Serveur Académique Lausannois SERVAL serval.unil.ch

Author Manuscript
Faculty of Biology and Medicine Publication

This paper has been peer-reviewed but dos not include the final publisher proof-corrections or journal pagination.

Published in final edited form as:

Title: Caspase-3 and RasGAP: a stress-sensing survival/demise switch.

Authors: Khalil H, Bertrand MJ, Vandenabeele P, Widmann C

Journal: Trends in cell biology

Year: 2014 Feb

Volume: 24

Issue: 2

Pages: 83-9

DOI: 10.1016/j.tcb.2013.08.002

In the absence of a copyright statement, users should assume that standard copyright protection applies, unless the article contains an explicit statement to the contrary. In case of doubt, contact the journal publisher to verify the copyright status of an article.

serval
serveur académique lausannois

Unil
UNIL | Université de Lausanne
Faculté de biologie et de médecine

Caspase-3 and RasGAP: a stress-sensing survival/demise switch

Hadi Khalil[1], Mathieu JM Bertrand[2,3], Peter Vandenabeele[2,3], Christian Widmann[1]

[1] Department of Physiology, University of Lausanne, Switzerland; [2] Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB, Gent, Belgium;

[3] Department of Biomedical Molecular Biology, Ghent University, Gent, Belgium.

Correspondence to Christian Widmann, Department of Physiology, Bugnon 7, 1005 Lausanne, Switzerland, Phone: +41 21 692 5123, Fax: +41 21 692 5505, E-mail: Christian.Widmann@unil.ch.

Keywords: Caspase-3, RasGAP, Cellular stress, Sensors

Abstract

The final decision on cell fate, survival versus cell death, relies on complex and tightly regulated checkpoint mechanisms. The caspase-3 protease is a predominant player in the induction of apoptosis. However, this protease is more than a blind killer. It can gauge the degree of cellular stress and damage by differential processing of p120 RasGAP to generate the appropriate cellular response (survival or cell demise). Partial cleavage of RasGAP by caspase-3 initiates the anti-apoptotic Akt pathway. In contrast, full cleavage of RasGAP abrogates this survival response, hence favoring cell death. Therefore, rather than relying on separate sensors, cells can modulate a given set of proteins to generate, depending on the intensity of the input signals, opposite outcomes (survival vs. death).

29 **Introduction**

30 Proper functioning of cells requires that they maintain their biochemical
31 parameters within rather narrow limits. This process, called homeostasis, is vital
32 for cells and they spend a considerable energy to maintain it. For example, the
33 appropriate gradient of sodium and potassium ion concentrations between the
34 inside and the outside of brain cells via the action of the sodium/potassium
35 ATPase pump consumes close to half of the ATP used at the basal state [1]. If the
36 extracellular conditions are changing too much or if the cellular biochemistry is
37 exceedingly altered, such as following viral infection [2,3] or oncogene
38 expression [4], than homeostasis maintenance is compromised. This
39 perturbation is sensed by cells as cellular stress. Situations and conditions that
40 can generate cellular stress are extremely diverse. They include environmental
41 pollutants, metabolic stress induced by toxic lipids in obese individuals, UV-
42 mediated stress generated by prolonged exposure of the epidermis to sun
43 exposure, endoplasmic reticulum stress caused by sustained chronic increase in
44 insulin production by pancreatic beta cells in type 2 diabetes patients, chemical
45 or ionizing stress that cancer patients experience when treated with chemo- or
46 radiotherapy, or physicochemical stress provoked by heat or cold exposure.

47 When encountering a stress condition, cells have two options, either to cope with
48 it, which requires an adaptive response, or to give up and die, which involves the
49 initiation of an active and programmed cell death process. Cells are in fact well
50 equipped to sense the extent of stress and this allows them to decide whether
51 they should try to restore homeostasis or whether they should commit suicide
52 because stress is evaluated as too extensive and potentially deleterious. Various
53 switch mechanisms can be used by cells to evaluate stress and insults to either

54 promote cell survival or apoptosis. We will focus here on a newly described
55 physiologic stress-sensing cellular system based on the differential cleavage of a
56 specific caspase-3 substrate.
57
58 **Caspase-3, from reaper to friendly protease**
59 At the end of the nineties, a consensus emerged that apoptosis (Box1) was
60 carried on by the executioner members of the caspase family (Box1) (caspase-3,
61 -6, and -7) [5-8]. Caspase-3 has been considered as the central executioner
62 protease due to its high catalytic turnover and greediness to cleave substrates
63 [9]. Gene targeting in mice has revealed a certain level of redundancy in the
64 function of executioner caspases in apoptosis induction [10], and more
65 importantly that these executioner caspases fulfill other non-apoptotic functions
66 as well [11].
67 Historically however, non-apoptotic functions of pro-apoptotic caspases were
68 first discovered for caspase-8, an initiator caspase. It was shown in 1998 that
69 mice lacking caspase-8 were embryonic lethal [12]. This lethality was later
70 shown to result from degeneration of the vasculature of the yolk sac [13]. This
71 suggested that caspase-8 is required for the proper development and/or the
72 maintenance of vascular structures in the early embryo, which is most certainly
73 distinct from its ability to mediate apoptosis via the extrinsic cell death pathway.
74 Also in 1998, it was shown that cell expressing CrmA, a caspase-8 inhibitor, were
75 paradoxically sensitized to necrotic cell death [14], suggesting that caspase-8
76 could also promote cell survival [15]. Non-apoptotic functions were then
77 progressively reported for other caspases and a series of reviews have been
78 describing these in the past decade [11,16-21]. Caspase-3 in particular appears

79 to regulate the differentiation of skeletal muscle cells, osteoblasts, lens epithelial
80 cells, neural stem cells, and several hematopoietic lineages (T and B
81 lymphocytes, erythrocytes, macrophages, platelets). Caspase-3 has also been
82 reported to participate in stem cell maintenance, inhibition of B-cell
83 proliferation, dendritic cell maturation, and proliferation of brain cells and
84 keratinocytes [22-25].
85 The levels of activated caspase-3 that stimulate the non-apoptotic biological
86 responses should obviously be lower than during apoptosis otherwise this cell
87 death process would not be avoided. Numerous substrates of caspase-3 have
88 been determined [9,26-28]. However their role in apoptosis has only been
89 explored in a few cases. Additionally, the impact of caspase-mediated cleavage
90 for the vast majority of substrates has not been explored by studying the
91 phenotype of cells or mice in which the wild-type substrate is replaced with a
92 non-cleavable knock-in form.
93 Different executioner caspases can target the same substrates while a given
94 caspase can cleave some substrates better than others [9,29]. In particular,
95 caspase-3 appears to cleave most substrates more efficiently than other caspases
96 [29]. It is conceivable that the high K_m value of caspase-3 for some of its
97 substrates is related to its non-apoptotic functions while apoptosis may imply
98 the less effective substrates. In other words, low activation levels of caspase-3,
99 leading to restricted cleavage of high affinity substrates, would allow survival
100 while higher levels of caspase-3 activation would result in the cleavage of a much
101 wider spectrum of substrates, including low affinity ones, ultimately leading to
102 the induction of apoptosis.

103 In addition to the possible differential affinity of caspase-3 toward the anti- and
104 pro apoptotic substrates, there are other mechanisms that can keep caspase-3
105 activation levels in check during stressful situations. One possibility is that cells
106 have a built-in system that prevents amplification of caspase-3 activation. The
107 best-known family of proteins that can negatively control executioner caspases
108 is the inhibitor of apoptosis (IAPs) protein family. IAPs are characterized by one
109 to three baculovirus IAP repeat (BIR) zinc-binding domain, which, at least for
110 some IAP family members, is required for their ability to bind caspases [30].
111 However, contrary to initially speculated, X-linked IAP (XIAP) is the only IAP that
112 can function as a direct inhibitor of caspase-3, -7 and -9 [31,32]. Cellular IAP1
113 and 2 (cIAP1/2) instead promote cell survival by functioning as E3 ubiquitin
114 ligases that promote the degradation of caspase-3 and -7 [33] and positively
115 regulate activation of the canonical NF-kB pathway [34-36]. In addition, cIAP1/2
116 function as regulators of survival signaling in cancer cells by preventing RIPK1
117 from becoming a pro-death stress sensing molecule [37]. Hence, IAP family
118 members allow cells to cope with stress to a certain limit if they are expressed at
119 sufficiently high levels either by activating NF-kB, preventing RIPK1 from
120 becoming a pro-death molecule or by keeping caspase-3 activity in check.
121 Another possibility that can explain why activation of caspase-3 does not lead to
122 apoptosis in all cases is that caspase-3 itself, when activated at low levels, turns
123 on an anti-apoptotic response that acts as a negative feedback loop preventing
124 further caspase-3 activation (i.e. an amplification of caspase-3 activity). A recent
125 study [38] provides genetic evidence for this second possibility. In the C57BL/6
126 background, mice lacking caspase-3 are viable and fertile [39]. The vast majority
127 of development programs occur normally in these mice. They do however

128 display abnormal inner ear development [40]. It is not known whether this
129 defect results from a defect in apoptosis or from alterations of other functions
130 controlled by caspase-3. Apoptosis in these mice can proceed normally or be
131 inhibited, depending on which tissues and organs are investigated. For example,
132 female germ cell apoptosis is not affected by the lack of caspase-3 while
133 granulosa cell apoptosis is not taking place during follicular atresia when this
134 caspase is missing [41]. Caspase-3 knock-out mice are unable or strongly
135 impaired in activating the anti-apoptotic Akt kinase (box 2) in response to a
136 variety of stresses such as UV-B exposure of the skin, doxorubicin-mediated
137 cardiac damage, and experimentally-induced colitis, an inflammation of the large
138 intestine. Akt activation is also compromised in wild-type mice injected with the
139 Q-VD-OPh caspase inhibitor suggesting that the activity of caspase-3, and not a
140 potential adaptor function of the protein, is required for its capacity to activate
141 Akt. As detailed below, the defect in Akt activation in mice lacking caspase-3 is
142 accompanied by increased cell death responses. Caspase-3 therefore appears to
143 stimulate survival responses in some situations.

144 As caspase-3 is a main executioner caspase, its absence generally compromises
145 or abrogates apoptosis induction by various stress inducing conditions. For
146 example, pancreatic beta cells lacking caspase-3 are fully resistant against
147 streptozotocin-induced death [42]. Even though absence of caspase-3 generally
148 reduces the apoptotic response, this does not mean necessarily that the death
149 response is lowered. For example, UV-B-induced apoptosis is decreased in the
150 epidermis of caspase-3 KO mice but the overall death response is not, as in the
151 absence of apoptosis induction cell death proceeds in caspase-independent cell
152 death with necrotic morphology [38].

153 In some cases, caspase-7 can compensate, at least partially by enhanced
154 expression levels in C57Bl6 strains for the lack of caspase-3 and allow apoptosis
155 to proceed [10]. Doxorubicin is an efficient anti-cancer drug that is currently
156 used against various tumors. However, this drug also induces serious cardiac
157 side effects. Cardiomyocytes are indeed rather sensitive to doxorubicin and
158 readily undergo apoptosis when exposed to the drug. Unexpectedly, caspase-3
159 knock-out mice experience even more cardiomyocyte apoptosis than wild-type
160 mice when treated with doxorubicin, leading to increased mortality [38]. This
161 suggests that the apoptotic role of caspase-3 is redundant while its prosurvival
162 role in stressed tissues and organs is not.
163
164 **Cellular sensor of caspase-3 activity**
165 There is only a handful proteins including p120 RasGAP[43], p27^{kip1} [44], Lyn
166 [45], Synphilin-1 [46], nucleophosmin [47], Rb [48], and the Drosophila IMD
167 protein [49] that may activate survival pathways once cleaved by caspases. The
168 first of these, RasGAP, is of particular interest in the present context because the
169 N-terminal moiety generated by cleavage of RasGAP at position 455 by caspase-3
170 activates Akt and protects cells *in vitro* [50,51]. In cells, RasGAP does not seem to
171 be cleaved by caspases other than caspase-3 [51]. RasGAP bears another
172 caspase-3 cleavage site at position 157 that is made accessible only following the
173 first cleavage event or that is much less efficiently recognized by caspases [43].
174 Consequently, the second site is not used when caspase-3 is mildly activated [52]
175 (Figure 1, upper left panel). However, after an apoptotic insult, concentrations of
176 activated caspase-3 further increases, reaching a threshold allowing the N-
177 terminal fragment of RasGAP to be cleaved at position 157, abrogating Akt

178 activation. The second RasGAP cleavage thus favors death [52] (Figure 1, upper
179 right panel). RasGAP is the only caspase-3 substrate that shows this differential
180 cleavage-mediated control of two opposite outcomes (survival vs apoptosis).
181 Interestingly, RasGAP has no obvious anti-apoptotic activities as a full-length
182 protein [53]. It only acquires cell protective functions when cleaved at position
183 455. The physiological role of caspase-3-mediated cleavage of RasGAP has
184 recently been assessed using a knock-in mouse strain homozygous for a
185 mutation in RasGAP at position 455 that prevents its cleavage by caspase-3. The
186 RasGAP knock-in mice phenocopied the caspase-3 knock-out stress-induced Akt
187 activation defect in response to various patho-physiological stresses (Figure 1,
188 lower panels). The knock-in mice also experienced increased tissue damage and
189 organ dysfunction in response to these insults in comparison to wild-type mice
190 [38]. These results provide genetic evidence for the importance of caspase-3-
191 mediated p120 RasGAP cleavage as a defense mechanism to protect organisms
192 against damage induced by diverse pathological conditions.
193 It can therefore be proposed that caspase-3 itself activates the anti-apoptotic Akt
194 kinase following certain types of stresses (e.g. starvation, DNA damage)[51] and
195 this seemingly operates via the efficient cleavage of only one of its substrates, the
196 ubiquitous p120 RasGAP protein. The model drawn for these studies is that
197 RasGAP bears two cleavage sites with differential sensitivities for caspase-3
198 proteolytic activity. RasGAP acts as a "sensor like" protein that reflects the levels
199 of caspase-3 activation, which correlates with the extent of cellular stress. This
200 differential cleavage aids determining the fate of stressed cells: activation of a
201 protective Akt-dependent pathway following the first cleavage of RasGAP or

202 inactivation of this protective pathway following the second cleavage of RasGAP
203 (Figure 1, upper panels).
204
205 **Perspectives: one set of signaling proteins, different outcomes**
206 There are other comparable decision-making systems assessing the magnitude
207 of cellular stress, where within a given signaling pathway, differential regulatory
208 events dictate distinct cell signaling events. These "sensor-like" responses
209 include the unfolded protein response (UPR) in the endoplasmic reticulum and
210 the activation of p53 transcription factor pathway by DNA damage. What
211 determines the cellular outcome once a given switch is triggered relies on the
212 duration and the extent of the stress that allows or not the accumulation of pro-
213 apoptotic genes, either as a consequence of differential stability of the anti- and
214 pro-apoptotic proteins or as a result of post-translational modifications of the
215 switch components (Figure 2). For instance, the goal of the UPR initially is to try
216 restoring the folding capacity of the ER while diminishing protein load in order
217 to reestablish ER homeostasis [54-56]. Only a strong and sustained ER stress
218 allows for the accumulation of pro-apoptotic labile proteins such as the
219 transcription factor CHOP [56,57]. Similarly, distinct post-translational
220 modifications (e.g. acetylation on lysine 150 or phosphorylation of serine 46)
221 generated by various degrees of DNA damage, determines whether p53
222 preferentially stimulates a repair pathway versus a cell death response [58-60].
223 From these examples one could propose a general paradigm were cellular stress
224 is sensed by molecules and pathways that stimulate either cell survival or
225 apoptosis based on the extent of their stimulation (as in the case of the UPR) or
226 as a result of stress-mediated differential post-translational modifications (e.g.

227 cleavage of RasGAP, acetylation/phosphorylation of p53). Hence, cells may use
228 the same set of sensor proteins to fine-tune an appropriate cellular response
229 either resulting in cell survival or cellular demise, rather than relying on
230 separate sensors for either response.

231 **Figure legends**

232

233 **Figure 1. RasGAP cleavage, a sensor of caspase-3-activity controlling the**
234 **survival and the death of cells.**

235 RasGAP bears two cleavage sites with different sensitivities towards caspase-3
236 activity. Site 1 at position 455 is used at low levels of caspase-3 activity, while
237 site 2, at position 157, is only recognized at high levels of caspase-3 activity.
238 **Upper left panel**. In situations of mild stress, caspase-3 is activated to low levels
239 and this leads to the partial cleavage of RasGAP into fragment C and fragment N.
240 The latter activates an Akt-mediated anti-apoptotic response that inhibits
241 further amplification of caspase-3 activity. **Upper right panel**. When cells are
242 facing higher stress, caspase-3 is more strongly activated resulting in further
243 cleavage of fragment N and abrogation of Akt activation. This favors cell death.
244 **Lower panels**. When cleavage of RasGAP cannot occur as a result of a mutation
245 at its first caspase-3 cleavage site, the prosurvival kinase Akt is not stimulated. In
246 this condition, the feedback loop that prevents an initially mild caspase-3 activity
247 is not activated and this results in an amplification of caspase-3 activity.
248 Consequently, mild stresses generate elevated caspase-3 activation comparable
249 to when cells experience stronger stresses. This eventually leads to cell death.

250

251 **Figure 2. Examples of mechanisms allowing single stress sensors to**
252 **activate survival or death responses**

253 A given protein or set of proteins can determine the fate of a cell (survival vs
254 death). This can result as a consequence of a differential cleavage of a protease
255 substrate (e.g. cleavage of RasGAP by caspase-3) (**upper panel**), the

256 accumulation above a certain threshold of a labile transcription factor (e.g CHOP
257 translation following ER stress) (**middle panel**), or differential post-
258 translational modifications (e.g. as occurring on p53 in response to varying
259 degrees of DNA damage) (**lower panel**).
260

261 **Box 1. Apoptosis and caspases**

262 Apoptosis (apo=for, ptosis=falling) is a type of cell death that was formally
263 described by Kerr, Wyllie and Currie in the early 1970s [61]. Apoptosis
264 participates in the control of tissue size and shape during development. It is
265 involved in the elimination of cells that may represent a threat to the organism
266 such as pre-malignant cells and activated immune T cells. Apoptosis is
267 characterized by a series of organized and finely regulated biochemical and
268 cellular modifications that process the dying cells for efficient elimination by
269 phagocytosis by neighboring cells or macrophages. This insures that no
270 cytoplasmic compounds leak from the cells that would otherwise generate an
271 inflammatory response [62]. The hallmarks of apoptosis include cell shrinkage,
272 membrane blebbing, nuclear and cytoplasmic condensation, DNA fragmentation,
273 cytoskeleton proteolysis and breakup of organelles. Cellular components are
274 sorted into a number of vesicles known as apoptotic bodies. Only a few of these
275 events are diagnostic for apoptosis (DNA cleavage, formation of apoptotic
276 bodies). Intact apoptotic cells display "eat-me" flags on their surface such as
277 phosphatidylserine that tag them as targets for phagocytosis. Phosphatidylserine
278 is usually confined to the inner side of the plasma membrane but becomes
279 exposed on the surface of apoptotic cells [63,64].

280 The cellular and biochemical features of apoptosis are triggered by members of
281 the caspase family of proteases. Caspases are a family of cysteine aspartic acid
282 proteases present in healthy cells as inactive precursor enzymes (zymogens). All
283 caspases have a similar domain structure (pro-polypeptide, large and small
284 subunits) [65]. Domains within the pro-polypeptide, such as caspase recruitment
285 domain (CARD) and death effector domain (DED), by homophylic interactions,

286 can recruit caspases to activation platforms. Not all caspases are implicated in
287 apoptosis but those that are can be divided into initiator (caspase-2, -8, and -9)
288 or executor caspases (caspase-3, -6, and -7). The former auto-activate when
289 brought together by receptors belonging to the death receptor family (e.g. Fas).
290 The latter are activated by the former by proteolysis and execute the proteolysis
291 events that are seen during the demolition phase of apoptosis [62,66].
292 Executioner caspases cleave hundreds of substrates during the process of
293 apoptosis but only for a minority of these is the physiological function of their
294 cleavage understood. These include ICAD, the inhibitor of CAD, the DNAse that
295 cleaves the DNA between nucleosomes. Caspase-mediated cleavage of ICAD leads
296 to CAD activation, generating ~180 base pair multimeric DNA fragments, the
297 diagnostic laddering in apoptotic cells.
298
299 **Box 2. The generally pro-survival Akt pathway**
300 Akt is a serine/threonine kinase involved in the regulation of cell survival,
301 proliferation, and metabolism and is activated by phosphorylation. Three
302 isoforms exist (1, 2, and 3) that all contain an N-terminal pleckstrin homology
303 (PH) domain, a central kinase domain containing a phosphorylation site within
304 the activation-loop (threonine 308 in human Akt1), and a conserved regulatory
305 serine phosphorylation site in a hydrophobic motif near the C terminus (position
306 473 in human Akt1). The interaction of the Akt PH domain with 3'-
307 phosphoinositides causes Akt translocation to the plasma membrane, inducing
308 conformational changes that allow Akt to expose its phosphorylation sites [67].
309 Phosphoinositide-dependent kinase-1 (PDK-1) is also recruited to the plasma
310 membrane after PIP3 generation. PDK-1 is the kinase that phosphorylates Akt on

311 threonine 308, which stabilizes the activation loop in an active conformation.
312 Serine 473 is then phosphorylated by the PDK2 kinase activity which is
313 predominantly carried out by mammalian target of rapamycin (mTOR) complex
314 2 (mTORC2) or DNA-PK. Phosphorylation of threonine 308 is a prerequisite for
315 kinase activation. Phosphorylation of serine 473 appears to further increase Akt
316 kinase activity. Recent evidence suggests that this phosphorylation event also
317 controls the target specificity of the kinase [67].
318 Among the Akt substrates that have been identified in mammalian cells, many
319 are regulators of apoptosis or cell growth [67]. The Akt substrates are
320 phosphorylated within the same basic motif RXRXXS/T. The anti-apoptotic
321 response induced by Akt involves NF-κB- or CREB-mediated up-regulation of
322 anti-apoptotic proteins such as c-IAP1/2, Mcl-1 and Bcl-2, or the direct
323 phosphorylation and inhibition of pro-apoptotic proteins such as Bad and
324 caspase-9. It is worth noting however that the Akt phosphorylation site in
325 caspase-9 is not conserved in mammals [68]. Akt also inhibits members of the
326 Forkhead family of transcription factors (FOXO transcription factors). This
327 prevents the expression of pro-apoptotic genes such as Fas ligand. Additionally,
328 murine double minute 2 (MDM2), an E3 ubiquitin ligase targeting p53 for
329 degradation, is stabilized by Akt-mediated phosphorylation. This will therefore
330 diminish p53-induced pro-apoptotic signaling in cells. Therefore, Akt promotes
331 survival in most cells either through direct phosphorylation of targets, or
332 through the induction of anti-apoptotic genes [67]. There are some cell types
333 however in which Akt activation can lead to death. In pancreatic beta cells for
334 example, Akt activation leads to NF-κB-dependent apoptosis [69].
335

336 Reference List

337

338 1 Astrup, J. *et al.* (1981) Oxygen and glucose consumption related to Na^{+}-K^{+}
339 transport in canine brain. *Stroke.* 12, 726-730
340 2 Barry, G. *et al.* (2010) Semliki forest virus-induced endoplasmic reticulum
341 stress accelerates apoptotic death of mammalian cells. *J. Virol.* 84, 7369-7377
342 3 Hosakote, Y.M. *et al.* (2009) Respiratory syncytial virus induces oxidative
343 stress by modulating antioxidant enzymes. *Am. J. Respir. Cell Mol. Biol.* 41, 348-
344 357
345 4 Halazonetis, T.D. *et al.* (2008) An oncogene-induced DNA damage model for
346 cancer development. *Science* 319, 1352-1355
347 5 Martin, S.J. and Green, D.R. (1995) Protease activation during apoptosis: death
348 by a thousand cuts? *Cell* 82, 349-352
349 6 Steller, H. (1995) Mechanisms and genes of cellular suicide. *Science* 267, 1445-
350 1449
351 7 Fraser, A. and Evan, G. (1996) A license to kill. *Cell* 85, 781-784
352 8 Thornberry, N. and Lazebnik, Y.A. (1998) Caspases: enemies within. *Science*
353 281, 1312-1316
354 9 Demon, D. *et al.* (2009) Proteome-wide substrate analysis indicates substrate
355 exclusion as a mechanism to generate caspase-7 versus caspase-3 specificity.
356 *Mol. Cell Proteomics.* 8, 2700-2714
357 10 Houde, C. *et al.* (2004) Caspase-7 expanded function and intrinsic expression
358 level underlies strain-specific brain phenotype of caspase-3-null mice. *J. Neurosci.*
359 24, 9977-9984

360 11 Lamkanfi, M. *et al.* (2007) Caspases in cell survival, proliferation and
361 differentiation. *Cell Death. Differ.* 14, 44-55
362 12 Varfolomeev, E.E. *et al.* (1998) Targeted disruption of the mouse *Caspase 8*
363 gene ablates cell death induction by the TNF receptors, Fas/Apo1, and DR3 and
364 is lethal prenatally. *Immunity.* 9, 267-276
365 13 Kang, T.B. *et al.* (2004) Caspase-8 serves both apoptotic and nonapoptotic
366 roles. *J. Immunol.* 173, 2976-2984
367 14 Vercammen, D. *et al.* (1998) Inhibition of caspases increases the sensitivity of
368 L929 cells to necrosis mediated by tumor necrosis factor. *J. Exp. Med.* 187, 1477-
369 1485
370 15 Oberst, A. and Green, D.R. (2011) It cuts both ways: reconciling the dual roles
371 of caspase 8 in cell death and survival. *Nat. Rev. Mol. Cell Biol.* 12, 757-763
372 16 Abraham, M.C. and Shaham, S. (2004) Death without caspases, caspases
373 without death. *Trends Cell Biol.* 14, 184-193
374 17 Garrido, C. and Kroemer, G. (2004) Life's smile, death's grin: vital functions of
375 apoptosis-executing proteins. *Curr. Opin. Cell Biol.* 16, 639-646
376 18 Kuranaga, E. and Miura, M. (2007) Nonapoptotic functions of caspases:
377 caspases as regulatory molecules for immunity and cell-fate determination.
378 *Trends Cell Biol.* 17, 135-144
379 19 Feinstein-Rotkopf, Y. and Arama, E. (2009) Can't live without them, can live
380 with them: roles of caspases during vital cellular processes. *Apoptosis.* 14, 980-
381 995
382 20 Yi, C.H. and Yuan, J. (2009) The Jekyll and Hyde functions of caspases. *Dev. Cell*
383 16, 21-34

384 21 Galluzzi, L. *et al.* (2012) Non-apoptotic functions of apoptosis-regulatory
385 proteins. *EMBO Rep.* 13, 322-330
386 22 Burguillos, M.A. *et al.* (2011) Caspase signalling controls microglia activation
387 and neurotoxicity. *Nature* 472, 319-324
388 23 Carlile, G.W. *et al.* (2004) Caspase-3 has a nonapoptotic function in erythroid
389 maturation. *Blood* 103, 4310-4316
390 24 Fernando, P. *et al.* (2005) Neural stem cell differentiation is dependent upon
391 endogenous caspase 3 activity. *FASEB J.* 19, 1671-1673
392 25 Fernando, P. *et al.* (2002) Caspase 3 activity is required for skeletal muscle
393 differentiation. *Proc. Natl. Acad. Sci. USA* 99, 11025-11030
394 26 Luthi, A.U. and Martin, S.J. (2007) The CASBAH: a searchable database of
395 caspase substrates. *Cell Death. Differ.* 14, 641-650
396 27 Timmer, J.C. and Salvesen, G.S. (2007) Caspase substrates. *Cell Death. Differ.*
397 14, 66-72
398 28 Demon, D. *et al.* (2009) Caspase substrates: easily caught in deep waters?
399 *Trends Biotechnol.* 27, 680-688
400 29 McStay, G.P. *et al.* (2008) Overlapping cleavage motif selectivity of caspases:
401 implications for analysis of apoptotic pathways. *Cell Death. Differ.* 15, 322-331
402 30 Gyrd-Hansen, M. and Meier, P. (2010) IAPs: from caspase inhibitors to
403 modulators of NF-κB, inflammation and cancer. *Nat. Rev. Cancer* 10, 561-574
404 31 Eckelman, B.P. and Salvesen, G.S. (2006) The human anti-apoptotic proteins
405 cIAP1 and cIAP2 bind but do not inhibit caspases. *J. Biol. Chem.* 281, 3254-3260
406 32 Eckelman, B.P. *et al.* (2006) Human inhibitor of apoptosis proteins: why XIAP
407 is the black sheep of the family. *EMBO Rep.* 7, 988-994

408 33 Choi, Y.E. *et al.* (2009) The E3 ubiquitin ligase cIAP1 binds and ubiquitinates
409 caspase-3 and -7 via unique mechanisms at distinct steps in their processing. *J.*
410 *Biol. Chem.* 284, 12772-12782
411 34 Mahoney, D.J. *et al.* (2008) Both cIAP1 and cIAP2 regulate TNFα-mediated NF-
412 κB activation. *Proc. Natl. Acad. Sci. U. S. A* 105, 11778-11783
413 35 Varfolomeev, E. *et al.* (2008) c-IAP1 and c-IAP2 are critical mediators of
414 tumor necrosis factor alpha (TNFα)-induced NF-κB activation. *J. Biol. Chem.* 283,
415 24295-24299
416 36 Bertrand, M.J. *et al.* (2008) cIAP1 and cIAP2 facilitate cancer cell survival by
417 functioning as E3 ligases that promote RIP1 ubiquitination. *Mol. Cell* 30, 689-700
418 37 Darding, M. and Meier, P. (2012) IAPs: guardians of RIPK1. *Cell Death. Differ.*
419 19, 58-66
420 38 Khalil, H. *et al.* (2012) Caspase-3 protects stressed organs against cell death.
421 *Mol. Cell Biol.* 32, 4523-4533
422 39 Leonard, J.R. *et al.* (2002) Strain-dependent neurodevelopmental
423 abnormalities in caspase-3- deficient mice. *J. Neuropathol. Exp. Neurol.* 61, 673-
424 677
425 40 Takahashi, K. *et al.* (2001) Caspase-3-deficiency induces hyperplasia of
426 supporting cells and degeneration of sensory cells resulting in the hearing loss.
427 *Brain Res.* 894, 359-367
428 41 Matikainen, T. *et al.* (2001) Caspase-3 gene knockout defines cell lineage
429 specificity for programmed cell death signaling in the ovary. *Endocrinology* 142,
430 2468-2480
431 42 Liadis, N. *et al.* (2005) Caspase-3-dependent β-cell apoptosis in the initiation
432 of autoimmune diabetes mellitus. *Mol. Cell Biol.* 25, 3620-3629

433 43 Yang, J.-Y. and Widmann, C. (2001) Antiapoptotic signaling generated by
434 caspase-induced cleavage of RasGAP. *Mol. Cell. Biol.* 21, 5346-5358
435 44 Eymin, B. *et al.* (1999) Caspase-induced proteolysis of the cyclin-dependent
436 kinase inhibitor p27Kip1 mediates its anti-apoptotic activity. *Oncogene* 18, 4839-
437 4847
438 45 Luciano, F. *et al.* (2003) The p54 cleaved form of the tyrosine kinase Lyn
439 generated by caspases during BCR-induced cell death in B lymphoma acts as a
440 negative regulator of apoptosis. *FASEB J.* 17, 711-713
441 46 Giaime, E. *et al.* (2006) Caspase-3-derived C-terminal product of synphilin-1
442 displays antiapoptotic function via modulation of the p53-dependent cell death
443 pathway. *J. Biol. Chem.* 281, 11515-11522
444 47 Guery, L. *et al.* (2011) Fine-tuning nucleophosmin in macrophage
445 differentiation and activation. *Blood* 118, 4694-4704
446 48 Rincheval, V. *et al.* (1999) Inhibition of Bcl-2-dependent cell survival by a
447 caspase inhibitor: a possible new pathway for Bcl-2 to regulate cell death. *FEBS*
448 *Lett.* 460, 203-206
449 49 Paquette, N. *et al.* (2010) Caspase-mediated cleavage, IAP binding, and
450 ubiquitination: linking three mechanisms crucial for Drosophila NF-κB signaling.
451 *Mol. Cell* 37, 172-182
452 50 Yang, J.-Y. and Widmann, C. (2002) The RasGAP N-terminal fragment
453 generated by caspase cleavage protects cells in a Ras/PI3K/Akt-dependent
454 manner that does not rely on NFκB activation. *J. Biol. Chem.* 277, 14641-14646
455 51 Yang, J.-Y. *et al.* (2004) Partial cleavage of RasGAP by caspases is required for
456 cell survival in mild stress conditions. *Mol. Cell Biol.* 24, 10425-10436

457 52 Yang, J.-Y. *et al.* (2005) Impaired Akt activity down-modulation, caspase-3
458 activation, and apoptosis in cells expressing a caspase-resistant mutant of
459 RasGAP at position 157. *Mol. Biol. Cell* 16, 3511-3520
460 53 Yang, J.-Y. and Widmann, C. (2002) A subset of caspase substrates functions as
461 the Jekyll and Hyde of apoptosis. *Eur. Cytokine Netw.* 13, 387
462 54 Hetz, C. (2012) The unfolded protein response: controlling cell fate decisions
463 under ER stress and beyond. *Nat. Rev. Mol. Cell Biol.* 13, 89-102
464 55 Jager, R. *et al.* (2012) The unfolded protein response at the crossroads of
465 cellular life and death during endoplasmic reticulum stress. *Biol. Cell*
466 56 Puthalakath, H. *et al.* (2007) ER stress triggers apoptosis by activating BH3-
467 only protein Bim. *Cell* 129, 1337-1349
468 57 McCullough, K.D. *et al.* (2001) Gadd153 sensitizes cells to endoplasmic
469 reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state.
470 *Mol. Cell Biol.* 21, 1249-1259
471 58 Dai, C. *et al.* (2011) Differential effects on p53-mediated cell cycle arrest vs.
472 apoptosis by p90. *Proc. Natl. Acad. Sci. U. S. A.* 108, 18937-18942
473 59 Rinaldo, C. *et al.* (2007) MDM2-regulated degradation of HIPK2 prevents
474 p53Ser46 phosphorylation and DNA damage-induced apoptosis. *Mol. Cell* 25,
475 739-750
476 60 Kracikova, M. *et al.* (2013) A threshold mechanism mediates p53 cell fate
477 decision between growth arrest and apoptosis. *Cell Death. Differ.* 20, 576-588
478 61 Kerr, J.F. *et al.* (1972) Apoptosis: a basic biological phenomenon with wide-
479 ranging implications in tissue kinetics. *Br. J. Cancer* 26, 239-257
480 62 Taylor, R.C. *et al.* (2008) Apoptosis: controlled demolition at the cellular level.
481 *Nat Rev Mol Cell Biol* 9, 231-241

482 63 Fadok, V.A. and Henson, P.M. (1998) Apoptosis: Getting rid of the bodies. *Curr.*
483 *Biol.* 8, R693-R695
484 64 Ravichandran, K.S. (2010) Find-me and eat-me signals in apoptotic cell
485 clearance: progress and conundrums. *J. Exp. Med.* 207, 1807-1817
486 65 Pop, C. and Salvesen, G.S. (2009) Human caspases: activation, specificity, and
487 regulation. *J. Biol. Chem.* 284, 21777-21781
488 66 Budihardjo, I. *et al.* (1999) Biochemical pathways of caspase activation during
489 apoptosis. *Annu. Rev. Cell Dev. Biol.* 15, 269-290
490 67 Hers, I. *et al.* (2011) Akt signalling in health and disease. *Cell Signal.* 23, 1515-
491 1527
492 68 Fujita, E. *et al.* (1999) Akt phosphorylation site found in human caspase-9 is
493 absent in mouse caspase-9. *Biochem. Biophys. Res. Commun.* 264, 550-555
494 69 Bulat, N. *et al.* (2011) RasGAP-derived fragment N increases the resistance of
495 beta cells towards apoptosis in NOD mice and delays the progression from mild
496 to overt diabetes. *PLoS. ONE.* 6, e22609
497
498
499

Cells or animals expressing wild-type RasGAP

Mild stress
Low caspase-3 activity
site 1
site 2
RasGAP
Frag-ment C
site 2
Frag-ment N
Akt
Survival

Strong stress (apoptotic stimulus)
High caspase-3 activity
site 1
site 2
RasGAP
Frag-ment C
N2
N1
No Akt stimulation
Death

Cells or animals expressing a caspase-3 cleavage-resistant RasGAP mutant

Mild stress
Low caspase-3 activity
RasGAP
No Akt stimulation
High caspase-3 activity
Death

Strong stress (apoptotic stimulus)
High caspase-3 activity
RasGAP
No Akt stimulation
Death

Figure 1

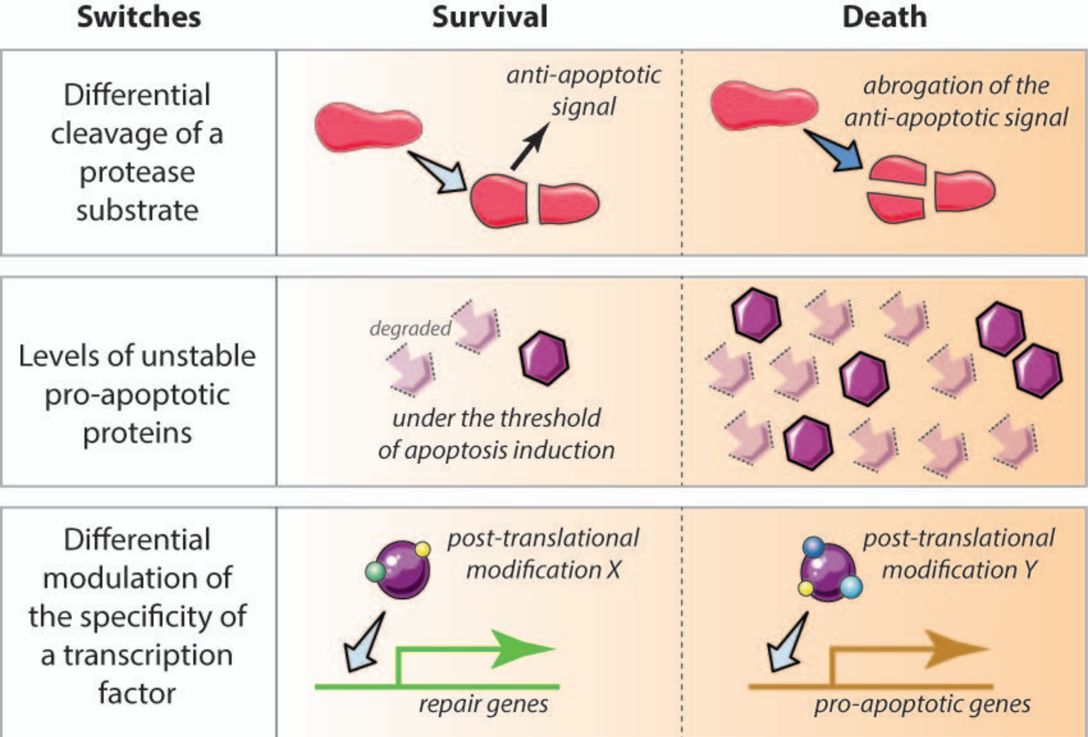

Figure 2