Mechanisms of Apoptosis in Spermatogenesis

by
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Because
men never ask for
directions!

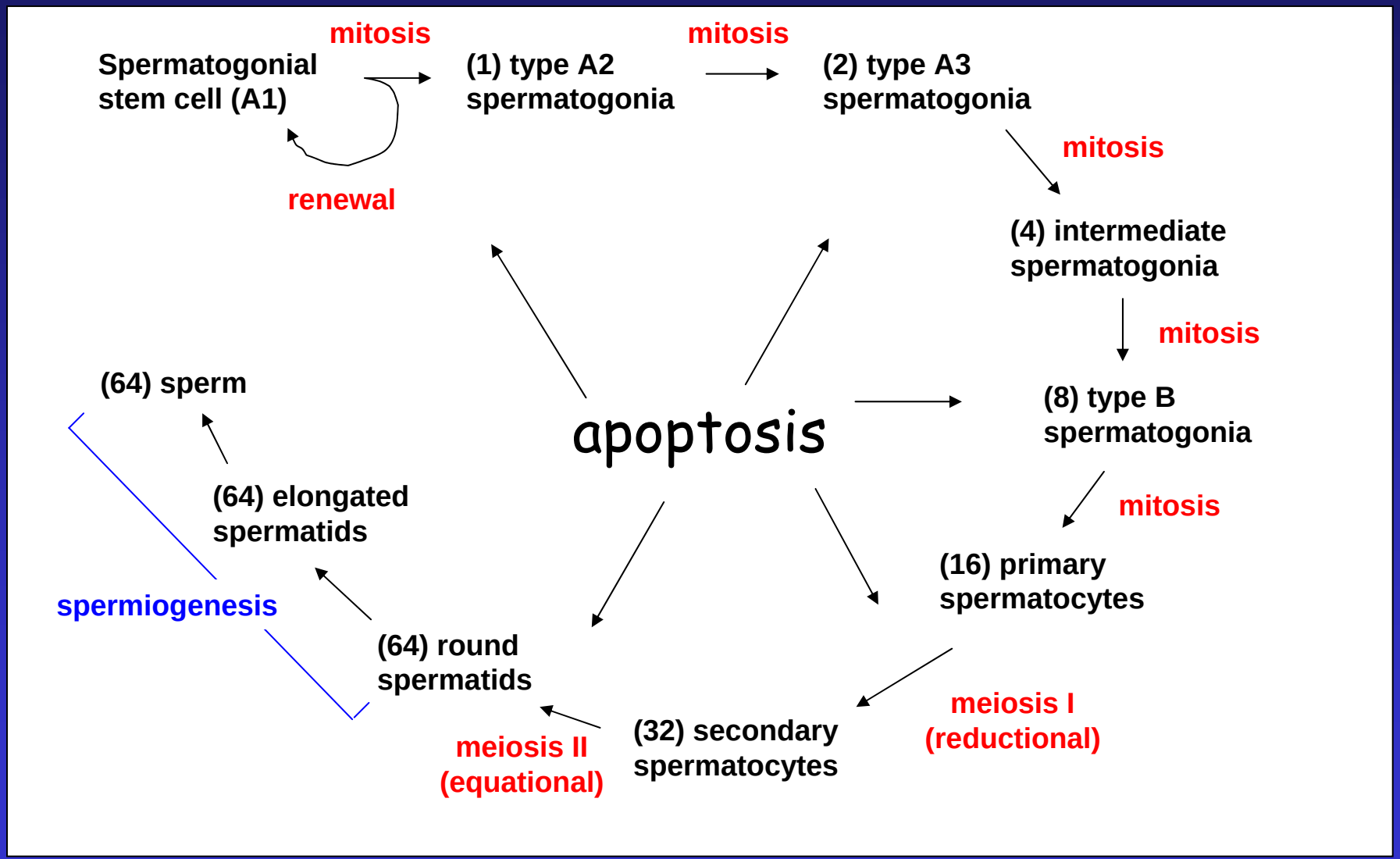
We know the way:

(Assisted Reproductive Technologies)

Why so much germ cell death during spermatogenesis?

- Limitation of nutritive function of Sertoli cells?
- Controlled proliferation, homeostasis?
- Increasing gene pool mixing and quality control?

Apoptosis in spermatogenesis



Spermatogenesis:
differentiation and apoptosis

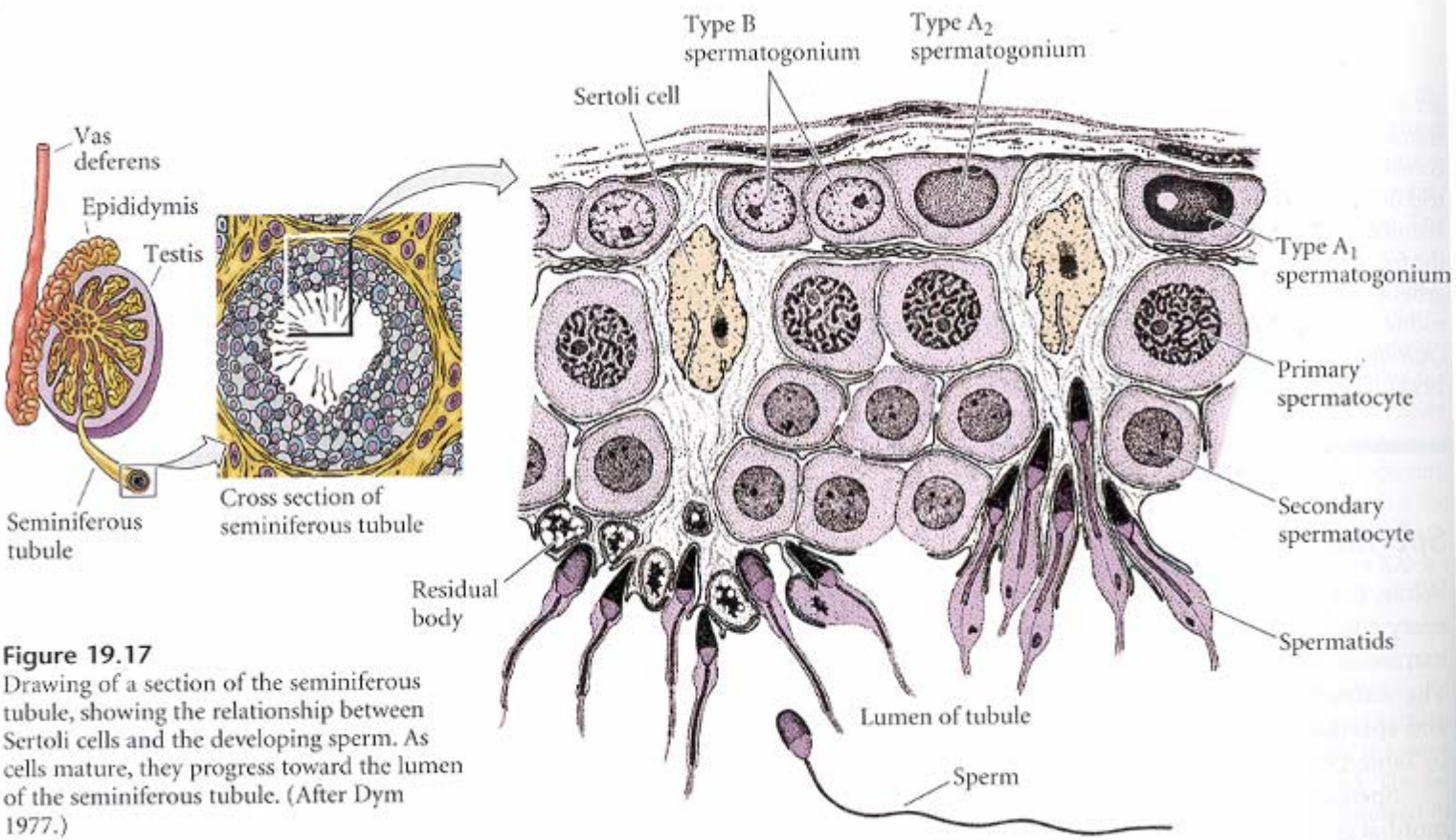
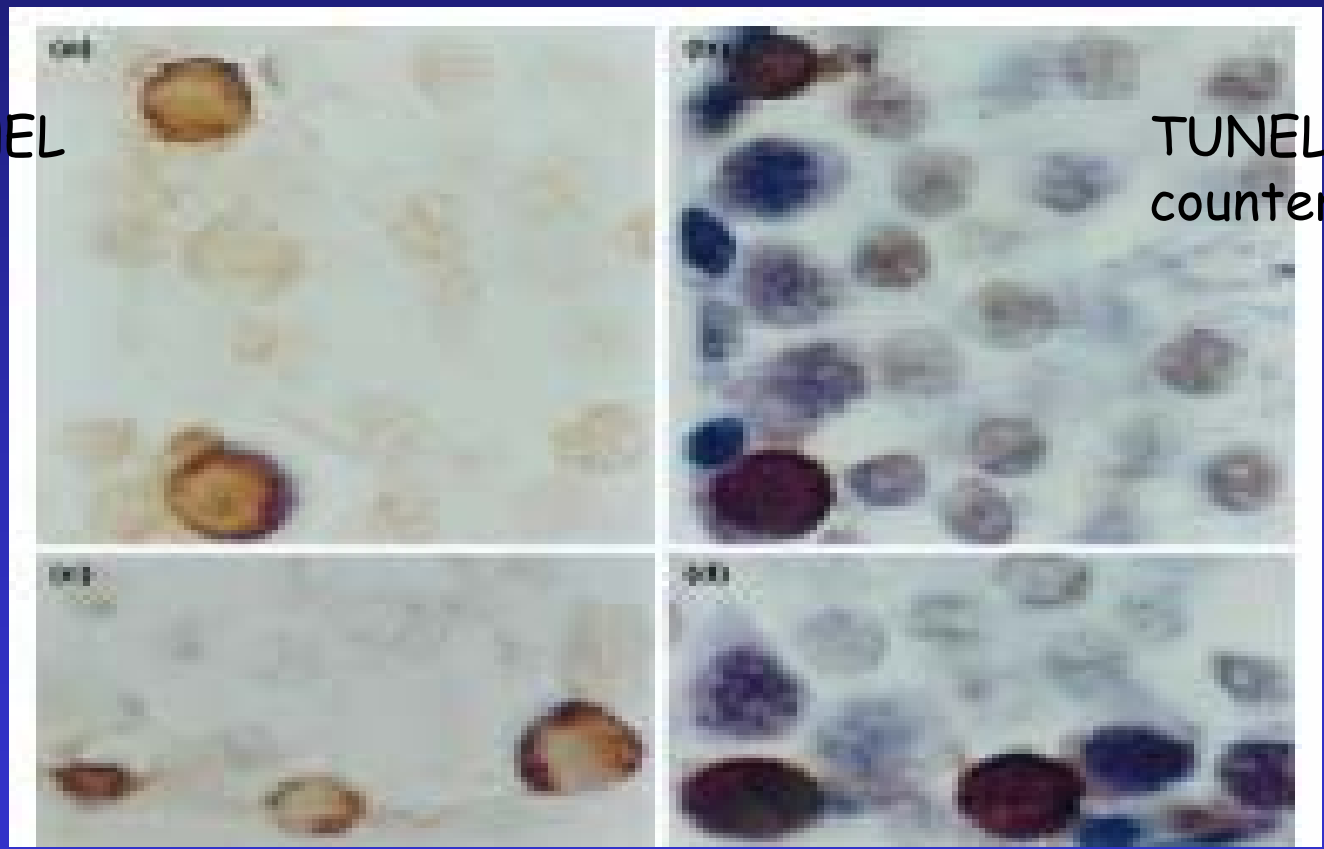


Figure 19.17
Drawing of a section of the seminiferous tubule, showing the relationship between Sertoli cells and the developing sperm. As cells mature, they progress toward the lumen of the seminiferous tubule. (After Dym 1977.)

Male germ cell apoptosis highlighted in by TUNEL assay

TUNEL



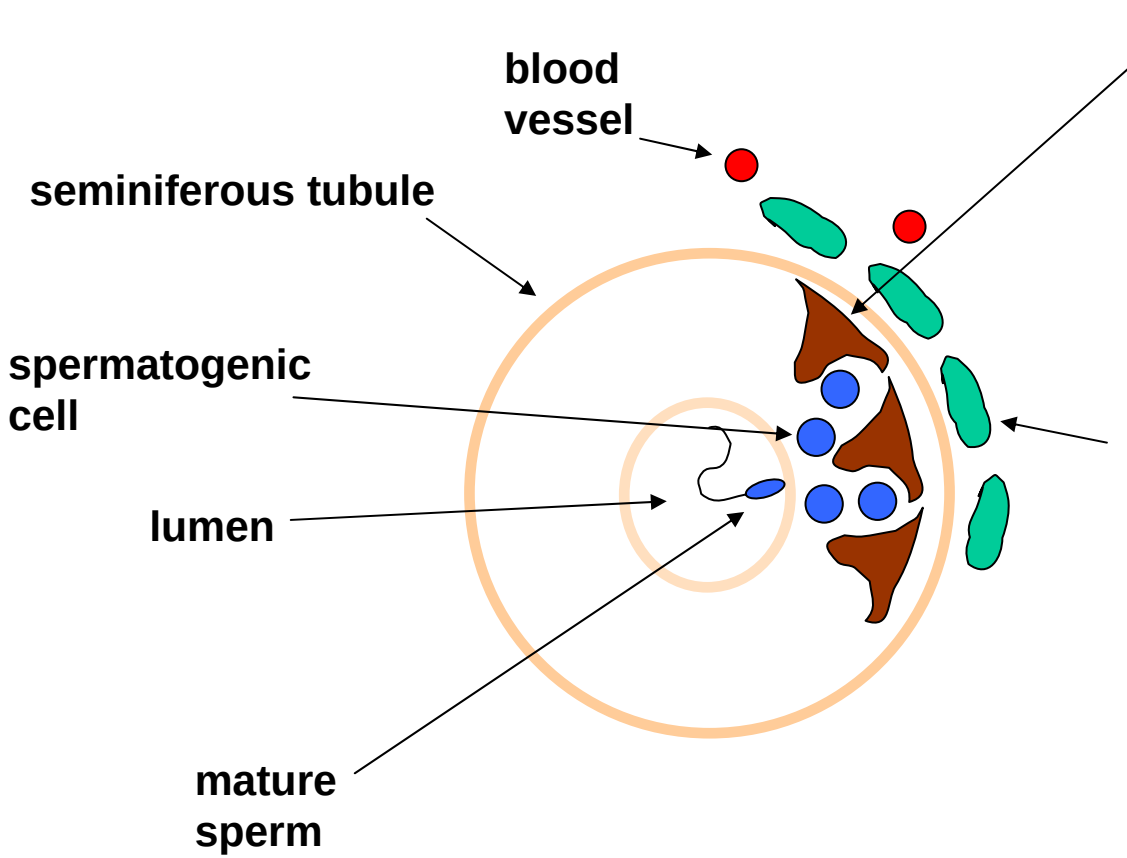
TUNEL and counterstain

What makes a cell decide to commit suicide?

The balance between:

- positive signals; that is, signals needed for continued survival
- the receipt of negative signals

Localization within of the seminiferous tubule



Sertoli cell

- -regulate spermatogenesis
- -form blood-testis barrier
- -analogous to ovarian granulosa cells

Leydig cell

- -produce testosterone
- -external to semin. tubules
- -analogous to ovarian thecal cells

Survival and death signals

Survival signals

The continued survival of most cells requires that they receive continuous stimulation from other cells

growth factors, hormones

Death signals

- increased levels of oxidants within the cell
- damage to DNA
  - x-rays
  - chemotherapeutic drugs
- apoptotic signaling molecules include:
  - Fas ligand (FasL), a molecule that binds to a cell-surface receptor named Fas (also called CD95)

3 mechanisms of apoptosis

1. generated by intra-cellular **signaling**
2. triggered by **death activators** binding to receptors at the cell surface

   TNF-a

   Fas ligand (FasL).
3. triggered by reactive oxygen species.

Apoptosis triggered by internal signals: the intrinsic or mitochondrial pathway

- The outer membranes of mitochondria express the protein **Bcl-2**
- **Bcl-2** is bound to a molecule of the protein **Apaf-1**.
- Internal **damage** to the cell causes Bcl-2
  - to release Apaf-1 to no longer keep **cytochrome c** from leaking out of the mitochondria
- The released cytochrome c and Apaf-1 bind to molecules of caspase 9.
- The resulting complex of
  - **cytochrome c**
  - **Apaf-1**
  - **caspase 9** (and ATP) is called the **apoptosome**

The apoptotic executors

- Caspase 9 is one of a family of over a dozen caspases.
- Caspases are proteases cleaving- mostly each other - at aspartic acid residues.
- Caspase 9 cleaves and, activates other caspases.
- The sequential activation of one caspase by another creates an expanding cascade of
- Proteolytic activity leads to
  - digestion of structural proteins in the cytoplasm
  - degradation of chromosomal DNA and
  - phagocytosis of the cell

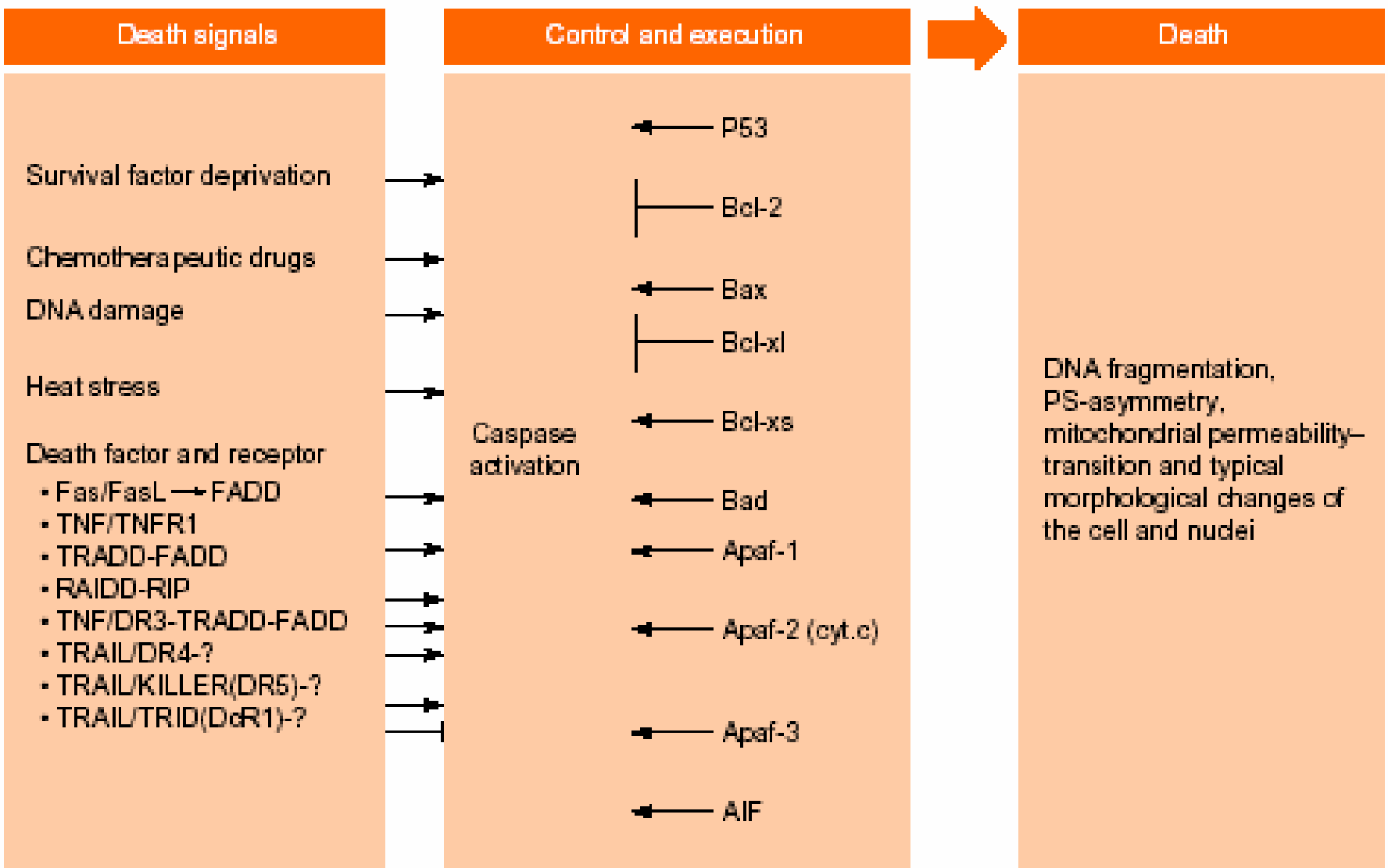
Apoptosis-Inducing Factor (AIF)

Apoptosis-inducing factor (**AIF**) is normally located in the intermembrane space of mitochondria. When the cell receives a death signal **AIF**

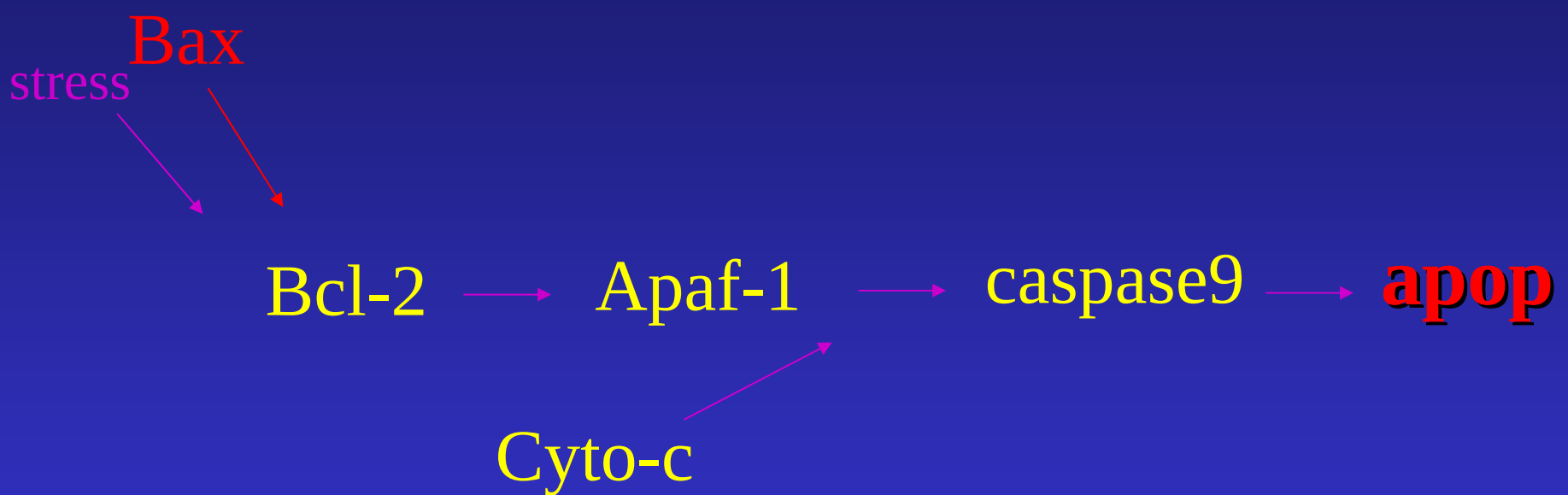
- is released from the mitochondria (like the release of cytochrome c in the first pathway)
- migrates into the nucleus
- binds to DNA, which
- triggers the destruction of the DNA and cell death.

Apoptosis: Dance of Death

Programmed cell death cascade



Sensitivity to survival and death signals



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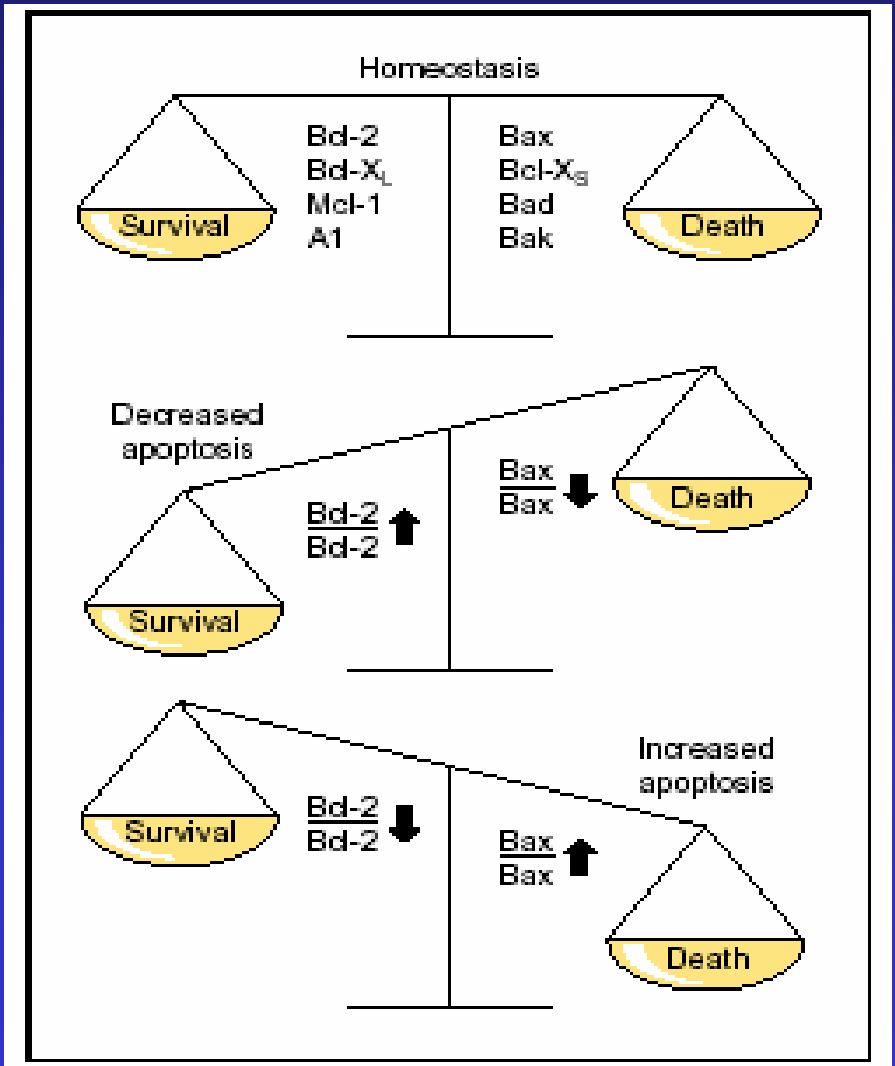
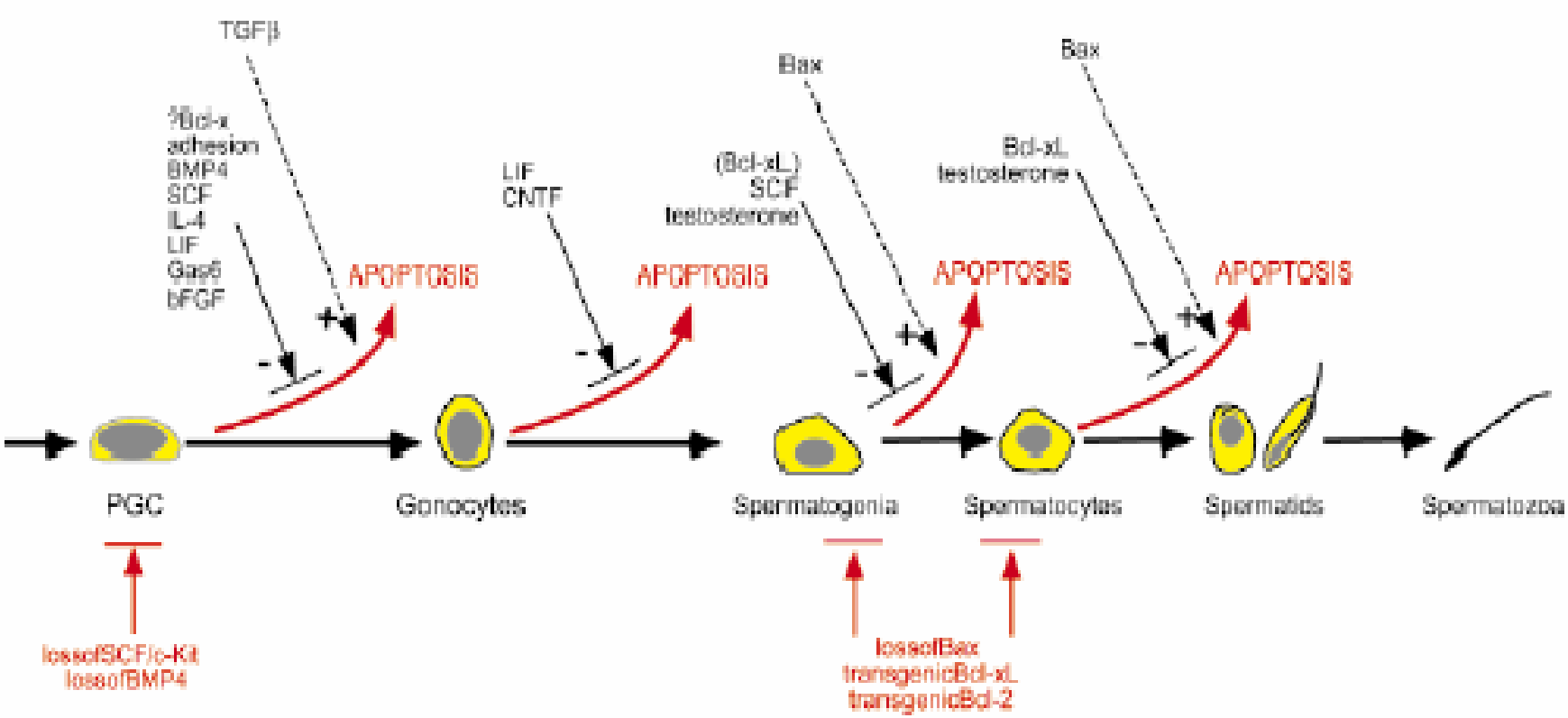
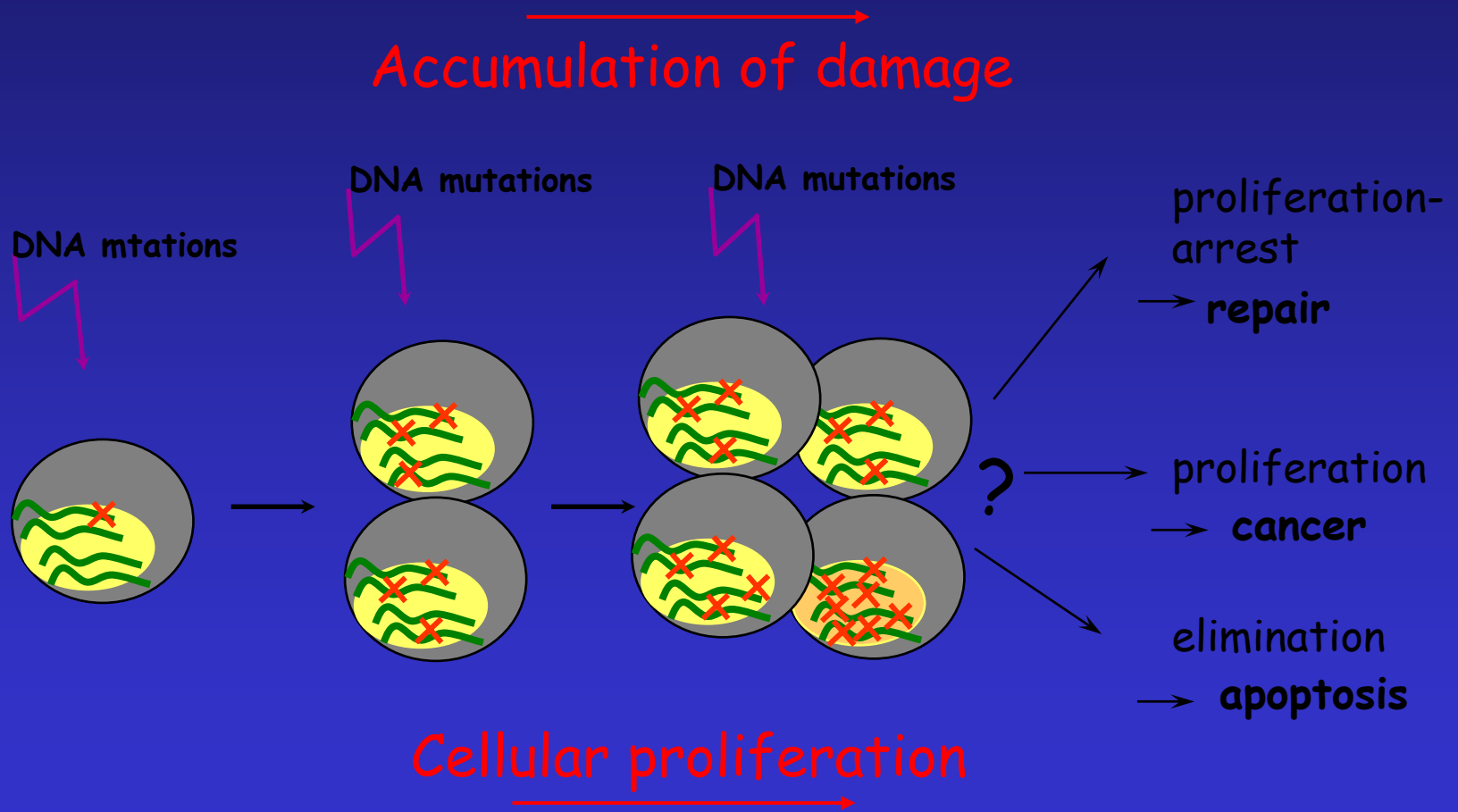


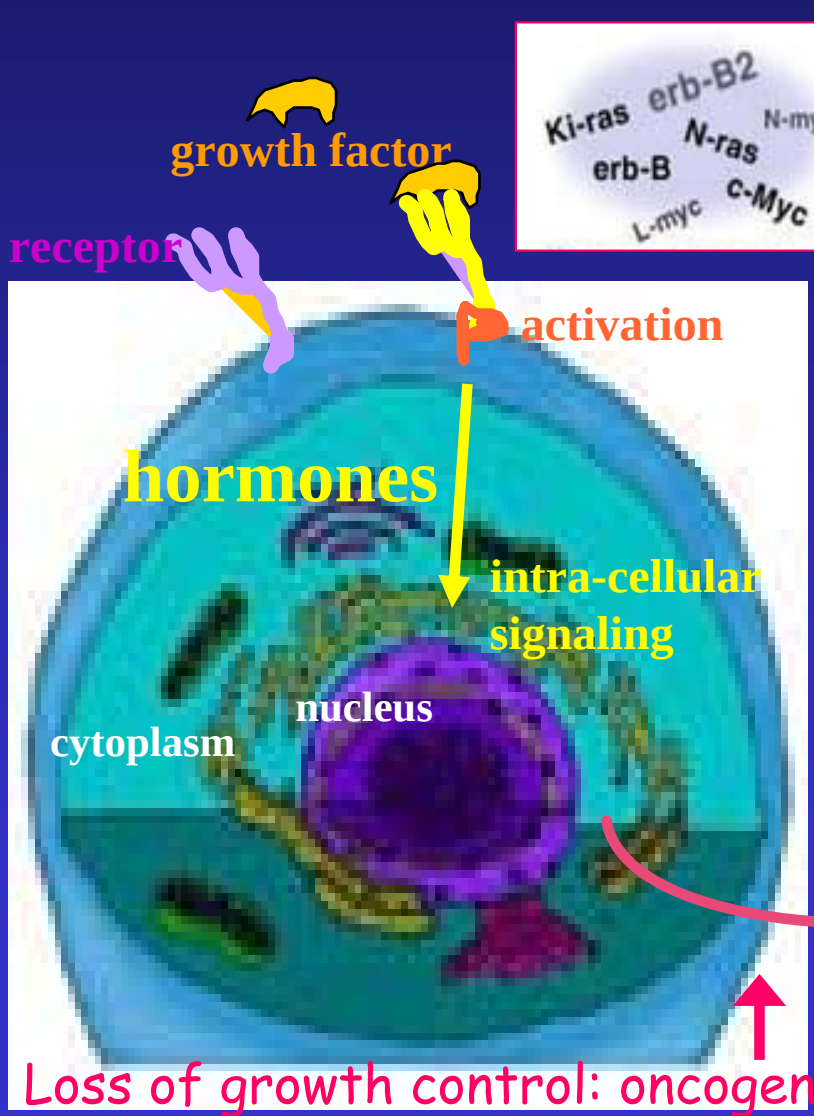
Survial and death signals at multiple steps of spermatogenesis

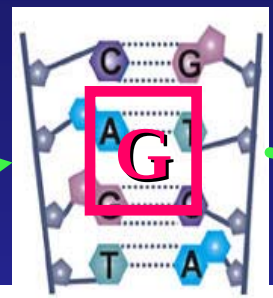


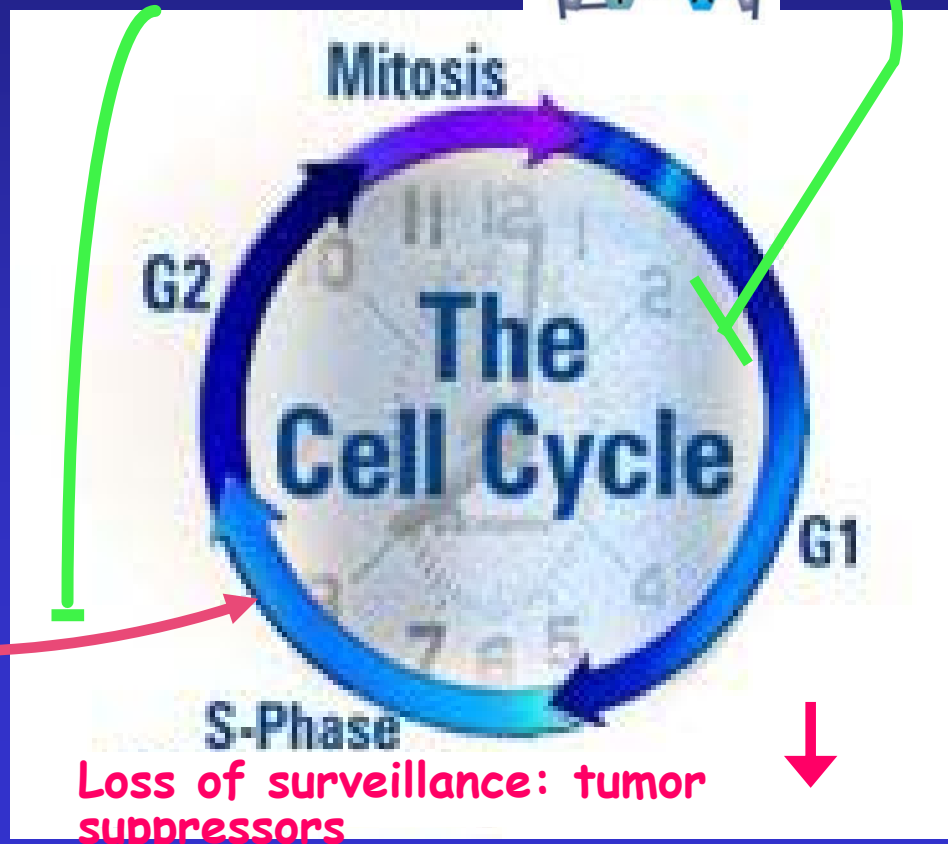
Proliferation accumulation of mutations and repair



Repair, or die







Mouse knock-outs affecting spermatogenesis

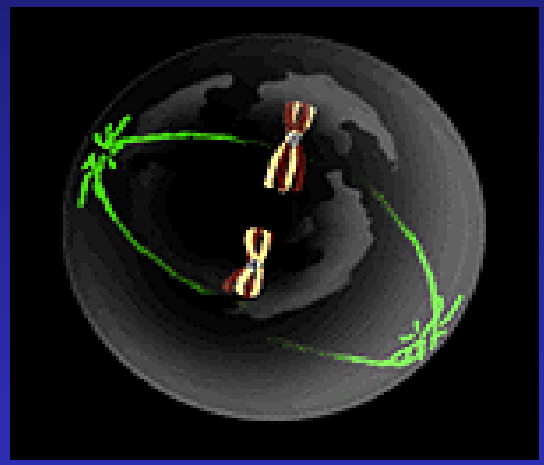
Table 2. Partial list of genes the deletion of which, in mice, results in defects in spermatogenes

Gene disrupted	Phenotype
Bax	Accumulation of atypical premeiotic germ cells but no mature haploid spermatozoa. Marked increase in germ cell apoptosis. Infertile.
CREM	Late spermatids are completely absent and there is a significant increase in germ cell apoptosis. Sterile.
HR6B	Severely impaired spermatogenesis with only small numbers (< 6% of controls) of predominantly abnormal spermatozoa. Marked increase in germ cell apoptosis. Defects in postmeiotic condensation of chromatids.
Hsp70-2	Failure of meiosis with a marked increase in spermatocyte apoptosis. Infertile.
ATM	Complete arrest at pachytene spermatocyte. Increased germ cell apoptosis. Infertile.
MLH-1	Complete arrest at pachytene spermatocyte stage. Accelerated germ cell apoptosis. Infertile.
A-myb	Arrest at pachytene spermatocyte stage. Complete absence of post-meiotic cells such as spermatids or spermatozoa. Infertile.
Dazla	Complete absence of meiotic (spermatocytes) and post-meiotic (spermatids or spermatozoa) germ cells. Infertile.
Bclw	Progressive depletion of germ cells through accelerated apoptosis to a Sertoli cell-only phenotype by approximately 6 months of age followed by a loss of Sertoli cells.
p53	Increased spermatogonial proliferation, decreased spermatocyte apoptosis and increased sperm output.

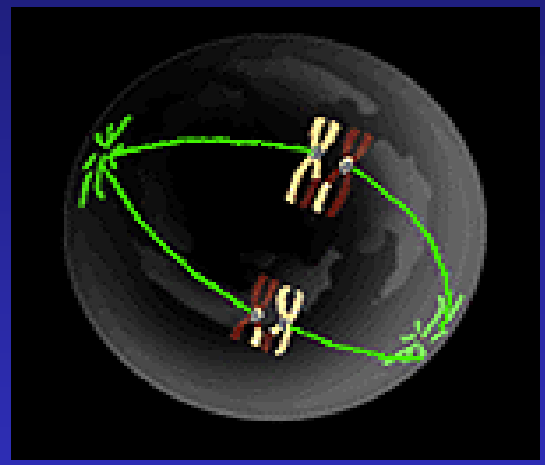
Male meiosis

Prophase I

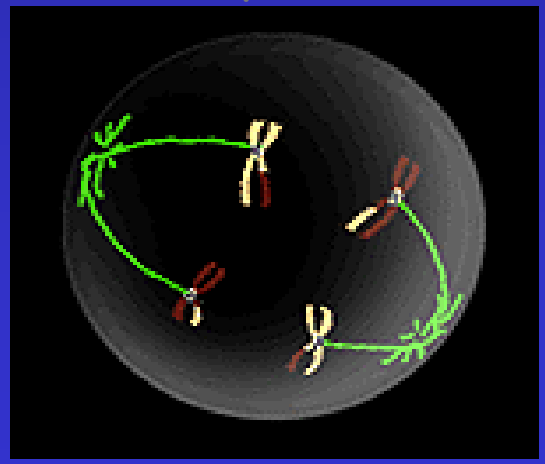
Prometaphase I



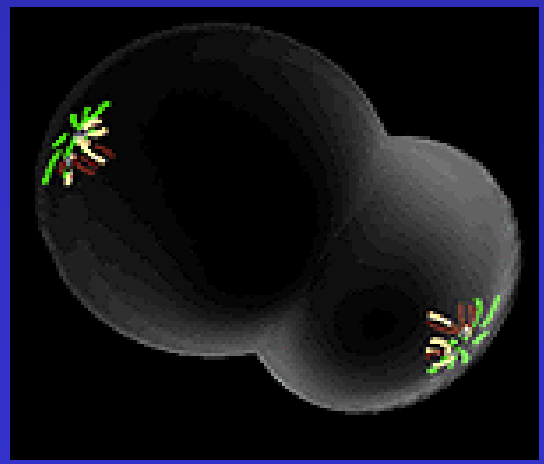
Metaphase I



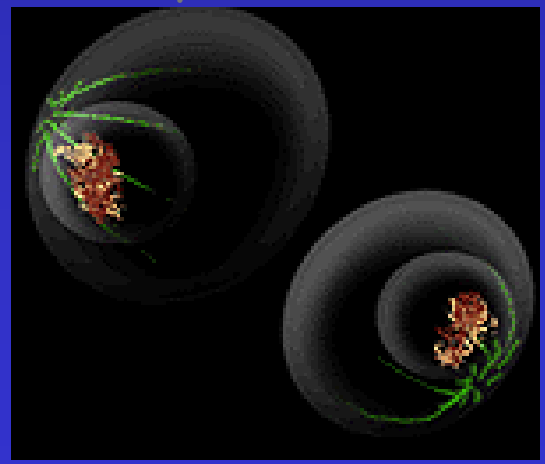
Anaphase I



Telophase I



Cytokinesis



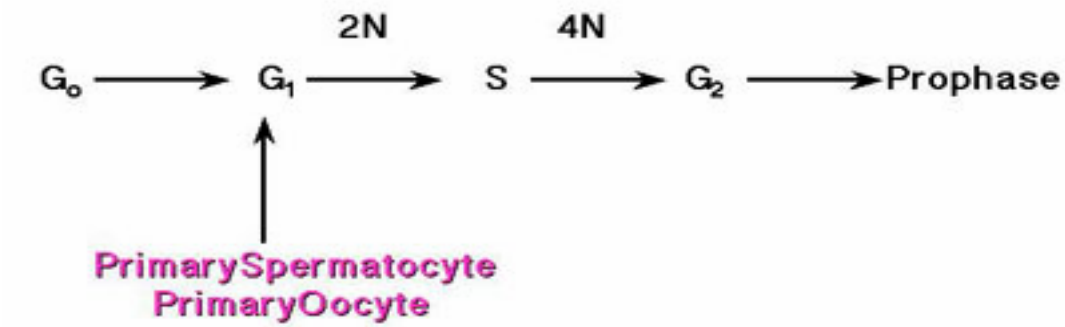
Meiosis a play ground for variability

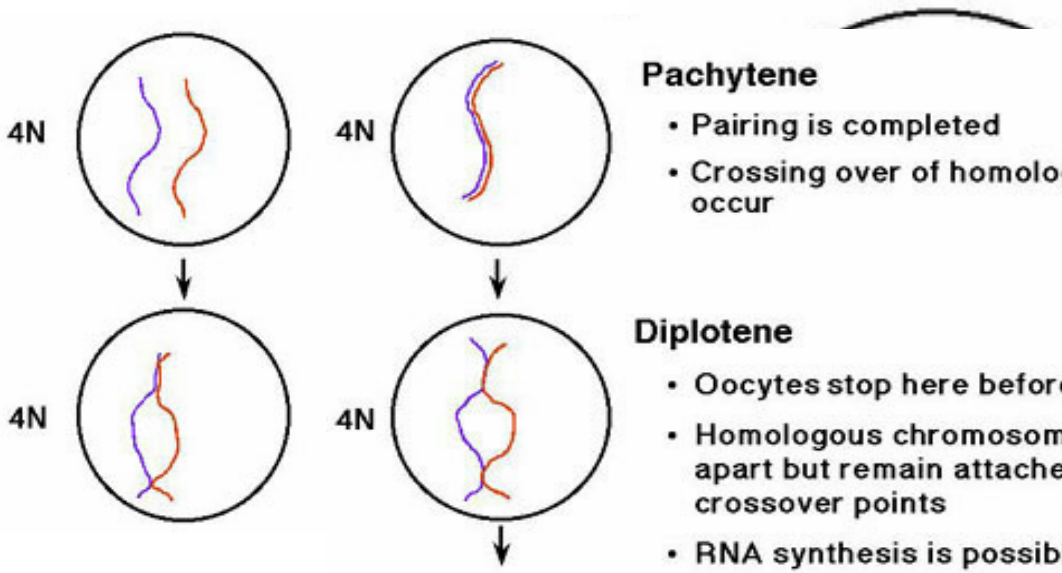
Players:

Synapsis
chiasma
recombination

Generation of variability

Meiosis





Pachytene

- Pairing is completed
- Crossing over of homologous occur

Homologous chromosomes move toward opposite poles and pair across from one another

Diplotene

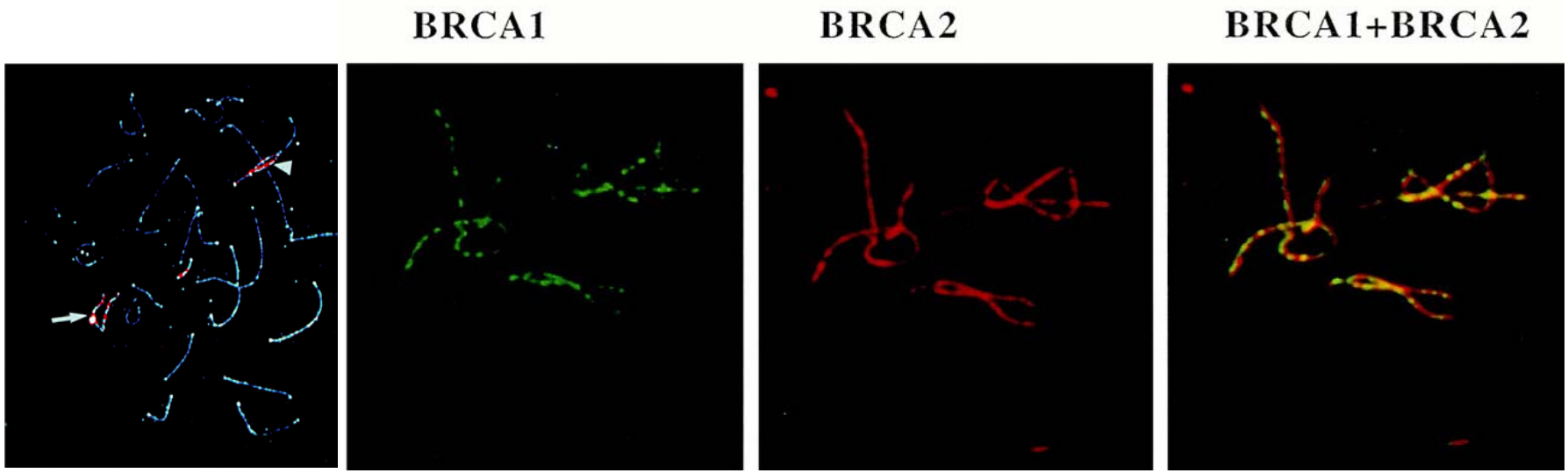
- Oocytes stop here before puberty
- Homologous chromosomes pull apart but remain attached at crossover points
- RNA synthesis is possible

logous
omes held
together via chiasma

Repair proteins function in meiosis

RAD51
ATM
BRCA1
BRCA2

BRCA1 functions in meiosis



BRCA1 and 2 localize to recombination nodules [Scully et al.,1997]

Meiotic errors

- **Nondisjunction**- homologues don't separate in meiosis 1

results in aneuploidy

usually embryo lethal

Trisomy 21, exception leading to Downs syndrome

Sex chromosomes

Turner syndrome: monosomy X

Klinefelter syndroms: XXY

- **Translocation and deletion**: transfer of a piece of one chromosome to another or loss of fragment of a chromosome.

Epigenetic changes

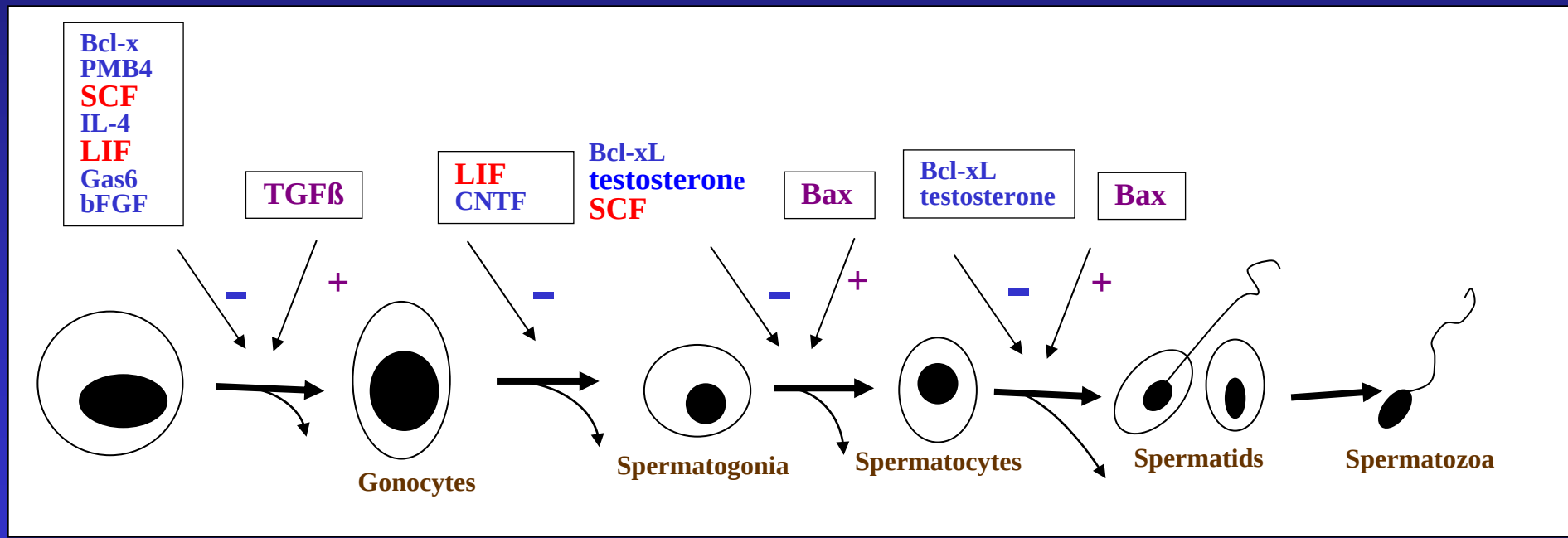
- **Genetics:** inheritance of information based on gene sequence
- **Epigenetics:** inheritance of information based on gene expression level
  - hypermethylation in GpC islands – effecting gene expression- silencing
  - hypomethylation derepression genome wide
- Epigenetics can be influenced by environmental factors

Methylation

- Methylation is a host defence mechanism for repression of parasitic DNA sequences
- Methylation cascade of events
  - Transcriptional silencing
  - Genome hypomethylation
  - Loss of imprinting
  - Defects in chromatin related genes> transformation

Epigenetic modifications are reversible=therapeutics

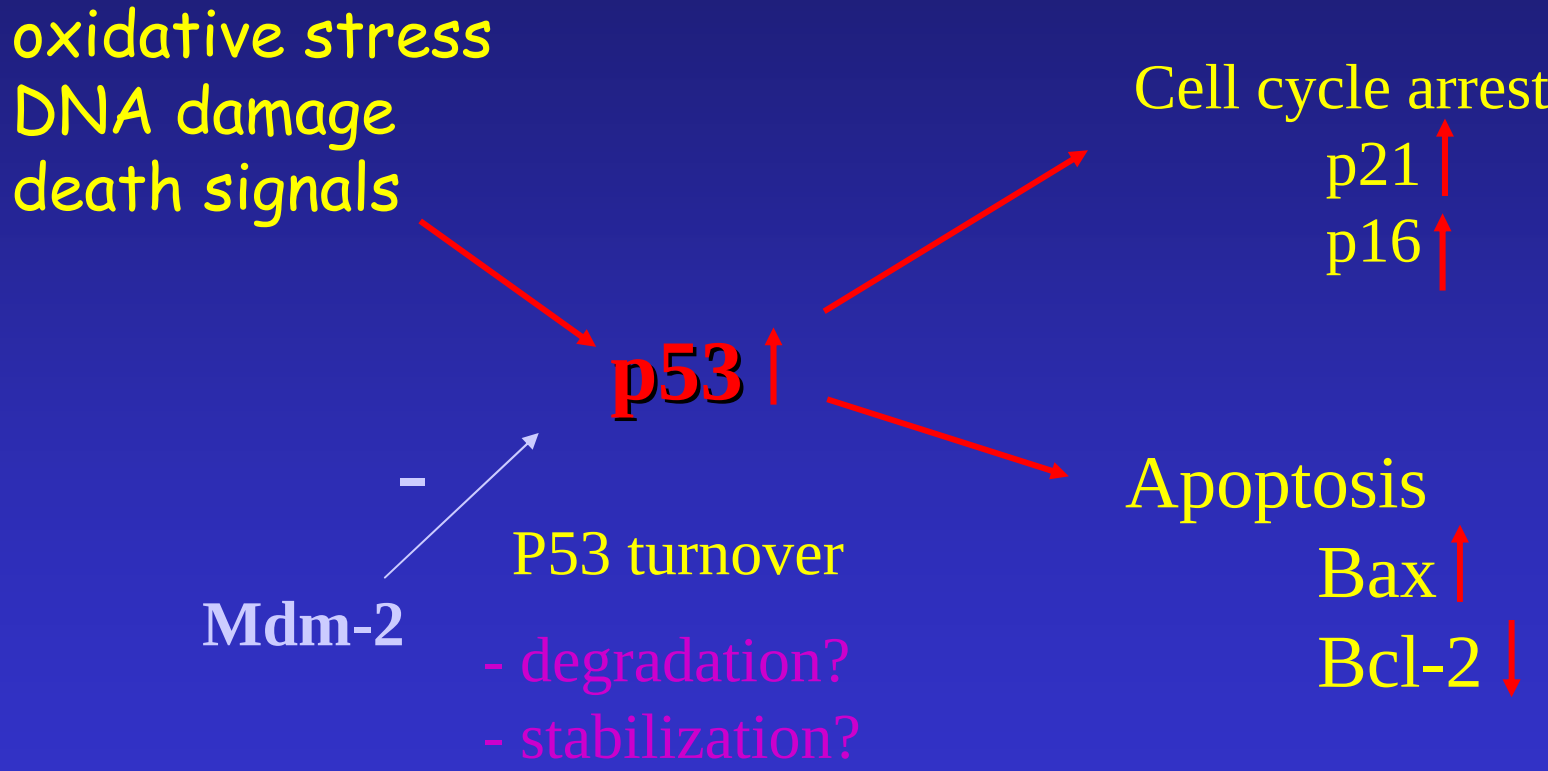
Integration of death and survival signals to suppress tumorigenesis



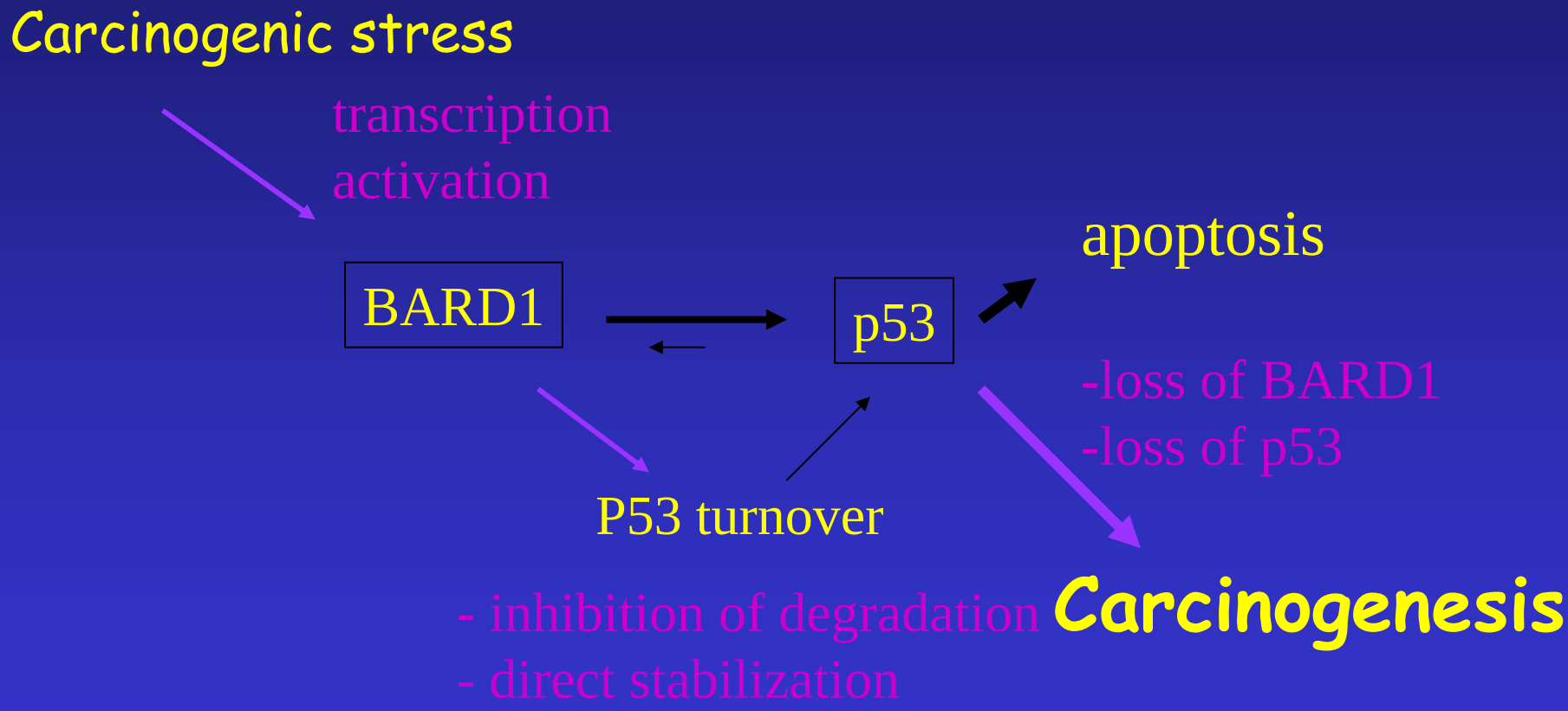
Male germ cells = stem cells - cancer cells

- germ cell-stem cell
  - immortal
  - LIF
  - SCF
  - stays undifferentiated
- Cancer cell
  - immortal
  - is (becomes) undifferentiated

Role of tumor suppressors in spermatogenesis and apoptosis



Role of BARD1 in apoptosis



Conclusion

- Rapid proliferation of germ cells (spermatogonia) needs homeostatic control and quality control?
  - Elimination by apoptosis before meiosis
- Generation of errors due to meiotic crossovers need repair or
  - Elimination by apoptosis
- Important integrator of repair and apoptotic signals **p53**