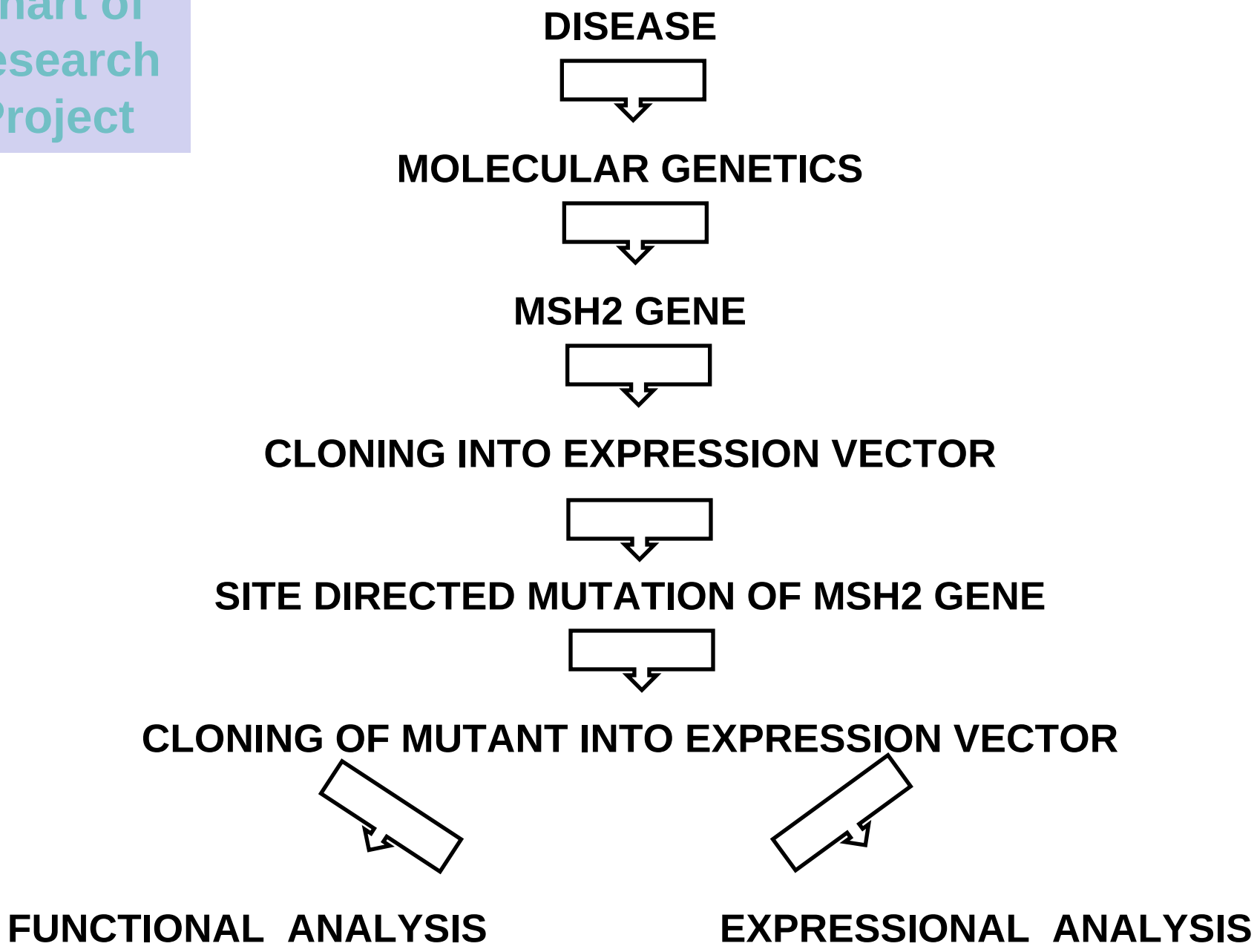
Chart of Research Project



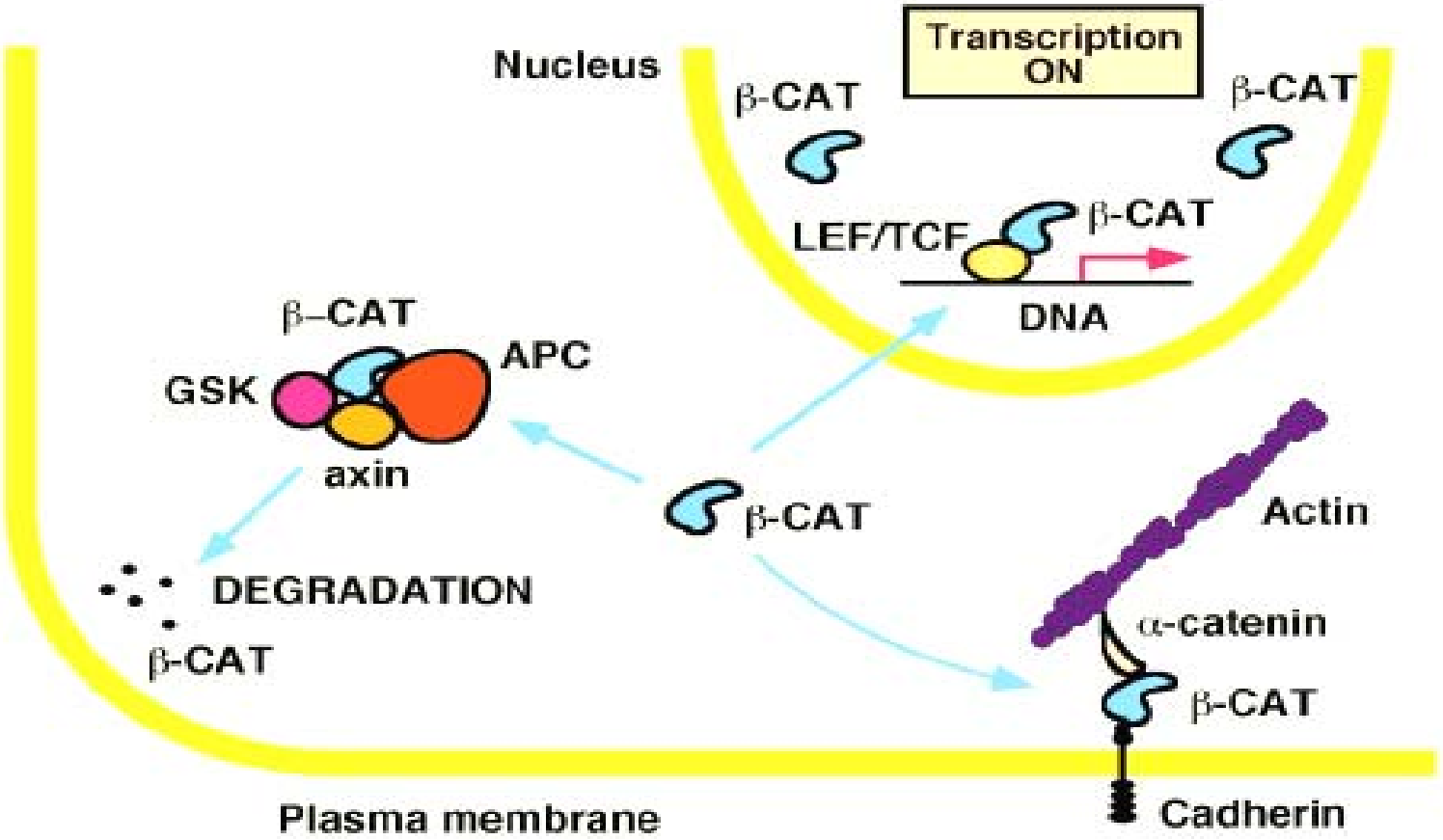
THE BIG PICTURE

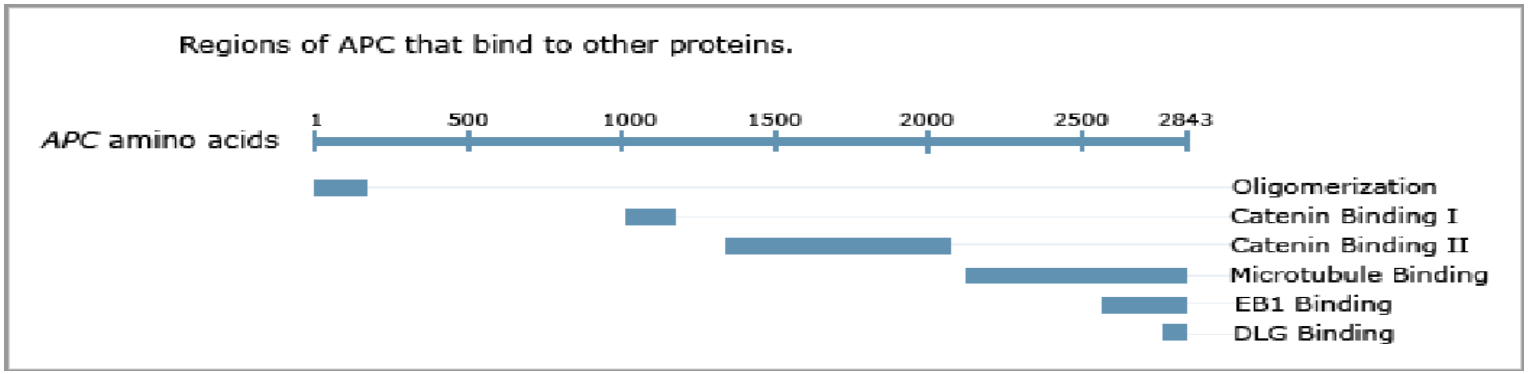
THEORY CONCEPTS BIOINFORMATICS

MSH2 IN MISMATCH REPAIR→
MUTATION→DISRUPTION OF CELL CYCLE →
COLON CANCER

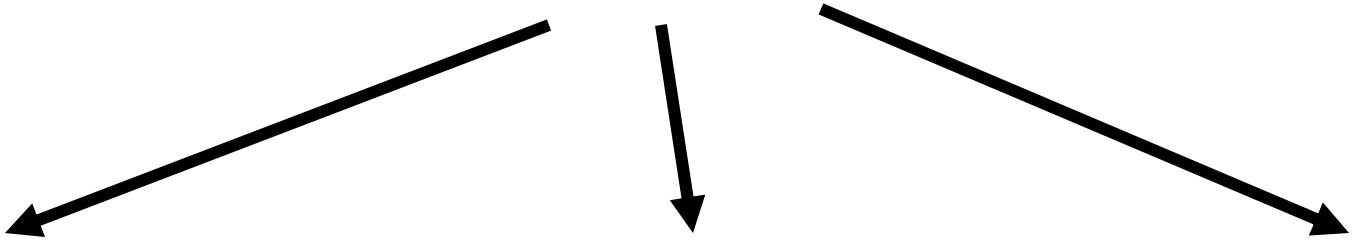
PLASMID PREPARATION
PRIMER DESIGN
PCR AMPLIFICATION
RESTRICTION ANALYSIS
DNA GEL ELECTROPHORESIS
YEAST TRANSFORMATION
TRANSFORMANTS IN COMPLEMENTATION ASSAY
TRANSFORMANT PROTEIN EXTRACTION
PROTEIN GEL ELECTROPHORESIS

Signaling by β-catenin





Applying The Cloning Strategies



Yeast Transformation (Expression analysis)

Amplify plasmids in yeast strains by PCR

Analyze PCR products by restriction fragmentation and agarose gel electrophoresis

Complementation Assay (Functional Analysis)

Grow and select yeast strains in selective medium

Grow yeast strains in fluoroorotic acid

Visually Assess survival

MSH2 Protein Analysis (Protein Fractionation)

Grow and select yeast strains in selective medium

Isolate yeast protein

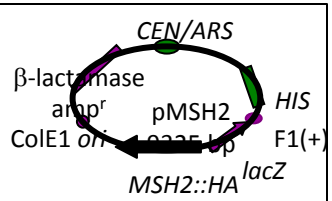
Identify MSH2 in yeast proteins by PAGE/Western Blot –OR- Immunoprecipitation

Molecular Biology

Genomics

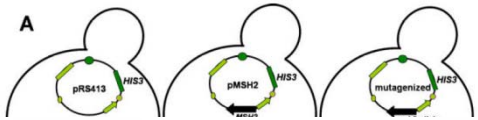
PCR and

Plasmid Constructs



Expected Outcomes From Project Outlined

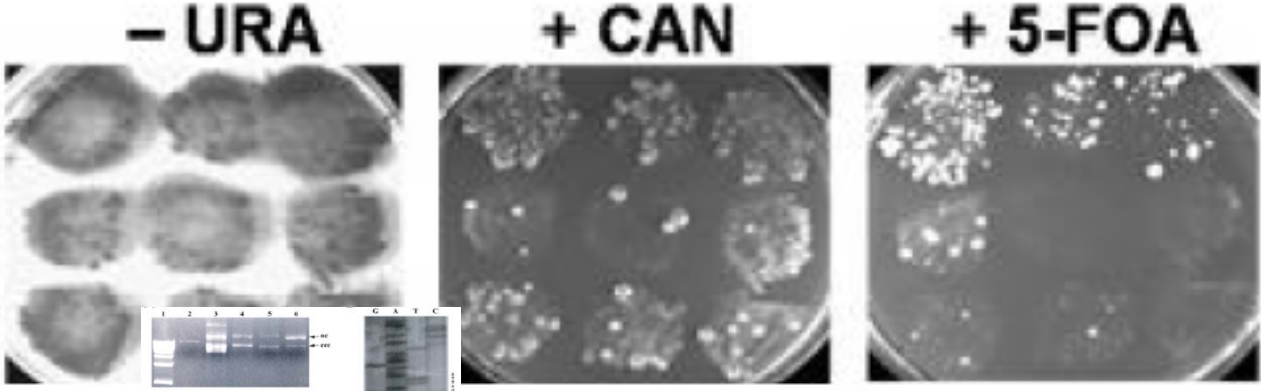
MSH2 Gene

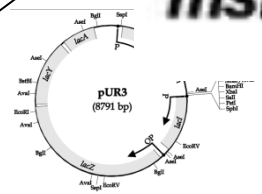


msh2Δ

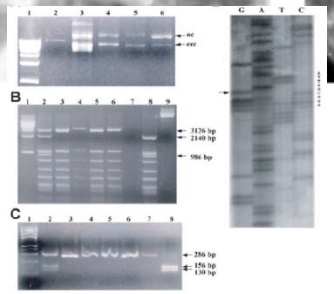
MSH2

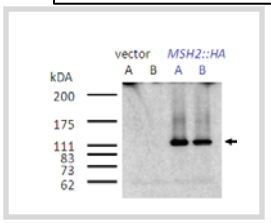
msh2-M707I

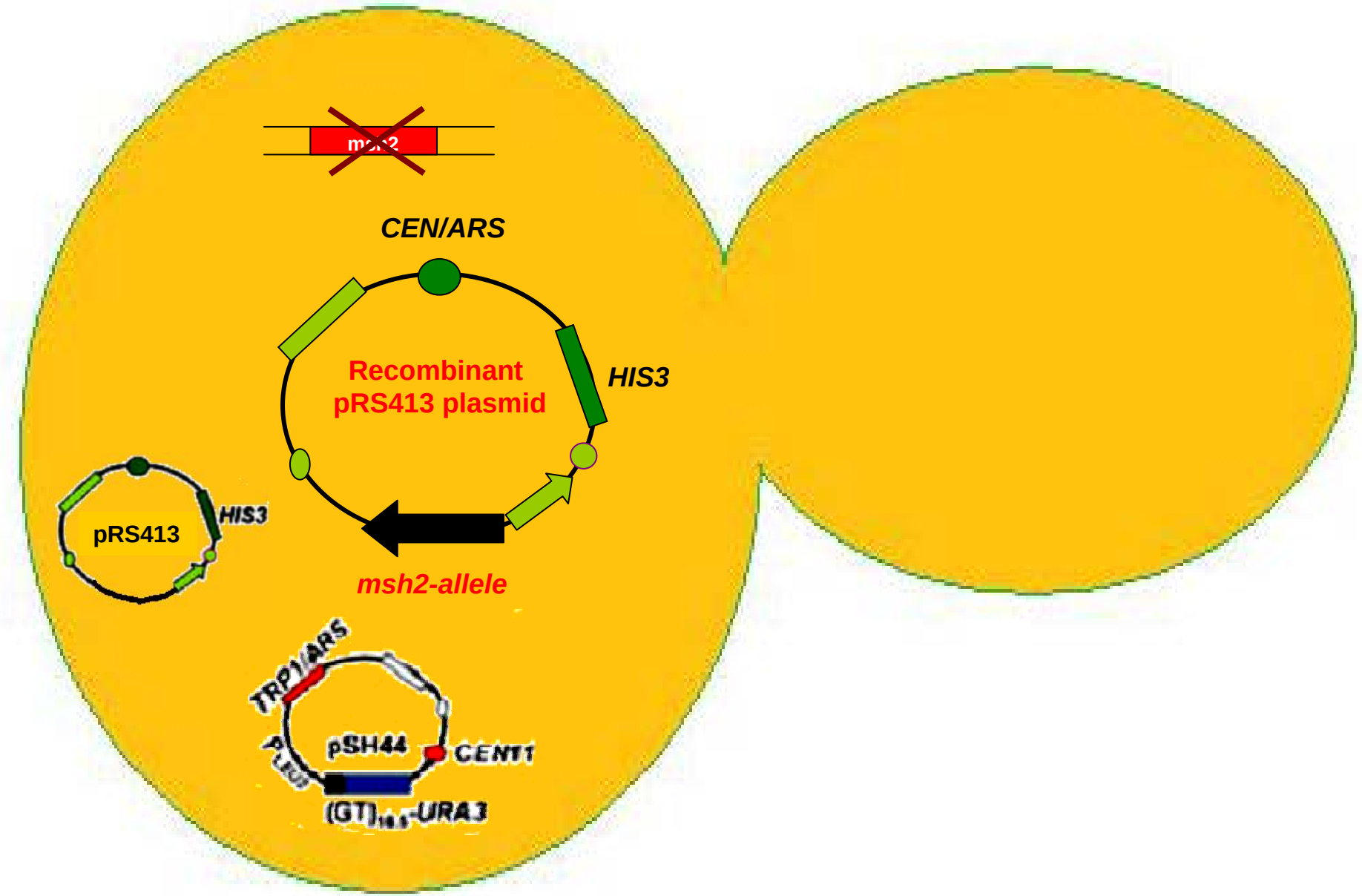




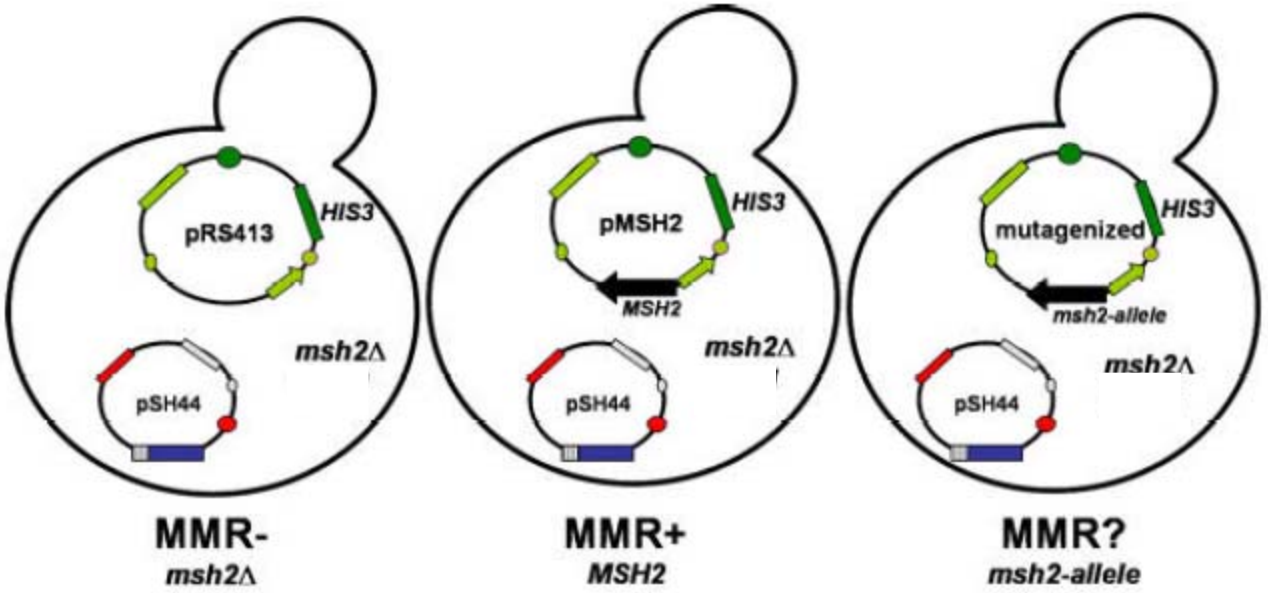
Plasmid Constructs





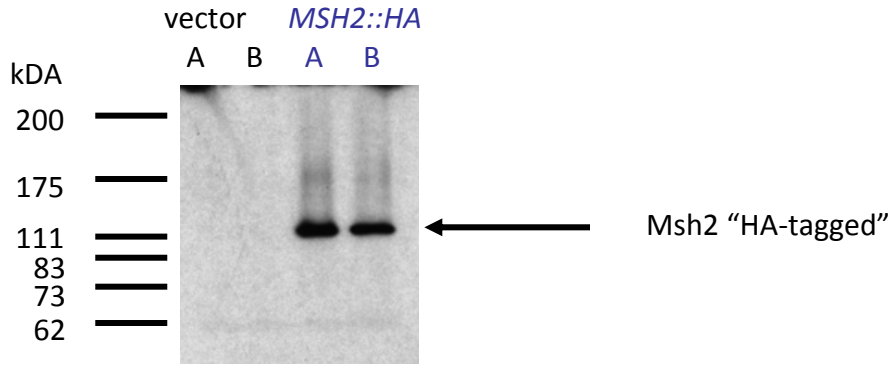

msh2
CEN/ARS
Recombinant
pRS413 plasmid
HIS3
pRS413
HIS3
msh2-allele
TRP1/ARS
pSH44
CEN1
(GT)16.5-URA3

Outline of Yeast Transformation Experiment

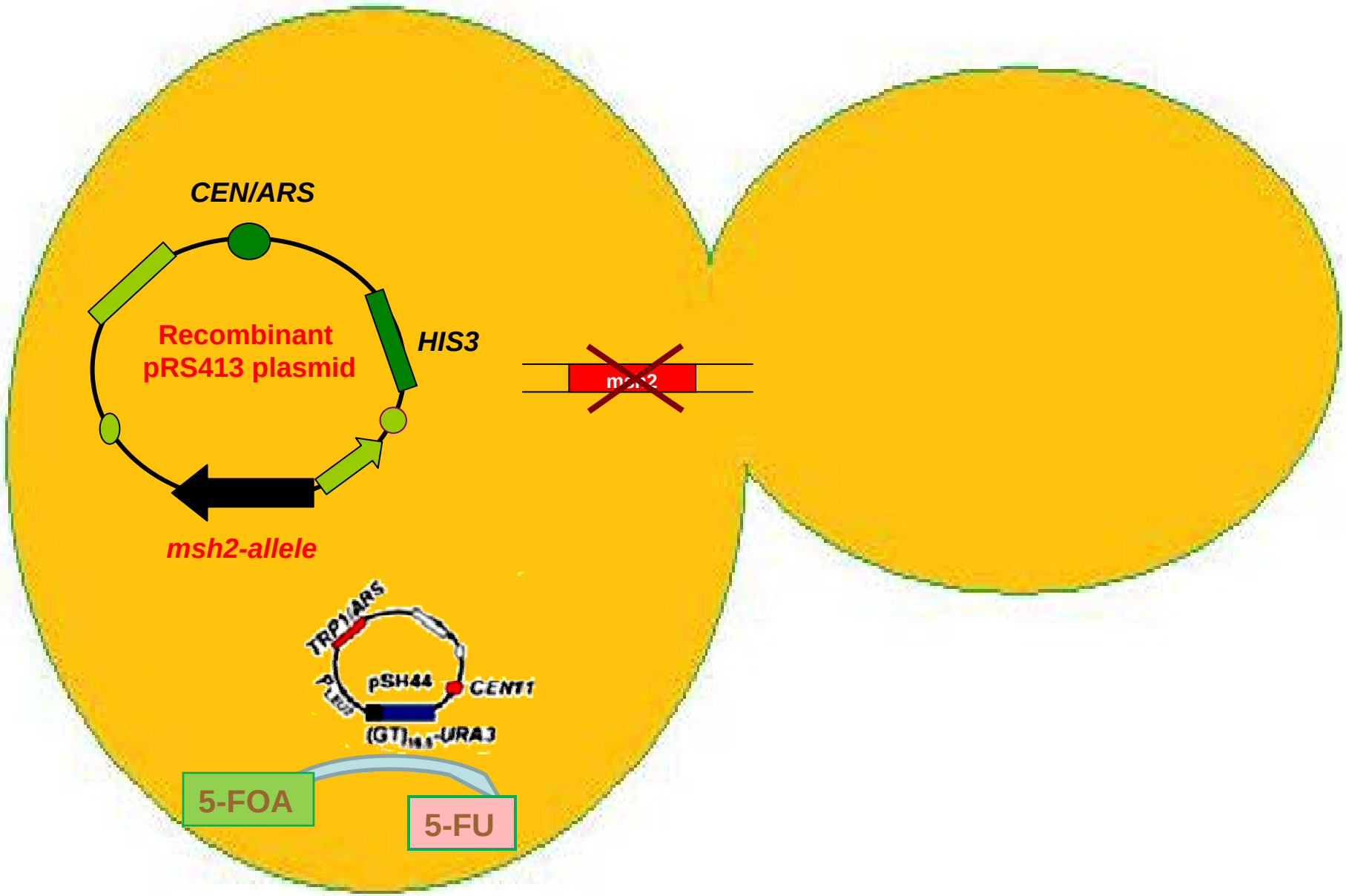


Functional Analysis

-produces a protein product of the expected molecular weight



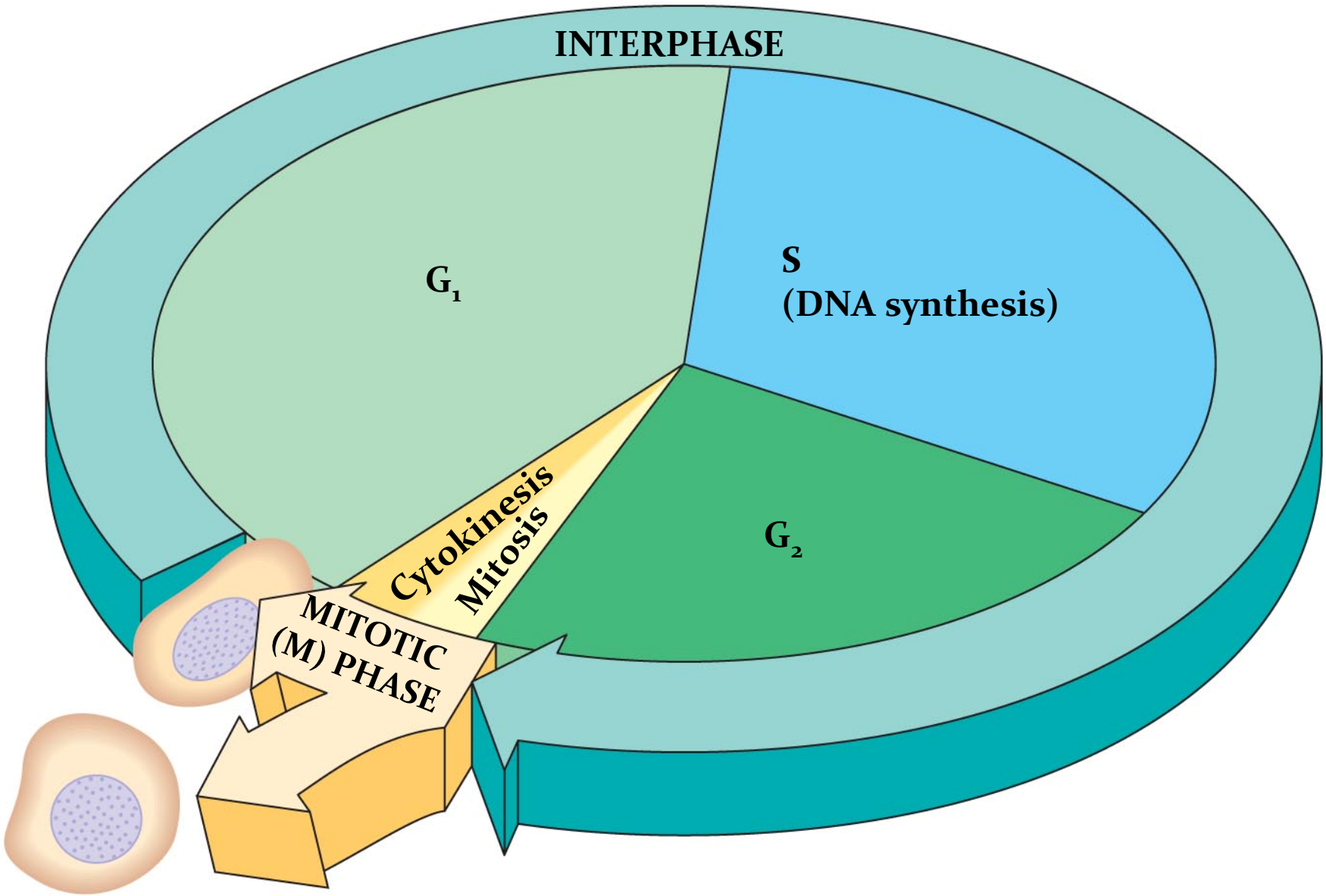
-complements *msh2*Δ defects, therefore the construct is functional


CEN/ARS
Recombinant
pRS413 plasmid
HIS3
msh2
msh2-allele
TRP1/ARS
pSH44
CEN11
(GT)
-URA3
5-FOA
5-FU

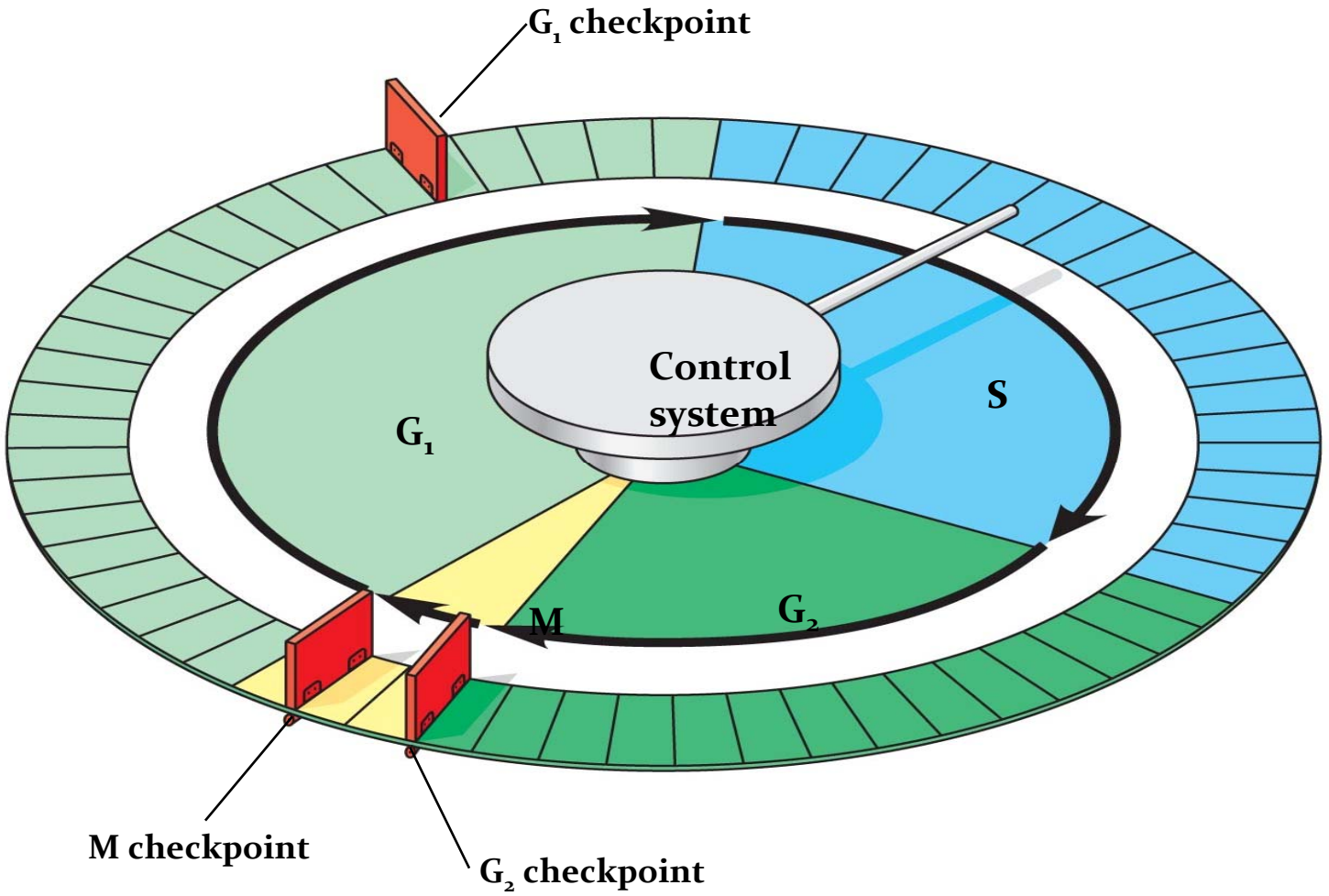
Mechanisms behind cancer development

Phases of the Cell Cycle

- The cell cycle consists of
  - Mitotic (M) phase (mitosis and cytokinesis)
  - Interphase (cell growth and copying of chromosomes in preparation for cell division)
- Interphase (about 90% of the cell cycle) can be divided into subphases:
  - G_1 phase (“first gap”)
  - S phase (“synthesis”)
  - G_2 phase (“second gap”)



Cell Cycle Checkpoints



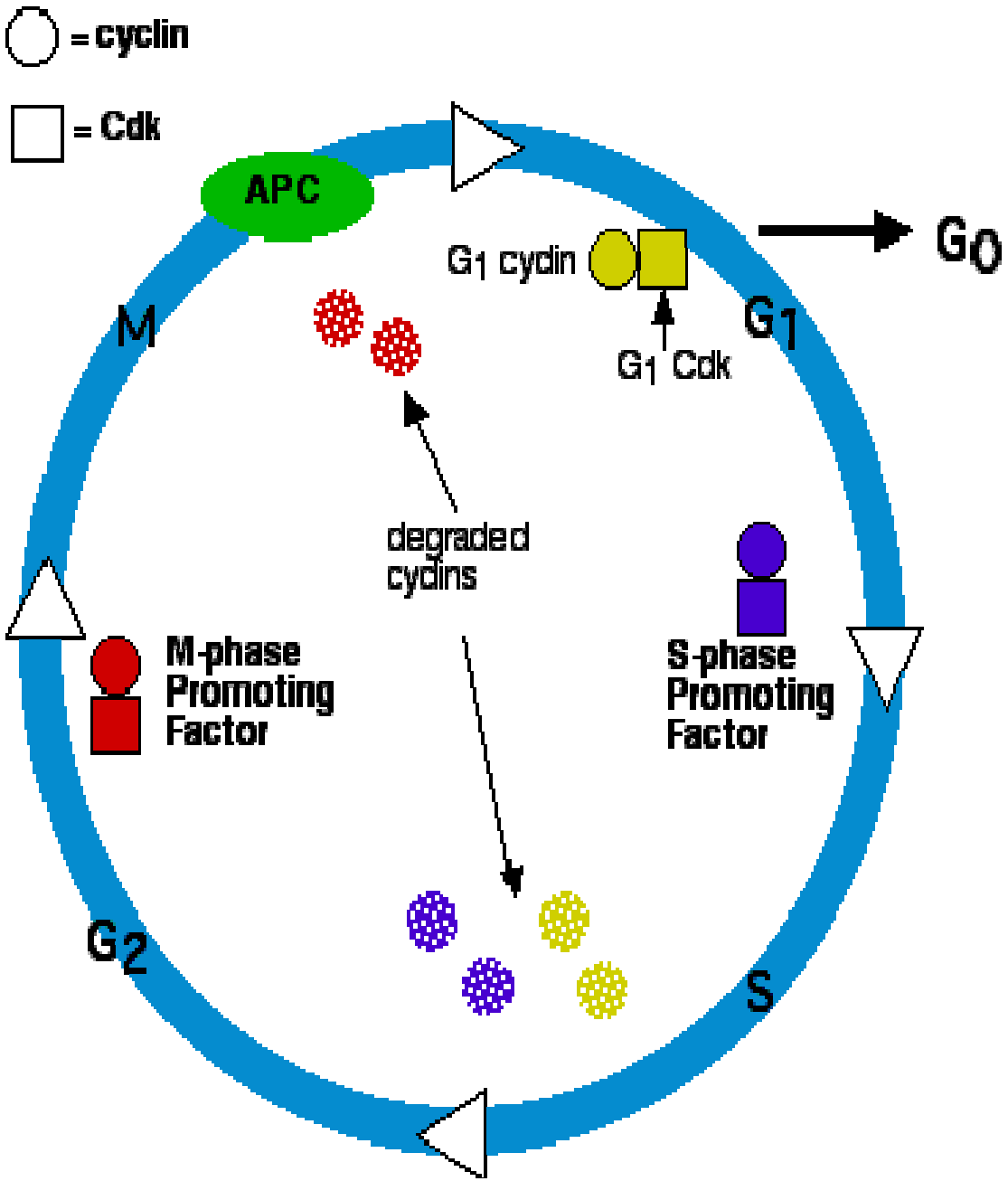
For cell cycle, Cyclin D binds CDK4/6. The active complex phosphorylates the tumor suppressor retinoblastoma (Rb), this relieves the inhibition of the transcription factor E2F, which causes the expression of cyclin E. Cyclin E interacts with CDK2 to allow for G1-S phase transition.

The first checkpoint (G1 checkpoint) is located at the end of the cell cycle's G_1 phase, just before entry into S-phase, making the key decision of whether the cell should divide, delay division, or enter a resting stage.

This first checkpoint involves p16, which inhibits Cyclin D-CDK4 binding.

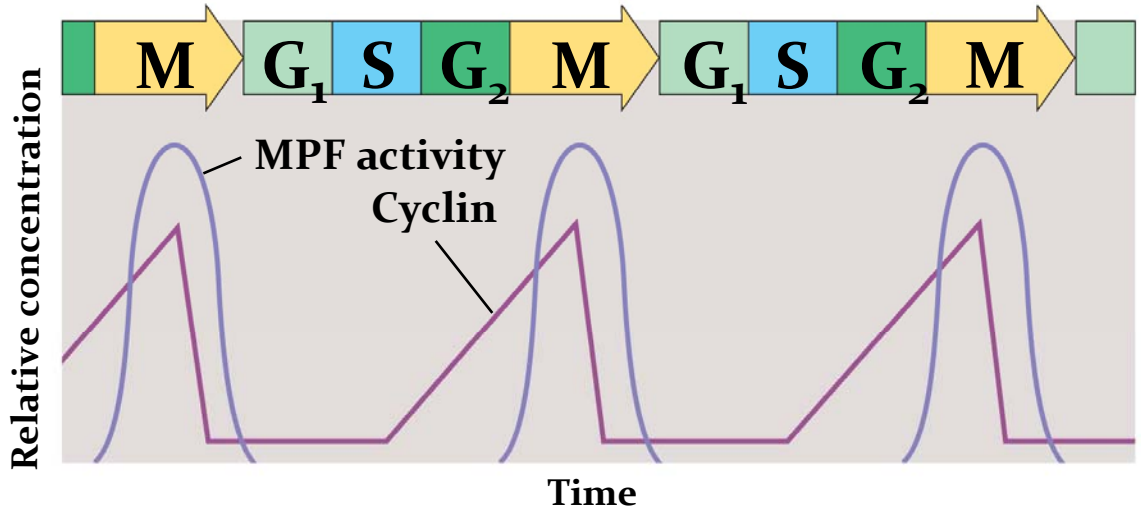
For the second checkpoint (G2 checkpoint), MPF activates the CDKs involved.

Then there is Replication checkpoint, and Mitosis checkpoint.



The Cell Cycle Clock: Cyclins and Cyclin-Dependent Kinases

- Two types of regulatory proteins are involved in cell cycle control: cyclins and cyclin-dependent kinases (Cdks)
- The activity of cyclins and Cdks fluctuates during the cell cycle



(a) Fluctuation of MPF activity and cyclin concentration during the cell cycle

Loss of Cell Cycle Control in Cancer Cells

- Cancer cells do not respond normally to the body's control mechanisms
- Cancer cells form tumors, masses of abnormal cells within otherwise normal tissue
- If abnormal cells remain at the original site, the lump is called a primary tumor
- Malignant tumors invade surrounding tissues and can metastasize, exporting cancer cells to other parts of the body, where they may form secondary tumors

Gene Categories Important in Cancer

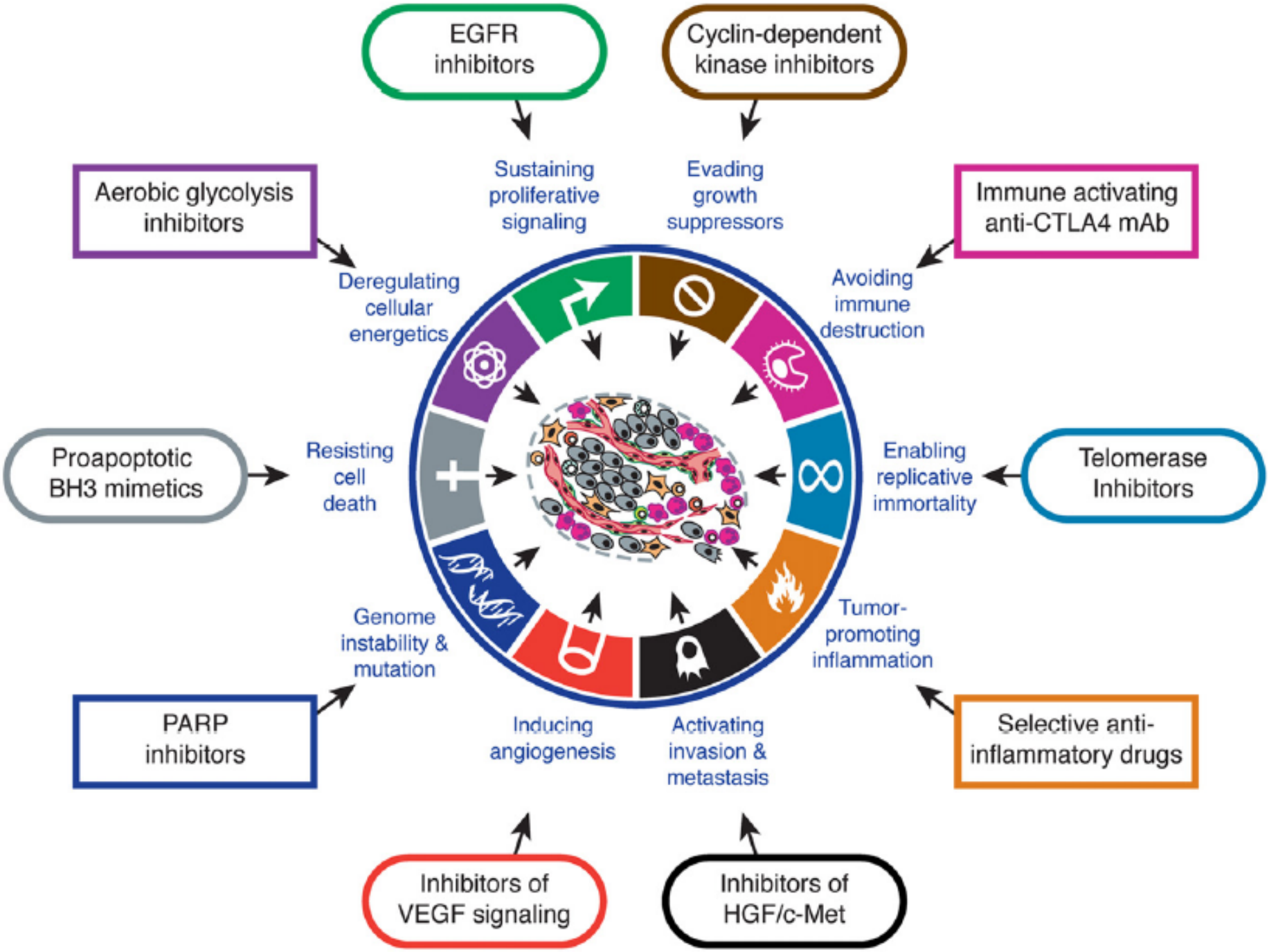
- **Tumor suppressor genes**-normally act to inhibit cell growth.
  - When damaged cell growth is no longer regulated
    - Mutation results in nonfunctioning gene (loss-of-function)
- **Proto-oncogenes** - normally act to regulate cell growth and division.
  - Oncogene is the defective version
    - Mutations results in constitutively active gene (gain-of-function)

Gene Categories Important in Cancer

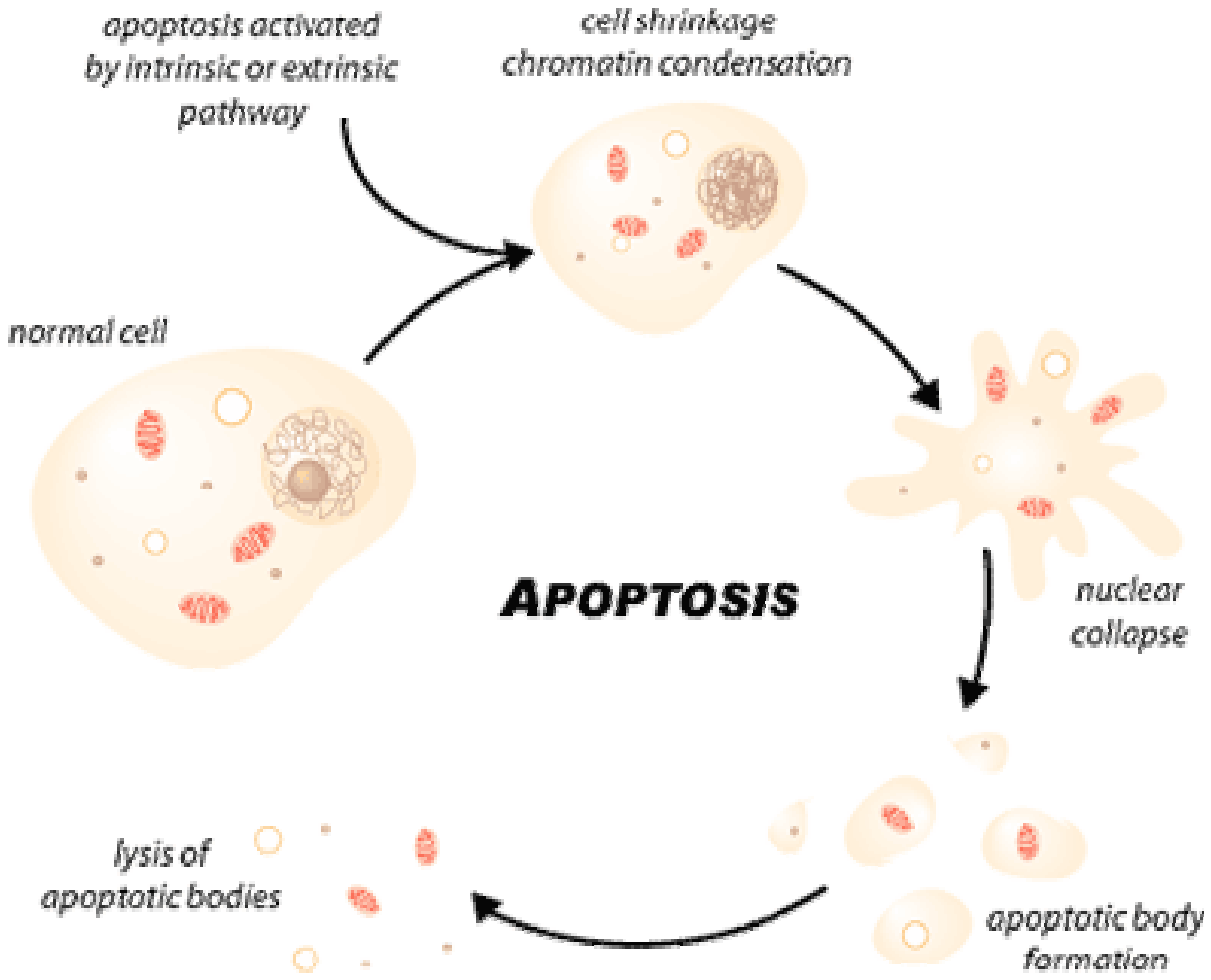
Genome stability gene - normally responsible for responding to and repairing DNA damage

- Mutations result in inactive/overactive protein

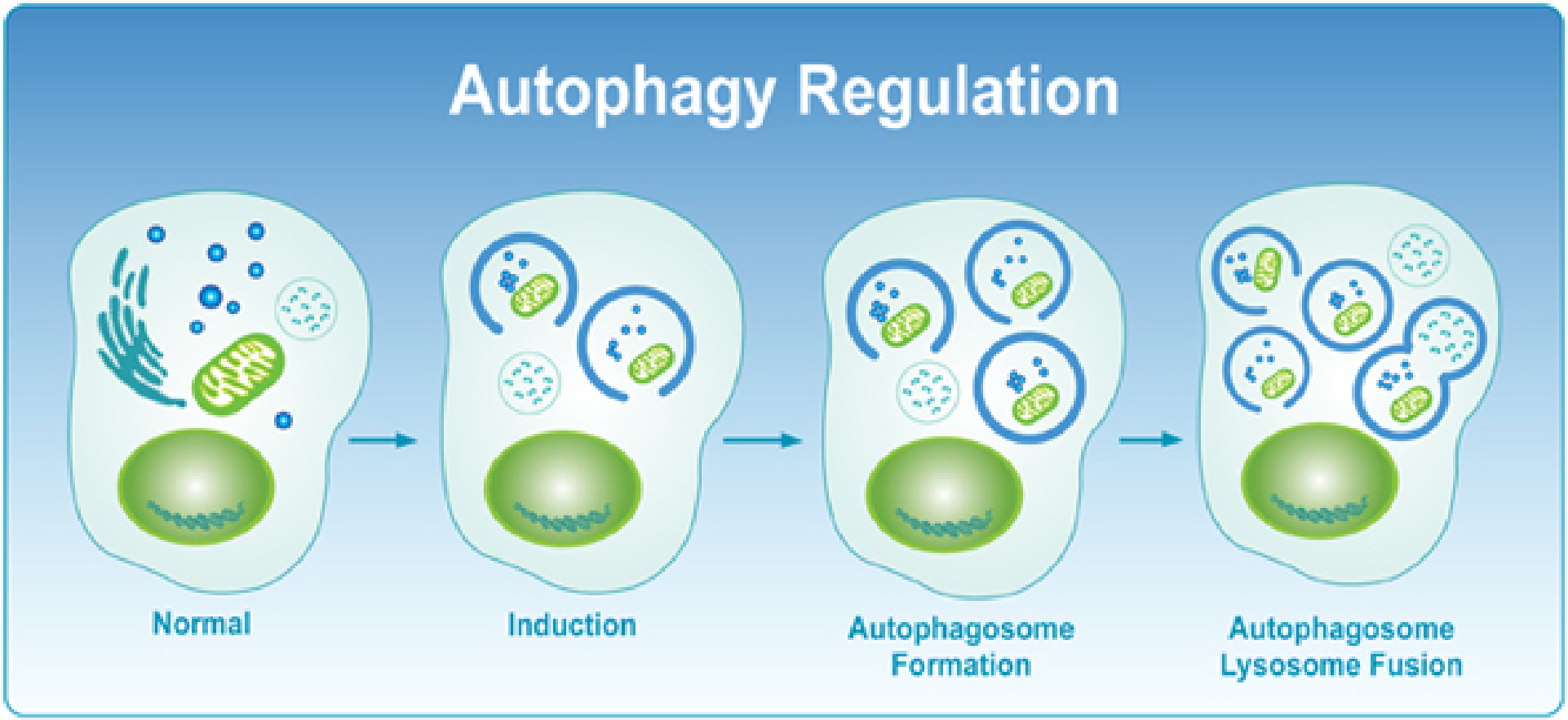
There is more to cancer than just cell cycle changes, tumor suppressors and proto-oncogenes

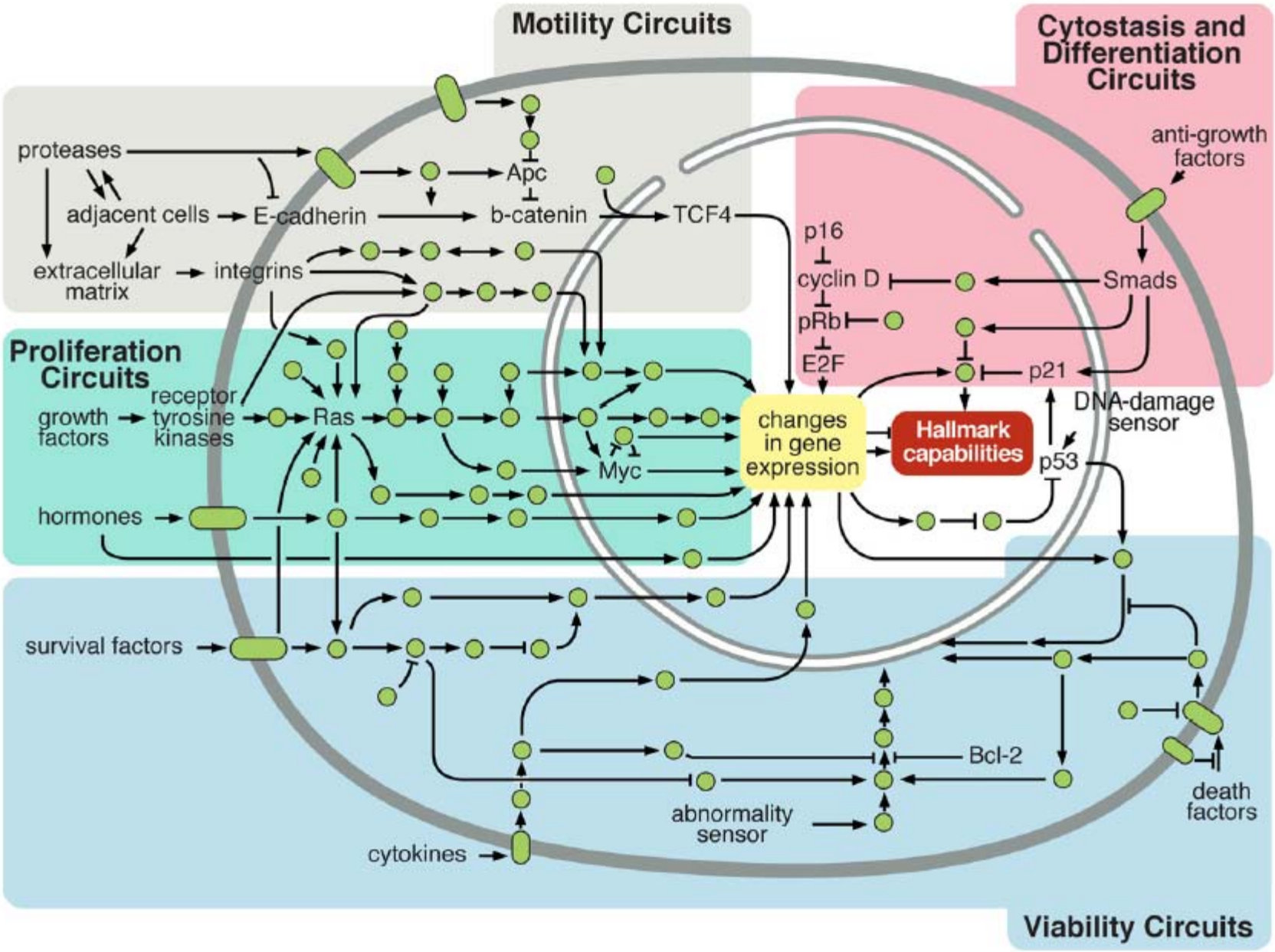

EGFR inhibitors
Cyclin-dependent kinase inhibitors
Sustaining proliferative signaling
Evading growth suppressors
Aerobic glycolysis inhibitors
Immune activating anti-CTLA4 mAb
Deregulating cellular energetics
Avoiding immune destruction
Proapoptotic BH3 mimetics
Resisting cell death
Enabling replicative immortality
Telomerase Inhibitors
Genome instability & mutation
Tumor-promoting inflammation
PARP inhibitors
Inducing angiogenesis
Activating invasion & metastasis
Selective anti-inflammatory drugs
Inhibitors of VEGF signaling
Inhibitors of HGF/c-Met

Apoptotic cell death and cancer

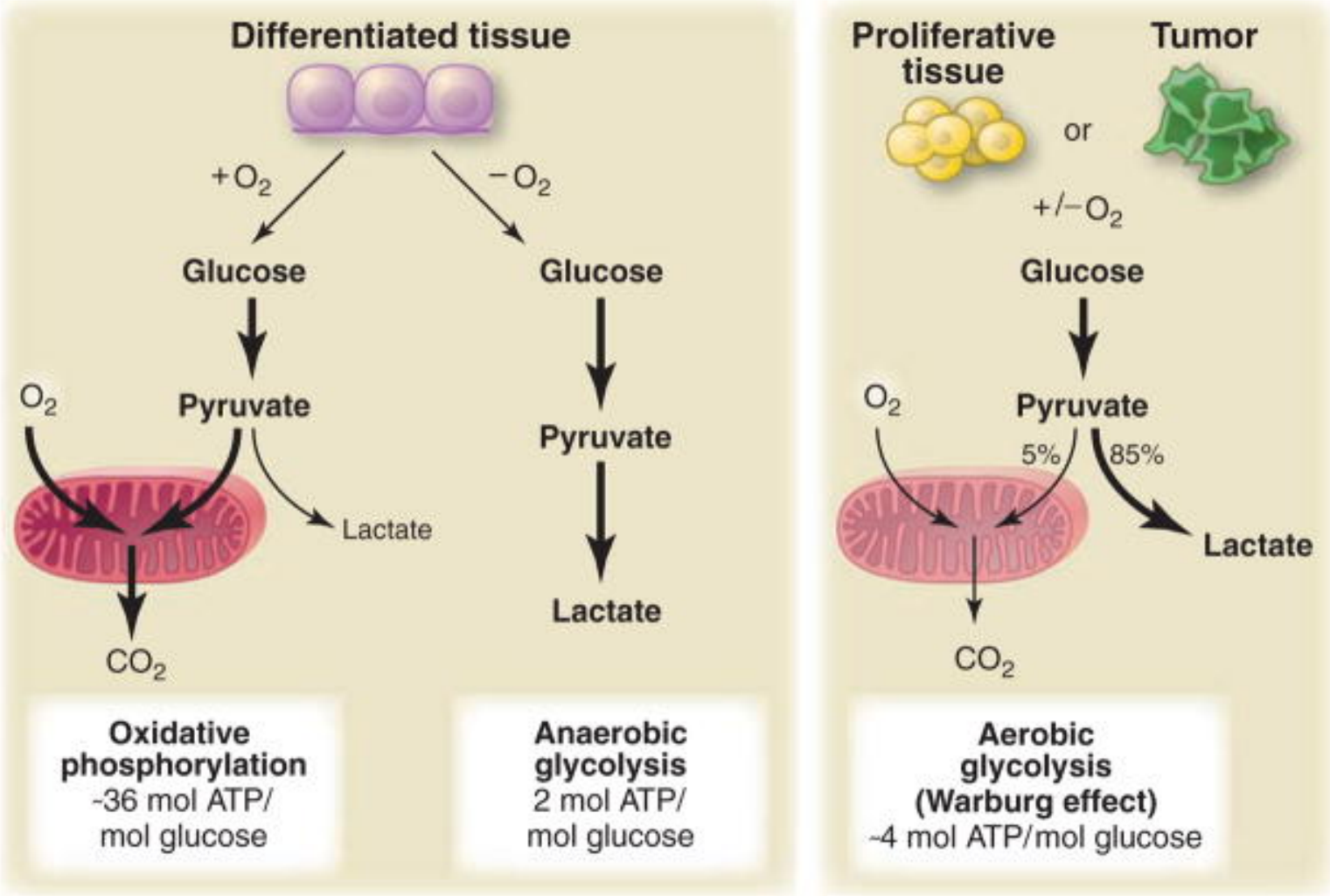


Autophagy may protect or promote cell death

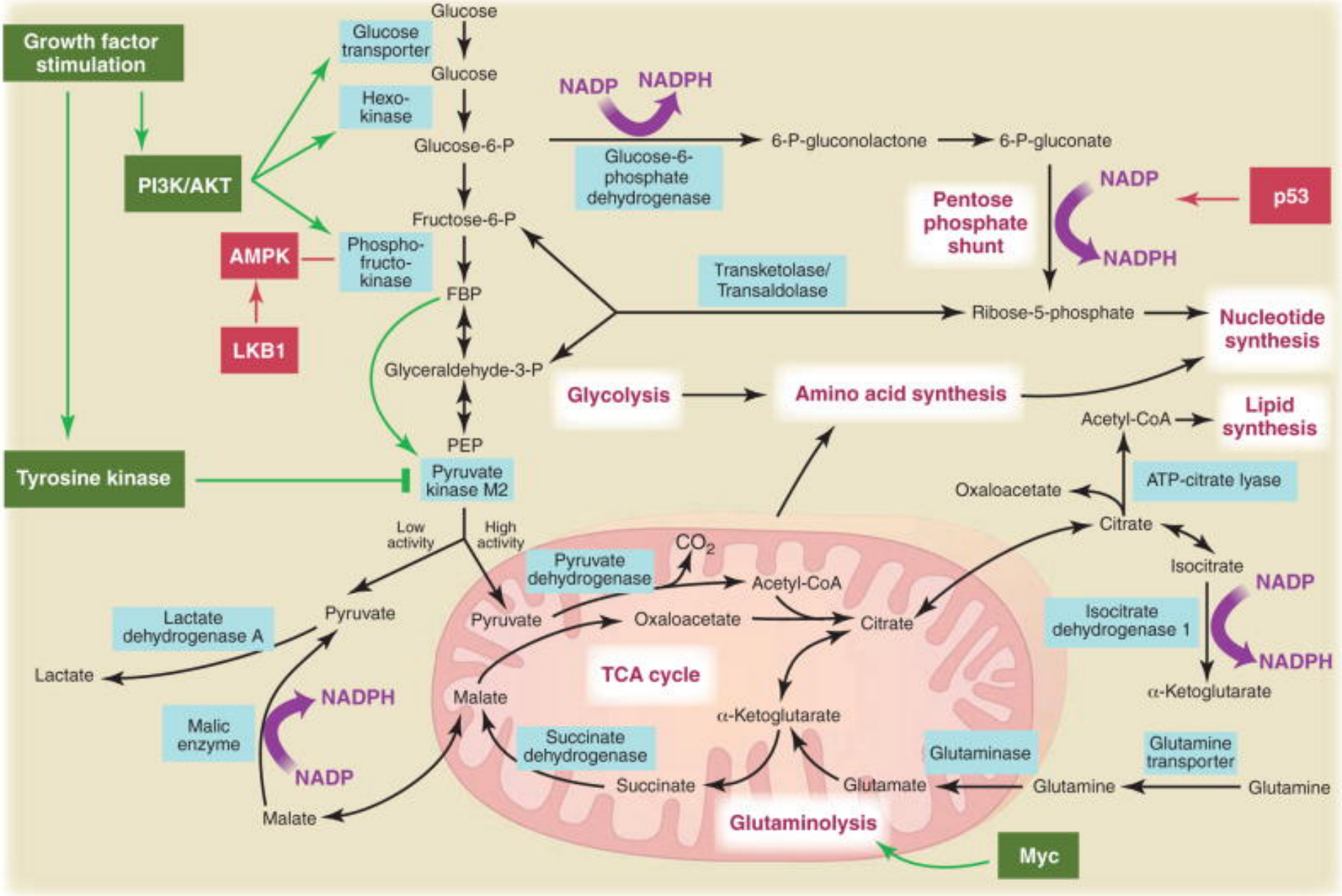



Motility Circuits
Cytostasis and Differentiation Circuits
Proliferation Circuits
Viability Circuits
proteases
adjacent cells
extracellular matrix
integrins
E-cadherin
Apc
b-catenin
TCF4
anti-growth factors
Smads
p16
cyclin D
pRb
E2F
p21
DNA-damage sensor
p53
growth factors
receptor tyrosine kinases
Ras
Myc
changes in gene expression
Hallmark capabilities
hormones
survival factors
Bcl-2
abnormality sensor
cytokines
death factors

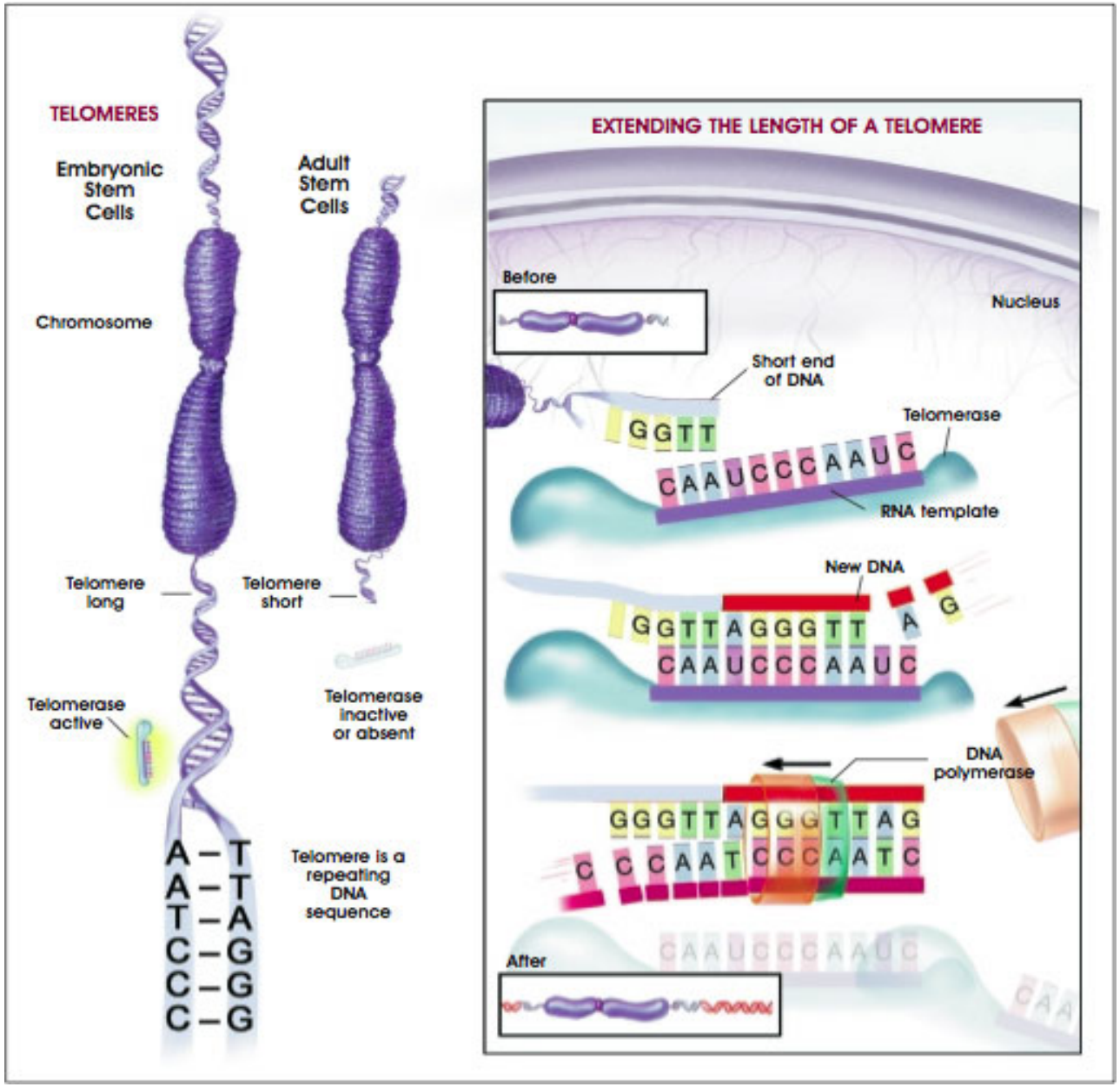
Changing energetics of the cell - Warburg effect in cancer tissue



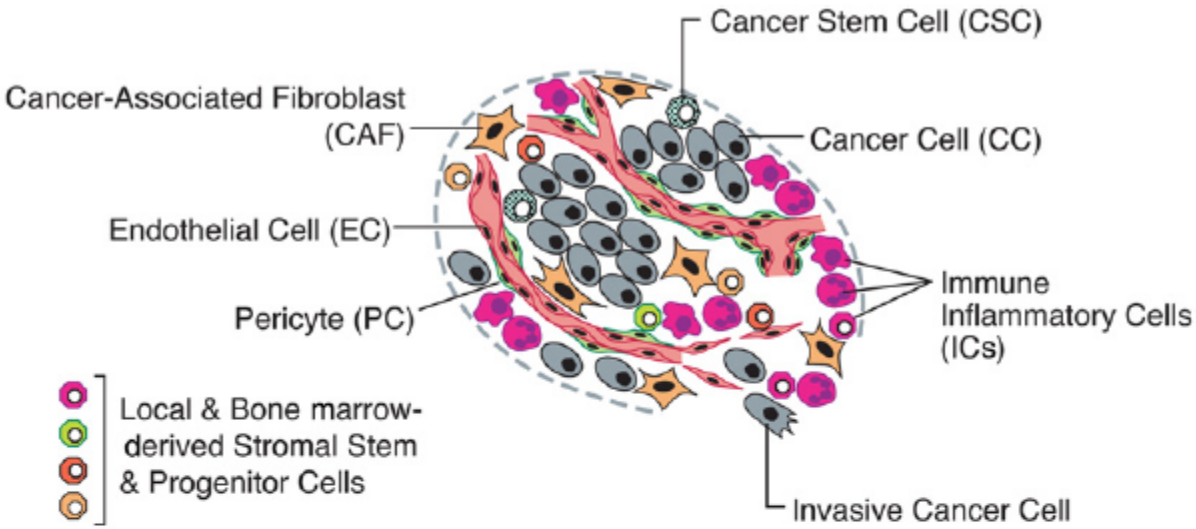
Redirecting metabolic pathways towards synthesis of lipids, nucleic acids and proteins

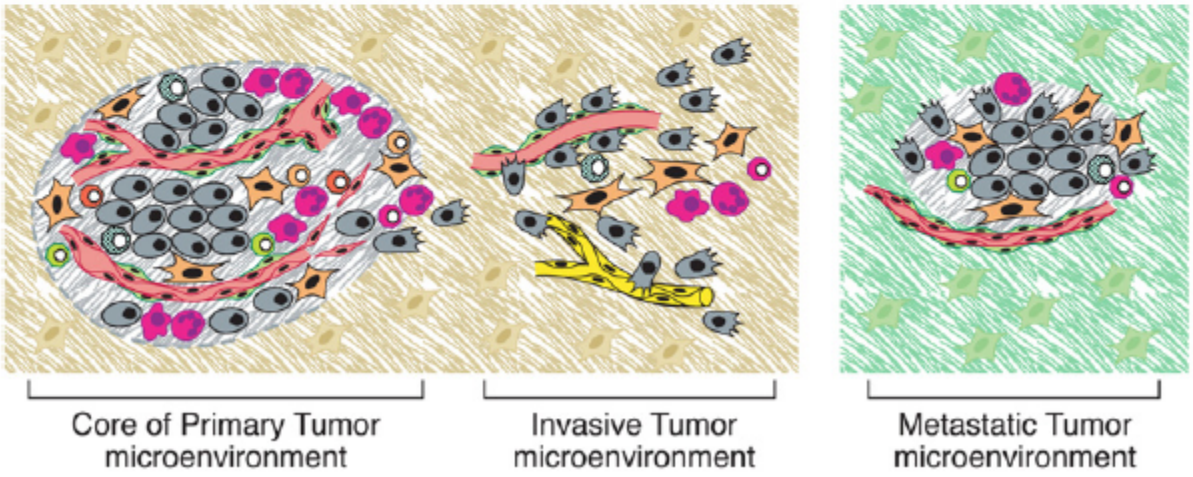


Telomerase activity



Tumor microenvironment

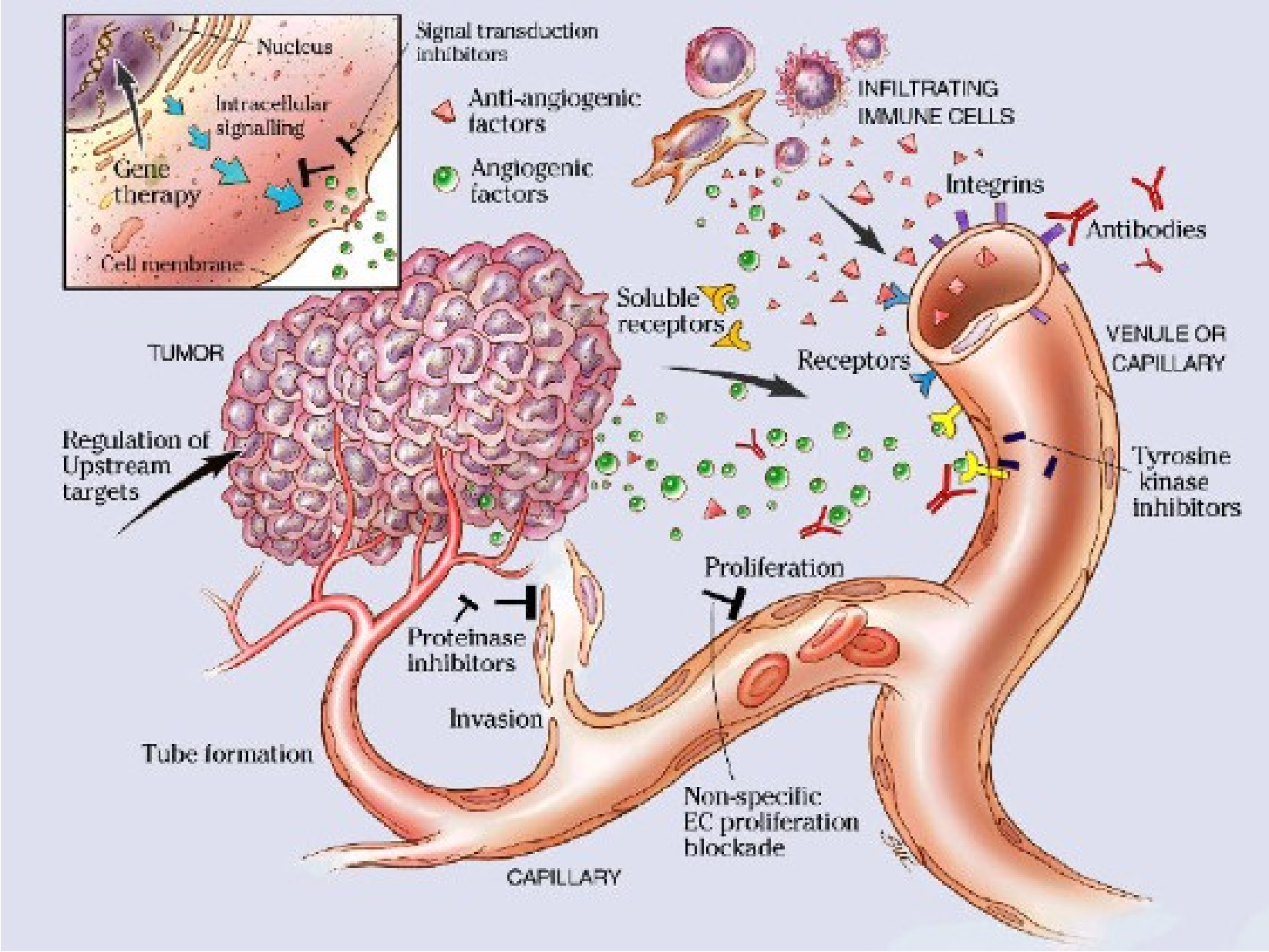




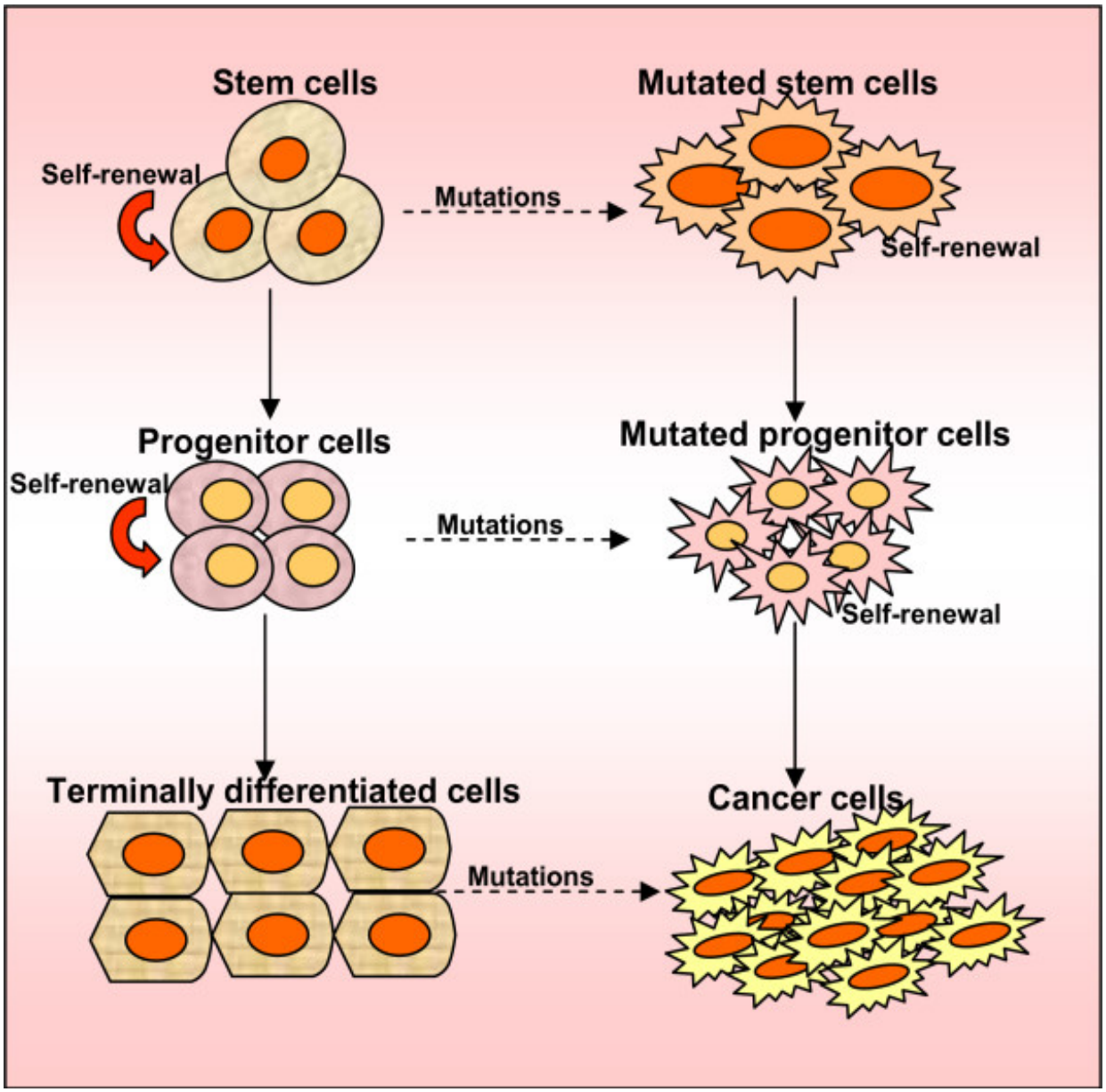
Upper) An assemblage of distinct cell types constitutes nost solid tumors. Both the parenchyma and stroma of umors contain distinct cell types and subtypes that collectively enable tumor growth and progression. Notably, the immune inflammatory cells present in tumors can include both tumor-promoting as well as tumor-killing subclasses.

Lower) The distinctive microenvironments of tumors. The nultiple stromal cell types create a succession of tumor nicroenvironments that change as tumors invade normal issue and thereafter seed and colonize distant tissues. The abundance, histologic organization, and phenotypic characteristics of the stromal cell types, as well as of the extracellular matrix (hatched background), evolve during progression, thereby enabling primary, invasive, and then netastatic growth. The surrounding normal cells of the primary and metastatic sites, shown only schematically, likely also affect the character of the various neoplastic nicroenvironments. (Not shown are the premalignant stages in tumorigenesis, which also have distinctive nicroenvironments that are created by the abundance and characteristics of the assembled cells.)

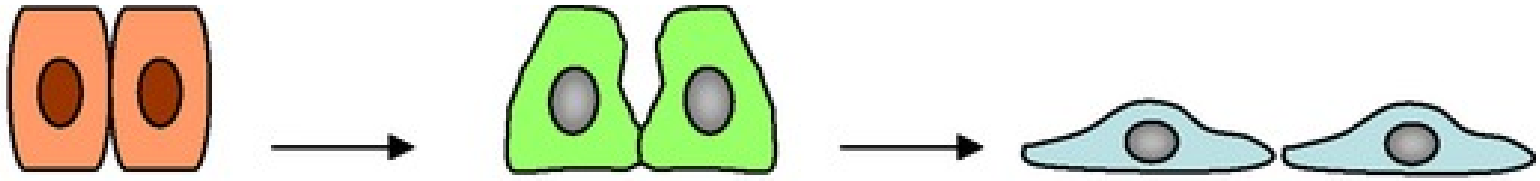
Angiogenesis and Metastasis



Cancer Stem Cells



Epithelial Mesenchymal Transition



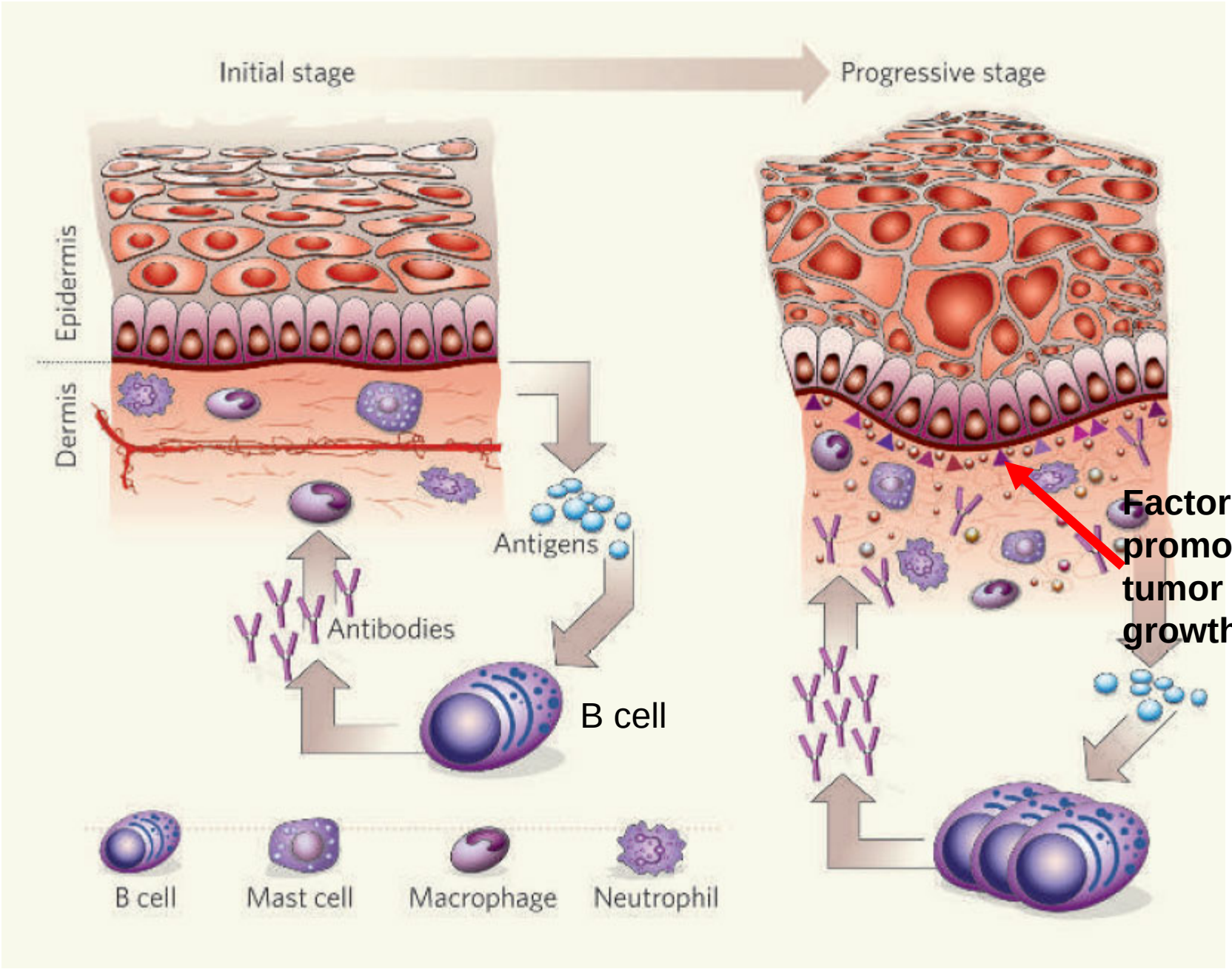
Epithelial markers
- E-cadherin
- Claudin
- Occludin
- Desmoglein
- Desmocollin
- Cytokeratins

Characteristics
- Cobblestone
- Non-motile
- Non-invasive

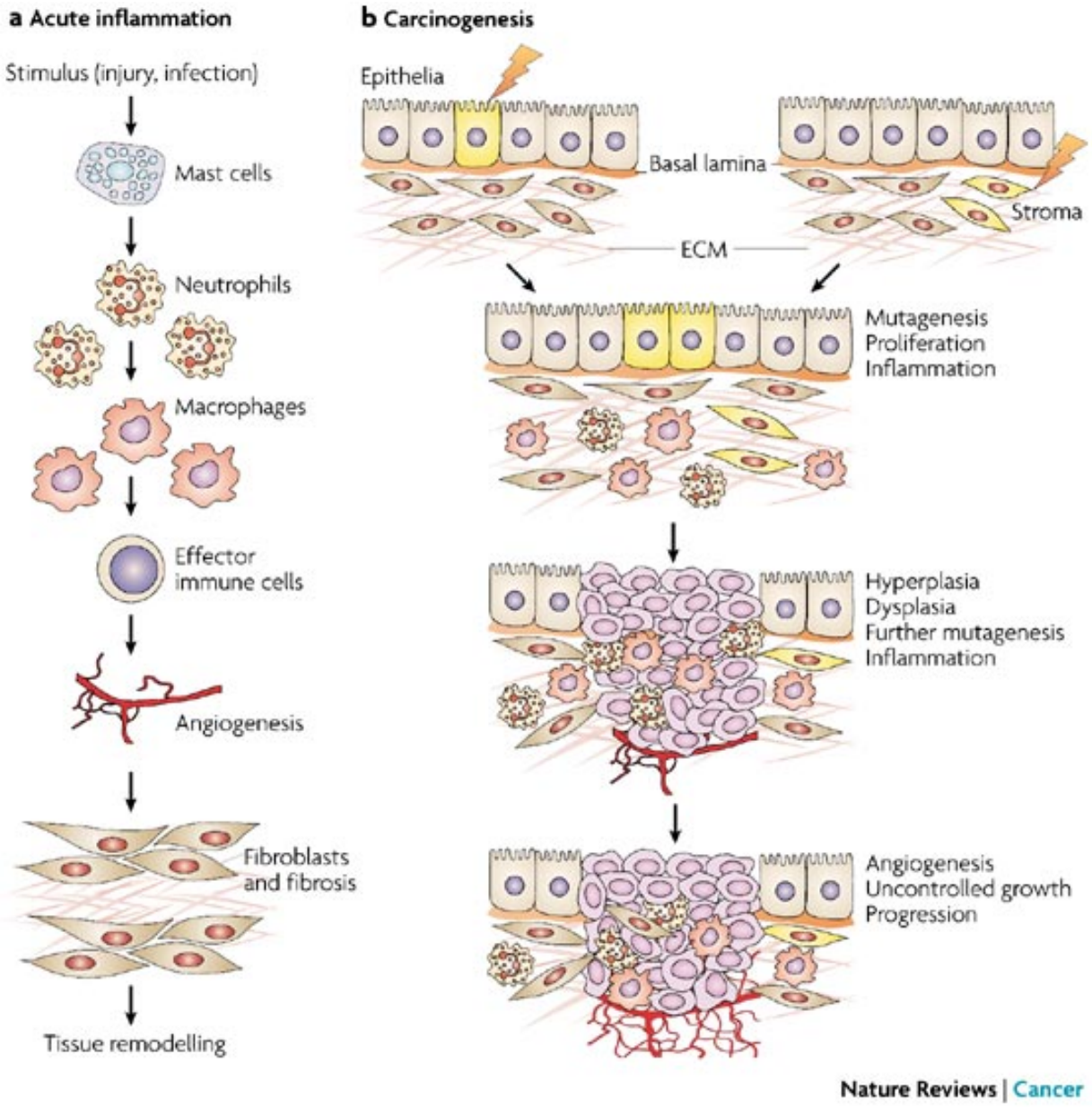
EMT effectors
Growth factors
Cytokines
ECM

Mesenchymal markers
- N-cadherin
- Vimentin
- Fibronectin
- Snai1/2
- FSP1
- Smooth muscle actin

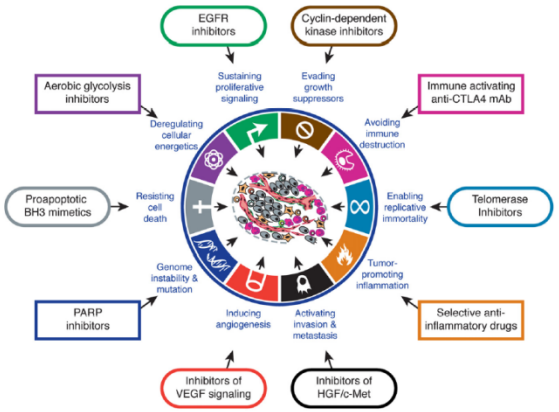
Characteristics
- Elongated
- Motile
- Invasive


Initial stage
Progressive stage
Epidermis
Dermis
Antigens
Antibodies
B cell
Factors promoting tumor growth
B cell
Mast cell
Macrophage
Neutrophil

Inflammation and cancer



Hallmarks of cancer



- Genome instability and mutation
- Continued proliferation:
  - Sustained signaling to promote proliferation
  - Evading growth suppressors
- Avoiding immune destruction
- Replicative immortality - increased telomerase activity
- Tumor promoting inflammation
- Resisting cell death (apoptosis, autophagy or necrosis)
- Deregulating cellular energetics
- Inducing angiogenesis
- Activating invasion and metastasis