Regulation of Apoptosis in Neural Cells: Two methods for overcoming asynchrony

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Abstract

Programmed cell death, or apoptosis, plays a major role in the development of the nervous system and in the pathogenesis of neurodegenerative diseases. Although many proteins that play a key role in apoptosis in other systems also appear to function in neurons, the mechanism that triggers apoptosis in neurons is unknown. Apoptosis occurs asynchronously in neural and differentiated neuronal cells, which makes biochemical studies difficult because a small number of cells are at a particular stage at any one time. Two strategies were devised to overcome asynchrony during neural cell death.

The first strategy was to separate rat pheochromocytoma (PC12) cells at different stages of commitment to cell death on the basis of cell density using equilibrium density gradient centrifugation. Three populations were defined. Cells in population 1 were the most dense and committed to cell death. They showed extensive loss of mitochondrial cytochrome c, DNA fragmentation, and chromatin condensation. Population 3 contained live cells that floated to the top of density gradients. Population 2 displayed some chromatin condensation, yet little DNA fragmentation and loss of cytochrome c. This population showed upregulation of the pro-death factor, c-Jun, and downregulation of pro-survival kinase, Akt. Importantly, these cells could be rescued from death by nerve growth factor (NGF) and thus represent an intermediate stage of apoptosis, upstream of irreversible commitment.

The second strategy was to create a cell-free system to reconstitute apoptosis. The addition of cytochrome c to human neuroblastoma (SY5Y) cell extracts activated caspase-9 and –3, and nucleolytic events in PC12 nuclei. Using this system, requirements for ATP and phosphatase activity for caspase activation and nuclear apoptosis were characterised. In addition, pro-survival molecules Akt and Creb were identified as caspase substrates during apoptosis *in vitro*.

To assess whether these events occurred *in vivo*, the kinase inhibitor staurosporine and the topoisomerase inhibitor camptothecin were used to induce apoptosis in intact SY5Y cells. The pro-survival signalling kinase Raf-1 was downregulated during both staurosporine- and camptothecin-induced apoptosis, but Akt was only downregulated by camptothecin. These studies illustrate the complex interactions of apoptosis and signalling mechanisms in neural cells.

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AD	Alzheimer's Disease
AIDS	Acquired Immune Deficiency Syndrome
Aif	Apoptosis inducing factor
Akap	A-kinase anchoring protein
ALPS	Autoimmune lymphoproliferative syndrome
ALS	Amyotrophic lateral sclerosis
Ant	Adenine nucleotide translocator
Apaf-1	Apoptosis promoting factor 1
App	Amyloid precursor protein
ATP	Adenosine triphosphate
Bad	Bcl-X_L/Bcl-2 associated death promoter
BDNF	Brain-derived neurotrophic factor
BH	Bcl-2 homology
BIR	Baculovirus IAP repeat
BSA	Bovine serum albumin
Cad/Dff40	Caspase-activated DNase/DNA fragmentation factor 40
CamkII or IV	Ca^{2+}/calmodulin dependent kinase II or IV
cAMP	cyclic adenosine monophosphate
CARD	Caspase recruitment domain
Caspase	Cysteine aspartic acid protease
Ced	Cell death abnormal
Ces	Cell death specification
CHEF	Clamped homogeneous electrical field
CNS	Central nervous system
CPT	Camptothecin
Creb	cAMP response element binding protein
CuZnSOD	Copper/zinc superoxide dismutase
Cyt. c	Cytochrome c

dADP	Deoxyadenosine diphosphate
ddATP	Dideoxyadenosine triphosphate
DcR	Decoy receptor
ddH_2O	Double distilled water
DEVD-CHO	acetyl-Asp-Glu-Val-Ala-aldehyde
Diap1	*Drosophila* inhibitor of apoptosis 1
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	DNA endonuclease
DR	Death receptor
DTT	Dithiothreitol
EDTA	Ethylenediamine tetraacetic acid
EGTA	Ethylene glycol-bis(β-aminoethyl ether N,N,N',N'-tetraacetic acid)
eNOS	Nitric oxide synthase enzyme
ER	Endoplasmic reticulum
Erk	Extracellular-signal regulated kinase (Erk1 = p44Mapk, Erk2 = p42Mapk)
Fadd/Mort	Fas-associated death domain
FasL	Fas ligand
FGF	Fibroblast growth factor
GAP	GTPase-activating protein
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
Gsk-3	Glycogen synthase kinase-3
GST	Glutathione S-transferase
GTP	Guanosine triphosphate
HD	Huntington's Disease
HSNs	Hermaphrodite specific neurons
Iap	Inhibitor of apoptosis protein
Icad	Inhibitor of caspase-activated DNase
Ice	Interleukin-1β-converting enzyme

IGF	Insulin-like growth factor
Ikk	IκB-kinase
Jnk	c-Jun amino-terminal kinase (Sapk)
kb	kilobase
kD	kilodalton
Mapk	Mitogen-activated protein kinase
Mek	Mapk/Erk kinase (Mek1 = Mkk1, Mek2 = Mkk2)
Mekk	Mapk/Erk kinase kinase
Mkk	Mapk kinase
Mkkk	Mkk kinase
mRNA	Messenger ribonucleic acid
Na_3VO_4	Sodium orthovanadate
NF-κB	Nuclear factor-κB
NGF	Nerve growth factor
Nik	NF-κB -inducing kinase
NO	Nitric oxide
NSMs	Neurosecretory motor neurons
NT	Neurotrophin
OKA	Okadaic acid
$p75^{NTR}$	p75 neurotrophin receptor
Pak	p21-activated kinase
Parp	Poly(ADP)-ribose polymerase
PBS	Phosphate-buffered saline
PC12	Rat adrenal pheochromocytoma cell line
PCD	Programmed cell death
PD	Parkinson's disease
PDGF	Platelet-derived growth factor
Pdk	Phosphoinositide-dependent kinase
PFGE	Pulse-field gel electrophoresis
PGB	PBS with glucose and BSA
PH	Pleckstrin homology domain

PI3-K	Phosphatidyl inositol 3-kinase
PKA	Protein kinase A
PKB	Protein kinase B
PKC	Protein kinase C
PLC	Phospholipase C
PMSF	Phenylmethylsulfonyl fluoride
PP2A	Protein phosphatase 2A
PT	Permeability transition
PtdIns	Phosphatidyl inositol
Rip	Receptor interacting protein
ROS	Reactive oxygen species
Rsks	pp90 ribosomal S6 kinases
Sapk	Stress-activated protein kinase
SAR	Scaffold attachment region
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Sek1	Sapk/Erk kinase 1 (Mkk4, Jnkk)
SH	Src homology
SODD	Silencer of death domains
STS	Staurosporine
SY5Y	SK-N-SH-SY5Y human neuroblastoma cell line
TBS	Tris-buffered saline
TCA	Trichloroacetic acid
TE	Tris-EDTA
TNF	Tumour necrosis factor
TNFR	Tumour necrosis factor receptor
Tradd	TNFR-associated death domain
Traf-2	TNFR-associated factor 2
Trk	Tyrosine receptor kinase
tRNA	Transfer RNA
Tween-20	Polyoxyethylenesorbitan monolaurate
Vdac	Voltage-dependent anion channel

v/v	volume/volume
w/v	weight/volume
z-VAD-fmk	benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone

Note on genetic nomenclature:

The conventions used for writing the names of genes and gene products is according to Murray & Hunt (1993). Gene names are always written in lower case letters and are italicised. Gene products are written with the first letter capitalised and without italics.

C. elegans	*Caenorhabditis elegans*
E. coli	*Escherichia coli*
S. cerevisiae	*Saccharomyces cerevisiae*
S. pombe	*Schizosaccharomyces pombe*

Abbreviations for amino acids

Amino acid	*Three-letter abbreviation*	*One-letter symbol*
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Iso	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

(from Stryer, 1988)

List of publications arising from this thesis

François, F. and Grimes, M.L. (1998) Stages in apoptotic commitment in PC12 cells. *Mol. Biol. Cell* **9,** 368a [abstract].

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