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IJBCB

The International Journal of Biochemistry & Cell Biology xxx (2004) xxx–xxx

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Review

Apoptosis, autophagy, and more

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Received 4 February 2004; received in revised form 16 April 2004; accepted 20 April 2004

Abstract

Cell death has been subdivided into the categories apoptosis (Type I), autophagic cell death (Type II), and necrosis (Type III). The boundary between Type I and II has never been completely clear and perhaps does not exist due to intrinsic factors among different cell types and the crosstalk among organelles within each type. Apoptosis can begin with autophagy, autophagy can end with apoptosis, and blockage of caspase activity can cause a cell to default to Type II cell death from Type I. Furthermore, autophagy is a normal physiological process active in both homeostasis (organelle turnover) and atrophy. "Autophagic cell death" may be interpreted as the process of autophagy that, unlike other situations, does not terminate before the cell collapses. Since switching among the alternative pathways to death is relatively common, interpretations based on knockouts or inhibitors, and therapies directed at controlling apoptosis must include these considerations.

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1. Introduction

Cell death is a field that has attracted much deserved attention in recent times, leading to several new

and important insights in cell biology, development, and pathology; but the recruitment of many new researchers to the field has led to some confusion in terms and sometimes overly precise dichotomies such as between apoptosis and necrosis, or between apoptosis and autophagic cell death. Often the biology is more complex or ambiguous. We therefore first define the terms and then summarize current understanding

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33 of their relationship. The field is moving so rapidly
34 and it is so massive that it is not possible to cover ev-
35 erything in a small review. We therefore refer to sev-
36 eral recent and more extensive summaries to which
37 the interested reader should refer.

38 The terms used in the field have all evolved in the
39 30–40 years since their first use, and it is more useful
40 to explain them than to attempt to preserve a definition,
41 since efforts to constrain language ultimately succumb
42 to popular understanding. In order to limit references
43 to more current items, the reader is referred to several
44 recent reviews that address older literature (Clarke &
45 Clarke, 1996; Lockshin, 1997; Lockshin, Osborne, &
46 Zakeri, 2000; Lockshin & Zakeri, 2001, 2002; Vaux,
47 2002) and more specific topics (Lockshin & Zakeri,
48 2004a). A summary of the relationships of cell deaths
49 is given in Fig. 1.

50 The term “*Programmed cell death*” (Table 1) was
51 from its inception an operational definition, referring
52 to the fact that one could document, in cells that were
53 doomed, a series of changes consistent with the im-
54 pending failure but at a period at which one could
55 experimentally prevent the death. The predictability
56 of the death arose from examples in development,
57 such as embryonic chick wings and the eponymous
58 death of intersegmental muscles in moths. In the chick

59 wing, programming was recognized by explantation
60 of the posterior necrotic zone, in which case it would
61 die on schedule in vitro, as opposed to transplanta-
62 tion to another locus, in which case it would heal in
63 place and survive. For intersegmental muscles, pro-
64 gramming was identified as early hormonal and neural
65 signals affecting the muscles and finally metabolic
66 changes, including expansion of the lysosomal com-
67 partment and activation of autophagic activities. When
68 the cells begin to deteriorate rapidly, there is a gen-
69 eralized proteolytic loss of most major proteins, cou-
70 pled with a rapid downregulation of most protein syn-
71 thesis but the survival and even upregulation of a
72 small number of proteins including ubiquitin (Haas,
73 Baboshina, Williams, & Schwartz, 1995; Schwartz,
74 Jones, Kosz, & Kuah, 1993; Schwartz, Kosz, & Kay,
75 1990; Schwartz, Myer, Kosz, Engelstein, & Maier,
76 1990; Wadewitz & Lockshin, 1988). Later, neurose-
77 cretory elements were identified, as was the activation
78 of proteasomal proteases. For insect muscle, tadpole
79 tail, and glucocorticoid-treated thymocytes, blockage
80 of mRNA and protein synthesis was found to prevent
81 or delay cell death. This observation is still valid, and
82 is being reconfirmed today though, surprisingly, the
83 requirement appears to persist even into an advanced
84 stage of cell death (Myohara, 2004).

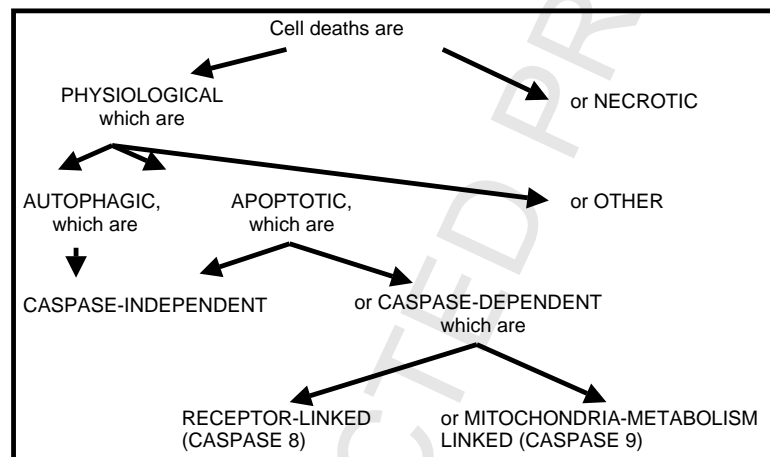


Fig. 1. Cell deaths fall into several categories, the boundaries of which are not always distinct. Deaths are controlled (physiological) or not. The controlled deaths frequently display substantial caspase-independent autophagy) or they are predominantly apoptotic. Most apoptotic deaths are caspase-dependent, but there are claims of apoptotic morphology in situations in which caspase activity is equivocal. Caspase activation can occur by means of ligation of a membrane-bound receptor or by means of metabolic changes resulting in depolarization of mitochondria and release of cytochrome *c* and APAF-1. Other metabolic means of activating apoptosis include “ER stress,” usually interpreted as overloading of the ER with misfolded proteins.

Table 1
Definitions

Term	Definition
Programmed cell death	Developmental A sequence of (potentially interruptible) events that lead to the death of the cell Now recognized to require the activity of specific genes (but often activation of pre-existing proteins, not necessarily transcription at time of death)
Necrosis	Apparently uncontrolled cell death Loss of ATP or membrane pumps Most commonly osmotic swelling of cell membrane and organelles, with extraction of contents and precipitation of proteins Inflammatory response
Apoptosis	A specific morphology, cell shrinkage and blebbing; organelles (other than ER) do not swell; nucleus fragments; chromatin marginates; no inflammation Degradation of DNA by 3' cleavage to nucleosome-sized fragments Exteriorization of phosphatidylserine Activation of caspases such as caspase 3
Lysosomal/Type II/autophagic cell death	Death characterized by formation of many large autophagic vacuoles Caspase activation very late if at all Primary proteases are cathepsins or proteasomal proteins DNA fragmentation very late if at all Exteriorization of phosphatidylserine No inflammation

85 This latter function proved not to be necessary in
86 most instances of cell death not directly associated
87 with development (see below). Nevertheless, the ob-
88 servations provided a hint toward what became the
89 most important discovery and change in the concept
90 of “programmed cell death,” the recognition that there
91 were a handful of genes that controlled substantially
92 all cell deaths in embryonic *Caenorhabditis elegans*.
93 Today “programmed cell death” carries the overtone
94 that cells possess the genes and hence the proteins for
95 their own destruction, and that almost all physiologi-
96 cal and most pathological cell deaths are managed and
97 ritualistic rather than chaotic.

98 “Necrosis” is today the catch-all term for any deaths
99 that do not fit in the other categories described here.
100 Typically, cells entering necrosis lose control of their
101 ionic balance, imbibe water, and lyse. Intracellular
102 proteins in new ionic milieus, often in the presence of
103 high ionic calcium and acid or other abnormal pH, of-
104 ten precipitate (Fig. 2). The lysis releases many intra-
105 cellular constituents, attracting (in vertebrates) Mast
106 cells and provoking an inflammatory response. Con-
107 sequently, the morphology of necrosis is variable and
108 poorly defined.

109 Many of these deaths may have some physiologi-
110 cal basis. For instance, the death of osteocytes in bone
111 is usually described as more necrotic in style, but it
112 is not clear to what extent the osteocyte participates
113 in its own death. Osteocytes may undertake a fair
114 amount of self-destruction before decaying, inaccess-
115 ible to phagocytes (Cerri, Boabaid, & Katchburian,
116 2003). Similarly, between fertilization and the mater-
117 nal-zygotic transition vertebrate eggs are considered
118 to be incapable of undergoing apoptosis. In our hands,
119 early zebrafish eggs exposed to cycloheximide indeed
120 die a necrotic death rather than the apoptotic death of
121 an older embryo, but they activate caspase 3. We con-
122 sider that these freshwater eggs lyse before they can
123 complete apoptosis (Negrón & Lockshin, in press).
124 In severely inflammatory situations, the number of
125 phagocytes clearing dead cells is likely to be lim-
126 iting, with apoptotic cells lying around like rotting
127 corpses until they lyse. When strong toxins are ad-
128 ministered to animals, for instance hepatotoxins, many
129 cells are identified that appear to have begun apopto-
130 sis but failed to complete it before becoming necrotic
131 (Ledda-Columbano et al., 1991). In tissue culture the
132 fate of a cell is always necrosis, because if a cell is

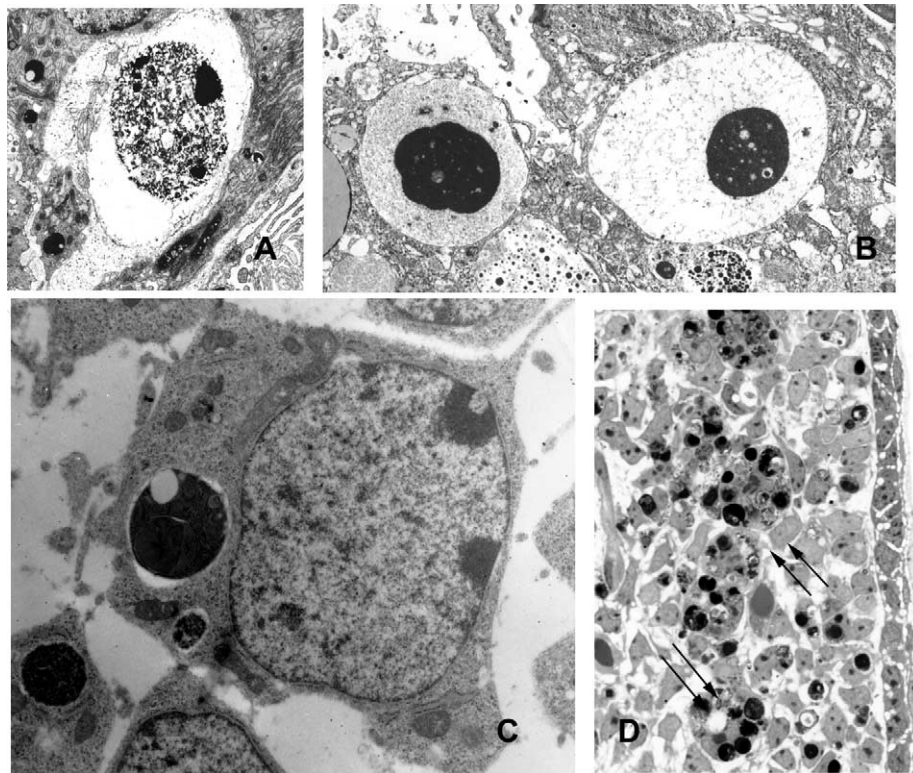


Fig. 2. Different forms of cell death. (A) A necrotic cell in the prostatic epithelium of a mouse 2 days after castration. Note the disorganized nucleus and cytoplasm. (B) Two apoptotic cells in mammary epithelium of a mouse 2 days after weaning. Note condensed nuclei and cytoplasm that is neither extracted nor precipitated. These cells have been phagocytosed by neighbouring epithelial cells. (C) Phagocytosed apoptotic nucleus in mouse embryo following exposure of the mother to cyclophosphamide. (D) Low power image from interdigital region of a day 12.5 mouse embryo, showing numerous apoptotic cells phagocytosed by macrophages or resident cells. The double arrows indicate cells that have phagocytosed several autophagic fragments. (A) and (B) are prepared in collaboration with Martin Tenniswood (currently at University of Notre Dame, Notre Dame, IN, USA); (C) and (D) in collaboration with Daniela Quaglino, University of Modena, Modena, IT.

133 not consumed by phagocytes it will ultimately lyse.
 134 Thus, the distinction between apoptosis and necrosis
 135 may be simply one of timing and severity of insult.
 136 There is evidence that necrosis may not be completely
 137 chaotic. There may even be defined pathways for
 138 necrosis (Yuan, Lipinski, & Degterev, 2003).

139 “Apoptosis” was first used to describe a particular
 140 morphology of death, common to the vast majority
 141 of physiological deaths, that was not readily explic-
 142 able by the assumption of loss of ionic control: shrink-
 143 age and blebbing of cells, rounding and blebbing of
 144 nuclei with condensation and margination of chromatin,
 145 slight shrinkage or morphologically undetectable
 146 changes in organelles, and phagocytosis of cell
 147 fragments without accompanying inflamma-

tory responses (Fig. 2). Later, active exteriorization of
 phosphatidylserine was identified as one of the signals
 for phagocytosis. The margination of the chromatin
 was associated with a controlled internucleosomal
 cleavage of DNA detectable by electrophoresis and
 in situ end labelling. Many of the other changes
 derived from activation, in apoptotic cells, of one or
 more specific proteases called caspases. In contrast to
 the developmental situations, protein synthesis was not
 required and in fact apoptosis was often experimen-
 tally induced by administration of cycloheximide. To-
 day the morphology and behaviour of apoptotic cells is
 largely explained by activation of caspases, and apo-
 ptosis is considered to be nearly synonymous with cas-
 pase activation. In most cells the machinery for killing

163 the cell is present but inactive long before the cell is
164 induced to die, and death appears to be a release from
165 inhibition. Here we assume that classical apoptosis is
166 a caspase-dependent form of cell death, whether trig-
167 gered by extrinsic (cell surface receptor) or intrinsic
168 (mitochondrial depolarization) means, and manifest-
169 ing any of several other markers including DNA lad-
170 dering as determined by electrophoresis; DNA frag-
171 mentation as determined by TUNEL or similar tech-
172 niques; sub-2N DNA as seen by FACS analysis; bleb-
173 bing and rounding of the cell; fragmentation of nuclei
174 with condensation and margination of chromatin; and
175 exteriorization of phosphatidylserine as detected by
176 annexin V binding (Table 1). There is also a profound
177 implication to our understanding of apoptosis. This is
178 that, in contrast to the developmental situations de-
179 scribed above, all or the vast majority of maturing or
180 mature cells possess the machinery for self-destruction
181 in the form of inactive proenzymes (pro-caspases) as
182 well as machinery for regulating or adjusting the level
183 at which the proenzymes can be activated. Cells nor-
184 mally hold the machinery in abeyance, and default to
185 its activation when any of numerous conditions define
186 an imperfect situation for the cells. The fact that cells
187 are programmed to self-destruct should inform our in-
188 terpretations of sequences leading to death.

189 In some situations, the machinery can be used in
190 partial or targeted fashion, in which parts of apoptotic
191 cells are preserved for other physiological purposes.
192 These situations are described as “partial apoptosis”
193 and include maturation of lens fibres, keratinocytes,
194 spermatocytes, and mammalian erythrocytes. Here
195 major organelles are discarded, usually in a process
196 that involves one or more components of apoptosis,
197 but other parts of the cell persist or survive. The
198 means by which apoptosis is rendered selective can
199 potentially teach us much, but our current understand-
200 ing is limited (Allombert-Blaise et al., 2003; Cerri
201 et al., 2003; Gandarillas, Goldsmith, Gschmeissner,
202 Leigh, & Watt, 1999; Ishizaki, Jacobson, & Raff,
203 1998; Lippens et al., 2000; Mammone et al., 2000;
204 Weil, Raff, & Braga, 1999).

205 2. Lysosomal/Type II/autophagic cell deaths

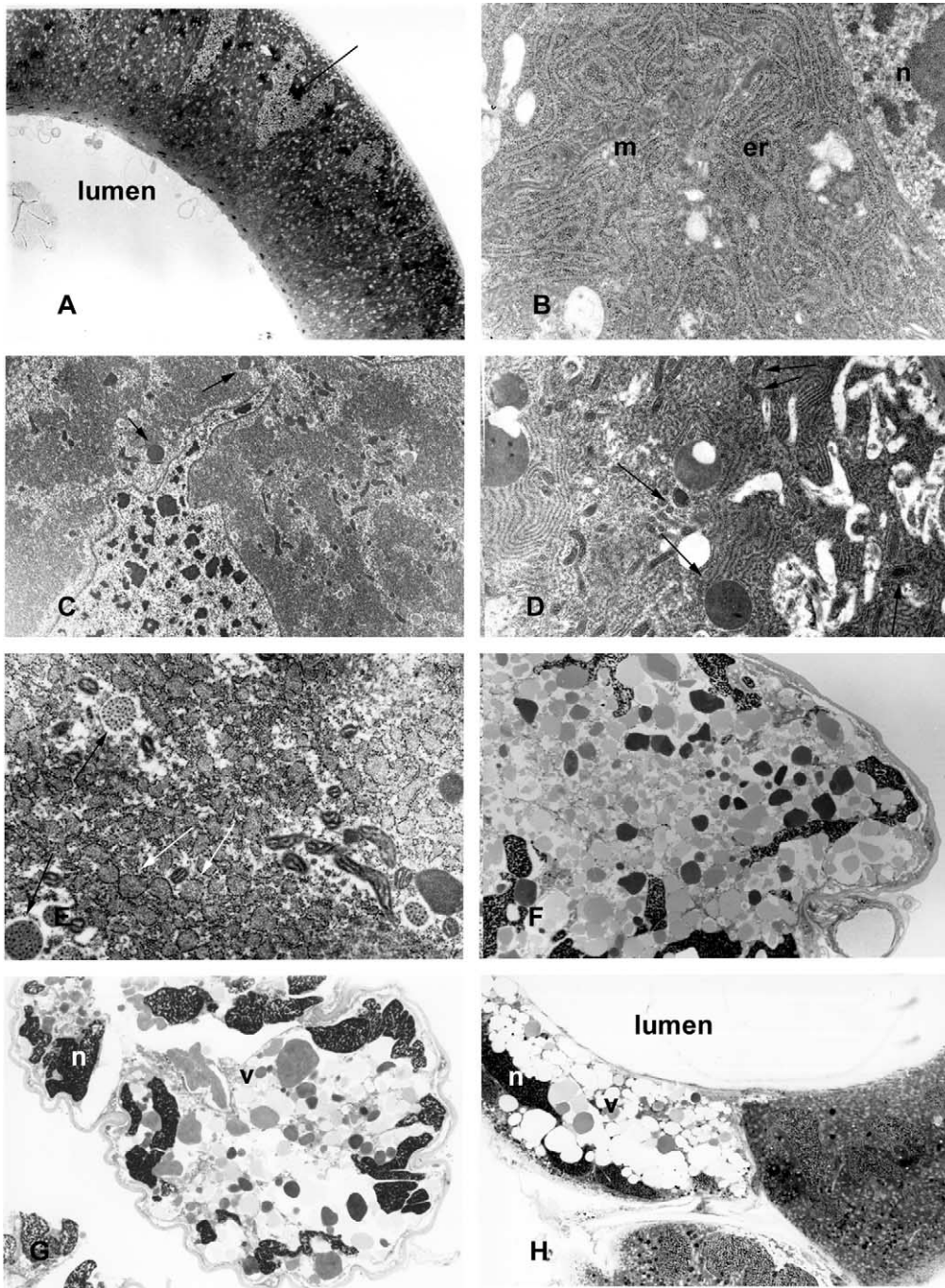
206 In 1980, apoptosis became a centrepiece of atten-
207 tion and within a few years apoptotic cell death and

208 activation of caspases dominated our understanding
209 of cell death. However, before the discovery of the
210 caspase family of proteases, most cell deaths were
211 considered to be lysosomal (Lockshin, 1969), or
212 “Type II” (Schweichel & Merker, 1973) requiring
213 activation of the lysosomal compartment. The term
214 “autophagic cell deaths” was applied later, as the
215 relationship between primary lysosomes, autophagic
216 vacuoles, and autophagosomes became more appar-
217 ent. In insect larval muscles and salivary glands, the
218 bulk of the cytoplasm is removed. In salivary gland
219 (*Drosophila*) or the homologous labial gland (*Man-
220 duca*, Lepidoptera) (Lockshin & Zakeri, 2001) the
221 formation of autophagic vacuoles is most prominent.
222 In insects, cell death at metamorphosis is typically
223 autophagic, and blocking autophagy is a puparia-
224 tion lethal (Juhász, Csikos, Sinka, Erdelyi, & Sass,
225 2003). In insect muscles, the myofilaments are re-
226 moved by a predominantly proteasomal mechanism,
227 while organelles such as mitochondria are last seen in
228 autophagic vacuoles.

229 This autophagic type of death, which was typi-
230 cally seen in large, cytoplasm-rich post-mitotic or only
231 slowly mitotic cells, was characterized by autophagic
232 capture of organelles and particles, substantial expan-
233 sion of the lysosomal compartment including primary
234 lysosomes, autophagic vacuoles, and secondary lyso-
235 somes, and belated collapse of the nucleus. Often or-
236 ganelles appeared to be eliminated in waves, for in-
237 stance one wave in which mitochondria were seen in
238 autophagic vacuoles and afterwards nearly eliminated,
239 and another in which ribosomes or glycogen particles
240 were the primary occupants of autophagic vacuoles
241 (Locke & McMahan, 1971) (Fig. 3). Interest in au-
242 topathagic cell deaths has recently revived and is now
243 recognized to occur in many situations. In some in-
244 stances the lysosomes that had been detected proved
245 to reside in attacking phagocytes. In others, most no-
246 tably insect intersegmental muscles and silk glands,
247 post-lactational mammary glands, and post-castration
248 prostate, the sequestration and digestion of cell or-
249 ganelles such as mitochondria was well documented,
250 with any apoptotic morphology delayed until the cy-
251 toplasm was nearly completely destroyed. This death
252 appeared to be distinct from apoptosis and is now gen-
253 erally called autophagic cell death. It is far more com-
254 mon than most researchers recognize, and is conse-
255 quently under-investigated.

256 Insect cells, which figure heavily in this definition,
 257 are in many ways different from mammalian cells, in-
 258 cluding the prominence of remodelling of surviving
 259 cells during metamorphosis and the greater effective-

ness of the barrier effect of the basement membrane 260
 (Locke, 2003), and one could argue that insects are 261
 a special case. However, in many tissues, autophagy 262
 is a means of reducing cell mass prior to apoptosis



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263 (Bursch, Ellinger, Gerner, & Schulte-Hermann, 2004;
 264 Lockshin & Zakeri, 2001, 2004b). It may also be
 265 used in situations in which conventional apoptosis is
 266 blocked or limited by mutation or other controls, as in
 267 MCF7 cells, which lack caspase 3, or in which mas-
 268 sive cell death overwhelms the ability of phagocytes
 269 to clear the terrain.

270 3. Autophagy is a normal physiological process 271 that does not necessarily lead to cell death

272 The biggest constraint to the theory of autophagic
 273 cell death is the realization that most cells manifest-
 274 ing substantial autophagy do not die. Autophagy is a
 275 well-known physiological process involved in routine
 276 turnover of cell constituents. It is an evolutionarily
 277 ancient process, well documented in species as simple
 278 and diverse as Dictyostelium (Cornillon et al., 1994;
 279 Levraud et al., 2004; Olie et al., 1998) and yeast
 280 (Klionsky & Emr, 2000) and has often been de-
 281 scribed in metamorphosing insects. It functions in
 282 normal physiology (Mizushima, Yamamoto, Matsui,
 283 Yoshimori, & Ohsumi, 2004) and is a major mech-
 284 anism regulating turnover of many proteins and or-
 285 ganelles (Yoshimori, 2004). The autophagic pathway
 286 is used for bulk proteolysis, and the ubiquitin for fine
 287 control (Kadowaki & Kanazawa, 2003). It is likewise
 288 used for recycling of materials during starvation; au-
 289 tophagy is induced by starvation and by catabolic
 290 hormones (Wang & Klionsky, 2003). Autophagy
 291 eliminates abnormal proteins, such as regulating pro-
 292 cessing of N terminal huntingtin fragments (Qin et al.,
 293 2003). Blocking autophagy leads to accumulation of
 294 small mitochondria, suggesting that these are normally

295 processed by autophagy. Such results suggest that
 296 changes in autophagy probably determine changes
 297 in aging post-mitotic cells (Terman, 1995; Terman,
 298 Dalen, Eaton, Neuzil, & Brunk, 2003). Though overall
 299 metabolism slows with aging, entraining a slowing of
 300 specific elements of metabolism such as apoptosis and
 301 autophagy, evidence from both genetically long-lived
 302 *Caenorhabditis* and dietary-restricted mammals sug-
 303 gests that an active autophagic system is essential for
 304 maintenance of youthful characteristics and extended
 305 life.

306 Unused, starving, or hormone-deprived cells can
 307 atrophy, removing the bulk of their cytoplasm by au-
 308 tophagy or other means; and yet the cells survive and
 309 in appropriate circumstances can be resuscitated. At
 310 what point does autophagy become autophagic cell
 311 death? It is conceivable that the autophagy is a pro-
 312 tective mechanism, attempting to reduce metabolic
 313 demand by the cell to stave off death, much as
 314 a starving organism will consume first labile and
 315 then less labile muscle proteins. In this instance
 316 the death may yet be apoptotic, ensuing when au-
 317 tophagy has reached its limit (Fig. 4). Evidence for
 318 such an argument exists. For instance, Tolkovsky and
 319 her collaborators find that the death of a neuronally
 320 differentiated PC-12 cell following withdrawal of
 321 NGF becomes irreversible only when autophagy
 322 eliminates mitochondria (Tolkovsky, Bampton,
 323 & Goemans, 2004; Tolkovsky, Xue, Fletcher, &
 324 Borutaite, 2002; Xue, Fletcher, & Tolkovsky, 1999,
 325 2001). Stemming from the identification of autophagy
 326 genes in yeast, there are now means of mutating
 327 or otherwise interfering with parts of autophagic
 328 machinery and observing the consequences to the
 survival of cells (particularly post-mitotic cells such

Fig. 3. Autophagic cell death in the labial gland of the tobacco hornworm, *Manduca sexta*. (A) Low magnification image of transverse section of gland just before involution. The arrow points to a large stellate nucleus with considerable heterochromatin, typical for this polyploidy insect tissue. (B) High magnification of such a gland, illustrating cytoplasm rich in endoplasmic reticulum (er) and small mitochondria (m). (C–H) Successive stages in erosion of the gland. (C) First day, showing localized erosion and small autophagic vacuoles (arrows). (D) Second day, showing continuing erosion and larger autophagic vacuoles (arrows) and mitochondria (double arrow). (E) Also second day, showing autophagic vacuoles filled with ribosomes (black arrows) and swollen, fragmented endoplasmic reticulum (white arrows). Mitochondria still appear to be relatively normal, and respiration does not collapse until the third day. (F) Third day. Cytoplasm is entirely vacuolar; most of the vacuoles are acidic and contain lysosomal enzymes. Nuclei are condensing but heterochromatin patches are still identifiable. Traces of a DNA ladder can be detected at this time; the tissue becomes TUNEL positive and annexin V-positive; and oxidative respiration decreases markedly. (G) Third day. Vacuoles (V) are disappearing and nuclei continue to condense. Remnant materials including basement membrane are picked up by phagocytes in the vicinity. (H) There is some asynchrony among cells. The final collapse is relatively rapid, and during the third day cells are seen in different stages. By the fourth day nothing remains but highly condensed, TUNEL positive nuclei. Electron micrographs by Daniela Quaglino, University of Modena, in a collaborative project.

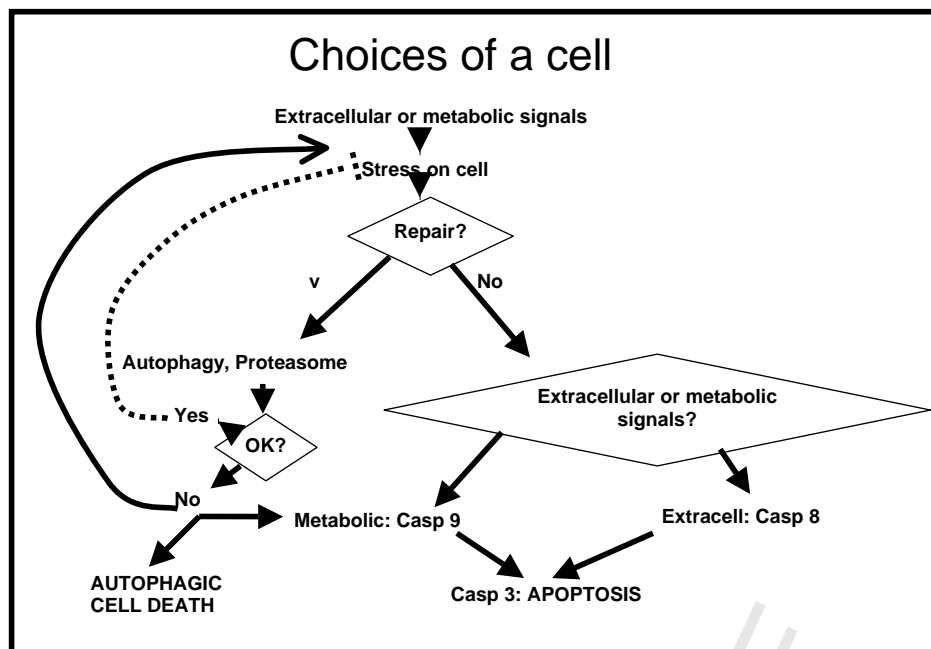


Fig. 4. Suggested physiological maintenance mechanisms: a cell under stress can determine that maintenance is not possible, and activate apoptosis machinery (right branch). If the stress is initiated by extracellular ligands such as Fas or TNF, it will use the extrinsic (caspase 8) pathway; if the stress derives from metabolic changes, it will use the intrinsic (mitochondrial, caspase 9) pathway. If the stress is initially less severe, the cell will attempt to cope by activating the autophagic or proteasomal salvage pathways (left branch). This may suffice (dotted line) and the cell recovers). If not, autophagy may continue in a cyclical fashion (leftmost curved arrow). Ultimately, if the condition is not compensated, the cell will be too severely drained and will die. It may elect, belatedly, to exit via the metabolic route to apoptosis, or it may simply destroy all of its contents to disappear via autophagic cell death. These latter two options may not be readily distinguishable.

329 as neurons or muscle fibres) and the organism. The
 330 result is that there is considerable controversy as to
 331 whether autophagy protects cells or is a means to
 332 their destruction. The most reasoned arguments sug-
 333 gest that the role of autophagy depends on the status
 334 or history of the cell, that autophagy (which can be
 335 subdivided into macroautophagy, microautophagy,
 336 and chaperone-mediated autophagy) is initially pro-
 337 tective but ultimately results in the accumulation of
 338 indigestible materials (Cuervo, 2003, 2004) or the
 339 destruction of vital components of the cell (Fig. 4).

340 When does autophagy, used to reduce cell volume,
 341 become autophagic cell death, in which the cell be-
 342 comes non-recoverable? In insects this conundrum is
 343 quite common: one can augment autophagy in the
 344 salivary gland of *Drosophila* by starving the larva, in
 345 which case the cells atrophy; but they die by autophagy
 346 at metamorphosis. In the blood-sucking Hemipteran
 347 *Rhodnius prolixus* the intersegmental muscles atro-

348 phy after each moult but are destroyed at metamor-
 349 phosis. Our technology provides new challenges. Al-
 350 though we have genetic markers or inhibitors of au-
 351 tophagy, particularly in yeast, we have very poor mark-
 352 ers of autophagy (Mizushima, Ohsumi, & Yoshimori,
 353 2002), and little ability to measure the origin of au-
 354 tophagy and its metabolic control. Likewise, we need
 355 to improve our analysis of the means by which iso-
 356 lation membranes recognize and target organelles for
 357 ingestion, or the consequences of blockage or failure
 358 of autophagy. An effort to identify genes associated
 359 with autophagic cell death, as opposed to autophagy,
 360 has begun (Inbal, Bialik, Sabanay, Shani, & Kimchi,
 361 2002; Kimchi, 2001; Lee et al., 2003). For instance,
 362 the molecular interactions of the autophagy-promoting
 363 DAP kinase that connect it to phosphorylation of the
 364 myosin light chain and membrane blebbing as well to
 365 calmodulin activity are being established. As for other
 366 mechanisms of cell death, autoinhibition keeps these

367 death-promoting kinases silent in healthy cells (Inbal
368 et al., 2002; Kimchi, 2001).

369 We also need to design experiments to establish
370 whether there is any difference between autophagy
371 as physiological regulation and autophagy leading to
372 cell death, and if it is possible to reverse “autophagic
373 cell death” by supplying nutrients, energy sources,
374 or other materials. We also need to understand more
375 thoroughly what activates autophagy. Current impres-
376 sions are that organelles are targeted by some fail-
377 ure in their metabolism. In other words, in a situa-
378 tion such as obtains in insect metamorphosis, a re-
379 markable stage-specific removal of organelles such
380 as mitochondria reflects a stage-specific injury to or
381 failure of mitochondria, rather than an aggressive at-
382 tack on healthy mitochondria by isolating membranes.
383 In Parkinsonism, dysfunctional mitochondria are de-
384 stroyed by autophagy, resulting in activation of ERK
385 signalling pathways and eventually activating apop-
386 tosis (Zhu, Guo, Shelburne, Watkins, & Chu, 2003).

The question nevertheless remains, what change has
activated the autophagy, and what brought about this
change?

4. There is overlap between autophagic and apoptotic cell deaths

It has long been clear that not all deaths can be
neatly categorized, and that different types overlap
(Lockshin & Zakeri, 2004b, Fig. 5). Recently, several
laboratories have reported that molecules previously
defined as intermediaries in the activation of apop-
tosis also function as intermediaries in the activation of
autophagy, thus calling into question the primacy of
the roles of both apoptosis and autophagic cell death
in these situations as well as our ability to distinguish
the processes by use of inhibitors. For instance, in
a model of the formation of lumens in mammary
acini, the pro-apoptotic TRAIL mediates autophagy

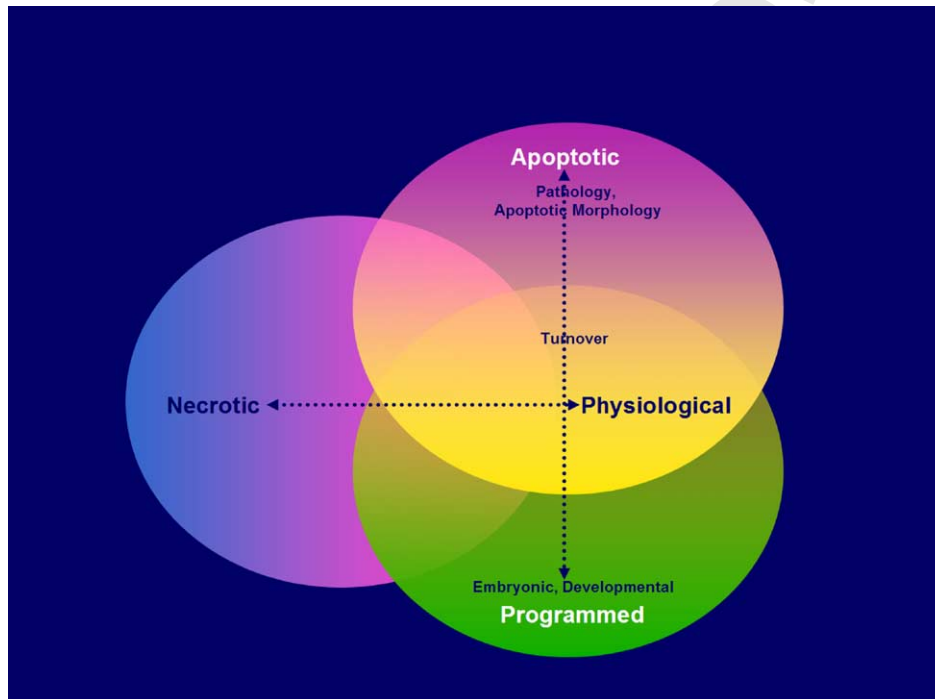


Fig. 5. Overlap of types of cell death. Deaths are frequently clearly physiological or uncontrolled (necrotic) but because of temporal or other factors the distinction may not be precise. Among the physiological deaths, programming involving protein synthesis is most clearly seen in developmental situations, whereas most pathological or induced cell deaths are pre-programmed and can activate apoptosis without protein synthesis. Cell turnover is for the most part not documented but is presumed to be apoptotic in nature.

(Lockshin & Zakeri, 2004b). In neural precursor cells, deprivation of growth factors leads to an autophagic cell death, which can be blocked by the anti-apoptotic Bcl-2 (Cardenas-Aguayo et al., 2003), an involvement that has also been recognized in autophagic cell death induced by HSPin1, a molecule first identified as interacting with Bcl2/Bcl-xL (Yanagisawa, Miyashita, Nakano, & Yamamoto, 2003). Ceramide, which has been considered by many researchers to participate in the activation of apoptosis, is effective in establishing macroautophagy (Scarlati et al., in press). The endosome–lysosome system appears to be activated early in Alzheimer’s disease, in which death is ultimately apoptotic (Cataldo, Hamilton, Barnett, Paskevich, & Nixon, 1996). In *Drosophila* salivary glands, caspase-like enzymes are needed for needed for autophagy (Baehrecke, 2003; Martin & Baehrecke, 2004); the same is true for death of neurons in *Manduca* at metamorphosis (Weeks, 2003).

One of the means of interaction between autophagy and apoptosis is the possibility that lysosomal activity can activate apoptosis. Taking advantage of newer inhibitors and fluorescent markers, Kroemer and co-workers have established that lysosomes can activate classical apoptotic pathways (Boya et al., 2003a,b; Ferri & Kroemer, 2001). Several laboratories have observed that damage to the lysosomal compartment, like other serious injuries to cells, can activate apoptosis. Their findings, as discussed below, indicate that lysosomal misbehaviour can trigger apoptosis, operating through the mitochondrial pathway. Others have argued that autophagy is a precursor and even initiator of apoptosis (Uchiyama, 2001). This adds a new question to those listed above: How does activation of the lysosomal system, or damage to the lysosomal system, activate apoptosis (Boya et al., 2003b)?

Alternatively, since release of materials from mitochondria triggers the intrinsic pathway to apoptosis, sequestration of mitochondria in autophagic vacuoles might protect cells against Type I cell death. At least one laboratory has postulated that sequestration of mitochondria may delay the release of cytochrome *c* (Bauvy, Gane, Arico, Codogno, & Ogier-Denis, 2001) and therefore interfere with apoptosis; autophagy would therefore become anti-apoptotic and protective. Surprisingly, but similar to the findings for caspase 3, it appears that limited release of lysosomal

enzymes into the cytoplasm is not necessarily lethal (Boya et al., 2003a,b; Perfettini & Kroemer, 2003). Typically, lysosomal enzymes function at very acid pH and would be expected to be ineffective in the cytoplasm, but the pH curves for cathepsins B and H extend into ranges that might be approached in hypoxic cells. As for caspase 3, these proteases are potentially extremely damaging, especially to proteins required for cell structure or enzymes at key points of metabolism, and the preponderance of cell effort is to constrain their activities. It appears that the activation of most cellular proteases is a very carefully orchestrated release from inhibition.

5. Perhaps autophagic cell death is open-ended autophagy

It is common today to contrast apoptosis with “autophagic cell death” but there are compelling reasons to question whether this is truly a qualitative difference. It is currently not possible to distinguish among autophagy as a routine mechanism of turnover of organelles, autophagy as a response to organelle injury or cell starvation, and autophagic cell death. Until we can, it remains conceivable that, if autophagy is a means by which a cell can temporize in difficult times, then autophagic cell death may be the result of the inability of the cell, for whatever reason, to terminate the autophagy. The question may therefore turn to the issue of why the cell cannot recover—inability of the cell to recognize or respond to growth factors, lack of a crucial substrate, biochemical or physical impediment to its ability to process oxygen, or other reason. One clue might be the following: during the programmed cell death of metamorphosing labial glands in *Manduca sexta* or of the salivary gland of *Drosophila*, the first 90% of the collapse is autophagic, with no sign of activation of caspases or other indicator of cell death. Finally, once virtually all the cytoplasm has been removed by autophagy, the cell manifests many of the criteria of apoptosis including exteriorization of phosphatidylserine, mitochondrial depolarization, and, more equivocally, activation of a caspase-like enzyme. One question that deserves far more attention is whether the end of autophagic cell death is ultimately apoptosis, with the autophagy being the prelude that leads to apoptosis (Fig. 3).

496 6. Other proteolytic processes in cell death

497 In addition to lysosomes and caspases, other means
 498 exist to destroy cells. The maintenance of cell vi-
 499 ability is heavily dependent on its shape, ability to
 500 conduct intracellular trafficking, and its communica-
 501 tion with extracellular matrix and neighbouring cells.
 502 Evidence exists that ubiquitination–proteasome sys-
 503 tem and of matrix metalloproteases can function in
 504 cell death. Proteasomes were first connected to cell
 505 death when Schwartz et al. documented their promi-
 506 nent role in the destruction of myofilaments during
 507 the programmed cell death of the intersegmental mus-
 508 cles of moths (Grimm, Goldberg, Poirier, Schwartz,
 509 & Osborne, 1996; Schwartz et al., 1990). Several labo-
 510 ratories have made similar observations. However,
 511 ubiquitination can also destroy pro-apoptotic pro-
 512 teins, and, in a convoluted way, become protective. In
 513 *Drosophila*, the inhibitor DIAP can be degraded by
 514 a caspase, which necessarily binds to it. The caspase
 515 activity however creates a substrate for ubiquitination,
 516 entraining the caspase as well into ubiquitination and
 517 destruction and thereby limiting apoptosis. Such a
 518 mechanism potentially applies also IAPs in neurons
 519 (Ditzel et al., 2003; Varshavsky, 2003). In healthy
 520 cells, the balance favours anti-apoptotic complexing
 521 partners (IAPs) over pro-apoptotic complexing part-
 522 ners and the modest levels of spontaneously-activated
 523 caspases are not sufficient to induce apoptosis.

524 Finally, three laboratories have argued that a cell's
 525 interaction with its matrix helps define its survival.
 526 Joining more theoretical and conceptual arguments
 527 related to anoikis, Tenniswood and colleagues sev-
 528 eral years ago identified, using differential display, a
 529 few genes that were upregulated in both autophagic
 530 death and apoptosis. One of these was a matrix met-
 531 alloprotease (Guenette, Mooibroek, Wong, Wong, &
 532 Tenniswood, 1994). More recently, in two gene
 533 screens using very different systems, different labo-
 534 ratories found that among the most prominent upreg-
 535 ulated genes in autophagy and apoptosis were matrix
 536 metalloproteases (Hu, Fink, & Mata, 2002; Lee et al.,
 537 2003). These interesting findings suggest that release
 538 of the affected cell from its environment (if this is the
 539 function of the MMPs) is a central feature of many
 540 types of cell death. However, as intriguing as these
 541 studies are, one must remember that many events of
 542 different types of cell death, including activation of

543 caspases and formation of isolation membranes, are
 544 not controlled at the transcriptional level and will not
 545 be identified in gene screens using microarrays.

546 7. When the death of a cell is inevitable, a cell 547 will take any available route to death

548 Many of our interpretations of the role of autophagy
 549 or its role vis-à-vis apoptosis depend on experiments
 550 involving the blockage of one or more pathways. How-
 551 ever, it is not sufficient, for instance, to block caspase 3
 552 and measure cell survival using markers of apoptosis,
 553 since a cell with a severe defect or deficit may still die
 554 by other means. Four common overlapping fallacies
 555 often produce conflicting claims concerning the role
 556 of particular pathways in cell death. The first is that
 557 inhibiting or knocking out a given pathway prevents
 558 cell death when (particularly in the case of effector
 559 caspases) it prevents the development of apoptotic
 560 morphology or another marker of apoptosis, but not
 561 the death itself (which may be evaluated by various
 562 tests of function or reproduction). The second is that
 563 if upregulating a specific pathway results in apoptosis,
 564 then the pathway is part of the functional sequence
 565 of apoptosis. The third is the assumption that if one
 566 blocks a single pathway and the cell dies by another
 567 pathway, that the connection between the pathways is
 568 meaningful. Finally, the assumption is risky that the
 569 response of a cell under extremely arduous conditions
 570 reflects its normal biology. Many comments about
 571 apoptosis and autophagy fail to acknowledge that a
 572 cell under extreme conditions—penurious medium,
 573 lack of growth factors, exposure to cycloheximide,
 574 staurosporine, or other toxic media—is likely to die.
 575 For most cells, the preferred means is the controlled
 576 death for which it is predisposed and prepared, the
 577 activation of pre-existent procaspases and apoptosis.
 578 If the apoptotic pathway is blocked for any reason,
 579 for instance interference with effector caspases or
 580 caspase activation, upregulation of antiapoptotic fac-
 581 tors such as bcl-2, or protection of mitochondria, the
 582 cell can still die, perhaps using autophagic pathways
 583 or others. Autophagy is most likely, in evolutionary
 584 terms, more ancient than apoptosis (Cornillon et al.,
 585 1994; Klionsky & Emr, 2000; Levraud et al., 2004;
 586 Olie et al., 1998) and is a function possessed by al-
 587 most all cells. It is likely that, although activation of

588 apoptosis takes precedence for a cell in trouble, if
 589 apoptosis fails the cell can fall back on autophagy.
 590 For instance, though mammary epithelium normally
 591 can undergo apoptosis, in vivo milk-filled epithelium
 592 can become heavily autophagic, and MCF-7 cells,
 593 which lack caspase 3, die by autophagy (Bursch et al.,
 594 2004; Zakeri, Bursch, Tenniswood, & Lockshin,
 595 1995). Autophagy likewise becomes an alternative
 596 pathway to death when caspases are inhibited in neu-
 597 rons (Lang-Rollin, Rideout, Noticewala, & Stefanis,
 598 2003) and when protein synthesis is inhibited in de-
 599 veloping retina (Guimaraes, Benchimol, Amarante-
 600 Mendes, & Linden, 2003). In aging, both apoptosis
 601 and autophagy generally decrease, though apoptosis
 602 may increase in cells of hematopoietic origin (Ahn
 603 et al., 2003; Bergamini, Cavallini, Donati, & Gori,
 604 2003; Birge, 2004; Bossy-Wetzel, Barsoum, Godzik,
 605 Schwarzenbacher, & Lipton, 2003; Brunk & Terman,
 606 2002; Cho et al., 2003; Cuervo & Dice, 2000; Del
 607 Roso et al., 2003; Dirks & Leeuwenburgh, 2004;
 608 Donati et al., 2001; Ermak & Davies, 2002; Fraker
 609 & Lill-Elghanian, 2004; Nixon, Cataldo, & Mathews,
 610 2000; Terman et al., 2003; Warner, 2004). Mainte-
 611 nance of autophagy extends the life of *Caenorhabditis*
 612 (Melendez et al., 2003; Riddle & Gorski, 2003).

613 8. Conclusions

614 The threshold at which a cell commits to die is set
 615 by many metabolic and structural features of cells.
 616 The permeabilization or depolarization of mitochon-
 617 dria can release to the cytoplasm cytochrome *c*, but
 618 the threshold at which it does so is surely adjusted
 619 by metabolic and respiratory factors, which are them-
 620 selves adjusted by other activities of the cell. As the
 621 cell commits to death, there is considerable crosstalk
 622 among metabolic pathways. Cytoplasmic or lysoso-
 623 mal proteases other than caspases can affect the ac-
 624 tivation of effector caspases, most frequently proba-
 625 bly contributing to the activation. Matrix metallopro-
 626 teinases are often upregulated in dying cells, assuring
 627 the release of the dying or apoptotic cell from its at-
 628 tachments, and perhaps playing other important roles
 629 in the death of the cells. In some cells there is consid-
 630 erable autophagy prior to or instead of apoptosis. This
 631 autophagy might be defensive and protective, reducing
 632 metabolic demand, generating maintenance resources,

or even sequestering mitochondria and preventing re- 633
 lease of cytochrome *c*; it could remove enough mito- 634
 chondria to commit a cell to death; or it could be an 635
 automatic, mechanical response to building failures in 636
 a cell already condemned and for which maintenance 637
 mechanisms have been shut down. Most commonly, 638
 cells follow an apoptotic route to death, but they have 639
 many options can divert to or accentuate autophagic 640
 or other catabolic pathways. We also do not fully un- 641
 derstand the extent to which cells can switch among 642
 pathways. If apoptosis is blocked, can a sufficiently 643
 challenged cell default to autophagic cell death, or 644
 vice versa? When, if at all, is autophagy protective, as 645
 opposed to being the prodromal phase of an apoptotic 646
 or other cell death? To what extent can the metabolic 647
 history of a cell, including nutritional reserves, ac- 648
 cumulated oxidative damage, and prior and current 649
 stress to the endoplasmic reticulum—in other words, 650
 the conversation among organelles—affect the thresh- 651
 old at which the cell commits to death, or the pathway 652
 that it follows to death? The cell operates more as an 653
 ecosystem than as a collection of individual enzymatic 654
 pathways. Efforts to establish therapies that are based 655
 on the control of apoptosis will eventually incorporate 656
 these considerations. 657

Uncited reference 658

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