REVIEW ARTICLE

Plant memory: a tentative model

M. Thellier1 & U. Lüttge2
1 Rue de la Chézine, Nantes, France
2 Department of Biology, Technical University of Darmstadt, Darmstadt, Germany

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Correspondence
U. Lüttge, Department of Biology, Technical University of Darmstadt, Schnittspahnstr. 3–5, D-64287 Darmstadt, Germany.
E-mail: luettge@bio.tu-darmstadt.de

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INTRODUCTION

Definitions of memory of living organisms are given by Trewavas (2003) as: ‘... ... an ability to access past experience so that new responses incorporate relevant information from the past’, and ‘... ... information storage of previous signalling, with the ability to retrieve the information at a much later time.’ Memory is a basic property of living beings. Any kind of hysteresis, the phenomenon of a lag in responses to changed conditions due to previous experience, shows the operation of memory. A simple experiment in plant physiology is recording of light saturation curves of photosynthesis. When irradiance on leaves coming out of darkness is increased step-wise to very high photosynthetic photon flux densities (PPFD), photosynthesis follows a saturation curve, but at the highest densities may be photoinhibited. This involves protective molecular changes within the photosynthetic apparatus (Osmond & Grace 1995). When PPFD is subsequently decreased, the rate of photosynthesis declines again, but in this hysteretic curve the rates of photosynthesis for any given PPFD are smaller than before when irradiance was increased.

Memory is among a list of key terms used by Trewavas (2003) when he advocates ‘plant intelligence’. These key terms can be listed as: (i) plants are unitary organisms having individuality (see also Lüttge 2012); (ii) plants are capable of intra- and inter-plant communication (see also Baluška & Ninkovic 2010); (iii) plants show phenomena of learning with storage of experience in memory; (iv) plants have foresight; and (v) plants develop intentions. It is entertaining and stimulating to read the kaleidoscope of examples used by Trewavas to illustrate this. Examples abound in plant biology and possibly one might be able to easily come up with a different and similarly voluminous set of examples. However, the conceptual question is whether it is really helpful to let the first three points [(i) to (iii)] culminate in the last two points [(iv) and (v)], and to speak of plant neurobiology and consider all kinds of cellular communication in plants as synapses (Baluška et al. 2004, 2005). Is one not passing the borders here between homologies and analogies? Homologies comprise precise actual mechanisms shared by animals and plants. Analogies inferring conclusions based on the application of terms, such as foresight and intention, belonging to the realm of cognitive animal behaviour, unavoidably merge with speculative philosophical extrapolation. It appears to be wiser to avoid inflationary widening of terminology and to maintain its specificity for the sake of precision. The resulting challenge then is to find a term comprising what is homologous in plants and animals. The task needs to be resolved. A preliminary suggestion for the moment is ‘information’. In this essay we attempt to understand memory as a basic feature of life, without unjustified homologies to cognitive performance. In fact, memory has a molecular basis that can be found in all organisms.

WHOLE PLANT MEMORY

Sensitivity to stimuli and stressors

Often the stark distinction is made between organisms endowed with sensitivity (i.e. animals and humans) and plants, which are considered inert and insensitive. However, this intuitive belief is not correct. Sensitivity is not a criterion for distinction between animate and inanimate life. Plants are sensitive to many kinds of biotic or abiotic stimuli. The biotic stimuli include competition or cooperation with other plants,
wounds inflicted by herbivorous animals and attack by pests. The abiotic stimuli comprise any factor of the environment, such as light intensity and quality, water, temperature, gravity, wind and mechanical stimuli, pollutants, etc., as also listed e.g. in Goh et al. (2003). According to the biological stress concept (Selye 1973; Levitt 1980; Larcher 1987; Beck & Lüttge 1990), any factor can become a stressor if its intensity is either too high or too low. Such sensitivity is given for all organisms. It is based on various signal receptor molecules, mostly at the membrane level of cell organisation. As a testament to plant sensitivity, there is now ample evidence that plants react to such stimuli by immediately increasing their level of cytosolic free Ca$^{2+}$ (Knight et al. 1991) and by responding, in the longer term, by movements [that may be rapid (e.g. Mimosa, Venus flytrap)], by morphogenetic changes and by modification of their metabolism. Signal transduction in whole plants involves different mechanisms, such as chemical (phytohormones, Ca$^{2+}$, H$^+$ and pH), hydraulic and electrical ones (reviewed in Lüttge 2012). All of this is fairly general textbook wisdom. However, in relation to memory of previous stress, it has become essential to design experiments to dissect pathways allowing accumulation and realization of stimulation obtained, i.e. whether complex molecular storage and recall networks or direct habituation or priming-like pathways are involved.

There may actually be complex molecular storage and recall networks. Alternatively, there may also be more linear pathways, which we can call ‘habituation’ or ‘priming’. We have used the term ‘habituation’ earlier for the form of memory in which perception of previous stimuli modified the transduction of a new stimulus (for review, see Thellier 2011). We realise the problems of terminology. Authors interested in the ‘learning’ behaviour of lower animals use the term ‘habituation’ as a synonym for ‘familiarisation’. Thus, another term in the general context is ‘learning’. However, this has a strong anthropomorphic touch, although ‘learning’ is also used for computer programs and other machines. In the botanical literature on phytopathology and herbivory the term ‘priming’ is used (Baluška & Ninkovic 2010). In this essay we use the terms ‘habituation’ and ‘priming’ synonymously, and it should be clear that in this way we distinguish this type of memory from storage/recall networks (STO/RCL); experimental distinction of the two is reviewed in sections Examples of the STO/RCL form of plant memory and Examples of the ‘habituation’ (or ‘priming’) form of plant memory, respectively.

**Distinction between the ‘STO/RCL’ and ‘habituation’ forms of memory**

At the beginning of the 1980s, plants were shown to be able to ‘memorise’ morphogenetic information following an appropriate stimulus (Thellier et al. 1982). Since then, this memorisation ability of plants has been confirmed by many authors using a variety of plants and of types of stimulation. The various cases studied were distributed into two different categories (Trewavas 2003): the first involves storage of information and recall of that information at a later time, and the second, where the perception of one or several stimuli changes the way the plant transduces one or several subsequent stimuli. These have been termed the ‘STO/RCL’ and ‘habituation’ forms of memory, respectively, while the term ‘priming’ has often been used in preference to ‘habituation’ in defence physiology (Bruce 2010; Heil 2010; van Hulten et al. 2010). For reviews giving examples of the two forms of plant memory, see for instance: Thellier et al. 2000, 2012; Trewavas 2003; Taffreau et al. 2006; Ripoll et al. 2009; Thellier 2011.) In our following text we will retain this distinction between two forms of plant memory because it is a convenient way to describe the experimental observations. However, in future work, it might be interesting to investigate experimentally if these two forms really involve the operation of specific molecular mechanisms, or if one form is simply a particular case of the other form. The reason is that cases exist in which the RCL function of a STO/RCL form of memory can become blocked ‘on’ under appropriate circumstances (see section Calcium condensation/decondensation and the operation of the RCL function) and, in such cases, the distinction from an ‘habituation’ form of memory becomes less clear than when the RCL function can be switched ‘on’ and ‘off’.

**Examples of the STO/RCL form of plant memory**

The STO/RCL form of memory has been especially studied by two groups from the French universities of Clermont and Rouen, using three experimental seedling systems. These systems will be termed STO/RCL1 to STO/RCL3 in the following text. Moreover, a fourth system (termed STO/RCL4), which was studied by a second group from the university of Clermont, can probably also be related to the STO/RCL form of memory.

In STO/RCL1 (Desbiez et al. 1983, 1987; Thellier 2011; Thellier et al. 2012), seedlings of Bidens pilosa L. (or of other plants) were subjected to a stimulus (cotyledon pricking or deposition of droplets of appropriate solutions on the cotyledons) shortly after germination. This did not change the rate of elongation of the hypocotyl as long as the seedlings were left in a classical nutrient solution. As soon as the seedlings were transferred into pure water, the rate of hypocotyl elongation was significantly reduced (while transferring the seedlings from nutrient solution to water had no important effects on hypocotyl elongation compared with non-stimulated seedlings). The interpretation was that the pricking stimulus induced the storage of hypocotyl elongation–inhibition information (STO function) and that transfer of the seedlings to water enabled them to recall stored information and let it take effect in the control of hypocotyl elongation (RCL function).

In STO/RCL2 (Desbiez et al. 1991a,b; Thellier 2011; Thellier et al. 2012), 2- to 3-week-old seedlings of B. pilosa (or of other plants) were grown under conditions of limited mineral nutrition and light. When the terminal bud was removed (seedling decapitation), usually one of the cotyledonary buds started to grow significantly before the other one (thus breaking the symmetry of bud growth). Defining one cotyledon of each seedling as being cotyledon A and the other as cotyledon B, with the cotyledonary buds a and b, one can introduce a symmetry index, $g$, with

\[ g = \frac{(n_b - n_a)}{n} \]

in which $n_b$ and $n_a$ are the numbers of seedlings where it is bud $b$ or $a$, respectively, that is the first to start to grow in a population of $n$ seedlings. If $g \approx 0$, this means that the seedling population is globally symmetrical with respect to bud growth, whereas $-1 \leq g < 0$ and $0 < g \leq 1$ means that the seedling population is asymmetrical in favour of bud $a$ or $b$, respectively. When the seedlings are maintained in the absence of any
asymmetry, as could be expected, the observed g-values are always close to zero. When the seedlings have been asymmetrically stimulated (e.g. stimulation of only cotyledon A), the g-values are now close to zero when seedlings were decapitated in the middle of the day, and become positive when seedlings were decapitated at the onset of daylight. This also supports the idea that the recall function may be associated with biological timing in the seedlings (section Memory and the Biological Clock). Moreover, the addition of treatments that do not lead to any asymmetry per se (e.g. conditions of seedling decapitation, symmetrical stimulation of the cotyledons, thermal treatment) can cause g to shift from zero to positive values, or vice versa. The interpretation is that the asymmetrical stimulation has induced the storage of bud growth symmetry-breaking information (STO function), while the experimental conditions or the addition of symmetrical treatments enable/disable the seedlings to recall stored information and let it take effect in the control of bud growth (RCL function).

In STO/RCL3 (Verdus et al. 1997, 2007, 2012; Tafforeau et al. 2002a,b, 2004), when flax seedlings were subjected, shortly after germination, to a variety of physical stimuli immediately followed by a transient depletion of Ca\(^{2+}\), epidermal meristems were produced in the seedling hypocotyls during the following 2–3 weeks. When the transient depletion of Ca\(^{2+}\) was delayed relative to stimulation, the production of meristems was correspondingly delayed. The interpretation again involves the intervention of two functions: the stimulus induces storage of meristem production information (STO function) and the transient depletion of Ca\(^{2+}\) enables the seedlings to recall stored information and let it take effect in the promotion of meristem production (RCL function).

In STO/RCL4 (Boyer et al. 1979; Bourgeade et al. 1989), tissue cultures of calli were derived from stimulated bryony (Bryonia dioica Jacq.) internodes. When a growing internode of a bryony plant was stimulated through repeated, gentle rubbing, this tended to increase several peroxidase activities in this internode. These increased activities were maintained in a few successive callus subcultures, and then decreased back to the level in non-stimulated internodes after a number of subcultures (one subculture per month), dependent on the type of peroxidase activity under consideration. In that case, it is assumed that the rubbing stimulus induces the storage of peroxidase activity-increase information (STO function), while the stimulated internode and the first calli derived from it would spontaneously be able to recall the stored information and let it take effect through increasing peroxidase activities (RCL function spontaneously ‘on’); but it is not known whether the final decrease of peroxidase activities corresponds to a progressive fading of stored information or to the eventual switching ‘off’ of the RCL function.

The 13 characteristics listed below for the STO/RCL form of memory can be inferred from the combination of data obtained in a large number of experiments carried out with systems STO/RCL1 to STO/RCL4 (see the original publications for detail).

1. A great diversity of stimuli is liable to induce information storage (STO/RCL1 to STO/RCL4): touching, wounding, pricking, drought, cold shock, slow cold treatment, simple plant manipulation, deposition of droplets of solutions of diverse (but not all) types of molecules and even electromagnetic radiation such as used in mobile phones. Plant sensitivity to electromagnetic radiation was unexpected because mobile phones are too recent for plants to have had the time to become adapted to them; but this sensitivity has now been confirmed and studied in great detail by Alain Vian, Françoise Paladian and co-workers in Clermont (Roux et al. 2006; Vian et al. 2006). The authors made sure that the effect of the electromagnetic radiation, under their experimental conditions, was not due to heating. A possible interpretation might lie in the photon ability to interact with molecules possessing chemical bonds with a binding energy close to that of the impinging photons.

2. Pharmacological agents preventing the transient elevation of cytosolic Ca\(^{2+}\) that follows stimulus perception also prevent information storage (STO/RCL3).

3. There is a restricted period of vulnerability to these pharmacological agents, which is less than 2 min after the stimulus for mechanical stimuli and over 5 min for other abiotic stimuli. After that period of time, information is firmly stored, whether the pharmacological agents are still present or not (STO/RCL3). In this respect, plant memory somehow resembles memory in higher animals, which is not immediately acquired in a stable form but undergoes a process of consolidation over time (Dudai 2004; Lesburguères et al. 2011).

4. The perception of a stimulus by plants and the corresponding information storage is accompanied by rapid and often transient proteome modifications [including protein phosphorylation (STO/RCL3)] that are specific for the stimuli perceived [(STO/RCL3); Henry-Vian et al. 1995; Tafforeau et al. 2002a]. Substances controlling progression of the cell cycle may be involved (Desbiez et al. 1998) and perhaps also some ‘memory metabolites’ (Ueda & Nakamura 2006).

5. However, what are memorised by the plants are not the stimuli themselves but the types of metabolic/morphogenetic response that the plant should make to these stimuli. This is extremely different from the memory in higher animals and humans, in which the very facts, events and feelings are memorised. Under the (rather artificial) experimental conditions described above, plants stored information of hypocotyl elongation—inhibition, bud growth symmetry breaking, meristem production or peroxidase activity increase. Under natural conditions, in which it is crucial for a plant to optimise the relative allocation of its resources to growth and defence (Herms & Mattson 1992; Gayler et al. 2006, 2008; Gayler 2010), it may be expected that plants will memorise the optimal balance of their resources between the requirements for growth and defence.

6. There is an apparent contradiction between the data obtained with STO/RCL2 (in which memorisation appeared as an all-or-nothing process) and those with STO/RCL1 and STO/RCL3 (stored information dependent of the number and intensity of the stimuli perceived). However, it may be noted that in STO/RCL2, the response (as characterised by the g-index) corresponds to a population of plants and not to each individual plant; therefore the all-or-nothing character of that response is probably more in the definition and use of the g-index than in the properties of the biological material. A simpler explanation
may be based on the fact that the g-value was either close to zero (symmetrical response) or different from zero (asymmetrical response). The result therefore had necessarily an all (symmetrical)-or-nothing (non-symmetrical) character. However, this would still need to be tested statistically. From the relevant sets of data (STO/RCL1 and STO/RCL3), we may infer that plant memory consists of storing information governing the integrated response to be made to the variety of signals (and their fluctuation) perceived over the course of time.

7 When the site perceiving the initial stimulus was different from that where the final response took place, information initiated by the stimulus was transported from the first to the second site at a rate of ~100 μm·s⁻¹, and was finally stored in this second site (STO/RCL1 and STO/RCL2). In STO/RCL2, a slow electrical wave was particularly well correlated with the transfer of information from the stimulated to the reacting area. Electrical signalling thus is probably involved in information migration in plants. However, since laying a droplet of a solution of diverse substances on a cotyledon had an effect similar to that of pricking this cotyledon (STO/RCL1 and STO/RCL2), this could mean that these substances play a part in the perception of wound signals and their migration via the dragging of such substances in the sap flow.

8 The ‘storage period’ (i.e. lapse of time between the perception of the initial stimulus inducing information storage and the recall and expression of stored information) can always be long in the STO/RCL form of memory: it has been shown to be at least equal to 2 days in STO/RCL1, 8 days in STO/RCL3, 2 weeks in STO/RCL2 and up to several months in STO/RCL4.

9 Plants are enabled/disabled to recall stored information (RCL function turned ‘on’ or ‘off’) by treatments specific to each individual case under consideration: plant growth and the closure of a leaf of the Venus flytrap (Hab5) can be initiated by a series of sub-threshold stimuli also cause closure when their sum equals the threshold value (Volkov et al. 2008). The history of phosphate levels (Hab6) in the media affects the way that the uptake system adapts when Anabaena variabilis cells are transferred from phosphate-poor to phosphate-rich medium (Falkner & Falkner 2003).

10 The RCL function is easily turned from ‘off’ to ‘on’ or vice versa (STO/RCL1 to STO/RCL3), at least as long as the storage period is not too long (≤ 2 days in STO/RCL2); with longer stimulus–response periods (e.g. 14 days in STO/RCL2), the RCL function was blocked ‘on’ (i.e. the seedlings were always able to recall stored symmetry-breaking information). In STO/RCL4, the stimulated bryony internode and the first calli derived from it were spontaneously able to recall stored information (RCL function spontaneously ‘on’).

11 Stored information can be repeatedly recalled (STO/RCL2 and STO/RCL3).

12 In STO/RCL2, the oscillation of the g-values as a function of the lapse of time between the application of an asymmetric and a symmetric stimulus, and the sensitivity of the RCL function to the time of day when seedling decapitation occurred, suggest that the RCL process may be related to internal rhythms of the plants.

13 Whether plants were stimulated (STO ‘on’) before or after being enabled to recall stored information (RCL ‘on’) did not change the final response (STO/RCL1 to STO/RCL3). This means that the RCL and STO functions can be activated independently of one another.

**Examples of the ‘habituation’ (or ‘priming’) form of plant memory**

In these cases, stimulus perception modifies the way in which plants transduce subsequent stimuli, either at the level of the early elevation of cytosolic Ca²⁺ or at that of the final response. The effect is sometimes due to a single initial stimulus or to a series of stimuli. Six examples of the habituation form of memory (termed Hab1 to Hab6) will be considered below.

1. In Nicotiana plumbaginifolia Viv. seedlings (Hab1), a wind stimulus causes cytosolic Ca²⁺ to rapidly increase; but repeated wind stimuli make the plant cells refractory to further Ca²⁺ signalling for ~1 min (Knight et al. 1992).

2. In Arabidopsis thaliana (Hab2), hyperosmotic stress pretreatment increases the elevation of cytosolic Ca²⁺ due to hyperosmosis, while an oxidative stress pretreatment reduces it (Knight et al. 1998).

3. Again in A. thaliana (Hab3), cold pretreatments attenuate the increase of cytosolic Ca²⁺ due to cold shock (Plieth et al. 1999).

4. In graminaceous coleoptiles (Hab4), gravitropic stimulation results in stabilisation to counter-stimulation of equal strength when the time lapse between the two stimuli exceeds 90 min (Nick & Schäfer 1988).

5. The closure of a leaf of the Venus flytrap (Hab5) can be initiated by an electrical stimulus above a threshold value; but a series of sub-threshold stimuli also cause closure when their sum equals the threshold value (Volkov et al. 2008).

6. The history of phosphate levels (Hab6) in the media affects the way that the uptake system adapts when Anabaena variabilis cells are transferred from phosphate-poor to phosphate-rich medium (Falkner & Falkner 2003).

In these examples of the ‘habituation’ form of memory, information storage clearly occurs, as it did in the STO/RCL form. We have not found any mention of the intervention of a RCL function; it is as if the plants were always able to recall stored information; but this is not necessarily a major distinction with the STO/RCL form where we have seen that RCL was sometimes blocked ‘on’ (STO/RCL2 and STO/RCL4).

However, the storage periods seem to be much smaller in the habituation form than in the STO/RCL form: of the order of minutes (Hab1 and Hab4) instead of days, weeks or months (STO/RCL1 to STO/RCL4). Therefore it is still difficult to decide if the habituation form of plant memory is simply a particular case of the STO/RCL form, or whether these two forms correspond to basically different processes.

Elementary forms of memory have been associated with ‘learning’ in lower animals. Two complementary variants have been described. The first may be termed ‘familiarisation’, a process through which an animal learns to ignore a harmless stimulus after repeated perception of that same stimulus. The second, opposite to the preceding definition, is the ‘sensitisation’, in which the repetition of an unpleasant or painful stimulus induces increasingly violent responses (Lodish et al. 2000). The ‘habituation’ form of memory is somehow equivalent in plants to the learning memory of animals, and behaviours...
comparable to familiarisation (Hab1 and Hab3) and sensitisation [response to hyperosmotic treatment in (Hab2)] also occur.

**CALCIUM AND THE OPERATION OF WHOLE-PLANT MEMORY FUNCTIONS**

**Calcium waves and operation of the STO function**

*Calcium waves*

In the cell cytosol, the Ca²⁺ concentration is normally very low, but the cell possesses Ca²⁺ stores (cell wall, vacuole, etc.) that are connected with the cytosol via inositol-3-phosphate (IP₃)-dependent channels. IP₃ is a signalling molecule that is produced from cell membrane constituents under the effect of a particular enzyme, termed phospholipase C. The channels are normally closed. Some of them open under the effect of an electrical potential, membrane stretch and a variety of other external or internal signals. Other channels then open according to an autocatalytic process involving the binding of both IP₃ and Ca²⁺: briefly, the Ca²⁺ released at the mouth of an open channel favours the opening of neighbouring channels. A wave of Ca²⁺ thus enters the cytosol. This Ca²⁺ increase in turn activates ATPases that pump Ca²⁺ back to the stores, and the channels close. Depending on their action on the respective rates of IP₃ formation and degradation, on the location of the phospholipase C and other enzyme molecules, on the characteristics of Ca²⁺ distribution, etc., different signals will induce Ca²⁺ waves of different shape, form and kinetics. Ca²⁺ waves thus react specifically to the signals perceived; then they induce downstream processes such as the opening of K+ and Cl⁻ channels or the Ca²⁺-dependent expression of certain genes (Trewavas 1999). Calcium waves are known to play a crucial role in a variety of cellular functions in animal physiology, from the initial activation of the fertilised egg to the final, irreversible failure of Ca²⁺ wave generation in the heart as a frequent cause of death (Trewavas 1999). Ca²⁺ waves have also been shown to occur in plant cells (Trewavas 1999), for instance in pollen tubes (Franklin-Tong et al. 1996), Fucus rhizoids (Taylor et al. 1996) and guard cells (Gilroy et al. 1991; Grabov & Blatt 1998). Ca²⁺ waves provide a logical explanation for the transient elevation of cytosolic Ca²⁺ that follows stimulus perception by plants.

*Information storage in plant cells*

Combining various sets of data, information storage by plants may be understood as follows. Shortly after stimulus perception, the plant reacts through a transient elevation of its cytosolic Ca²⁺ (the Ca²⁺ wave). The amplitude and duration of this transient elevation of cytosolic Ca²⁺ differ between different stimuli and are thus specific to the type of stimulus perceived.

In the case of a memory of the type ‘learning’ (habituation, priming), this is thought to orient the plant directly toward a final response adapted to the stimulus that has been perceived (Dolmetsch et al. 1997; Knight et al. 1998; Mcainsh & Hetherington 1998). This permits plants to respond rapidly to a stimulus that they have still not experienced; moreover, upon repetition of the same stimulus, an advantage of this type of memory is that the plants can progressively (i) decrease the intensity of their response (and thus correspondingly economise on their resource use) if the stimulus finally appears to be innocuous (familiarisation), or (ii) increase the intensity and rapidity of their response, thus becoming better and better adapted to resist an event that they have already experienced to be dangerous (sensitisation).

In the case of a memory of the type STO/RCL, the transient elevation of cytosolic Ca²⁺ results in the storage (but not necessarily in the immediate expression) of morphogenetic information relative to the final response to be made to the stimulus that has been perceived (Verdus et al. 2007). Moreover, when different stimuli are perceived successively, it is likely that the information that was stored after the first stimulus is modulated according to subsequent stimuli. Hence, the advantages of the STO/RCL type of memory are (i) that it permits the plant to progressively organise an averaged response adapted to the entirety of the environmental stimuli and stresses that it perceives over the course of time at the site where it is located, and (ii) that it prevents the plant from effectively responding unless the environmental conditions are appropriate for the RCL function to be switched ‘on’.

*Calcium condensation/decondensation and the operation of the RCL function*

An interesting peculiarity of the RCL function is that it is often easily switched ‘on/off’ (or vice versa), but may also sometimes be blocked ‘on’ or ‘off’. The exact process involved in the control of such abilities is still not known, although Ca²⁺ condensation/decondensation appears as a good candidate for that (Fig. 1).

*Calcium condensation/decondensation*

Consider (Manning 1969) a 1-D structure bearing negative charges that is bathed in a solution containing co-ions and counter-ions that are either divalent or univalent. (i) When the density of anionic sites, ξₒ, on the structure is low, the co- and counter-ions diffuse freely in a form of an ionic cloud. (ii) If some event causes ξₒ to increase to a value, ξₑ, which is above a critical value, ξₑ, counter-ions ‘condense’ on the 1-D structure until ξₑ decreases back to the critical value, ξₑ. Divalent ions (e.g. Ca²⁺) condense before univalent ions. Condensed ions are free to move along the charged 1-D structure but, even at infinite dilution, they remain in close vicinity to this structure.

![Fig. 1. De-condensation A: and condensation B: as a threshold phenomenon at a negatively charged one-dimensional structure. Divalent cations, e.g. Ca²⁺ (++), monovalent cations (+) and counter anions (−).](image-url)
This threshold-dependent phenomenon does not obey mass-action law and may result in abrupt modifications to the relative concentration condensed/decondensed Ca\textsuperscript{2+} (Fig. 1).

Information recall in plant cells

Cells contain networks of ionised, negatively charged 1-D structures (e.g. nucleic acids, cytoskeleton, 1-D assemblies of proteins) that may engage in the process of counter-ion condensation (Ripoll et al. 2004). Moreover, via pH modifications or chemical reactions such as protonation/deprotonation, methylation/demethylation, etc., the events acting on the activation/deactivation of the RCL function can begin by modifying the $\xi$-value of such structures.

We assume, as a working hypothesis, that Ca\textsuperscript{2+} condensation/decondensation plays a decisive part in the recall of stored information by plants via the activation/inactivation of Ca\textsuperscript{2+}-dependent processes (including kinases and phosphatases). When Ca\textsuperscript{2+} is condensed, the Ca\textsuperscript{2+}-dependent processes that are linked to the charged 1-D structures are activated, while those in the bulk are inactivated; and the reverse is true when Ca\textsuperscript{2+} is not condensed. A modification of the $\xi$-value (rendering it larger or smaller than $\xi_c$) will thus act as a switch in the overall functioning of the system. Moreover, when the $\xi$-value is not very different from $\xi_c$, $\xi$ can easily be rendered reversibly larger or smaller than $\xi_c$; hence, the RCL function can be easily turned on/off or vice versa. In contrast, when $\xi$ is much above or much below $\xi_c$, it becomes more difficult (or even impossible) to cause $\xi$ to decrease below or increase above $\xi_c$, respectively; the RCL function will then seem to be blocked (either ‘on’ or ‘off’).

The possible role of Ca\textsuperscript{2+} condensation in the control of plant responses

Since the STO function can be switched from ‘off’ to ‘on’ and the RCL function from ‘off’ to ‘on’ or vice versa, some sort of switch must exist in the organisation of each of these two functions. A Ca\textsuperscript{2+} wave almost systematically occurs as an early switch must exist in the organisation of each of these two functional processes that are linked to the charged 1-D structures are activated, while those in the bulk are inactivated; and the reverse is true when Ca\textsuperscript{2+} is not condensed. A modification of the $\xi$-value (rendering it larger or smaller than $\xi_c$) will thus act as a switch in the overall functioning of the system. Moreover, when the $\xi$-value is not very different from $\xi_c$, $\xi$ can easily be rendered reversibly larger or smaller than $\xi_c$; hence, the RCL function can be easily turned on/off or vice versa. In contrast, when $\xi$ is much above or much below $\xi_c$, it becomes more difficult (or even impossible) to cause $\xi$ to decrease below or increase above $\xi_c$, respectively; the RCL function will then seem to be blocked (either ‘on’ or ‘off’).

Molecular memory

The ubiquitous molecular nature of memory

Memory generally has a strong molecular nature. Not only plants and animals, but certainly all living organisms that have (i) biomembranes, (ii) polynucleotides and (iii) proteins can develop memory. Bases for building up memory that are shared among organisms, for example, are electrical phenomena, epigenetics with histone and chromatin methylation and acetylation (see section Molecular model of epigenetic memory), enzyme modifications with phosphorylation/dephosphorylation (protein kinases/phosphorylases), changing redox poise (thioredoxins) and effects of Ca\textsuperscript{2+}. Transient protein phosphorylation shortly after a stimulus was related to the STO/RCL memory (Tafforeau et al. 2006).

That prokaryotic bacteria develop memory can be readily illustrated from studies of substrate induction, which led to the regulator–operator theory of gene regulation of François Jacob and Jacques Monod (Nobel Prize in 1965). They showed that in Escherichia coli the β-galactosidase as a substrate could induce expression of the gene for the enzyme β-galactosidase required for lactose metabolism through inactivating a repressor. When the substrate is removed, the cells will remember that they can metabolise lactose for a certain time until turnover extinguishes this again. The regulator–operator mechanism is a general concept and evidently implies a particular type of molecular memory.

Epigenetic stress memory

History and definition of epigenetics

The basic idea of epigenesis, as a principle producing the gestalt of organisms, dates back to Johann Friedrich Blumenbach (1752–1840; see Gierer 1998). It was picked up again by Conrad Hal Waddington (1905–1975) in the early 1930s, who gave it more precision. He used the metaphor of an ‘epigenetic landscape’. This is a hilly landscape where balls may roll down following various slopes into different valleys where they always end up at the deepest point at the bottom of the respective slope. Each of the points offers a particular programme of differentiation and development.

In this vein, molecular epigenetics is a system of reading the genetic information of DNA. A naturally occurring example is observed in populations of the ubiquitous ruderal plant Linaria vulgaris Mill., characterised by bilateral symmetry of its yellow flowers. There is a rare different form with radial symmetry of the flowers. Charles Linné took it for a different genus, which he named Peloria. We now know that both have identical DNA profiles. The only difference is methylation of the promoter DNA of a single gene (cycloidea, Lcy) in Peloria (Paulsen 2007; Daxinger & Whitehall 2010). The Lcy DNA sequence is the same in both forms, which are distinguished by a heritable epimutation involving DNA methylation in the peloric phenotype (Cubas et al. 1999).

The molecular mechanism of epigenetic regulation is based on the structure and conformation properties of chromatin modulated by acetylation and methylation, respectively, of
DNA and nucleosomal histones. In the DNA, the cytosine groups are methylated. In the histone proteins, the lysine and arginine residues are post-translationally modified (Yaish et al. 2011), i.e. through acetylation/methylation (Grunstein 1997; Zhang & Reinberg 2001), ADP ribosylation (Tanigawa et al. 1984), glycosylation (Cervantes-Laurean et al. 1996), phosphorylation (Lo et al. 2001) or ubiquitination (Sridhar et al. 2007).

Modulation of the DNA if stable for shorter or longer periods constitutes a molecular memory, which we call here epigenetic memory. In the state of acetylation DNA is accessible for regulator molecules of gene activation or deactivation due to the larger size of the acetyl group as compared to the smaller methyl group. Methylation leads to repression of gene transcription, and the genetic information is silenced (Chinnusamy & Zhu 2009). A molecular definition is that epigenetics is ‘The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence’ (V. E. A. Russo, R. A. Martienssen & A. D. Riggs, 1996, quoted from Bird 2002). For quantifying histone and DNA methylation, various methods are available. A comprehensive methylation map of the entire genome of *A. thaliana* as a model has been obtained, presenting the DNA ‘methylo-sequence’ (Richards 2006; Zhang et al. 2006; Zhu 2008). A functional analysis with high-resolution genome-wide characterisation of DNA methylation in *A. thaliana* underlines the overarching role that gene methylation exerts in the control of biological functions of genes (Zhang et al. 2006).

**Molecular model of epigenetic memory**

Nevertheless, the precise molecular mechanism of epigenetic memory function and the mediators of epigenetic memory expression are still largely unknown (Saze 2008). However, progress in the vigorously unfolding field of epigenetics already offers a number of building blocks that can be used to develop a scheme for the operation of epigenetic stress memory. This is provided in Fig. 2. Epigenetic modifications comprise methylation of both histones and chromatin. Histones play a key role in chromatin structure (Zhang 2008; Chinnusamy & Zhu 2009); thus, while histone modifications also modulate chromatin modifications, for the sake of simplicity, in the following we only consider chromatin. Describing the various modules of the scheme, we follow the numbers given in Fig. 2.

1. **Epigenetic variations can be triggered by external stimuli and environmental cues** (Jablonska & Lamb 1989; Boyko & Kovalchuk 2008; Alvarez et al. 2010; Chen et al. 2010; Yaish et al. 2011). There is increasing evidence that both histone and chromatin methylation patterns are strongly modified by environmental stress (Molinier et al. 2006; Bond & Finnegan 2007; Chinnusamy & Zhu 2009; Adams 2010; Daxinger & Whitelaw 2010; Verhoeven et al., 2010), such as salt stress (Wang et al. 2010), drought stress (Baek et al. 2011), nutrient stress, e.g. nitrogen deficiency (Kou et al., 2011), and chemical induction of anti-herbivore and anti-pathogen defences (Verhoeven et al. 2010).

2. The step following stress reception is signalling for chromatin modification. This may involve chemical signals such as by phytohormones, electrical signals and Ca^{2+} waves (Trewayas 2003; Thellier et al. 2012). These signals may be transcribed into particular RNA signals through stress-induced expression of micro-RNAs, for example under salinity, drought and cold (Sunkar & Zhu 2004; Shen et al. 2010; Yaish et al. 2011). It is a specific feature of epigenetics that small interfering RNAs (siRNAs) having a length of 24–26 nucleotides direct DNA methylation and histone modification (Richards 2006; Zhang et al. 2006; Bond & Finnegan 2007; Saze 2008; Zhang 2008; Chinnusamy & Zhu 2009). Small RNAs are mobile in the symplast via plasmodesmata and in the phloem. They can be transmitted within plants and function as systemic signals produced following stress (Saze 2008).

3. **Modified chromatin is the basis for epigenetic memory** (Bond & Finnegan 2007).

4. A central issue of the scheme is transfer of the epigenetic stress memory. A requirement for retaining methylation as the stress memory is that stress-induced methylation patterns are not reset to the basal level when the stress is relieved (Chinnusamy & Zhu 2009). Transfer could occur through cell divisions, both mitotically and meiotically; both appear to be possible (Molinier et al. 2006). Mitotic transfer constitutes a more short-term epigenetic stress memory. Nevertheless, in perennial plants, including long-lived trees, memory can be retained for as long as the change in seasons. Carry-over effects of acid rain and ozone on photosynthesis have been observed from one season to new flushes of loblolly pine seedlings (*Pinus taeda* L.) in the following season, before experience of any stress in the new season (Sasek et al. 1991). Such ‘ozone memory’ was also demonstrated in *Pinus sylvestris* L. and *Picea abies* (L.) Karst. The visible symptoms appearing during spring emergence of a new flush following the previous season with ozone exposure included premature shedding of needles. The stress metabolite catechin is known to be involved (Langebartels et al. 1998). Analysis of methylation of the genes of catechin metabolism might potentially open an avenue for assessing the involvement of epigenetic memory. A rather long-term trans-generational stress memory is given as meiotic transfer of the methylation state (Saze 2008). With meiotic transfer, epigenetic information of stress received by plants can be transferred to subsequent generations. Stress-induced methylation changes that are not reset can be transmitted through the germ-line and are mostly heritable. They can be transferred through several generations (Jablonska & Lamb 1989; Bird 2002; Molinier et al. 2006; Bond & Finnegan 2007; Saze 2008; Verhoeven et al. 2010). In plants this is facilitated because reproductive lineages or germ lineages are developed from vegetative or somatic lineages late in development, and therefore, resetting of methylation patterns is less extensive than in animals (Richards 2006). Transfer is possible through both female and male gametates (Molinier et al. 2006); however, differences are also seen due to the differences in chromatin structure in the gametates as a consequence of the different ways in which the chromatin is packaged in sperm/spermatozoa and egg (Jablonska & Lamb 1989). This may lead to parental genomic imprinting and epigenetic asymmetry in inheritance of male and female parental alleles marking their parental origin (Guitiérrez-Marcos et al. 2006). The mechanism for transfer during mitotic and meiotic cell divisions is based on the function of maintenance of cytosine-DNA methyltransferases (Henderson & Jacobsen
For gene expression creating phenotypes, it is noted that i.e. regulated by methylation and demethylation the methylation status of modified chromatin as it is dynamically regulated by methylation and demethylation via methyl transferases and demethylases, respectively (Gong et al. 2002; Saze 2008). A question is whether the transfer carrying the epigenetic stress memory onwards is: (i) through the methylation status is transferred, i.e. propagated (Numbers annotating modules and functions are explained in the text.), and (ii) after the transfer if the changed epigenetic marker is inherited with their epigenetic information intact (Bond & Finnegan 2007), or (ii) only through the changed markers on genes. The former (i) is supported by observations where transcriptionally silent states (i.e. methylated states) are predominantly propagated (Richards 2006). De novo methylation/demethylation must establish the information (i) before the transfer if genes are inherited with their epigenetic information intact (Bond & Finnegan 2007), i.e. the methylation status is transferred, and (ii) after the transfer if the changed epigenetic marker is transferred.

For the output, i.e. producing phenotypes during cell divisions and differentiation in development, continuous maintenance of methylation is required.

For gene expression creating phenotypes, it is noted that when promoters are methylated this is tissue-specific, and when coding regions are methylated the impact is rather at higher organisational levels (Zhang et al. 2006). Non-coding RNAs can provide a feedback to the stress signal transduction chain. Small RNAs might also be involved as a form of chaperone in recruiting the methyl transferases to target promoters or genes, although this is still debated (Matzke et al. 2001, 2007; Pikaard 2006; Zhang et al. 2006; Zhu 2008). Small RNAs also have functions in demethylation, as they bind protein required for DNA demethylation (Zheng et al. 2008). Besides RNA-effected methylation, there is also RNA-independent methylation (Zhu 2008).

**MEMORY AND THE BIOLOGICAL CLOCK**

Does the biological clock participate in memory functions? If so, how does it contribute? A clock allows measuring the flow of time, but as such it is not memory. However, a clock immediately becomes part of structure and function of memory if it contains set points. For example, an alarm clock set on a specific point in time is a reminder: it causes us to remember.

The output of biological clocks is rhythmicity based on endogenous oscillations. Most important are the circadian clocks reflecting the natural day–night rhythms with period lengths close to 24 h when running free under constant environmental conditions (Lüttge 2002). It is often said that such endogenous circadian rhythmicity is important for fitness, because it provides preparedness or alertness for regularly changing conditions in the day–night rhythm. While this appears logical common sense, the scientific evidence for it is actually quite meagre. There is largely still only one study, which is regularly cited in support of the argument (Dodd et al. 2005). In addition, we recall the much less cited work of S.S. Golden and collaborators with the cyanobacterium *Synechococcus elongatus* PCC7942. They grew mutants having different circadian periods of their endogenous clocks in co-culture under different external light/dark cycle periods. Endogenous circadian rhythms that resonated with the selective pressure of altered external light/dark cycle periods. A competitive advantage disappeared in constant environments (Ouyang et al. 1998; Johnson & Golden 1999; Woelfle et al. 2004). With a similar rationale, Yerushalmi et al. (2011) used an approach of crossing *A. thaliana* mutants with different circadian period lengths and studied the F2 and F3 generations, which they subjected to the selective pressure of altered external light/dark cycle periods. Endogenous circadian rhythms that resonated with the environmental ones were positively selected, so that the circadian clock proved to be an adaptive mechanism in *A. thaliana* that increases fitness. These are very nice studies; however they...
remain astonishingly solitary many years after the appearance of the Dodd et al. (2005) paper (see also Green et al. 2002; Hotta et al. 2007; Yerushalmi & Green 2009). Evidence for circadian rhythmicity supporting day-to-day fitness remains rare.

In contrast, overwhelming evidence for the absolute necessity of the biological clock for plant fitness comes from a huge amount of literature on photoperiodism and phenology. Plant growth and development, frost hardiness, flowering and seed production are subject to regulation by daylength or photoperiod, where phase adjustment of the biological clock is an essential mechanism in photoperiod perception (e.g. Frankhauser & Staiger 2002; Roden et al. 2002; Love et al. 2004; Ogudì et al. 2004; Fujiwara et al. 2008; Niwa et al. 2009; Ibáñez et al. 2010). In the STO/RCL3 system (see section Examples of the STO/RCL form of plant memory) meristem production reveals a seasonal rhythm (Verdus et al. 1997). Hence, we can conclude that seasonal phenological memory is stored in the clock. This means, as argued above, that there are marked set points in the clock.

Therefore, the question arises, what is the molecular basis of such set points? The molecular structure of the clock of higher plants is best studied in Arabidopsis thaliana (Nakamichi 2011). There are master genes of the clock, which are expressed in the morning (morning genes: CIRCADIAN CLOCK ASSOCIATED, CCA1 and LATE ELONGATED HYPOCOTYL, LHY) and in the evening (evening genes: TIMING OF CHLOROPHYLL a/b BINDING, TOC1), and there are further morning and evening elements functioning as transcription factors (e.g. Harmer & Kay 2005; Kikis et al. 2005; McClung 2006; Nakamichi 2011); these genes determine the phases of the clock. Downstream of these genes a vast number of other genes are controlled by the clock, the so-called clock controlled genes (CCGs). A plethora of plant functions are under regulation of the clock, including complex processes such as growth (Farre´ 2012).

In terms of memory storage, the key question, however, is how an upstream feedback might work to change existing and introduce new set points for the memory and reminder functions of the clock. This does not appear to be studied. What is investigated, though, is resetting of the clock, which we always experience after jet lag or which in more general terms is the basis of new entrainment after changed environmental phases of rhythmicity. Therefore, it is important for understanding of the relations between clock and memory to examine genes giving phase information (Michael & McClung 2002), phase variations in populations (Darrah et al. 2006) and phase mutants (Onai et al. 2004). In Arabidopsis an OUT OF PHASE 1 gene has been identified (Salomé et al. 2002).

In conclusion, it appears theoretically justified to consider the clock as a putative pathway from external cues via signalling to the STO/RCL memory (see section Modelling Plant Memory). The recall function of the STO/RCL memory breaking the symmetry of bud growth after stimulation of cotyledons (as described with the g-index explained in section Examples of the STO/RCL form of plant memory) is dependent on diurnal timing. This experimental observation is also a hint for involvement of the clock. It appears rewarding to pursue this in future investigations.

MODELLING PLANT MEMORY

With the elements discussed above, i.e. the experimentally documented STO/RCL and habituation functions (section Distinction between the ‘STO/RCL’ and ‘habituation’ forms of memory), Ca\(^{2+}\) waves (section Calcium and the Operation of Whole-Plant Memory Functions), epigenetics (section Molecular Memory) and the biological clock (section Memory and the Biological Clock), we can now attempt to construct a model of plant memory, as described in Fig. 3. This model integrates the various elements involved in the emergent functions of memory as follows:

1. The information of stimuli and environmental cues is stored so that the genes involved in the storage of the information change from a locked to an unlocked state. This may occur through direct signalling or signalling via the epigenetic memory (section Epigenetic stress memory) or the biological clock (section Memory and the Biological Clock).

2. On the basis of the unlocked genes, we distinguish habituation memory or priming (section Distinction between the ‘STO/RCL’ and ‘habituation’ forms of memory/Examples of the ‘habituation’ (or ‘priming’) form of plant memory) and storage/recall memory (STO/RCL) (see section Distinction between the ‘STO/RCL’ and ‘habituation’ forms of memory/Examples of the STO/RCL form of plant memory). Possibly the input to the habituation memory through the effectors is more direct, while the input to the STO function seems to require the network of the epigenetic memory and/or the biological clock. Nevertheless, in the model the paths via the epigenetic memory and the biological clock feed into the genes of both habituation and STO/RCL. Epigenetic stress memory is first acquired in individuals [section Molecular model of epigenetic memory points (1) to (3)], and habituation is a property of individuals. However, epigenetic stress memory can also be transmitted in a lineage of offspring [section Molecular model of epigenetic memory point (4)]. Thus, a function of memory with a rather long time constant is involved, which we might relate to the STO/RCL form. As stated above (section Distinction between the ‘STO/RCL’ and ‘habituation’ forms of memory), it remains difficult to decide if habituation and STO/RCL are fundamentally different forms or the former is only a special case of the latter, and epigenetic memory may govern both.

3. Signalling can affect ionisable site – Ca\(^{2+}\) condensation/decondensation patterns (see section Calcium and the Operation of Whole-Plant Memory Functions) activating RCL genes.

4. The RCL gene products activate the store genes.

5. The gene products of the habituation/priming memory are generated through new stimulation.

6. The gene products of the STO/RCL memory are obtained from the activated STO genes.

CONCLUSIONS

Basically, in all kinds of memory functions of living organisms, molecular mechanisms are involved, namely in reception and transformation (signal perception and transduction), and in storage and recall of information. Hence, it is not surprising that some form of memory can be found in organisms at all levels of organisation, from prokaryotes to the highest mammals. Notwithstanding the fundamental barrier between plants and cognitive animals, the molecular, biochemical and
biophysical memory functions of plants are quite sophisticated and based on complex networks. Plants have typical learning (habituation, priming) and complexly integrated store/recall systems of memory. By possessing the two sorts of memory, a plant can optimise its metabolic and morphogenetic behaviour through adapting its phenotype to the climatic and other environmental conditions prevailing at the site where it grows (STO/RCL memory), and by becoming both insensitive to harmless stimuli and more and more efficient in resisting harmful stimuli (‘learning memory’).

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