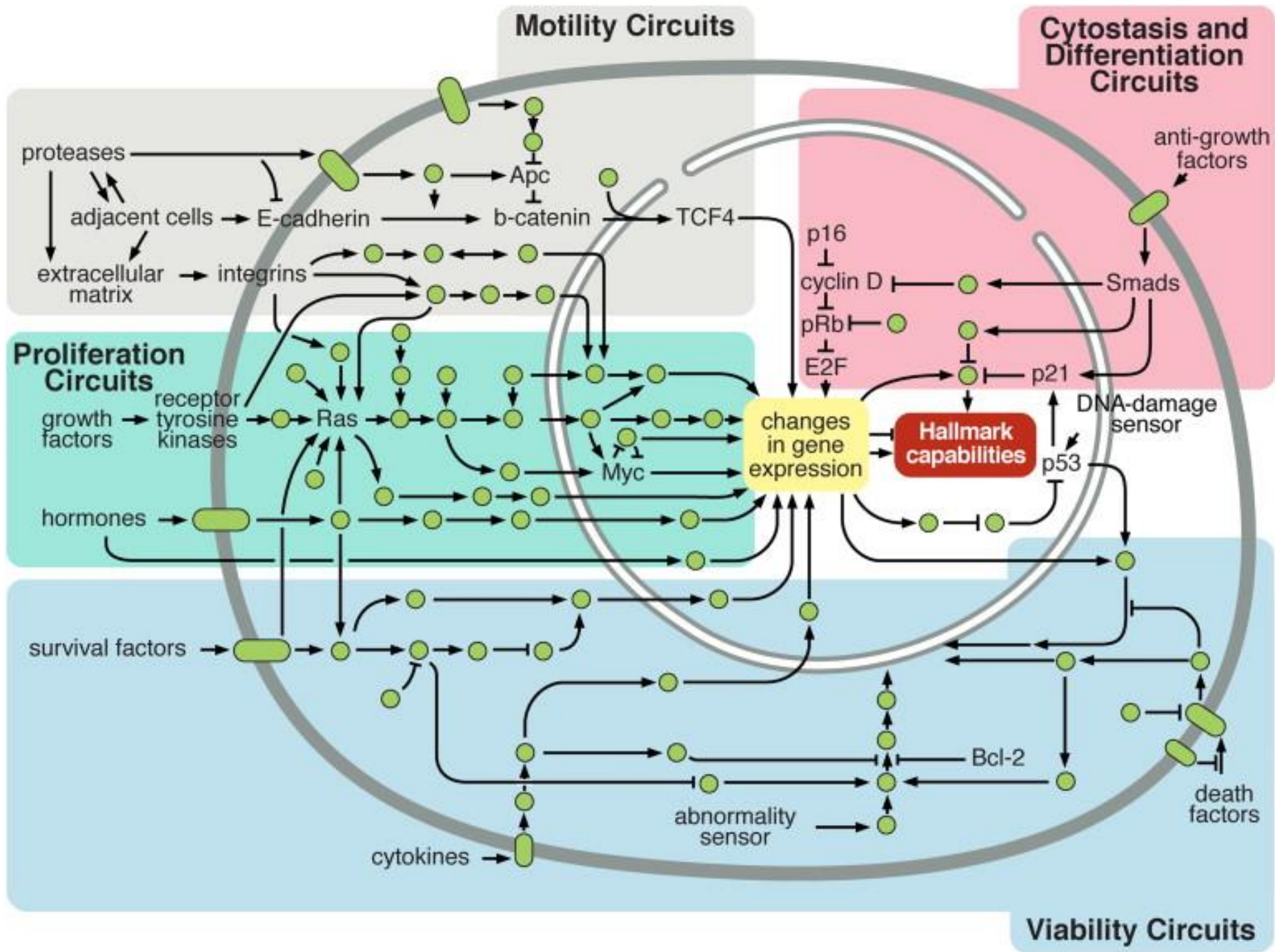
Coding region:

- **Point mutations**
 - Missense
 - Nonsense
 - Silent
- **Frameshift mutations**
 - Insertions
 - Deletions
 - (Indels)

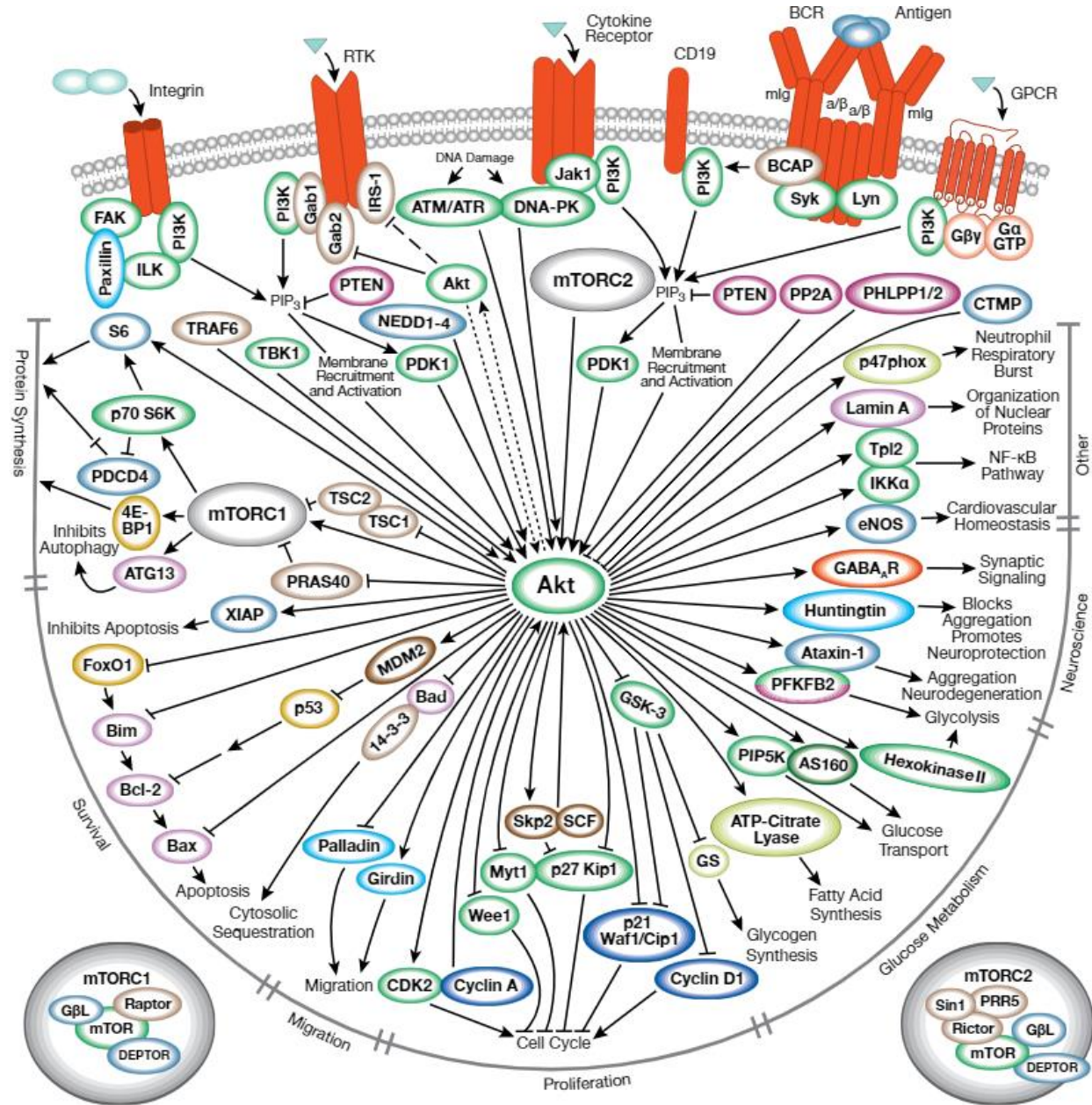


Tumor suppressor p53 - the guardian of genome

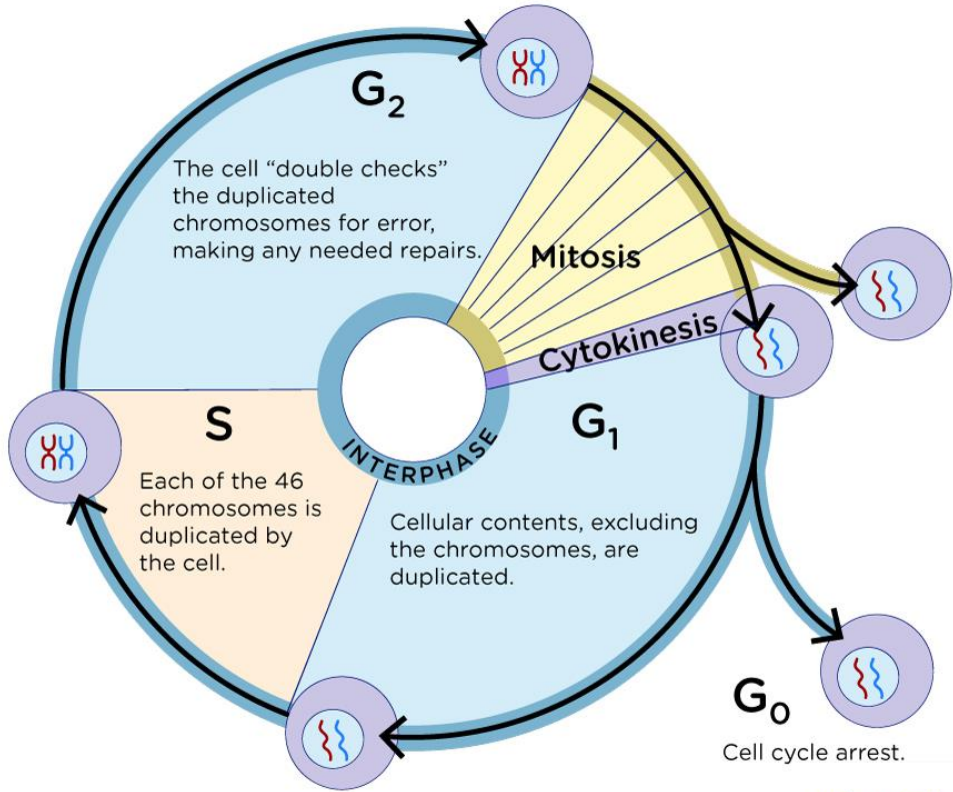




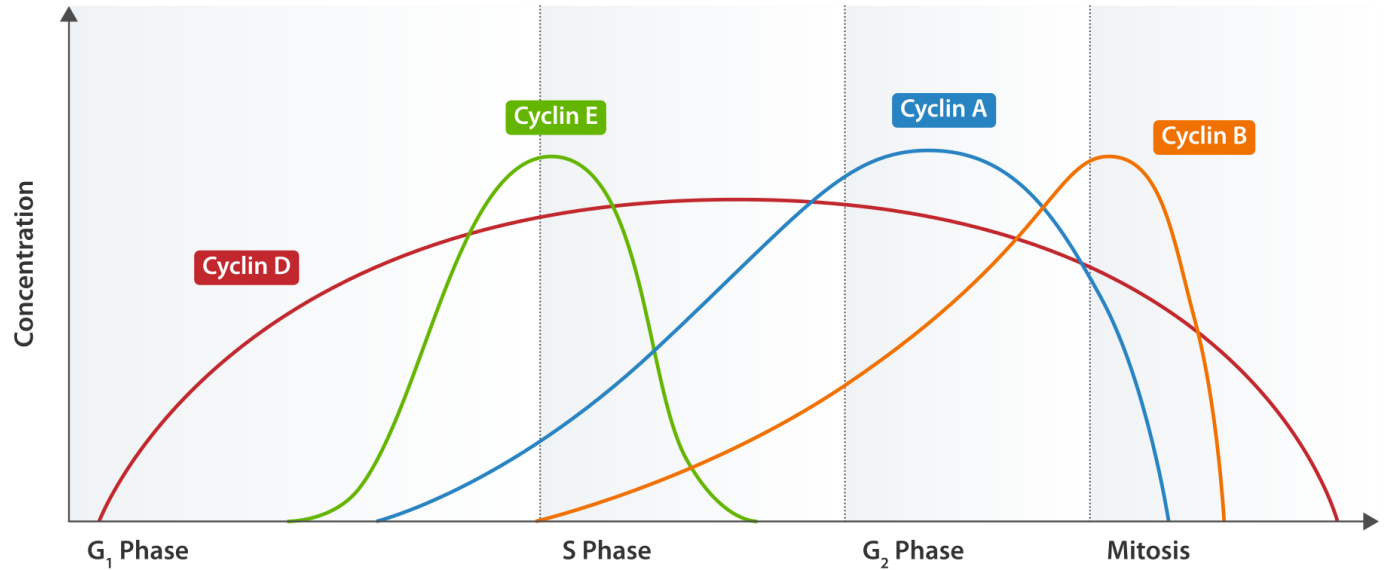
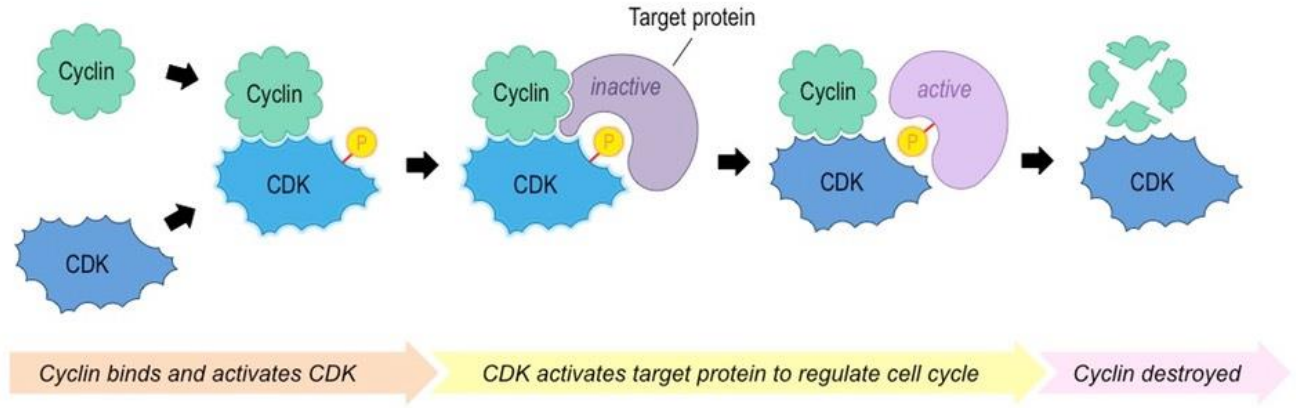
Regulation of growth and protein synthesis



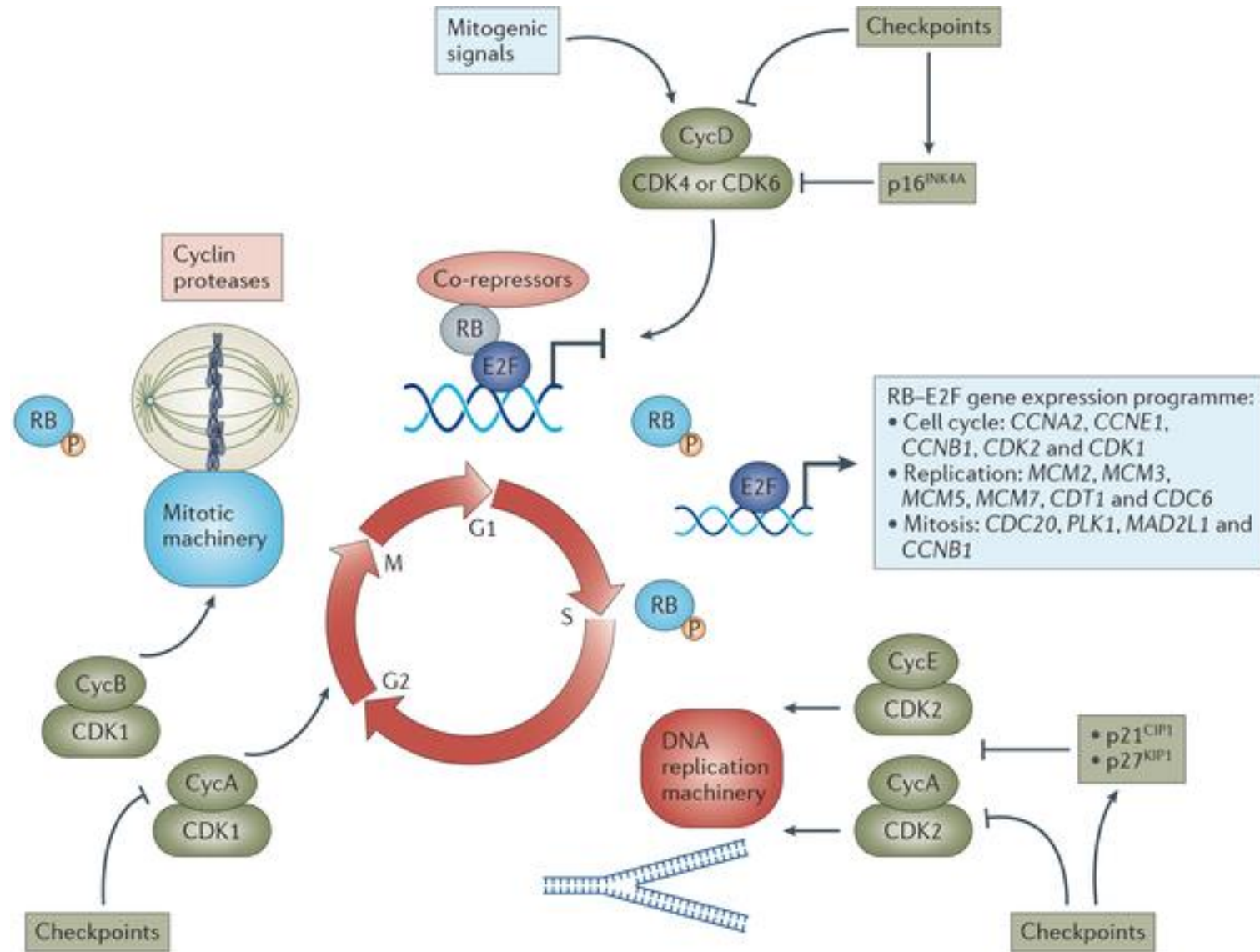
Cell cycle is regulated by Cyclin dependent kinases (CDKs) and cyclins



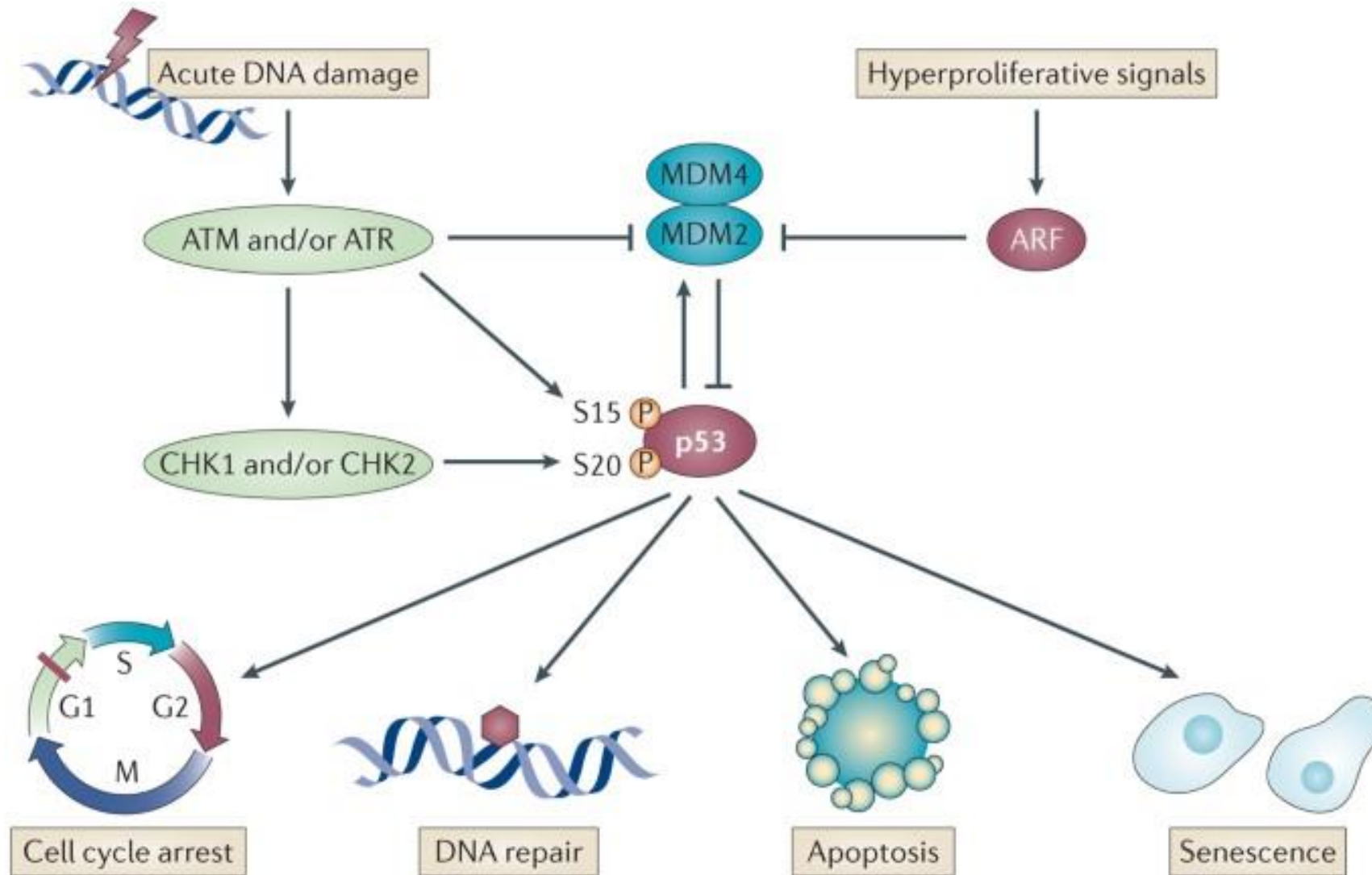
Jack Westin



How CDK/cyclins regulate cell cycle



Tumor suppressor p53 - the guardian of genome



The most frequently altered genes in cancer

Tumour suppressor genes:

- p53 – transcription factor
- RB – pocket protein regulating transcription factors
- P16/INK4a – CDK inhibitor
- PTEN – phosphatase, inhibits AKT signalling

Gatekeepers

Oncogenes:

- Transcription factors / inhibitors of transcription
 - Myc (N-Myc, C-Myc, L-Myc)
 - E2F1
 - Beta-catenin
 - MDM2, MDMX, E6, E7
- Growth factor receptors (receptor tyrosin kinases)
 - HER2/Neu
 - PDGFR
 - EGFR
- Growth factor/ proliferation signalling
 - RAS
 - RAF
 - PI3K
- Cell cycle
 - Cyclins/CDKs

The most frequently altered genes in cancer

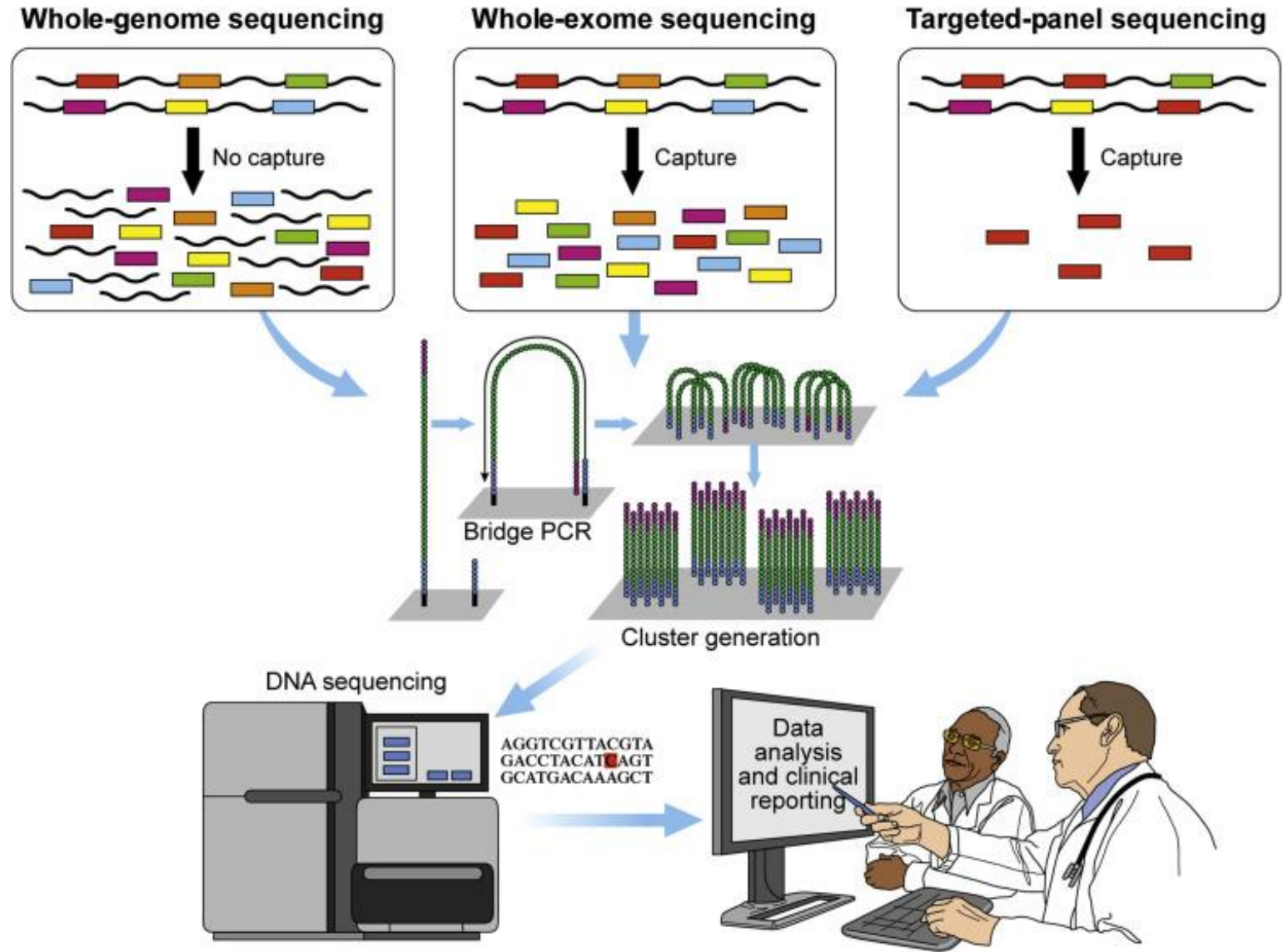
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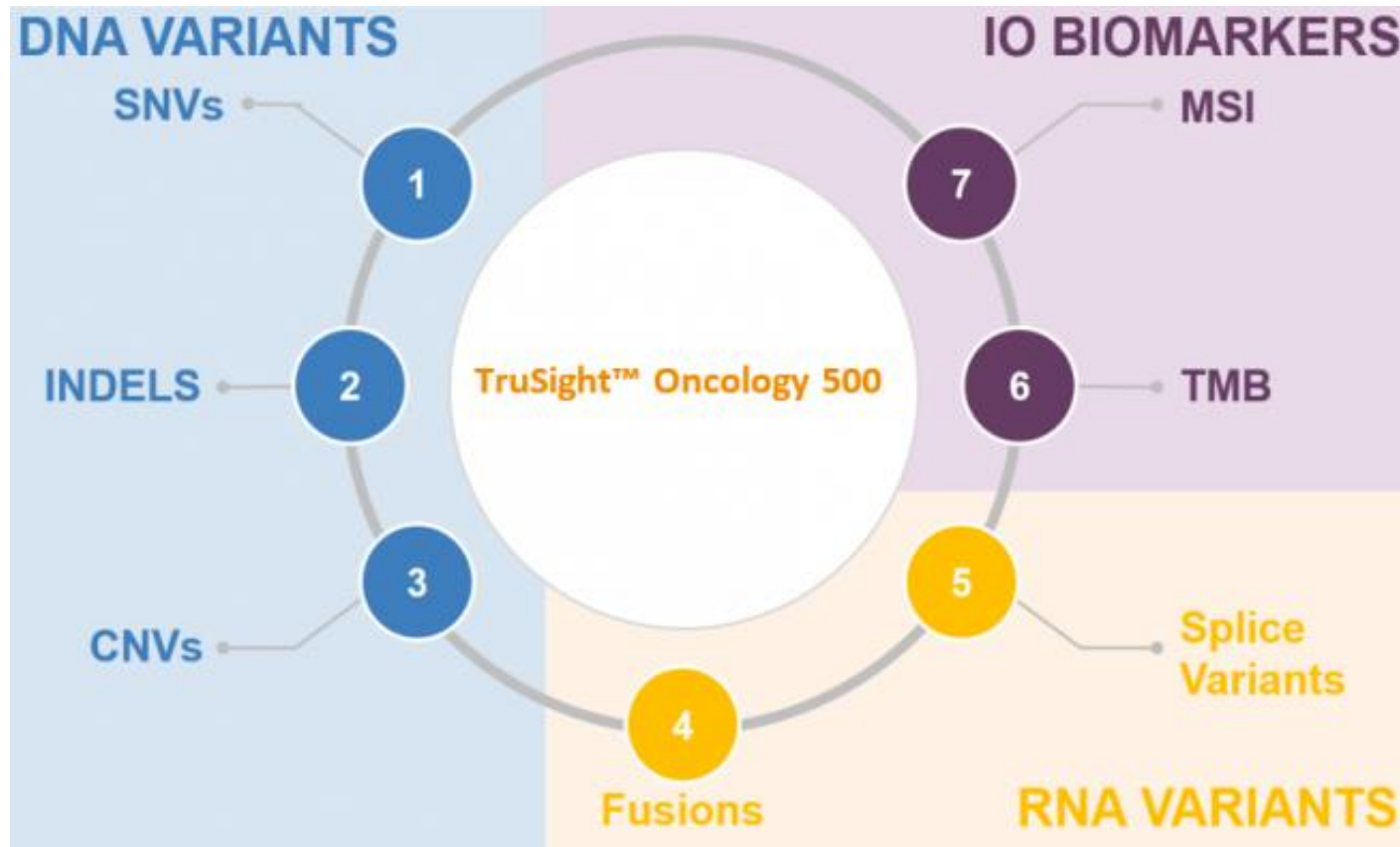
Caretakers




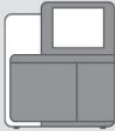


NGS

Next Generation Sequencing



TruSight™ Oncology 500



Illumina					PierianDx
FFPE specimens	Sample	Library prep and enrichment	Sequence	Variant calling	Interpretation and reporting
					
Supports multiple tissue types	Commercial DNA/RNA* extraction kits	TruSight Oncology UMI kit DNA Probes (523 genes) RNA* Probes (55 genes) Automatable workflow	NextSeq® System	TruSight Oncology 500 Local App	Powered by PierianDx Clinical Genomics Workspace

Thank you for your attention