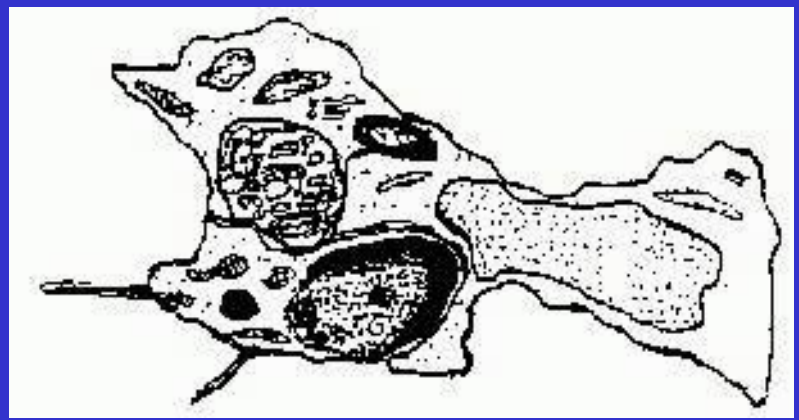
APOPTOSIS DURING PREIMPLANTATION EMBRYO DEVELOPMENT



Marie-Noël Bruné

CELL DEATH

"For every cell, there is a time to live and a time to die"

There are two ways in which cells die:

- Killed by injurious agents
- Induced to commit suicide

Death by injury: Necrosis

- Mechanical damage
- Exposure to toxic chemicals

Characteristic series of changes:

- Swelling
- Cell contents leak out

WHAT IS APOPTOSIS?

Synonyms: necrobiosis

From Greek: Dropping off or falling off (*Lodish et al, 2000*)

Definition:

A normal series of events in a cell that lead to its death (and then replacement)

Cells that are induced to commit suicide:

- Shrink
- Mitochondria break down releasing cytochrome c
- Nuclear chromatin (DNA and protein) degraded
- Break into small, membrane-wrapped, fragments
- Phagocytosis

WHY SHOULD A CELL COMMIT SUICIDE?

1. Programmed cell death is as needed for proper development as mitosis is

Examples:

- Resorption of the tadpole tail
- Formation of the fingers and toes of foetus

2. Programmed cell death is needed to destroy cells that represent a threat to the integrity of the organism.

Examples:

- Cells infected with viruses
- Cells with DNA damage

WHAT MAKES A CELL DECIDE TO COMMIT SUICIDE?

The balance between:

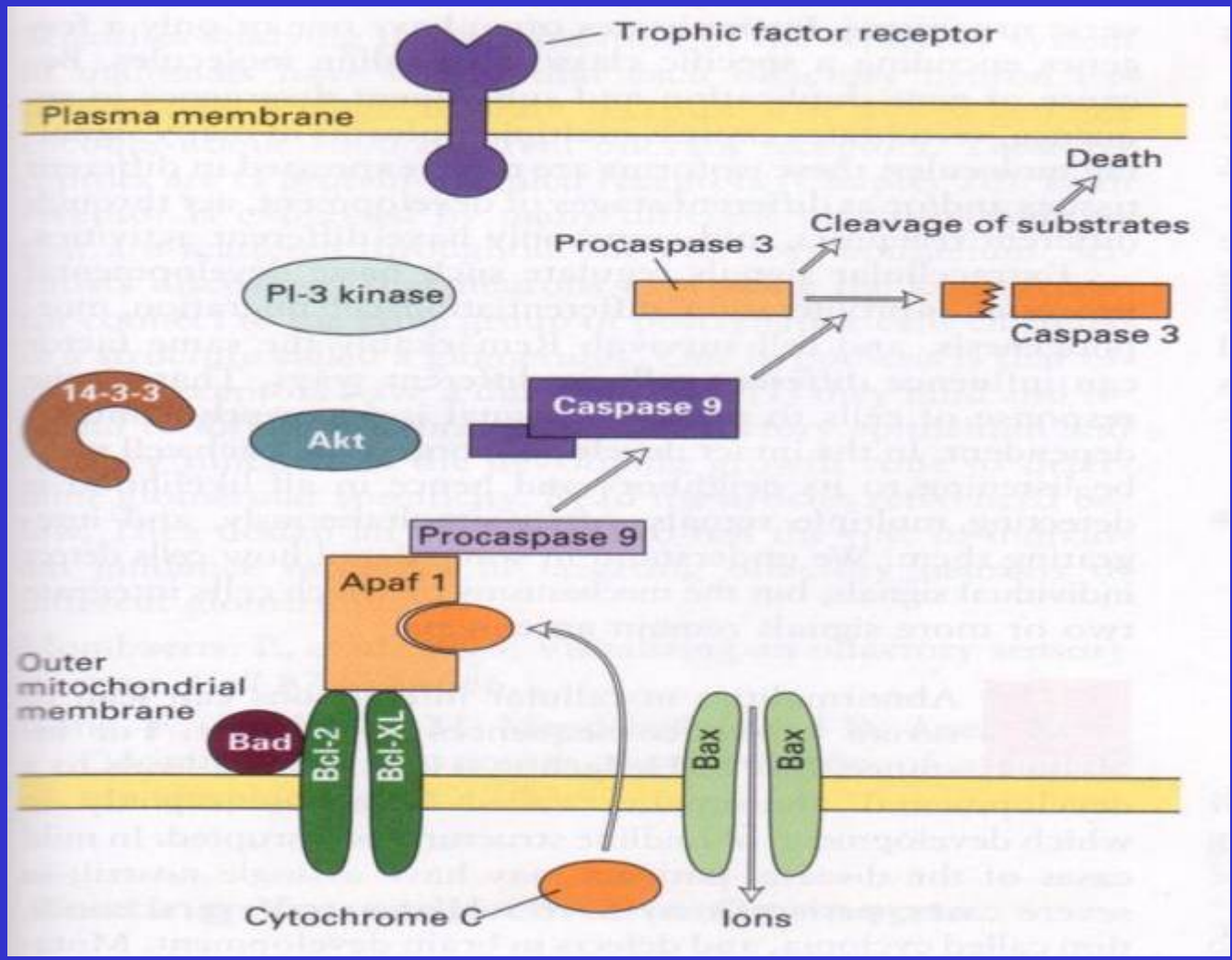
- **Withdrawal of positive signals**: trophic factors (neurotrophins for neurons)
- **Receipt of negative signals**: death activators bind to receptors (cell surface) and activate caspases

Others:

- Increased levels of oxidants within the cell
- Damage to DNA by oxidants or other agents

INTRACELLULAR APOPTOTIC PATHWAY

Pettman et al, 1998



HOW CAN WE MEASURE APOPTOSIS?

- **Conventional morphological observation:**
  - Haematoxilin / eosin stained routine sections
  - Confocal microscopy
- **Other methods:** COMET
  - Single cell microgel electrophoresis of embryos
  - Comet-like appearance
  - **Visualization:** Fluorescent staining of DNA

HOW CAN WE MEASURE APOPTOSIS?

Apoptosis Detection Kits – ApopNexin (Serologicals Inc)

-Detects translocation of phosphatidylserine to outer membrane surface (early event in apoptosis)

-Distinguish apoptosis from necrosis in live cells.

-Results in less than one hour

-Visualization:Microscopy/image analysis systems

HOW CAN WE MEASURE APOPTOSIS?

Caspase Activity Detection - CaspaTag (Serologicals Inc)

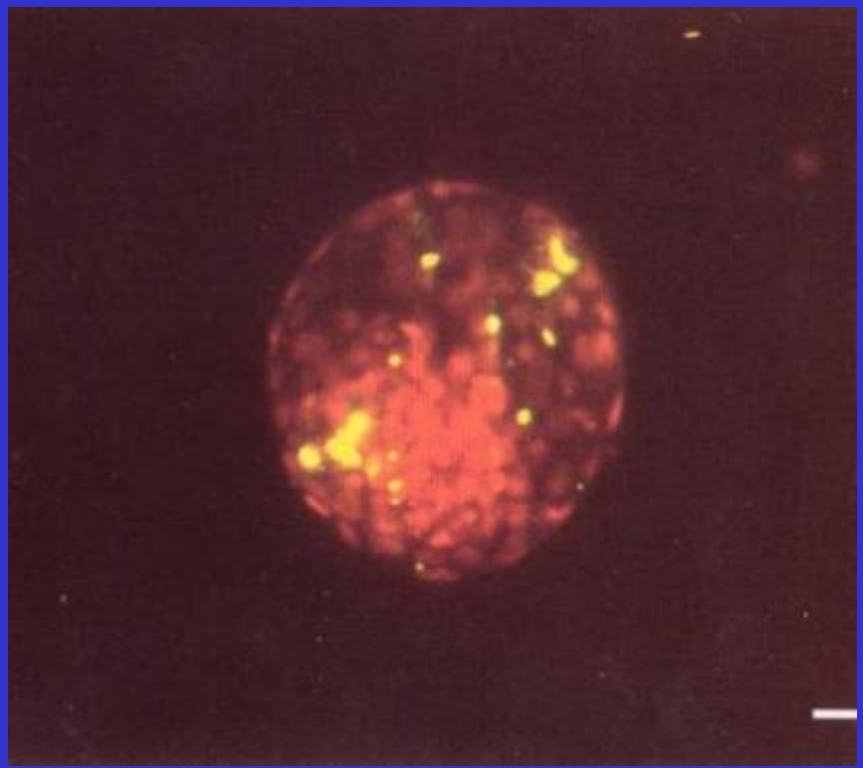
- Detects active caspases in live cells
- Carboxyfluorescein labelled caspase inhibitor irreversibly binds to active caspase
- **Fast**: Completed assay within 70 minutes
- **Safe**: Non-toxic and cell permeable with live cells
- **Visualization**: fluorescence microscopy

HOW CAN WE MEASURE APOPTOSIS?

TUNEL: [TdT]-Mediated [dUTP] Nick End Labelling

- Uses TDT enzyme
- Shows broken DNA ends by addition of labelled nucleotides
- **Visualization**: Fluorescence (*Mori C., et al.1994*)
- **Disadvantages**: False positive and negative results due to variable time in fixation (Upstate Biotechnology Inc., USA)

HOW CAN WE MEASURE APOPTOSIS?



Apoptosis Analysis of Nuclei in Bovine Embryos by TUNEL

Day 7 expanded blastocyst - Apoptotic nuclei can be seen in yellow

(*Byrne A.T et al, 1999*)

APOPTOSIS IN PREIMPLANTATION EMBRYO

- DNA fragmentation: a consequence, not a cause of embryo arrest
- Cell death depends on embryo quality
- Elimination of abnormal cells
- Elimination of nuclear and chromosomal abnormalities
- Removal of embryos that fail to activate the embryonic genome
- In blastocyst: elimination of ICM cells (potentially trophoectodermic)

FACTORS THAT CAN INDUCE APOPTOSIS

- **Oxidative stress:**
  - Senescence
  - Arrest of DNA repair

- **Low quality embryos (IVF):**

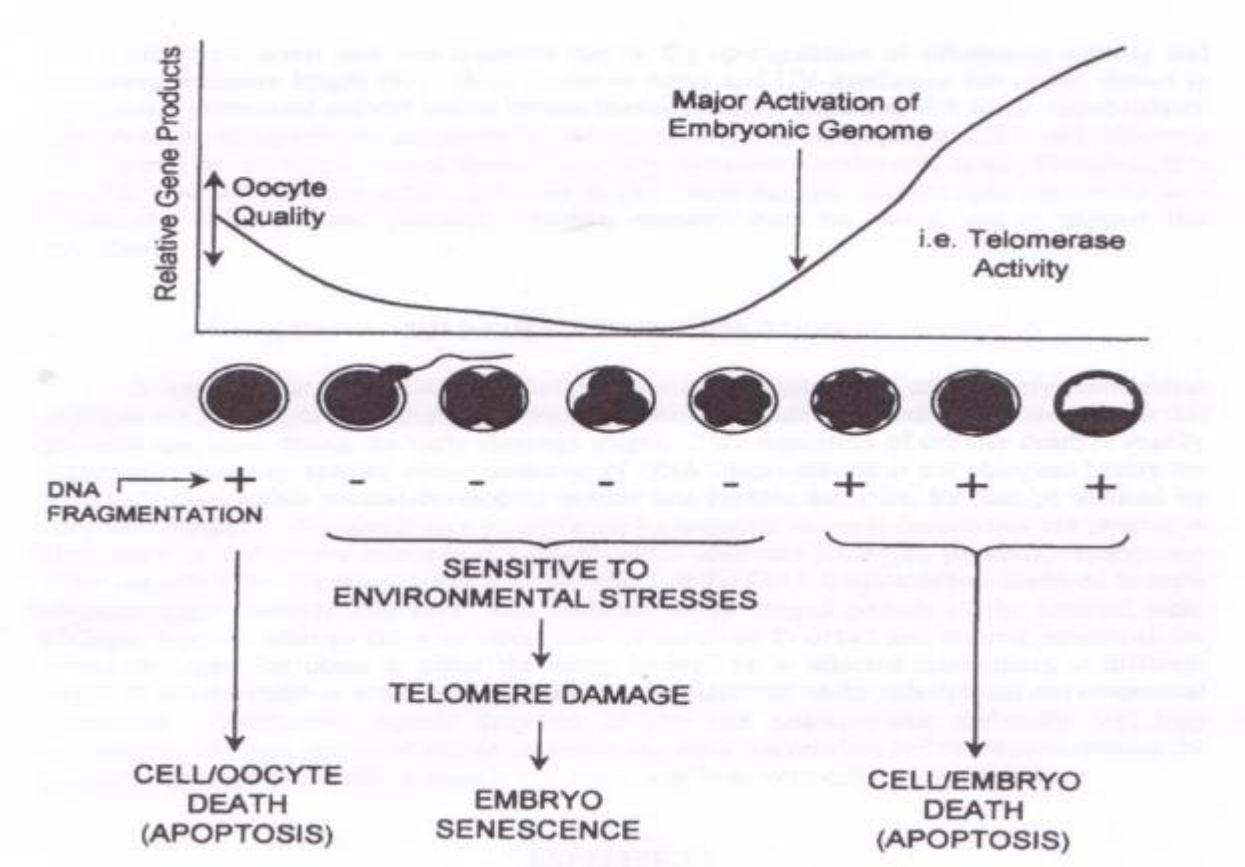
  Sub-optimal in vitro conditions:

  -TGF-alpha or IGF-I – positive

  -TNF-alpha – more fragmentation

  -FBS?

MODEL FOR EMBRYO DEATH AND ARREST



Betts et al (2001)

SURVIVING EMBRYOS

- Apoptosis may be inhibited by maternal survival factors (*Betts et al, 2001*)

- Need for genetic detection of pro and anti-apoptotic molecules

- Ironically: inverse correlation between cell death and survival of organism (*Moley K.*)