THE BIOCHEMISTRY OF NEURONAL NECROSIS: ROGUE BIOLOGY?

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When stressed beyond their tolerance, cells undergo necrosis, an acute, non-apoptotic form of cell death. Necrosis is crucial to the damage that injury and disease inflict on the nervous system. Recent discoveries have shed light onto the molecular requirements for necrosis, and provide new evidence that, as is the case for apoptosis, the mechanisms of necrotic cell death are conserved from nematodes to humans. But in contrast to apoptotic mechanisms, necrotic mechanisms did not evolve specifically to carry out necrosis. Instead, under extreme circumstances, normal cellular activities are destabilized with devastating consequences for the cell. Here, we review the mechanisms that are implicated in necrosis and discuss the events that transform them to catastrophic for cell survival.

ISCHAEMIA
The insufficient supply/flow of blood, usually due to a blocked vessel. Ischaemia in the brain can lead to a stroke. About 80% of all strokes are ischaemic. Most blockages in the cerebral blood vessels are due to a blood clot, often in an artery narrowed by plaque.

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Operationally, cell death can be categorized into appropriate and inappropriate, depending on whether it should happen by design or not. Appropriate cell death in most cases is programmed and takes the form of apoptosis[1–3]. The apoptotic program, which is hard-wired into the genetic material of every cell, is activated only in those cells destined to die at a given point in time. These doomed cells are destroyed through a series of orderly events that is carefully orchestrated by dozens of genes[4,5]. Far from being detrimental, appropriate cell death is essential for the correct development of organs and tissues. Failure to accurately undergo apoptosis can cause severe anomalies, ranging from developmental defects to cancer[6–8].

Inappropriate cell death on the other hand, is the unanticipated destruction of a cell that, under normal circumstances, was not destined to die. Inappropriate cell death can take many forms, displaying either necrotic or apoptotic characteristics, or a combination of both. For example, apoptosis, when implemented erroneously under certain stressful or otherwise abnormal conditions, results in cell death that can have detrimental consequences to tissues and organs[7,9–12].

The most notable form of inappropriate cell death is necrosis. As is the case for apoptosis, necrosis was originally described as an important type of cell death with distinct morphological characteristics[13,14]. Cells suffer necrotic death when exposed to extreme stress. Different cell types can withstand different degrees of stress, ranging from mild to severe. Although all cells possess elaborate homeostatic mechanisms that can buffer environmental fluctuations and maintain a stable internal milieu, the ability of the cell to maintain its integrity when challenged is not unlimited. Intense, adverse conditions that exceed the buffering capacity of the cell's protective systems will irreversibly compromise homeostatic mechanisms, extensively damage the cell and ultimately cause cell death. So, adverse environmental conditions such as lack of oxygen or essential nutrients (for example, in the case of ISCHAEMIA after stroke), elevated temperature, contact with toxic compounds and excessive mechanical strain (such as trauma[15,16]), all are potent necrosis initiators. Necrosis can also be triggered by abnormalities such as those that underlie many neurodegenerative disorders[17].

Despite the devastating impact of necrosis on human health, and contrary to the progress in deciphering the biochemistry of apoptosis, characterization of the molecular mechanisms that bring about necrotic cell death has proceeded at a relatively slow pace. There are two principal reasons for this slowness. First, unlike apoptosis, necrosis seemed to lack a well-defined core set of hallmark features tipping-off a robust underlying

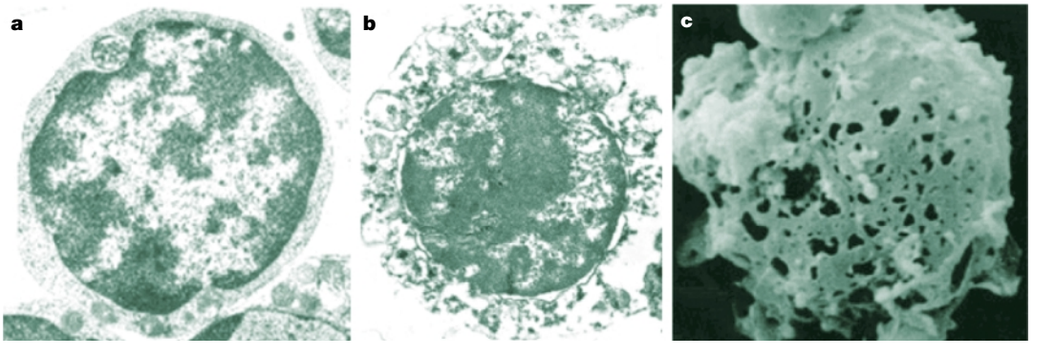


Figure 1 | **Necrotic cell death.** Electron micrographs of a normal cell (**a**) and a cell undergoing necrotic cell death (**b**). Extensive distortion of the cytoplasm and the plasma membrane is evident. The scanning electron micrograph shown in **c** illustrates the marked lesions that appear on the surface of the plasma membrane at late stages of necrotic cell death. Adapted, with permission, from REF. 181 © (1997) MMK Holdings Inc. in association with Purdue University Cytometry Laboratories.

programme[14,18]. Rather, necrosis was considered an inexorable, chaotic breakdown of cells under intolerable conditions[19]. Second, until relatively recently, simple and reliable animal models that faithfully reproduce aspects of necrosis were lacking. The use of such models greatly accelerated the discovery of molecular mechanisms and characterization of biochemical pathways of apoptosis[3,4,20,21]. These limiting factors are now being overcome, and several recent studies are beginning to transform our understanding of necrotic cell death. Many models of necrosis and neurodegeneration are now available in organisms ranging from the simple nematode worm *Caenorhabditis elegans*, to the fruitfly *Drosophila melanogaster* and the mouse[22–31]. Moreover, evidence is accumulating that necrotic cell death might not be as chaotic as initially thought, instead adhering to certain regular patterns[32,33].

Is there a core biochemical pathway that takes on the dismantlement of the cell during necrosis? To answer this question, we review the current state of the knowledge in the field of necrotic cell death in an effort to describe the molecular mechanisms that are involved in necrosis. We delineate the biochemical pathways that participate in cell death and the physiological parameters that affect this process. In addition, we discuss methodologies that are aimed at blocking or ameliorating necrotic cell death. Such approaches are not only potentially useful in attempts to counter neurodegeneration associated with human pathologies, but also provide valuable probes to further dissect necrotic cell death mechanisms.

The apoptosis–necrosis continuum

The morphological features of necrosis are markedly different from those of apoptosis and were first described in early studies of cell death[13,34]. Cells undergoing necrotic death do not show the characteristic macroscopic, ultrastructural and physiological hallmarks of apoptosis, such as nuclear compaction, chromatin condensation, internucleosomal cleavage of DNA, blebbing of the plasma membrane and disintegration of the cell into multiple vesicles[14,18]. Instead, necrosis is accompanied by mitochondrial swelling, dilatation of endoplasmic reticulum and extensive vacuolation of the cytoplasm (FIG. 1). There is no extensive plasma membrane blebbing, and cells swell and eventually lyse without the formation of vesicles[32]. As the cell is dying, the cytoplasm becomes ill defined — the chromatin pattern becomes coarse and clumpy, and this change is followed by loss of nuclear staining and KARYOLYSIS. Cellular contents are liberated into the intercellular space, often damaging neighbouring cells and inducing inflammatory responses[19]. By contrast, apoptosis typically inflicts minimal damage on the surrounding cells, and it is generally not accompanied by inflammation[18,35,36]; after gradual dismantling, cellular remains are assimilated by surrounding cells and tissues[37]. Unlike apoptotic cells, which are usually scattered throughout tissues, necrotic cells are commonly found in contiguous sheets[18].

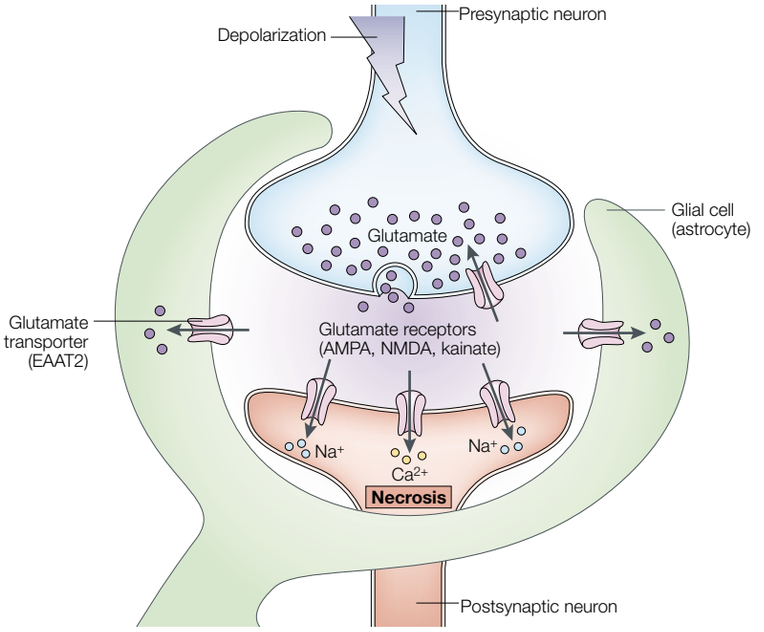
Whereas the distinction between necrosis and apoptosis is obvious in certain situations, in others — including some human pathologies such as stroke — the dividing line is less clear[15,38,39]. Detailed investigation of the molecular aspects of death mechanisms indicates that the initial distinction between apoptosis and necrosis was an over-simplification[32]. For example, alternative morphological death profiles such as PARAPTOSIS have been described, and certain dying cells show distinctive features of both apoptosis and necrosis[40,41]. Likewise, specific cellular markers of death are expressed by both apoptotic and necrotic cells[42]. Moreover, the same cells can undergo either necrotic or apoptotic cell death in response to different stimuli[43]. So, it is becoming progressively clear that cell fate is determined by the intensity of insult[44]. An additional factor is the temporal distribution of the death-initiating condition — acute insults generate responses that are different to those induced by similar insults that have been delivered to the cell over prolonged periods of time[45].

The emerging theme is that, instead of distinct types of cell death, there is a continuum of responses. These responses orchestrate the cellular destruction that is manifest in stereotypical macroscopic morphological patterns[32,38].

KARYOLYSIS
Disintegration of the nucleus.

PARAPTOSIS
An alternative form of cell death that does not seem to involve caspases. In addition, paraptosis induces changes in cellular morphology that are distinct from those generated by apoptosis and bear similarity with features of necrosis. For example, there is no prominent chromatin condensation, whereas there is extensive cytoplasmic vacuolation. Unlike necrosis, however, paraptosis requires *de novo* protein synthesis, similarly to apoptosis.

Box 1 | **Excitotoxic cell death**



Excitatory neurotransmitters such as glutamate are released from synapses on depolarization after the arrival of an action potential. The release process is carefully controlled and build-up of excessive neurotransmitter at the synapse is prevented by the action of dedicated transporters that clear the synaptic cleft. However, many deleterious conditions can converge to induce unrestrained glutamate release at synapses, initiating a cascade of events that leads to death of the postsynaptic cell[51]. For example, catastrophic depolarization occurs during hypoxia or hypoglycaemia, which compromise energy production and therefore the ability of the cell to maintain a membrane potential[168,169]. Overstimulation of neurons during seizure has the same effect on glutamate release[170,171].

Glutamate binds to and opens specific ionotropic receptor channels on postsynaptic neurons (AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NMDA, *N*-methyl-D-aspartate). Gating of these channels provokes an influx of calcium ions inside the cell either directly (through glutamate receptors that conduct both calcium and sodium) or indirectly (through the secondary activation of voltage-gated calcium channels[172]). The sharp increase of intracellular calcium concentration is a principal death-signalling event that is involved in both necrosis and apoptosis. The contribution of each type of death to excitotoxicity correlates with the severity and the abruptness of the increase in intracellular calcium concentration[82]. More profound changes initiate necrosis, whereas relatively mild increases preferentially induce apoptosis[15,57]. During stroke, the area immediately affected by restricted blood flow is usually the focal point of necrosis[44]. However, both necrotic and apoptotic cell death occur within the surrounding tissue, which suffers less from oxygen and nutrient deprivation[48]. EAAT2, excitatory amino acid transporter 2.

DEGENERINS
A group of proteins first described in *Caenorhabditis elegans*, which can mutate to cause neurodegeneration. Degenerins are ion channels with roles in sensory transduction and ionic homeostasis. Nematode proteins share sequence similarity with vertebrate epithelial sodium channels (ENaCs) and some *Drosophila* proteins such as Ripped Pocket (RPK) and Pickpocket (PPK).

Triggering necrosis

A wide range of factors can trigger necrotic cell death. Both extrinsic and intrinsic signals can initiate necrotic cell death; for example, hostile environmental conditions or mutated genes[18,46]. Different triggers impinge on different aspects of cellular physiology and provide valuable probes to investigate the capacity and tolerance of various cellular systems and the mechanisms that underlie cell demise.

Acute energy depletion is one of the most potent necrosis-triggering conditions in neurons[43,45]. Energy depletion can rapidly develop during ischaemic or hypoglycaemic episodes[44,47,48]. Without the energy necessary to sustain ionic gradients, the resting potential of neurons collapses. The ensuing depolarization results in release of a massive amount of the excitatory neurotransmitter glutamate at synaptic clefts[49,50]. Energy shortage also impairs re-uptake of glutamate by the high-affinity transporters of surrounding glial cells and neurons. Excessive build-up of glutamate at synapses induces hyperexcitation and, eventually, necrotic death of downstream synaptic target neurons, a phenomenon known as excitotoxicity[15,51] (BOX 1). A similar fatal outcome is observed during seizure episodes, when prolonged hyperexcitation of neurons initiates excitotoxic death[52,53].

Exposure of cells to toxic substances and harsh environments — as occurs during trauma — elicits necrosis. For example, exposure to strong detergents, acids and oxidants, extreme heat or cold, and excessive mechanical strain has detrimental effects on cell viability[18,54]. Excessive accumulation of reactive oxygen species (ROS), which are generated as by-products of normal and aberrant metabolic processes that use molecular oxygen, can trigger necrosis[55,56]. These factors normally inflict death by overwhelming regulatory and homeostatic mechanisms, or by compromising the structural integrity of the cell.

A diverse group of neurodegenerative disorders such as Alzheimer's, Huntington's and Parkinson's diseases, amyotrophic lateral sclerosis (ALS), spinocerebellar ataxias and transmissible spongiform encephalopathies have been associated with pathological necrotic cell death, in addition to apoptosis[16,17,57,58].

Necrotic cell death can be readily triggered in both *C. elegans* and *Drosophila*. Toxic mutations in several genes induce degeneration of specific types of neurons or other cells in the nematode. The most thoroughly characterized case involves deleterious, gain-of-function mutations in genes encoding specific ion channel proteins called DEGENERINS, such as DEG-1 and MEC-4 (REFS 25,59). Degenerins bear sequence similarity to mammalian epithelial sodium channels[60] (ENaCs). Cells that express the mutant genes undergo late-onset necrotic cell death, which is mechanistically and morphologically reminiscent of excitotoxic cell death in mammals[61] (BOX 1).

Progression of necrosis caused by mutant degenerins has been studied at the ultrastructural level in *C. elegans*[61]. Although degenerin mutations kill different groups of neurons depending on where the mutant gene is expressed, the morphological features of the cell death they induce are the same. First, the nucleus and body of the affected cell seem distorted. Then, the cell swells to several times its normal diameter, resembling the morphology of mammalian cells undergoing necrotic cell death. Under the electron microscope, cells dying as a consequence of mutant DEG-1 and MEC-4 expression show some notable features. The earliest detectable abnormality is the formation of small, tightly wrapped membrane whorls that seem to originate at the plasma membrane. These whorls are internalized and seem to coalesce into large, electron-dense membranous structures. Large internal vacuoles form, and distortion of the nucleus by these vacuoles is associated with chromatin clumping (FIG. 2). Cell volume can increase

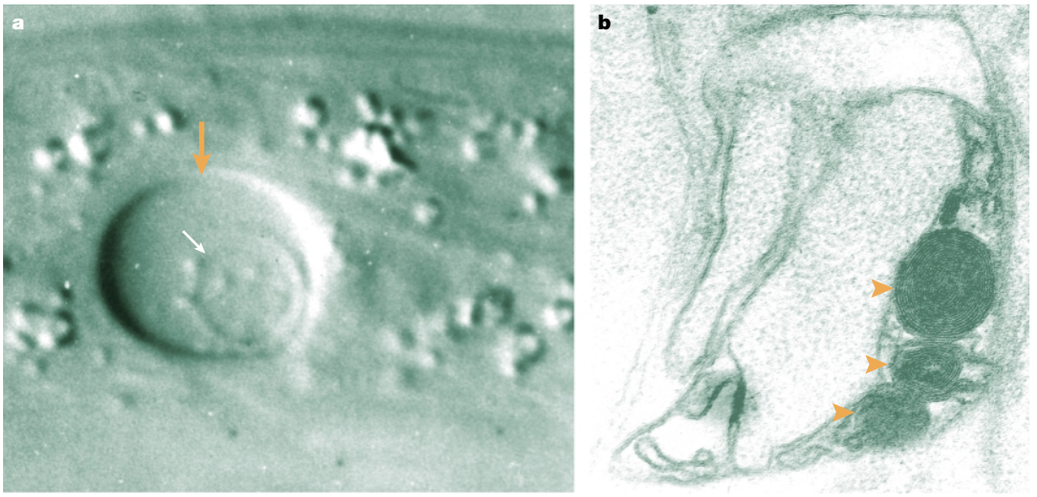


Figure 2 | **Neurodegenerative disease in *Caenorhabditis elegans*.** A nematode neuron undergoing necrosis as a result of degenerin ion channel hyperactivation. In panel **a**, the degenerating cell (orange arrow) seems swollen to several times its normal diameter, whereas the nucleus (white arrow) is distended and has a distorted morphology. Panel **b** shows the peculiar electron dense membranous circumvolutions (arrowheads) that accompany cellular destruction, under the electron microscope. Modified, with permission, from REF. 61 © (1997) Society for Neuroscience.

about 100-fold during this process. Last, organelles and cytoplasmic contents are degraded, usually leaving a membrane-enclosed shell. The prominent internalized membranous inclusions indicate that intracellular trafficking might contribute to degeneration. Interestingly, in some mammalian degenerative conditions, such as NEURONAL CEROID LIPOFUSCINOSIS (in, for example, the *MND* MOUSE), cells develop vacuoles and whorls that look similar to the internalized structures in dying *C. elegans* neurons[62,63]. Similar structures are observed in the *WOBBLER* MOUSE[30,64,65]. Furthermore, disrupted trafficking has been implicated in Alzheimer's disease[66–69], Huntington's disease[26,70] and ALS[71–73]. Together, these observations indicate that some degenerative processes might be similar in nematodes and mammals.

In addition to mutant degenerins, hyperactivating mutations in *deg-3*, the gene that encodes the α-subunit of the nicotinic acetylcholine receptor (nAChR), as well as mutations in a hyperactivated G-protein α-subunit, induce neurodegeneration in *C. elegans*[74–76]. Apart from these genetic insults, hypoxic conditions imposed either by shortage of oxygen or by chemical inhibitors of the respiratory chain (such as sodium azide) can induce cellular dysfunction and necrotic cell death in the nematode[77].

The availability of both genetic and environmental means that robustly elicit necrotic cell death in simple animal models provides the essential tools that are required to explore the molecular mechanisms of necrosis. Harnessing the exceptional experimental potential of these organisms has greatly facilitated the identification of crucial factors that modulate necrotic cell death[78,79].

Modulators and mechanisms of necrosis

Numerous investigations of necrosis caused by various conditions in a plethora of organisms converge to highlight a limited array of factors and biochemical mechanisms that mostly influence or mediate necrosis. Comprehensive genetic screenings for suppressors and enhancers of necrosis in *C. elegans* have revealed key players in the process of cell death (REF. 78 and N.T., P.S. and C. Samara, unpublished observations). Such studies show the importance of a limited set of common execution mechanisms during necrosis[33,79]. This is an exciting realization, indicating that in most cases, necrosis follows relatively specific patterns and involves common pathways. In the following sections, we review the contributions of important physiological parameters and factors to the manifestation of necrosis, and delineate relevant biochemical processes.

Ion homeostasis. Intracellular and extracellular ion homeostasis has been implicated in many cases of necrotic cell death in a range of organisms, from nematodes to mammals[78,80–82]. Examples include neurodegeneration in *C. elegans*, the morphologically related excitotoxic cell death in mammals, and several human neurodegenerative disorders. We outline the evidence for the involvement of ion homeostasis in each case, and highlight commonalities that point to conserved and pervasive basic mechanisms of ion regulation.

In *C. elegans*, gain-of-function mutations in degenerin genes such as *deg-1* and *mec-4* induce necrotic cell death of the specific neurons in which the mutant genes are expressed[83]. The time of onset of degenerative death correlates with the initiation of degenerin gene expression, and the rapidity of death progression correlates with the dose of the toxic allele[61]. The deleterious, gain-of-function mutations that confer neurodegeneration *in vivo* also stimulate sodium ion influx through the degenerin channel in ectopic expression studies[84,85]. Furthermore, homologous, neuronally expressed mammalian proteins engineered to encode amino-acid substitutions analogous to those of toxic degenerins, induce degeneration when expressed in *Xenopus* oocytes and in embryonic hamster

NEURONAL CEROID LIPOFUSCINOSIS
Condition caused by lack of the enzyme palmitoyl-protein thioesterase, which is involved in the catabolism of lipid-modified proteins. The absence of this protein is thought to be responsible for the disease by allowing a waste product (ceroid lipofuscin) to accumulate in neurons.

MND MOUSE
Motor neuron degeneration mouse. A naturally occurring mutant mouse that shows abnormalities similar to those of the human neuronal ceroid lipofuscinosis.

WOBBLER MOUSE
The *wobbler* mutation causes muscle weakness due to motor neuron degeneration and a defect in spermatogenesis. The *wobbler* mouse is used as an animal model for human spinal muscular atrophies.

kidney cells[86]. Degenerin-induced cell death in these systems is reminiscent of vertebrate excitotoxic cell death. So, vertebrates and *C. elegans* share a death mechanism that involves hyperactivation of ion channels. These observations are consistent with the hypothesis that breaching a threshold level of ion influx is needed to initiate the degenerative process. Early work established that specific mutations in the *mec-6* gene are general suppressors of degenerin-induced cell death in the nematode[25,83,87]. The *mec-6* gene encodes a membrane-spanning protein with limited similarity to PARAOXONASES. The biochemical role of the MEC-6 protein is not clear, but MEC-6 physically interacts with MEC-4 and is thought to be specifically required for operation of the degenerin channel[88].

Mutation in genes other than degenerin can perturb ion homeostasis and induce the degeneration of various types of *C. elegans* neurons. The nAChR subunit DEG-3 was originally identified by a dominant allele, *deg-3(u662)*, which causes neuronal degeneration of selected nematode neurons[74]. This degeneration is partially suppressed by loss-of-function mutations in the *des-2* gene. This gene also encodes a nAChR subunit, and is necessary for DEG-3-dependent channel activity *in vivo*[89]. Pharmacological analysis of DEG-3/DES-2-receptors expressed in *Xenopus* oocytes showed that this receptor is highly calcium-permeable. The degeneration-causing mutant DEG-3 is much more toxic to oocytes than the wild-type channel, indicating that necrosis is triggered by excess calcium influx through a constitutively activated channel[90].

Neurodegeneration in *C. elegans* can also be initiated by gain-of-function mutations in the *gsa-1* gene, which encodes a heterotrimeric G-protein subunit $G\alpha_s$. The *gsa-1* gene is essential and is ubiquitously expressed in the nervous system and muscle cells of *C. elegans*. A conditionally active mutant $G\alpha_s$ protein, bearing a mutation that inhibits GTPase activity and locks the protein in the GTP-bound active conformation, induces swelling of muscles of the body wall and vacuolization of a specific subset of neurons (specifically, the ventral nerve cord motor neurons, and some neurons in the head and tail ganglia of the nematode[75]). Similarly, a rat DNA encoding the homologue $G\alpha_s$ subunit and harbouring the same mutation causes neurodegeneration when expressed in specific *C. elegans* neurons. $G\alpha_s$-induced neurotoxicity is distinct from apoptosis and similar in morphology to the neuronal degeneration that is observed in degenerin mutants[76].

Screens for extragenetic suppressors of activated $G\alpha_s$-induced neurodegeneration in *C. elegans* have identified mutations in the *acy-1/sgs-1* gene, which encodes an adenylyl cyclase[76,91]. The *acy-1/sgs-1* gene is expressed in virtually all neurons and body muscles, but loss-of-function mutations do not result in severe developmental or behavioural phenotypes. This indicates that *acy-1/sgs-1* has a subtle, but non-redundant, role in the nervous system. Adenylyl cyclases are a family of signalling molecules that generate cyclic AMP (cAMP). In mammals, all adenylyl cyclase isoforms are stimulated by $G\alpha_s$. Given the morphological similarity between activated $G\alpha_s$-induced neurotoxicity in *C. elegans* and degenerin-induced cell death, the finding that mutations in *acy-1/sgs-1* suppress this type of cell death indicates that $G\alpha_s$-induced neurodegeneration in this organism is mediated by changes in intracellular cAMP through activation of ion channels and consequent alterations of ion homeostasis. Increased levels of cAMP can either directly modulate cAMP-gated ion channels or can modulate ion channels through activation of a cAMP-dependent protein kinase A (PKA)[76].

Alterations of cellular ionic homeostasis contribute to necrotic neuronal death owing to excitotoxicity after ischaemic events. Excessive calcium influx through several channel- and transporter-mediated routes leads to intracellular calcium overload[92] (FIG. 3). Sodium influx amplifies acute neuronal swelling and facilitates calcium entry through voltage-gated channels and the Na^+/Ca^{2+} exchanger[82]. Cell injury and death can also be induced by disturbances of calcium homeostasis in the endoplasmic reticulum (ER)[68,81,93] — the main compartment for calcium storage in the cell. Sequestration of calcium into the ER is mediated by the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA), and release back to the cytoplasm is controlled by ryanodine (RyR), and inositol-1,4,5-trisphosphate receptors ($Ins(1,4,5)P_3R$)[81,94]. Within the ER, calcium binds to molecular chaperones such as calreticulin and calnexin[95,96]. Under conditions of extreme cellular stress, ER calcium stores are rapidly mobilized, boosting the massive increase of intracellular calcium concentration and signalling cell demise[46].

Oxidative stress, which develops as a consequence of exposure to ROS, induces rapid increases in intracellular Ca^{2+} levels by stimulating Ca^{2+} influx from the extracellular environment and efflux from intracellular stores, leading probably to calpain activation[97,98]. There is mounting evidence for a potential role of ROS in acute neurological events, such as ischaemia, and chronic neurodegenerative disease, such as Alzheimer's disease, Parkinson's disease and ALS[99,100]. Aggregates containing the enzyme superoxide dismutase (SOD) are found in motor neurons of ALS patients[101]. Interestingly, heterologous expression of five human SOD alleles that are associated with familial ALS in a *Drosophila Sod*-null background resulted in increased oxidative stress, accompanied by physiological impairment of abrupt onset and decreased life span of the mutant fly[102]. Pharmacological treatments or mutations that inhibit calcium release from the ER have a strong protective effect against necrotic cell death[78,103]. By contrast, treatment with chemicals such as thapsigargin, which promotes the discharge of calcium from intracellular stores by specifically inhibiting SERCA, induces necrotic cell death[78,104].

Accumulation of unfolded proteins in the ER, provoked by alterations in ER calcium homeostasis, can elicit ER stress responses by increasing transcription of genes encoding ER-resident chaperones (such as GRP78/Bip) to facilitate protein folding and protect cells. This system is termed the unfolded-protein response[105,106]. However, prolonged ER stress leads to cell death and is linked to the pathogenesis of some neurodegenerative disorders[107].

PARAOXONASE
A serum protein that is bound to high-density lipoproteins (HDLs), made in the liver and delivered to the bloodstream. The physiological function of paroxonase is unknown, but a role in lipid metabolism has been postulated.

SERCA
Sarco-endoplasmic reticulum calcium ATPase. A pump that sequesters calcium to the endoplasmic reticulum at the expense of ATP. SERCA, which comprises one of the main mechanisms for maintaining calcium homeostasis in the cytoplasm, is inhibited by the drug thapsigargin, which is extracted from the seeds of the plant *Thapsia garganica*.

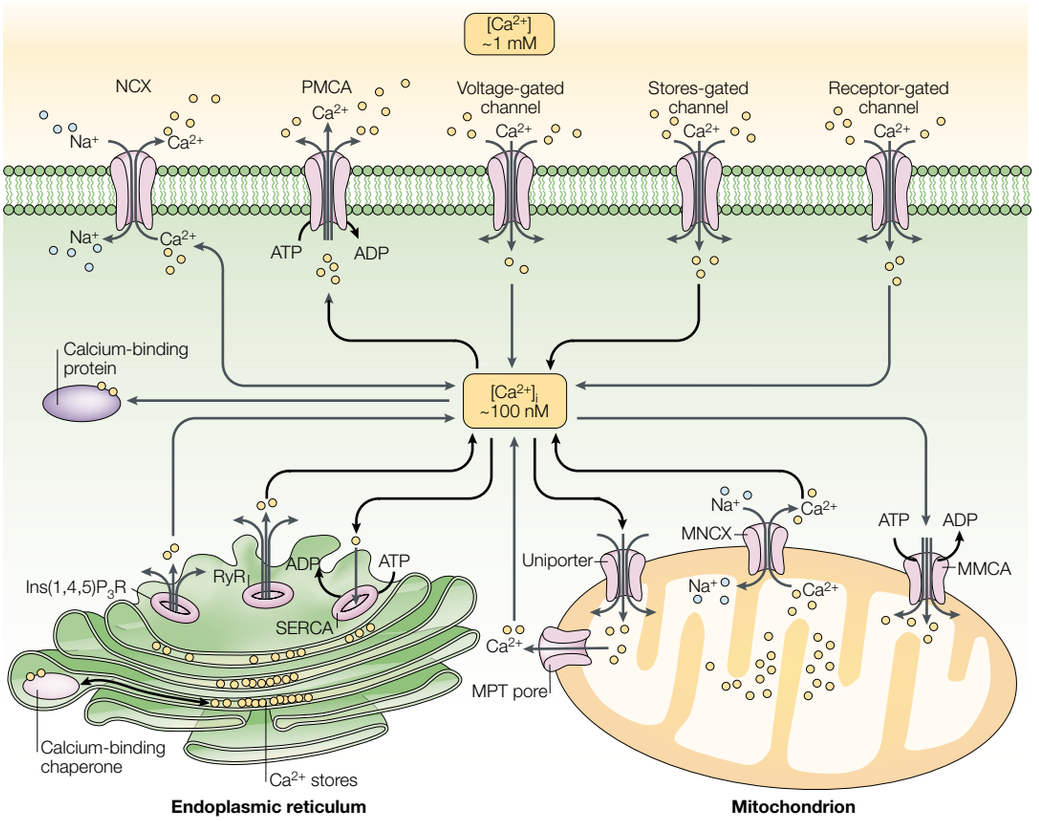


Figure 3 | **Calcium homeostasis mechanisms.** Intracellular calcium concentration is tightly regulated within narrow limits. Under pathological conditions however, regulatory mechanisms are overwhelmed and intracellular calcium concentration ($[Ca^{2+}]$) increases through calcium influx from extracellular pools through various channels (voltage-, ligand- or concentration-gated channels) and, under extreme circumstances, through the sodium/calcium exchanger (NCX). Under normal conditions, NCX is the main pathway for calcium efflux, but it can also contribute to Ca^{2+} influx (reverse mode exchange) especially during strong depolarization, and with increased intracellular sodium[182]. Calcium concentration can also increase through release from endoplasmic reticulum stores, through the ryanodine (RyR), and inositol-1,4,5-trisphosphate receptors ($Ins(1,4,5)P_3R$). Counterbalancing mechanisms fight to halt calcium concentration increase in the cytoplasm. The plasma membrane calcium pump (PMCA), NCX and sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) function to restore normal calcium levels. Increased intracellular calcium concentration drives calcium overload at mitochondria, through PMCA, and relaxed specificity channels (Uniporter). In turn, calcium overload triggers secondary release of calcium from mitochondrial stores, through the mitochondrial NCX (MNCX) and mitochondrial pores opened during MITOCHONDRIAL PERMEABILITY TRANSITION (MPT)[136,183–185]. Calcium-binding proteins in the cytoplasm and in the endoplasmic reticulum offer additional calcium buffering capacity. MMCA, mitochondrial membrane Ca^{2+} ATPase.

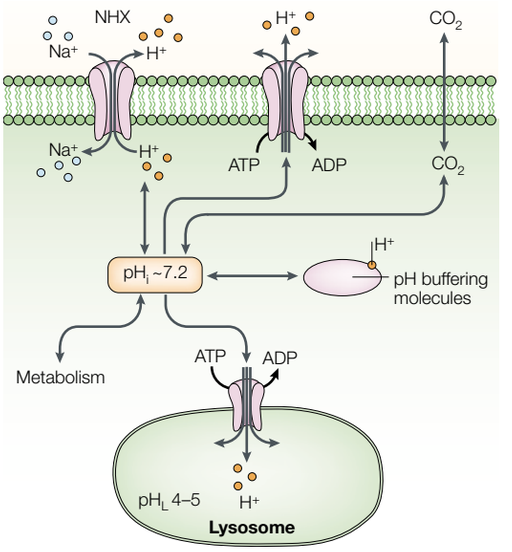
MITOCHONDRIAL PERMEABILITY TRANSITION (MPT). A non-specific increase in the permeability of the inner mitochondrial membrane that occurs when matrix calcium is greatly increased, especially under oxidative stress and adenine nucleotide depletion. MPT is associated with the opening of a non-specific pore in the mitochondrial inner membrane, which transports molecules that are smaller than 1,500 Daltons.

HYPERCAPNEA
A state of increased partial pressure of CO_2 in the blood. Hypercapnea is usually accompanied by a decrease of oxygen in the bloodstream.

Two other ions that are involved in necrosis are magnesium and zinc. Magnesium entry through the NMDA (*N*-methyl-D-aspartate)-receptor channel and the drop in intracellular pH that follows NMDA-receptor-mediated calcium influx exacerbate necrotic neuronal death[108–110]. Zinc, which serves in the central nervous system as a neurotransmitter and neuromodulator, plays an important part in determining the mode of death during excitotoxic attacks. Similarly to glutamate, cataclysmic release of zinc into the extracellular space in certain disease states might be responsible for neuronal death[111]. Excessive subsequent influx of zinc through voltage-gated calcium channels, NMDA-receptor channels and calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors can induce either apoptosis or necrosis, depending on the intensity of exposure (with more intense exposure causing necrosis, and less intense causing apoptosis)[112,113].

Intracellular and extracellular pH have a profound impact on the initiation and progression of necrosis[38,44,114]. An elaborate array of homeostatic mechanisms maintains intracellular and organelle pH within narrow physiological limits (BOX 2). During the development of pathological conditions such as ischaemia and trauma, the intracellular pH of brain transiently acidifies to 6.2–6.8 (from 6.8–7.6 under physiological conditions)[54,115]. Ischaemia is associated with both hypoxia and acidosis owing to increased glycolysis, production of lactic acid and decreased intracellular pH, but the role of hypoxia in ischaemia-mediated cell death is unclear. *In vivo*, increased lactate acidosis is associated with increased ischaemic injury, providing additional evidence that acidosis contributes to neurotoxicity during stroke and trauma. The hyperglycaemia and HYPERCAPNEA that precede ischaemia also result in severe acidosis and neuronal loss[116,117]. Early effects of acidosis include depression of

Box 2 | **Cellular pH homeostasis**



Tight regulation of intracellular pH and subcellular organelle proton concentration is paramount for the normal function and survival of the cell. For example, fibroblasts in cultures go into cell quiescence (G0 phase of cell cycle) with a pH change as little as 0.2 units[173]. Under such conditions, gene transcription stops, DNA synthesis ceases, rates of metabolism and protein synthesis decrease, and the cell does not grow or divide until the pH is brought back to normal levels. Given that most molecules cannot function outside a certain pH range, cells have a battery of specialized homeostatic mechanisms to keep their internal pH (pH_i) at a constant level (usually around 7.2, in the cytoplasm). These mechanisms can be divided into active, which require the use of ATP, and passive, which do not require ATP.

Cells passively regulate their pH with an overabundance of buffer molecules[174]. Buffers sequester excess protons under conditions of low pH, and release protons if the cytosolic pH becomes too high. The most important and abundant cytosolic buffer is phosphoric acid (H_3PO_4), which has a pKa of 7.2, helping to maintain a pH of 7.2. Amino acids are also important pH buffers. Lysine, arginine, and histidine have basic side chains with amino groups, and aspartate and glutamate have acidic carboxyl groups on their side chains. Free amino acids have additional amino and carboxyl groups, which can add to their buffering capabilities. Normal cellular metabolism also creates buffer molecules. These include acetic, lactic and citric acids, and the production of carbon dioxide (CO_2). Passive pH regulatory mechanisms are augmented by energy-dependent processes that maintain cytoplasmic and organelle pH at its optimum value. Many ion exchangers regulate intracellular pH, by letting one charged ion into the cell while releasing another charged ion. These 'antiporters' draw energy from electrochemical gradients across the plasma or organelle membranes to drive the electro-neutral exchange of protons for Na^+ or K^+ ions[175]. Dedicated pumps such as the vacuolar H^+ ATPase also regulate cellular pH at the expense of ATP by forcing protons out of the cytoplasm to the extracellular space or into subcellular organelles[176]. The vacuolar ATPase acidifies lysosomes, generating a low pH that is necessary for the optimum activity of lysosomal hydrolytic enzymes such as cathepsin proteases. NHX, sodium/proton exchanger; pH_L, lysosomal pH.

neuronal activity, cell swelling and enhanced production of free radicals. Unless acidosis is severe, these effects are reversible.

Two additional factors that influence intracellular pH are DNA damage and the insulin-like growth factor (IGF) signalling pathway. DNA damage rapidly activates the nuclear enzyme poly(ADP-ribose) polymerase (PARP), which forms polymers of ADP-ribose from the substrate NAD, releasing protons as a by-product of the reaction[118]. *In vitro*, the PARP activation reaction causes cellular acidification and consequent necrotic cell death after extensive DNA damage[48].

Activation of the phosphatidylinositol 3-kinase (PI3K) by insulin, which in turn activates protein kinase C (PKC), stimulates the Na^+/H^+ exchanger NHE1 in human erythrocytes, promoting acidosis[119]. Interestingly, mutations in the *daf-2* gene, which encodes an insulin/IGF receptor homologue, prevent hypoxia-induced cell death in *C. elegans*[77]. This indicates that acidosis might also be an important component of hypoxic death in the nematode. However, mutant animals carrying mutations in the PI3K gene, *age-1*, are only mildly resistant to hypoxia[77].

Protein-degradation mechanisms. One of the most distinctive features of apoptosis is the involvement of executioner caspases. Necrosis seems to also be heavily dependent on proteolytic systems[79,120]. So, lysosomal and cytoplasmic proteases, including caspases, have been implicated in the execution of necrotic cell death (FIG. 4).

The lysosomal system is a key player during the final stages of cellular destruction. Lysosomes contain a plethora of hydrolytic enzymes, including non-specific proteases. Erroneous delivery of cellular contents to the lysosome, or spillage of hydrolytic enzymes from lysosomes into the cytoplasm can induce necrosis. These lysosomal mechanisms, which are similar to what is found in AUTOPHAGY, have been implicated in necrotic cell death after ischaemic injury of both heart and brain[121]. Two classes of lysosomal proteolytic enzymes seem to be the most active in the process — aspartyl (cathepsin D) and cysteine (cathepsin B, H and L) proteases[122]. Aspartyl proteases are characterized by the presence of a catalytic aspartic acid residue at their active site, and normally mediate the intracellular and extracellular degradation of proteins. This includes digestion of food and processing of peptide hormones, antigens and immunoglobulins. Several studies indicate that cathepsin D and cathepsin E (an aspartyl protease) mediate the execution of neuronal death induced by ageing, transient forebrain ischaemia and excessive stimulation of glutamate receptors during excitotoxicity[123]. Increased cathepsin D expression and immunoreactivity have been observed in the brain of rats treated with kainate, particularly in regions that

AUTOPHAGY
A catabolic process by which cells degrade and digest their own cytoplasmic constituents, usually through the action of lysosomal enzymes. One of the most distinguishing features of autophagy is the dynamic rearrangement of cellular membrane to sequester cytosol and organelles into autophagosomes for delivery to the lysosome or vacuole. Autophagy is crucial for cell maintenance and development, and has also been linked to a growing number of human diseases, including neurodegenerative conditions, cardiovascular disease and breast cancer.

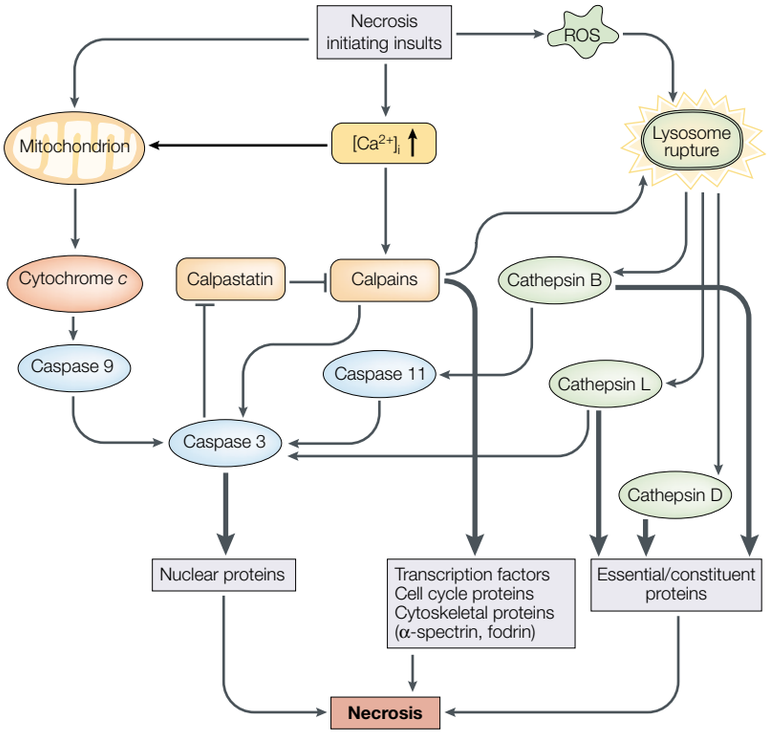


Figure 4 | **Proteases effecting necrosis.** Necrotic insults, either directly or indirectly, trigger the activation of proteolytic activities that participate in dismantling the cell. Calcium-activated calpain proteases together with lysosomal cathepsins liberated in the cytoplasm and caspases contribute to necrosis. Destructive proteolytic events are shown with bold arrows. ROS, reactive oxygen species.

showed features of neurodegeneration[124]. At the cellular level, increased cathepsin-D immunoreactivity was found in both neuronal and glial cells. In *C. elegans*, conditions that decrease cathepsin-D activity protect against neurodegeneration inflicted by various insults, including hyperactivated degenerins, the nAChR subunit DEG-3 and the G-protein subunit $G\alpha_s$. Two specific cathepsins that act synergistically, ASP-3 and ASP-4, seem to mediate most necrotic cell death[79].

Lysosomal cathepsins B and L have been implicated in delayed neuronal cell death after global and focal cerebral ischaemia. For example, increased amount and activity of cathepsin B have been reported in hippocampal neurons after global ischaemia. Specific inhibitors of cathepsins B and L effectively reduce ischaemic cerebral damage in such situations[125].

Lysosomal changes correlate spatiotemporally with ageing and age-associated neurodegenerative pathologies. The endosome–lysosome system in the rat brain begins changing early in adulthood, as indicated by increased numbers of lipofuscin-positive lysosomes and disturbances of lysosomal chemistry. Immunocytochemical analysis in rat brain revealed that non-lysosomal cathepsin E was barely detectable in embryonic tissues, in contrast to the relatively high levels of lysosomal cathepsin D. However, after birth, both forms of cathepsin were expressed in brain tissues at increasing levels with age. Experimentally induced lysosomal disturbances trigger the production of characteristic features of the aged human brain[126]. Ageing and experimentally induced lysosomal dysfunction are accompanied by a gradual leakage of cathepsin D into the cytoplasm of cultured hippocampal slices, and generation of TAU fragments with microtubule-binding capacity that could potentially interfere with normal tau–tubulin interactions[127]. Furthermore, certain genetically determined lysosomal dysfunctions result in conditions that are remarkably similar to those associated with pathological ageing, such as the hyperphosphorylated tau and NEUROFIBRILLARY TANGLES found in subjects with type C NIEMANN–PICK DISEASE[128,129]. Lysosome numbers and the concentration of cathepsin D increase in neurons that are vulnerable to Alzheimer's disease before the onset of pathology[123,130,131]. Cathepsin D has also been proposed to function as γ-secretase, converting the amyloid precursor protein (APP) into β-amyloid[132].

Studies of age-related changes in the subcellular localization of cathepsin D and the morphological features of cathepsin-immunoreactive neurons in rat cerebral cortex indicate that leakage of cathepsin D into the cytoplasm in old rats is closely associated with neurodegeneration. In cerebral cortical neurons of young rats, cathepsin D was observed mainly in lysosomes, whereas in aged cerebral cortex cathepsin D was prominently localized in the cytosol as diffuse granules[133].

What causes spillage of destructive cathepsins and other hydrolytic enzymes from lysosomes? Two mechanisms have been proposed. First, the lysosomal membrane becomes damaged by free radicals generated under conditions of extreme oxidative stress. Second, injury to the lysosomal membrane is inflicted enzymatically by the action of specific hydrolases. The 'calpain–cathepsin' hypothesis, whereby lysosome damage or rupture is mediated by activation of calpain proteases, is an attractive mechanism to account for this injury (BOX 3). In primates, calpains rapidly localize to lysosomal membranes after the onset of ischaemic episodes[122]. In *C. elegans*, two specific calpains — TRA-3 and CLP-1 — that function upstream of cathepsins ASP-3 and ASP-4 are required for neurodegeneration by various necrosis initiators[79] (FIG. 5). In addition to freeing hazardous enzymes, calpains facilitate cell destruction by other mechanisms. On activation by elevated levels of intracellular calcium, calpains cleave FODRIN, causing the collapse of the cytoskeleton[134]. Fodrin hydrolysis accompanies hypoxia and acidosis, two conditions that trigger necrosis through perturbation of calcium homeostasis. Inhibition of ion pumps such as the Na^+/H^+ exchanger, the Na^+/Ca^{2+} exchanger and the ER Ca^{2+}-ATPase (which facilitate increases of cytoplasmic calcium concentration either directly or indirectly) reduces fodrin proteolysis and cell death[135]. Inhibitors of PI3K, an enzyme that promotes metabolic acidosis, also have a neuroprotective effect.

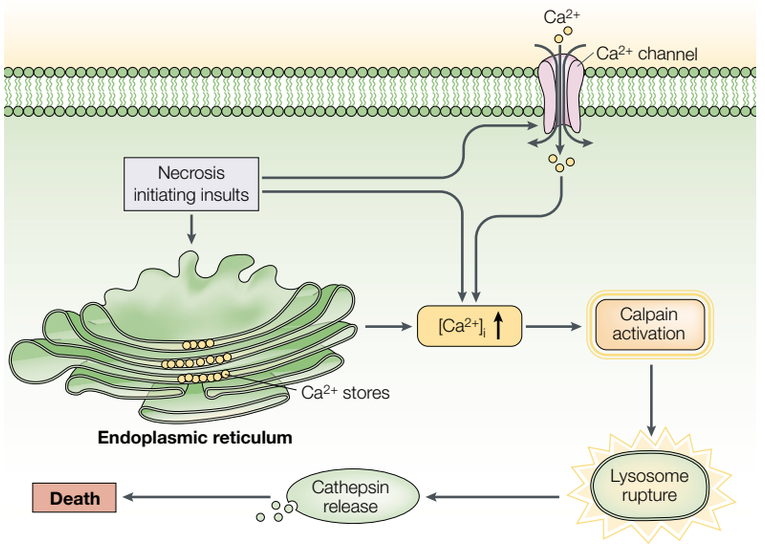
Paradoxically, caspase-mediated proteolysis also seems to have a role in necrosis. In addition to activating calpain proteases, excess intracellular calcium elicits mitochondrial damage and release of cytochrome *c*, activating caspases[112,136]. Caspases might also become

TAU
A neuronal protein that binds to microtubules, promoting their assembly and stability.

NEUROFIBRILLARY TANGLES
Large filamentous tau aggregates within neurons, usually prominent in the cerebral cortex, and hippocampus. Neurofibrillary tangles are common in the brains of patients with Alzheimer's disease.

NIEMANN–PICK DISEASE
A recessive metabolic disorder of lysosomal storage that results in a build-up of sphingomyelin and cholesterol.

Box 3 | **The calpain–cathepsin hypothesis**



This hypothesis was formulated on the basis of observations in mammalian systems[122,177] and encompasses two central players as key mediators of cellular destruction during necrosis: calpains and cathepsins. Calpains become activated when calcium concentration is elevated. Increases in intracellular calcium concentration occur either directly or indirectly in response to many diverse necrosis-initiating stimuli, and have been implicated as principal death-inducing signals in various organisms. Cathepsin proteases are liberated in the cytoplasm after activated calpains compromise the integrity of lysosomal membranes. Lysosomes contain over 80 types of hydrolytic enzymes, including cathepsins. Although, the mechanism of calpain-mediated rupture of lysosomes is unclear, spilling of hydrolytic enzymes from lysosomes into the cytoplasm owing to injury or rupture of lysosomal membranes has been implicated in necrotic cell death after ischaemic injury to both heart and brain[178]. In the cytoplasm, these enzymes degrade cellular structures and interfere with normal metabolism — death is unavoidable. This process is reminiscent of autophagy, and supports de Duve's original categorization of lysosomes as the cell's 'suicide bag'[179]. The mechanism by which overactivation of autophagy causes cell demise is not clear. A probable scenario is that cell death is triggered by severe energy depletion following destruction of mitochondria[121,180]. Genes that encode proteins involved in cellular calcium homeostasis, as well as genes for lysosomal and calpain proteases, have been detected in genetic screens as suppressors of neurodegeneration in *C. elegans*, a result that is consistent with observations in cultured mammalian neurons[78,79].

activated by calpain proteases[122] or might indirectly activate calpains by mediating degradation of calpastatin, an endogenous inhibitor of calpain[137] (FIG. 4). Interestingly, calpastatin is degraded in rat brain cells after ischaemia[138]. Furthermore, cathepsins activate caspases, directly and indirectly[46]. Recent findings indicate that the plasma-membrane calcium pump in neurons is a substrate for caspase-mediated cleavage and inactivation[139]. This, in turn, disrupts intracellular calcium homeostasis, resulting in calcium overload and ultimately necrotic cell death. Expression of non-cleavable mutant forms of this calcium pump markedly delays necrosis, as do caspase inhibitors during brain ischaemia.

Regardless of the mechanism, caspase activation during necrotic death is probably an event that is isolated from the rest of the apoptotic cascade and is, therefore, qualitatively different. Caspases are inappropriately activated and contribute to necrotic death just as any other deregulated cellular process does, without conferring any discernible apoptotic morphology.

Interfering with necrosis

The traditional view has been that, once necrotic cell death is triggered, it is inevitable — escaping death would only be possible by timely removal or neutralization of the initiating stimulus[32,140]. However, necrosis-initiating conditions are by definition totally unpredictable. Consequently, the challenge of escaping death without removing the initial trigger becomes a vital component of strategies to generally defend against necrosis. The development of broad-spectrum intervention strategies aimed at fortifying cells against insults that would otherwise be lethal is particularly important, considering that necrotic cell death represents a significant problem in human health[44]. Apart from many neurodegenerative disorders that have a necrotic component, the neuronal cell death that accompanies the oxygen deprivation after stroke is a principal contributor to death and disability. Although there are extreme insults that will unavoidably lead to cell death (such as those experienced during severe trauma), most necrotic damage is caused by relatively mild, chronic offences such as slowly progressing neurodegenerative disorders and limited occlusion of blood vessels. In such cases, it is conceivable that cells could be reinforced through genetic engineering or pharmacological treatments to withstand degenerative conditions[115]. Recent research on the common mechanisms of necrosis inflicted by unrelated initiators indicates that such a goal is within reach[33]. The molecules that enact these common biochemical events constitute global effectors of necrosis and make excellent therapeutic targets.

Intracellular calcium is recognized as a central effector of necrosis. Preventing increases of intracellular calcium concentration after excitotoxicity by augmenting compensatory mechanisms has been shown to reduce death due to hyperexcitation. For example, stimulation of outwardly rectifying potassium channels or overexpression of components of the glutamate-gated chloride channel, two molecules that oppose membrane depolarization, prevent neurotoxicity[115,141]. Necrotic pathways initiated by elevated calcium concentration in the cytoplasm can also be blocked at specific points to prevent or delay death. For example, controlling cytoplasmic calcium levels with specific calcium chelators has neuroprotective effects in both *C. elegans* and mammalian neurons[78,142]. Similarly, overexpression of the calcium-sequestering protein calbindin D28K protects cultured cells and neurons from necrotic insults[143]. Dantrolene, a potent ryanodine receptor antagonist, also ameliorates necrosis by reducing calcium efflux from the endoplasmic reticulum to the cytoplasm and is neuroprotective in models of epileptic seizures[103]. Inhibitors of calcium-activated calpain proteases favour survival in several models of necrosis, as do inhibitors of lysosomal catabolic enzymes such as CA-074, E64c and pyridoxal (vitamin B6), which inhibit thiol-proteases (cathepsins B and L,

FODRIN
A non-erythroid cell, spectrin like protein. Fodrin forms a two-dimensional mesh beneath the plasma membrane and seems to be involved in stabilizing membrane structures, maintaining cell shape and linking the cytoskeleton to plasma membrane or intracellular vesicles. In particular, fodrin mediates the association of actin filaments with the plasma membrane and is a well-known substrate for calpain.

Figure 5 | **Deadly proteolytic cascades in the nematode.** Execution of necrotic cell death in the nematode requires the activity of both calpain and cathepsin proteases. Two specific calpain proteases TRA-3 and CLP-1 function redundantly upstream of aspartyl proteases ASP-3 and ASP-4 to mediated necrotic death[79]. Such an arrangement is consistent with a function of calpains in the activation of non-specific acidic proteases such as cathepsin D, in accordance with the calpain–cathepsin hypothesis[122].

calpains)[144–146]. Mutations that reduce or abolish aspartyl protease activity have a similar effect[79].

Studies of the mechanisms of excitotoxic cell death during ischaemic episodes and seizures have provided several possible points of intervention. Energy depletion due to shortage of essential nutrients is one immediate consequence of reduced or diminished blood flow, and leads to uncontrolled release of glutamate[49]. Overexpression of the Glut1 glucose transporter has been shown to protect neurons from excitotoxic insults, perhaps by increasing the capacity for glucose absorption under conditions of glucose deprivation[115]. Clearing synapses of excess glutamate by enhancing its uptake through overexpression of synaptic glutamate transporters could provide additional protection. However, it is possible that this strategy could have adverse effects — the direction of transport can be reversed owing to collapse of the ion gradient, increasing glutamate extrusion and further aggravating the insult[50,115].

Although considerable progress has been made in combating specific instances of necrotic cell death, more general strategies might not be straightforward, given the complex interplay of cell death mechanisms in chronic neurodegenerative diseases. However, the effectiveness of the approaches described above to interfere with necrosis emphasizes the importance of understanding the intricacies of this death process for the development of successful intervention methodologies in humans.

Conclusions and perspectives

Studies in recent years have contributed to provide a more coherent and detailed picture of the mechanisms that are involved in necrosis. In spite of the diversity of conditions that initiate necrotic cell death and of the cellular responses that are involved, several commonalities are starting to emerge, which indicate the existence of a limited repertoire of necrotic mechanisms. This is encouraging, considering that necrosis was once regarded as a totally disordered and highly varied process of cellular breakdown. However, there is still a long way to go before our understanding of necrosis is comparable to that of apoptotic cell death. The availability of well-established models of necrosis in *C. elegans* and *Drosophila*, coupled with the sophisticated genetics and molecular biology available for study of these organisms, should allow detailed and systematic dissection of the necrotic process[24,28,33]. The power of this approach has already been shown for apoptotic cell death by the many ground-breaking discoveries in *C. elegans*[147,148]. Similarly, extensive genetic screens aimed at identifying suppressors and enhancers of necrosis provide new insights into the molecular mechanisms of necrotic cell death. Progress is encouraging and holds promise that missing pieces of the puzzle will soon be found.

There is debate about the actual role of necrosis in neurodegenerative diseases that are associated with the formation of large protein aggregates[149–151]. Genetic studies associate several neurodegeneration disorders with mutations in genes that encode the proteins that are found in aggregates, which indicates that protein aggregation is causative rather than a symptom of cell death[152]. The mechanisms underlying the cytotoxicity of aggregates are not completely understood, and two principal conceptual models have been advanced[152].

First, the mutant proteins that aggregate have simply lost functionality and cannot carry out their essential normal functions, therefore promoting cell death. Second, protein aggregation becomes toxic because the aggregates interfere with normal cellular processes and generate stress, because they trigger necrotic and inflammatory responses[153], or because such aggregates trap other essential proteins, interfering with cellular processes that are crucial for cell viability[154,155]. The development of both invertebrate and mammalian animal models has greatly facilitated investigations into the molecular mechanisms that are responsible for cell demise induced by protein aggregation[24,156,157]. Ectopic overexpression of both human and nematode torsin proteins in *C. elegans* has the capacity to reduce the formation of protein aggregates[158]. Torsins share sequence similarity with the large and diverse family of AAA+ ATPases (ATPases associated with diverse cellular activities) that include heat-shock proteins, proteasome subunits, transcriptional regulators and other molecular chaperones. The distant sequence similarity between torsins and chaperones indicates a possible role for torsins in protein maturation, and underlines the importance of chaperones and effective management of protein misfolding in neurodegenerative disorders. Indeed, increased activity of several heat-shock proteins, which serve as molecular chaperones and aid the maturation of other proteins, protects against the cytotoxic effects of protein aggregates[156,157,159–165].

A puzzling observation that deserves more experimental attention is the variation between cell types in resistance to similar necrotic insults. For example, degenerin hyperactivation in *C. elegans* can kill neurons but does not seem to affect muscle and certain hypodermal cells to the same degree[83]. Diverse mammalian cell types also show varying vulnerability to extreme conditions[38,166]. What makes one cell type resistant to necrosis while another succumbs to the same insult? Understanding the aspects of cellular physiology that underlie these differences will provide valuable insight into the molecular mechanisms and pathways involved in necrosis.

It is important to note that, contrary to apoptosis, necrosis does not involve the mobilization of molecular mechanisms that evolved to specifically facilitate cell death. Rather, death is effected by cellular mechanisms that operate within the cell under normal conditions; under exceptional conditions or when extensive damage

is inflicted, these mechanisms turn 'rogue' and demolish the cell. In addition to understanding the physiological functions of these biochemical pathways, it is necessary to elucidate the means by which these are deregulated. We need this knowledge if effective intervention strategies aimed at countering neurodegeneration are to be developed.

As necrotic mechanisms are mostly malfunctioning biochemical pathways that fulfil normal functions in 'unstressed cells', manipulating them in an effort to ameliorate necrosis could have detrimental effects, and this must be taken into account when developing therapeutic strategies. Although it might not always be possible to prevent necrosis, intervention that switches cell fate towards apoptotic death could be beneficial, particularly as, unlike necrosis, apoptosis evokes minimal or no inflammation of surrounding tissues[167]. In most cases that are pertinent to human health, necrotic cell death results from exposure to relatively mild offences[38], and data from several experimental systems indicate that it is feasible to shield cells against such insults[33]. So, there is plenty of scope for further research into how certain cells defy necrotic insults, which should lead to the identification of vital cellular activities that could be reinforced in necrosis-susceptible cells to increase their resistance to potentially fatal challenges.

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