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REVIEW ARTICLE

Defining senescence and death

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Abstract

This article evaluates features of leaf and flower senescence that are shared with, or are different from, those of other terminal events in plant development. Alterations of plastid structure and function in senescence are often reversible and it is argued that such changes represent a process of transdifferentiation or metaplasia rather than deterioration. It may be that the irreversible senescence of many flowers and some leaves represents the loss of ancestral plasticity during evolution. Reversibility serves to distinguish senescence fundamentally from programmed cell death (PCD), as does the fact that viability is essential for the initiation and progress of cell senescence. Senescence (particularly its timing and location) requires new gene transcription, but the syndrome is also subject to significant post-transcriptional and post-translational regulation. The reversibility of senescence must relate to the plastic, facultative nature of underlying molecular controls. Senescence appears to be cell-autonomous, though definitive evidence is required to substantiate this. The vacuole plays at least three key roles in the development of senescing cells: it defends the cell against biotic and abiotic damage, thus preserving viability, it accumulates metabolites with other functions, such as animal attractants, and it terminates senescence by becoming autolytic and facilitating true cell death. The mechanisms of PCD in plants bear a certain relation to those of apoptosis, and some processes, such as nucleic acid degradation, are superficially similar to aspects of the senescence syndrome. It is concluded that, in terms of physiological components and their controls, senescence and PCD are at best only distantly related.

Key words: Cell death, flower, leaf, senescence.

Introduction

'Music may be what we think it is; it may not be'
(Bohlman, 1999)

Discussions on terminal events in plant development often end up in a semantic Babel. There is no general agreement about what are the boundaries and overlaps between ageing, death, senescence, ripening, post-harvest deterioration, hypersensitivity, lesions, chlorosis, necrosis and so on. The present review aims to define some of these terms and clarify their interrelationships from the perspective of leaf and flower senescence.

Senescence is a transdifferentiation process

Plastids are the defining organelles of plant cells and the interconnecting network of plastid forms (etioplast, chloroplast, amyloplast etc.) is a centre-piece of classical developmental cell biology (Thomson and Whatley, 1980). Chloroplasts typify healthy leaf tissues; the German microscopist Sitte coined the useful term 'gerontoplast' to describe the organelle of senescing, formerly green tissues (Parthier, 1988). The ripening of carotenoid-accumulating fruit such as tomato is defined by the differentiation of chloroplasts into chromoplasts. It is reasonable to think of the senescence of mesophyll cells as a homologous process, leading from chloroplasts to gerontoplasts. The chloroplast-to-gerontoplast transition predates, and may even be the origin of, chloroplasts-to-chromoplasts in evolution. If so, reproductive structures (petals, fruits) that owe their colours to accumulated carotenoids are really modified senescing leaves (Thomas

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and Sadras, 2001; Thomas *et al*., 2001). Conversion of chloroplasts to chromoplasts occurs well before flower opening and thus before the reproductive function of the flower commences. It is commonplace in evolution for novelty to arise through a pre-existing differentiation event becoming displaced in time within the normal developmental schedule of a tissue or organ, and certainly much more likely than evolving a completely new event *ab initio*. The concept of the petal as a neotenic senescing leaf may well be disputable, but the case for regarding the kind of senescence/ripening behaviour associated with plastid remodelling as a process of cellular differentiation rather than degeneration is defensible. It is made more convincing still by the observation that the conversion of chloroplasts to gerontoplasts is reversible in the leaves of many, perhaps all, species (Zavaleta-Mancera *et al*., 1999*a*, *b*). Similarly, there are many reports of the reversion of chromoplasts to chloroplasts, for example in *Citrus flavedo* (Caprio, 1956; Huff, 1983), pumpkin skin (Devidé and Ljubesic, 1974) and carrot root (Grönegress, 1971). Although there are no reports of experimentally-induced reversion of petal chromoplasts to chloroplasts, such modulations do occur naturally in certain orchids (Curtis, 1943) where, following pollination, the perianth regreens and becomes photosynthetic.

There is a gradation in plasticity of senescence from flowers to leaves with flower longevity being brief and, for the most part, unidirectional, fruit ripening having more temporal variation than flowers and leaf senescence showing maximal temporal variation (often environmentally controlled) and an amenability to being stopped in its tracks and even reversed (Molisch, 1938). The flower functions strictly to advertize the reproductive potential of the organ, and once this task is complete and pollination has taken place the organ can be pushed into the senescence programme. Understanding the mechanistic basis of senescence reversibility is a key to resolving the relationship between senescence and death. A plausible hypothesis is that, as the balance of metabolic turnover becomes displaced away from biosynthesis, and/or the import of essential translocated materials declines, tissues become depleted of some critical component(s) with age and effectively 'wear out'. The hypothesis is consistent with the observation that removing the upper shoot results in the regreening of lower leaves; similarly, removal of young floral buds increases the longevity of the remaining flowers (Chanasut *et al*., 2003). The reversibility seen in leaf senescence and some fruit ripening may have evolved as an add-on to this underlying mechanism in order to maximize the efficiency of nutrient usage in changing environments. Alternatively, plasticity may be a characteristic of the ancestral senescence syndrome which flowers have largely lost.

Plant cell differentiation without growth is a special case and earns its own terminology—transdifferentiation (defined by the *Dictionary of Cell and Molecular Biology Online* as 'change of a cell or tissue from one differentiated state to another'). An alternative term is metaplasia, used in animal pathology to describe 'a reversible change in the character of a tissue from one mature cell type to another' (Underwood, 2000). There is a strong case for treating senescence as a cellular transdifferentiation or metaplastic process in the course of which plastid structure and function are remodelled.

Pre- or post-mortem?

It follows from the conclusion reached above that the physiological changes that occur during senescence are those of viable cells and tissues. Indeed, the most effective way to stop senescence is to render the tissue non-viable, which accounts for the arrest of herbarium specimens and frozen spinach in the pre-senescent state. On the other hand it is often difficult to tell whether the chemical and structural changes observed during the various types of cell death are pre- or post-mortem events. In particular, oxidations, free radical cascades and the cleavage of macromolecules may be the agents that lead to cell death; but they are also what happens to dead organic material in the early stages of necrotrophic and necrochemical attack. The idea of genetic programming has to be applied with caution to events that occur in cells in the intermediate state between living and dead. Thomas (1987) pointed out that cyanide is fatal because the proteins of the respiratory chain have a particular reactivity towards it as a consequence of their genetically-determined structures; but this emphatically does *not* justify the assertion that death by cyanide poisoning is genetically *programmed*. Many gerontological processes that appear to have a genetic basis may do so simply because of the built-in obsolescence of macromolecules, which is genetically determined only in the sense that durability may not be a high priority in the design specifications of critical cell structures. Much of the evidence for and against a genetic basis for ageing and death is open to this kind of criticism. Of course, post-mortem changes will certainly have a purposeful, non-random appearance. The structures of cells, membranes, nucleic acids or proteins will collapse in a semi-reproducible fashion because they are built the way they are. Any study claiming to define mechanisms of (programmed) cell death should provide evidence that the events observed are physiological and biochemical rather than necrochemical and entropic. Senescence defined as metaplasia clearly satisfies this requirement.

How much of what happens in terminal processes is transcription-dependent?

Thomas and Stoddart (1980) reviewed the genetic and metabolic features of the initiation and progress of

senescence and concluded that it was controlled largely at the post-transcriptional level. Developments in proteomics and other methodologies for studying post-transcriptional regulation now present new opportunities to revisit these conclusions. So far differential, subtractive and genomics approaches to the identification of senescence-specific genes have predominated (Smart, 1994; Buchanan-Wollaston, 1997; Nam, 1997; Quirino *et al*., 2000; Chandlee, 2001) and the focus has been almost exclusively on transcription, the factors that regulate it and the signalling pathways that orchestrate their interplay. Empirically, plant breeders have always known that variation in the timing of senescence initiation is selectable and studies of the inheritance of this and related characters were first carried out almost a century ago (reviewed by Thomas and Smart, 1993). The syndrome as a whole is under genetic control, so there is nothing surprising or contentious about the idea of transcription-level induction. It also explains why the most successful transgenic modifications have moved the entire syndrome about by, for example, intervening in the hormonal signalling pathways. In one influential study, leaves of plants transformed with isopentenyl transferase, coupled to a senescence-associated promoter, were induced to produce cytokinins when senescence was triggered and in doing so their senescence was reversed (Gan and Amasino, 1995). In the same way enhanced cytokinin levels in petunia and tobacco delay flower senescence (Zubko *et al*., 2002). Disruption of the ethylene-signalling pathway similarly delays the onset of leaf senescence (John *et al*., 1995), fruit ripening (Klee, 2002) and flower senescence (Wilkinson *et al*., 1997) in a number of species.

Nevertheless, the transcriptional level is not the only one at which a genetically-programmed pathway can be regulated, and the approaches described above have not yet helped with dissecting and analysing the (mostly mysterious) cellular processes that make up the syndrome. It is (or should be) worrying that it is still not known how protein is mobilized from senescing leaves, even though it is an agronomically, economically and environmentally critical process (Thomas and Donnison, 2000). Transgenic and mutational approaches have not been much help in solving this mystery. Similarly, until recently the pathway of chlorophyll degradation was also unknown. There is now enzymological and cell biological evidence that a metabolic sequence is activated during senescence, all except one, or perhaps two, of the steps of which are constitutive (Matile *et al*., 1999; Thomas *et al*., 2001; Kräutler, 2002). Remobilization of nutrients from senescing flower tissues occurs and is believed to contribute either to the development of the ovary or to that of new flowers. It has even been suggested that changes in source:sink relationships are the trigger for floral senescence (Nichols and Ho, 1975*a*, *b*). Moreover, trimming of flowers from flowering spikes increases longevity of the remaining flowers, again implying that source:sink relationships influence longevity and senescence (Chanasut *et al*., 2003). All in all it looks likely that signalling and transcriptional networks are coarse regulators of when and where senescence starts, whereas execution, fine control and integration of constituent processes require cascades of activation and inactivation with a strongly post-transcriptional element. Moreover, unlike the propagating wave of destruction characteristic of cell death, these non-transcriptional mechanisms must be under *play–pause–stop–rewind* control until extremely late in senescence. This adds another critical feature to the list that places senescence in a distinct group within the taxonomy of terminal processes.

Senescence schedules of different cells and tissues

Programmed cell death (PCD) is just that: a means by which cells die individually. Senescence in the sense discussed here is an organ-level phenomenon. Leaves and floral organs are not uniform structures. Typically, only about half of the cells in a mature leaf contain chloroplasts (Pyke, 1994), but research on the molecular basis of senescence has almost always treated leaf tissues as if they are made up exclusively of mesophyll cells. Although senescence-associated genes are assumed to be expressed and active in senescing green tissues, in few cases has this been verified. One such gene for which there is information about tissue distribution of expression is that encoding the cytosolic nitrogen mobilization enzyme, glutamine synthetase I. It has been located not in senescing mesophyll but in the vascular tissue (Kamachi *et al*., 1992). Is mesophyll senescence cell-autonomous? Helen Ougham, Sue Dalton and Phil Morris (unpublished data) have had some limited success with isolated mesophyll protoplasts, observing yellowing in wild-type *Lolium temulentum* and retention of pigment in a stay-green isoline. The transdifferentiation of *Zinnia* mesophyll cells into tracheids begins with senescence-like changes in plastids (Fukuda, 1996). It would be interesting to compare these events (rather than the later processes of xylogenesis and cell death, which have been the focus of attention to date) with what happens in senescing *Zinnia* leaves. A promising single-cell model for mesophyll senescence is *Chlorella*. Degreening in *Chlorella* can be induced by changing culture conditions and there is good evidence that the enzymic step in chlorophyll degradation that is activated by this treatment is the same one that is stimulated in leaves at the initiation of senescence. Protein mobilization also takes place, and some of the up-regulated genes are similar to those found in senescing terrestrial plant tissues (Hörtensteiner *et al*., 2000). Degreening in *Chlorella* is also reversible (Aoki *et al*., 1965). The evidence, soft and indirect as it is, points towards a capacity for senescence within each individual

mesophyll cell, just as cell autonomy is a characteristic of PCD.

Flower cell death is equally complex; in the few detailed anatomical studies made it is clear that mesophyll cell degradation occurs before outward symptoms of senescence are visible to the casual observer. In *Alstroemeria* (C. Wagstaff *et al*., unpublished results), for example, the mesophyll cells at the petal margins are completely degraded by the time the flower opens. A similar situation occurs in other short-lived flowers such as *Hemerocallis* (Stead and van Doorn, 1994) and *Iris* (Bailly *et al*., 2001). In *Sandersonia*, however, a similar collapse of the mesophyll cells occurs but not until the corolla wilts, and it is not clear that the collapse of mesophyll cells near the petal margins occurs earlier than that of other mesophyll cells (O'Donoghue *et al*., 2002). This again illustrates the dangers of considering whole organs as being composed of a collection of homogeneous cells undergoing development and senescence in synchrony with one another, and of assuming that senescence of a given tissue proceeds in an identical manner in different organisms.

Autolysis versus transdifferentiation

Senescence has been defined here in terms of plastid transitions, but another organelle has a cryptic though probably a critical part to play in terminal processes of cell development—the vacuole. If plastid interconversions are characterized by the unmasking or accumulation of isoprenoids, the phenylpropanoid pigments of fruits such as strawberry, and of anthocyanin-accumulating leaves, are the signatures of the vacuole's participation in senescence and ripening. In flowers the accumulation of vacuolar anthocyanins typically precedes senescence and occurs before, or at least concomitant with, the reproductive phase of the flower's life. This may be another instance of evolution of floral features by displaced timing of terminal events in the development of a foliar progenitor. The vacuole is also the destination of the final products of chlorophyll catabolism (Matile *et al*., 1999; Thomas *et al*., 2001). There is evidence that the sequestration of pigments and catabolites in the vacuole is an adaptation for direct or indirect protection from photodamage (Matile *et al*., 1999; Feild *et al*., 2001). The vacuole has a critical role in defences against pathogens and pests. Significant numbers of senescence up-regulated genes are also pathogenesis-related (Hanfrey *et al*., 1996) and others, such as the cysteine endopeptidases that turn up in just about every collection of senescence-related cDNAs (Thomas and Donnison, 2000), perhaps have a defence rather than a protein remobilization role. The pH optima for many classes of proteases suggest vacuolar localization. A third function for the vacuole is in (auto)lysis. Older ideas about senescence resulting from the release of vacuolar hydrolases into the cytosol, or plastid engulfment, have not stood the test of time, but such processes seem to be relevant for the death phase of *Zinnia* tracheid transdifferentiation (Fukuda, 1996). Recent work on protein storage vacuoles (Jiang and Rogers, 2001) reveals a more complex mode of biogenesis and organization than was formerly suspected, and it is likely that plant vacuoles, in general, will turn out to be more than combinations of dustbins and bags of 'disasterases'. So the definition of senescence as a type of metaplasia includes a protective role for the vacuole during transdifferentiation, followed by an autolytic function which terminates senescence and cauterizes the tissue.

PCD mechanisms

The very term programmed cell death implies control at the transcriptional or translational level and indeed several genes specific to PCD have been isolated from animal tissues. As yet few, if any, of these have homologues in plants; genes encoding molecules similar to repressors of animal PCD have, however, been found in *Arabidopsis* and rice (Kawai *et al*., 1999). Moreover, the expression of human negative regulators of PCD (*Bcl-2* and *Bcl-xl*) in plants confers pathogen resistance and delays DNA laddering (Dickman *et al*., 2001). Evidence from studies of animal apoptosis suggests that entry into PCD is dependent upon de-repression of pro-apoptotic genes such as *BAX* that are located in the mitochondrial membrane (Green and Reed, 1998). In animal systems this leads to a release of cytochrome *c*, shortly after which caspase activity is up-regulated followed by DNA laddering. Cysteine proteases are the closest functional homologue to caspases in senescing plant tissues and these are commonly found during leaf and petal senescence (Buchanan-Wollaston, 1997; Griffiths *et al*., 1997; Wagstaff *et al*., 2002). The occurrence of laddering is also frequently reported in a variety of senescing plant tissues as is TUNEL labelling of degrading nuclei (Kawai and Uchimiya, 2000; Oraez and Granell, 1997), though, as discussed in the following section, these observations must be treated with caution. Release of cytochrome *c* from the mitochondria has been reported for only one, rather bizarre, plant cell type. Tapetal cells have a very short life span and for the most part are fully functional while being enucleate. Death of tapetal cells was shown to feature the hallmarks of animal PCD, namely cell condensation, cleavage of nuclear DNA, chromatin separation, and release of cytochrome *c* from the mitochondria into the cytosol of tapetal cells (Balk and Leaver, 2001). The final activity of these cells is to deposit their remaining cell contents onto the developing pollen exine, with the only remaining identifiable organelles at this stage being the mitochondria (A Stead, unpublished results). Perhaps these cells, along with the transdifferention of parenchyma cells into tracheids, show the closest similarity to animal PCD or apoptosis.

Be careful walking under ladders

Finally, returning to the subject of nucleic acids in senescence and cell death: plants are ruthless recyclers and do so by employing senescence and death for resource reallocation. Phosphorus presents nutritional problems for plants, since it is not very soluble or mobile in soils and usually has to be mined, hence the wide distribution of mycorrhizal associations. For a plant running a marginal P economy, nucleic acids represent an expensive investment. This is particularly true for species with large genomes, and for the many plants that indulge in somatic endoreduplication. It is accepted that a protein like rubisco can not only have an enzymic function but can also act as a storage protein, the N of which is redistributed during senescence. If the sacred role of DNA and RNA as the carriers of genetic information is set aside, nucleic acids could be regarded as potentially valuable P storage compounds. In a dramatic demonstration of the determined reuse of P during plant development, Helen Ougham (unpublished results) grew *Lolium temulentum* from seed on a hydroponic medium lacking phosphorus and obtained a mature, albeit extremely stunted, plant bearing a single fertile seed—all achieved by unrelentingly turning over the P that was present in the original grain through successive leaves and into the new seed. Although RNA is the primary target for P remobilization, many plant nucleases are capable of using both RNA and DNA as substrates (Yupsanis *et al*., 1996; Kefalas and Yupsanis, 1995). When DNA is attacked by P-mobilizing nucleases, nicks and ladders are quite likely to occur. The DNA fragmentation patterns and/or TUNEL staining observed as a consequence may be similar to those used as indicators of apoptotic or programmed death-like mechanisms in animal cells, but it would be inappropriate to conclude that they necessarily signify functional similarities between apoptosis and the terminal processes, including senescence, which occur in plants. Indeed Lee and Chen (2002) conclude that cell death during rice leaf senescence does not proceed via an apoptosis-like pathway as seen in animals.

In conclusion

Putting together current and long-established information on the molecular, cell and comparative biology of senescence identifies a set of defining characteristics that distinguish it from other terminal, death-related processes. A similar critical evaluation of, for example, the hypersensitive response, developmental lysigeny or lesion mutations would contribute towards building a taxonomy that might help avoid fruitlessly chasing one-size-fits-all explanations of mechanism and control.

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