



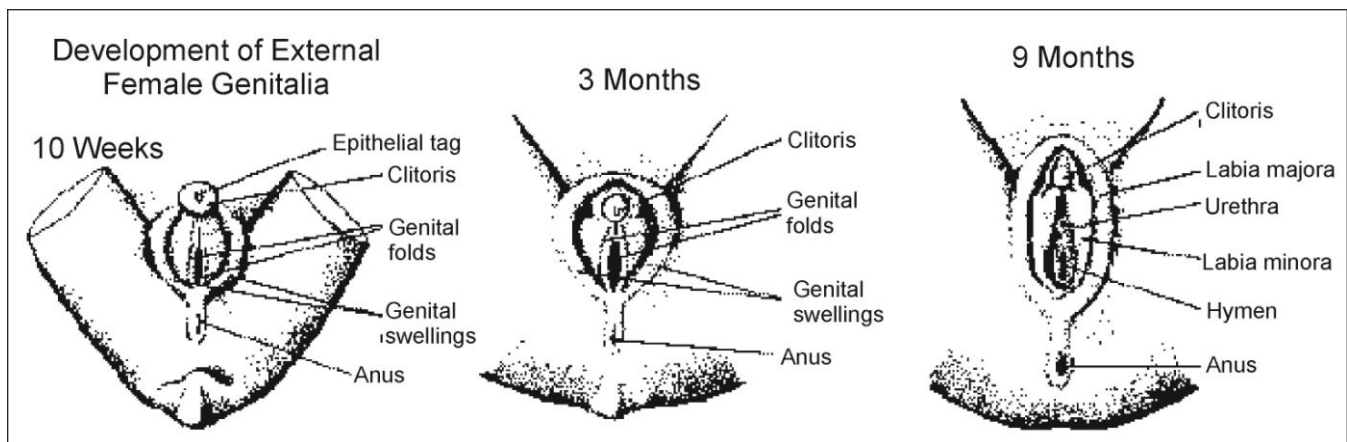
## Life & Death in the Ovary

### Introduction

The female produces eggs from primordial germ cells (covered in 2nd lecture) that have entered her genital ridges during embryonic development. The process of egg formation thus begins in the embryo and continues into the adult. In this lecture, we will first examine the structure of the female anatomy finally focusing in upon the ovaries where oogenesis (egg formation) occurs. We will examine the development of the egg follicle and the layers of cells and extracellular material that surrounds the egg prior to ovulation. We'll then quickly remind you about the important role of hormones in the reproductive cycle of females, a topic that is covered in detail in physiology courses. The role of hormones in sexual maturation and reproduction is not covered in detail in this course.

### Development of the External Genitalia

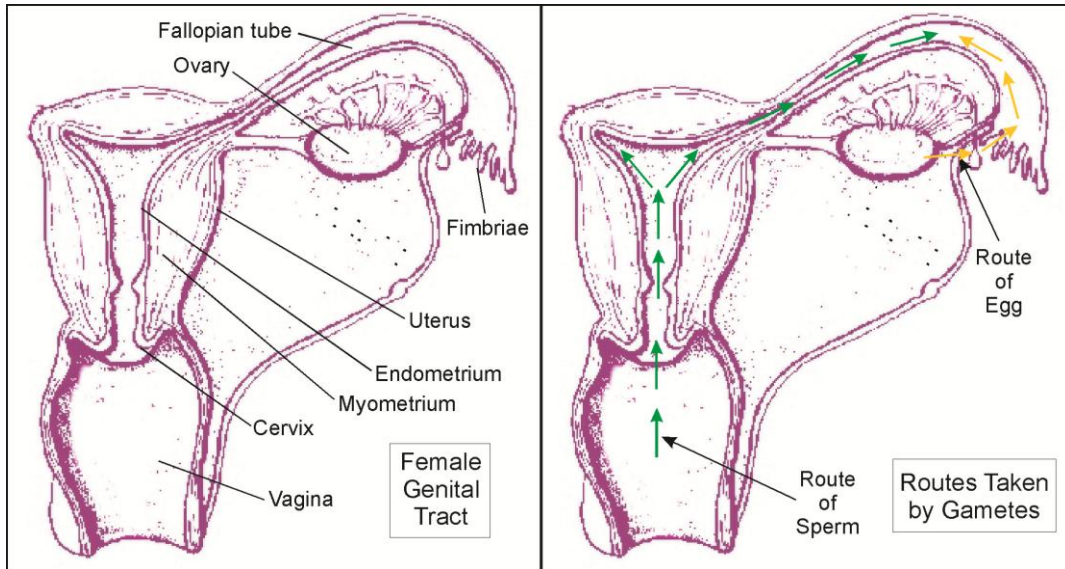
While most students understand the fundamentals of human anatomy, not everyone does. For this reason and because it is important to put every process and event into perspective, it is important for us to know the reproductive anatomy of the female if we are to first appreciate where and how eggs are formed and later released prior to fertilization. Before we look at the mature female anatomy, let's take a quick look at the development of the external genitalia by looking at the figure below. Later, when we look at the early development of the male genitalia we'll become aware of some very basic similarities between the two processes.



### Female Genital Tract

The picture below right shows the route the eggs and sperm will take through the female genital tract. Fertilization, as will be discussed in a future lecture, occurs in the upper region of the fallopian tubes. Follow the pathway for sperm entry into the genital tract (green arrows) and that of the egg after release from the ovary (gold arrow) and its movement into and down the fallopian tube. Fertilization commonly occurs approximately where the brown and orange arrows meet.

## Life & Death in the Ovary

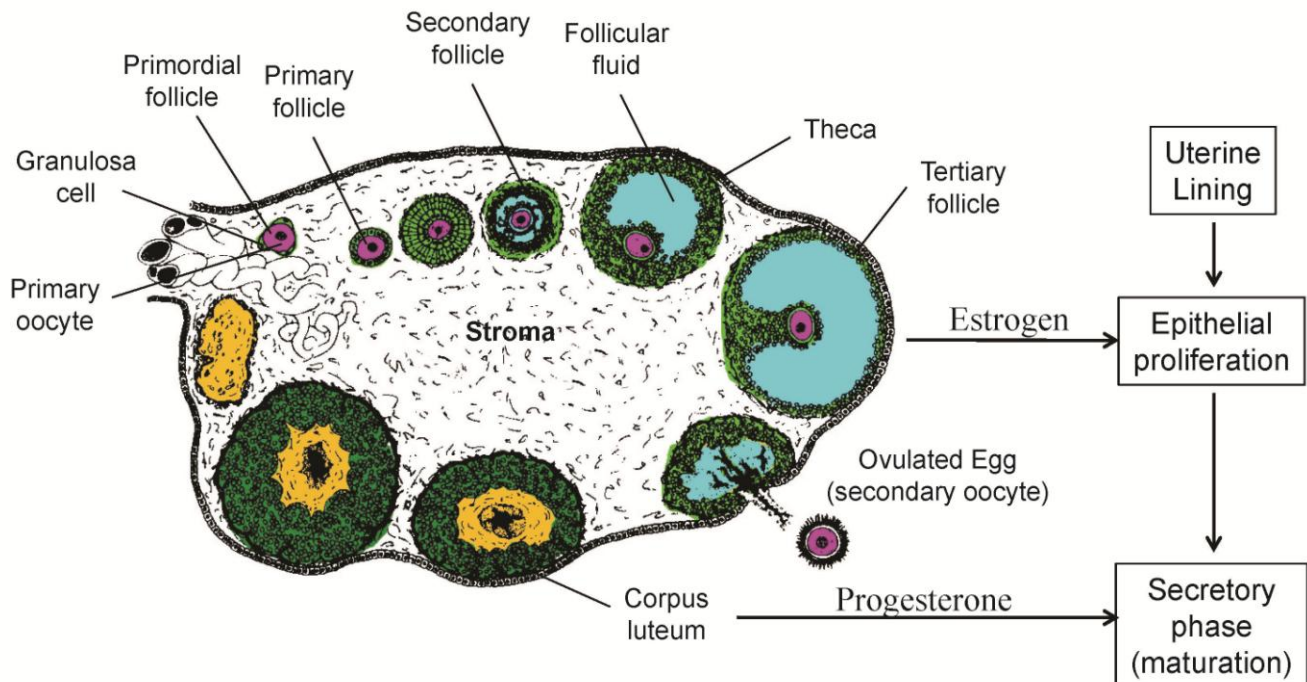


**The female genital tract is specifically designed to:**

- Produce Ova
- Accept Sperm
- Control the Process of Fertilization
- Provide a Site for Implantation of the Egg
- Provide Essentials for Fetal Development to Term

### The Ovary & Follicle Development

The ovary doesn't appear to be a very organized structure. Each stage of oogenesis and follicle maturation is haphazardly strewn throughout both the left and right ovaries. However, since it is not our goal to be reproductive anatomists, we will use a typical textbook picture which sorts things out for us:



## Life & Death in the Ovary

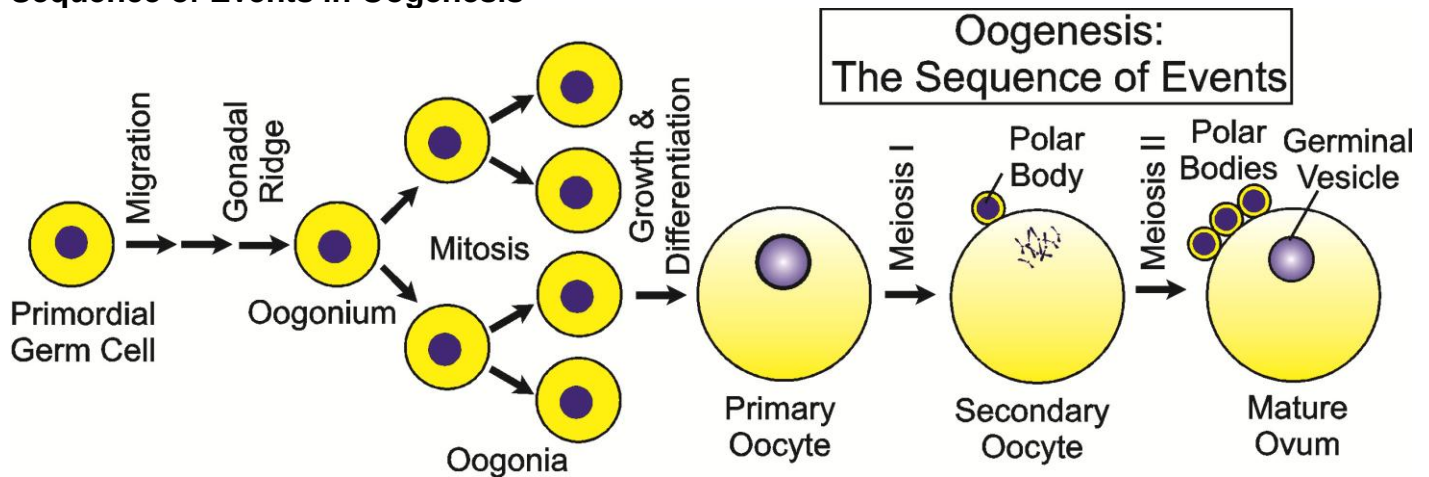
The above figure also emphasizes the events in follicle and egg development. During embryogenesis the first follicles that form are called primordial follicles: the granulosa is 1 cell layer thick and the oocyte is arrested (has stopped developing) at prophase I of meiosis. Over time there is an increase in the number of granulosa cells and they become stratified and are now referred to as the theca. As well, the fluid filled cavity or antrum around the oocyte continues to fill with follicular fluid. In keeping with these changes, the follicles are progressively named primary, secondary and tertiary (or mature) follicles. In the figure, the follicle cells (green), follicular fluid (cyan) and developing oocyte (magenta) have been coloured to clearly demonstrate the changes in the follicle as the egg matures and is finally ovulated. Subsequently, the follicle undergoes changes as it becomes a hormone producing corpus luteum (gold, dark green).

Estrogen, released from the maturing follicles, causes the uterine epithelial lining to proliferate in preparation for a fertilized egg. Progesterone released from the corpus luteum will further mature the uterine lining causing it to enter the secretory phase which will be able to interact with the blastocyst should fertilization occur. These hormonal changes plus many others controlled by the pituitary lead to the monthly menstrual cycle of females, a topic covered in detail in physiology courses.

### Gametogenesis in the Female

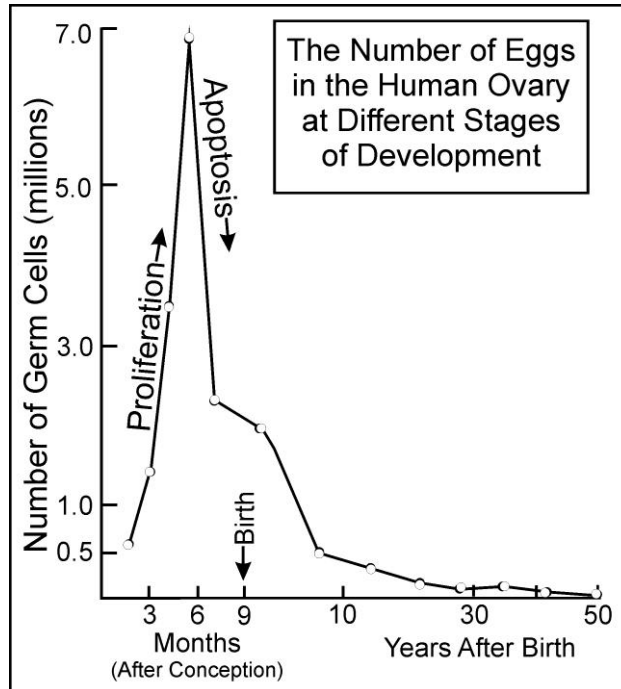
- Oogonia undergo mitosis only in embryo
- Oogenesis ends at menopause
- Two meiotic blocks occur: Prophase I & Metaphase II

### Sequence of Events in Oogenesis



- Primordial Germ Cells migrate into gonadal ridge
- Each PGC becomes an oogonium
- Oogonia multiply by mitosis
- Oogonia enter a phase of growth & differentiation
- Primary Oocyte grows and specializes
- Nucleus is called germinal vesicle
- Meiosis I generates Secondary Oocyte + Polar Body
- Meiosis II produces Mature Ovum + Polar Body

### Changes in Germ Cell Numbers



- Increases to ~7 million by 6 months *in utero*
- Then # decreases due to apoptosis
- Only ~2 million at birth
- ~0.5 million at puberty
- Continual decline until menopause: ovulation and death.
- In females, germ cells (oogonia) typically undergo a total of ~24 cell divisions
- In males, the germline cells (spermatogonia) divide continuously throughout life

### Apoptosis: Cell Death is Good For You

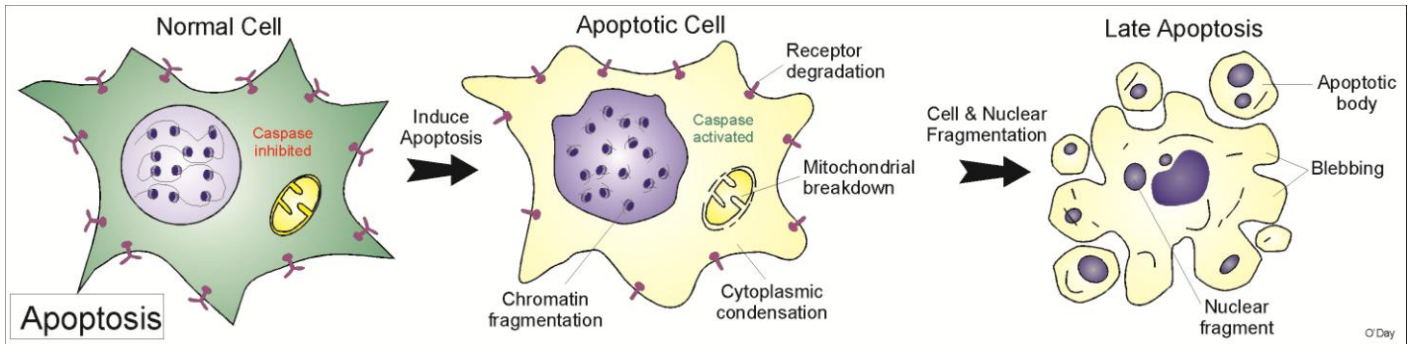
The death of cells is an important part of human development. It occurs during oogenesis, brain development and the formation of our toes and fingers, to name a few events that will be discussed in this course.

Apoptosis is the formal name for the controlled regulation of cell death. It involves the activation of specific genes and signal transduction pathways that underlie the cell death program. Unlike tissue necrosis caused by external damage, it is a controlled process in which cells show a precise breakdown in a series of well-defined steps that are under active study. Apoptosis allows the body to remove specific cells without damaging surrounding cells and tissues. Here we present a short outline of apoptosis.

#### Apoptosis is characteristic by specific morphological features

1. Cell shrinkage: cells become smaller and lose cell-cell contacts
2. Chromatin condensation: chromatin initially condenses to the periphery of the nucleus and ultimately nuclear fragmentation occurs. During these events DNA is digested in specific ways leading to what is called "laddering" in DNA gels.
3. Cell membrane blebbing (small bulges on cell surface) occurs
4. Cell fragmentation ("apoptotic bodies" are formed) and phagocytosis of these by macrophages.

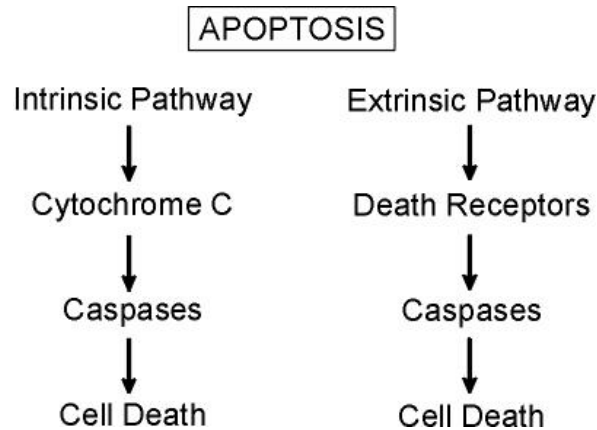
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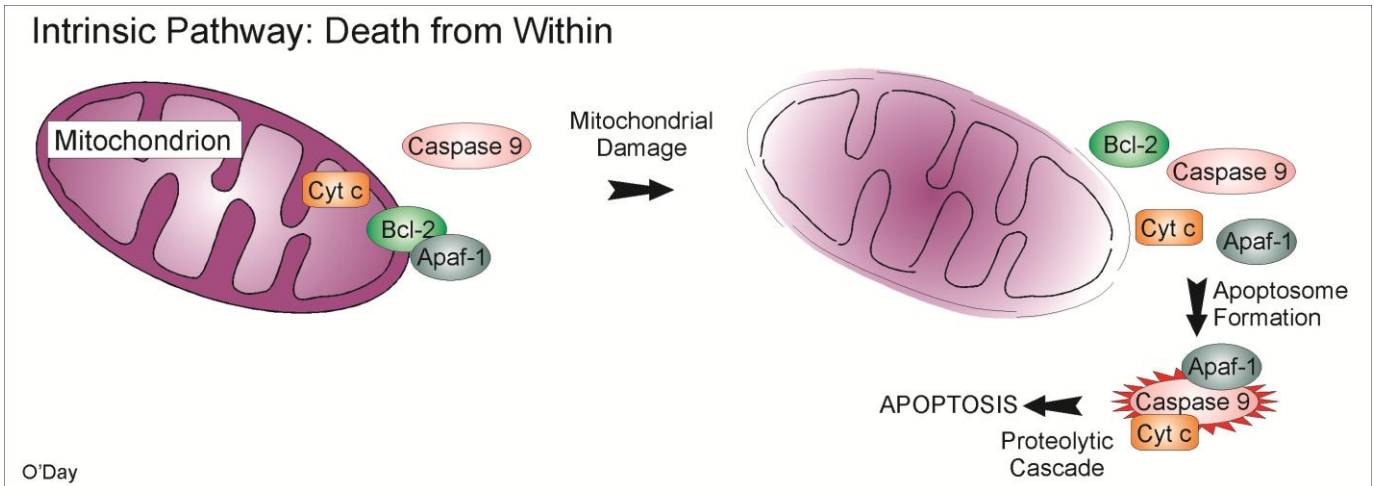
Apoptosis is regulated by a diversity of signalling pathways all of which involve caspases. There is a large family of caspases in humans that exist in an inactive form (pro-caspase) that becomes activated by limited proteolysis: **procaspase** → **active caspase**.

Caspases are a family of cysteine proteases, protein-digesting enzymes that cleave proteins after aspartic acid residues. The caspases work in cascades (a number of caspases working in sequence) digesting a diversity of proteins that underlie specific apoptotic events. Typically initiator caspases (e.g., caspase-2, 8, 9, 10) activate effector caspases (e.g., caspase-3, 6, 7) that digest specific proteins or activate other specific caspases (e.g., caspase-1, 4, 5, 11, 12, 13, 14) that have roles in inflammation. Effector caspases are sometimes called executioner caspases.

### Caspase Cascade: Initiator Caspases → Effector/Excutioner Caspases

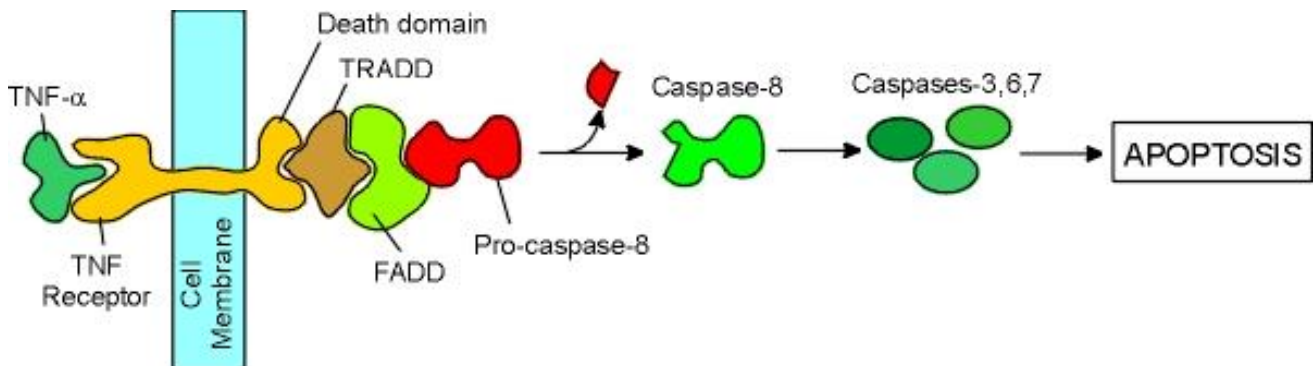


There are many variations on how apoptosis is regulated. Apoptosis can be initiated by internal events (i.e., "Intrinsic Pathway") involving the disruption of mitochondria and the specific enzyme cytochrome C, in turn leading to the downstream activation of caspases. In the following example, when mitochondria are disrupted, cytochrome C (located on the inner mitochondrial membrane, gets released allowing it to bind to and activate caspase 9. Similarly Apaf-1 (Apoptotic activating factor-1) is bound to mitochondria via Bcl-2 (beta-cell lymphoma-2). Bcl-2 is found in a number of cancers where it suppressed apoptosis. Mitochondrial disruption leads to the release of Apaf-1 which also binds to caspase 9 activating it. This is just one example of many intrinsic pathways.



Alternatively, surface receptors can be activated by specific ligands that bind to "death receptors" (i.e., "Extrinsic Pathway"). Death receptors are members of the tumour necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily. They make up a subfamily characterized by the intracellular death domain (DD). The extrinsic pathway is typically mediated by immune cells, to initiate intracellular signaling and the downstream activation of relevant caspases. Some work suggests both Intrinsic and Extrinsic Pathways mediate the apoptosis during oogenesis and likely of aging eggs after fertilization.

The following diagram shows some of the signaling events that are initiated when tumor necrosis factor alpha (TNF- $\alpha$ ) leads to apoptosis. It should be noted that TNF- $\alpha$  also mediates other signaling pathways involved in normal cellular functions.



The binding of TNF- $\alpha$  to its receptor (TNF-receptor or TNFR) makes the receptors intracellular death domain available for binding to TRADD (TNFR-associated death domain). TRADD is an adaptor that in turn directs the binding of FADD (Fas-associated death domain) another adaptor that mediates the binding of pro-caspase-8 to this multiprotein complex. This leads to the proteolytic processing of the inactive pro-caspase-8 into the active caspase-8 enzyme. Caspase-8 is an initiator caspase that in turn proteolytically activates several other caspases. The activated caspases-3,6 and 7 are effector caspases that proteolytically digest a number of target proteins ultimately leading to apoptosis. There are a number of other apoptosis-specific pathways each of which involves unique sets of adaptor proteins and caspases and each of which is designed to direct apoptosis at a specific place or time in human development or other aspects of cell function.

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