

# **CELL DEATH DURING DEVELOPMENT**

**LLOYD A. GREENE**  
**DEPT OF PATHOLOGY AND CELL BIOLOGY**

**DECEMBER 3, 2007**

# KEY DEVELOPMENTAL PROCESSES

**CELL PROLIFERATION**

**CELL MIGRATION**

**CELL DIFFERENTIATION**

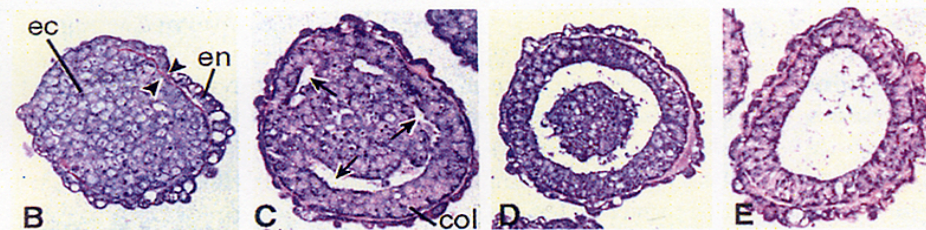
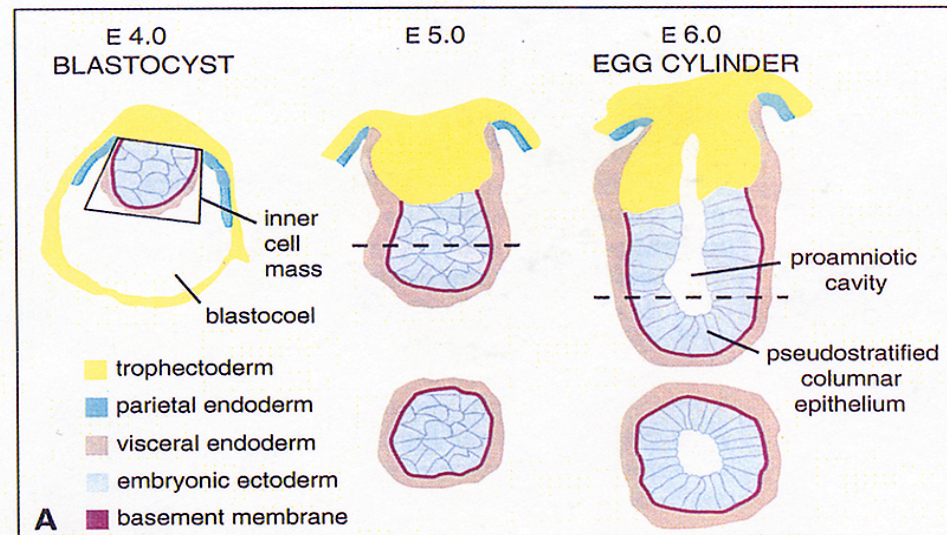
**CELL DEATH**

# FUNCTIONS OF DEVELOPMENTAL CELL DEATH

## A. MORPHOGENESIS: SCULPTING/SHAPING STRUCT

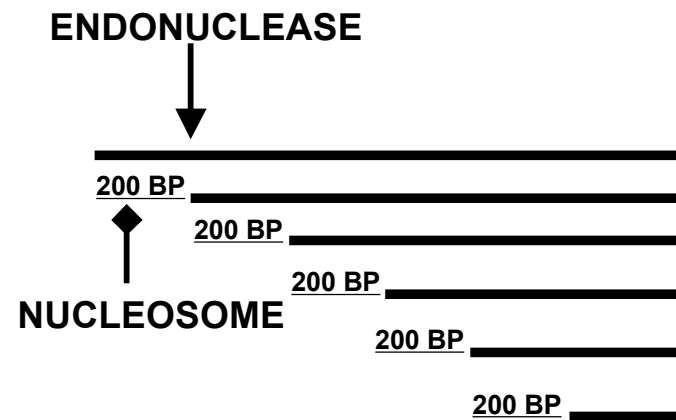
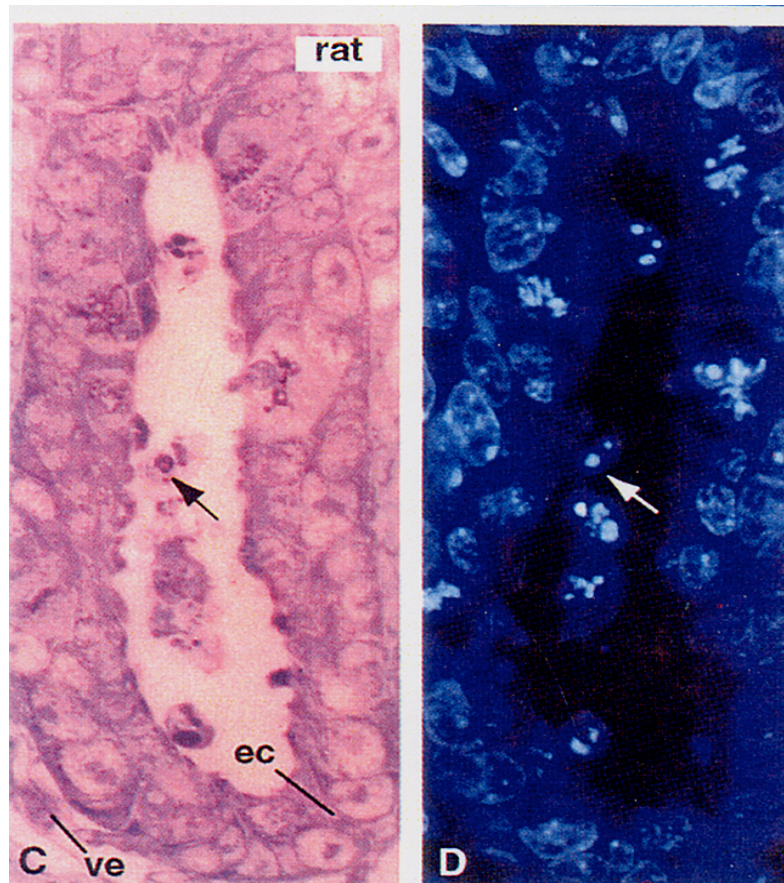
CREATION OF CAVITIES AND TUBES

# CELL DEATH AND FORMATION OF THE PROAMNIO CAVITY FROM THE BLASTOCYST 1



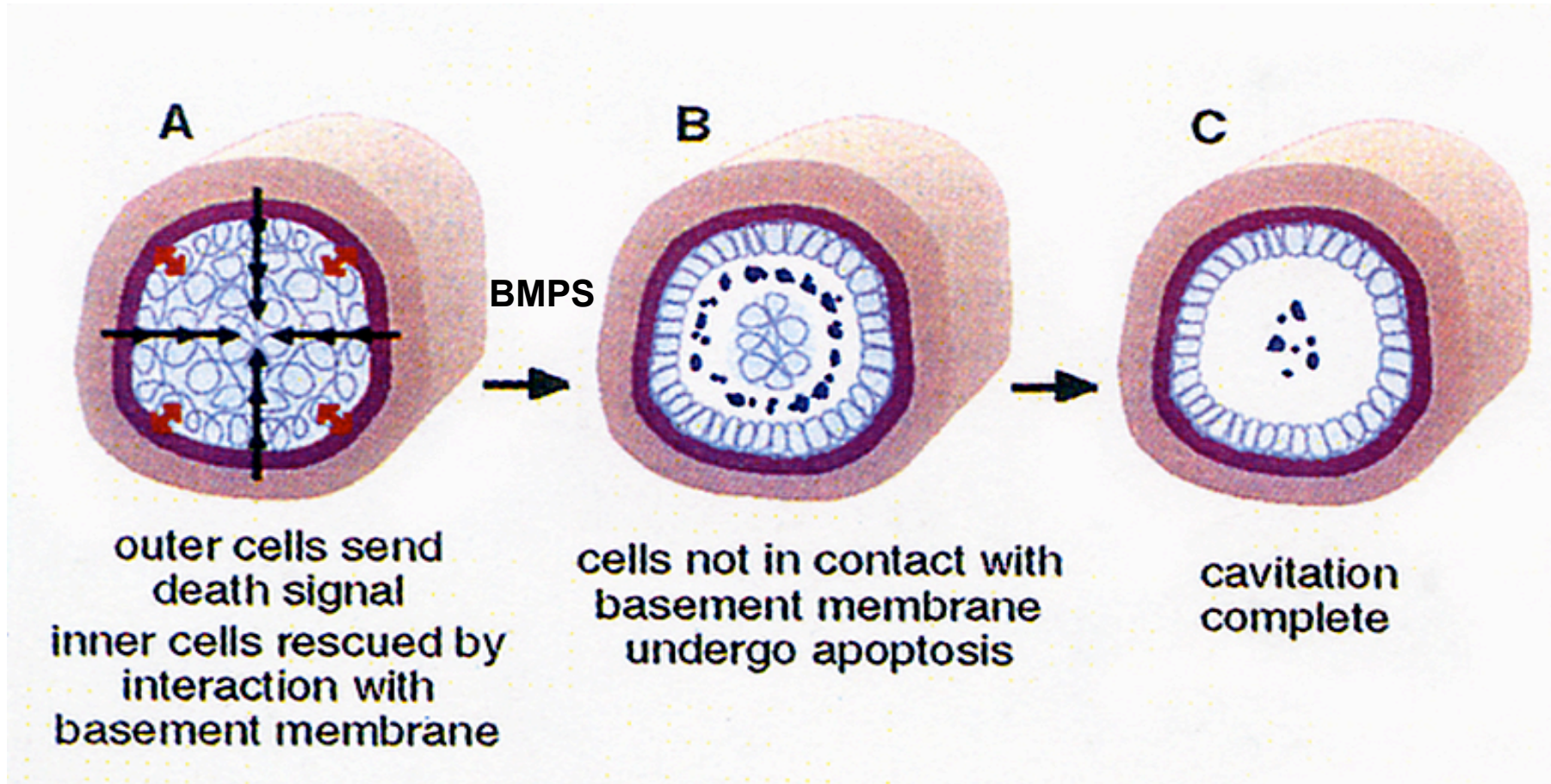
FROM: Coucouvanis and Martin. Cell 83: 279-287 (1995)

# CELL DEATH AND FORMATION OF THE PROAMNIO CAVITY FROM THE BLASTOCYST 2



FROM: Coucouvanis and Martin. Cell 83: 279-287 (1995)

# CELL DEATH AND FORMATION OF THE PROAMNIO CAVITY FROM THE BLASTOCYST 3



FROM: Coucouvanis and Martin. Cell 83: 279-287 (1995)

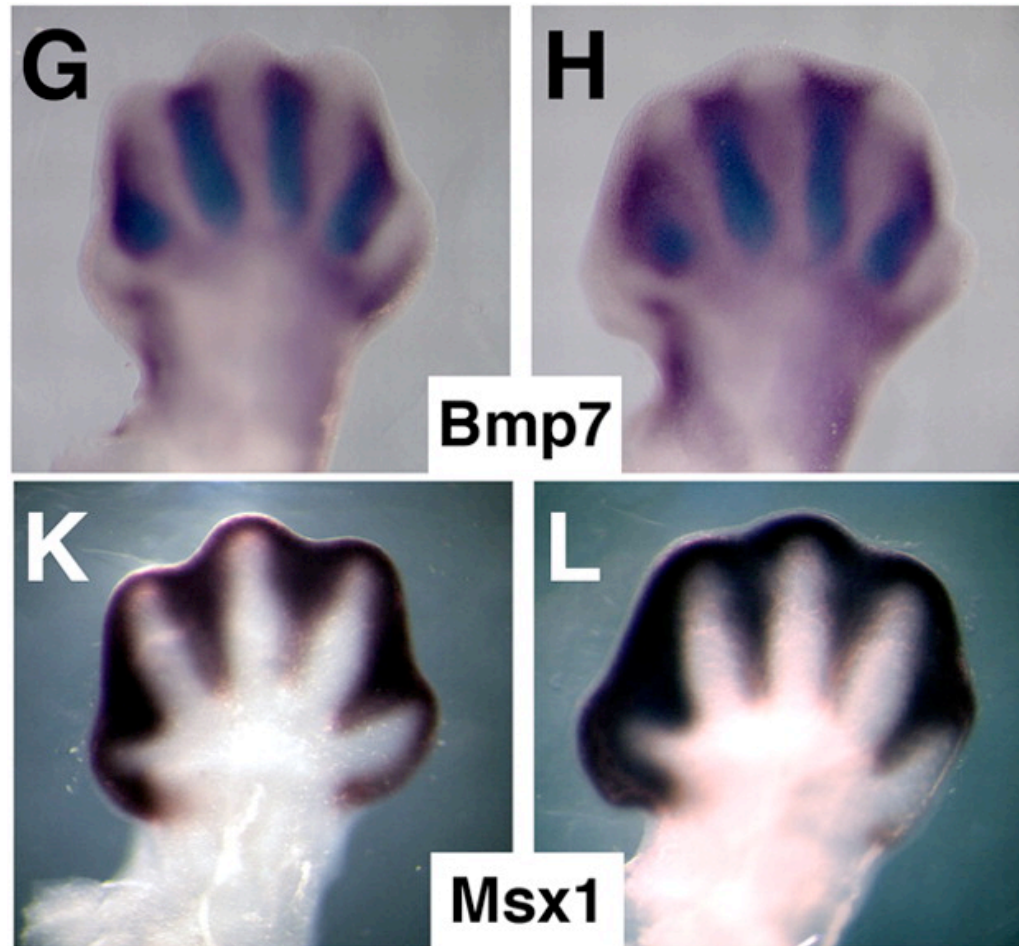
# FUNCTIONS OF DEVELOPMENTAL CELL DEATH

## A. MORPHOGENESIS: SCULPTING/SHAPING STRUCT

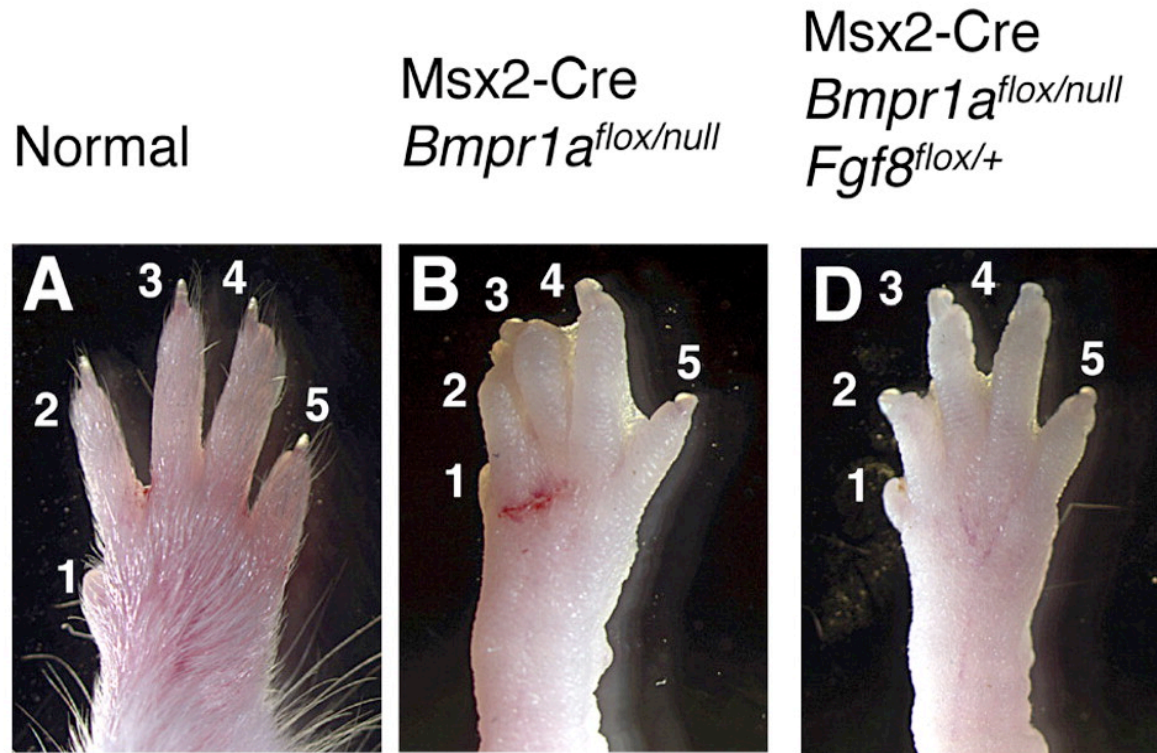
CREATION OF CAVITIES AND TUBES

CREATION OF FORM (DIGITS)

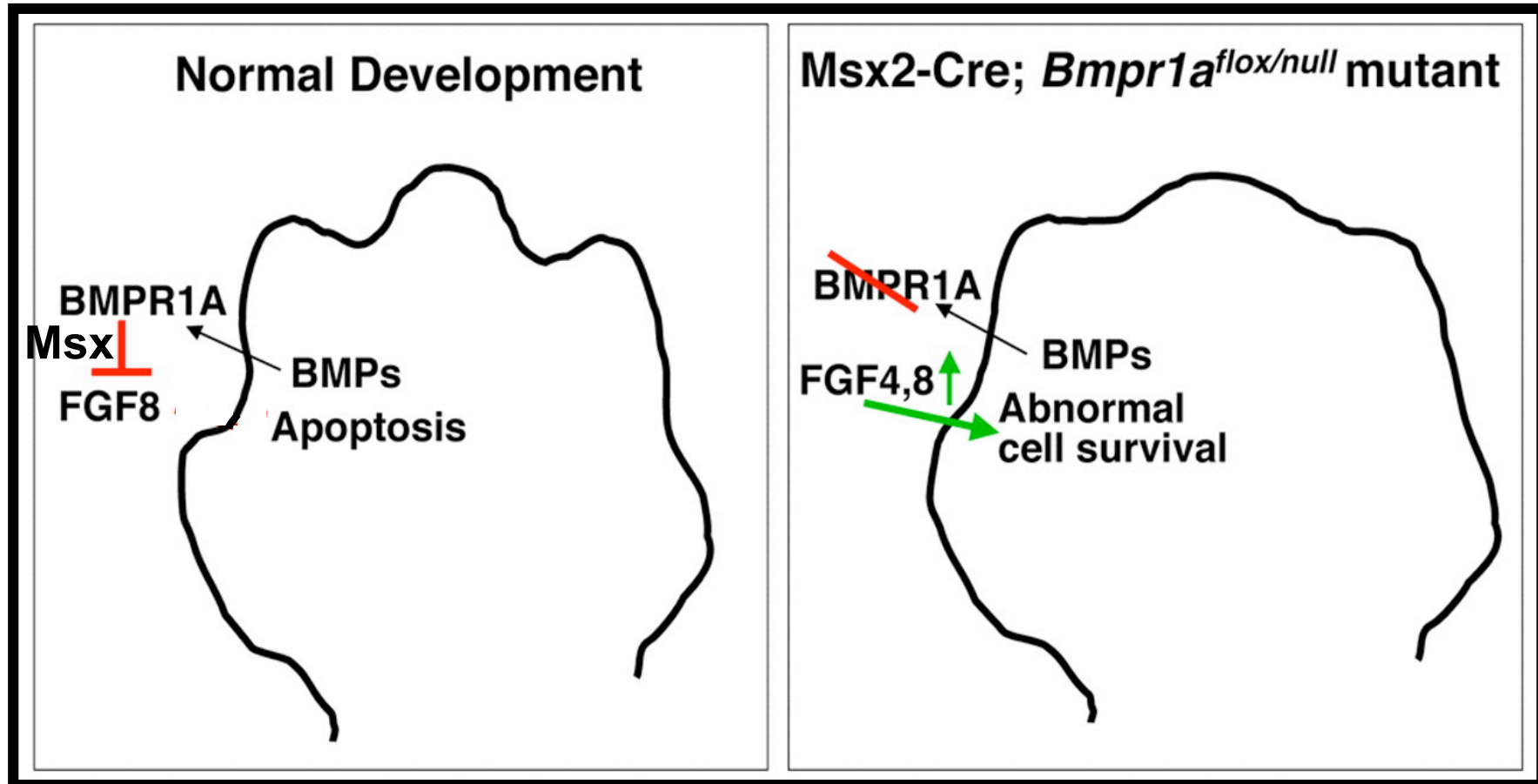
# CELL DEATH AND FORMATION OF DIGITS 3



# BMP AND MSX CAUSE CELL DEATH IN THE INTERDIGITAL ZONE BY INHIBITING FGF SIGNALING



# MODEL FOR HOW BMPs PROMOTE CELL DEATH IN THE EMBRYONIC INTERDIGITAL ZONE

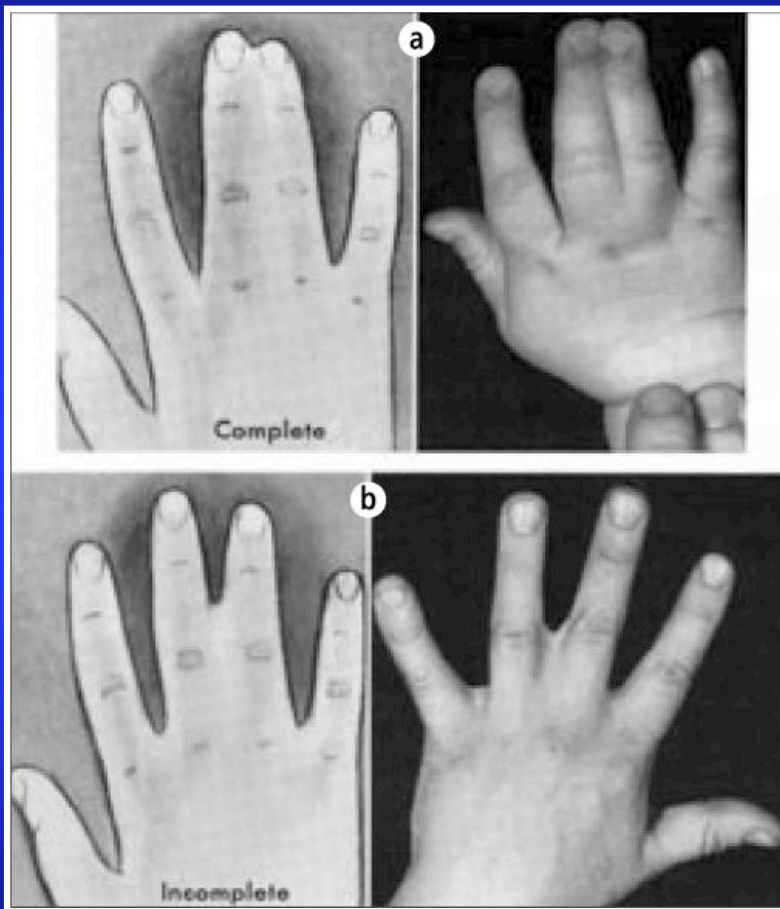


# HUMAN SYNDACTYLY

**SIMPLE**

**COMPLEX**

**FOR EXAMPLE: ONE  
FEATURE APERT  
SYNDROME - CAUSED BY  
ACTIVATING MUTATIONS IN  
FGF2 RECEPTORS**



From: Flatt AE. Proc (Bayl Univ Med Cent). 2005 Jan;18(1):26-37.

# FUNCTIONS OF DEVELOPMENTAL CELL DEATH

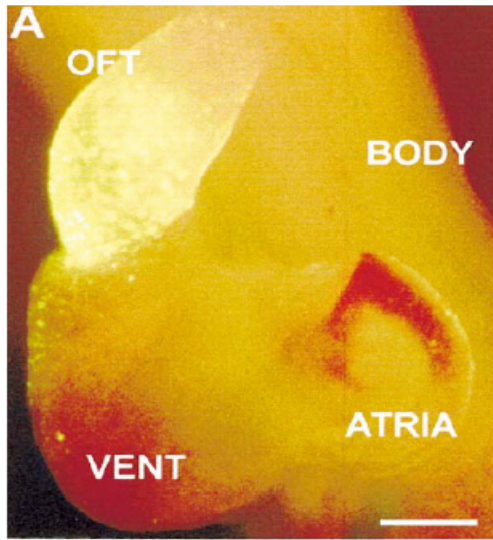
## A. MORPHOGENESIS: SCULPTING/SHAPING STRUCTURE

CREATION OF CAVITIES AND TUBES

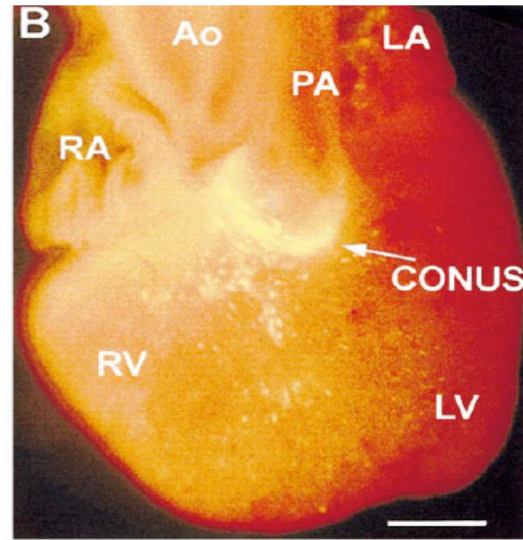
CREATION OF FORM (DIGITS)

TISSUE REMODELING (CARDIAC OUTFLOW TRACT)

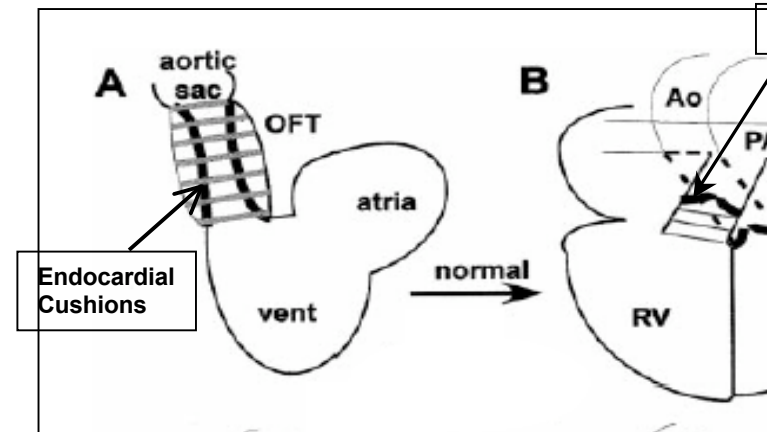
# CELL DEATH AND CARDIAC MORPHOGENESIS



STAGE 23

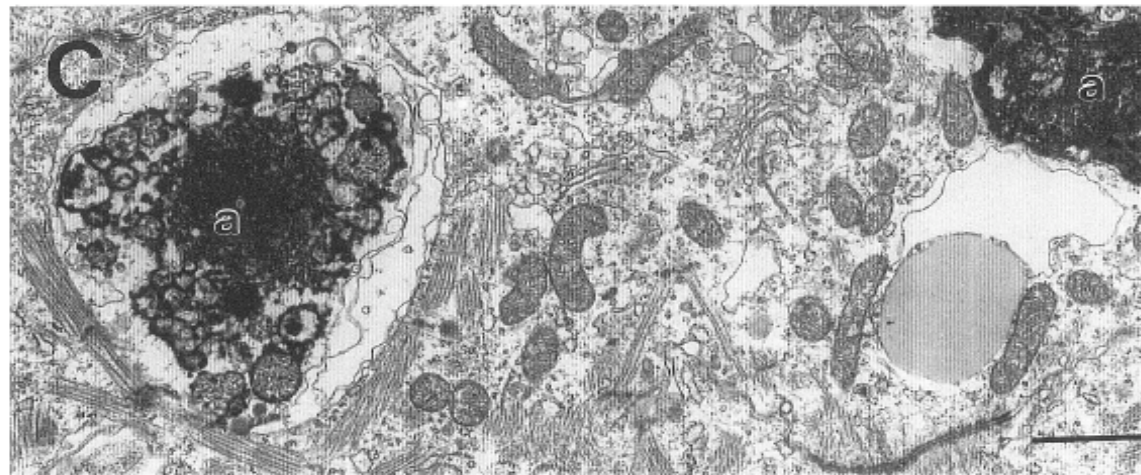


STAGE 35

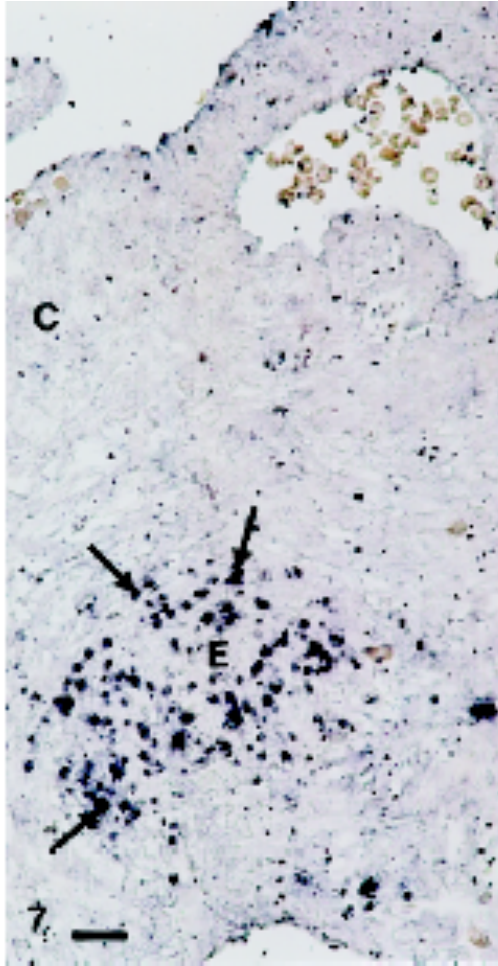


OTF = Outflow Tract  
 RA = Right Auricle  
 RV = Right Ventricle  
 LA = Left Auricle  
 LV = Left Ventricle  
 PA = Pulmonary Artery  
 Ao = Aorta

a = Apoptotic Cardiomyocyte

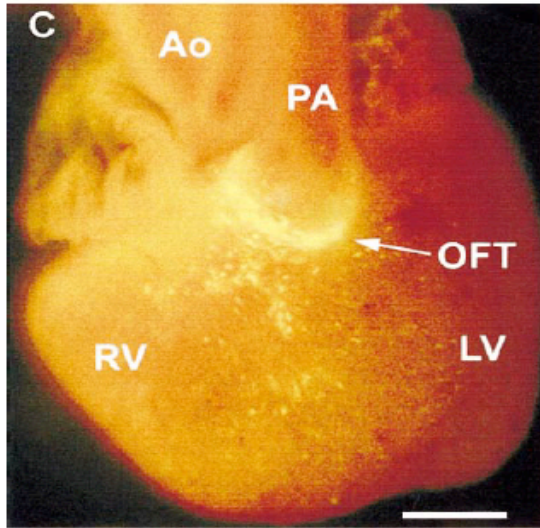


# CELL DEATH AND CARDIAC MORPHOGENESIS

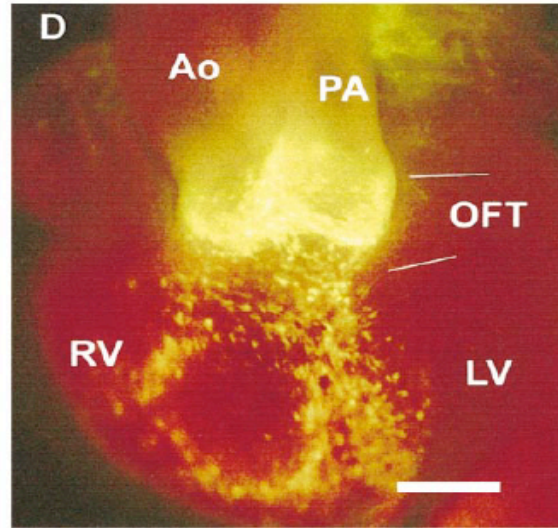


CELL DEATH IN CARDIAC  
OUTFLOW TRACT OF E13 MOL  
HEART AS REVEALED BY  
TUNEL STAINING (ARROWS)

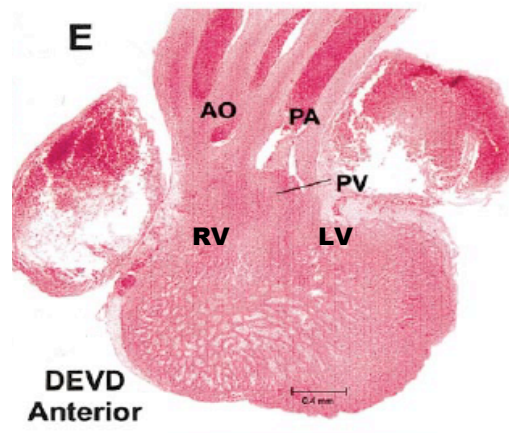
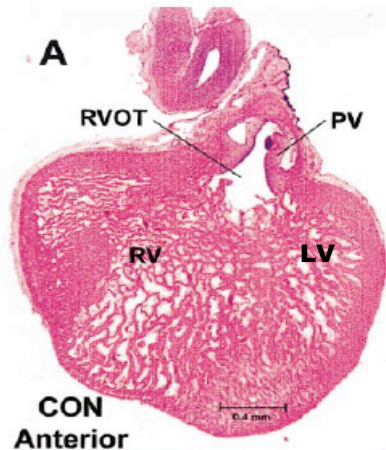
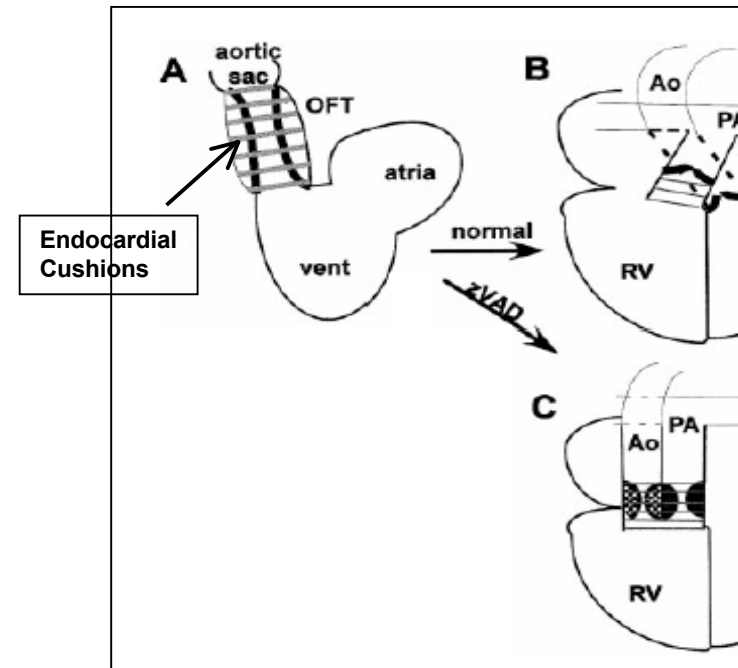
# BLOCKADE OF DEATH IN DEVELOPING HEART OFT LEADS TO DOUBLE OUTLET RIGHT VENTRICLE (DORV)



**CONTROLS**



**zVAD-fmk**



PV = Pulmonic Valve  
 RVOT = Right Ventricular Outflow Tract  
 Ao = Aorta  
 PA = Pulmonary Artery  
 OFT = Outflow Tract

From: Watanabe et al. Dev. Bio. 240: 274-288 (200

# **FUNCTIONS OF DEVELOPMENTAL CELL DEATH**

## **B. DELETION OF UNNEEDED STRUCTURES**

**KIDNEY: PRONEPHROS AND MESONEPHROS**

**BRAIN: CORTICAL SUBPLATE NEURONS**

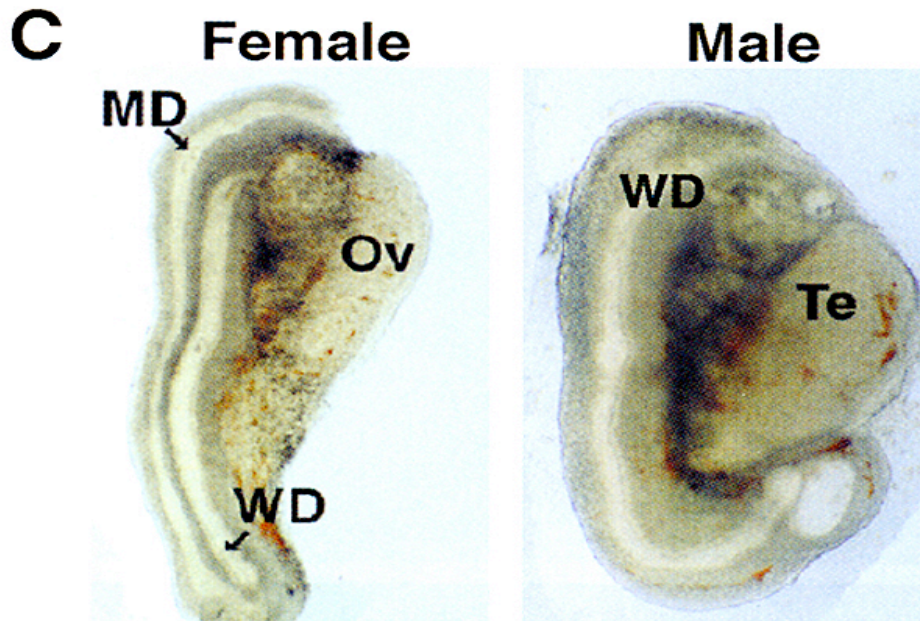
**UROGENITAL SYSTEM: WOLFFIAN AND MÜLLER DUCTS**

# REGULATION OF REPRODUCTIVE TRACT DEVELOPMENT 1

EMBRYONIC RAT GENITAL  
RIDGES SHOWING:

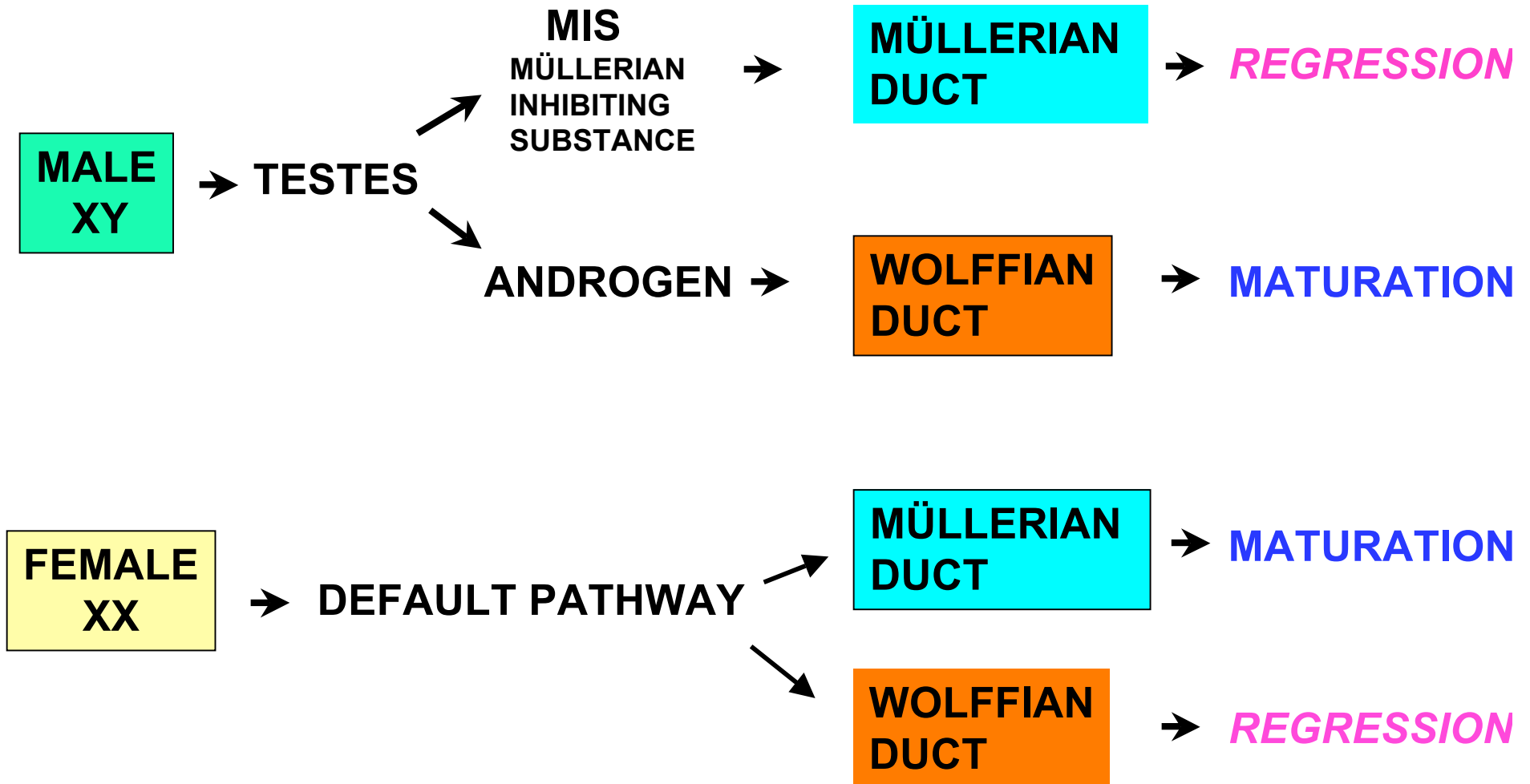
MÜLLERIAN DUCT (MD)  
WOLFFIAN DUCT (WD)

OVARY (Ov)  
TESTES (Te)

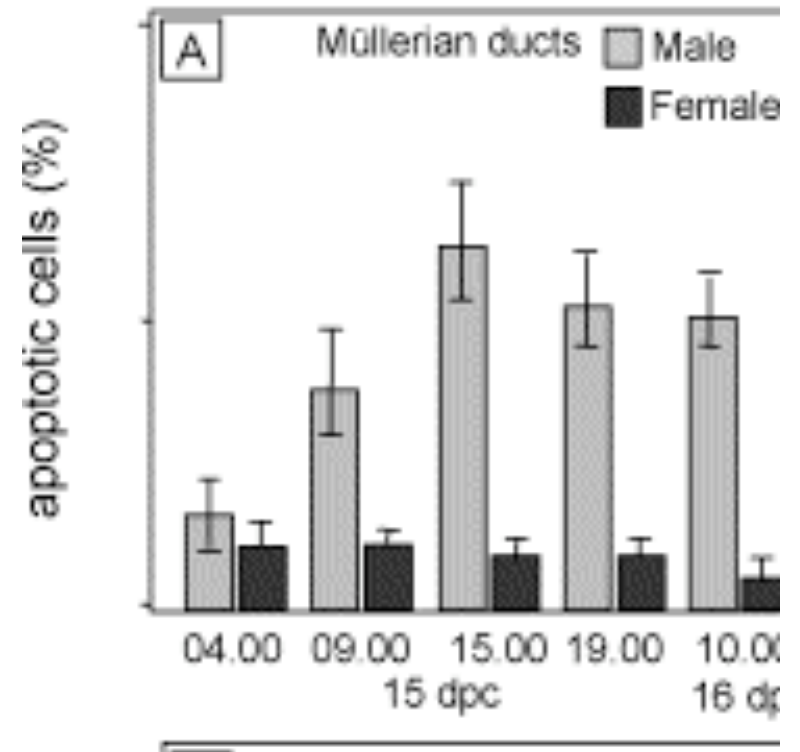
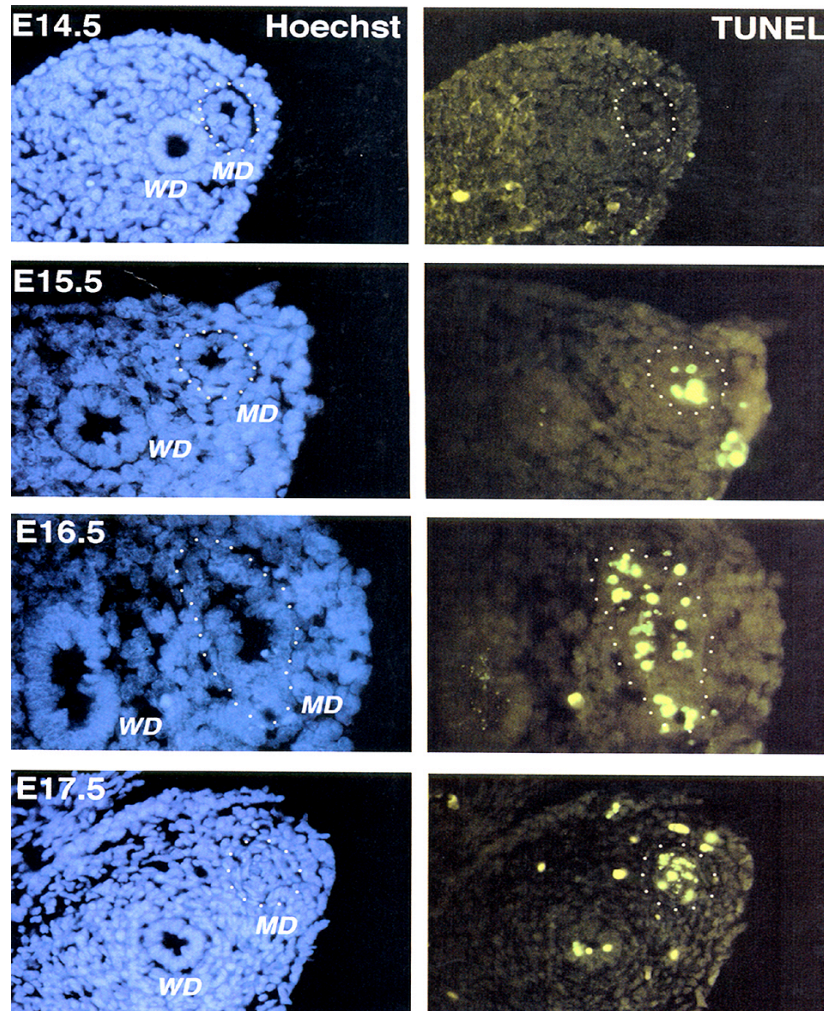


FROM: Roberts et al., Devel. Bio. 208: 110 (1999)

## REGULATION OF REPRODUCTIVE TRACT DEVELOPMENT 2



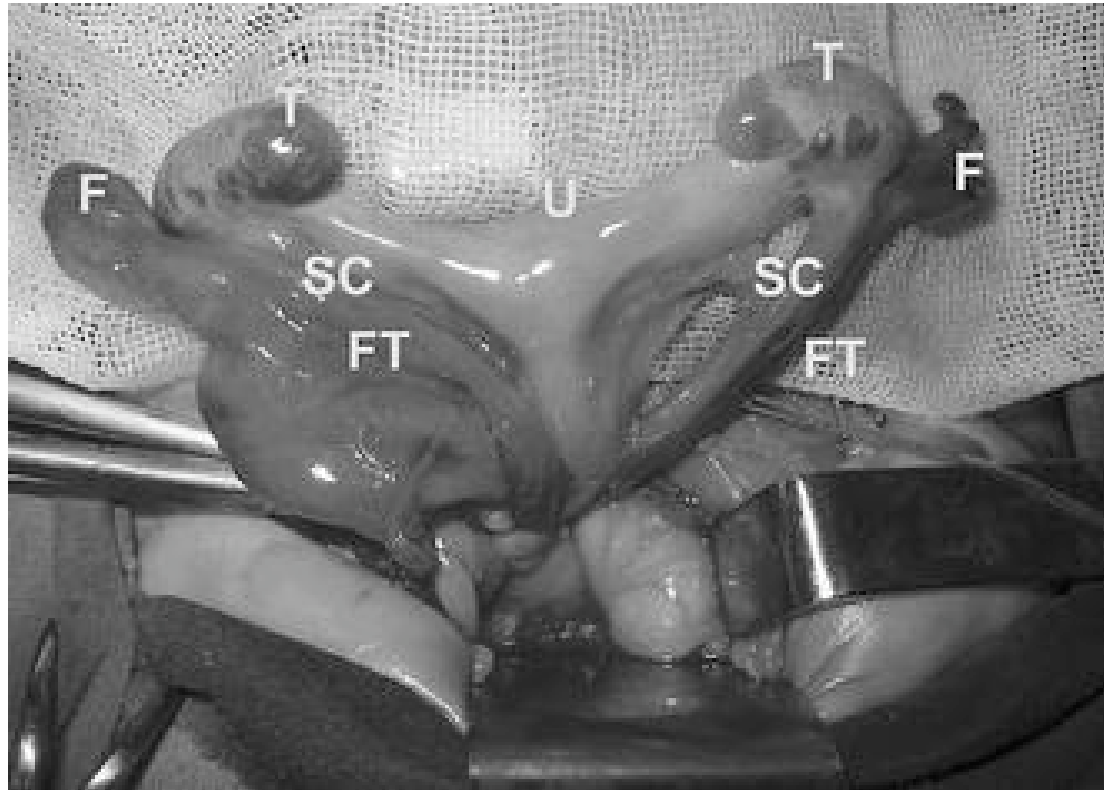
# REGULATION OF REPRODUCTIVE TRACT DEVELOPMENT 3 CELL DEATH DURING MÜLLERIAN DUCT REGRESSION



FROM: Roberts et al., *Devel. Bio.* 208: 110 (1999)

FROM: Xavier and Allard *Mol Cell Endocrin*

# FAILURE OF MÜLLERIAN DUCT REGRESSION: PERSISTENT MÜLLERIAN DUCT SYNDROME



**Intraoperative appearance of a 15-month-old male with persistent müllerian duct syndrome**  
**T: Testis, SC: Spermatic cord, FT: Fallopian tube, F: Fimbria, U: Uterus**

From: Boleken et al., Int Urol Nephrol. March 1, 2000

# FUNCTIONS OF DEVELOPMENTAL CELL DEATH

## C. REGULATION OF CELL NUMBERS

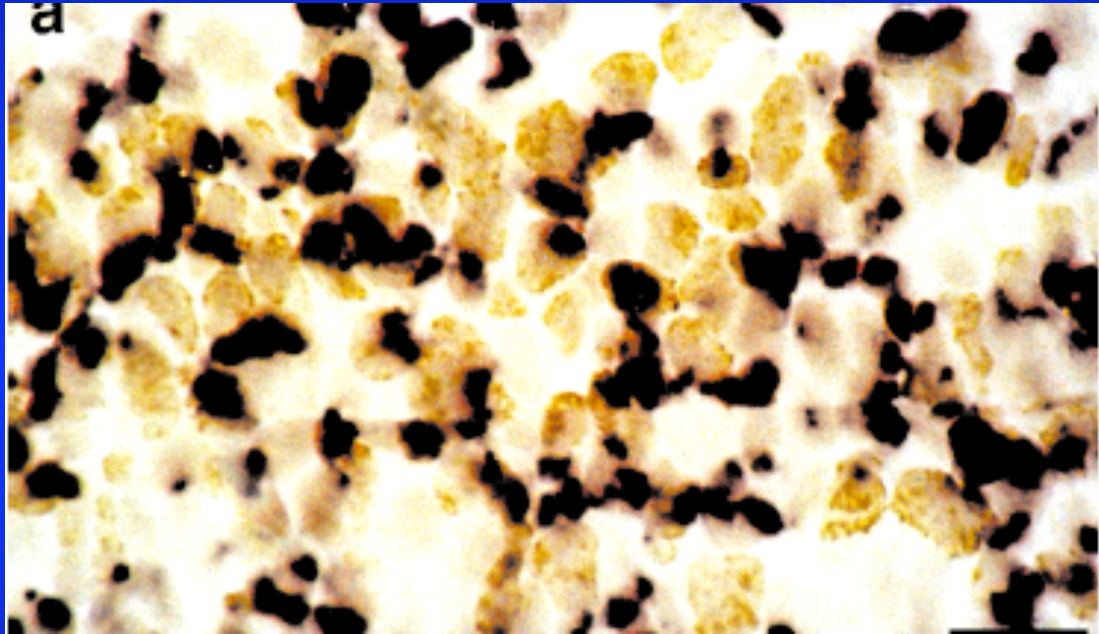
### NERVOUS SYSTEM:

→ GENERATION OF CORRECT NEURON NUMBER

MATCHING NEURONS AND TARGETS

MATCHING SCHWANN CELL AND  
OLIGODENDROCYTES WITH AXONS

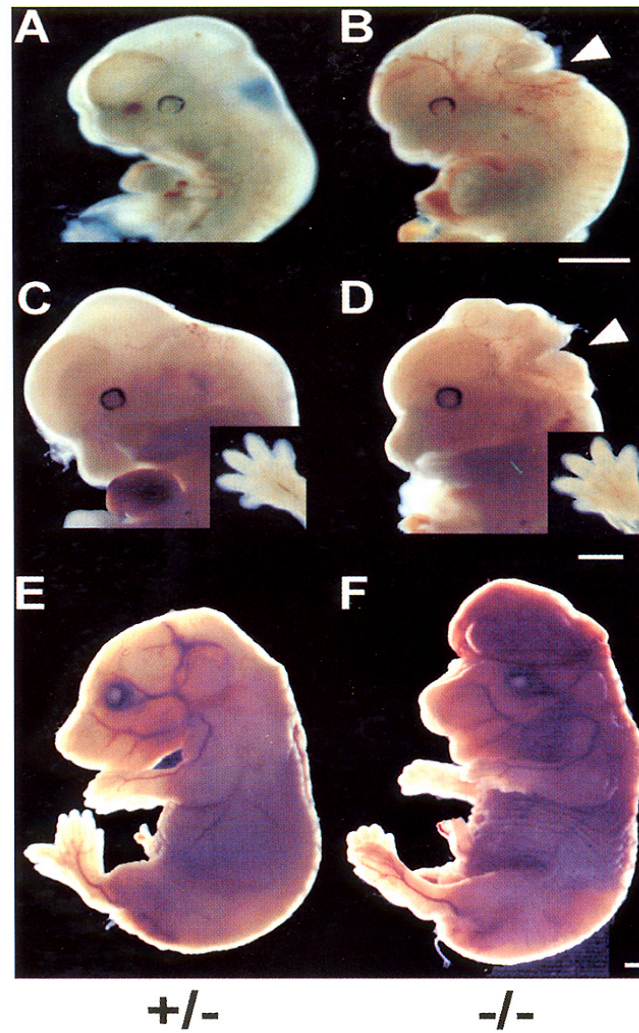
# DEATH OF PROLIFERATING VZ CELLS IN E14 MOUSE CEREBELLUM



Tan = BrDU; Purple = ISEL/dying

Blaschke et al J Comp Neurol 1998

# EMBRYOGENIC DEFECTS IN A MOUSE LACKING CASPASE-



From: Kuida et al Cell:94: 325-337, 1998

# **FUNCTIONS OF DEVELOPMENTAL CELL DEATH**

## **C. REGULATION OF CELL NUMBERS**

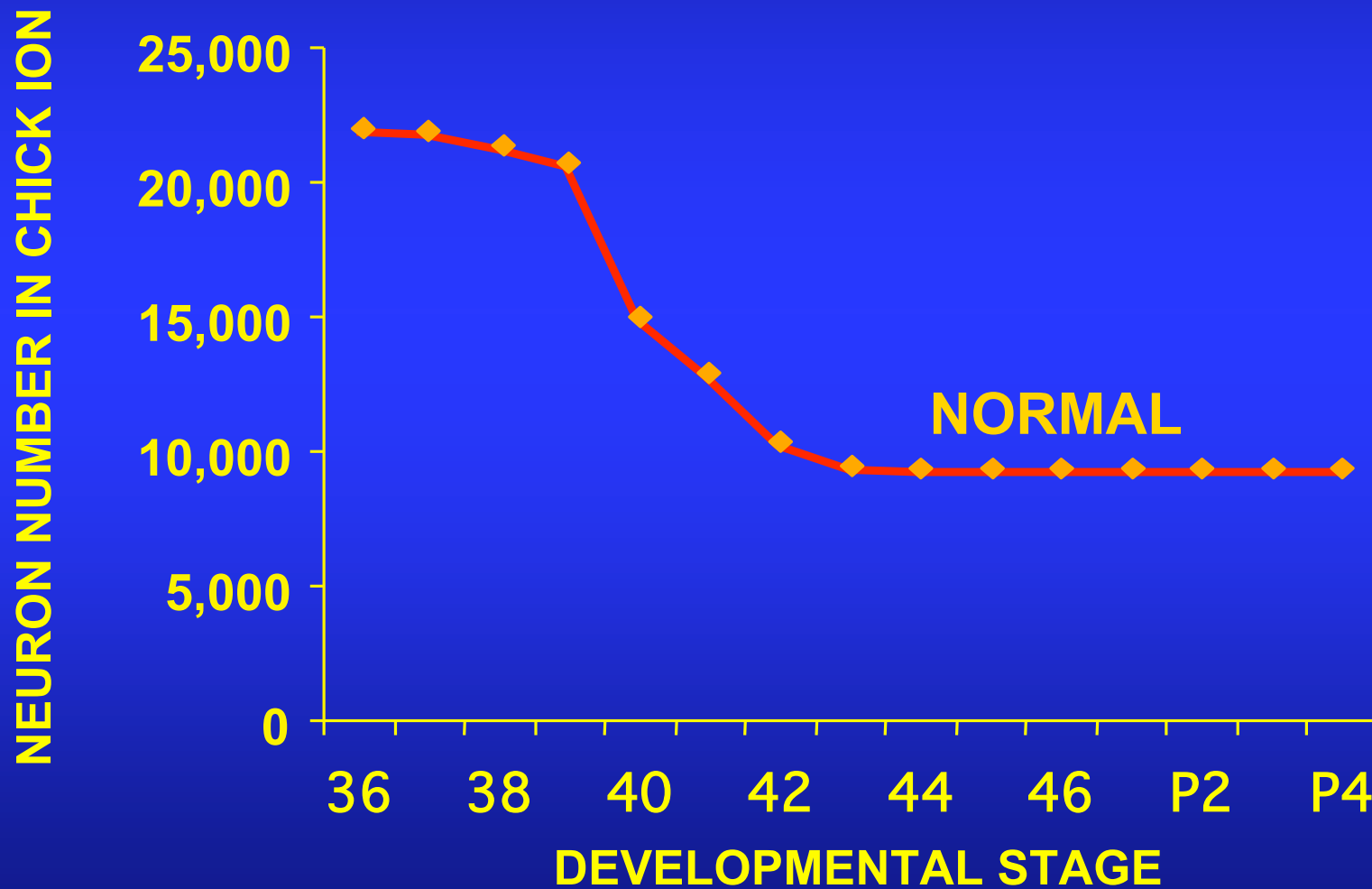
### **NERVOUS SYSTEM:**

**GENERATION OF CORRECT NEURON NUMBER**

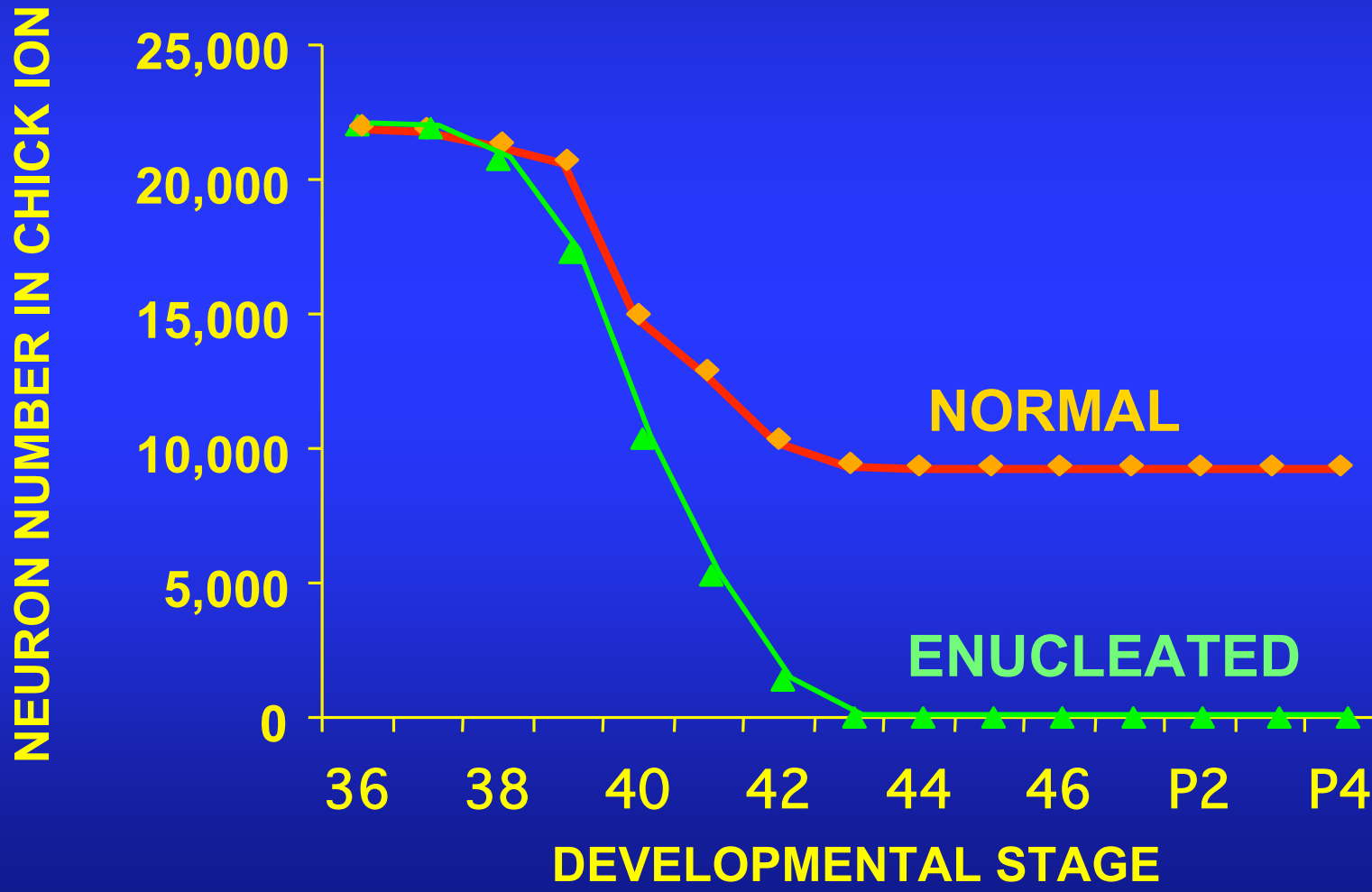
**→ MATCHING NEURONS AND TARGETS**

**MATCHING SCHWANN CELL AND  
OLIGODENDROCYTES WITH AXONS**

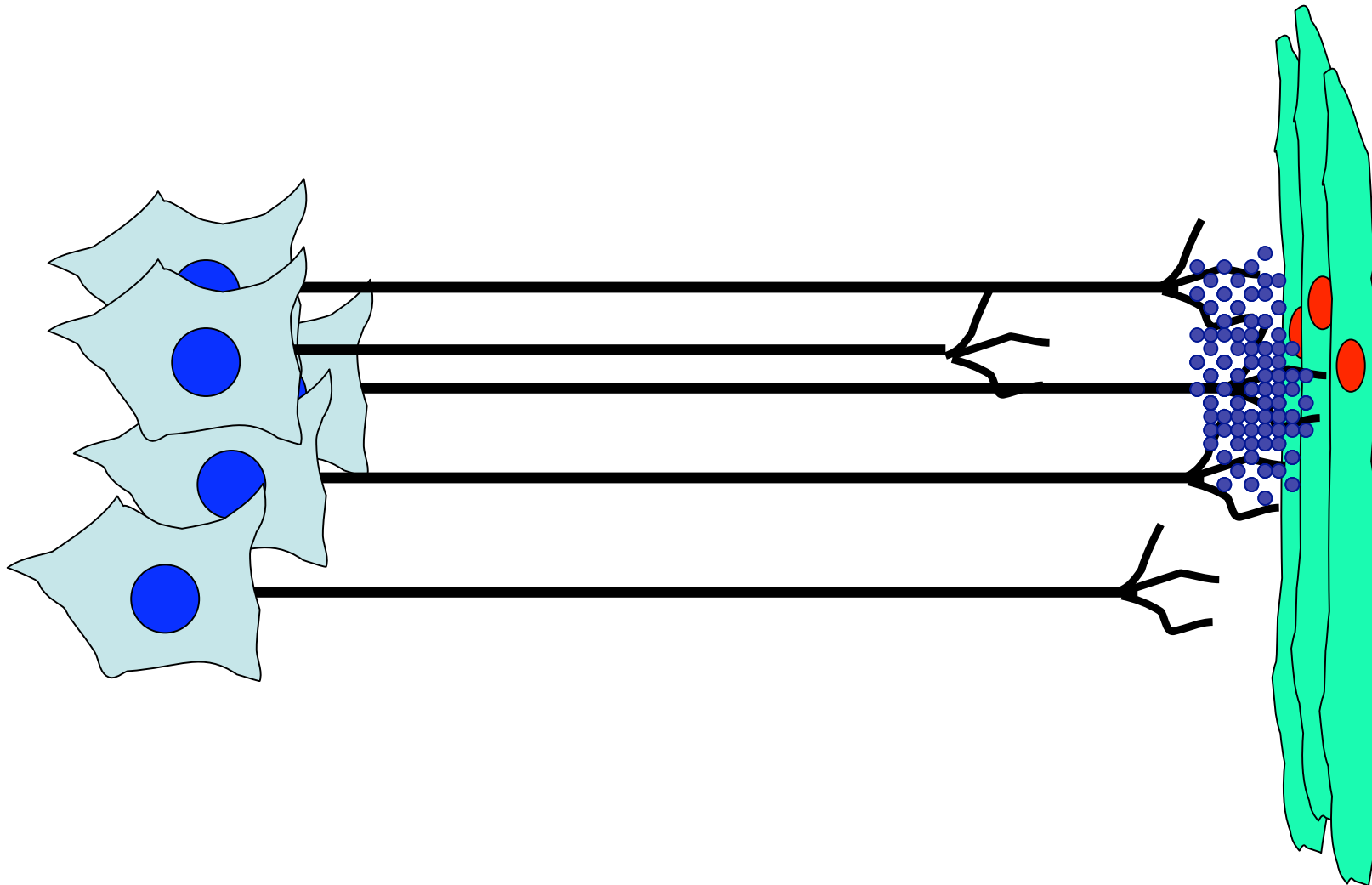
# NORMAL DEVELOPMENTAL NEURONAL DEATH OCCURS AND IS REGULATED BY TARGET DERIVED TROPHIC FACTORS



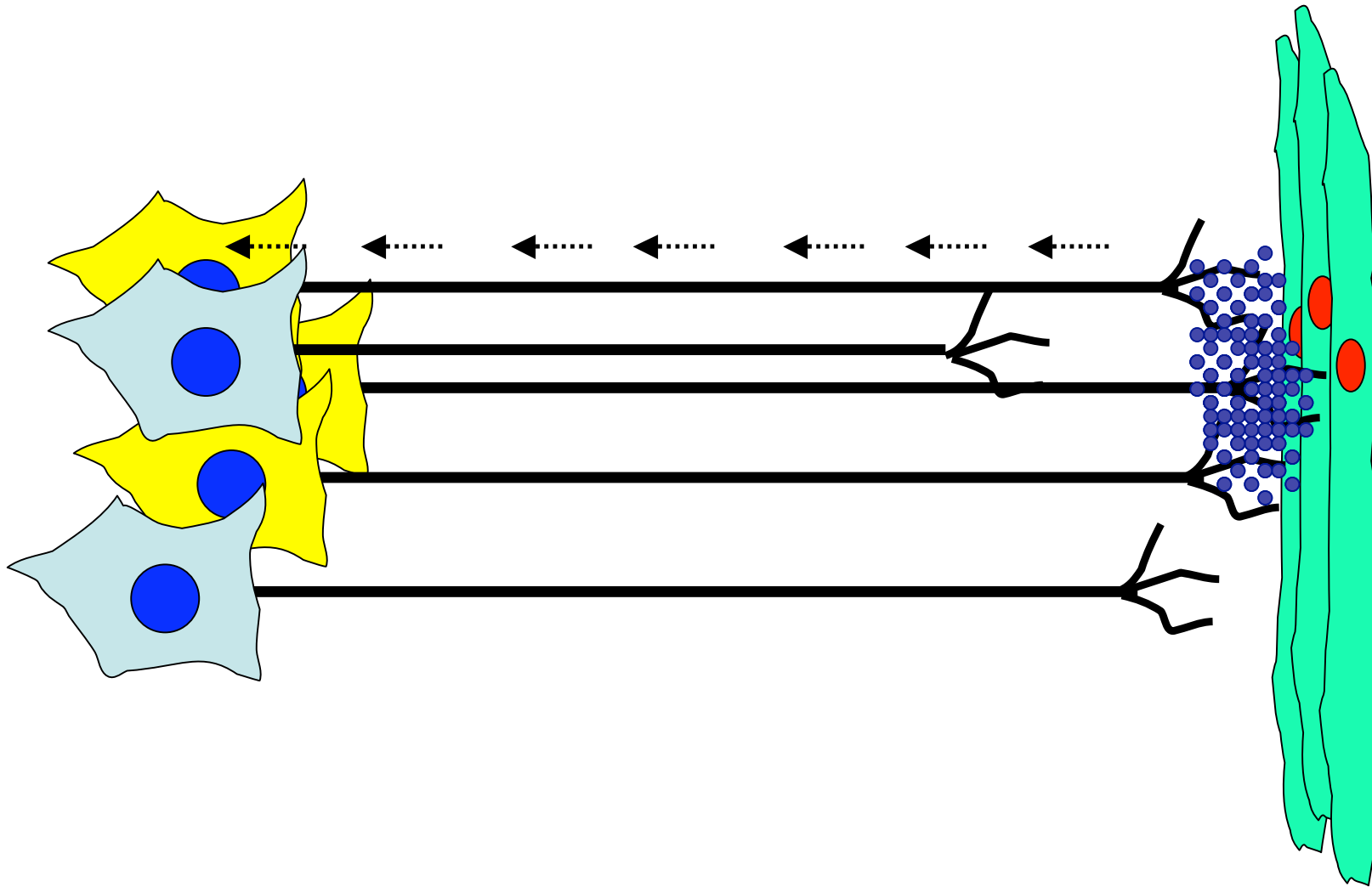
# NORMAL DEVELOPMENTAL NEURONAL DEATH OCCURS AND IS REGULATED BY TARGET DERIVED TROPHIC FACTORS



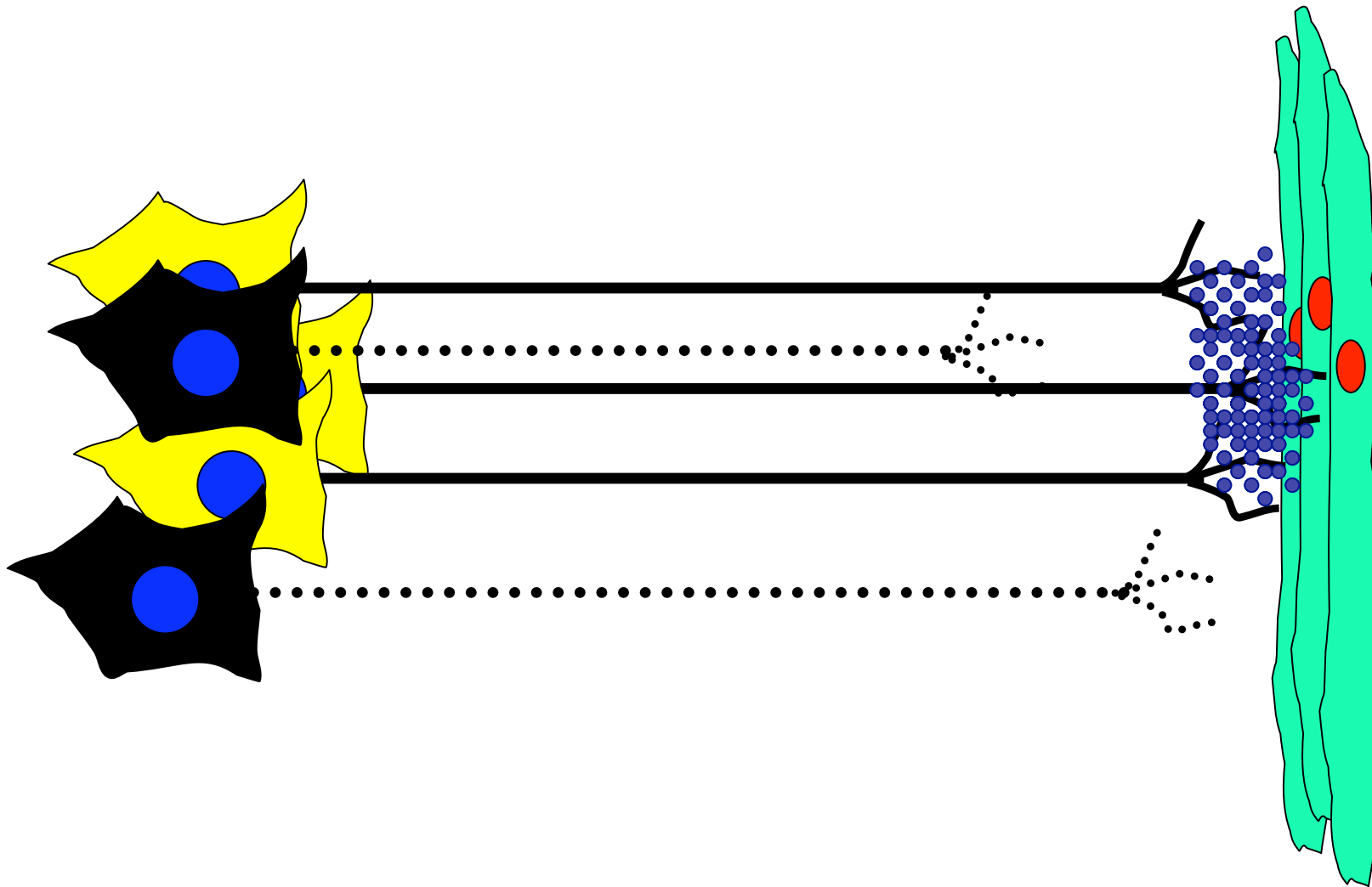
# NEURONAL CULLING AS REGULATED BY COMPETITION FOR TARGET-SUPPLIED TROPHIC FACTOR



# NEURONAL CULLING AS REGULATED BY COMPETITION FOR TARGET-SUPPLIED TROPHIC FACTOR

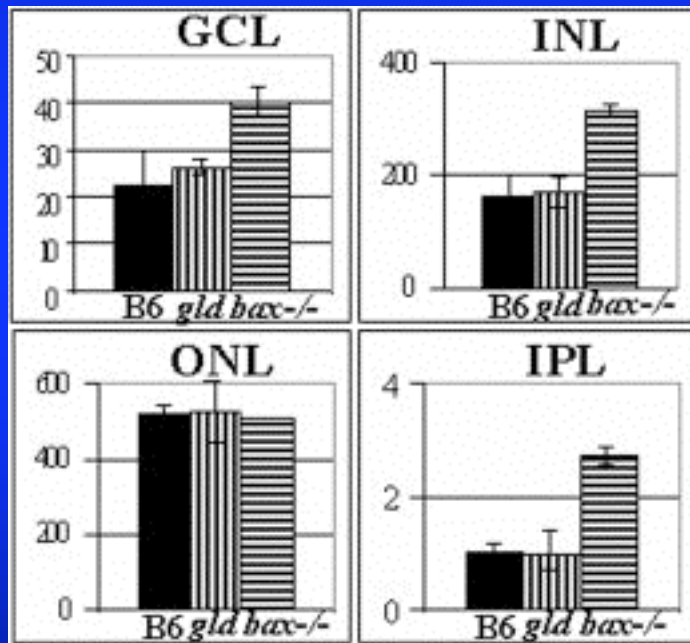


# NEURONAL CULLING AS REGULATED BY COMPETITION FOR TARGET-SUPPLIED TROPHIC FACTOR

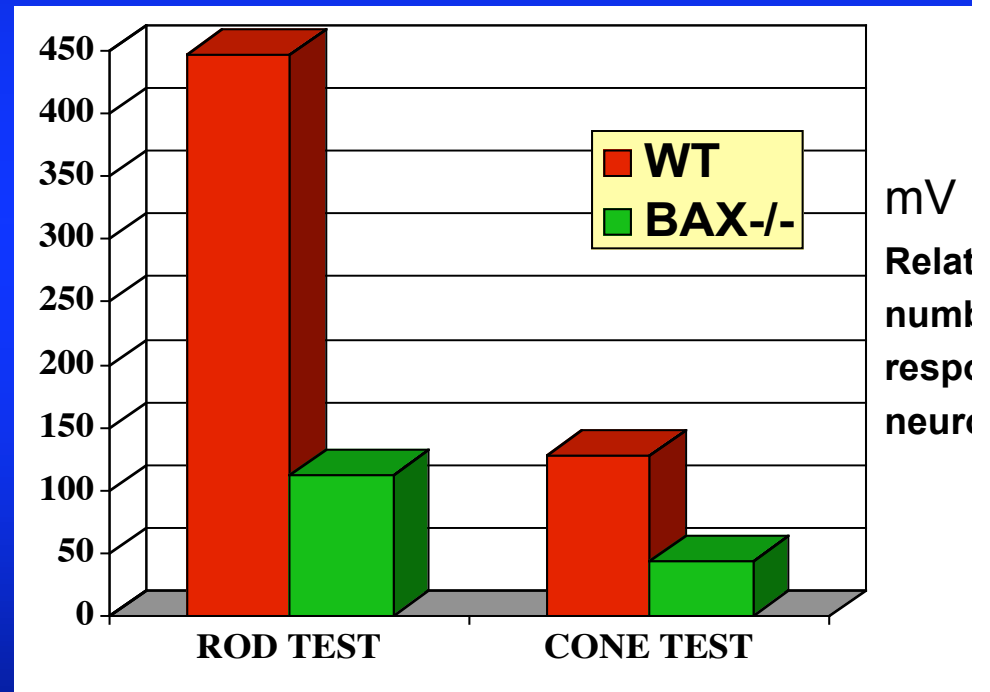


# RETINAL NEURON RESPONSES TO LIGHT CHANGE IN ABSENCE OF NORMAL DEVELOPMENTAL CELL DEATH

NEURON NUMBER



GCL=ganglion cell layer  
 INL=inner nuclear layer  
 ONL=outer nuclear layer  
 IPL=inner plexiform layer



Péquignot et al. Dev Dyn 2003

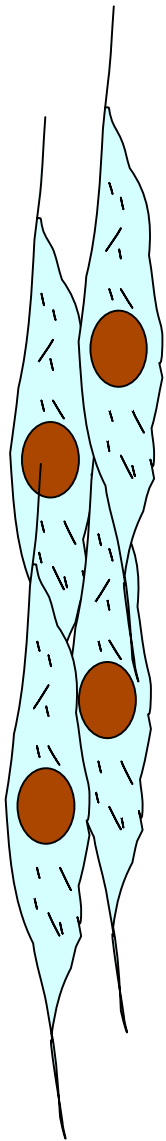
# **FUNCTIONS OF DEVELOPMENTAL CELL DEATH**

## **D. PRODUCTION OF STRUCTURES WITHOUT ORGANELLES**

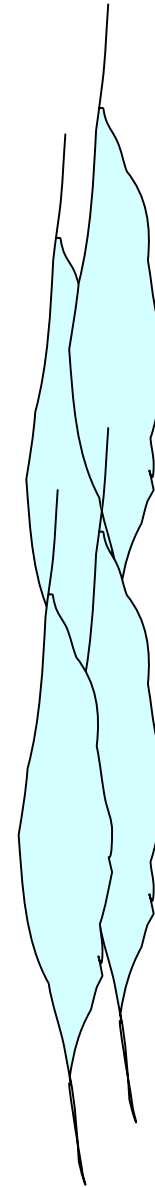
**SQUAMOUS EPITHELIUM FROM KERATINOCYTES**

**FORMATION OF LENS FROM LENS FIBER CELLS**

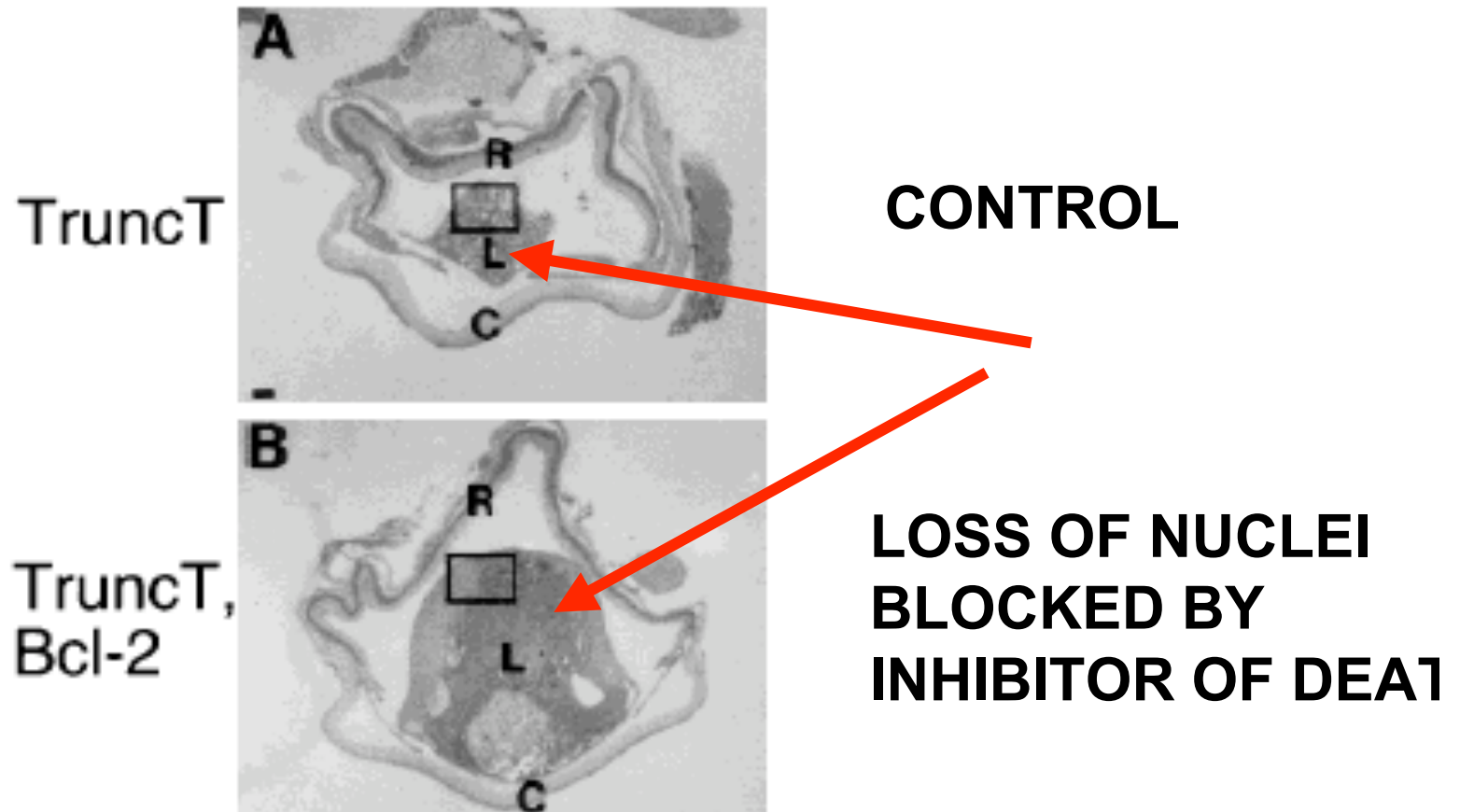
# FORMATION OF CLEAR LENS FROM LENS FIBER CELLS



**DEGRADATION OF NUCLEI  
AND ORGANELLES BY  
DEATH-LIKE MECHANISM**

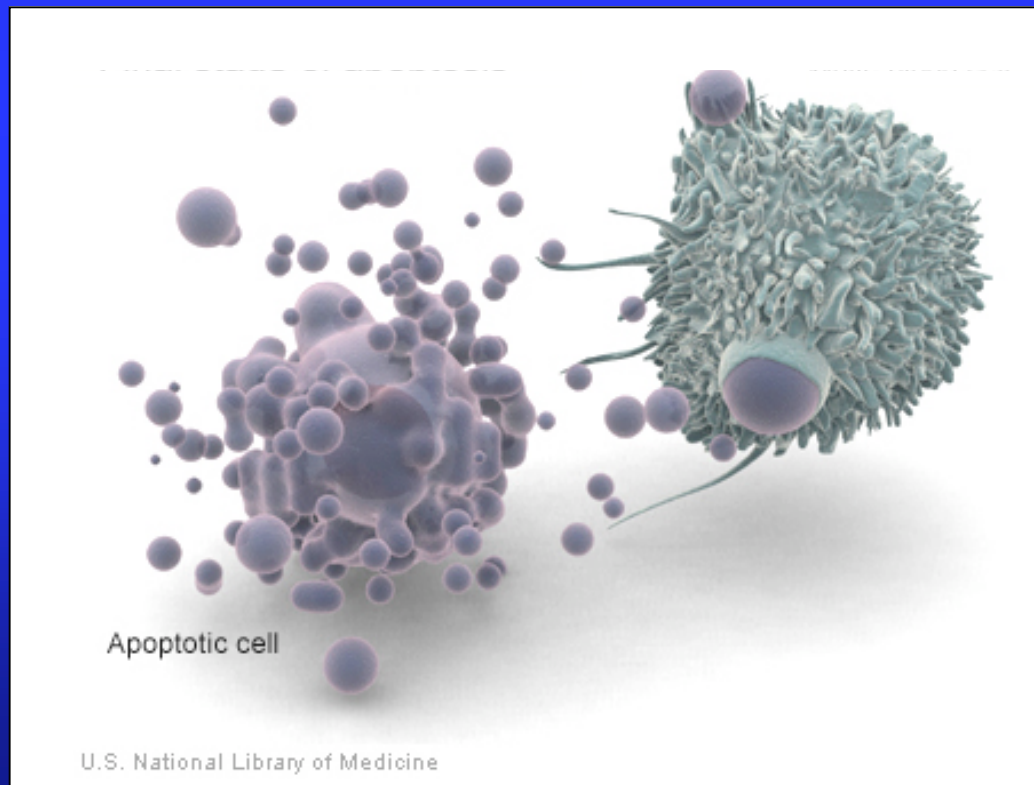


**EXPERIMENTAL BLOCKADE OF DEATH-LIKE ACTIONS  
IN DEVELOPING LENS FIBER CELLS PERMITS THEIR  
ABNORMAL PROLIFERATION**



FROM: Fromm et al., Dev Genetics 20: 296 (1997)

# HOW DOES DEVELOPMENTAL CELL DEATH OCCUR?



## APOPTOTIC DEATH

vs

## NECROTIC DEATH

PRESENT IN DEVELOPING TISSUES

RESPONSE TO CELL INJURY, TO

CYTOPLASMIC BLEBBING

CELLULAR & NUCLEAR PYKNOSIS

CELL & NUCLEAR SWELLING

CHROMATIN CONDENSATION

DNA DEGRADATION BY ENDONUCLEASES  
(FORMATION OF DNA LADDER)

RANDOM DNA DEGRADATION

FORMATION OF MEMBRANE-LIMITED  
APOPTOTIC BODIES

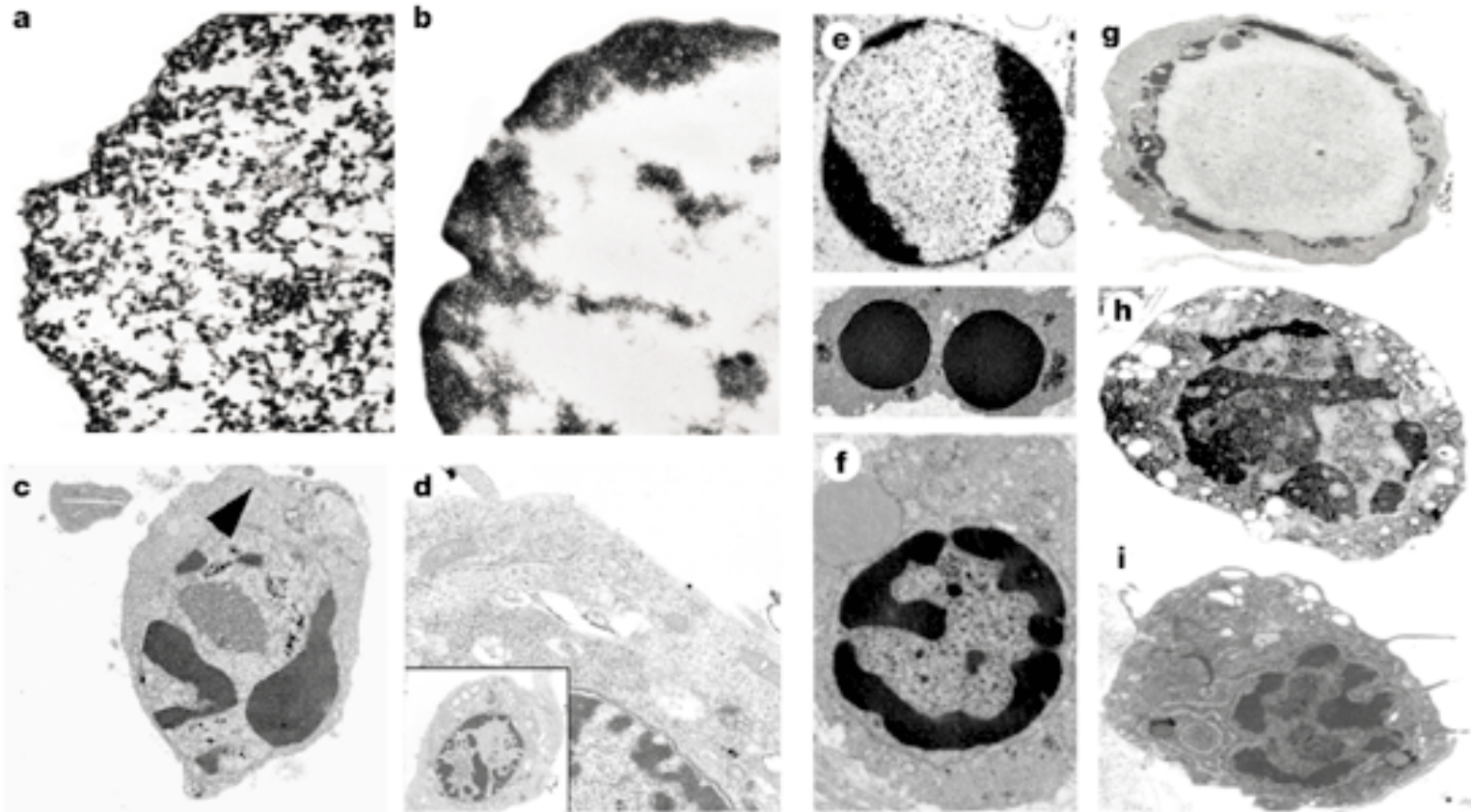
LOSS OF MEMBRANE INTEGRITY  
& LOSS OF CYTOPLASMIC CON

PHAGOCYTOSIS OF APOPTOTIC BODIES

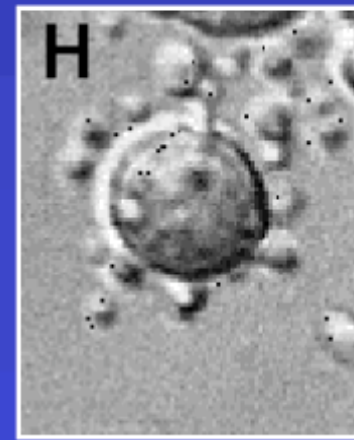
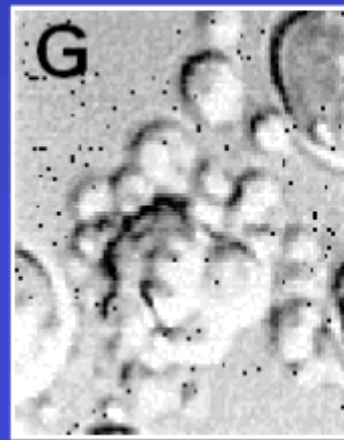
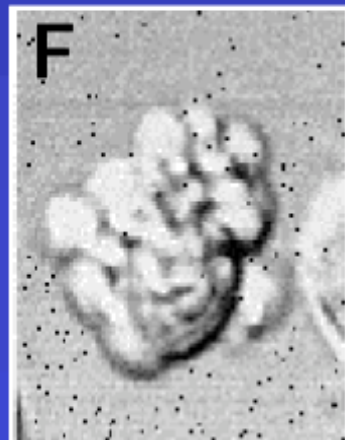
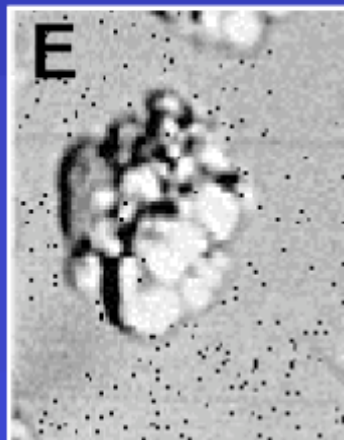
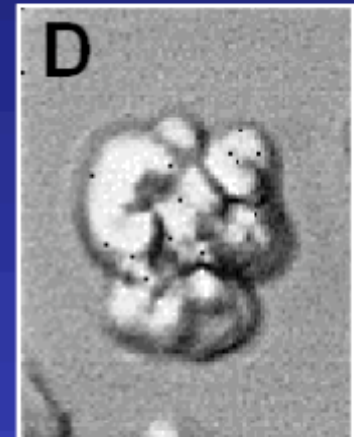
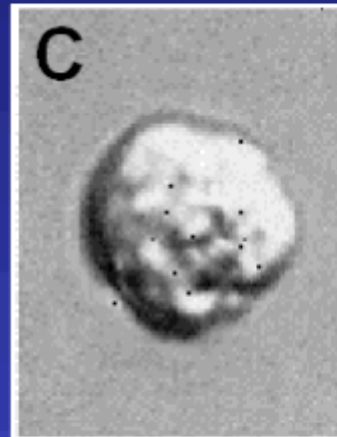
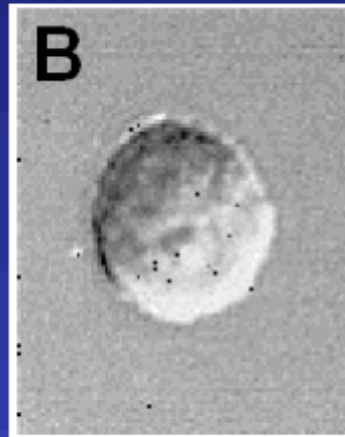
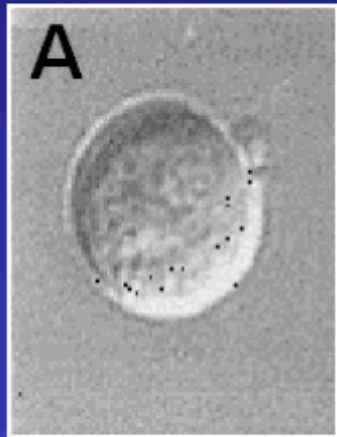
ABSENCE OF INFLAMMATORY RESPONSE

INFLAMMATORY RESPONSE

# ELECTRON MICROSCOPIC VIEWS OF NORMAL (a) AND APOPTOTIC (b-i) NUCLEI

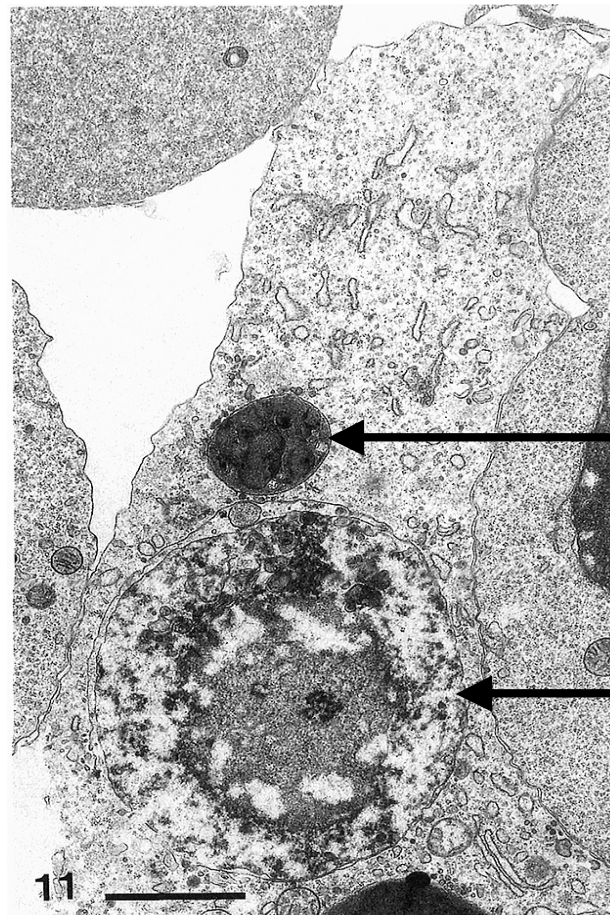


# TIME LAPSE IMAGES OF A CELL UNDERGOING APOPTOSIS



# APOPTOTIC BODIES AND NUCLEI ARE CLEARED BY PHAGOCYTOSIS

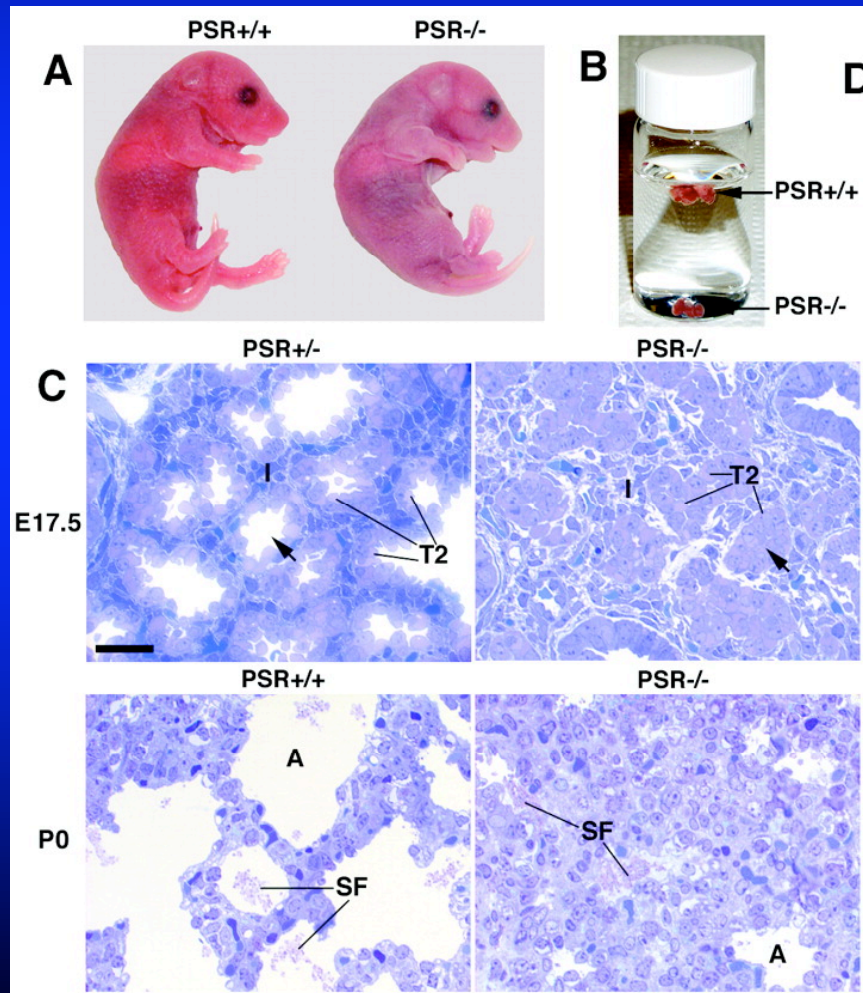
**EM OF A MACROPHAGE  
ENGULFING AN  
APOPTOTIC BODY AND  
APOPTOTIC NUCLEUS  
IN THE VENTRICULAR  
WALL OF THE  
DEVELOPING MOUSE  
HEART**



**APOPTOTIC BODY**

**APOPTOTIC NUCLEI**

# MICE MUTANT FOR PHAGOCYTOSIS SHOW DEFECTIVE LUNG DEVELOPMENT AND RESPIRATORY SYMPTOMS SIMILAR TO THOSE IN HUMAN RESPIRATORY DISTRESS SYNDROME (RD)



From: Li et al., Science: 302 (

# WHAT ARE THE MECHANISMS BY WHICH CELLS DIE DURING DEVELOPMENT?

THERE ARE EVOLUTIONARILY CONSERVED MECHANISMS  
THAT GOVERN DEVELOPMENTAL CELL DEATH

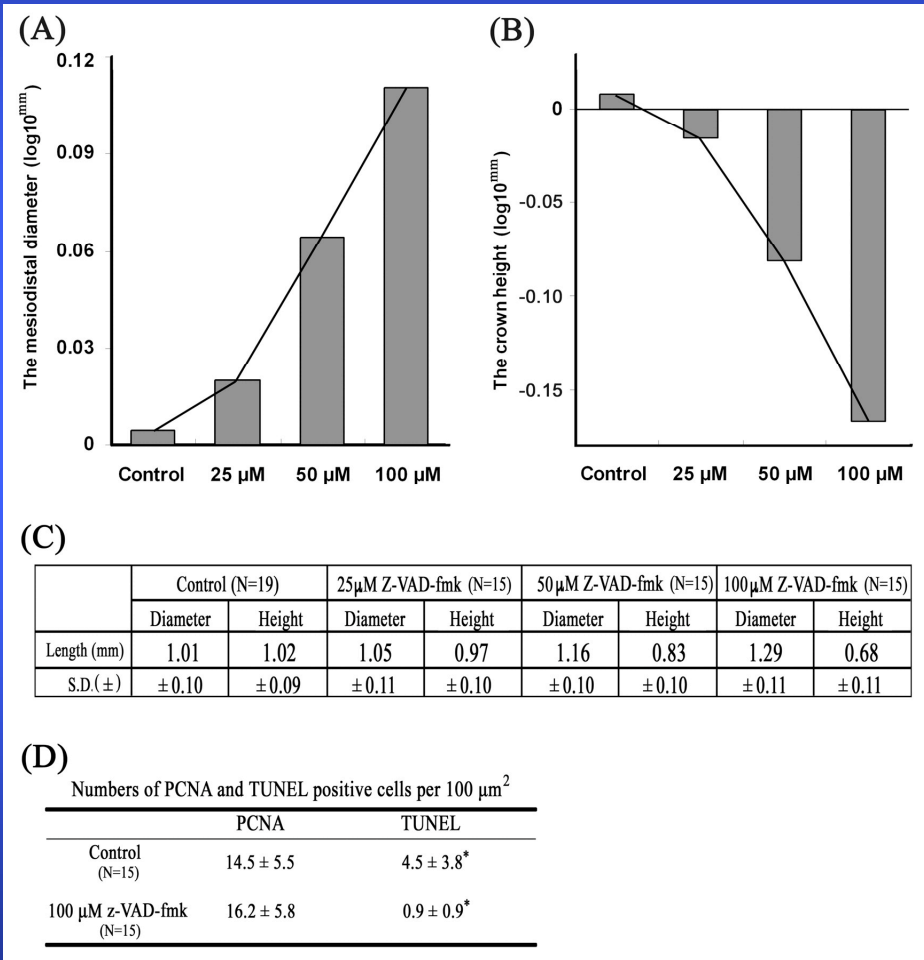
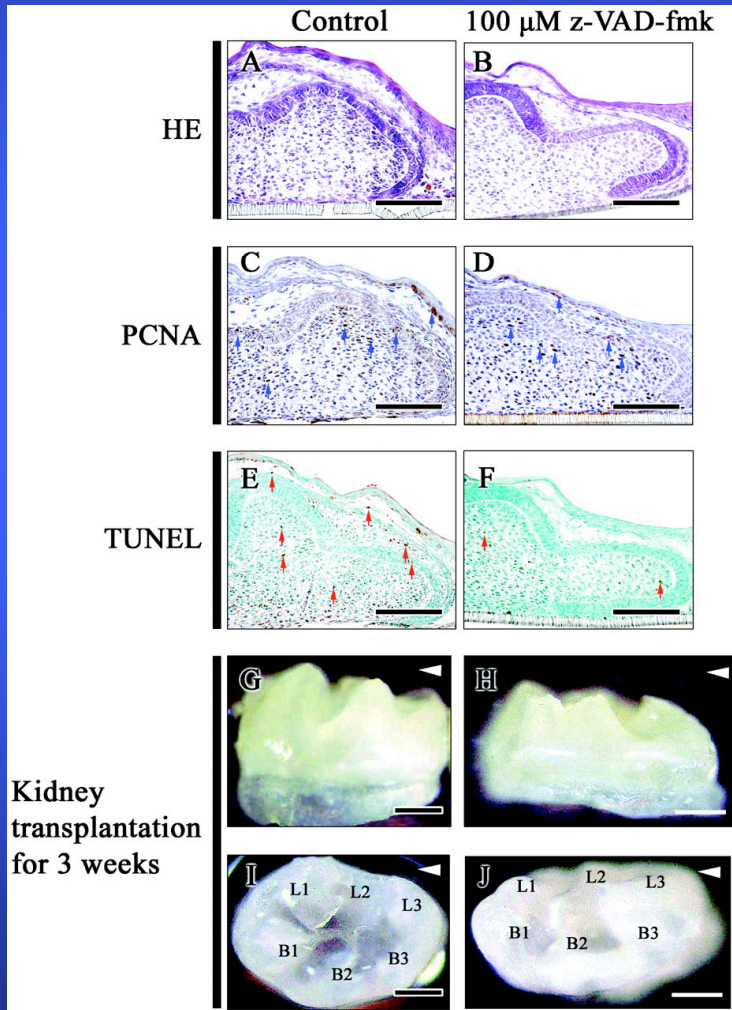


**Robert Horvitz**

# CASPASES

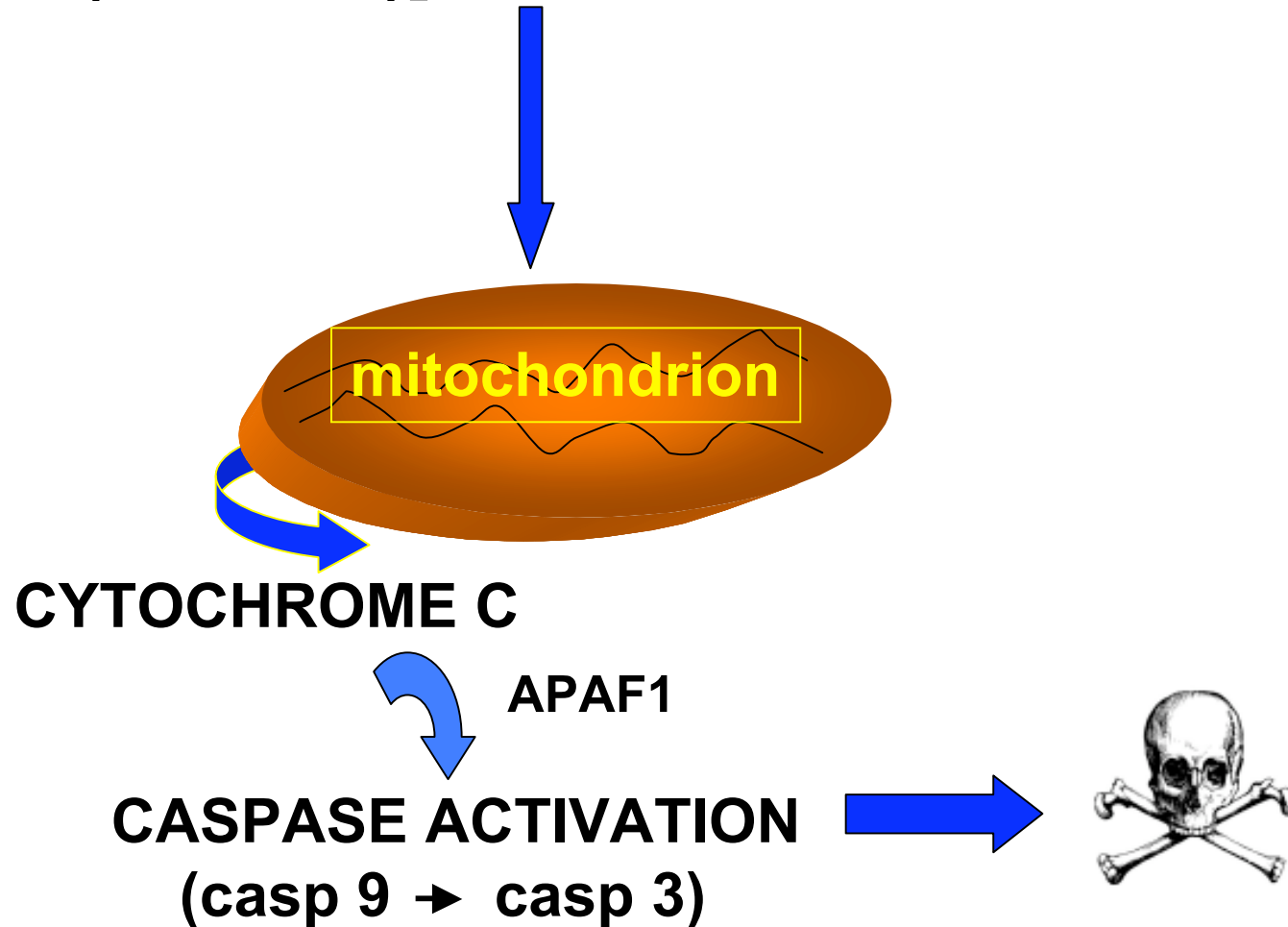
- FAMILY OF EXECUTORS OF APOPTOTIC DEATH
- CYSTEINE PROTEASES THAT CLEAVE AFTER ASP
- CONSTITUTIVELY PRESENT AS INACTIVE FORMS
- ACTIVATED BY CLEAVAGE OR CONFORMATIONAL CHANGE VIA INTERACTION WITH COFACTORS SUCH AS APAF1 AND CYTOCHROME C
- WHEN ACTIVATED, CLEAVE CELLULAR SUBSTRATES, LEADING TO APOPTOTIC DEATH

# BLOCKING CELL DEATH WITH CASPASE INHIBITORS AFFECTS TOOTH DEVELOPMENT



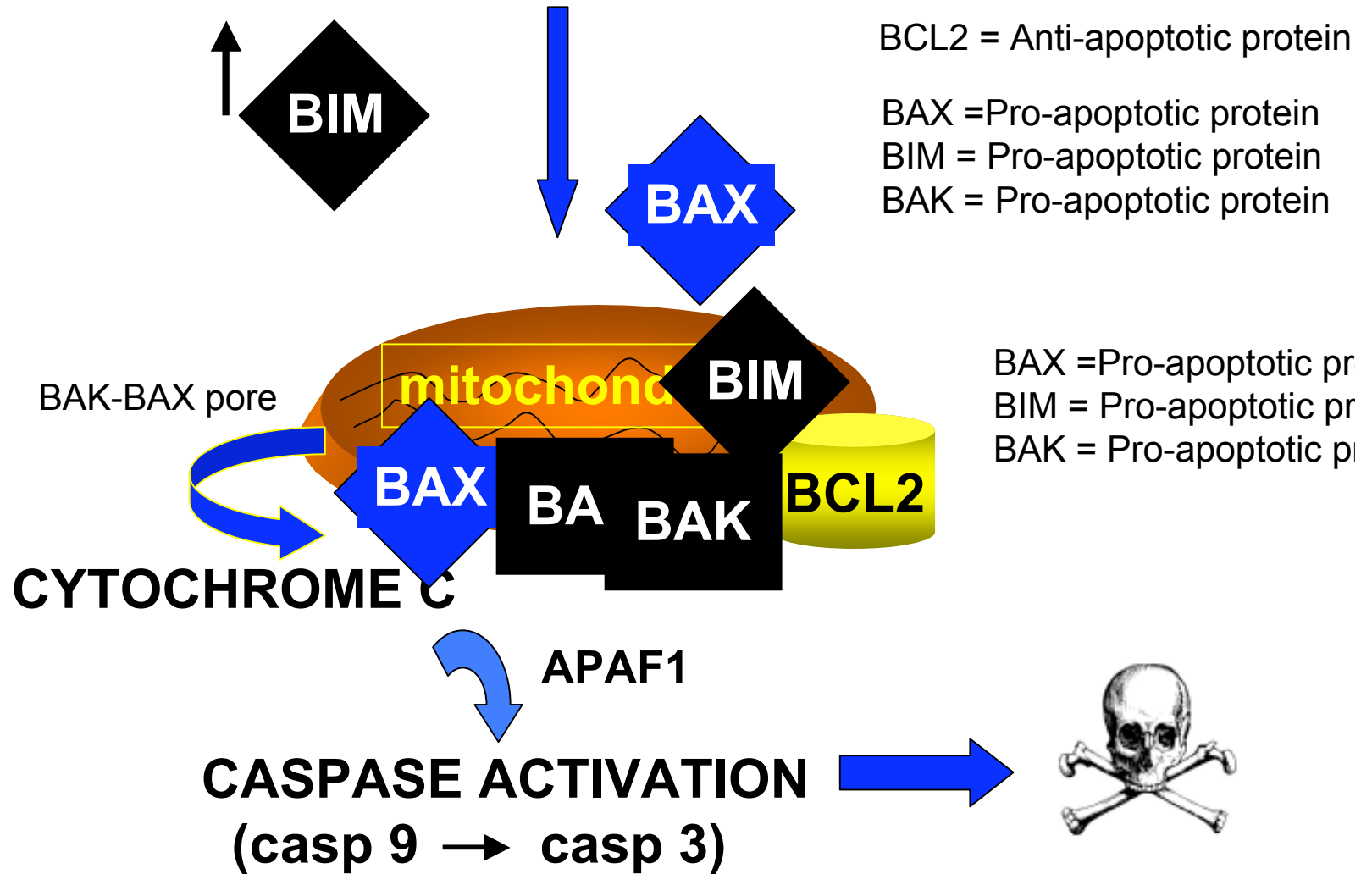
# THE MITOCHONDRIAL PATHWAY OF APOPTOTIC DEATH -

**APOPTOTIC STIMULI [absence of survival factors (i.e., FGF)]**

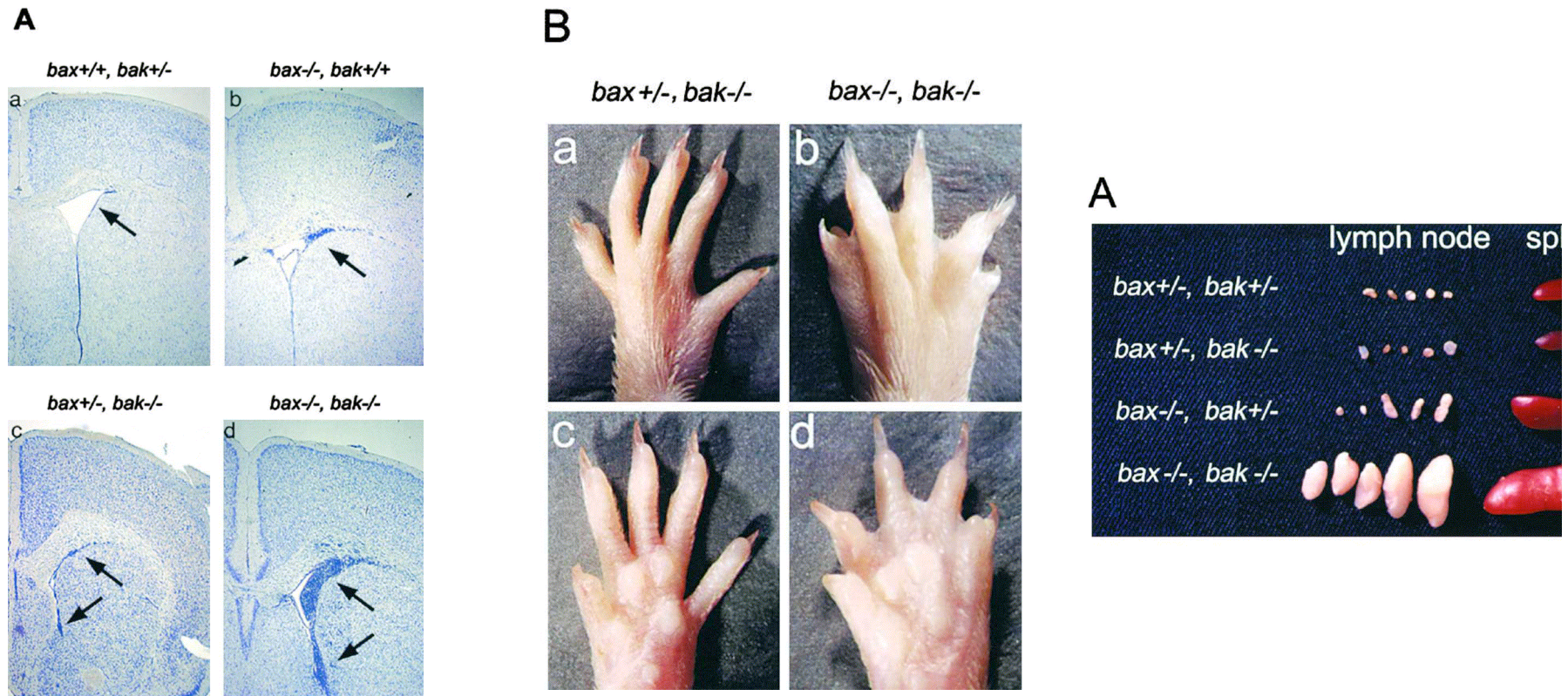


# THE MITOCHONDRIAL PATHWAY OF APOPTOTIC DEATH -

## APOPTOTIC STIMULI



# ABNORMALITIES IN MICE LACKING BAX AND BAK



Arrow shows neuroprogenitors  
in ventricular zone of embryonic brain

# DEATH PROMOTING RECEPTORS AND LIGANDS THE EXTRINSIC PATHWAY TO APOPTOTIC DEATH

## LIGAND

TNF $\alpha$

FAS ligand

TRAIL

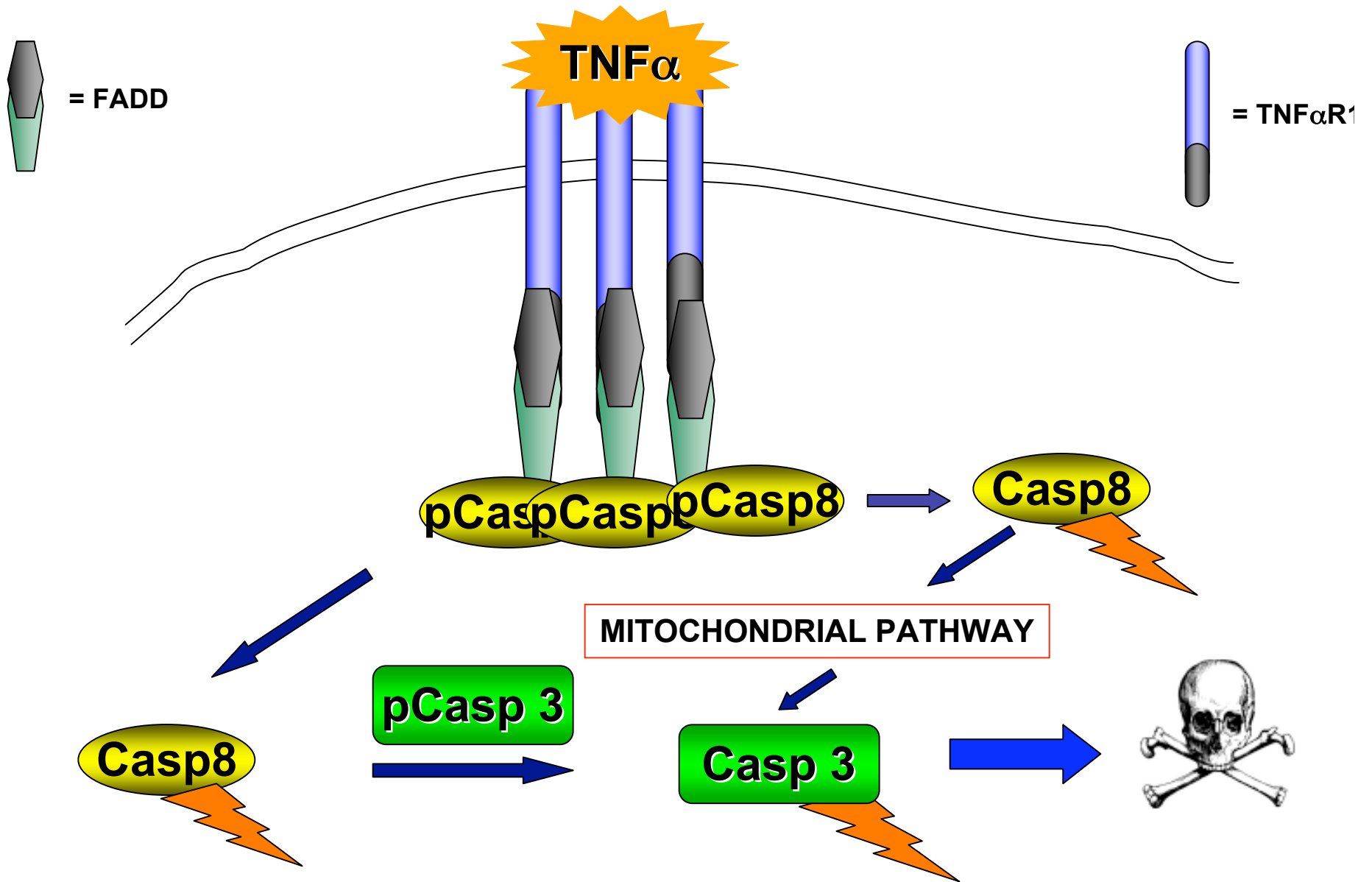
## RECEPTOR

TNF $\alpha$ R1

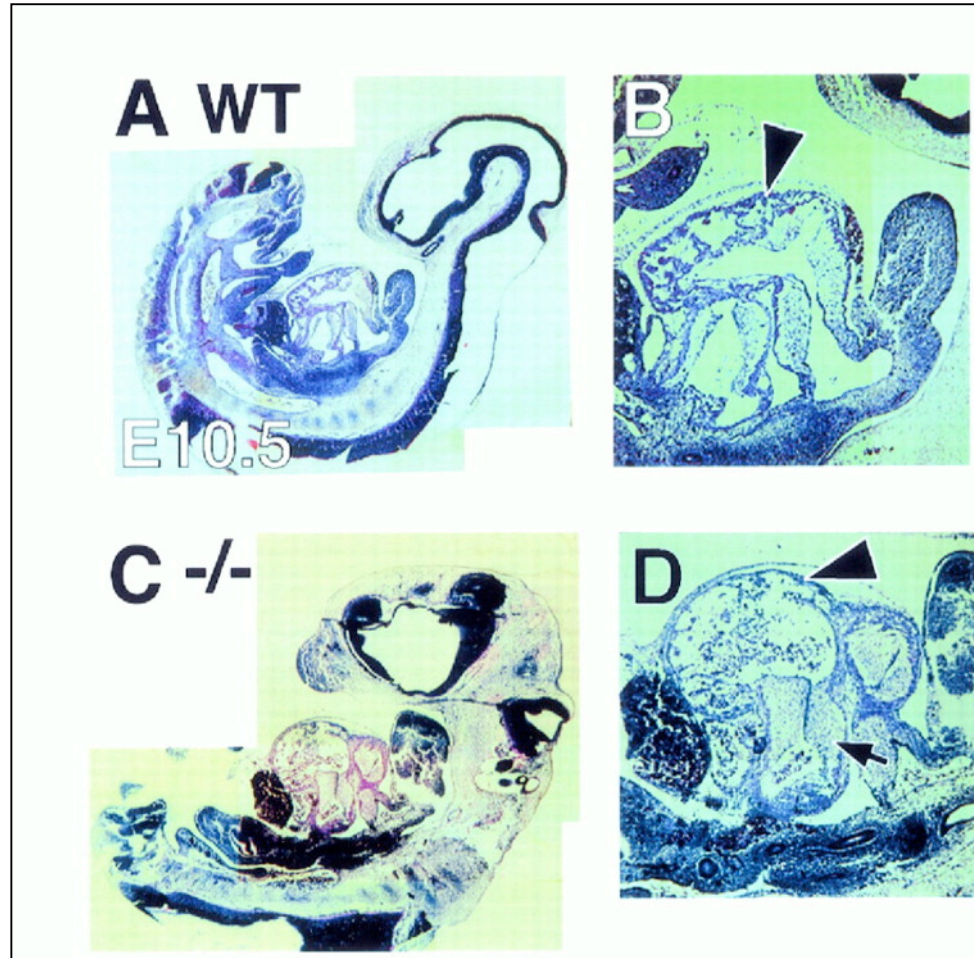
FAS

TRAIL-R

# THE RECEPTOR-MEDIATED PATHWAY OF APOPTOTIC DEATH



# MICE LACKING FADD DIE DURING EMBRYOGENESIS AND HAVE MULTIPLE DEFECTS



Low power view:  
A,C

B,D: Ventricular  
Myocardium

Arrowhead shows  
abnormal development of  
trabeculae; arrow shows  
normal endocardial  
cushion.

# CONCLUSIONS

- CELL DEATH IS A MAJOR REGULATOR OF NORMAL EMBRYOGENESIS
- IT OCCURS AT ALL STAGES OF EMBRYONIC DEVELOPMENT AND IN MULTIPLE ORGAN SYSTEMS AND PLAYS A VARIETY OF ROLES
- FAILURE OF NORMAL CELL DEATH DURING EMBRYOGENESIS LEADS TO A VARIETY OF DEVELOPMENTAL DEFECTS
- DEVELOPMENTAL CELL DEATH IS GENERALLY APOPTOTIC IN NATURE
- THE GENERAL MECHANISMS OF APOPTOTIC CELL DEATH ARE BECOMING UNDERSTOOD AND ARE EVOLUTIONARILY CONSERVED
- MUTATION OF SPECIFIC APOPTOTIC GENES LEADS TO DEVELOPMENTAL ABNORMALITIES