Lec. 13

Apoptosis

Distinguishing features of apoptosis and necrosis

- During embryonic and postnatal development, and throughout adult life, many cells in the nervous system die.
- Such cell deaths are called ‘programmed’ because they are normal, being either adaptive or neutral with regard to their impact on the function of the nervous system.
- A particular form of programmed cell death (PCD) is apoptosis

Apoptosis vs. Necrosis

- Apoptosis:
- May be “programmed”
- Cell shrinkage
- Maintenance of ATP levels
- Condensation/fragmentation of nuclear chromatin
- Requires synthesis of death effector proteins
- Membrane surface blebbing
- Phagocytosis of cellular remnants/does not adversely affect neighbor cells
- Normal apoptosis occurs during development (in *C. elegans* development, 15% of neurons die)

- Necrosis:
- Cell Swelling
- Organellar swelling and damage
- Depletion of ATP
- May result from acute trauma or injury (stroke)
- Rapid lysis of cellular membranes
- Nuclear lysis
- Cessation of protein synthesis
- No activation of endogenous cell death program
- Promotes death of neighbor cells

Apoptosis

- Knowledge of the cellular and molecular mechanisms of apoptosis is necessary for a full understanding of how the brain and other regions of the nervous system develop
- Additionally, there is increasing evidence that apoptosis and related forms of cell death occur in a range of neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's disease and stroke.

Adaptive apoptosis occurs in developing and adult nervous system

- During embryonic and early postnatal development, many cells undergo apoptosis in most regions of the mammalian nervous system- this process is believed to be adaptive in nature in that it eliminates cells that are not integrated into neuronal circuits and are therefore unnecessary for the proper function of the nervous system.
- There appear to be two major waves of apoptosis, one occurring early and involving neuronal progenitor cells and another occurring later and involving neurons that are in the process of forming synaptic connections with target cells.
- While neuronal apoptosis is well established, the extent to which glial cells undergo apoptosis during development is unclear.

Adaptive apoptosis occurs in developing and adult nervous system-2

- Developmental processes continue in at least some locations in the adult nervous system.
- The signals that determine whether neural stem cells and their progeny survive or undergo apoptosis include growth factors, cytokines, and cell adhesion molecules.
- Neurons that do not receive sufficient trophic support from molecules such as those above, undergo apoptosis.

Apoptosis occurs in acute neurological insults

- Traumatic and ischemic injury to the nervous system are common events that result in considerable morbidity and mortality.
- Many neurons may undergo apoptosis in response to traumatic injury to the brain, spinal cord or peripheral nerves, an indicated by various *proapoptotic* proteins including caspases.
- In particular, neurons adjacent to the necrotic region of severe trauma are prone to apoptosis.
- Triggers of ischemic apoptosis include oxygen free radicals, glutamate receptor activation, calcium influx and release from intracellular stores, and lipid mediators.
- Mediators of ischemic neuronal apoptosis may include cytochrome c, apoptosis-inducing factor (AIF), and caspases.

Apoptosis occurs in neurodegenerative disorders

- Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS) are four prominent fatal neurodegenerative disorders that involve the death of specific populations of neurons.
- -In Alzheimer's abnormalities in processing of the amyloid precursor protein, due to gene mutations or age-related factors, results in accumulation of neurotoxic forms of amyloid beta peptide (Fig. 35-3).
- -In Parkinson's age-related increases in oxidative stress and possibly environmental neurotoxins cause selective degeneration of dopaminergic neurons in the substantia nigra of the brain.
- -In Huntington's disease, inheritance of a mutant huntingtin protein causes degeneration of striatal neurons.

Apoptosis occurs in neurodegenerative disorders-2

- The spatial and temporal patterns of neuronal death in Alzheimer's, Parkinson's, Huntington's, and ALS are consistent with apoptosis in that neurons in vulnerable brain regions do not die in unison; instead, individual neurons die on a progressive basis.
- Examinations of brains of Alzheimer's, Parkinson's, Huntington's and ALS patients have revealed evidence for activation of caspases and upregulation of apoptotic proteins such as ***Bax***.

There are many triggers of apoptosis (a few examples)

- Insufficient trophic support
- Death receptor activation
- DNA damage
- Oxidative and metabolic stress

Examples of apoptosis

- Insufficient trophic support

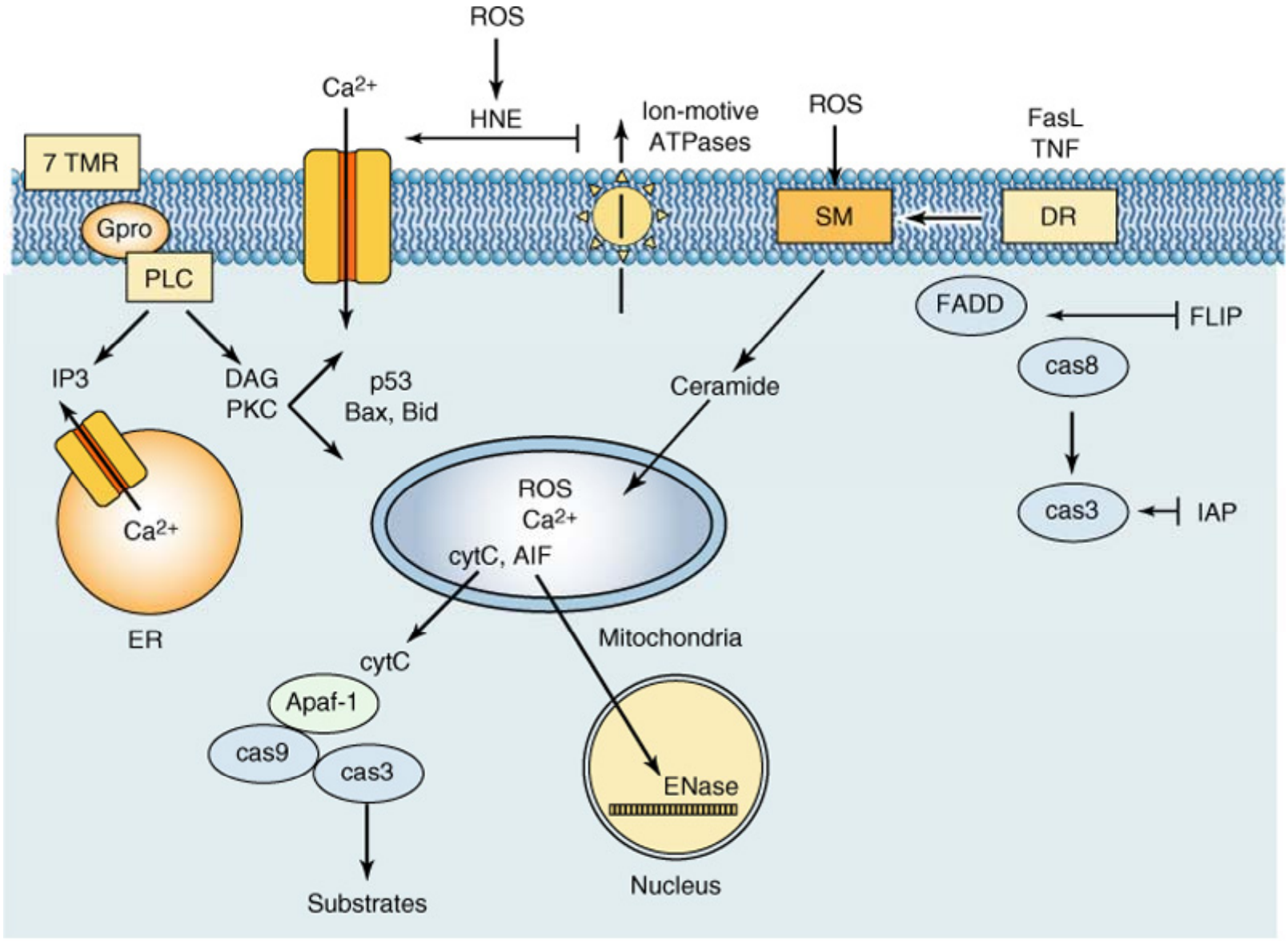
- When developing neurons are deprived of neurotrophic support, either by withdrawal of the neurotrophic factor in cell culture or by removal of the target cells for the neurons *in vivo*, they undergo apoptosis.
- For example, when sympathetic neurons in culture are deprived of NGF they upregulate **Bax**, release **cytochrome c** from mitochondria, activate **caspases** and exhibit cell body shrinkage and chromatin condensation and fragmentation.
- Depletion of neurotrophic factors may contribute to neuronal death in Alzheimer's, Parkinson's, and Huntington's diseases

Examples of apoptosis-2

- Death receptor activation

- Several different ligands can induce apoptosis of neural cells including certain cytokines (Fas ligand and interleukin-1β) and the neurotransmitter glutamate.
- Like tumor necrosis factor (TNF) receptors, Fas is coupled to downstream death effector proteins that ultimately induce caspase activation (ultimately, caspase-3). (Figure 35-4).

Examples of plasma-membrane initiated cell death cascades



Examples of apoptosis-3

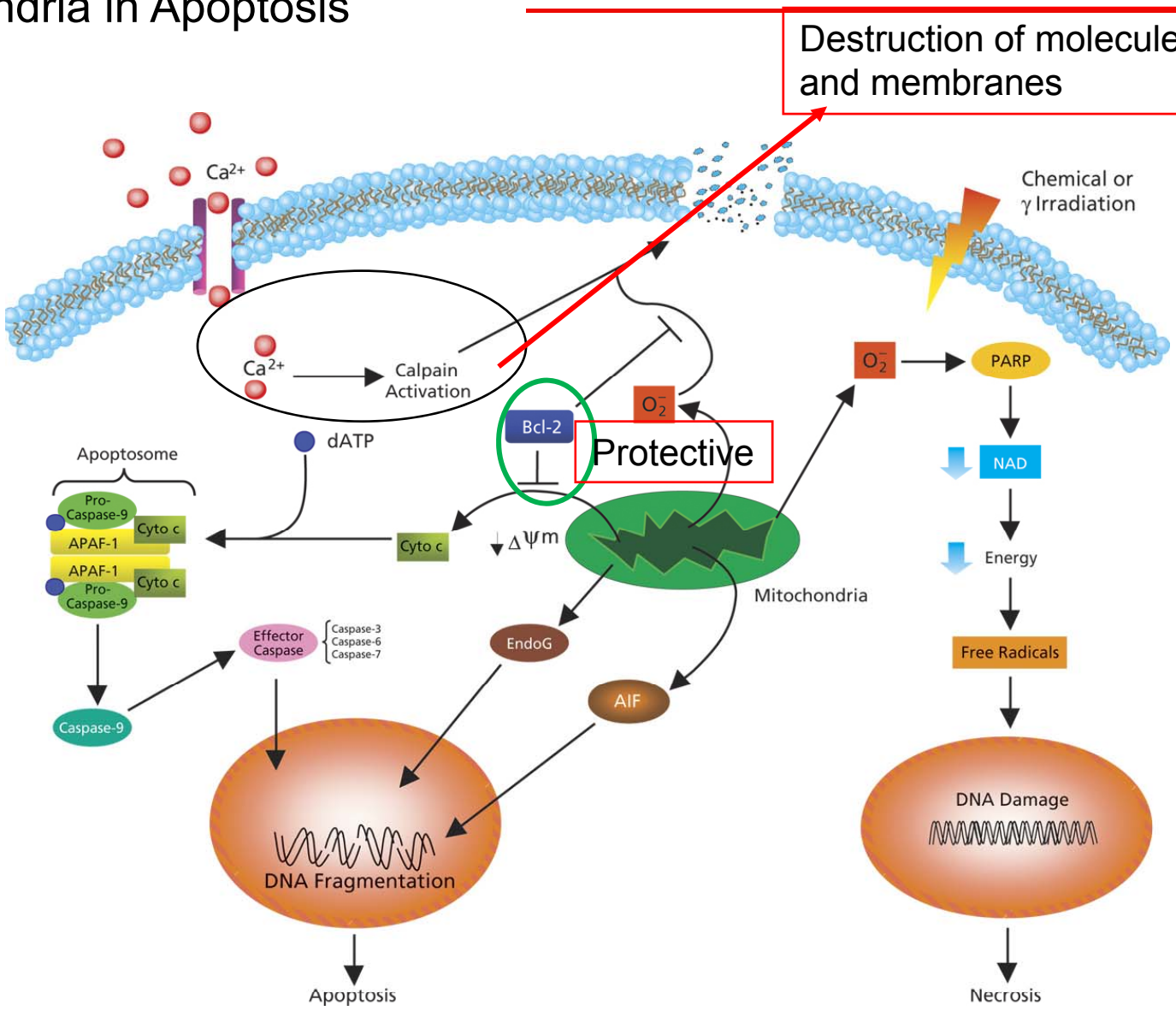
- DNA damage

- Considerable evidence suggests that DNA damage may be a pivotal trigger of apoptosis in both physiological and pathological settings. Neurons undergoing developmental apoptosis (programmed cell death), exhibit DNA damage and upregulation of DNA-damage responsive proteins. The causes for this process in development may include increased oxygen radical production, reduced trophic support, and ***impaired*** *DNA repair mechanisms*.

Apoptosis and Mitochondria

- In many cases of apoptosis, proteins and/or lipid mediators that induce changes in mitochondrial membrane permeability and calcium regulation are produced or activated. For example, the pro-apoptotic Bcl-2 family members **Bax, Bad,** and **Bid** may associate with the mitochondrial membrane and modify its permeability.
- Cell Membrane derived lipid mediators such as ceramide can induce mitochondrial membrane alterations that are critical for the execution of apoptosis.
- The presence and amounts of caclium-binding proteins and antioxidants such as glutathione and vitamin E can shift the threshold for activation of the cell death cascade by different apoptotic triggers.
- Events occurring in the endoplasmic reticulum (ER) have been shown to induce, prevent, or modify apoptotic cascades at a *premitochondrial* step; calcium ion release and uptake by the ER appears to be particularly important in this regard.

Mitochondria in Apoptosis



The post-mitochondrial events of apoptosis include activation of the Caspases

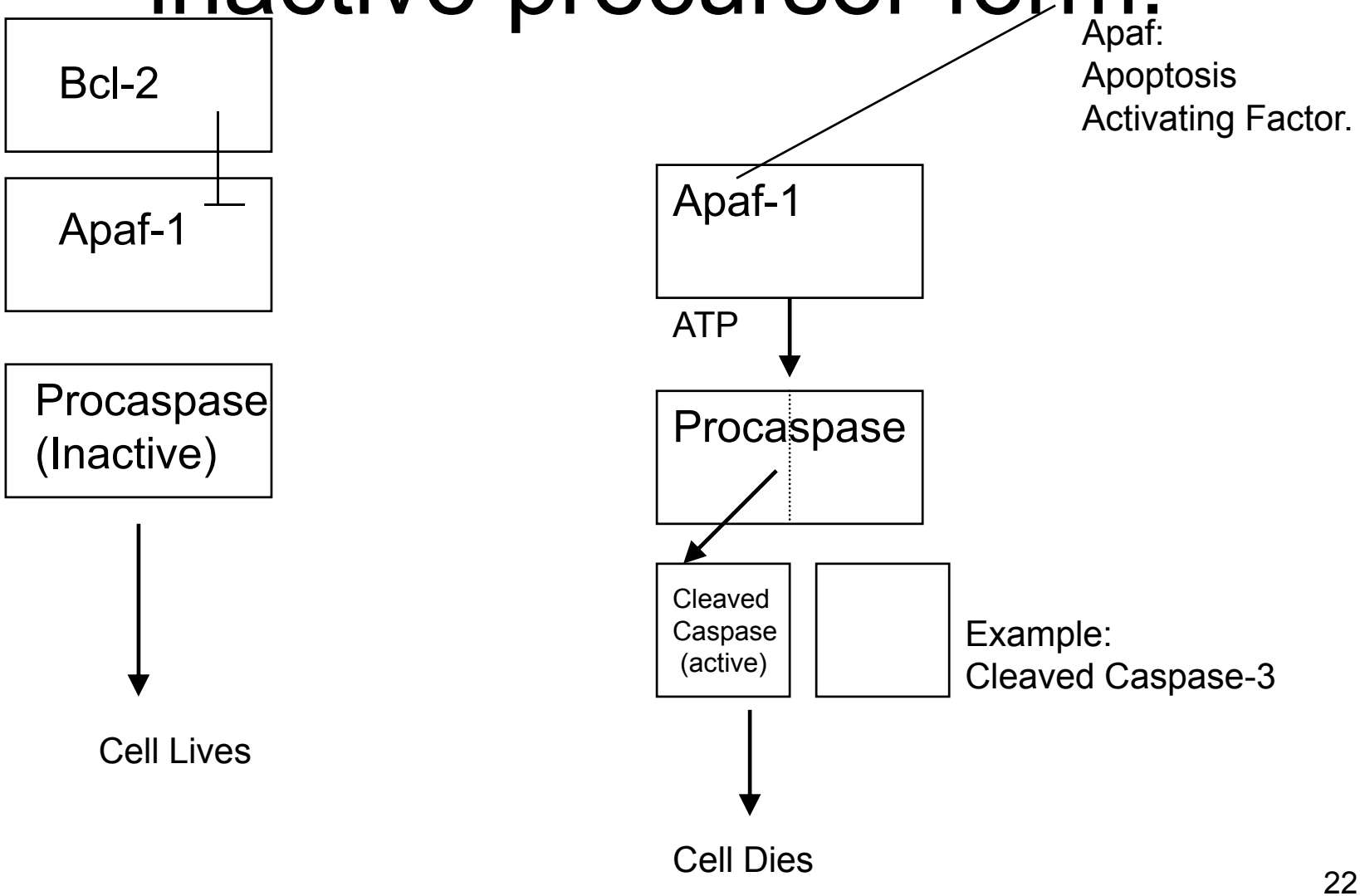
- Once cytochrome c leaks out of mitochondria during apoptosis, it binds to the protein Apaf-1 in the cytosol, resulting in the recruitment and activation of caspase-9, which in turn activates caspase-3.
- 18 different mammalian caspases (2015), have been identified and each may play a key role in apoptosis depending upon the cell type and the nature of the specific cell death stimulus.
- Numerous caspase substrates have been identified.

Caspases are cysteine proteases-Examples of Caspase Substrates

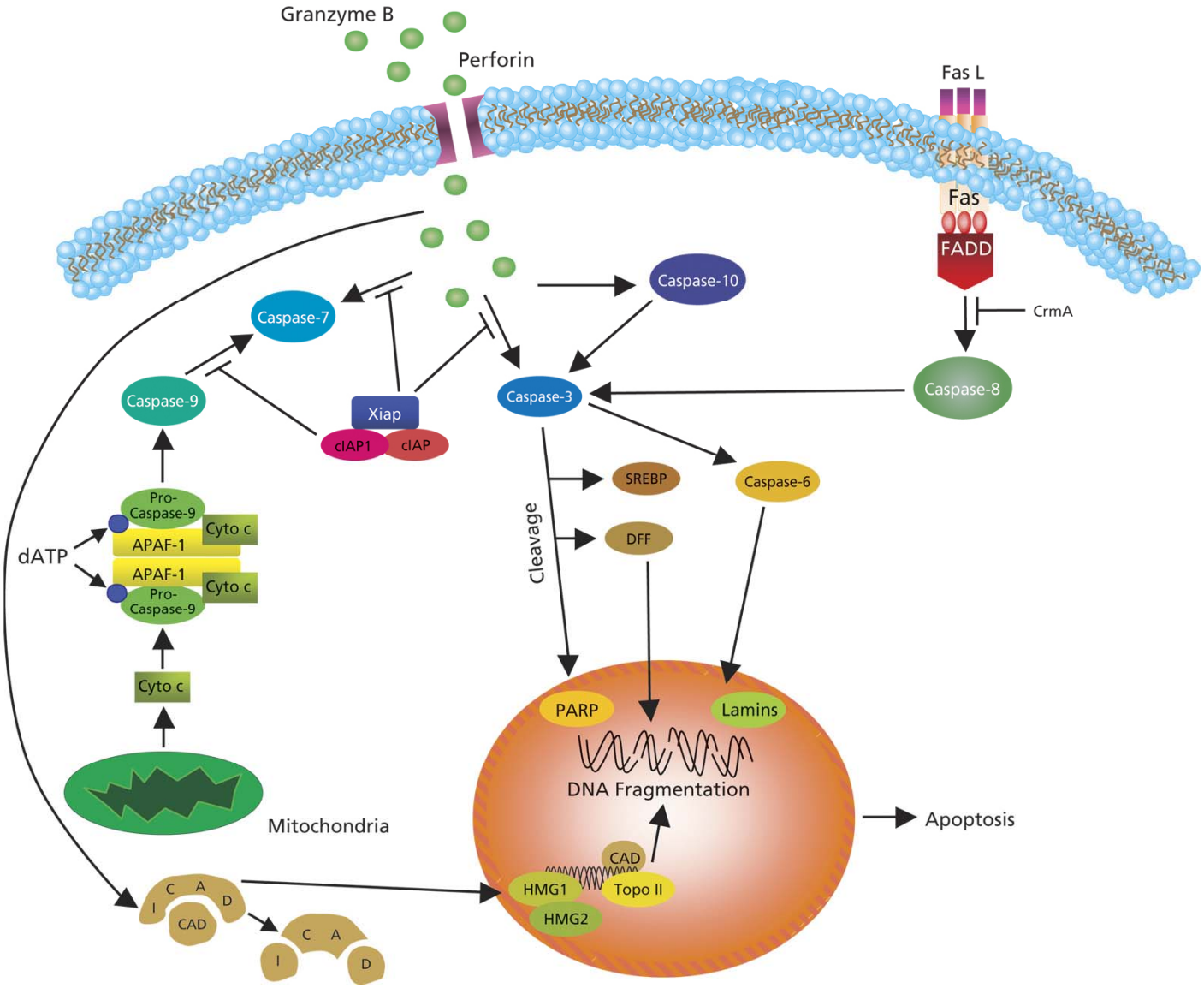
Caspase substrates thus far identified

Substrate	Reference
Gelsolin	Ohtsu et al., 1997
Actin	Mashima et al., 1997
Catenin	Fukuda et al., 1999
αII spectrin	Nath et al., 1996
βII-spectrin	Wang et al., 1998
Vimentin	Hashimoto et al., 1998
PARP (DANN repair enzyme)	
ICAD (inhibitor of caspase-activated DNAse)	Sakahira et al., 1998
APP (amyloid precursor protein)	Ishimura et al., 2002
Bcl2	Cheng et al., 1997
Bcl-xL	Clem et al., 1998
Calpastatin (calpain inhibitor)	Wang et al., 1998
AMPA-receptor	Chan et al., 1999
Presenilins	Kim et al., 1997
Signal transductions proteins	
PKCδ	Ghayur et al., 1996
PKCθ	Datta et al., 1997
PKB/Akt	Widmann et al., 1998
PKN	Takahashi et al., 1996

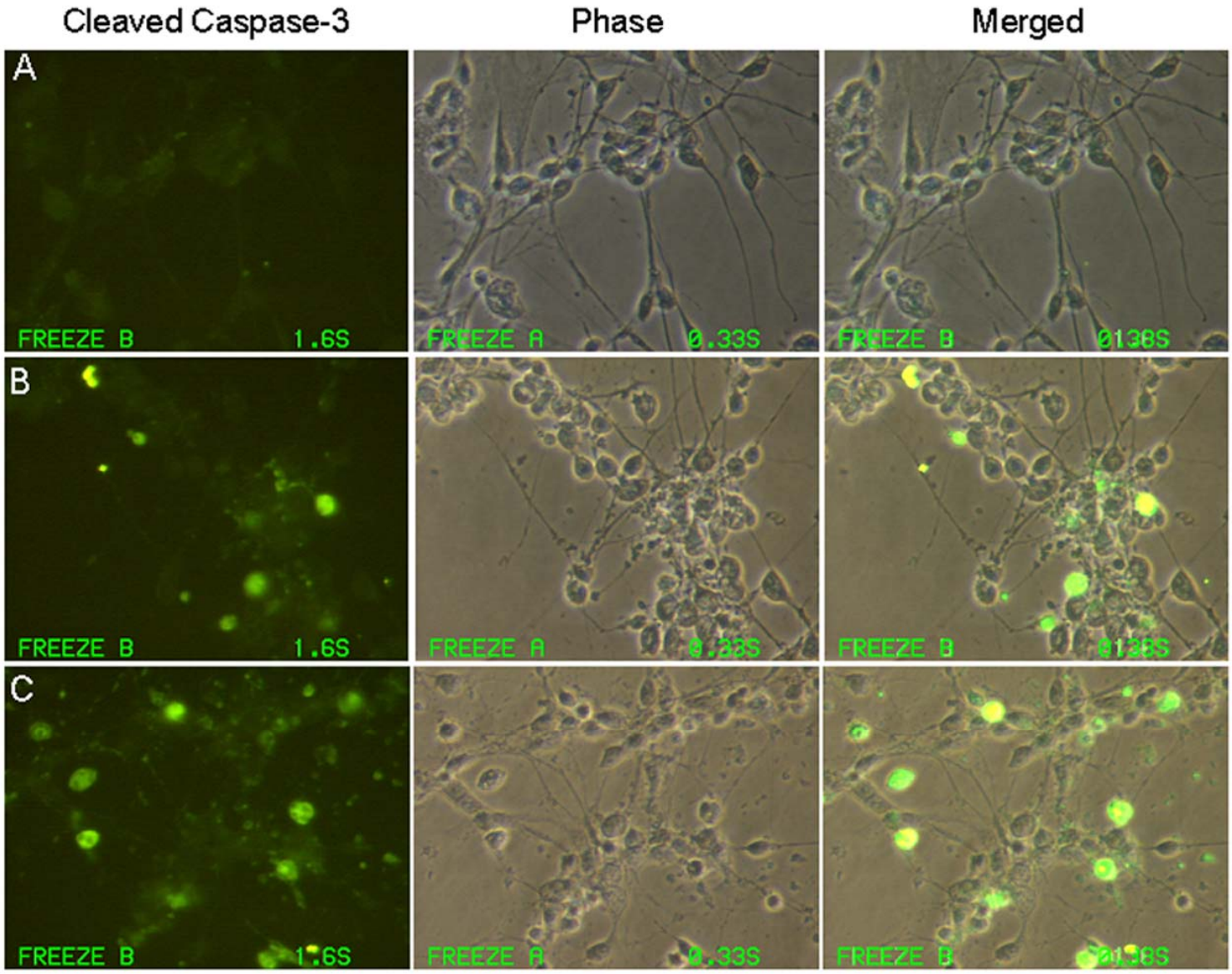
Caspases are generated in an inactive precursor form:



Caspase Cascade

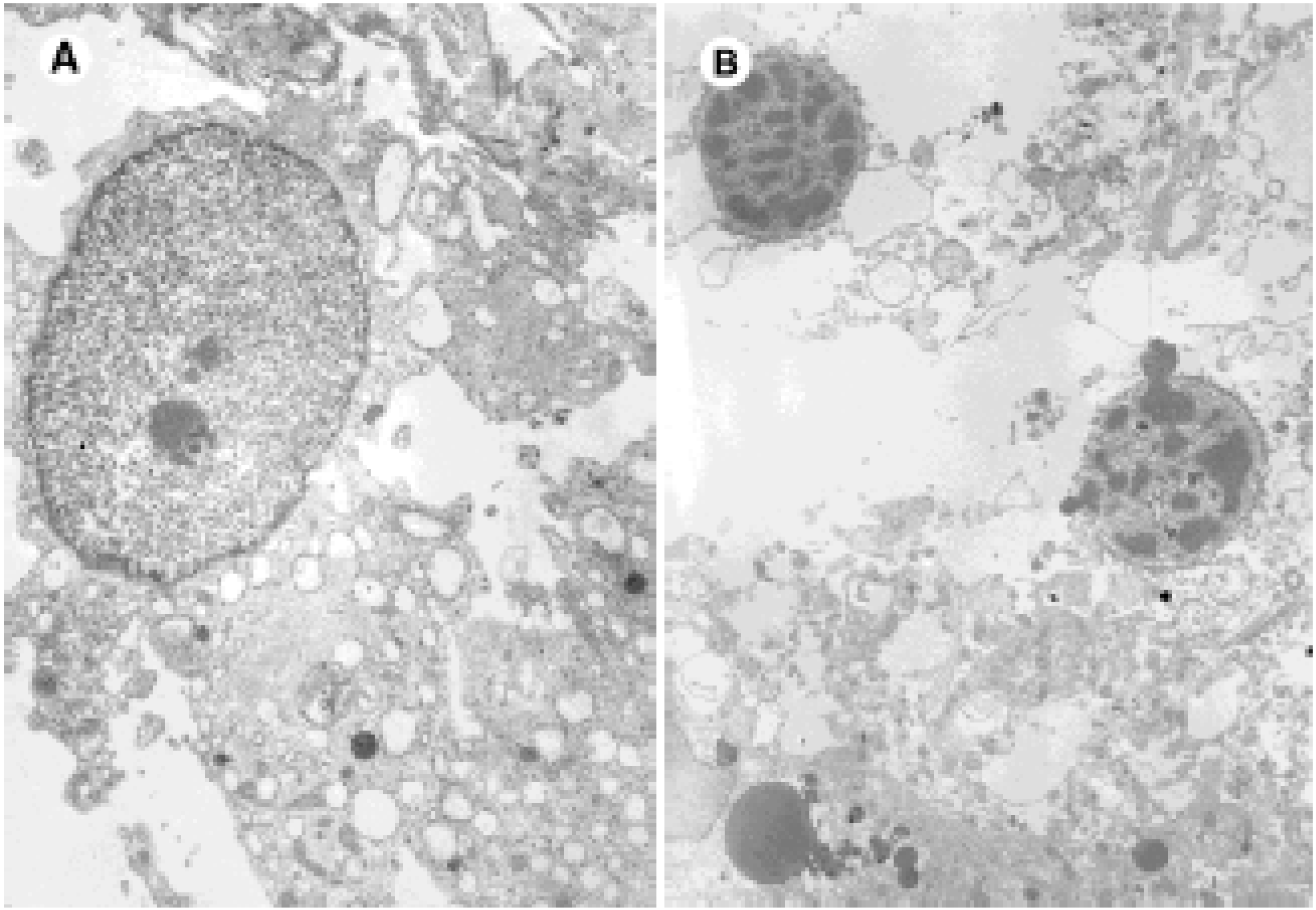


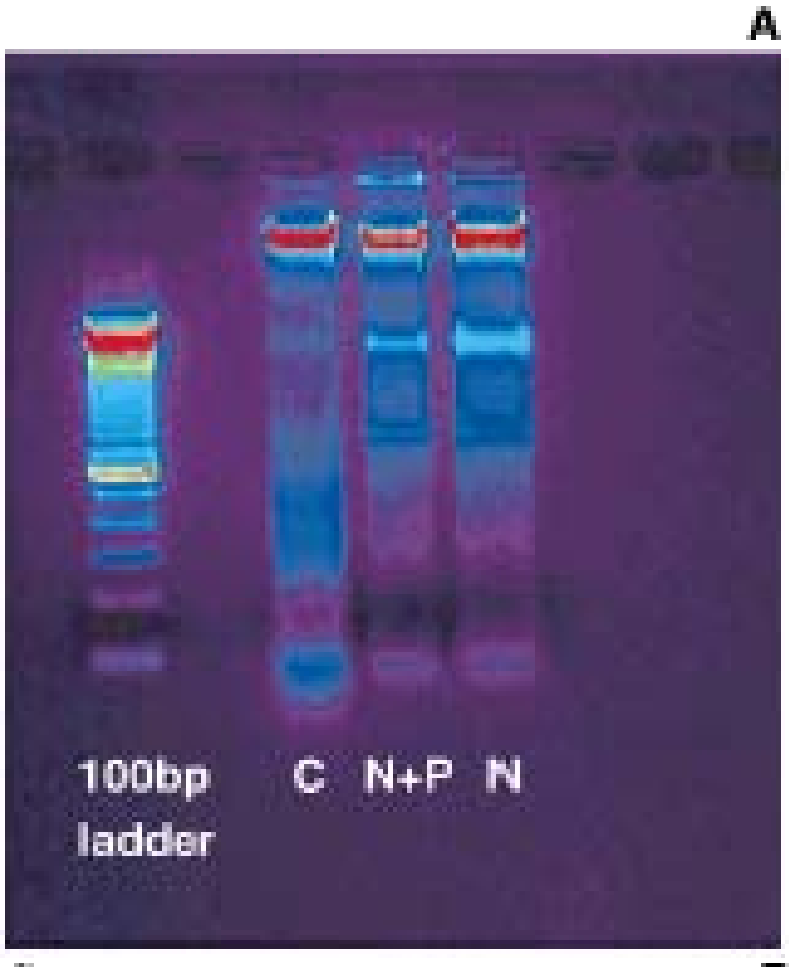
Mechanisms: How does $sPLA_2$ cause apoptosis?



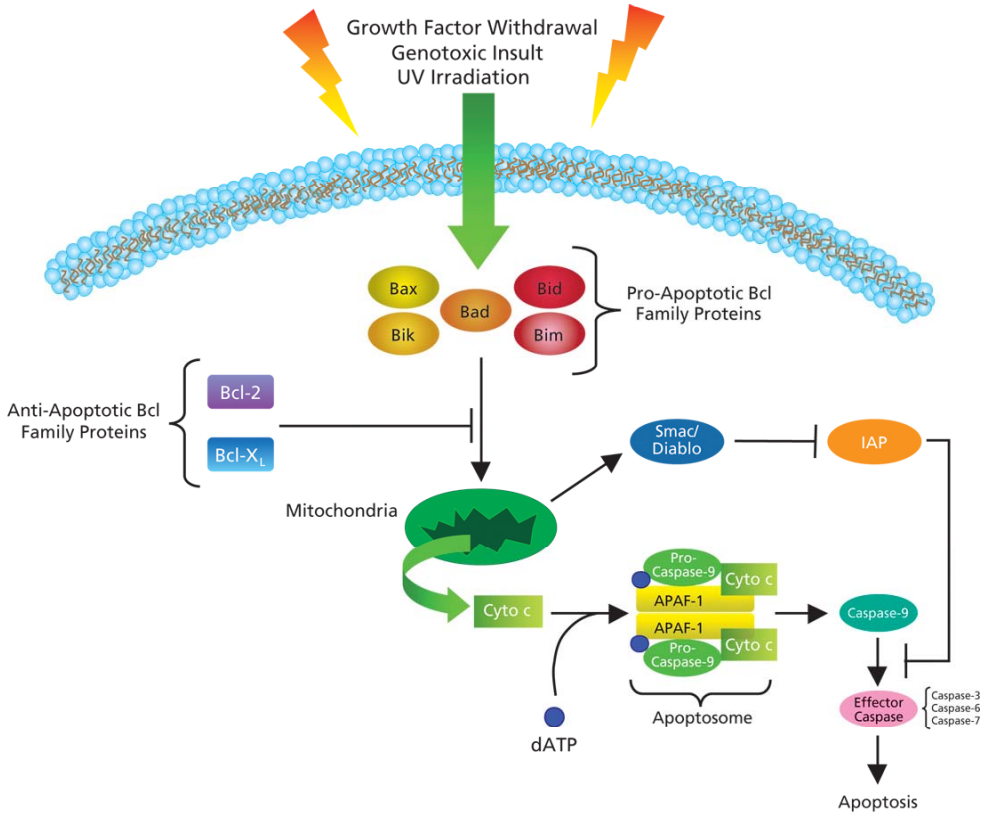
Nuclear chromatin condensation and fragmentation in apoptosis

- Caspases may activate endonucleases, resulting in DNA fragmentation and chromatin condensation


A
B


A
100bp
ladder
C
N+P
N

Programmed Cell Death



Modified from:

Necrosis

- Necrosis is a dramatic and very rapid form of cell death in which essentially every compartment of the cell disintegrates.
- Necrosis is characterized by dysregulation of ion homeostasis resulting in cell swelling, dilation of mitochondria and the ER, and the formation of vacuoles in the cytoplasm.
- Proteases play an important role in the degradation of cells during necrosis.

Calpain, a calcium-dependent neutral protease

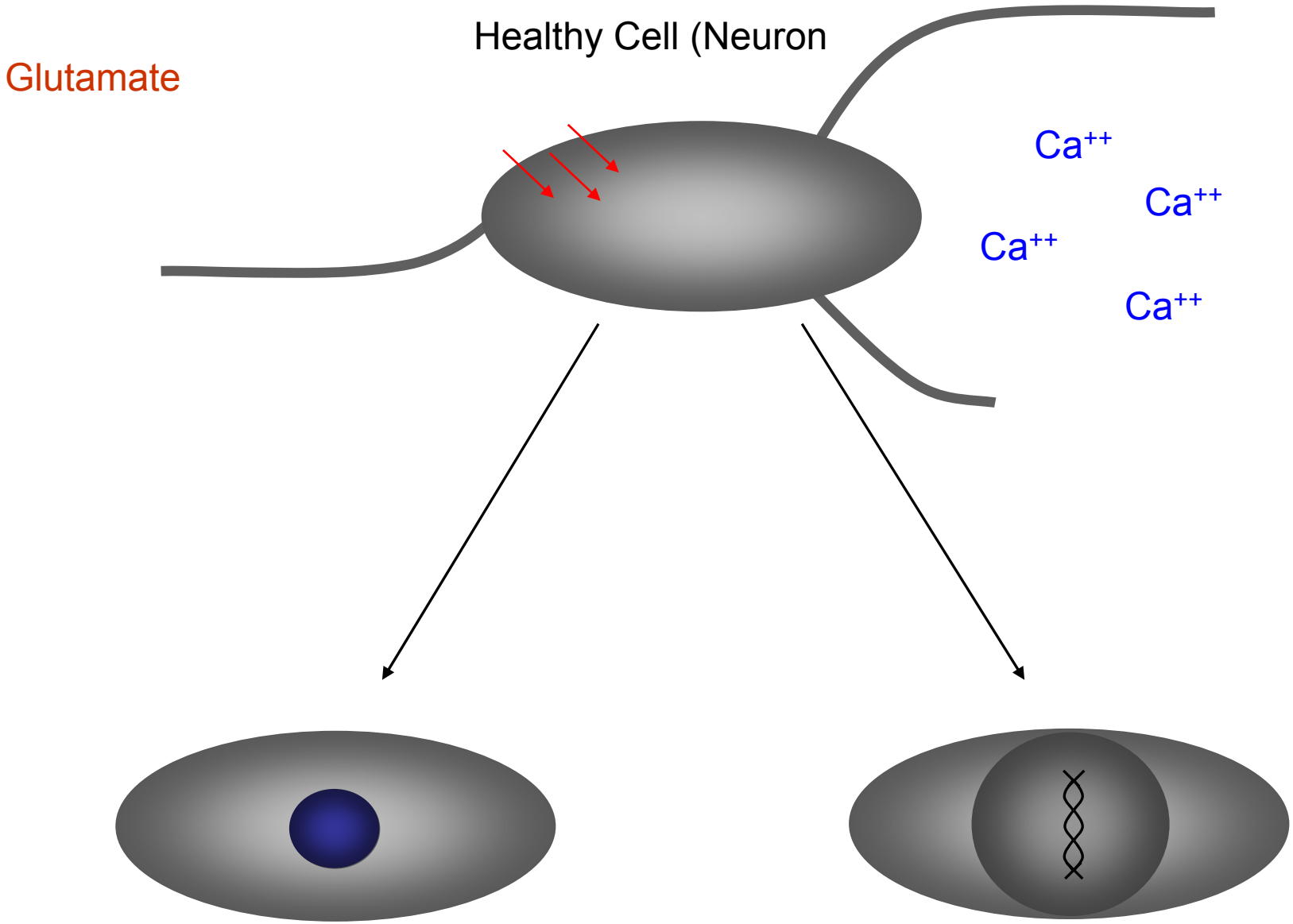
Table 1
Calpain substrates thus far identified

Substrate	Reference
Actin	Villa et al., 1998
Actinin	Brown et al., 1997
APP (amyloid precursor protein)	Siman et al., 1989
Adrenergic receptor	Parr et al., 1992
AMPA receptor	Bi et al., 2000
Ankyrin	Yoshida, 2000
Bax	Thomas et al., 1996
Calcium ATPase	Molinari et al., 1995
Caldesmon	Kakkar et al., 2000
Calponin	Yoshimoto et al., 2000
Connexin 32	Elvira et al., 1993
CREB	See and Loeffler, 2001
Desmin	Elamrani et al., 1995
c-*Fos*/c-*Jun*	Carillo et al., 1994
IP3 receptor	Haug et al., 1996
MAP-2	Billger et al., 1988
P53	Kubbutat and Vousden, 1997
PARP	McGinnis et al., 1999
Progesterone	Shiba et al., 1997
G-proteins	Greenwood and Jope, 1994
Spectrin (αII and βII)	Siman et al., 1990
Tau protein	Johnson, 1997
Troponin	Gao et al., 1997

Necrosis-2

- In necrosis, the cell ultimately lyses, releasing its contents into the extracellular compartment, where the contents may damage neighboring cells and induce inflammatory responses.

- ATP is rapidly depleted in cells undergoing necrosis.


Glutamate
Healthy Cell (Neuron
Ca++
Ca++
Ca++
Ca++
Necrosis
LDH Release
Apoptosis
DNA Damage

End!