Letters

Senescence: developmental program or timetable?

The concept of the 'program' is widely used by developmental biologists and generally everyone knows what it means. However, with the advent of Systems Biology there is an influx into the biological sciences of researchers from other disciplines, such as computing, mathematics and engineering, in which 'program' is also a technical term. If Systems Biology is to keep its promises, it is important to ensure that everyone engaged in the analysis of programmed processes in living cells is talking the same language. Arising from discussions in two recent conferences (Wingler, 2007; Thomas, 2008), this Letter takes a critical look at the notion of a program as conceived and studied by plant developmental biologists, focusing particularly on our area of interest, leaf senescence.

A program is a number of events that occur in a predetermined way, and developmental programs are believed to behave, by and large, like computer .exe files: signal molecules, kinases and transcription factors are often activated in sequence, leading to the development of, for example, an organ or a metabolic state. The plasticity of plant development, however, shows that developmental programs are not fixed but are instead continuously modulated by external and internal factors, to yield a plant body well adapted to its environment.

Developmental programs have often been studied by analysing pathway mutants, but in recent years profiling methodologies, such as DNA microarrays, have become the techniques of choice for dissecting the sequence of events during a developmental process (Schmid *et al.*, 2005). Every approach has its inherent problems, and we will, in this contribution, argue that, at least when leaf senescence is considered, the concept of a developmental program raises fundamental questions.

Senescence: pigment loss and differentiation without growth

Leaf senescence is postmitotic and essentially a process of transdifferentiation in fully grown cells (Thomas *et al.*, 2003). It occurs in, and uses the biochemical and cellular architecture of, mature cells and its main purpose is to degrade cellular components and remobilize them in order to re-use them elsewhere. Leaf senescence is therefore very different from the rapidly executed process of programmed cell death (PCD); paradoxically so because apoptosis, the common name for Type I PCD, is derived from the Greek term for leaves falling from a tree (Kerr *et al.*, 1972). Senescence involves chlorophyll loss via metabolism. Pathological bleaching, occurring after virus infection, for example, is not the same as senescence. In fact, these processes could be viewed as conflicting processes that are regulated by different sets of genes. Cell death has to be prevented until all mobilizable nutrients have been rescued (Hörtensteiner, 2004; Ougham *et al.*, 2008).

In some species, one way of distinguishing physiological and pathological yellowing is to demonstrate reversibility (Zavaleta-Mancera *et al.*, 1999a,b), a characteristic of senescence that fundamentally distinguishes it from nonphysiological bleaching. Reversibility is one of the aspects of senescence that does not fit with the concept of a program (Thomas *et al.*, 2003). Failure to make the distinction between the two possible fates of pigments (physiological and pathological) also contributes to confusion in the literature and a lack of consensus about what constitutes the core set of senescence processes.

How do we know if the process under study is truly senescence? One way is to use a mutant with a lesion in physiological chlorophyll degradation. If a particular treatment results in yellowing of wild-type but not of the staygreen, it is likely to have evoked true physiological senescence. If both genotypes lose the green colour, the senescence is pathological (Thomas & Matile, 1988; Ougham *et al.*, 2008). Physiological senescence, if not subject to suspension or reversal, will eventually be superseded by terminal cell death. Overlapping timetables in species with a rapid life cycle – such as Arabidopsis – make it difficult to identify the definitive elements in developmental programs, and encroachment of death into the senescence phase compromises the analytical separation of different patterns of gene expression and metabolism. Longer-lived species, with more extended developmental schedules and clearer temporal separation between phases, have advantages in this regard, even if they are experimentally less convenient.

Mutation and pathological disturbance are exceptional circumstances; normally the photodynamic dangers inherent in chlorophyll degradation during senescence are controlled by balancing catabolism with other senescence-related metabolic mechanisms that utilize or quench incoming light energy. For this reason, yellowing is more than a cosmetic index of senescence, it is a sensitive and convenient measure of the progress of the syndrome as a whole (Kingston-Smith *et al.*, 1997; Ougham *et al.*, 2008).

Ripeness to senesce

A leaf has to acquire competence to senesce, and this potential may exist before it is actually evoked. This is equivalent to an

old concept in developmental biology, proposed in 1918 by Klebs: *ripeness to flower* (see Bopp, 1996). In the same way, a seasonally quiescent species has to develop a competence to become truly dormant (endodormant; Vegis, 1964). The common feature of *ripeness* behaviours is that competence may be induced by different developmental and environmental influences from those that trigger the finally expressed syndromes.

This imposes another level of complexity. Imagine that the environmental factor triggering senescence initiation is present, but competence has not yet been acquired (Jing *et al.*, 2003, 2005). Senescence will not occur until conditions arise that develop competence and it will appear as if the factors that induce competence are primary inducers of senescence.

Regulation can operate at many levels, from the epigenetic unmasking of promoters and genes in chromatin, to post-translational protein modification or compartmentalization (Wingler, 2007). Early ideas about senescence were based on evidence that development of *ripeness to senesce* depends primarily on transcription, whereas the senescence trigger and subsequent mechanism may be largely post-transcriptional (and even post-translational) events (Thomas & Stoddart, 1980; Smart, 1994; Sullivan *et al.*, 2003; Thompson *et al.*, 2004; Hopkins *et al.*, 2007). This makes the notion of a 'senescence switch' conceptually and experimentally difficult.

Development as an amplifier

Because senescence is a terminal process, it is on the receiving end of the amplifier effect in plant development. A small perturbation early in development can have considerable consequences for the subsequent expression of senescence. This is apparent in Arabidopsis, where most growth and flowering mutants also have disturbed leaf senescence (Ellis *et al.*, 2005; Riefler *et al.*, 2006). This is part of the allometric control of senescence and life-history, which has been discussed by Ougham *et al.* (2007) and Marbà *et al.* (2007). Thus, genes for plastid assembly are, in the broad sense, senescence genes because a chloroplast has to be built in its characteristic way before it transdifferentiates into a gerontoplast.

Arising from the early classical molecular biology approaches of differential cloning (Smart, 1994; Buchanan-Wollaston, 1997) through to contemporary omics methods (Buchanan-Wollaston *et al.*, 2003; Guo *et al.*, 2004), knowledge of the variety of gene classes associated with senescence has revealed that the syndrome subsumes a wider range of cellular and physiological processes than might have been expected. Collections of senescence-associated genes typically comprise a number of transcription factors and other regulators – examples include WRKY factors, leucine zipper proteins, SARK and SIRK receptor kinases, calmodulin-binding proteins, MYBs, zinc fingers, MADS boxes, chromatin architecture-controlling AT-hook proteins and NAC factors (Hinderhofer & Zentgraf, 2001; Buchanan-Wollaston *et al.*, 2003; Lim *et al.* 2003, 2007; Lin & Wu, 2004). This adds up to a picture of the senescence program as a rather loose assemblage of transcriptional, post-transcriptional, epigenetic and allometric modules, which is difficult to convert into a coherent mechanistic framework.

Timetable or program?

What is the difference between a timetable and a program? A timetable is a record of events occurring in sequence, whereas a program requires the events to occur in a given order. While mutant studies may provide data about a program, profiling techniques, such as DNA microarrays, record instead a developmental timetable. In order to obtain reproducible data, plants are passed through the developmental stages under highly controlled conditions, and consequently development follows a certain trajectory. The search for senescence-associated genes, with differential expression, by using this approach (Lin & Wu, 2004; Guo *et al.*, 2004; Buchanan-Wollaston *et al.*, 2005) is motivated by a hope that some of these genes may be important for senescence, or at least could be markers of certain stages of senescence.

If senescence is not much of a program, even finding marker genes for senescence stages could be problematic. An illustration of this is the results from transcript profiling in autumn leaves of a free-growing aspen (*Populus tremula* L.). Senescence in this tree, measured as chlorophyll degradation, is initiated around 10 September, regardless of the weather conditions, and is therefore under photoperiodic control (Keskitalo *et al.*, 2005). Further studies of a range of aspen ecotypes (see Luquez *et al.*, 2007) have shown that the onset of senescence in the glasshouse, under natural photoperiod but otherwise controlled environmental conditions, is synchronized with free-growing ramets of the same clones, confirming that senescence in this system is triggered by the light environment alone. We performed transcript profiling using DNA microarrays over the period of initiation of senescence using leaf samples harvested from the same free-growing tree over 4 yr (Keskitalo *et al.*, 2005, Y. Fracheboud *et al.*, unpublished). There are indeed limitations in this approach. Leaf-to-leaf variation within a single tree, and the fact that the arrays used covered only *c.* 40% of the genome, will certainly reduce the precision of an analysis. Nevertheless, the data constitute a sufficiently large and representative sample of the entire senescence-associated transcriptome (Bhalerao *et al.*, 2003) to permit conclusions to be drawn. Even if critical genes that become induced and start the senescence program are absent from this analysis, a change in expression of a significant fraction of the arrayed genes would be expected if the term 'program' is to be justified. The expectation was to find that gene expression altered during this period and that a major shift in gene expression should occur before, at, or after, the initiation of senescence.

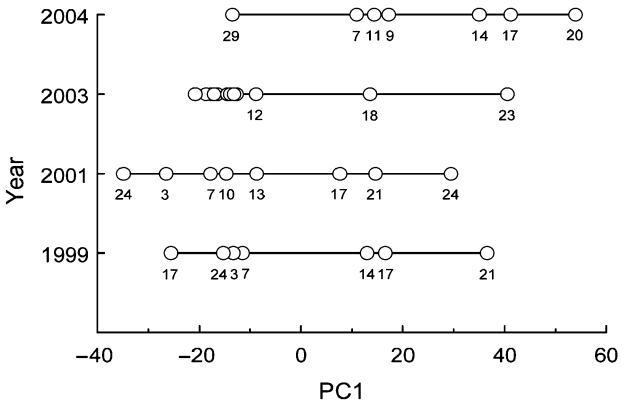

Fig. 1 Gene expression during initiation of autumn leaf senescence in an aspen (*Populus tremula*) grown at the Umeå University campus. The total pattern of gene expression from August to September in 1999, 2001, 2003 and 2004 was analysed on POP1 and POP2 DNA microarrays using a common reference (Andersson *et al*., 2004). Samples (a pool of > 20 leaves from each time point) were taken from a single tree at noon every day over several years. RNA preparation, microarrays and array analysis, including post-processing of the data, are described in Sjödin *et al*. (2006) and are stored in UPSC-BASE (www.upscbase.db.umu.se) where data are available under experiments UMA-0050 and UMA-0054. Principal component (PC) analysis was performed in SIMCA-P 11.5 (Umetrics, Umeå, Sweden). The first principal component, explaining 54% of the total variation in gene expression, is shown. This first principal component is, within each year, a description of date; numbers on the axes denote dates of leaf harvest, starting with fully green leaves (to the left) in August and ending in late September at the stage when leaves are so senescent that sufficient quantities of high-quality RNA for array analysis could not be obtained.

Indeed, major modulations occurred (downregulation of photosynthesis genes, for example) but, surprisingly, the shift did not coincide with a senescence stage, using extent of chlorophyll loss as the measure of physiological state between initiation and completion of senescence. Instead, the total pattern of gene expression in 2004, analysed using principal component analysis (PCA), was most similar to gene expression at later time points in the other years. In fact, the samples from 9 and 11 September 2004 had a transcriptome that were 'later' than those of 18 September 2003 and 17 September 2001 (Fig. 1, Y. Fracheboud *et al*., unpublished). Apparently, gene expression was governed by factors other than senescence and although it is obvious that, for a tree in the field, many other influences may modulate gene expression, the search for genes or gene-expression patterns that correlated with the onset of senescence was in this case unsuccessful.

We believe that the explanation for this may be that transcriptional patterns during leaf senescence merely represent a timetable and not a program. If senescence is initiated in leaves grown under identical environmental conditions in the laboratory, the transcriptome responds in a reproducible way, indicating that certain gene-expression patterns may correspond to specific stages of senescence and even that the expression of certain key genes could cause senescence. On the other hand, if senescence is initiated under different conditions, these relationships may not hold true.

If there is a senescence trigger, what could it be?

If senescence is not directly invoked by changes in gene expression, what is the trigger? Changes in the leaf metabolite profile, perhaps related to sink–source relationships, may be important in this respect, especially bearing in mind the key role of leaf senescence in nutrient recycling (Diaz *et al*., 2005; Hikosaka, 2005; Ougham *et al*., 2005). If leaf senescence first evolved in annuals or in perennials in a climate that did not undergo dramatic seasonal changes, its original role would have been to move mineral nutrients out of leaves that did not contribute much to photosynthesis and into leaves better positioned, or into other strong sinks like developing seeds (Thomas *et al*., 2000; Thomas & Sadras, 2001). In those deciduous trees that start senescing by the calendar (Keskitalo *et al*., 2005), however, one must postulate that photoreceptors could influence metabolism without transcriptional changes.

Senescence: programmed, but not a program

It is easy become confused about what is a program and what is programmable. Senescence is conditioned by genetic and environmental predispositions: an amplified outcome of a complex array of proximal and distant inputs. Very few of its constituent genetic, metabolic, cellular or physiological components have, however, been proven to be indispensable. We believe that our current knowledge of leaf senescence does not qualify it to be called a developmental program, like an .exe file. Perhaps senescence can instead be programmed according to the timetable set by development or the environment; that is, it behaves less like a fixed suite of propagating actions set in motion by a triggering event and more like a permissive operating system. Senescence may be better conceived of as a set of modelling routines where the nature of the inputs determine which modules are run, how they loop and interact, and which outputs follow.

Alternatively, we might simply be too ignorant to see the program and the 'Master Controller'. The search for the controller that makes leaves competent to senesce, and those that trigger senescence in competent leaves, will certainly continue. To what extent there may be a confusion between programs and timetables when other plant developmental processes are studied is hard for us to tell, but we believe that the understandable desirability of designing omics experiments to minimize the type of season-to-season environmental variation represented by Fig. 1 may have the unintended consequence of making it difficult to distinguish between a program and a timetable.

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References

Andersson A, Keskitalo J, Sjödin A, Bhalerao R, Sterky F, Wissel K, Tandre K, Aspeborg H, Moyle R, Ohmiya Y *et al.* 2004. A transcriptional timetable of autumn senescence. *Genome Biology* **5**: R2.

Bhalerao R, Keskitalo J, Sterky JF, Erlandsson R, Björkbacka H, Birve SJ, Karlsson J, Gardeström P, Gustafsson P, Lundeberg J *et al.* 2003. Gene expression in autumn leaves. *Plant Physiology* **131**: 430–442.

Bopp M. 1996. The origin of developmental physiology of plants in Germany. *International Journal of Developmental Biology* **40**: 89–92.

Buchanan-Wollaston V. 1997. The molecular biology of leaf senescence. *Journal of Experimental Botany* **48**: 181–199.

Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpour S, Page T, Pink D. 2003. The molecular analysis of leaf senescence – a genomics approach. *Plant Biotechnology Journal* **1**: 3–22.

Buchanan-Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG, Lin J-F, Wu S-H, Swidzinski J, Ishizaki K *et al.* 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in Arabidopsis. *Plant Journal* **42**: 567–585.

Diaz C, Purdy S, Christ A, Morot-Gaudry J-F, Wingler A, Masclaux-Daubresse C. 2005. Characterization of markers to determine the extent and variability of leaf senescence in Arabidopsis. A metabolic profiling approach. *Plant Physiology* **138**: 898–908.

Ellis CM, Nagpal P, Young JC, Hagen G, Guilfoyle TJ, Reed JW. 2005. AUXIN RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral organ abscission in *Arabidopsis thaliana*. *Development* **132**: 4563–4574.

Guo Y, Cai Z, Gan S. 2004. Transcriptome of Arabidopsis leaf senescence. *Plant, Cell & Environment* **27**: 521–549.

Hikosaka K. 2005. Leaf canopy as a dynamic system: ecophysiology and optimality in leaf turnover. *Annals of Botany* **95**: 521–533.

Hinderhofer K, Zentgraf U. 2001. Identification of a transcription factor specifically expressed at the onset of leaf senescence. *Planta* **213**: 469 – 473.

Hopkins M, Taylor C, Liu Z, Ma F, McNamara L, Wang T-W, Thompson JE. 2007. Regulation and execution of molecular disassembly and catabolism during senescence. *New Phytologist* **175**: 201–214.

Hörtensteiner S. 2004. The loss of green color during chlorophyll degradation – a prerequisite to prevent cell death? *Planta* **219**: 191–194.

Jing H-C, Hille J, Dijkwel PP. 2003. Ageing in plants: conserved strategies and novel pathways. *Plant Biology* **5**: 455–464.

Jing H-C, Schippers JHM, Hille J, Dijkwel PP. 2005. Ethylene-induced leaf senescence depends on age-related changes and OLD genes in Arabidopsis. *Journal of Experimental Botany* **56**: 2915–2923.

Kerr JFR, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer* **26**: 239–257.

Keskitalo J, Bergquist G, Gardeström P, Jansson S. 2005. A cellular timetable of autumn senescence. *Plant Physiology* **139**:1635–1648.

Kingston-Smith AH, Thomas H, Foyer CH. 1997. Chlorophyll a fluorescence, enzyme and antioxidant analysis provide evidence for the operation of an alternative electron sink during leaf senescence in a stay-green mutant of *Festuca pratensis*. *Plant, Cell & Environment* **20**: 1323–1337.

Lim PO, Kim Y, Breeze E, Koo JC, Woo HR, Ryu JS, Park DH, Beynon J, Tabrett A, Buchanan-Wollaston V *et al.* 2007. Overexpression of a chromatin architecture-controlling AT-hook protein extends leaf longevity and increases the post-harvest storage life of plants. *Plant Journal* **52**: 1140–1153.

Lim PO, Woo HR, Nam HG. 2003. Molecular genetics of leaf senescence in Arabidopsis. *Trends in Plant Science* **8**: 272–278.

Lin J-F, Wu S-H. 2004. Molecular events in senescing Arabidopsis leaves. *Plant Journal* **39**: 612–628.

Luquez V, Hall D, Albrectsen BR, Karlsson J, Ingvarsson P, Jansson S. 2007. Natural phenological variation in aspen (*Populus tremula*): the SwAsp collection. *Tree Genetics and Genomes* **4**: 1614–2942.

Marbà N, Duarte CM, Agustí S. 2007. Allometric scaling of plant life history. *Proceedings of the National Academy of Sciences, USA* **104**: 15777–15780.

Ougham H, Armstead I, Howarth C, Galyuon I, Donnison I, Thomas H. 2007. The genetic control of senescence revealed by mapping quantitative trait loci. *Annual Plant Reviews* **26**: 171–201.

Ougham H, Hörtensteiner S, Armstead I, Donnison I, King I, Thomas H, Mur L. 2008. The control of chlorophyll catabolism and the status of yellowing as a biomarker of leaf senescence. *Plant Biology* (in press).

Ougham HJ, Morris P, Thomas H. 2005. The colors of autumn leaves as symptoms of cellular recycling and defenses against environmental stresses. *Current Topics in Developmental Biology* **66**: 135–160.

Riefler M, Novak O, Strnad M, Schmülling T. 2006. Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *Plant Cell* **18**: 40–54.

Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Schölkopf B, Weigel D, Lohmann JU. 2005. A gene expression map of *Arabidopsis thaliana* development. *Nature Genetics* **37**: 501–506.

Sjödin A, Bylesjo M, Skogstrom O, Eriksson D, Nilsson P, Ryden P, Jansson S, Karlsson J. 2006. UPSC-BASE – Populus transcriptomics online. *Plant Journal* **48**: 806–817.

Smart CM. 1994. Gene expression during leaf senescence. *New Phytologist* **126**: 419–448.

Sullivan JA, Shirasu K, Deng XW. 2003. The diverse roles of ubiquitin and the 26S proteasome in the life of plants. *Nature Reviews Genetics* **4**: 948–958.

Thomas H. 2008. Systems biology and the biology of systems: how, if at all, are they related? *New Phytologist* **177**: 11–15.

Thomas H, Matile P. 1988. Photobleaching of chloroplast pigments in leaves of a nonyellowing mutant genotype of *Festuca pratensis*. *Phytochemistry* **27**: 345–348.

Thomas H, Ougham H, Thomas HM. 2000. Annuality, perenniality and cell death. *Journal of Experimental Botany* **51**: 1–8.

Thomas H, Ougham HJ, Wagstaff C, Stead AJ. 2003. Defining senescence and death. *Journal of Experimental Botany* **54**: 1127–1132.

Thomas H, Sadras VO. 2001. The capture and gratuitous disposal of resources by plants. *Functional Ecology* **15**: 3–12.

Thomas H, Stoddart JL. 1980. Leaf senescence. *Annual Review of Plant Physiology* **31**: 83–111.

Thompson JE, Hopkins MT, Taylor C, Wang T-W. 2004. Regulation of senescence by eukaryotic translation initiation factor 5A: implications for plant growth and development. *Trends in Plant Science* **9**: 174–179.

Vegis A. 1964. Dormancy in higher plants. *Annual Review of Plant Physiology* **15**: 185–224.

Wingler A. 2007. Transcriptional or posttranscriptional regulation – how does a plant know when to senesce? *New Phytologist* **175**: 1–4.

Zavaleta-Mancera HA, Franklin KA, Ougham HJ, Thomas H, Scott IM. 1999a. Regreening of senescent *Nicotiana* leaves. I. Reappearance of NADPH-protochlorophyllide oxidoreductase and light-harvesting chlorophyll a/b-binding protein. *Journal of Experimental Botany* **50**: 1677–1682.

Zavaleta-Mancera HA, Thomas BJ, Thomas H, Scott IM. 1999b. Regreening of senescent *Nicotiana* leaves. II. Redifferentiation of plastids. *Journal of Experimental Botany* **50**: 1683–1689.

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