

CELL DEATH DURING DEVELOPMENT

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DEPT OF PATHOLOGY AND CELL BIOLOGY

April 9, 2009

KEY DEVELOPMENTAL PROCESSES

CELL PROLIFERATION

CELL MIGRATION

CELL DIFFERENTIATION

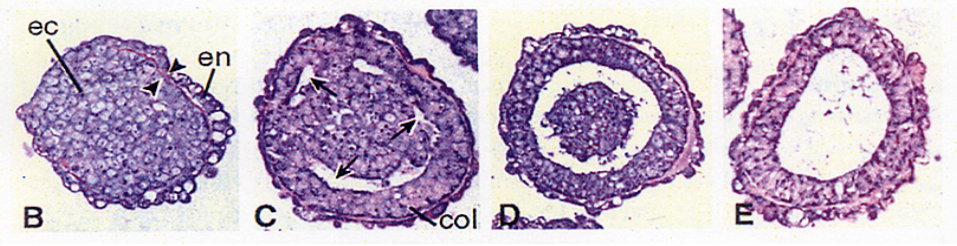
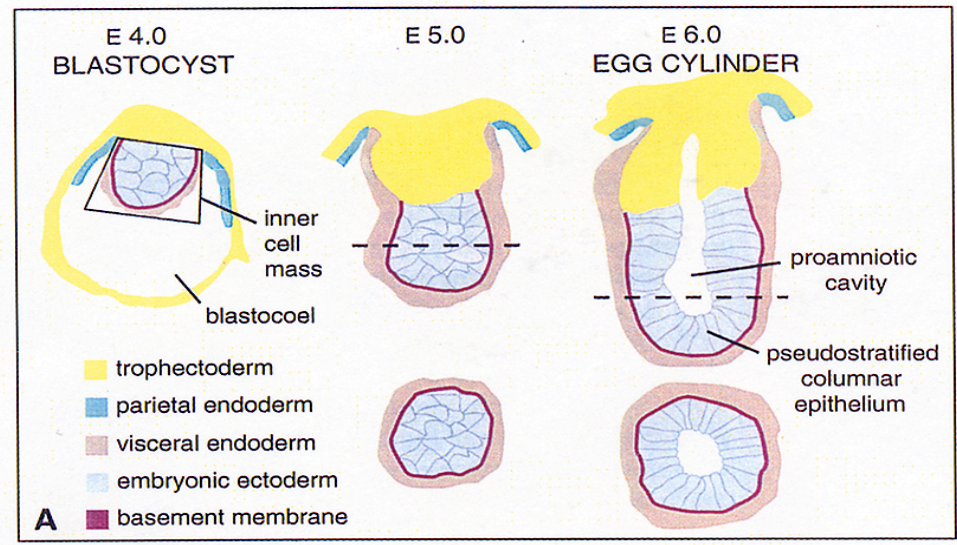
CELL DEATH

FUNCTIONS OF DEVELOPMENTAL CELL DEATH

A. MORPHOGENESIS: SCULPTING/SHAPING STRUCT

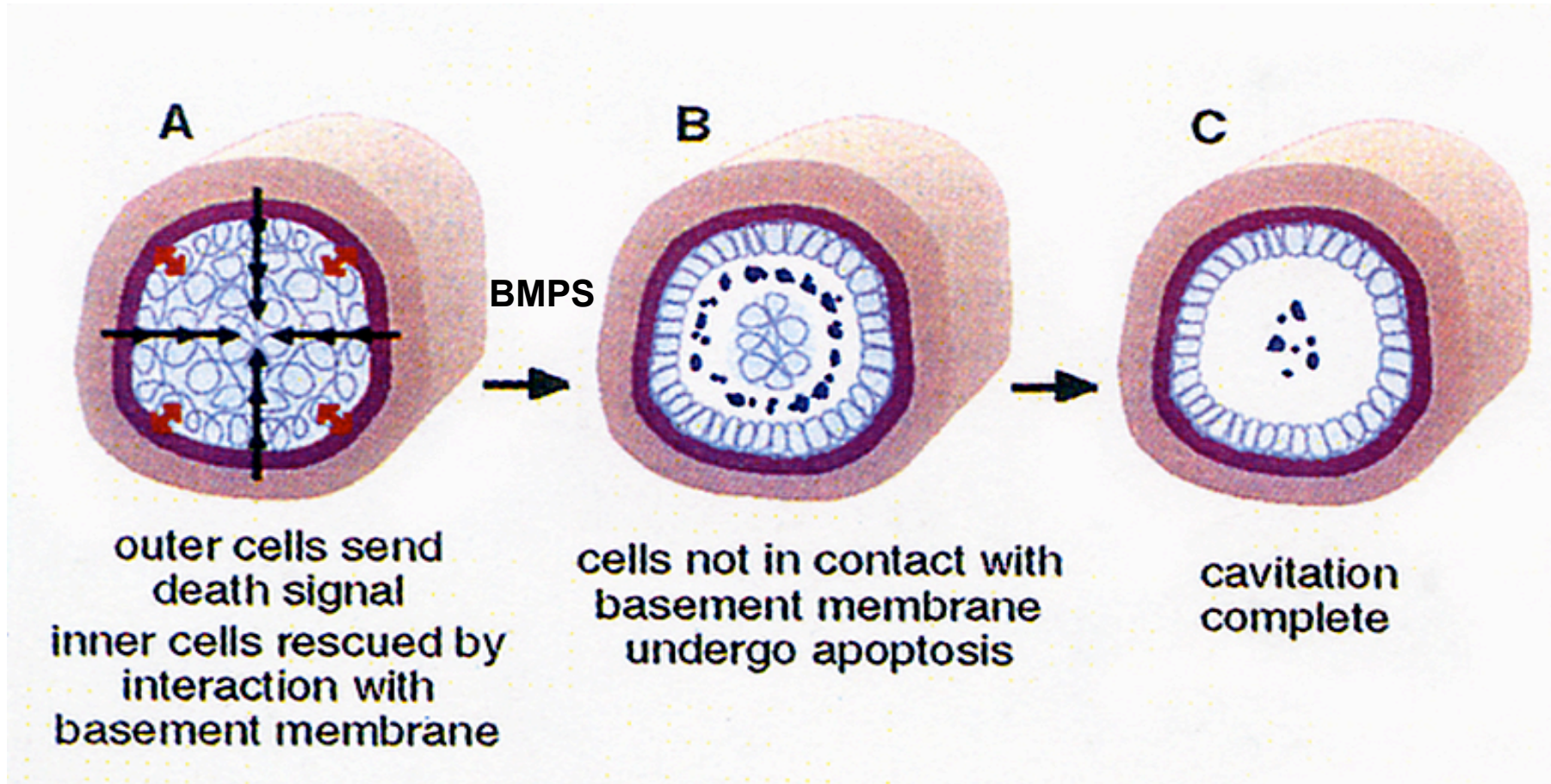
CREATION OF CAVITIES AND TUBES

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 3



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

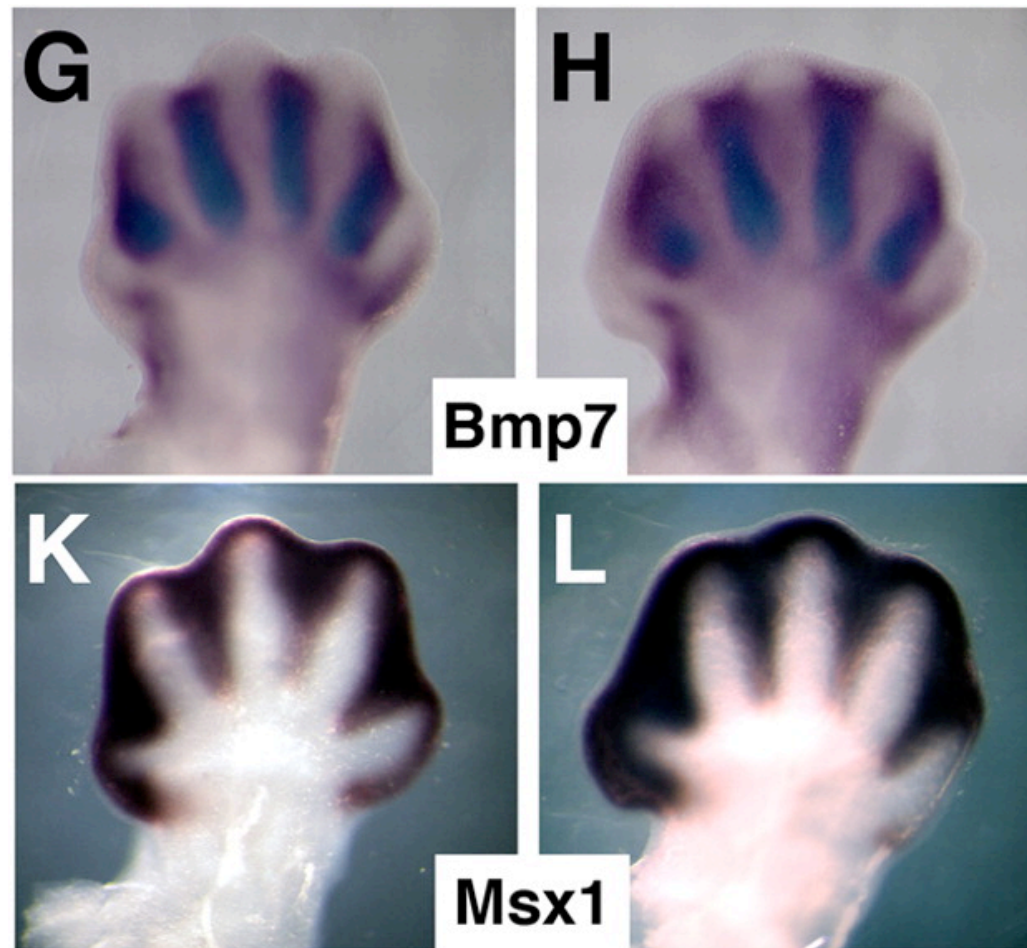
FUNCTIONS OF DEVELOPMENTAL CELL DEATH

A. MORPHOGENESIS: SCULPTING/SHAPING STRUCT

CREATION OF CAVITIES AND TUBES

CREATION OF FORM (DIGITS)

CELL DEATH AND FORMATION OF DIGITS 3



REGULATION OF INTERDIGITAL CELL DEATH

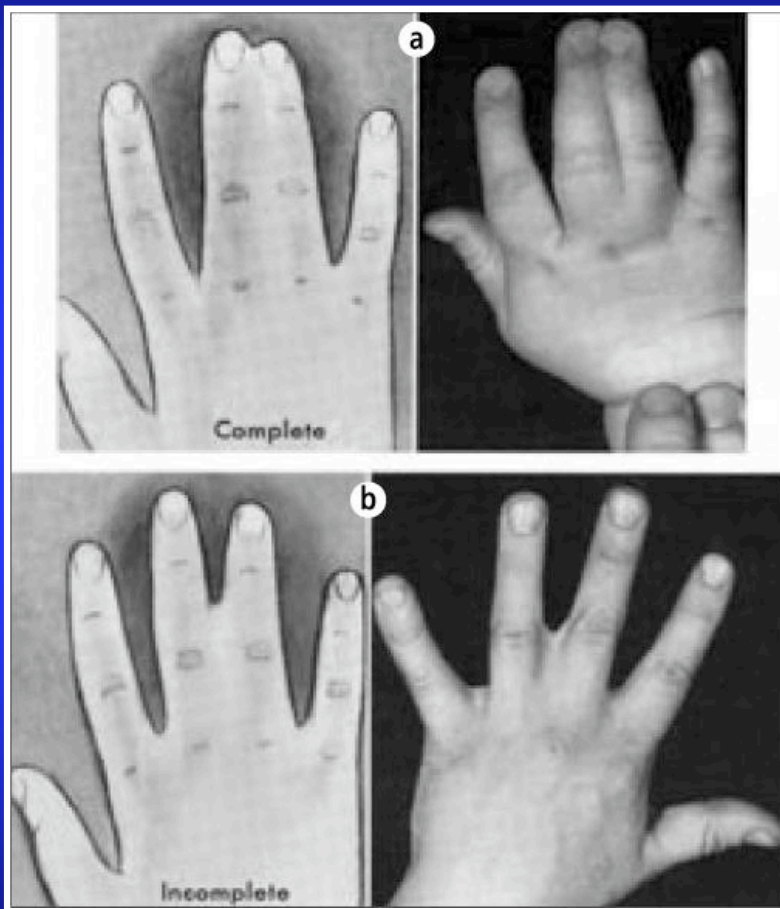


HUMAN SYNDACTYLY

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

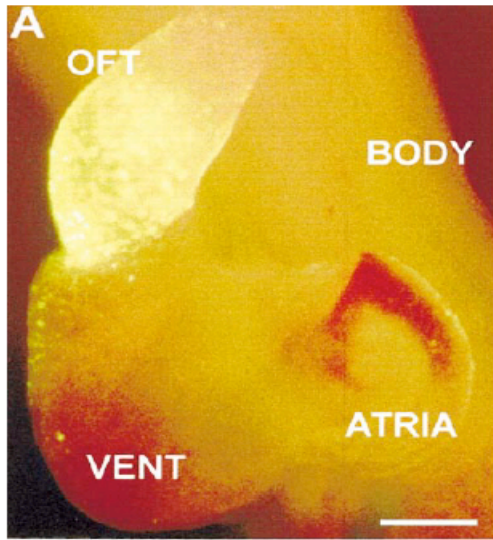
A. MORPHOGENESIS: SCULPTING/SHAPING STRUCTURE

CREATION OF CAVITIES AND TUBES

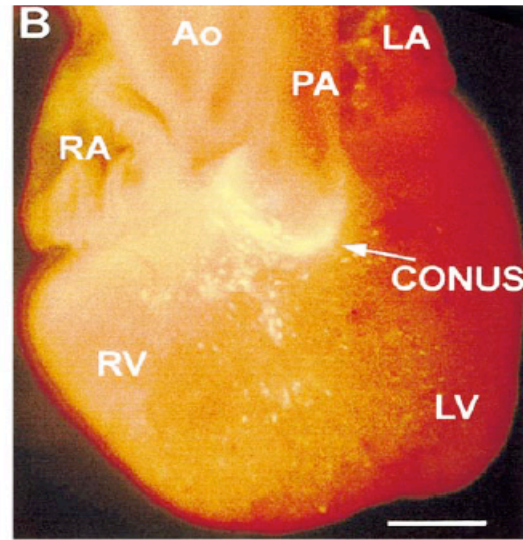
CREATION OF FORM (DIGITS)

TISSUE REMODELING (CARDIAC OUTFLOW TRACT)

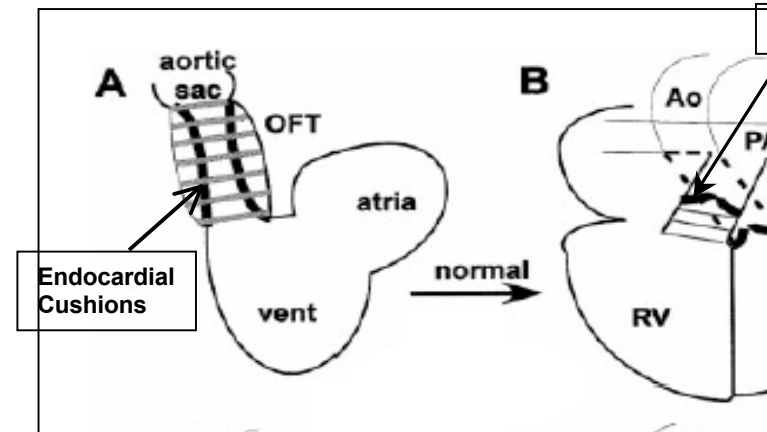
CELL DEATH AND CARDIAC MORPHOGENESIS



STAGE 23

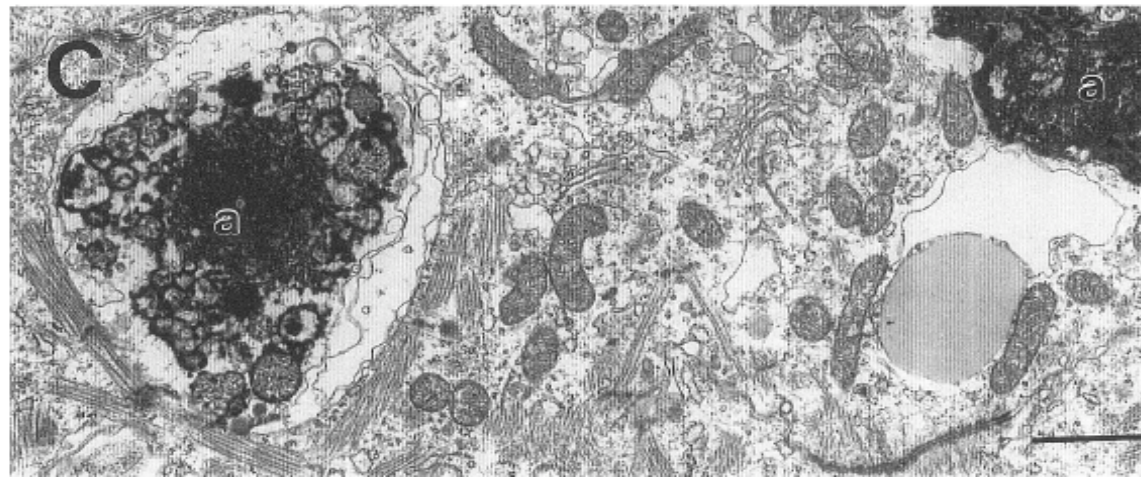


STAGE 35

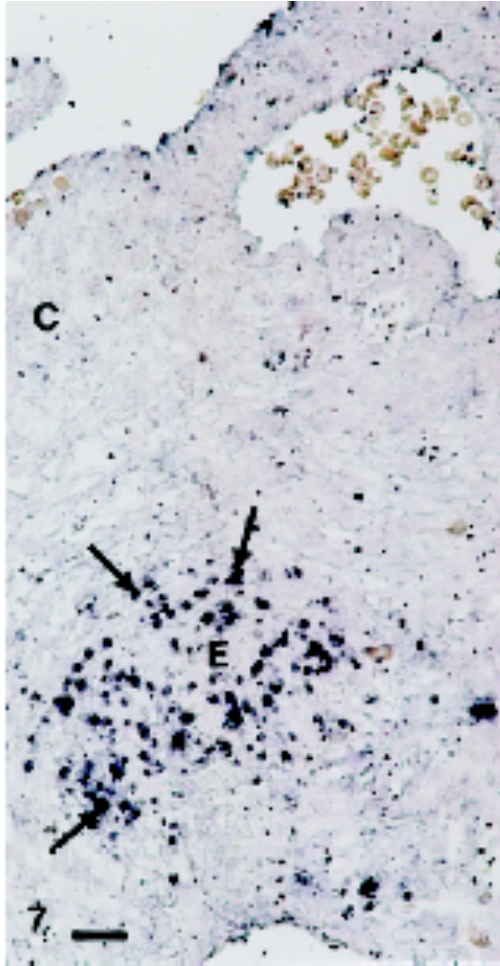


OTF = Outflow Tract
 RA = Right Auricle
 RV = Right Ventricle
 LA = Left Auricle
 LV = Left Ventricle
 PA = Pulmonary Artery
 Ao = Aorta

a = Apoptotic Cardiomyocyte



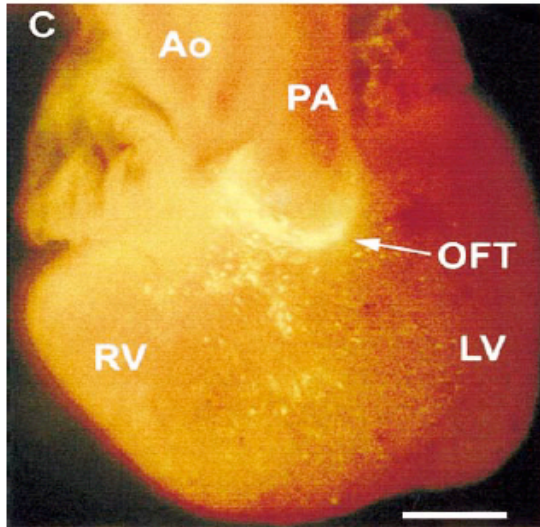
CELL DEATH AND CARDIAC MORPHOGENESIS



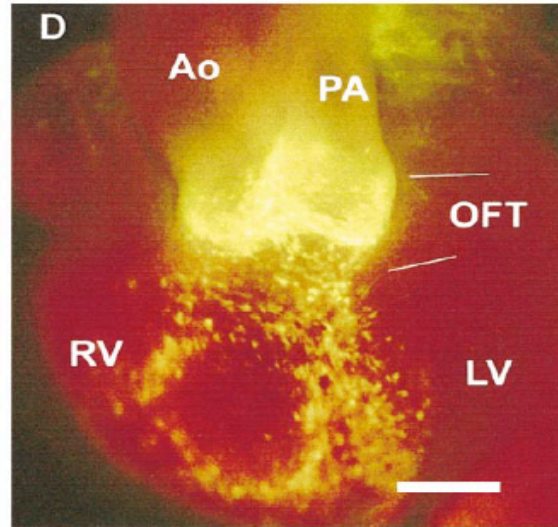
CELL DEATH IN CARDIAC
OUTFLOW TRACT OF E13 MOLF
HEART AS REVEALED BY
TUNEL STAINING (ARROWS)

Terminal deoxynucleotidyl transferase dUTP nick end lab

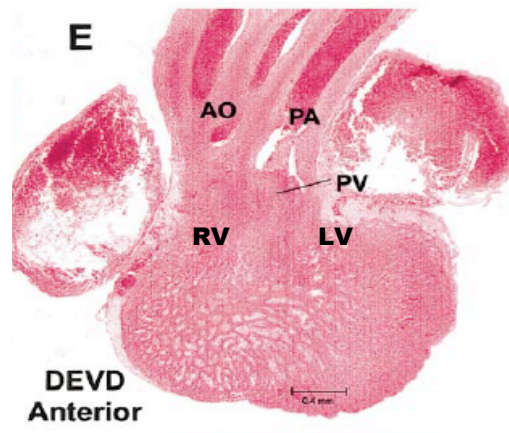
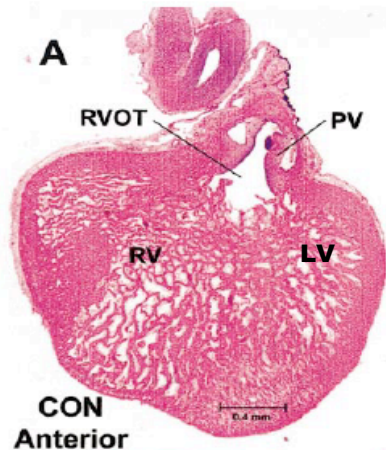
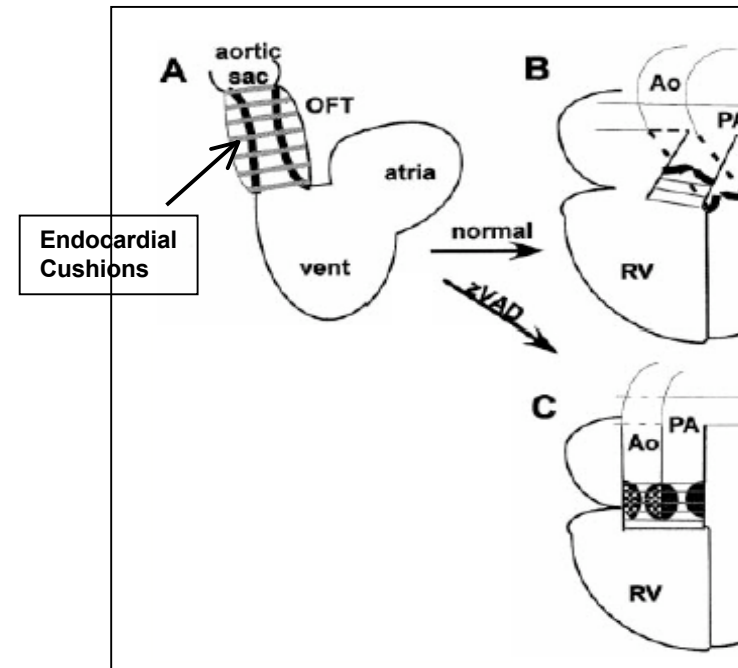
BLOCKADE OF DEATH IN DEVELOPING HEART OFT LEADS TO DOUBLE OUTLET RIGHT VENTRICLE (DORV)



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

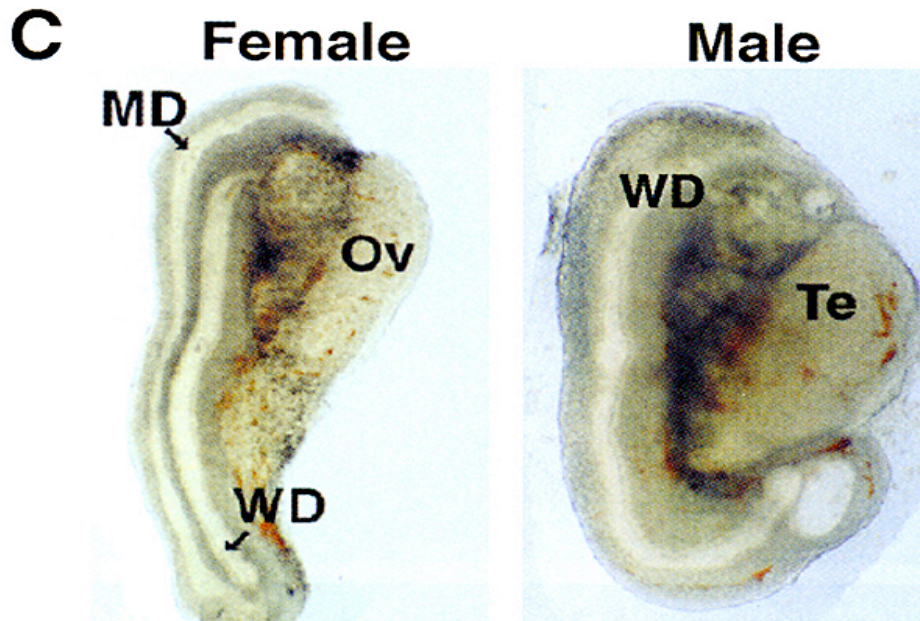
UROGENITAL SYSTEM: WOLFFIAN AND MÜLLER DUCTS

REGULATION OF REPRODUCTIVE TRACT DEVELOPMENT 1

EMBRYONIC RAT GENITAL
RIDGES SHOWING:

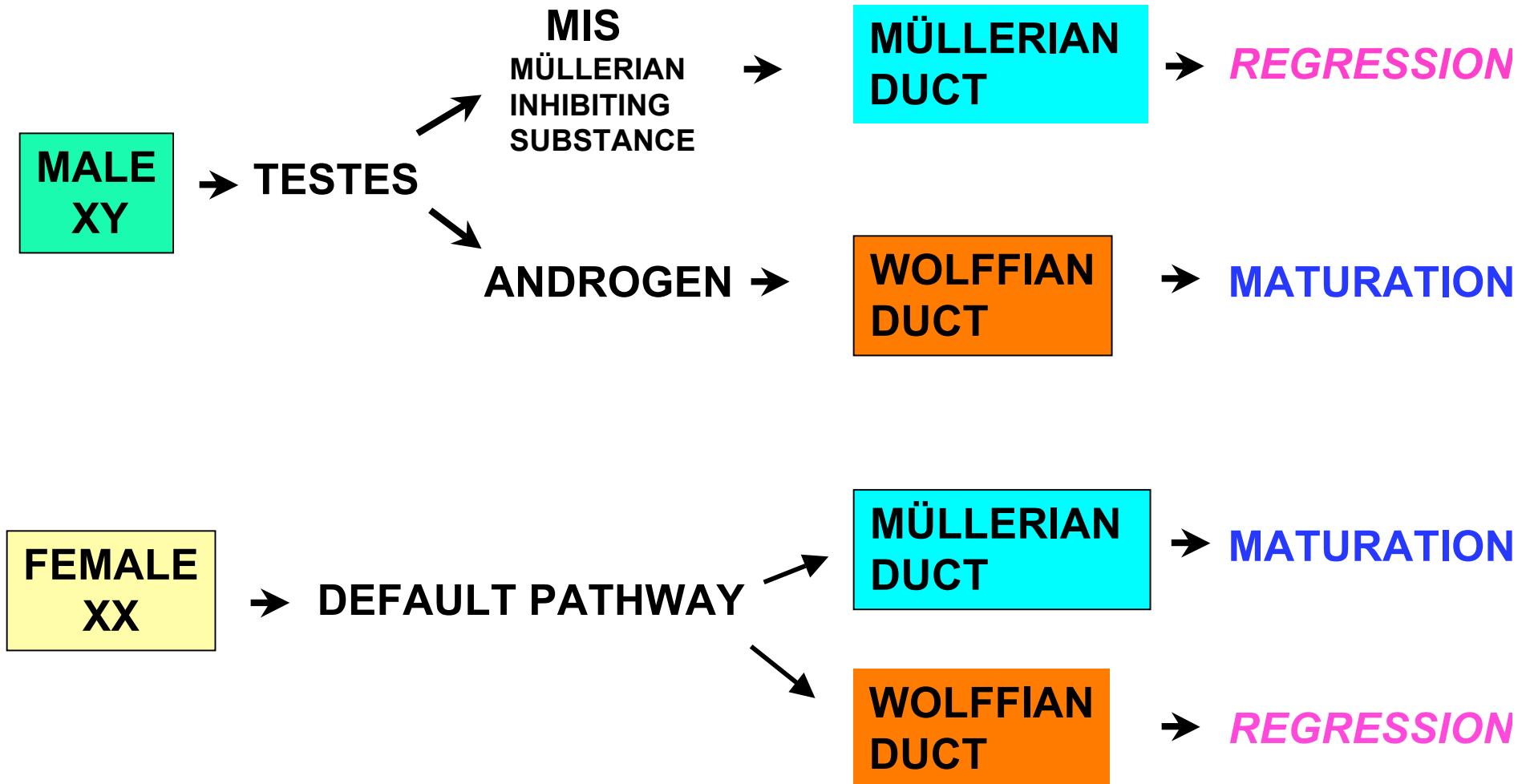
MÜLLERIAN DUCT (MD)
WOLFFIAN DUCT (WD)

OVARY (Ov)
TESTES (Te)

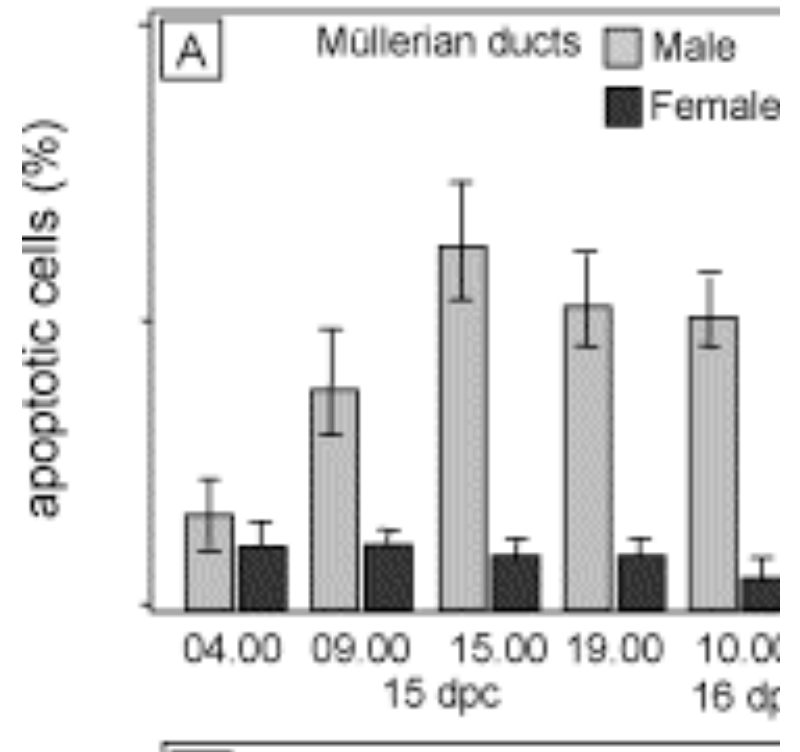
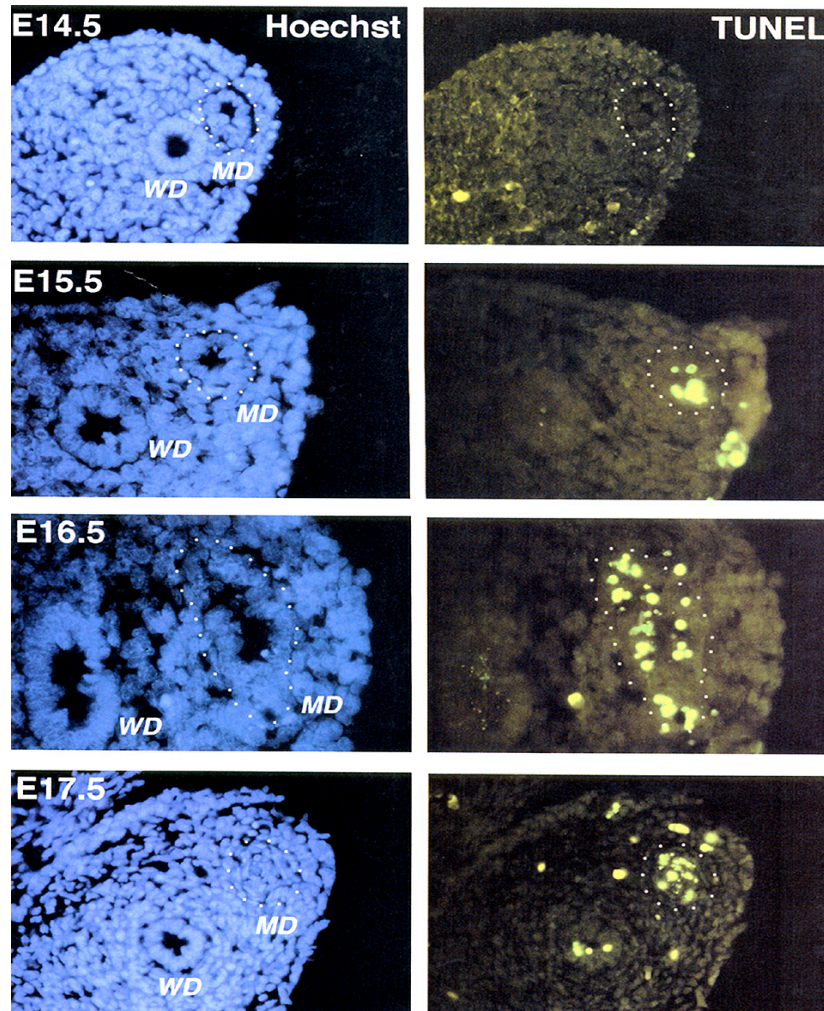


FROM: Roberts et al., Devel. Bio. 208: 110 (1999)

REGULATION OF REPRODUCTIVE TRACT DEVELOPMENT 2



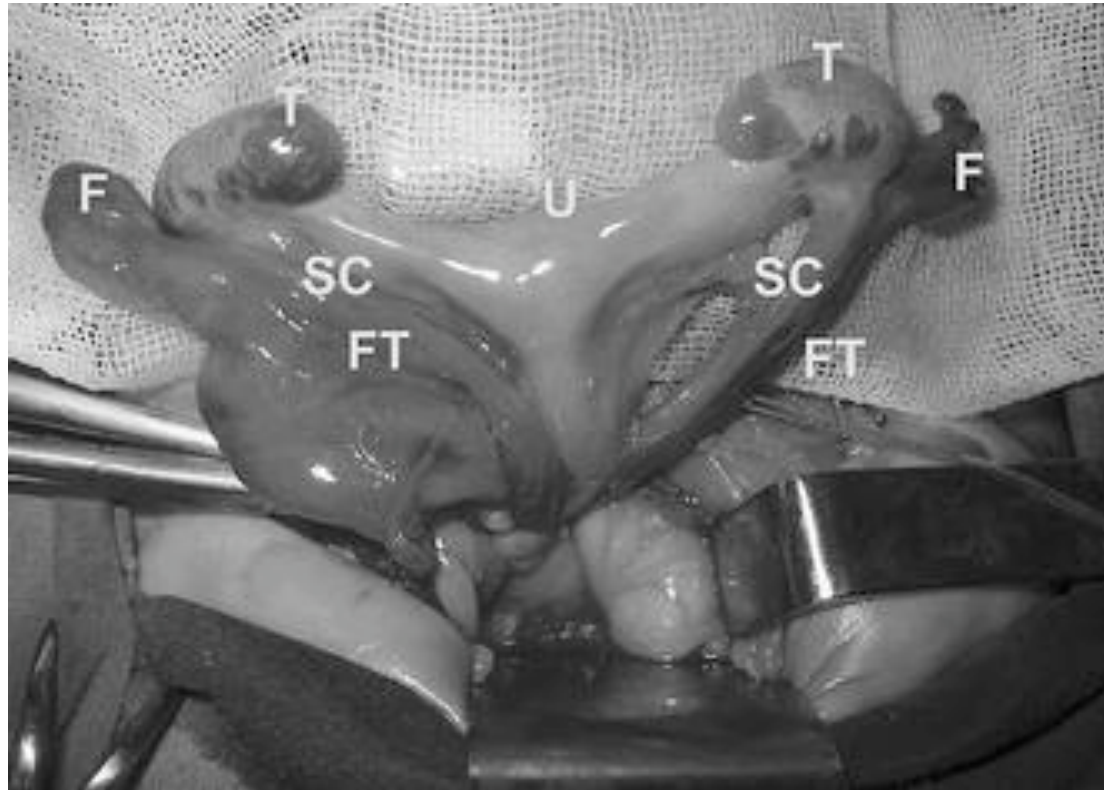
REGULATION OF REPRODUCTIVE TRACT DEVELOPMENT 3 CELL DEATH DURING MÜLLERIAN DUCT REGRESSION



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 synd
T: Testis, SC: Spermatic cord, FT: Fallopian tube, F: Fimbria, U: Uterus

From: Boleken et al., Int Urol Nephrol. March 1, 2

FUNCTIONS OF DEVELOPMENTAL CELL DEATH

C. REGULATION OF CELL NUMBERS

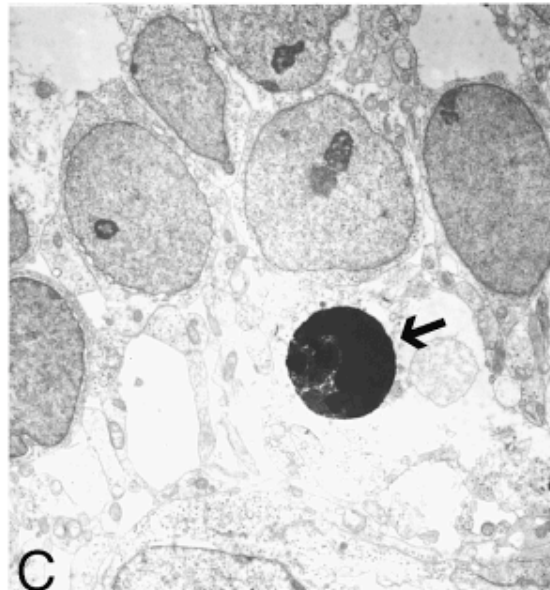
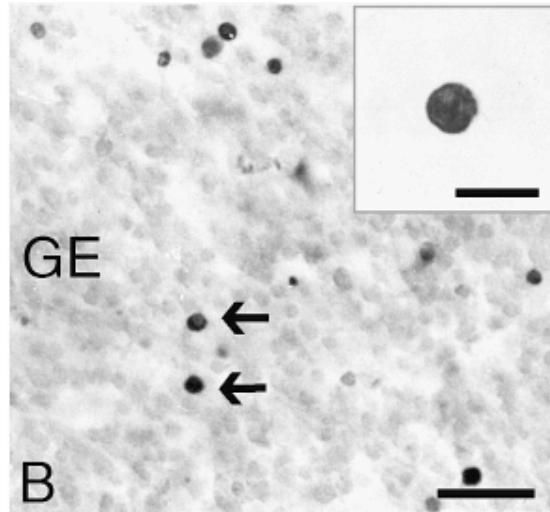
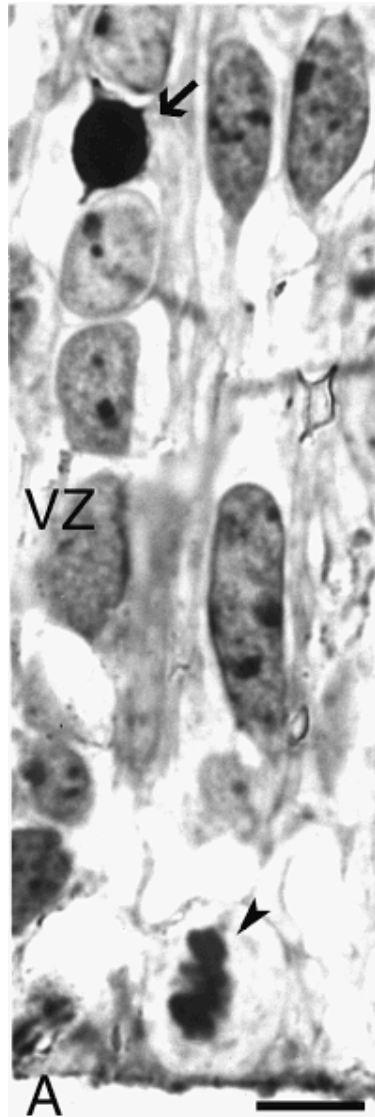
NERVOUS SYSTEM:

→ GENERATION OF CORRECT NEURON NUMBER

MATCHING NEURONS AND TARGETS

MATCHING SCHWANN CELL AND
OLIGODENDROCYTES WITH AXONS

DEATH OF NEUROPROGENITOR CELLS IN DEVELOPING HUMAN NERVOUS SYSTEM



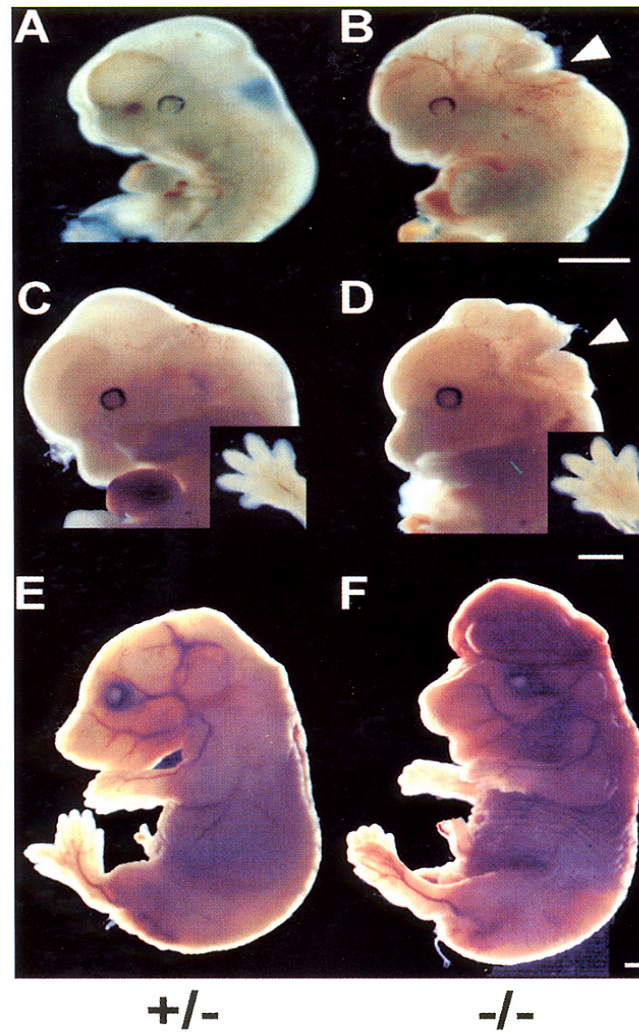
A. 4.5 gw Ventricular Zone.
Apoptotic nucleus (arrow)

B. 17 gw Ganglionic Eminence
TUNEL staining (arrow)

C. 4.5 gw Neural Tube.
EM of apoptotic cell

*FROM: Rakic S and Zecevic M
Eur J Neurosci. 12:2721-34 2000*

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:

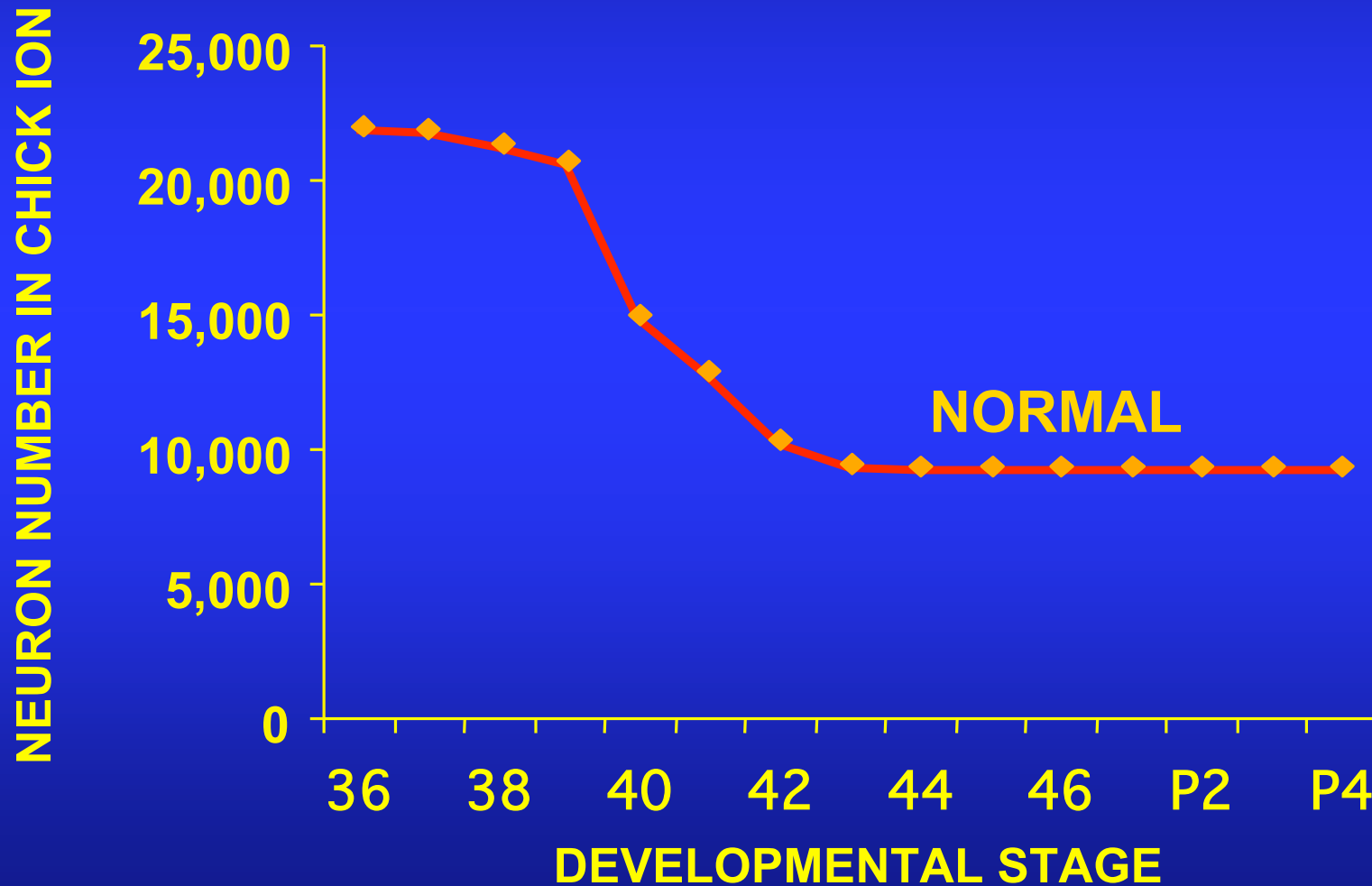
GENERATION OF CORRECT NEURON NUMBER



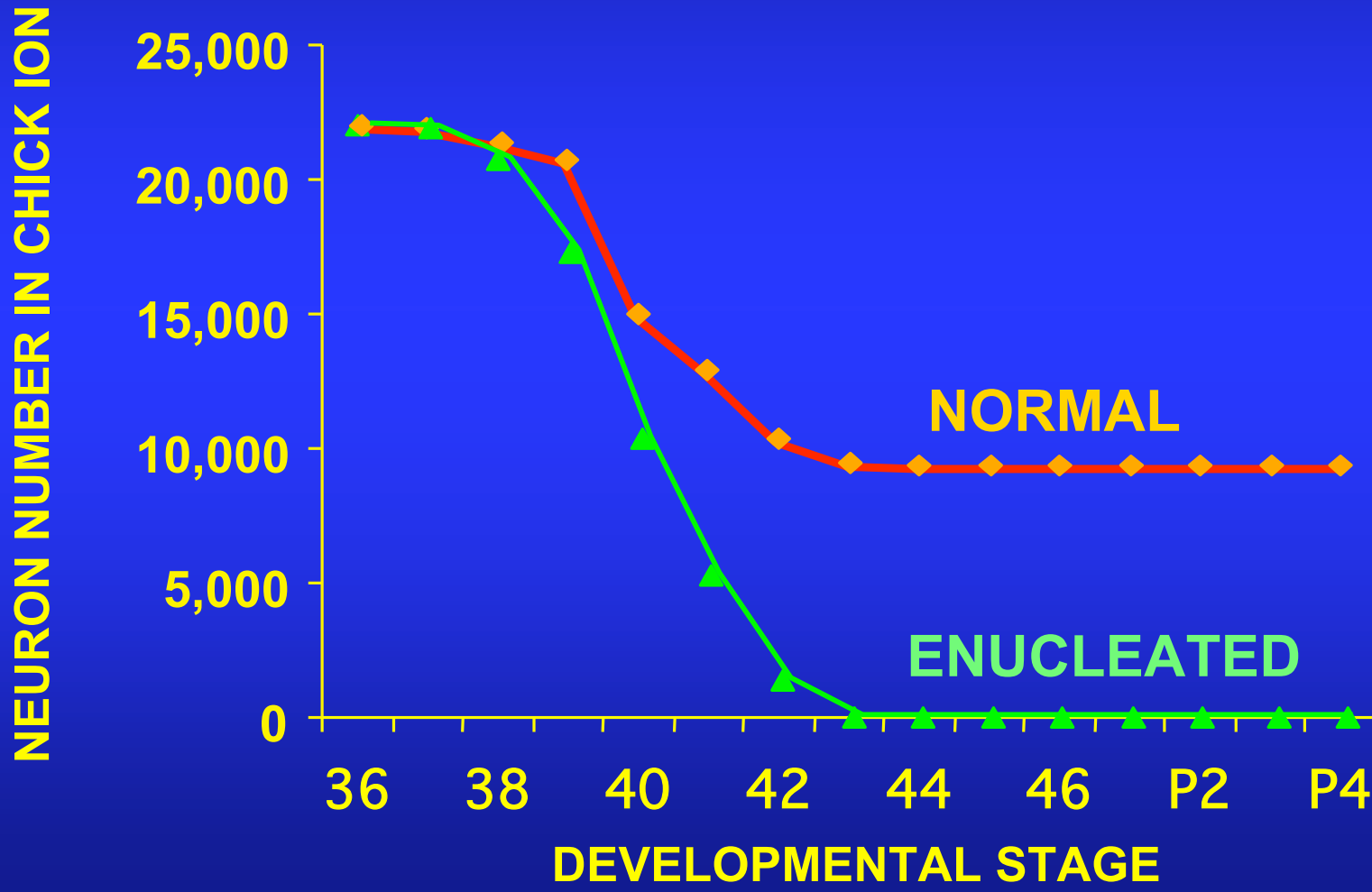
MATCHING NEURONS AND TARGETS

**MATCHING SCHWANN CELL AND
OLIGODENDROCYTES WITH AXONS**

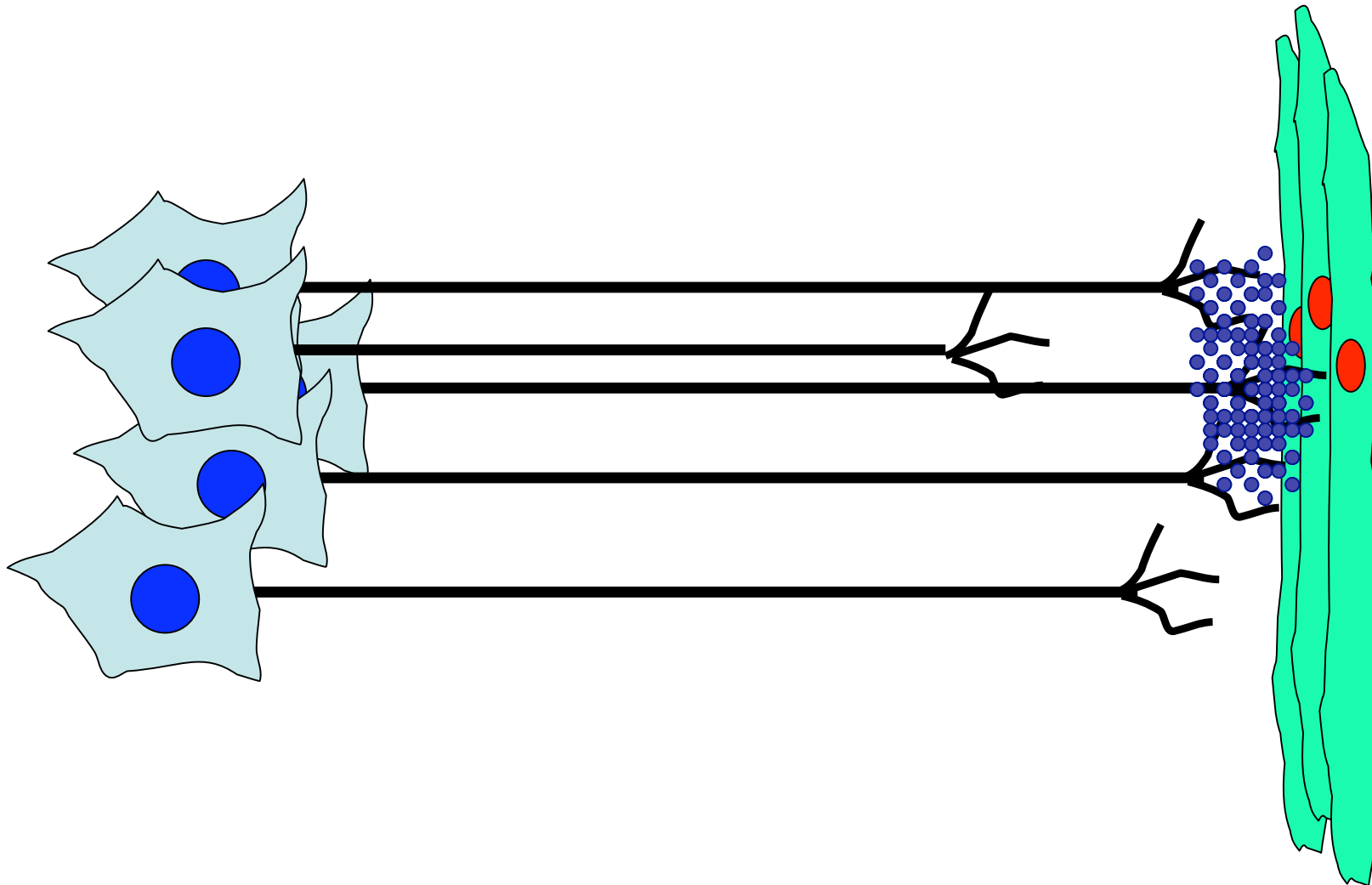
NORMAL DEVELOPMENTAL NEURONAL DEATH OCCURS AND IS REGULATED BY TARGET DERIVED TROPHIC FACTORS



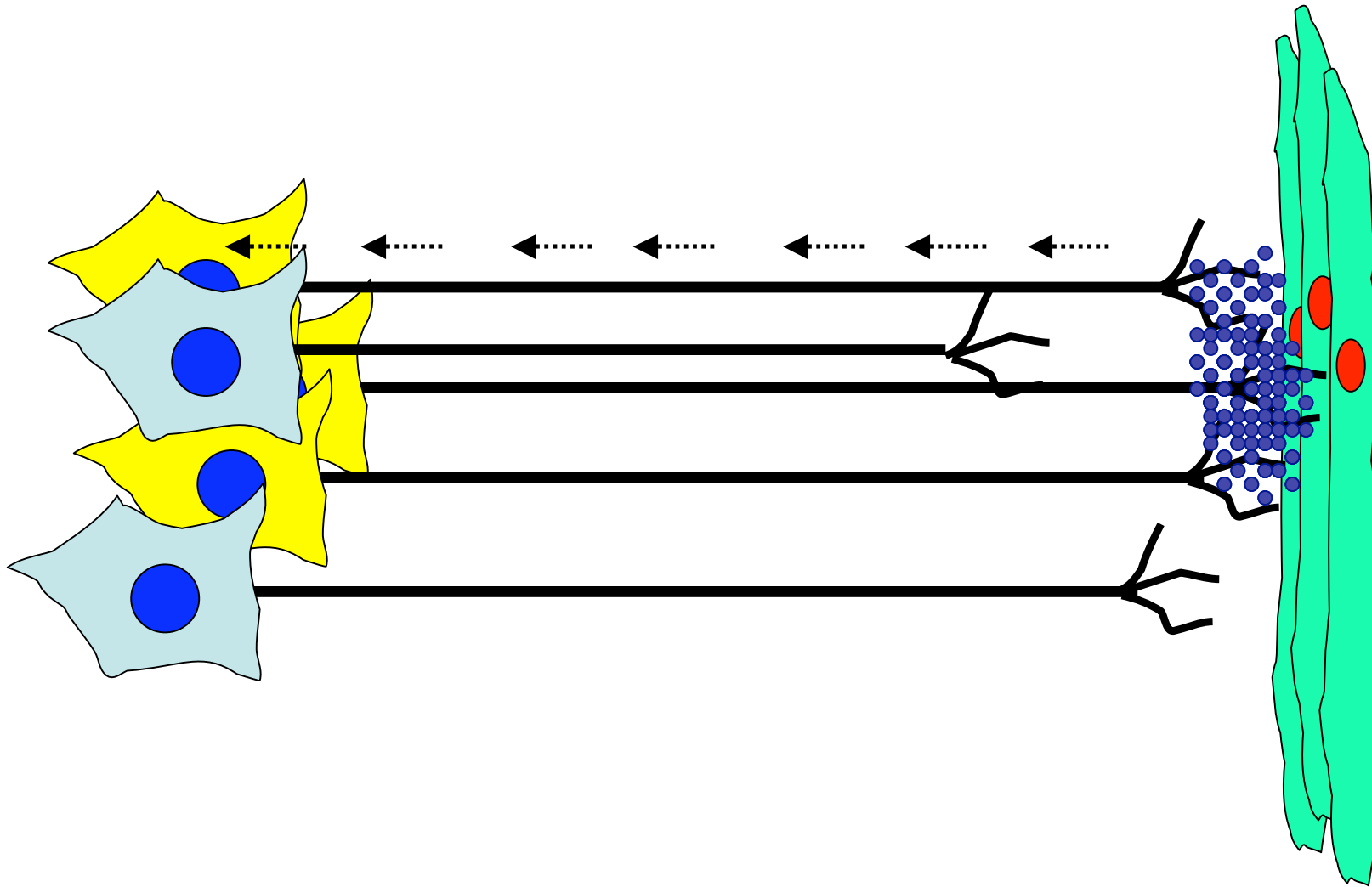
NORMAL DEVELOPMENTAL NEURONAL DEATH OCCURS AND IS REGULATED BY TARGET DERIVED TROPHIC FACTORS



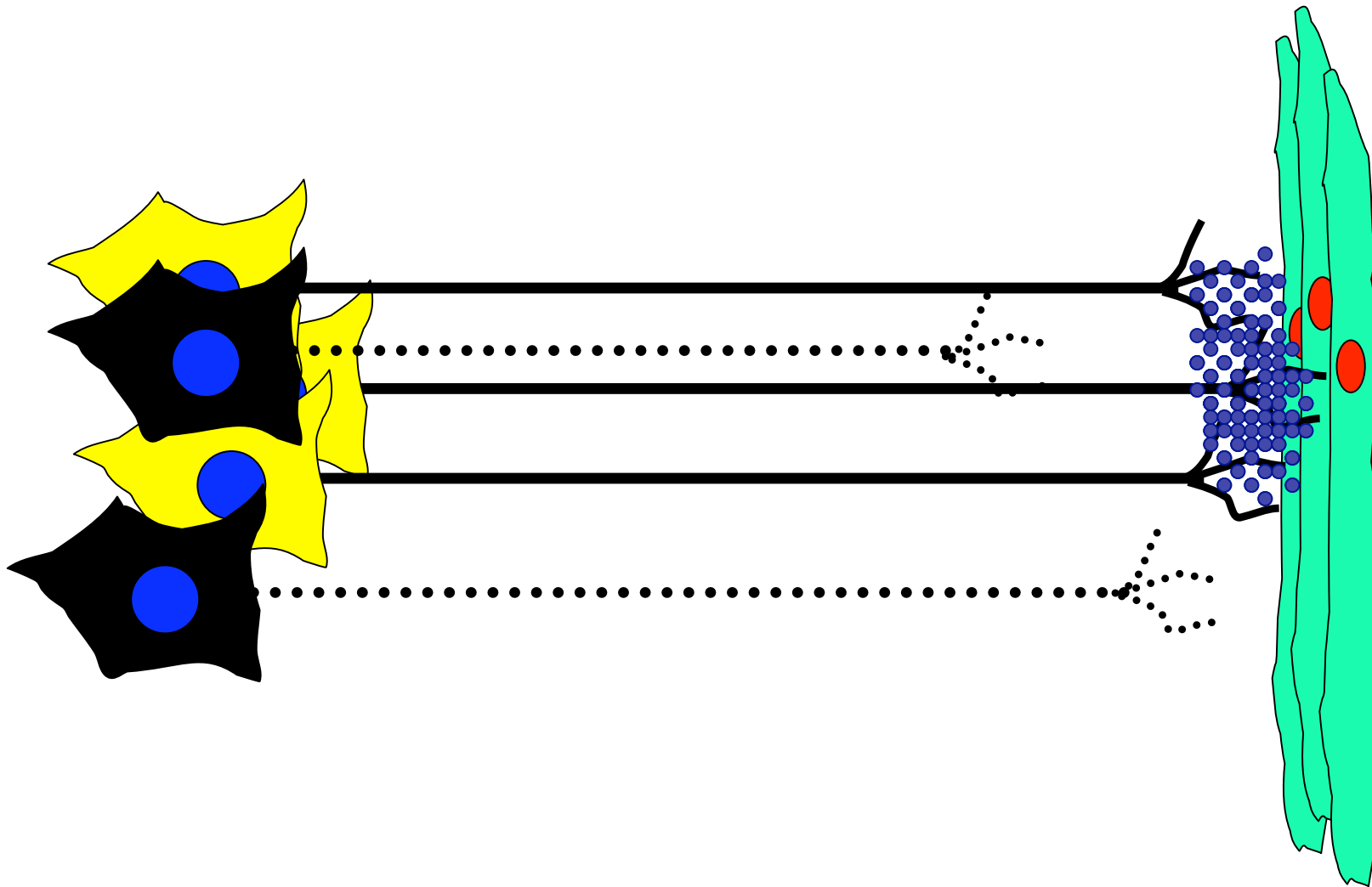
NEURONAL CULLING AS REGULATED BY COMPETITION FOR TARGET-SUPPLIED TROPHIC FACTOR



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

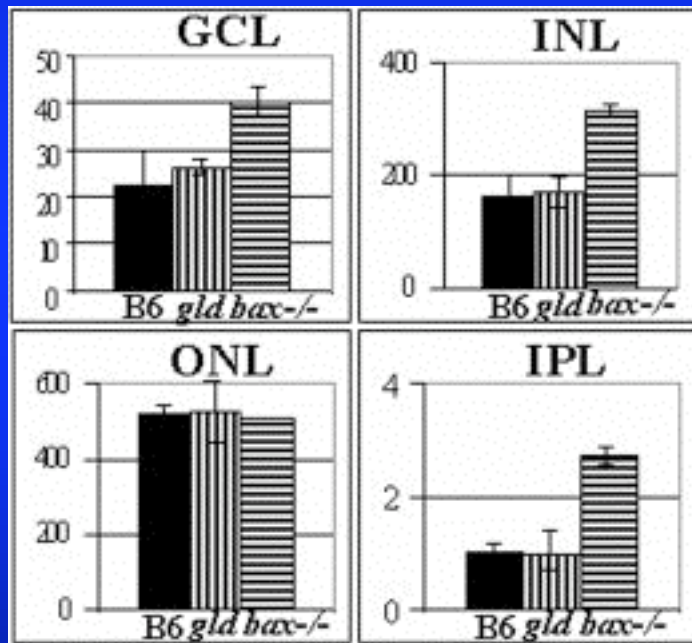


NEURONAL CULLING AS REGULATED BY COMPETITION FOR TARGET-SUPPLIED TROPHIC FACTOR

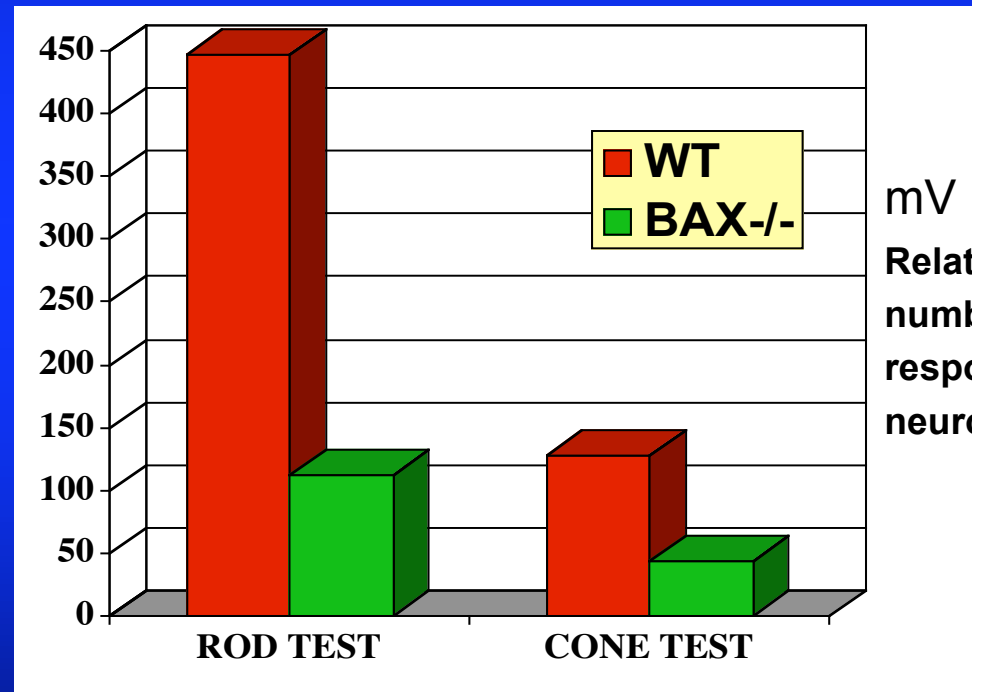


RETINAL NEURON RESPONSES TO LIGHT CHANGE IN ABSENCE OF NORMAL DEVELOPMENTAL CELL DEATH

NEURON NUMBER



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



Péquignot et al. Dev Dyn 2003

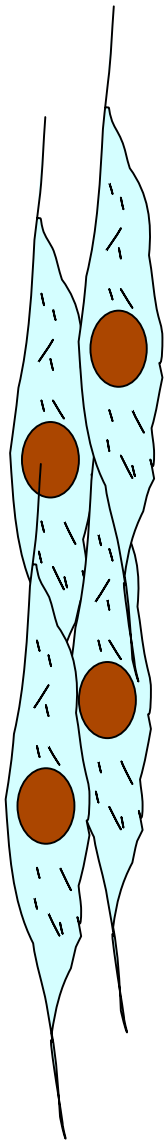
FUNCTIONS OF DEVELOPMENTAL CELL DEATH

D. PRODUCTION OF STRUCTURES WITHOUT ORGANELLES

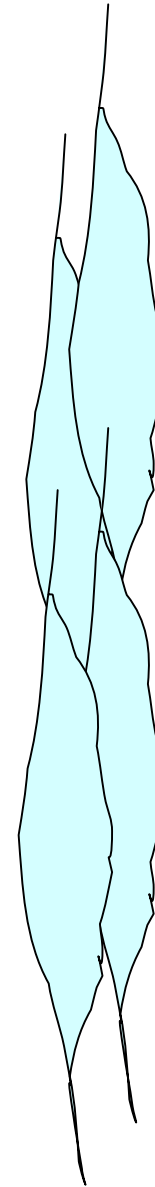
SQUAMOUS EPITHELIUM FROM KERATINOCYTES

FORMATION OF LENS FROM LENS FIBER CELLS

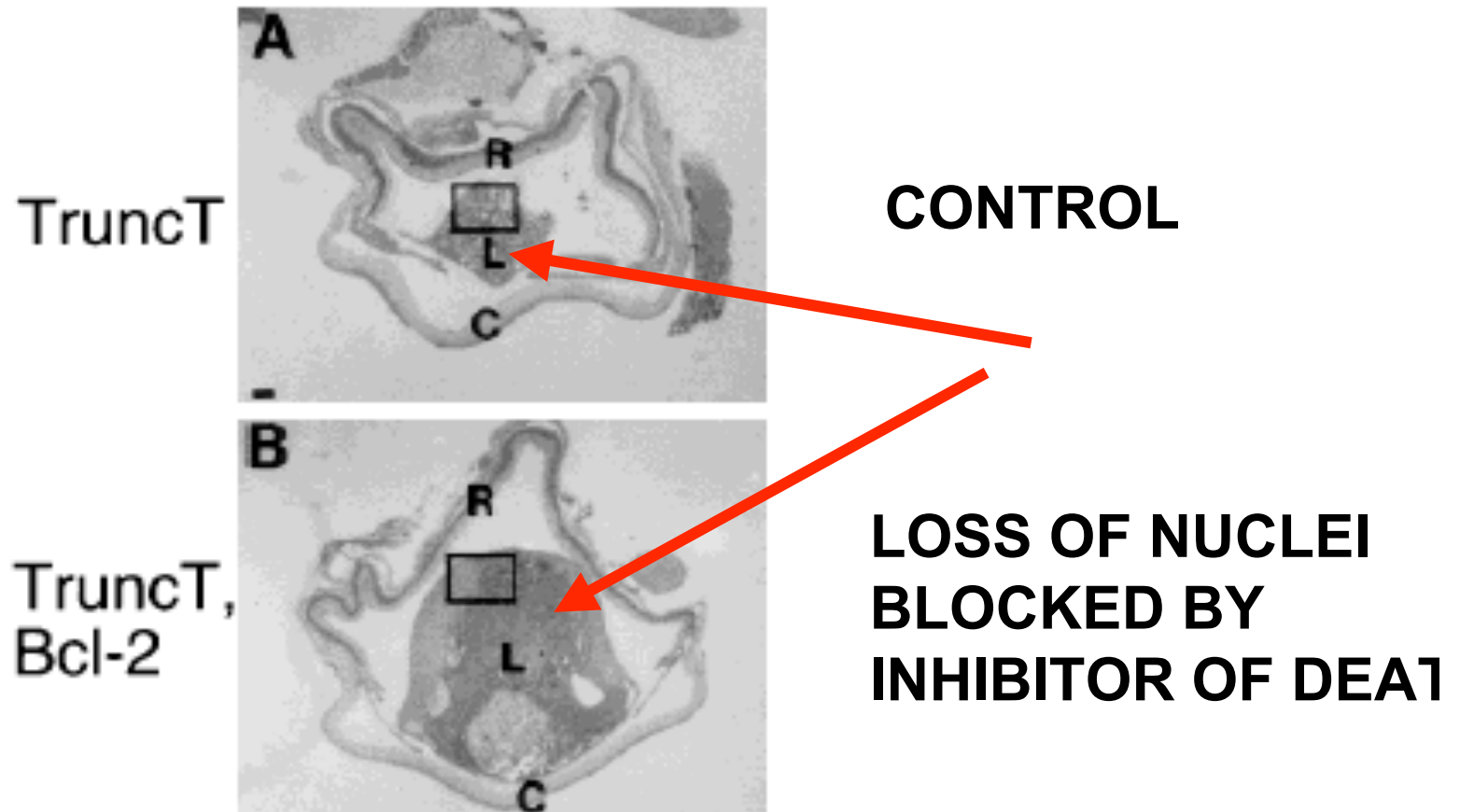
FORMATION OF CLEAR LENS FROM LENS FIBER CELLS



**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)

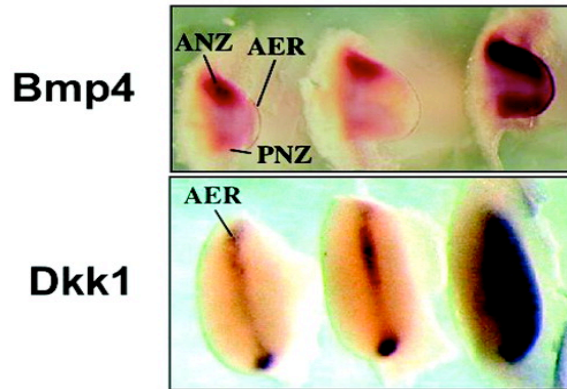
TERATOGENS AND CELL DEATH

THALIDOMIDE: USED TO TREAT MORNING SICKNESS IN EUROPE, CANADA, JAPAN AND AUSTRALIA IN 1950'S RESULTING IN 10,000 BABIES BORN WITH BIRTH DEFECT INCLUDING MALFORMED LIMBS (PHOCOMELIA)

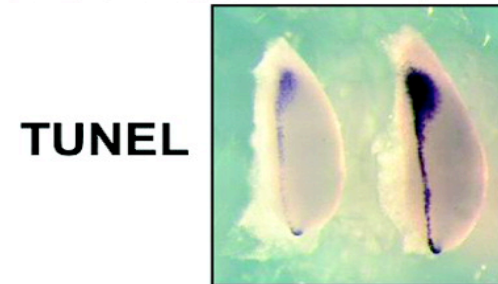


TERATOGENS AND CELL DEATH

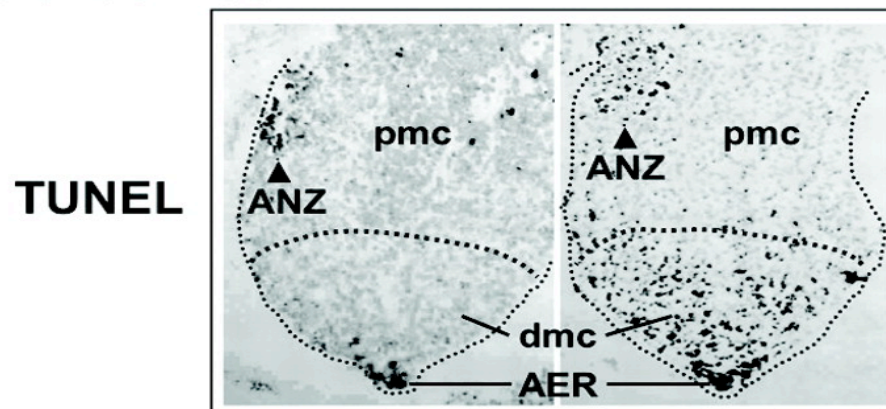
thalidomide	-	-	+
DMSO/PBS	-	+	+



thalidomide	-	+
-------------	---	---



thalidomide	-	+
-------------	---	---

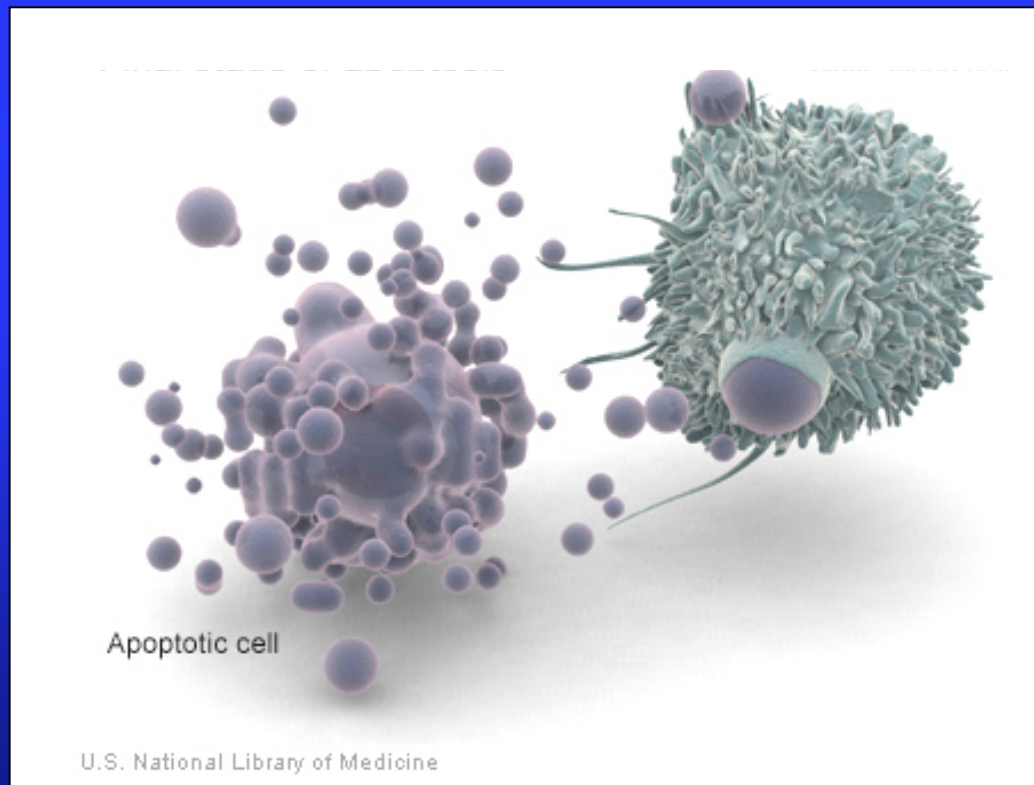


AE R= Apical ectodermal ridge

dmc = distal mesenchyme;

pmc = proximal mesenchyme.

HOW DOES DEVELOPMENTAL CELL DEATH OCCUR?



APOPTOTIC DEATH

vs

NECROTIC DEATH

PRESENT IN DEVELOPING TISSUES

RESPONSE TO CELL INJURY, TOXICITY

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 CONTENT

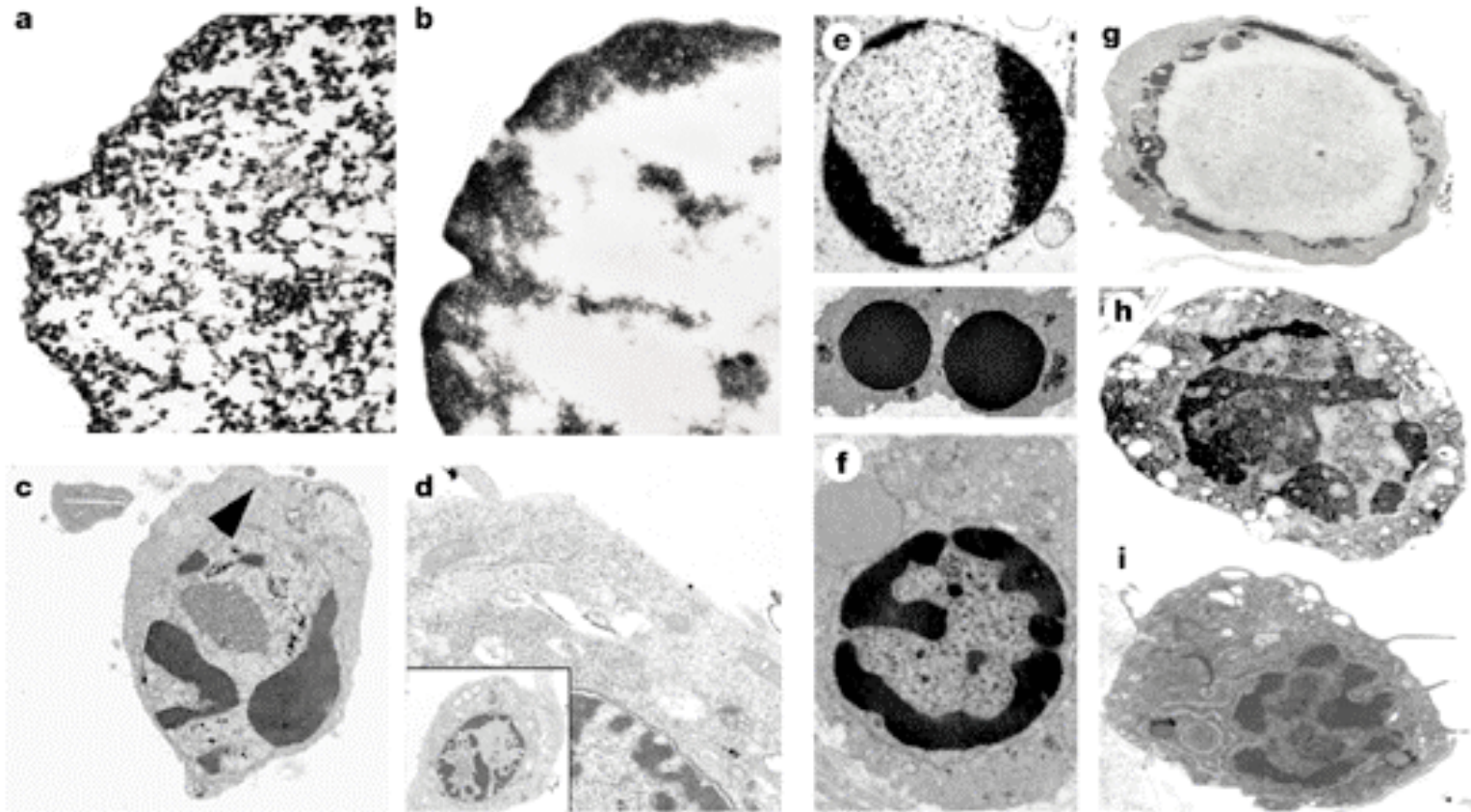
PHAGOCYTOSIS OF APOPTOTIC BODIES

ABSENCE OF INFLAMMATORY RESPONSE

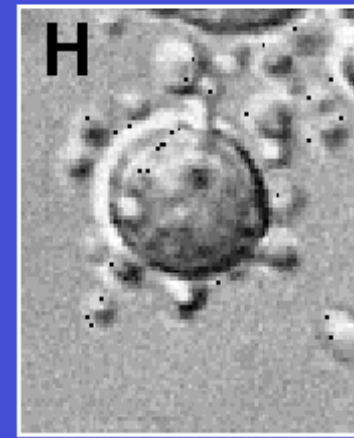
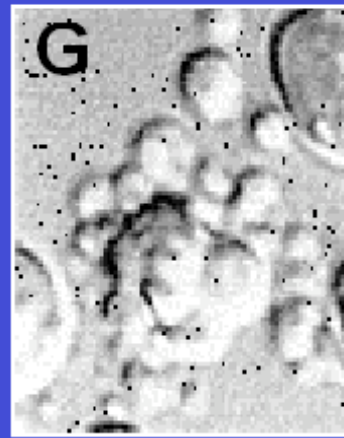
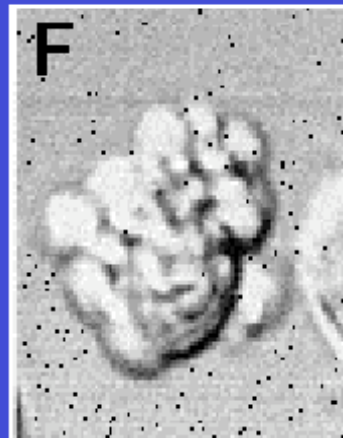
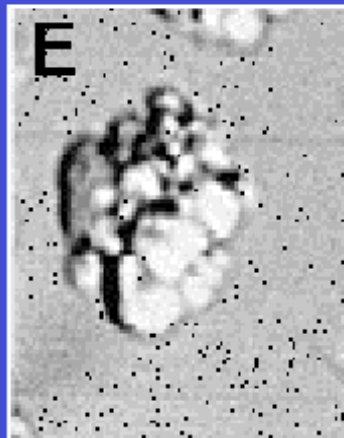
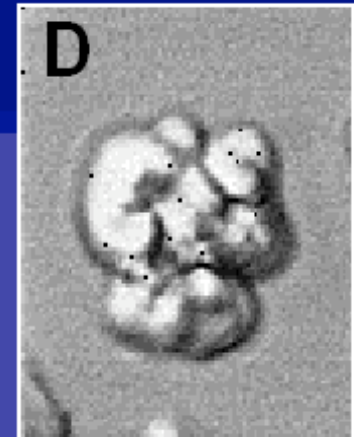
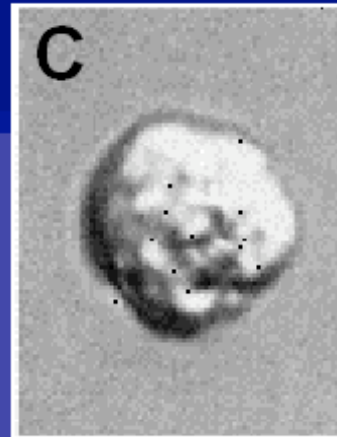
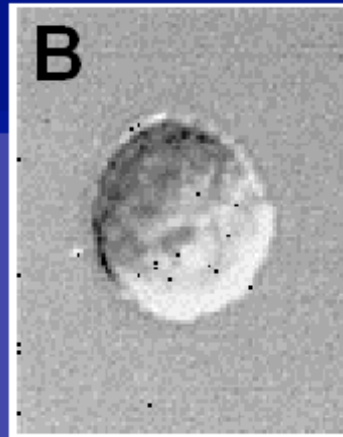
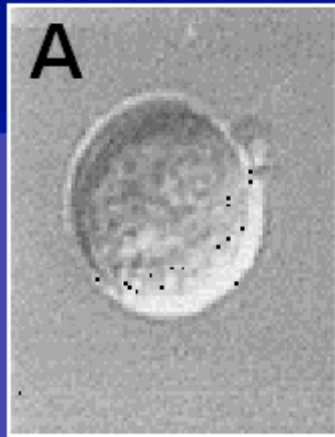
INFLAMMATORY RESPONSE

Kerr, Wylie and Currie

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

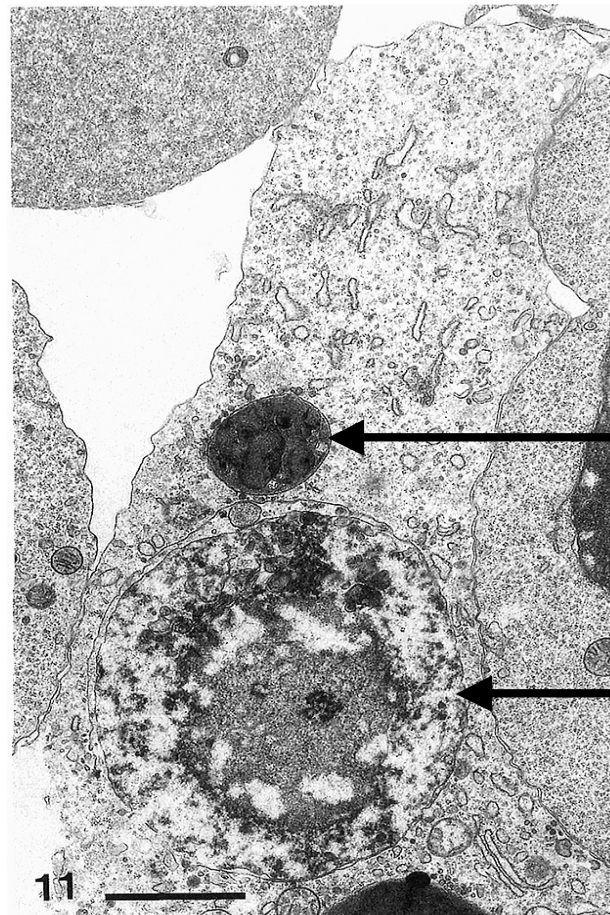


TIME LAPSE IMAGES OF A CELL UNDERGOING APOPTOSIS



APOPTOTIC BODIES AND NUCLEI ARE CLEARED BY PHAGOCYTOSIS

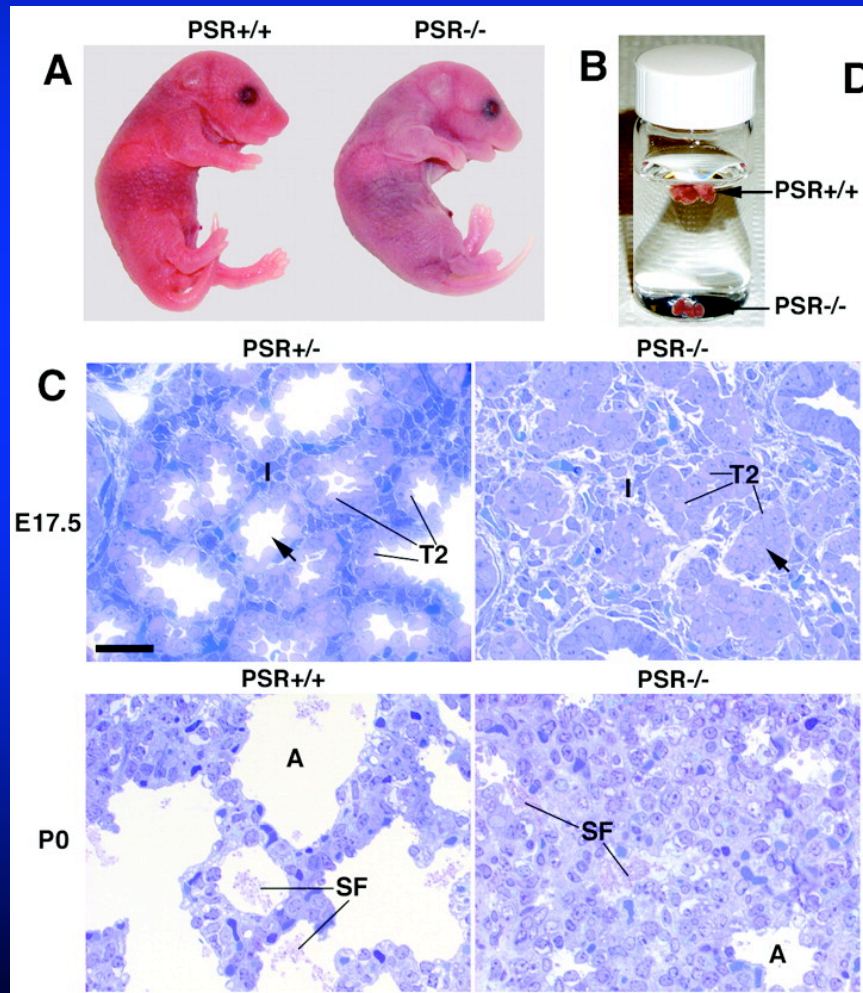
**EM OF A MACROPHAGE
ENGULFING AN
APOPTOTIC BODY AND
APOPTOTIC NUCLEUS
IN THE VENTRICULAR
WALL OF THE
DEVELOPING MOUSE
HEART**



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

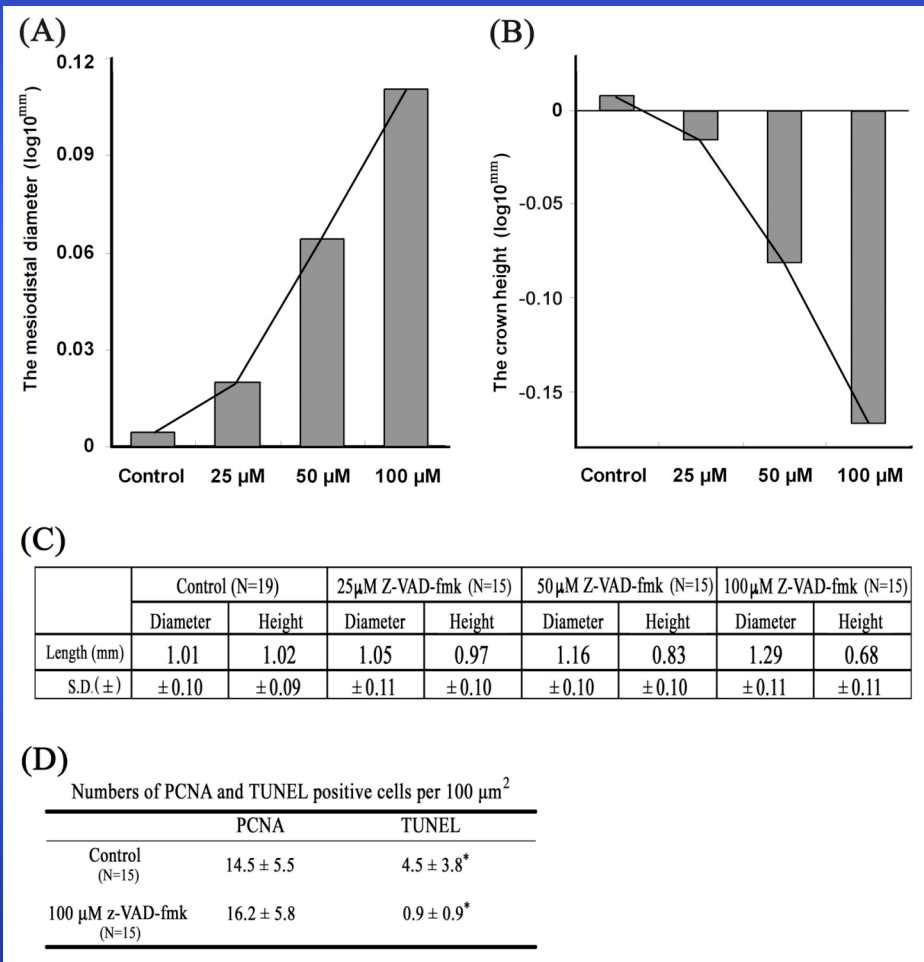
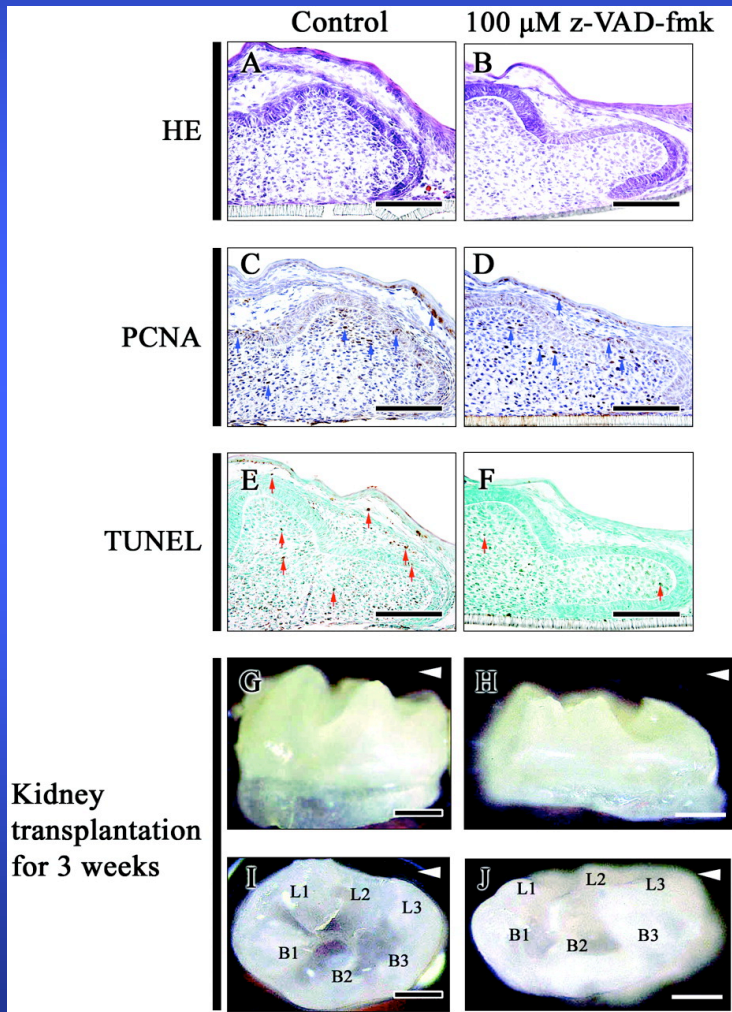


Robert Horvitz

CASPASES

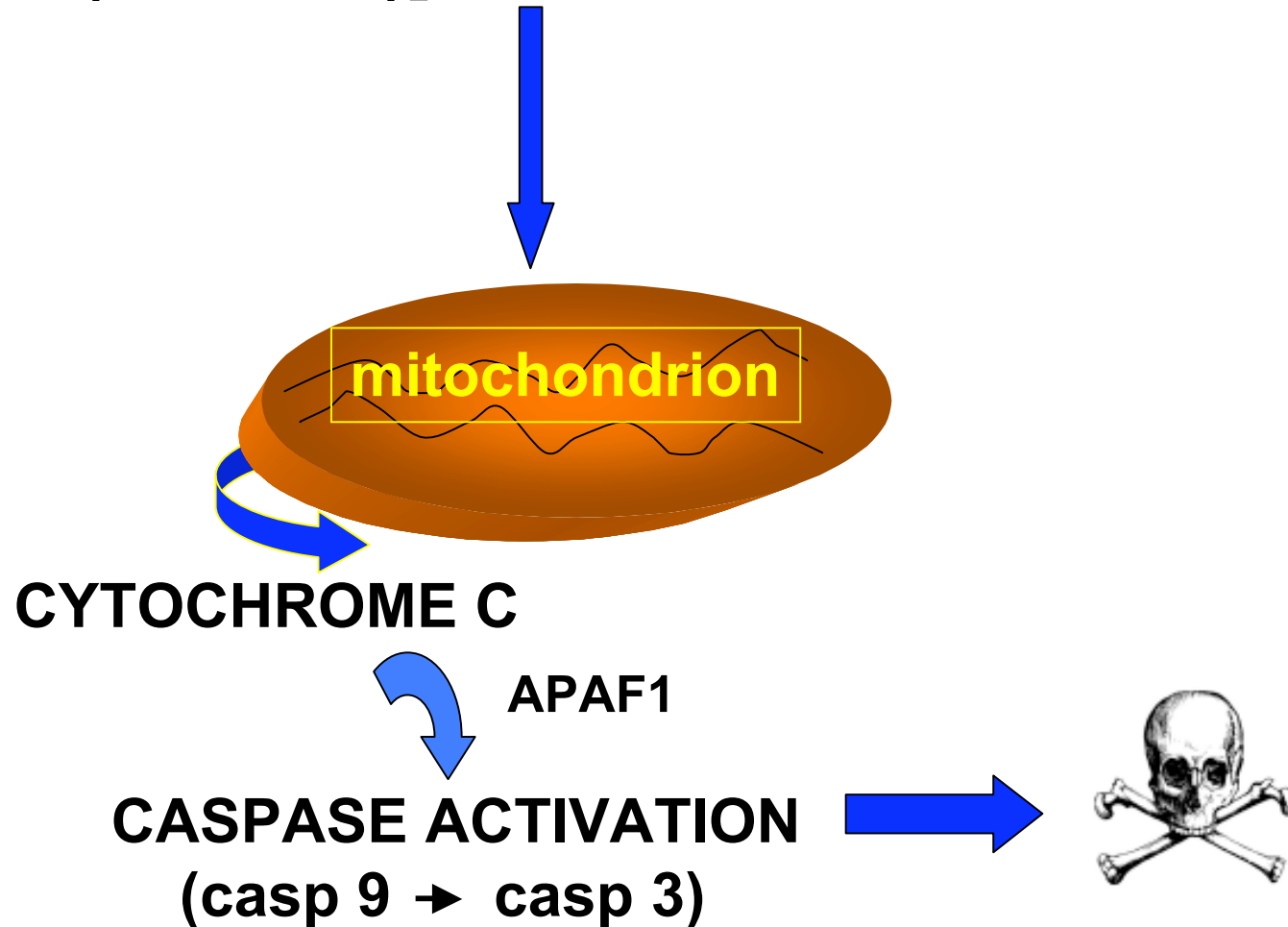
- FAMILY OF EXECUTORS OF APOPTOTIC DEATH
- CYSTEINE PROTEASES THAT CLEAVE AFTER ASP
- CONSTITUTIVELY PRESENT AS INACTIVE FORMS
- ACTIVATED BY CLEAVAGE OR CONFORMATIONAL CHANGE VIA INTERACTION WITH COFACTORS SUCH AS APAF1 AND CYTOCHROME C
- WHEN ACTIVATED, CLEAVE CELLULAR SUBSTRATES, LEADING TO APOPTOTIC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

BLOCKING CELL DEATH WITH CASPASE INHIBITORS AFFECTS TOOTH DEVELOPMENT



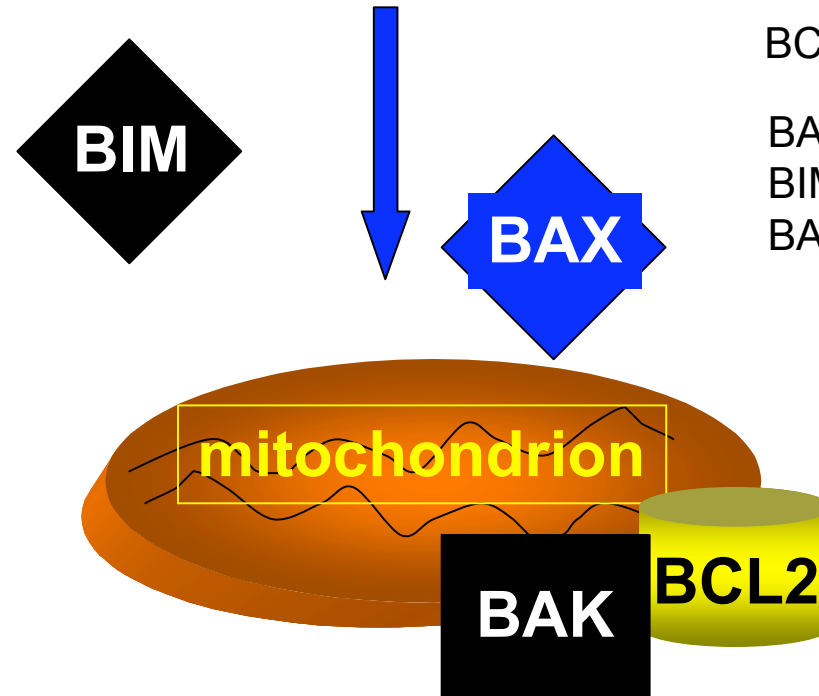
THE MITOCHONDRIAL PATHWAY OF APOPTOTIC DEATH -

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



THE MITOCHONDRIAL PATHWAY OF APOPTOTIC DEATH -

APOPTOTIC STIMULI



BCL2 = Anti-apoptotic protein

BAX = Pro-apoptotic protein

BIM = Pro-apoptotic protein

BAK = Pro-apoptotic protein

THE MITOCHONDRIAL PATHWAY OF APOPTOTIC DEATH -

APOPTOTIC STIMULI

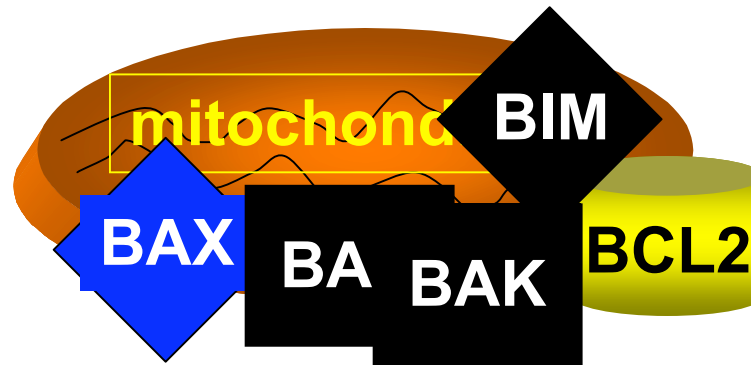


BCL2 = Anti-apoptotic protein

BAX = Pro-apoptotic protein

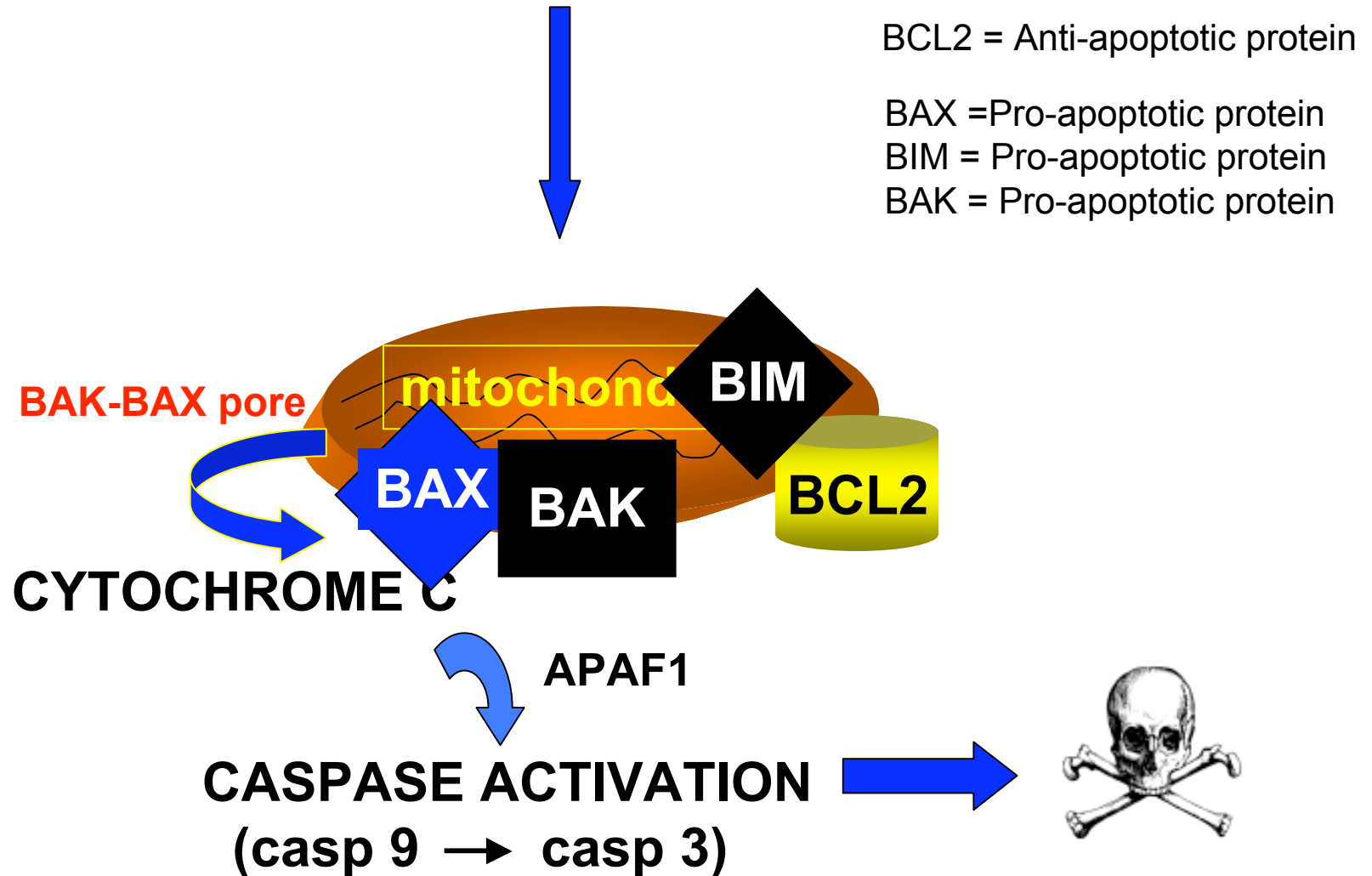
BIM = Pro-apoptotic protein

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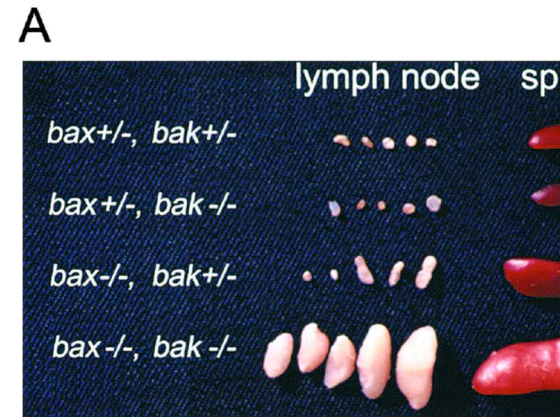
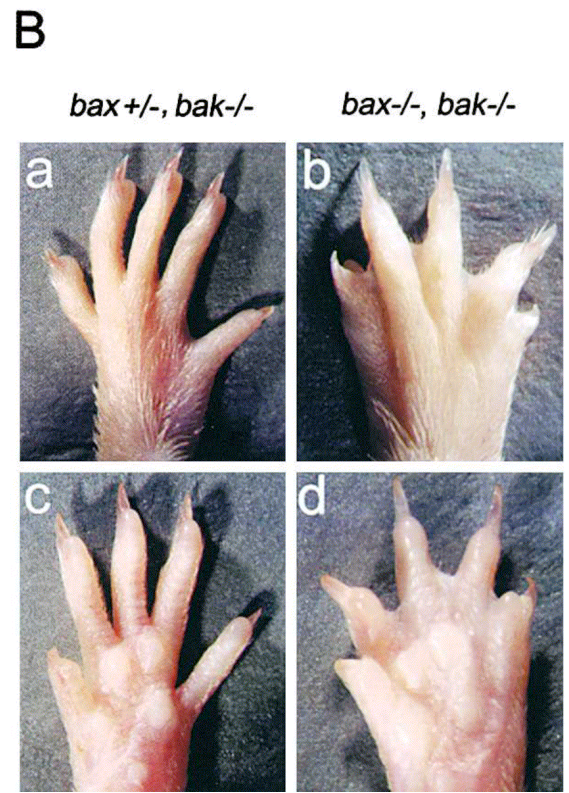
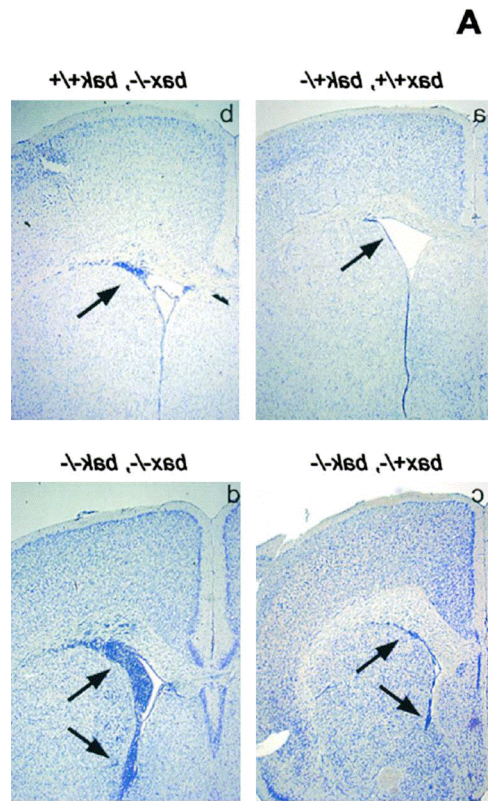


THE MITOCHONDRIAL PATHWAY OF APOPTOTIC DEATH -

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 α

FAS ligand

TRAIL

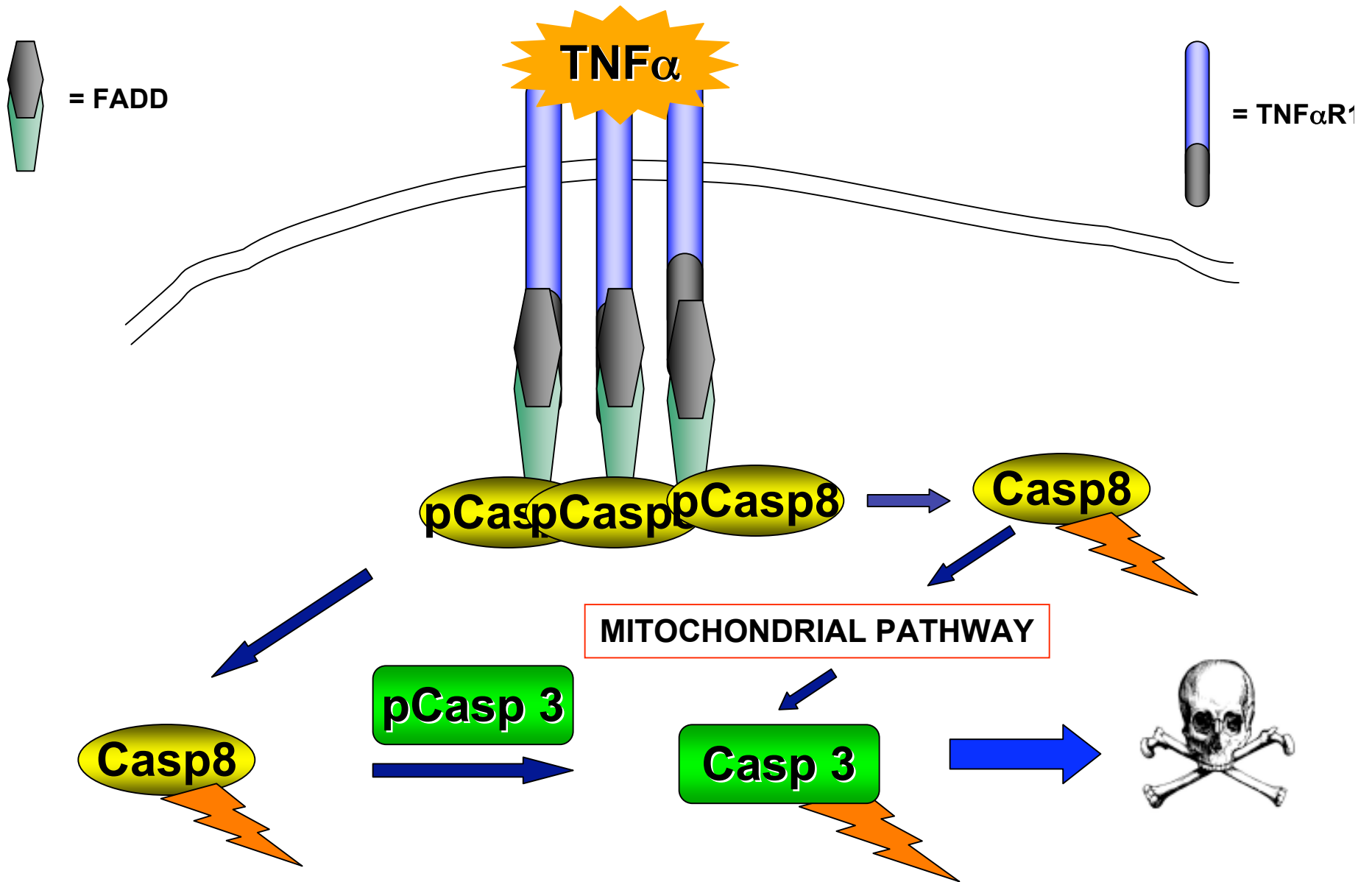
RECEPTOR

TNF α R1

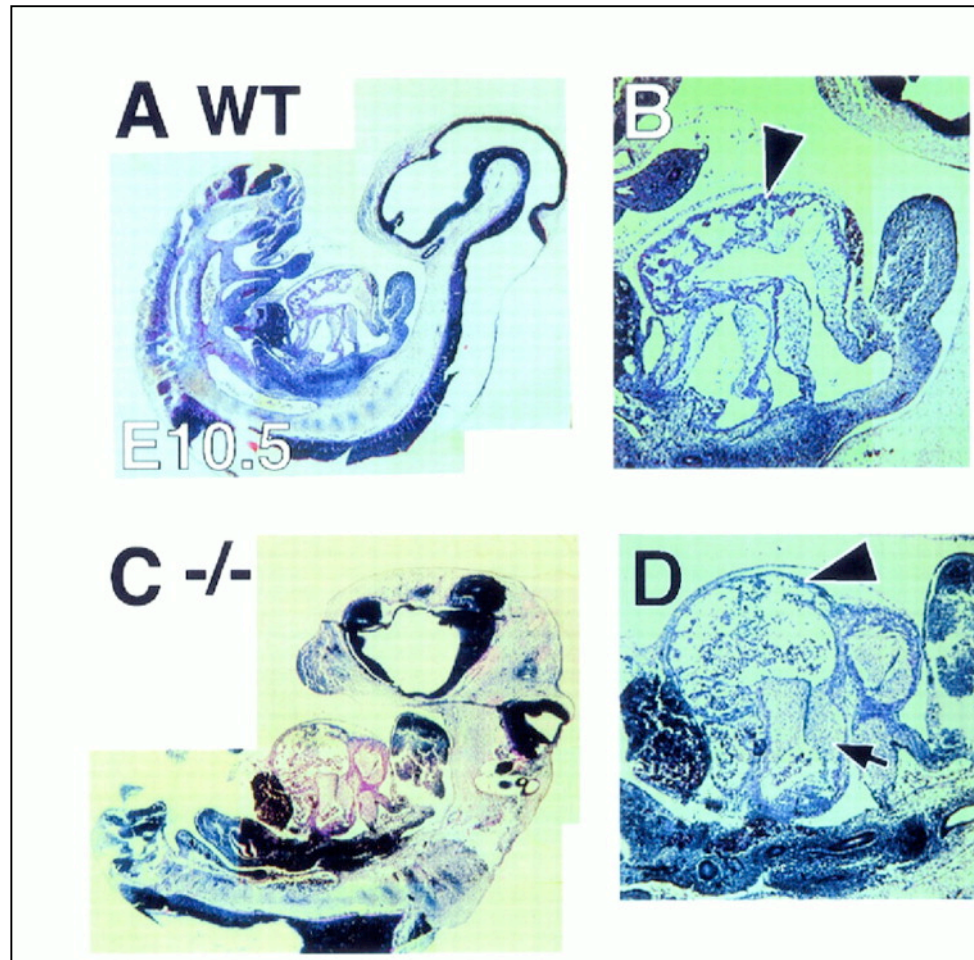
FAS

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