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Programmed cell death: similarities and differences in animals and plants. A flower paradigm

Review Article

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Summary. After an overview of the criteria for the definition of cell death in the animal cell and of its different types of death, a comparative analysis of PCD in the plant cell is reported. The cytological characteristics of the plant cell undergoing PCD are described.

The role of plant hormones and growth factors in the regulation of this event is discussed with particular emphasis on PCD activation or prevention by polyamine treatment (doses, timing and developmental stage of the organism) in a Developmental cell death plant model: the *Nicotiana tabacum* (tobacco) flower corolla. Some of the effects of polyamines might be mediated by transglutaminase catalysis. The activity of this enzyme was examined in different parts of the corolla during its life span showing an acropetal trend parallel to the cell death wave. The location of transglutaminase in some sub-cellular compartments suggests that it exerts different functions in the corolla DCD.

Keywords: Programmed cell death – Polyamines – Transglutaminase – Flower – Cell wall – Tobacco

Abbreviations: AZ, abscission zone; CD, cell death; DCD, developmental cell death; GFP, green fluorescen protein; PAs, aliphatic polyamines; PCD, programmed cell death; SAG, senescence-associated genes; TGase, transglutaminase

Definition of programmed cell death in animals and plants

The size, shape and well being of a pluricellular organism are determined by an equilibrium between signals for cellular destruction and others that drive cell proliferation and/or differentiation. Cell death is one of the most difficult events to study because, as a consequence of this process, the cells are continuously being destroyed and removed. Moreover, often it affects only individual, isolated cells. In addition, cells die as a consequence of a series of morpho-functional pathways, which do not follow a unique common programme.

A generally accepted nomenclature for animal cell death (CD) has not yet been agreed, because many historically used terms, like apoptosis, necrosis, autophagy etc. are based mainly on morphological descriptions of events frequently observed only in in vitro cultures, without a clear reference to underpinning biochemical mechanisms. The editors of Cell Death and Differentiation have established the Nomenclature Committee on Cell Death which proposed unified criteria for the definition of cell death and formulated several caveats against the misuse of words and concepts that slow down progress in the area of cell death research (Kroemer et al., 2005). A relevant fact is the distinction between ''dying'' which is a process and ''death'' which is the end point.

Dying cells can engage in a process that is reversible up to when the 'point-of-no-return' is trespassed. However the point-of-no-return has yet to be defined. This process is not synchronous in a population, thus individual cells will be at different dying stages. According to an acceptable definition, an animal cell is considered dead when: it has lost integrity of the plasma membrane; its cytoplasm and nucleus are fragmented into separate bodies and the cell or its fragments have been engulfed by adjacent cells.

The Committee also observed that the term programmed cell death (PCD) implies the concept of a ''genetically programmed'' process which occurs during development and ageing, as opposed to ''accidental cell death''. Neverthe-

less, sometimes it is difficult to discriminate between programmed and accidental phenomena, thus, it is suggested to replace PCD by more detailed expressions describing the causative agent and the parameters that are measured.

Types of death having distinct mechanisms and morphologies are: 1) Apoptosis, based on morphological description, not always presenting DNA fragmentation and caspase cascade; 2) Autophagy, presenting vacuolization of the cytoplasm; 3) Necrosis and oncosis, with a similar morphology; 4) Mitotic catastrophe (not a widely used terminology), which occurs during or after an irregular mitosis; 5) Anoikis occurring when cells have lost the substrate attachment; 6) Excitotossicity, occurring in neurons when the calcium channels are opened; 7) Wallerian degeneration, occurring in neurons, by axon degeneration; 8) Cornification or keratinisation, which are specific of epidermis. Thus, rather than formalizing nomenclature, the use of functional terms should be encouraged, like for example, ''developmental cell death'', ''cell death induced by osmotic shock'', ''etoposide-induced cell death'', etc.

In the present paper the term PCD is used in a general sense, whereas a more detailed term is used for specific processes.

It is obvious that some PCD of animals are specifically related to the cytological characteristics of animal cells, among which adhesion, etc., or to the type of cell (epidermal, neurons etc.) or to the general organisation of the organism.

In plants, studies on PCD are much less advanced than in animals and some attempts have been made to classify the types of PCD and frequently terms from the animal literature are used. Nevertheless, in plant developmental biology, there is a rather fundamental confusion especially centred on the application of the terms senescence and PCD (van Doorn and Woltering, 2004) which, according to the different authors, may be considered separate, partially overlapping or even identical events (Thomas et al., 2003; van Doorn and Woltering, 2005; Rogers, 2006). There is no general agreement about the boundaries and overlaps among ageing, death, senescence, ripening, postharvest deterioration, hypersensitivity lesions, chlorosis, necrosis and so on. Possibly, part of the confusion arises from the attribution of these terms to individual cells, specific groups of cells, organs or, finally, entire organisms.

The above definition of dead cell attributed to animal ones, can be only partially applied to plant cells. In fact, a plant cell is dead when it has lost the integrity of the plasma membrane, but not always the cell and its nucleus are fragmented in separate bodies and the cell or its fragments are never engulfed by adjacent cells.

Some features of PCD in plants are different from those in animals, because of the presence of specific cell compartments, specific mediators (i.e. plant hormones) and for the absence of external phagocytosis events (Greenberg, 1996; Pennel and Lamb, 1997). On the basis of plant cytological events, the point of no return in plants could be defined as the step, in which not only mitochondria but also chloroplasts are involved, beyond which the cell is irreversibly committed to die (van Doorn, 2005).

In plants, frequently there are events such as autophagocytosis (Liu et al., 2005), mummification (for example in the seed endosperm) (Dangl et al., 2000) and abscission of the entire organ. An explicative example is the vacuolar autophagocytosis occurring during the PCD caused by pathogen attack, when only the vacuoles of infected cells are autodigested; on the contrary, in uninfected cells the presence of intact vacuoles is necessary to allow a functional defence response (Seay and Dinesh-Kumar, 2005).

Developmental cell death (DCD) is a terminal stage of plant cell differentiation, so the dead cell plays specific functions itself (e.g. vascular tissues, fibres, trichomes, etc.) or by contrast, cells die after having accomplished their role. In reproductive organs, various parts undergo DCD and, according to the type of flower, could abscise or not, on the other hand the abscission is not always preceded by senescence (Hilioti et al., 2000). Usually, in flowers both petals and stamens abscise, whereas sepals and ovary remain in situ and the latter transforms into fruit. The petal senescence, an active, complex, highly regulated developmental phase, is controlled by growth factors and hormones, like ethylene, cytokinins and abscissic acid (Mayak and Halevy, 1978; Orzaez et al., 1999; Rogers, 2006). In the long lived flowers pollination acts as a signal for senescence, while in the short lived flowers this event is independent from the pollination.

It is reported that plants, before abscising some organ, like their leaves, remobilizes most of the nutrients during senescence. Leaves are specialized photosynthetic organs and the plant invests much energy and nutrient in leaf production. Leaves, after a period of production of photosyntates, enter their last stage of development, senescence, that results in the co-ordinated degradation of macromolecules and the subsequent mobilisation of components to other parts of the plants. Yellowing, the visible sign of senescence, is due to the preferential degradation of chorophyll over carotenoids. Proteins, lipids and nucleic acids are degraded and amino acids, nitrogen (glutamine), phosphorous and metals are reallocated to younger leaves and seeds or stored. Senescence-enhanced genes

are involved in these processes, whereas the expression of many other genes is switched off.

There is evidence that some plant proteases digest the substrates of caspases and plant PCD is inhibited by caspase inhibitors. In plants some groups of caspase-like, the metacaspase I e II are known (Woltering, 2004), whereas in microrganisms the presence of paracaspase is reported. These proteases seem to be the ancestors of caspases in animals. Moreover in plants there are cys-proteases, with specificity for Asn and Asp, that share homology with a structural domain of caspase 1 (Asp pocket) and they are located in the vacuole as inactive precursors and released in the cytosol during the last phase of PCD (Hatsugai et al., 2006). Another group are the saspase (serine proteases), with specificity for Asp, involved in PCD as processive enzymes (not degradative), that share structural homologies with subtilisin (Coffeen and Wolpert, 2004). In conclusion, the plant caspase-like activities share some similitude with caspase activity from animal because: 1) the proteolytic cutting occurs always on Asp residue, 2) generic inhibitors of cysteine and serine protease are ineffective, 3) they are involved in PCD.

Cytological events in plants

PCD in plants is accompanied by nuclear condensation, membrane blebbing and, in some cases, DNA fragmentation and cysteine proteases activity (Pennel and Lamb, 1997; Serafini-Fracassini et al., 2002). At the subcellular level, mitochondria may play a central role in both kingdoms (Desagher and Martinou, 2000) but the molecular mechanisms may be different. For example, in *Arabidopsis*, the DNA laddering activity induced by mitochondria is insensitive to caspase and other protease inhibitors, moreover it is unclear if the released cytochrome *c* can induce PCD in plants (Balk et al., 2003). Mitochondria retain their function during senescence, since respiration continues, whilst chloroplasts become inactive and are dismantled. Increase of ROS production and protease and nuclease activities have also been reported to occur in the plant PCD.

Other organelles typical of plants, such as chloroplasts, vacuoles, and possibly also cell walls, play a role in the induction or execution of PCD, as reported during the leaf senescence (Quirino et al., 2000; Rubinstein, 2000). Chloroplasts, in fact, swell and re-differentiate into gerontoplasts characterised by the dismantling of thylakoidal membrane organization in grana and intergrana and accumulation of lipids. During these events proteins, chlorophylls, lipids as well as nucleic acids are degraded and the photosynthetic activity decreases; products of this degradative activity are redistributed to other organs, whereas mitochondria catabolise lipids deriving from thylakoids. An initial step in the dismantling of thylakoids is the degradation of the chlorophyll that take place from the detachment of the tetrapyrrolic group and its conversion throughout a series of reactions to a not fluorescent catabolite that leaves the chloroplast to enter in the vacuole, by an ABC transporter, where it is accumulated.

Gerontoplasts can revert to chloroplasts in the leaves of many species, as well as chromoplasts to chloroplasts. In petals and fruits, chloroplasts are differentiating in chromoplasts, responsible for the yellow-red colours of these organs.

Vacuoles, which represent the lytic compartment of the plant cell, play relevant roles in the degradative metabolisms, as above exemplified for chlorophyll, and finally rupture of tonoplast membrane takes place causing the release of degradative enzymes.

Cell walls of some specialised cells, before the cell die, frequently undergo secondary modifications, like for example lignification, suberification and gelification.

Hormones and plant DCD

DCD in plants is a natural event (Jones, 2001; Greenberg, 1996), in which perception of specific signals and induction of cascades of gene expression are regulated by signals due to hormones and phytoregulators (ethylene, cytokinins, ABA, jasmonate, polyamines, etc.) as required in morphogenesis and sex determination. Little is known about the mechanism involved either in the initial signalling or subsequent co-ordination of the process. Senescence can only be initiated when cytokinins are below a threshold level. The maintenance of this cytokinin level inhibits transcriptional regulation of senescence genes. On the other hand, ethylene alters the timing of senescence and increased levels of this hormone enhance the rate of senescence progression only in leaves already programmed to start senescence. Ethylene probably represses gene involved in photosynthesis. During senescence the sensitivity for this hormone increases. Ethylene also stimulates senescence-associated genes (SAG), which share sequence similarity with genes of proteases, RNases, and glutamine synthetase. The gene 'defender against apoptotic death', *Dad*-1, is an evolutionarily conserved inhibitor of PCD, downregulated by ethylene. Dad-1 declines dramatically in pea petals after anthesis and correlates with an increase of DNA fragmentation.

Aliphatic polyamines (PAs) at difference with the plant hormones, are growth substances present in plant and animal kingdoms and in both of them are involved also in PCD.

Polyamines as modulators of DCD

In general, the molecular mechanism of PA action is related to several factors involved in their metabolism. In fact, PAs, not only form cytotoxic oxidative products, but also act as radical scavengers and interact with several important cell molecules. These interactions may occur by both electrostatic linkages causing conformational stabilisation/destabilisation of DNA, RNA, chromatin and proteins (enzymes, receptors, onco-proteins) and by covalent linkages giving rise to formation of hypusine, cytotoxic derivatives and 'cationisation' or cross-link formation of many proteins.

The PA involvement in apoptosis of animal cells has been recently reviewed by Seiler and Raul (2005): 'Activation and prevention of apoptosis due to polyamine depletion are reported for several cell lines. Elevation of polyamine concentrations may lead to apoptosis or to malignant transformation. Contradictory results and incomplete information blur the picture and complicate the interpretation'.

This panorama of the state of the art in animals possibly derives from the complexity of the PCD mechanism and by the fact that only small segments of it have been explored. In addition, the research is limited only to a few cell lines, which can react differently to the different PAs supplied and also those metabolised by the cell itself.

In plants, senescence of different organs can be delayed by PAs, which have a well-established role in the cell division, senescence and growth (Altman and Bachrach, 1981; Bagni and Pistocchi, 1988; Hanzawa et al., 2000). Previous reports mainly suggest that PAs allow a prolonged survival of excised organs. The necessity to have the most simple and natural model of PCD suggested to concentrate studies on the *Nicotiana tabacum* (tobacco) corolla model and to observe therein the changes in the levels of PAs, either free or conjugated.

Nicotiana corolla: a model for plant DCD

Petals, which are modified leaves, have in general a vexillary role, namely they favour pollination and, once completed this role, they enter senescence and fall or in some cases, e.g. *Nicotiana*, remain in situ to protect the initial growth of the ovary. *Nicotiana tabacum* petals are histologically rather homogenous consisting of parenchyma, thin veins and a single protecting layer of epidermis. Corolla senescence follows a visible acropetal gradient, which is completed by the death of the entire corolla at stage 10 (Fig. 1). The term DCD, applied to the *Nicotiana* petals, is used to define the terminal process of development constituted by senescence and the cell death phases. The growth and development of the corolla of *Nicotiana* is well characterised. This plant model involves gradual stages (from 1 to 10) of development, from flower growth to senescence and death (Fig. 1).

Different morpho-functional parameters were analyzed to characterise the onset of corolla senescence and cell death. Whereas protein and chlorophyll content decreased gradually, proteases were particularly active only during a short period simultaneously with the first appearance of DNA laddering, nuclear blebbing, rupture of the tonoplast membrane, anthocyanin pigment decrease and modification of cell walls (Serafini-Fracassini et al., 2002).

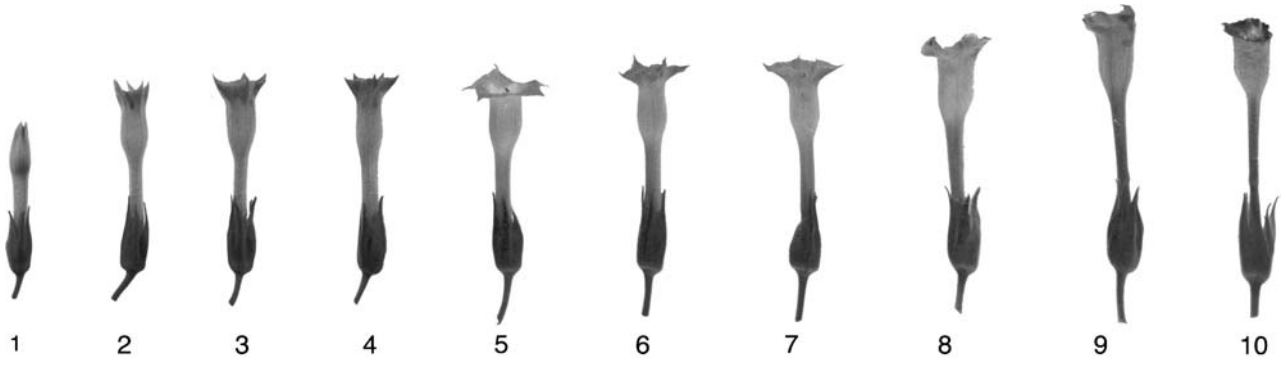

Fig. 1. The corolla life span is divided in ten stages. *1–4* Developing flower; *5* maximum opening of the corolla and the basal portion of the corolla does not show visible modifications; *6* transition stage in which the flower appears to be in good health, but the "point-of-no-return" is marked by the appearance of a low mechanical resistance ring at the corolla base corresponding to the abscission zone (AZ) shown in Fig. 3. *7–9* Senescence progression, the corolla, despite the abscission, remains in situ; *10* death of the entire corolla, which is completely dry and papyraceous

The earliest visible sign of the onset of DCD in the entire corolla is the appearance at its base of a 'ring' of dying cells, which become detached from each-other. This zone is named abscission zone (AZ) whose resistance sharply decreases at stage 6 (Fig. 1), although at this stage the flower otherwise appears to be in good health. Several parameters (membrane integrity, protease activity and DNA laddering, as reported by Serafini-Fracassini et al., 2002), confirm that the 'point of no return' occurs at this stage. Nevertheless preparatory events could occur in previous stages, as suggested by chlorophyll and protein decrease (stages 4 and 5) and a dramatic water loss measured in the proximal part between stages 5 and 6. Cells underwent nucleus blebbing and cell wall modification at stage 6 only in the proximal part (Serafini-Fracassini et al., 2002). These data are in agreement with the observation performed in *Alstroemeria* by Wagstaff et al. (2005). The senescence proceeds acropetally along the *Nicotiana* corolla, and concludes with the teeth curling (Fig. 1). Senescence is followed by a gradual DCD during lifetime and along the corolla, like an acropetal death wave, which ends with the death of the entire corolla at stage 10. Thus, corolla represents a good paradigmatic model.

Polyamine effect on corolla DCD

Results obtained by supplying the three main PAs, putrescine, spermidine and spermine, added at different concentrations, at precise stages of the flower life and for different supply times, confirm that all these factors are determinant in the PCD prevention or promotion. These experiments could represent a study model to explain some of the contradictory results reported in the literature about the PA roles in PCD.

Petal DCD can be delayed by PA supply to excised flowers, with spermine being particularly efficient; the presence of intracellular free and conjugated putrescine and spermidine, increased concomitantly to the treatment, due to an active PA metabolism (Serafini-Fracassini et al., 2002). Spermine and its derivatives, when analysed in the flowers, were also detected in bound form, namely as TCA-insoluble and soluble forms. In the latter PAs are mainly conjugated to hydroxycinnamic acids, particularly abundant in tobacco. The supply of 5 mM spermine delayed chlorophyll degradation, indicative of undamaged chloroplasts and enhanced petal colour due to anthocyanin synthesis, indicative also of undamaged vacuoles. In addition spermine delayed for at least 24 h the DNA laddering.

Transglutaminases and PCD

The molecular mechanism of action of PAs is not completely clarified, even though their non-covalent interaction with nucleic acids or other molecules, has been recognised for a long time. We focused our attention to PA conjugated to proteins by catalysis of transglutaminases (TGases) a family of calcium-dependent enzymes.

TGases catalyse linkages between glutamyl residues of proteins and amine donors, like lysyl residues or PAs, forming cross-links. The linkage of terminal amino-groups of PAs gives rise either to *mono*-(γ-glutamyl)-PAs or *bis*-(γ-glutamyl)-PAs (Folk et al., 1980) (Fig. 2). TGases form bridges between specific proteins, including those of the cytoskeleton or the animal extracellular matrix, and are involved in the regulation of cell growth and differentiation (Folk et al., 1980; Ichinose et al., 1990; Griffin and Verderio, 2000).

Several TGase activities have been detected both in higher and lower plants. TGases have been reported to be present in different organs, like leaves, tubers, shoots, roots, flowers (reviewed by Del Duca and Serafini-Fracassini, 2005). Plant TGases have not yet been classified and only one enzyme located mainly in the microsomes of *Arabidopsis thaliana* has been sequenced and found to contain the typical catalytic domain of the TGase superfamily (Della Mea et al., 2004a). In plant cells the roles of TGases are similar to those in animals, however their presence in particular plant compartments and the nature of their substrates suggest that they fulfil additional roles. TGases were found in some compartments of various types of cells, like the chloroplasts of several higher plants and some algae, where they possibly stabilize and protect antenna complexes and Rubisco, thus possibly being involved in the regulation of the photosynthetic process (Margosiak et al., 1990; Della Mea et al., 2004b; reviewed by Serafini-Fracassini and Del Duca, 2002). In some lower organisms, like *Chlamydomonas* (Waffenschmidt et al., 1999) and some fungi, TGase activity has been found in the cell walls, where the enzyme has a structural role or acts as the defence elicitors of higher plants (reviewed by Del Duca and Serafini-Fracassini, 2005). TGases are also present in the cytoplasm where they probably exert a structural role, for example during the dramatic cytoskeleton re-arrangement which occurs during the rapid growth of the pollen tube (Del Duca et al., 1997).

TGases are one of the relevant factors of PCD in animals; in fact in several animal cells, the presence and the activity of TGases are considered markers of apoptosis (Fesus et al., 1987; Melino and Piacentini, 1998; Fesus,

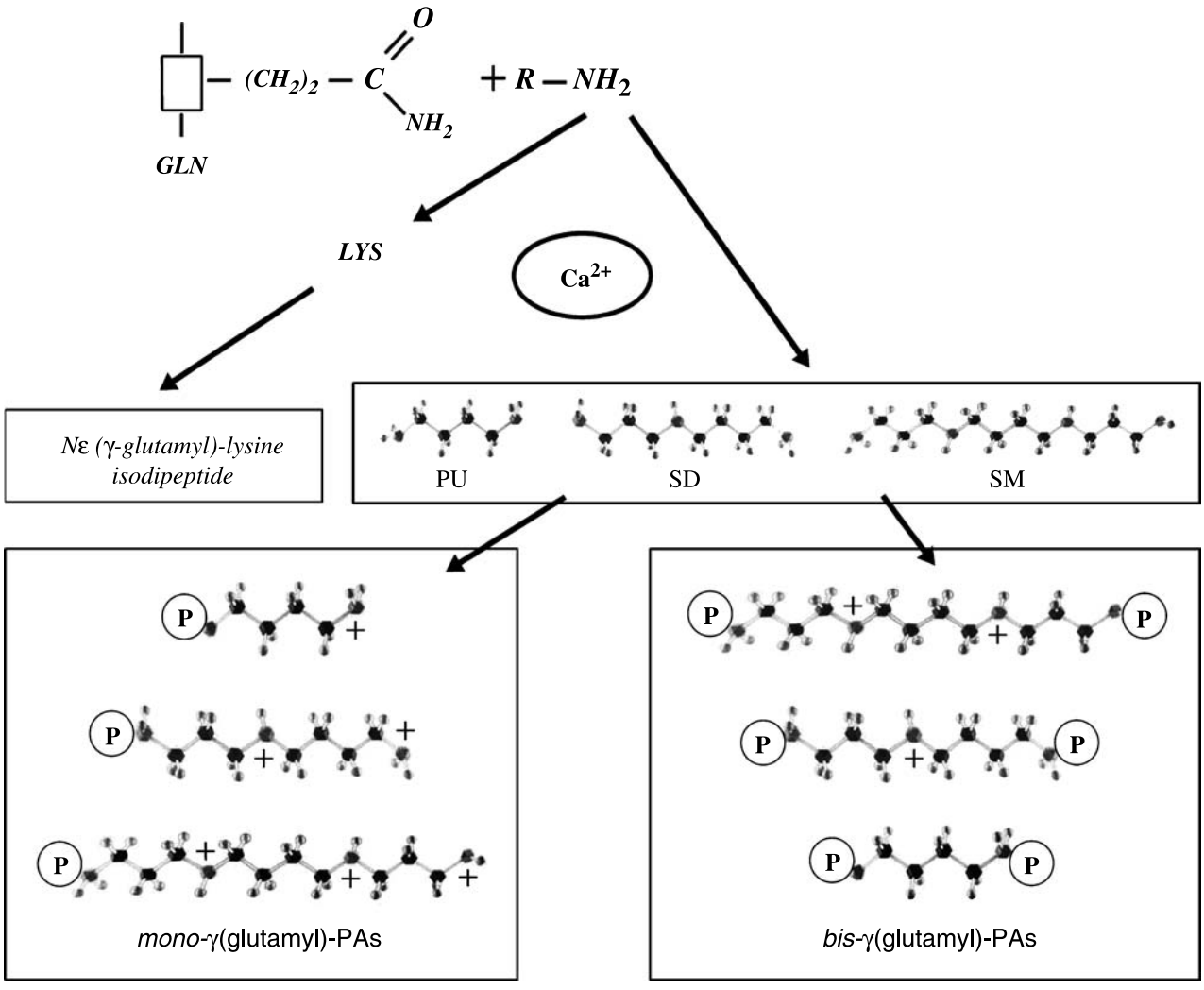

Fig. 2. TGases catalyse linkages between an acyl acceptor glutamyl residue and amine donors, like lysyl residues or PAs, forming cross-links within the same or between different proteins. PAs act as physiological substrates of TGases: the terminal amino-group binds one or two glutamyl residues giving rise either to *mono*-(γ-glutamyl)-PAs or *bis*-(γ-glutamyl)-PAs (Folk et al., 1980)

1999; Griffin and Verderio, 2000). Although at present it is not possible to establish with certainty a connection between TGases and apoptosis (Griffin and Verderio, 2000; Verderio et al., 1998), experimental evidences confirm that the expression or the accumulation of the enzyme accompanies PCD (Candi et al., 2005); moreover, proteins modified by TGases are more protected from protease digestion (Chen and Metha, 1998).

In contrast to the overwhelming evidences of the involvement of TGases in the mammalian PCD, only little information is available for that in plants. A calcium-dependent TGase was immunorecognised in *Nicotiana* by three antibodies raised against both plant and animal TGases. The TGase activity, tested on endogenous proteins, was assayed either with added calcium or without, the latter in order to assay the activity in conditions more similar to those in nature. It is known in fact that during DCD calcium increases, being released from its storage compartments. In this condition, TGase activity increases during DCD with the maximum at the no-return point (stage 6). It was observed that protein-conjugated PAs change their relative ratios during the sequential stages of DCD and that their level significantly differs if measured before or after DNA laddering. Bis-PU was the most abundant PA-derivative except in late senescence. The bis-PU and bis-SD levels showed a significant decreasing trend with increasing corolla age, whereas mono-PU significantly increased at late stages. The mono-PU/bis-SD ratio was around 1 and increased starting from the stages in which senescence is clearly evident and dehydration

set in. A similar result was observed also in detached flowers confirming that the relative proportion among the three derivatives is different in non-senescent and senescent corolla. The observed changes in TGase activity are thus related to developmental changes of the corolla, suggesting a specific PA requirement according to DCD progression.

The activity was also studied either in the presence of the endogenous substrates alone or by adding a constant amount of a specific recombinant mammal TGase exogenous substrate, namely His6-X Press-green fluorescent protein (GFP) (Furutani et al., 2001); the modifications of both substrates were also studied by analyzing the changes in their electrophoretic migration and the PA glutamyl-derivatives produced. The recombinant GFP is demonstrated to be a good substrate for plant TGase, because its electrophoretic shift changes in a similar way after modification by animal and plant TGases.

The activity of the corolla extracts shows an increase at senescence with the maximum at the no-return point whose occurrence shifts from proximal to distal part according to the stage progression; this wave-like pattern precedes that of senescence, suggesting a connection with DCD. The incorporation of [^{14}C]-spermine into corolla endogenous substrates was qualitatively similar in all stages, but more marked at late stages, when in addition high molecular mass and probably degradation labelled products are present.

The fact that the antibody raised against *Arabidopsis* TGase recognises some proteins of *Nicotiana*, also immuno-detected by two animal antibodies, and that plant and animal TGases present similar molecular weights and modify GFP in a similar way, would suggest a similarity among these enzymes. Even though there is no sequence homology between animal TGases and that of *Arabidopsis*, the presence in the latter of the typical catalytic triad and its structural homology with Factor XIII (Tasco et al., 2003) could be the cause of its immuno-recognition by heterologous antibodies.

During senescence progression TGase activity, examined in three parts of the corolla, basal, medial and distal part, clearly shows an acropetal progression of its maxima preceding a similar acropetal senescence gradient. The distribution of immuno-detected TGase in the three parts of the corolla clearly shows that a main 58 kDa form is always prevalent in the medial green part. The distribution of a 38 kDa putative enzyme is particular: it appears early in the proximal/medial parts and later in the distal one, where, in the late phases, it is exclusively present, in agreement with the acropetal wave of TGase activity.

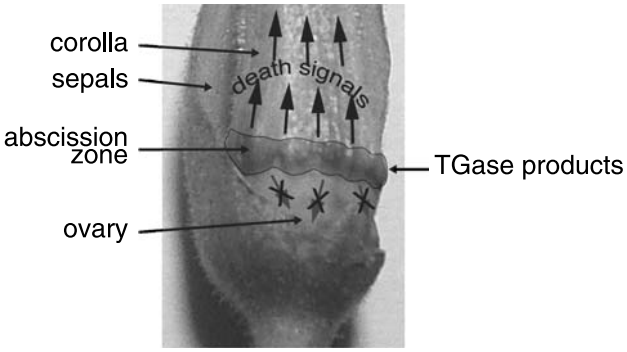

Fig. 3. Basal part of the flower of *Nicotiana*. Model of the possible TGase involvement in DCD progression. The diffusion death signals starting from the abscission zone and extending only acropetally to the entire corolla are preceded by the acropetal TGase activity. The abscission zone appears at the base of the corolla that is covered by sepals, here cut to expose it

As the earliest visible sign of the onset of DCD is the appearance at the base of the corolla of the abscission zone, it could be hypothesised that a diffusible 'one-way' death signal starts from this zone and extends acropetally to the entire corolla. TGase activity precedes the appearance of the death wave and could be one of the molecular signal to death arising from the dying cells of the abscission zone (Fig. 3).

TGases are differently compartimentalised in the corolla cells and their activities differ according to their life span. In the plastid, microsomal and cell wall fractions a 58 kDa band is the more represented immunorecognised protein and the TGase activity is present in all fractions. A 52 kDa band, observed only in the soluble fraction, might derive by post-translational modifications of the 58 kDa enzyme. In this fraction the activity is present only in the senescent corolla basal part, when, as previously reported (Serafini-Fracassini et al., 2002), cell membranes begin to be degraded and proteases activated. Then, this soluble activity could be due to a TGase release from microsomal vesicles; a similar mechanism exists in petals of *Arabidopsis*, in which vesicles containing cysteine proteinases precursors are delivered to the vacuole, where these enzymes participate in the protein disassembly during senescence (Hayashi et al., 2001).

The presence of a microsomal TGase in tobacco is in accord with the presence of a TGase, provided with a Golgi putative signal sequence, in the microsomal fraction of *Arabidopsis thaliana* (Della Mea et al., 2004a).

In the plastid fraction a 38 kDa form is localized, in addition to the more represented 58 kDa one; both enzymes are active and calcium dependent. The 58 kDa protein seems to be involved in photosynthetic complexes stabili-

sation (reviewed by Del Duca and Serafini-Fracassini, 2005). The considerably increased activity of chloroplast TGase might be necessary in *Nicotiana* corolla senescing tissues to supply energy to sustain the morpho-functional active events of DCD (DNA laddering, protease activity, abscission ring formation, cell wall hardening, curl of teeth, etc.) accompanying the acropetal wave of senescence.

Until now, the presence of TGases and their activity has been described only in the cell walls of lower organisms (Waffenschmidt et al., 1999), and some unicellular fungi (reviewed by Del Duca and Serafini-Fracassini, 2005) even though, till now no data were published on the identification of TGase in the cell wall of higher plants. At the contrary, the presence of a TGase substrate, the PAs, is documented in the cell wall (Berta et al., 1997) and the inhibition of their biosynthesis induced modification of the structure, shape and size of the primary cell walls of *Nicotiana* thin layers. The strengthening between cell wall components by PAs could be due to their ionic interaction with pectic substances or/and to covalent TGase-mediated interactions with proteins. It is, in fact, possible to hypothesize a role of TGase in cell wall strengthening, by protein cross-linking; in fact, in senescence, the cell wall undergoes modifications evidenced by the increased autofluorescence (Serafini-Fracassini et al., 2002) and by the papyraceous aspect of the corolla.

When PAs are present in physiological concentrations, in the cell wall both the glutamine-lysine and glutamine-PA-glutamine cross links are permitted; if PAs are added at high concentration, the cross links can be inhibited due to the prevalent formation of mono glutamyl-PAs derivatives. The minor extent of cross links can reduce the strengthening of the cell wall and its modification processes related to senescence progression (Fig. 4).

Recent experimental evidence shows that in animals a significant part of the TGase cellular pool is present on the cell surface where it is involved in cell interactions with the extracellular matrix (Zemskov et al., 2006), which can be considered to be analogous to the plant cell wall, and in the repair of tissues after injury (Telci and Griffin, 2006).

On the base of biochemical and bioinformatic data it can be hypothesised that the TGase can be released in the

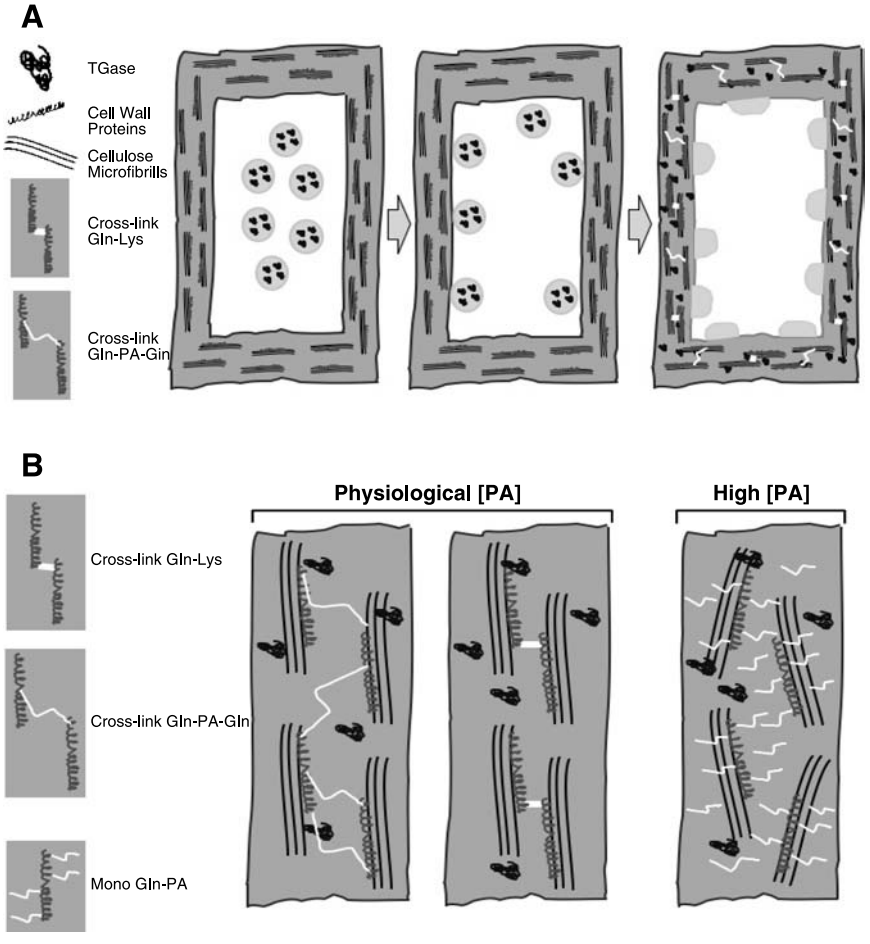

Fig. 4. **A** Schematic models of cells undergoing DCD. TGase is at first located in vesicles in the cytoplasm and then is secreted into the cell walls, where TGase catalyses the formation of cross links gln-lys and gln-PA-gln. **B** Detail of the cell walls. When PAs are present in physiological concentration, in the cell wall both the glutamine-lysine and glutamine-PA-glutamine cross links are permitted; if PAs are added at high concentration (5–10 mM), mainly *mono*-glutamyl-PAs derivatives are formed and the cross links can be inhibited, reducing the cell wall strength

cell wall by its transport via microsomal vesicles. The TGase compartmentalisation may be necessary, as TGase is potentially a 'dangerous' enzyme if free in the cytoplasm. The presence of TGase in *Nicotiana* cell walls is confirmed by confocal in situ immunostaining analysis (personal communication of C. Faleri and G. Cai, Siena University, Italy). Moreover, TGase might be involved in the cell wall structural modifications of differentiating cells located in the AZ, zone in which the increase in activity is considerable. A functional hypothesis to explain the TGase activity in the AZ is the prevention of uncontrolled release of toxic substances and pathogen defence after corolla abscission of the developing reproductive tissues, thanks to the cicatrisation of the AZ scar in which the TGase-mediated cross-links seem to be involved. The complex cascade of events in the tobacco corolla DCD can be mainly finalised to the protection of the developing ovary against external biological and physical-chemical factors by a suitable envelope.

In summary, the function of exogenous polyamines in the regulation of PCD depends on their concentration, precise stage of life of the treated cells and by the duration of the supply. All these factors are determinant in the PCD prevention or promotion.

In conclusion one of the most intriguing aspects of plant PCD, when compared with that in animals, is that PCD machinery and some inducing factors seem to be sometimes functionally conserved during evolution, however there is lack of homology at molecular level: for example, caspases and transglutaminases show scarce homology but similar functions. Moreover, the death strategies can be different dependent on the differences in organism and cytological organisation between plants and animals.

Note added in proof

Recent data here reported on *Nicotiana* DCD are published in: Della Mea M, De Filippis F, Genovesi V, Serafini Fracassini D, Del Duca S (2007) The acropetal wave of developmental cell death (DCD) of *Nicotiana tabacum* corolla is preceded by activation of transglutaminase in different cell compartments. Plant Physiology Preview. Published on April 13, 2007, as DOI: 10.1104/pp.106.092072.

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