Quadrant 1: Text

Apoptosis

1. General account

Activation of controlled intracellular programme by a cell leading to its own death is known as programmed cell death. This is a necessary biological phenomenon needed for elimination of damaged, unwanted or potentially detrimental cells from a living body. Cellular apoptosis is a death process characterised by cell shrinkage, condensation, DNA fragmentation and phagocytosis. **Caspases** are proteolytic enzymes and the key players of apoptotic cell death. This group of enzymes primarily cause cleavage of specific intracellular proteins. **Procaspases** are inactive precursors found in all nucleated animal cells. Activation complexes activate initiator procaspases which in turn cleaves and activates executioner procaspases leading to a downstream irreversible proteolytic cascade.

Activation of initiator procaspases takes place either by **extrinsic or intrinsic pathway**. In the extrinsic pathway, extracellular ligands bind to cell surface death-receptors; the ligand-receptor recognition being highly specific. The death receptors recruit procaspases-8 and 10 via adaptor proteins to form DISC (Death Inducing Signalling Complex). The intracellular pathway employs intracellular signals mainly involving cytochrome c which upon release from the mitochondrial intermembrane space activates Apaf 1, which self assembles into an apoptosome and activates procaspas-9. Anti-apoptotic and pro-apoptotic Bcl2 proteins regulate the intrinsic pathway by controlling release of mitochondrial proteins. IAP proteins inhibit activated caspases in cells where death is not beneficial to the organism. Thus, intracellular Bcl2 proteins and IAP proteins ensure death of cells that needs to be eliminated.

2. Programmed cell death in plants

Programmed cell death (PCD) has been defined as a sequence of (potentially interruptible) events that lead to the controlled and organized destruction of the cell.

In plant cell cultures an **apoptotic-like PCD (AL-PCD)** has been identified that is fairly rapid and results in a distinct corpse morphology which is visible 4–6 h after release of cytochrome *c* and other apoptogenic proteins. This type of morphology, distinct from autophagy and from necrosis, has also been observed in examples of plant development.

The term **autophagy** describes the process whereby cytoplasmic materials are degraded through the lysosomal machinery within the cell. It is characterized by the formation of autophagic vacuoles and also dilation of the mitochondria and endoplasmic reticulum and slight enlargement of the Golgi. However, cells with enlarged vacuoles and undergoing autophagy do not necessarily die as the process is involved in the routine turnover of cell constituents. It is important, therefore, to use the term ‘autophagy/type II cell death’ only when there is a fatal destruction of the cell.

In plants, PCD is a facet of a wide range of developmental programmes which vary from the beginning of the plant’s life cycle, through essential development (xylogenesis), right until the end of the plant life cycle (senescence). As with animals, it is involved in pathogen (hypersensitive response) and stress (e.g. aerenchyma formation) responses. Cell death is as fundamentally important to plants as cell division.

The most obvious feature of this morphology consisted of a retraction of the protoplast away from the cell wall. While there is no evidence for classical caspases in the *Arabidopsis* genome, caspase-like molecules are being discovered, and caspase substrates are cleaved during plant PCD. DNA is cleaved and several genes implicated in apoptosis seem to function in plant PCD. So, during some plant cell deaths there are striking similarities with apoptosis, but at the same time there are also differences. Therefore it has been suggested that this morphologically distinct type of PCD in plants should be termed apoptotic-like PCD (AL-PCD). There are similarities (that may have been evolutionarily conserved from single cell ancestors, between AL-PCD and apoptosis while recognizing there are distinct, and perhaps fundamental, differences. AL-PCD may respond to specific signalling molecules, gene regulation, caspase inhibitors, etc, while necrotic or autophagic cell death may not.

DNA cleavage is also a marker of AL-PCD as PCD-activated nucleases cleave DNA at linker sites between nucleosomes, resulting in DNA fragments that are multimers of approximately 180 bp and run as a ‘ladder’ pattern when separated by electrophoresis in agarose gels. Poly (ADP-ribose)

polymerase (PARP), which is involved in DNA repair and is a classic substrate for caspase-3 activity. Although AL-PCD morphology is commonly described during the hypersensitive response in plants during normal plant growth and developmental PCD much of the morphology described in the literature conforms to the mammalian Type II/autophagic PCD (i.e. formation of large vacuole which ruptures to release hydrolytic enzymes that degrade cellular contents. There is no evidence that the Bax/Bcl-2 family normally operates in plant cells although there is evidence for a mitochondrial release of PCD activating molecules.

Over-expression of a peroxidase gene has a significant effect on reactive oxygen species (ROS) levels in the cell and altering ROS levels can cause alive/PCD/necrosis threshold changes as it alters cellular stress levels. Hydrogen peroxide (H_2O_2) and other reactive oxygen species (ROS) have become recognized to be key modulators of PCD as well as many other biological processes such as growth, development, and stress adaptation. Although specific ROS receptors/sensors remain largely elusive, downstream components of H_2O_2 and ROS signal transduction networks controlling plant PCD have been identified, including protein kinases, protein phosphatases, and transcription factors.

Alterations in ion fluxes are one of the primary events of PCD initiation. Calcium influx is an early event (and possible trigger) in the cell death process. Release of apoptogenic proteins from the mitochondria is regarded as a hallmark feature of animal apoptosis and is also observed in AL-PCD. Cytochrome c is released from the mitochondria almost immediately following heat stress in cucumber cotyledons.

Caspase-like activity is also associated with AL-PCD mitochondrial permeability transition, occurred in a significant population of the cells' mitochondria.

3. <u>Types of PCD in plants</u>

3.1 Developmental PCD:

Developmental PCD is a terminal stage of plant cell differentiation. In some cases, the dead cells play specific functions (e.g., tracheary elements, fiber cells, trichomes), in other cases, cells must die to form organs with proper functions or shapes (e.g., unisexual reproductive organs in dicots, leaf shape in some plants, aerenchyma tissue), or cells die because they accomplished their function and/or are no longer required (e.g., petals and nectaries in some flowers after pollination, leaf senescence.

3.2 PCD in the interactions between plants and the environment:

A number of plant adaptation processes, including the hypersensitive response (HR) to pathogens, some plant–plant allelopathic interactions, and aerenchyma formation in response to oxygen deprivation, require PCD. In contrast, many unfavourable abiotic stress factors as well as necrotrophic pathogens trigger unwanted PCD. Thus, PCD both serves as positive and negative aspects of plant adaptation to the environment.

There are no core universal regulators and executioners of plant PCD analogous to the members of the animal Ced-9/Bcl-2, Ced-4/APAF1, and caspase families. From the emerging picture, it is becoming increasingly clear that most of the genes involved in the regulation of plant PCD are specific to the plant kingdom and this is most likely a reflection of the specific morphology and physiology of plants. Plant hormones like ethylene, brassinosteroids, and cytokinins together with other signaling molecules regulate PCD in a complex manner.

4. Enzymes involved in PCD:

A crucial role in programmed suicide of animal cells belongs to caspases, a family of highly specific cysteine proteinases that are activated in apoptosis, introducing single breaks in molecules of a restricted set of cellular proteins. Caspases have exclusive specificity of hydrolysis: they introduce break after an aspartic acid residue (D) localized within a certain amino acid context. Directed fragmentation of target proteins by caspases eventually leads to the ordered death of the cell. And, contrariwise, inhibition of caspases counteracts apoptosis.

Plants use PCD both in the course of development (for instance, during xylem formation, seed germination, prevention of self-pollination, and senescence) and in response to osmotic, thermal, and oxidative stresses and in defense from pathogens. Like in animals, PCD in plants takes various forms but a series of common PCD features can be traced in both kingdoms. These features include DNA fragmentation, cytochrome *c* release from mitochondria, cell shrinkage, generation of reactive oxygen species, exposure of phosphatidylserine, etc.

It is intriguing and significant that caspases, which generally fulfil PCD in animals, are absent in plants, as evident from sequencing of plant genomes. At the same time, much data suggests that inhibitors of animal caspases can suppress PCD development in plants. In connection with this, in plant PCD activation of unidentified caspase-like proteases is also observed and these can hydrolyze various peptide substrates of caspases. These data suggest that PCD in plants involves proteases that are functional analogs of animal caspases, but which are structurally different from caspases. In plants, phagocytosis of dying cells is lacking not only due to the absence of professional phagocytising cells, but also due to the presence of rigid cellulose walls separating the cells. The formation of apoptotic bodies has not been observed in plants either.

Table 1: Comparison of properties of aspartate-specific apoptotic animal and plant proteases: caspases and phytaspases:

Parameter	Caspases	Phytaspases
Type of protease	Cys- dependent, clan CD	Ser-dependent, subtilase family, S8A subfamily
Specificity of hydrolysis	Strictly Asp	Strictly Asp-specific
Preferred amino acid motif of recognition site	DEVD (caspase-3,7), VEID (caspase-6), IETD (caspase-8), LEHD (caspase-9), VDVAD (caspase-2)	VEID; less efficiently YVAD, VAD, IETD,LEHD, etc. Not recognized: DEVD
Substrate proteins	Different proteins of the cell and pathogens	VirD2 of *Agrobacterium tumefaciens*
Synthesized as	Proenzyme	Pre-proenzyme
Way of processing	Induced, autocatalytic, or performed by another caspase	Constitutive, autocatalytic
Mature enzyme	Dimer of heterodimers, subunits of ~12 and ~20 kDa	Monomer, ~80 kDa
Localization	Intracellular, mainly cytoplasmic	Extracellular in healthy tissues (apoplast); re-localizes into the cytosol during PCD induction
Role in PCD	PCD initiation and implementation	At early stages (before the involvement of mitochondria); fragmentation of foreign proteins

Phytaspases display similarity with the animal and yeast subtilisin-like proteases, so-called proprotein convertases, which belong to the S8B subfamily (which apparently is absent from plants), in the high selectivity of substrate hydrolysis.

Further characterization of the genes identified and their physiological functions in different aspects of plant development and response to environmental fluctuations will help to delineate the intricate network and elucidate the detailed mechanisms of specific checks and balances determined by levels and localization of various forms of ROS in all aspects of plant growth and development.

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