Information, Noise and Communication: Thresholds as Controlling Elements in Development

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Abstract Organisms are dependent on the continual transmission of information both within cells and from outside them. Information is concerned with the conveyance of signals that require both a transmitter and a receiver able to decide what is sent. Accuracy in transmission is degraded by noise, and the evidence that shows noisiness in genetic circuitry is described. Reliable noise coupled with positive feedback constructs probabilistic thresholds amongst a population. In higher plants, stochastic distribution of thresholds enables quantitative variation amongst cells, tissues or plants to variable strengths of signals. It is the function of information to be communicated, but the gel structure of the cytoplasm together with the ordering by structured water might instead increase noise in transmission by interfering with the necessary movement of molecules in signal transduction. To reduce potential noise in signal transmission and transduction, it is suggested that abrupt phase transitions in microdomains of the cytoplasmic gel structure are induced by cytoplasmic calcium, amongst other signals. Plasmodesmata also contain actin gels, and communication between cells may simply be controlled by abrupt gel phase transitions. Two threshold phenomena are thus seen in plant cells important during development. The first involves noise and positive feedback; the second, gel phase transition.

1 What Is Information?

Biological information is conveyed by particular sequences of signals and messages that originate within the cell or outside it. Information theory, first propounded by Shannon and Weaver (1949), stated that the information content of any message

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was determined by the probability of occurrence of a particular message as against others. Shannon and Weaver (1949) were concerned with the accuracy of transmission of messages through phone lines to a receiver and with maintaining the secrecy of transmission.

The critical components of information conveyance are firstly a transmitter of information and secondly an interpreter of the information transmitted. Following Aristotle's implied meaning towards information as 'surprise', it was argued that the more surprising the contents of the message, the greater its information content and in turn the lower the probability of its occurrence.

If, for example, the message transmitted was 'the sky is blue', little information is being conveyed since the information is of little surprise. If, on the other hand, the conveyed message is 'the sky is green and black striped', that is most certainly surprising, containing new unexpected information, thus increasing its novelty and in turn its information content. Surprise does suggest rarity, and rare things by definition occur infrequently and thus with low probability. Low probability messages are associated with strong constraints on the information transferred. These constraints can, in many cases, be related to the degree of detail in the message; the greater the detail, the greater the likely information content. The message 'the sky is deep blue interlaced with aeroplane vapour trails, there is a light warm wind and the smell of honeysuckle in the air' increases the information content too and constrains or limits the described scene compared to the first simple message, 'the sky is blue'.

Shannon drew attention to the possible relation of information content to entropy (Vedral 2010). Highly ordered systems have low entropy; disordered ones, high entropy. In terms of messages, comprehensible messages are very ordered whereas disordered messages can be uninterpretable. There are about half a million words in the Oxford English Dictionary. Only certain discrete combinations of words and in a particular order out of a truly enormous number of possible word combinations provide sensible information. For a five-word combination, there are at least 10^{25} possibilities. Five-word messages that make sense to a human receiver are probably of the order of a few thousand. Genuine messages are therefore by definition rare and note also the specific elements of interpretation that have to be present in the English-speaking receiver. A light signal is interpreted differently by a seedling stem compared to their leaves.

However, there are intrinsic problems with trying to determine the information content of any biological signal. If, for example, a plant growing in laboratory conditions, experiences a change in light intensity, that is expected information because such variations are normal for any growing plant. Variable cloud cover and sun specks lead to unexpected changes in light intensity. If, on the other hand, the change in light intensity is accompanied by a change in temperature, water availability and humidity, then the information content will be higher and may indicate the progress towards evening.

2 Noise in Transmission of Information Degrades Accuracy in Response

One of the major concerns of Shannon and Weaver (1949) was to try and estimate the accuracy of transmission of messages down phone lines. The degradation of information transmission is called noise. The effect of noise is usually to jumble or omit perceived words, and meaningless messages are more probable than meaningful ones. Thus, the relationship that Shannon developed, equating information to entropy. What he indicated is that noise in message transmission is disordering. Thus, if noise occurs in cellular messages, this may have serious consequences for either survival or interpretation of external signals.

2.1 Noise Is Likely Inevitable in Living Systems

Living cells use many thousands of chemical reactions and other molecular interactions. There is inevitable noise in such processes since many reactions are probabilistic, requiring two or more molecules to come together in a crowded cytoplasmic environment. Later in this chapter, I will indicate how the structure of the cytoplasm may interfere in these necessary events and increase unwanted biological noise. Life survives because the tendency of randomising processes at the single molecule level is however countermanded by correcting statistical forces. That is, a larger number of molecules working together tend, on average, to counteract individual stochastic events. Many control circuits have been constructed in cells to offset or reduce noise. Negative feedback is the commonest, providing information to the earlier part of the circuit to try and modulate or stabilise throughput. But one hazard of negative feedback is the delay in response and that, in itself, often makes the process noisy. Feedback really requires instant effects if it is to reduce noise substantially.

The simplest circuitry perhaps involves gene activation, transcription, translation and that immediately introduces probabilistic events that can destabilise control. DNA during transcription can change its structure; proteins necessary for transcription can drop off or change conformation and become non-functional for periods of time. In other transduction circuits, signalling complexes have to be formed from large numbers of soluble proteins aggregating together; delays and failures in construction must inevitably be common. Channels for ion signals, detected using patch clamp, are observably noisy. Noise is endemic, and the problem that arises is how individual living cells can manage and survive within that framework of noise.

2.2 Evidence for Noise in Genetic Circuitry

The evidence for noise in transcription/translation is extensive, and a variety of single bacterial, yeast and cultured cell systems have been used to demonstrate its presence. The methods developed to demonstrate noise have all marked milestones in technical achievement. Suitable fluorescent probes with some superb microscopy have enabled comparison of copy numbers of specific proteins and mRNAs between individual cells from the same culture. Lango and Hasty (2006) list 25 papers that have used this technology. The ultimate has been the imaging of the synthesis of individual protein and mRNA molecules.

The most common detection of noise has been to compare copy numbers of both specific mRNA and specific proteins between single cells. Greater noise between individual cells is to be expected in proteins that are expressed in small rather than large abundances, and this has proved usually to be the case (Federoff and Fontana 2002). Elowitz et al. (2002) defined two kinds of noise in protein copy numbers/cell that they observed. Intrinsic noise was defined as the variation in expression between two identical genes in the same cell. Extrinsic noise was considered global within the cell reflecting, for example, variations in polymerase numbers or other regulatory proteins affecting many transcription events. Intrinsic noise disappeared more quickly than extrinsic noise when cells were followed through cell cycles.

Lack of correlation between a specific mRNA level and its protein product are considered to originate from the differential stability of both; mRNA in bacterial cells, for example, decays stochastically within a few minutes, proteins are far more stable (Taniguchi et al. 2010). Transcription rates, regulatory dynamics and genetic factors all contribute to the amplitude of noise (Elowitz et al. 2002). Rosenfeld et al. (2005) measured the quantitative relation between transcription factor concentration and the rate of protein production from the downstream gene (so-called gene regulation function) and observed how the ratio between these two fluctuated dynamically, thus limiting the accuracy of genetic circuitry. Textbook models that picture transcription factors binding to DNA and protein synthesis continuing in an orderly level thereafter are clearly very misleading.

The range in copy number of a single protein species between individual cells can be enormous. Careful measurements using a technique that could measure individual protein molecules indicated up to 15-fold variation (Taniguchi et al. 2010). If the genetic circuitry incorporated positive feedback at some stage in their control sequence, then noise itself was sufficient to enable the induction of two distinct phenotypes. To and Maheshri (2010) introduced a promoter with a single binding site for an effector molecule or seven binding sites for the same effector in a system with positive feedback in the control loop and showed that noise was able to induce bistable states without any change at all in effector concentration; some cells were spontaneously switched on, others not.

2.3 Noise Can Spontaneously Induce Polarity and Ensure Each Cell Is Effectively a Unique Phenotype

Similar and significant results of To and Maheshri (2010) were found in the establishment of yeast polarity in an unpolarised cell. Membrane-bound signalling molecules able to recruit from a cytoplasmic pool with positive feedback and in limited cytoplasmic copy number (and thus noisy), spontaneously established a site of polarity in yeast (Altschuler et al. 2008). Such results have obvious significance for the establishment of polarity in many stages of plant development. Deterministic models would not of course predict this unexpected outcome that must result from noise-induced variations in the conformation of either the promoter or the membrane-binding protein, in this case, CDC42. In *E. coli*, a single-chance event, the spontaneous dropping of a repressor from DNA in the *lac* system can introduce a bistable condition in which lactose floods into the cell and switches on the lactose metabolising system (Pearson 2008).

Noise in an upstream gene due to transcription factor variation can be transmitted to downstream genes (Pedraza and van Oudernardene 2005). Further observations of complexity were made when a number of different gene products were all imaged in single cells at the same time. Analysis of 11 genes altogether indicated that each cultured cell produced its own unique pattern of gene expression, thus generating individual phenotypes (Levsky et al. 2002).

2.4 Transcription and Translation in Single Cells Takes Place in Brief Bursts

One surprising feature that has emerged from observations of single mRNA or single protein molecule production is that synthesis takes place in bursts rather than continuously, thus again contradicting textbook models. By constructing a special technology for visualising individual mRNA molecules for a single gene, Golding et al. (2005) were able to image the production of single mRNA species and found that throughout the period of observation, the gene was active in bursts producing between 1 and 8 molecules each time, but synthesis only occupied 10% of the observation period. By imaging the appearance of single protein molecules in a single bacterial cell by fluorescence, Yu et al. (2006) observed patterns of stochastic bursts in synthesis with long periods of inactivity. There were usually only 1–2 bursts in synthesis/cell cycle, and the numbers of molecules/burst followed a simple power series. Synthesis of p53 in human cells oscillated with different frequencies between single cells after stimulation by radiation (Geva-Zatorsky et al. 2006). Bursting characteristics in synthesis obviously tends to randomise production in time.

With a delay between the synthesis and degradation of any molecule, Pedraza and Paulsson (2008) observed that a simple memory was created. Sigal et al. (2006),

using human cells, observed that different proteins within one metabolic pathway showed less variation than between proteins in other pathways. They quantified the levels of some 20 different protein species and reported that the high or low noise variability could last at least between two cell cycles. Again, they indicate that this is a kind of molecular memory. The persistent memory for protein levels might induce cell individuality. Memory can only be present however if something has first been learnt. The learning mechanism involves the variable synthesis of specific proteins in this case, and such learning and memory capabilities are equally present in plant cells (Trewavas 1999).

Cells of the same type can again generate diverse physiological traits. A further study that labelled 2,500 proteins in yeast under different growth conditions found that there were dramatic specific-protein differences in noise that were correlated with function (Newman et al. 2006). However, these authors also reported that there was much greater noise in the proteins that respond to environmental signals, whilst those involved in protein synthesis were much quieter.

2.5 Is Noise Useful or If Not, Can It Be Reduced?

By engineering mutations into a control region of genes that confer antibiotic resistance in yeast, Blake et al. (2006) constructed two strains that differed in the noisiness of their expression. When incubated in a normally lethal concentration of antibiotic, the noisier strain survived much better. This is a kind of 'bet hedging' that noise can introduce to improve fitness. There will always be some variants that potentially can accommodate stressful circumstances better and thus ensure survival of the line. Noise must thus have value in variable environments. But on the other hand, noise will also cause cells to deviate from the optimum that they might have achieved in its absence. So noise may be useful only under certain less-than-usual circumstances. Clearly, there should be a trade-off between the control of noise and the need to optimise behaviour, and different organisms will alter the balance in this trade-off. Noise may also degrade biological signals and cause difficulties in perception and reduce appropriate sensitivity. But there may be ways around this by synthesising large numbers of critical proteins. Cells also get noisier as they get older, perhaps unsurprisingly.

Very low levels of electrical noise in neurons actually improved the response to weak signals (Collins et al. 1996). At that time, the phenomenon was called stochastic resonance. In these situations, a periodic signal inside cells that might normally be insufficient to be sensed is enhanced by the presence of noise. Elowitz et al. (2002) set up an oscillatory system using negative feedback on some of their gene circuitry and observed greater noise as a consequence. Proteins that respond to environmental signals are noisier than those that deal with protein synthesis that are relatively quiet (Newman et al. 2006).

So do cells have ways of reducing noise? The capacity of a cell to control its internal processes is obviously limited by information loss. Theoretical analyses

indicate that there are considerable limits to the possibility of reducing noise. To decrease the standard deviation of protein distribution by half between separate cells would require an increase of 16-fold in numbers of signalling proteins (Lestas et al. 2010). Cells can use brute force when necessary to reduce noise resulting in regulatory genes being transcribed tens of thousands of times/cell cycle.

In a cascade (e.g. MAP kinase cascade), information is obviously progressively lost from upstream events. Information transfer in cascades will be limited by the component(s) made in the lowest copy or activity numbers. A five-step linear cascade in gene circuitry, for example, requires at least 25 more bursts of synthetic activity than a single step to maintain the same capacity to reduce noise. 'The mechanisms for preventing noise propagation such as time averaging or kinetic robustness to upstream changes cause a greater loss of information; mechanisms that minimise information losses such as all-or-none, non-linear effects actually increase noise'. 'Making a decent job is 16 times harder than a half decent job' (Lestas et al. 2010).

Parallel signal and control systems can instead improve noise suppression because each pathway contributes independent information about the upstream state. However, the loss of information is determined by the number and frequency of signalling events, not their nature. There are physical constraints on the sensitivity with which external signals can be sensed and low impact signals will only be perceived with greater noise than larger ones (Bialek et al. 2005).

3 Consequences of Signal and Genetic Circuitry Noise for Plant Growth and Development Control

3.1 Relevance of Noise in Genetic and Transduction Circuitry for Plant Development

There are a number of significant conclusions for plant growth and development that can be drawn from the above studies. The above information was of necessity gained on single-cell systems, and it clearly applies to single eukaryotic cells. There are several single-cell systems in plants and for which noise might contribute to understanding their behaviour. These are guard cells, the fertilised embryo, pollen tubes and root hairs. Lateral roots and maybe even leaves and buds may be in this category too because they potentially originate from single cells. These tissues surely use positive feedback as part of their behavioural response to inducing stimuli and to carry development and plasticity in responses forwards. If there are errors or extrinsic noise in the progenitor cell such as the fertilised embryo, it is certainly feasible that these noise variations will be continued in the final seed by epigenetic processes that it is now known, can last through generations (Molinier et al. 2006).

In an article entitled 'Reliable Noise', Levens and Gupta (2010) point out that statistical fluctuations (i.e. noise) involving a weak promoter of a transcription

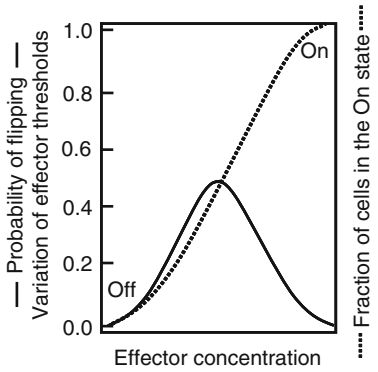

Fig. 1 The fraction of cells expressing a gene is a function of the concentration of an effector molecule. At very high or low effector concentrations, the population of cells has the gene either On or Off (as in the system used by To and Masheri 2010 and described in the text). At intermediate concentrations of the effector, some cells are 'On' and others are 'Off'. The right axis (*dotted line*) indicates the fraction of cells expressing the gene at different effector concentrations. The left axis (*continuous line*) represents the probability that a cell has flipped from the 'Off' state (no gene expression) to the full 'On' state of gene expression. The *continuous curve* also represents distribution of effector thresholds in the population of cells. Data redrawn from Levens and Gupta 2010

factor, can generate intrinsic noise. If the transcription factor is short lived, then the noise can be amplified by inducing extrinsic noise on each of the genes the transcription factor binds to; including, if so arranged, the original transcription factor itself. Dependent on the numbers of transcription sites as well as the potential variable strengths of promoters, different target genes may be tuned to switch to high output at different concentrations of the transcription factor. The consequence is a range of different phenotypes each with its own combination of gene products expressed to different degrees and responding differentially to a defined signal. Positive feedback of this kind can also fix the original gene into the 'on' position. Such stochastic switching will eventually generate a range of responses in unicellular organisms to a defined signal. Figure 1 summarises their thesis and is based on the observations of To and Maheshri (2010).

The mechanism described by Levens and Gupta (2010) in tissue responses is even more relevant if the initial gene(s) is concerned with controlling the synthesis of effectors. As indicated above, seeds could be an excellent example. Evidence that noise is an issue in plant cells and tissues and is observable between individual seeds was provided by Dahal et al. (1994) who reported variations of one enzyme up to a 1000-fold between individual seeds.

3.2 Synchronising Effects of Signals in Plant Cells

I have indicated previously that one of the most puzzling features of the effects of exogenously added plant growth regulators is that they appear to synchronise the

responding tissues (Trewavas 1982, 1987, 1991). Synchronisation suggests an underlying probabilistic mechanism. The classic example is in cell division in which cells have to cross a threshold before commencing division with the thresholds varying stochastically (noisily) amongst individual cells (Smith and Martin 1973). The effect of increasing the size of a cell division stimulus is simply to enable those cells whose threshold has now been exceeded to enter the division cycle. The thresholds are not necessarily fixed however, but can be lowered by various environmental or hormonal triggers. Most crucially, a system using variable thresholds enables a dose response to be constructed to variations in the concentration of the inducing stimulus. Smith and Martin (1973) considered that the construction of the threshold involved positive feedback mechanisms and thus the introduction of noise.

Figure 2a, b are taken from Bradford and Trewavas (1994). The symbols of Fig. 2a represent data points of the germination against time of a null gibberellin mutant of tomato when placed in different concentrations of gibberellin. Crucially, the impact of increasing the exogenous gibberellin concentration is to induce more seeds to cross the threshold from dormancy to germination. But a further effect of increasing the gibberellin stimulus is to increase a faster rate of germination in those seeds whose threshold has been exceeded. The lines in Fig. 2a were calculated by Kent Bradford from a simple model that contains both a threshold and a time

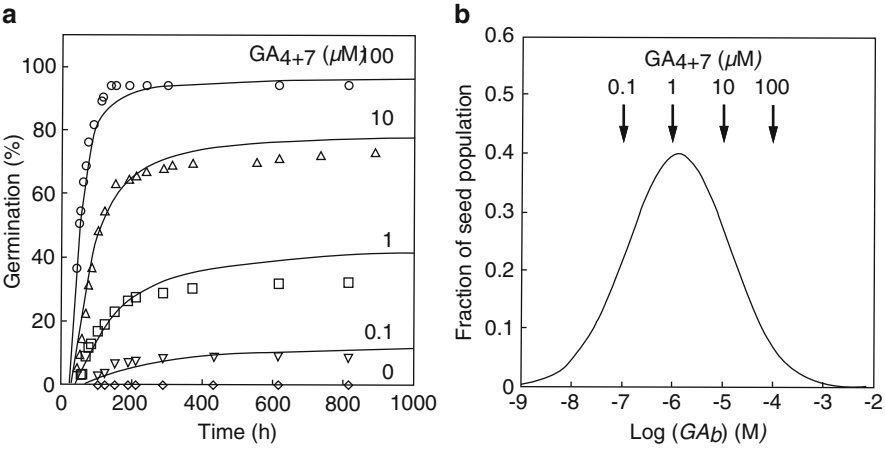

Fig. 2 Germination time courses and distribution of thresholds to gibberellin concentration in a population of tomato seeds. (**a**) Germination time courses (represented by symbols) of a GA-deficient mutant of tomato in different gibberellin concentrations from 0.1 μM to 100 μM. Increasing the gibberellin content of the medium increases the number of seeds germinating and shortens the time to germination too. The *solid lines* are the time courses predicted by a simple model incorporating both time and gibberellin concentration. (**b**) This graph shows the distribution of thresholds to gibberellin amongst the population of seeds. Only seeds with thresholds above the applied concentration will germinate. The extent to which gibberellin concentration exceeds the threshold increases the rate of germination. The distribution of thresholds is stochastic. Figures copied from Bradford and Trewavas (1994) with permission

component. Figure 2b indicates that there must be a Poisson (stochastic) distribution in thresholds amongst the individual seeds population. Note the similarity in character of response in Fig. 2b to Fig. 1 (probability in flipping and effector threshold variation axis).

The prediction here is that extrinsic noise in the fertilised cell is then stabilised by positive feedback and epigenetic processes, so that this initial noise variation is carried through to the mature seed. There is clearly a long-term memory in operation.

Figure 3a, b are modified from Gilroy and Trewavas (2001). Figure 3a reports the numbers of individual cereal aleurone cells that synthesise α amylase as gibberellin concentration in the medium is increased. There is clearly population variation in the thresholds of individual cells, as more cells cross their gibberellin threshold, more amylase is synthesised. The data shown as *triangles* and *filled circles* are plotted on the template of Fig. 1. The distribution of thresholds and thus the probability of cells to synthesise amylase is similar in character to Fig. 2b and to

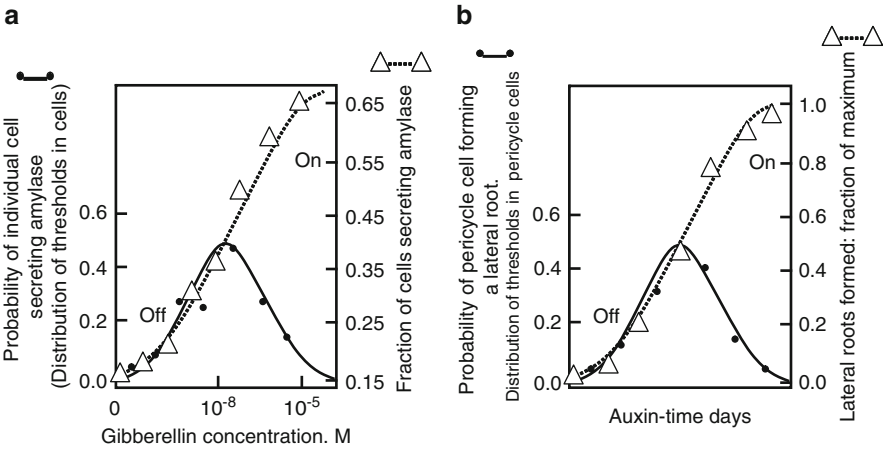

Fig. 3 Examples in plant cells showing that populations of tissue cells exhibit a stochastic variation in thresholds to inducing stimuli. (**a**) Reports the effect of variation in gibberellin concentration (from zero then 10^{-11} to 10^{-5} M in tenfold steps) on the numbers of individual aleurone cells synthesising amylase. The *triangles* represent the fraction of aleurone cells synthesising amylase, and the *closed circles* the probability of aleurone cells to synthesise amylase and thus the distribution of thresholds amongst the cell population. The symbols are plotted on the template of Fig. 1 and indicate the strong similarity in behaviour to Fig. 1. (**b**) Reports the effect of various lengths of time of treatment of root segments with auxin on numbers of lateral roots formed. Segments were incubated in auxin for variable periods of time and then removed and further incubated for a total period of 6 days in the absence of auxin. Lateral root numbers were then estimated. Lateral roots are formed from the pericycle. The triangles represent the fraction of lateral roots formed against the maximum number plotted against the total auxin treatment time in days. The *closed circles* represent the variation in thresholds to auxin amongst the population of pericycle cells and thus the probability that lateral roots will be formed. Again the data have been plotted on the template of Fig. 1 and indicates likely similarity in mechanism. Original data for (a, b) are to be found in Gilroy and Trewavas (2001)

Fig. 1. The distribution of thresholds is stochastic and likely resulting from the stochastic variation of noise coupled with a positive feedback mechanism during aleurone cell development.

Figure 3b shows numbers of lateral roots formed against auxin-time as the inducing stimulus. Root segments were incubated in auxin for variable periods of time before estimates of all treatments for lateral root number after 6 days. Again, the actual data are plotted as the symbols of *triangles* and *closed circles* on the template of Fig. 1. Since lateral roots are generally thought to develop from a single pericycle cell, the variation in thresholds again looks stochastic and presumably results from noise plus positive feedback during root and in particular pericycle cell development. As more cells cross their thresholds as the exposure to auxin increases, more lateral roots are formed.

By including this kind of mechanism involving noise and positive feedback in critical proteins, cells and tissues exhibit a dose-dependent response to the strengths of environmental or hormonal signals and to their duration. Other aspects of development where this mechanism may control is in seed dormancy breakage, leaf drop related to water deprivation in trees, bud break, root hairs, guard cells, etc. (Trewavas 1987, 2003). These examples indicate the importance of the threshold in understanding these phenomena.

4 Communication if Information Starts Within the Individual Plant Cell

4.1 Stochastic Responses Are Observed in Individual Plant Cells In Situ

One way to reduce noise is to use parallel changes that meet at some point and the result then averaged. It could potentially be seen as a basic reason why organisms became multicellular some two billion years ago, each cell receiving information and interpreting it with the necessary input of noise. With appropriate interaction, the noise level could be reduced. But this noise reduction will only work if the information from both cells is adequately and quickly transferred between cells and the subsequent response then being the average between the two cells. Does this actually happen in plant tissues?

That the stochastic, probabilistic response found in single cells above could be observed in single cells in situ in tissues was clearly shown by Nick et al. (1993). These authors used a microbeam of red light to switch on anthocyanin synthesis. They observed that there was considerable spottiness in response with patches of cells of varying sizes being switched on when using intermediate levels of illumination. They considered that the spottiness resulted from positive feedback in the transduction processes. Variation in individual cell thresholds is indicated. With saturating levels of red light, all cells respond. However, over the longer term, they observed a much slower inhibitory response that stopped anthocyanin synthesis.

Leaf patchiness in guard cell responses to closing signals is well established. However, the responses of individual cells to exogenously applied abscisic acid look distinctly stochastic and similar to the probabilistic response described above (Trewavas 2003). Variation in thresholds between individual guard cells is again implied. The speed with which patches of guard cell apertures change in response to closing signals however, suggests potential patch interaction issuing from another quicker source. A vapour phase–closing signal from the mesophyll is indicated (Sibbersen and Mott 2010). Excess short-term water loss from the leaf causes short-term stomatal closure by vapour phase signals. Prolonged water loss generates a slower ABA-dependent signal and now ABA-dependent closure and lasting for a much longer period.

4.2 Two Signals in Plant Development Change?

If this situation in guard cell closure can be generalised, and I consider it can be, the suggestion is that at least two signals are communicated in many aspects of plant behaviour. Growth regulators do not act as the initial inducers of behavioural change but as later signals that prolong and deepen the cellular change enabling its continuation for much longer periods of time and presumably reflecting the strength and depth of the signal. Certainly, recognition of this potential would mediate previous controversy based on the observable speed of cellular change as against the slower kinetics of changes in growth regulator concentration (Firn and Digby 1980). Perhaps a simple analogy from paper photography might suggest what is going on. Changes in development or behaviour are initially induced like the developer in photography; plant hormones act more like the fixative.

5 The Gel Nature of the Cytoplasm Provides for an Alternative Set of Threshold-Controlled Changes

5.1 Cytoplasm Is an Organised, Highly Structured Network

The cell is a highly structured entity. Although the basic outlines of the kinds of organelles, their structure, function and behaviour have been reasonably clear for many years, there is an area that is rarely referred to. The molecular structure of the cytoplasm is unclear apart from the generalised statement that some or all of it is gel-like in nature. Communication between cells and within cells is changed by the perception of the nature of cytoplasmic gels and their behaviour. In this context, then another controlling, threshold phenomenon appears separate from that indicated above.

The evidence for a defined structure of the cytoplasm at the molecular level comes from at least six sources.

1. The remarkable experiments of Zalokar (1960) and later Kaempner and Miller (1968). These authors respectively centrifuged whole cells of either a *Neurospora* hypha or the alga *Euglena gracilis*. Centrifugal segregation was accomplished in *Euglena*, for example, into the common fractions of starch grains, nucleus and large organelles, ER and a cytoplasmic soluble fraction. However, no macromolecules or enzyme activity were detected in the soluble fraction of the alga or fungal hyphae despite the retention of viability (Srere 2000). These observations confirmed earlier suppositions from the 1930s that cytoplasmic proteins are not free in the cytoplasm but attached to large subcellular structures that can be easily centrifuged.
2. The second indicator of structure comes from evidence for metabolons, integrated entities of enzymes that are responsible for metabolic pathways (Burbulis and Winkel-Shirley 1999; Winkel 2004). Metabolons encompass all the major metabolic pathways. The metabolon structure ensures that substrates in the pathway are not free but passed from one enzyme to another ensuring greater speed of metabolic output. Some metabolons may only transiently associate and may combine into different complexes. In signal transduction, large complexes of proteins are thought to form transiently around nucleation sites formed from PH or SH domains in membrane-bound proteins.
3. Polyribosomes have been shown to be localised to specific cytoplasmic regions and mislocalisation alters the phenotype (Luby-Phelps 2000). Even when cells were heavily permeabilised, enabling molecules of 400,000 molecular weight to penetrate, very few proteins were observed to leak out, indicating binding to the cellular contents.
4. Using two hybrid methods, large-scale networks of protein-protein interactions and co-expression networks in yeast and plant cells have been reported (Costanzo et al. 2010; Ficklin et al. 2010; Mutwil et al. 2010; Yu et al. 2008). These networks exhibit the typical small world, or scale free, network structure constructed of hubs and connectors.
5. Much of the cytoplasm is penetrated throughout by a network of microtubules and microfilaments and intermediate filaments to which other proteins can attach themselves. A complex of note is the peripheral cytoskeleton found underneath and attached to the plasma membrane that is about 100 times thicker than the plasma membrane (Alberts et al. 1983). It is known that it is this structure, and not the internal cortical matrix, that is responsible for governing specific aspects of morphological development in *Acetabularia* (Briere and Goodwin 1988; Goodwin and Pateromichelakis 1979 Goodwin et al. 1983; Mandoli 1998).
6. The experiments by Ling (1992) examined what happened to the potassium in the cells when they were cut in half. Although potassium is thought to be soluble in the cytoplasm, Ling (1992) observed that potassium only leaked out when proteins started to do so as the cell died. Some kind of structured binding of potassium to protein is indicated.

All these data suggest that the cytoplasm is a complex integrated network with perhaps microdomains specific for particular functions. The description of the cytoplasm as a gel capable of transition to a sol is of long standing and owes much to observations of organisms like *Amoeba* whose pseudopodial behaviour is constructed by swift changes between gel and sol. What then is known about gel structure?

5.2 The Design of Specific Synthetic Gels Is Intensely Researched

While the gel structure in organisms is the subject of intense research, good understanding may be gained by investigating the behaviour of synthetic gels. Artificial or synthetic gels are loosely described as two-component systems of a semisolid nature, but rich in liquid. There is intense industrial interest in the construction of 'intelligent polymer gel systems' for biotechnology, medicine and environmental issues (see references in Chen and Hoffman 1995). Gels with particular properties for drug delivery or for DNA transformation with the aim of delivery across the plasma membrane and directly into the nucleus have been constructed (Pack et al. 2005). A gel whose volume oscillates controlled by a non-linear reaction involving redox oscillations has been reported (Yoshida et al. 1999). These properties indicate the potential for biological gels constructed in different ways to have biologically interesting properties.

The cytoplasm contains anywhere from 20% to 40% protein, and it is some of these proteins, actin is a good example, that are likely responsible for cytoplasmic gel structure. However, with many proteins in the cytoplasmic gel and capable of gel formation, there is room for the construction of gel microdomains with different properties. Whatever structure is present in the cytoplasm, it cannot be fixed but must be capable of being changed in order to accommodate development and the response to signals. Flexibility in gel structure and behaviour becomes essential.

5.3 Synthetic Gels Indicate the Presence of Structured Water

The most familiar synthetic gel is the culinary jelly constructed from partly degraded collagen (gelatin). Such gels are formed at 5% collagen to water. Other gels using different polymers can form with a 1/1,000, polymer/water ratio. Such gels maintain their shape even though composed 95% or more of water. The water must clearly be in a form different from ordinary liquid water.

Each water molecule is an electric dipole with a δ+ charge on the proton and a δ− charge on the oxygen (Fig. 4a). H-bonds can form between different water molecules and enable the formation of non-covalent water structures and most

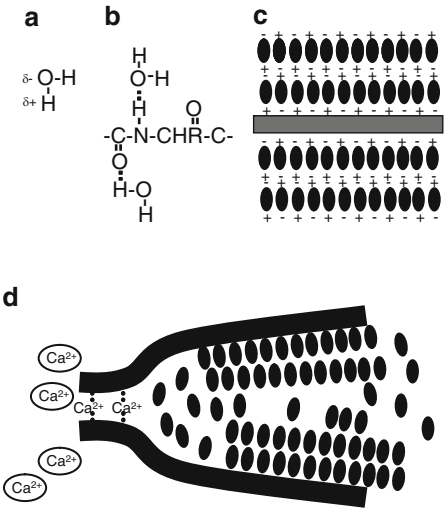

Fig. 4 Potential relation of structured water to gel formation in cells. (**a**) The water dipole, slight negative charge on the oxygen, slight positive charge on the hydrogen. (**b**) Potential hydrogen bonding of water molecules to the peptide bond. (**c**) On unfolded proteins, layers of water build up through initial hydrogen bonding to the peptide bond and then through hydrogen bonding to these vicinal water layers. The layers of structured water could be up to ten layers deep between adjacent polypeptide chains, thus linking them together in formation of a gel. (**d**) Ca^{2+} can cross-link adjacent polypeptide chains through negatively charged side chains and others and thus disrupt the structured water between them

certainly do so when ice forms. It was originally thought that unfolded, extended proteins would adopt a random coil configuration, but the three-dimensional structures of unfolded proteins, like partially degraded collagen, have turned out in contrast to be reasonably well defined. These configurations have been found to be stabilised by the interactions and structuring of water molecules around them. Although gelatin is an artificial gel, its structure has recently been clarified and may be representative of other unfolded globular proteins (Kozlov 1983; Carvagal and Lanier 2006).

Kozlov (1983) in early work indicated that water in gelatin existed in at least three distinguishable configurations. The first is now known to result from alignment of separate chains of collagen. In the proline-rich regions, the collagen molecules are cross-linked to adjacent chains through three or more water molecules. The first and third water molecules are hydrogen bonded through the carbonyl (−C=O) residues of the peptide bond of two adjacent chains of collagen. These two water molecules are then linked together by a third acting as a bridge (Carvagal and Lanier 2006). The second form of water is a tightly bound, usually single layer, of water molecules (vicinal water) responsible for hydration. There are several kinds known. Charged collagen side chains structure water around themselves. The water molecules structure initially through the dipoles and then to each other. Hydrophobic residues generate clathrate structures again around themselves.

However, if the protein is unfolded then in the open, neutral, polypeptide regions of collagen, water is attached through hydrogen bonds to the –imino (−NH) and (−C=O) carbonyl groups of many of the other peptide bonds (Fig. 4b). This second form of water does not freeze even at temperatures of −60°C.

The third more weakly bound water results from hydrogen bonding to the water molecules already attached to the open polypeptide regions and can form layers of structured-water attachment, four to even ten layers deep as intimated in Fig. 4c (Pollack 2001; Ling 2006). This structured water is in a form somewhere between the structure of ice and liquid, that is, in the structure expected of a gel. Not only will the viscosity be higher than pure water, but the diffusion rates of hydrated ions within structured water are proposed to be very much slower than in free liquid; rates of diffusion will be size-dependent.

Cytoplasmic, structured water (characterised as the restriction on freedom of motion of water molecules) has been detected with a variety of physical approaches such as NMR, frequency dielectric dispersion and quasi-elastic neutron scattering (Pollack 2001).

5.4 *Structured Water in the Cytoplasm May Affect Ion Fluxes*

Although charged molecules like K^+ or Cl^- could initially compete for the protein-charged groups as the gel is forming, the concentration of water is orders of magnitude higher. Thus, it is envisaged that initially it is water molecules that act to nucleate structured-water formation. Only later will hydrated K^+ or Cl^- penetrate structured water, bind to the charged protein side chains and remain held in the structured-water complex. If most cytoplasmic potassium is directly bound to the negatively charged, protein side chains inside the structured-water skin, then it may not be free in the conventional sense. Even when the plasma membrane is breached, potassium could remain bound until either the structured water is disorganised, and potassium becomes freely soluble, or the cell commences to lose both protein and potassium in agreement with observation (Ling 1992). Electrical integrity will thus be partly retained provided the structured water regions remain intact.

> The picture that emerges is that cell proteins exist in a semi-solid gel-like state and their water of hydration possesses unique solvent properties as a consequence of this organisation (Garlid 2000).

5.5 *The Impact of Structured Water for Cytoplasmic Functioning*

The presence of structured water does present problems for understanding cellular behaviour. Structured water will likely retard or inhibit direct interaction of cytoplasmic proteins, and yet, rapid transient protein-protein interactions are essential in our present understanding of signal transduction processes and indeed many other

processes that will involve inevitably structured water. Ling (1992) calculated that if only 5% of cell proteins are in an unfolded state, then virtually all cellular water would be structured.

Unfolded proteins organise the water dipoles into a low entropy structure along the polypeptide surface. However, low entropy structures contain stored energy that could be used to drive certain molecular processes. Pollack (2001) considers that cells can use the energy implicit in structured water to drive various cellular processes such as secretion, vesicle transport and actin/myosin-controlled movements.

Culinary jelly will resist freezing at −15°C. The ordered or structured water that presumably pervades the whole of the gel prevents the formation of the normal ice structure, which is itself dependent on a strict arrangement of hydrogen bonding between water molecules. Such observations suggest that a particular cytoplasmic gel state may account for freezing resistance in plants. The accumulation of low molecular weight antifreeze molecules commonly thought to account for this property would only lower freezing temperatures by a few degrees. Antifreeze proteins in animals adopt the same protein configurations and presumably structure water around themselves as does gelatin (Carvagal and Lanier 2006).

The importance of the nature of water inside cells has been highlighted by various researchers, and I quote only a few. For example, Watterson (1987) pointed to the observations that indicated that unfolded proteins like filamentous actin must be surrounded by clusters of water molecules. These are tightly bound water molecules and cannot be removed osmotically (Ito et al. 1991). Actin gels can be formed at concentrations as low as 0.1% actin/water. When ATP is added, the gel exhibits large contractions in volume and expels water. Watterson (1987) hypothesised that other proteins (at least 60 are known) bind to actin by mimicking the topological structure of water around actin and removing the structured water as a complete entity.

Wiggins (2002) pointed to the evidence of microdomains in the cytoplasm and that the properties of water in these domains differ substantially from liquid water. She pointed to two different kinds of water inside cells: high density and low density. High-density water can participate directly in peptide or polynucleotide hydrolysis through increased free OH^- or with the locally high concentrations of protons and hydroxyl ions. Low-density water can energise the removal of water in hydrolysis reactions. If the cytoplasm is structured in this manner, then it is to be expected that discrete areas of cytoplasm will be demarcated to perform specific functions as a result of prior localised protein and enzyme activities.

Finally, Pollack (2001) has suggested that structured water may play an important role in enabling water to easily rise to the top of tall trees.

5.6 Gels and Phase Transition Cooperativity: conformational spread

If the cytoplasm has the characteristics of a complex gel-like state, then to understand how cellular properties can be altered requires understanding of the potential

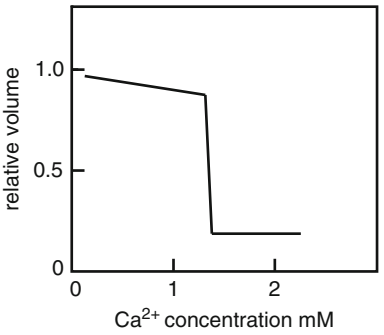

Fig. 5 Abrupt change in gel property with slight change in environment. Sodium polyacrylate gels were incubated in increasing concentrations of Ca^{2+} ions. At a critical concentration, a threshold is passed and the gel abruptly contracts. Data adapted from Tasaki and Byrne (1992)

changes in gel structure and behaviour. Again the properties of synthetic polymer gels are instructive (Pollack 2001).

Gelatin of course undergoes a phase change between two different states: liquid (sol) and solidified (gel), dependent on temperature and the conversion is reversible and usually abrupt. The transition in phase forms one of the basic properties of most non-covalently linked gels and the change can be induced by very subtle environmental alterations *once a threshold is exceeded* (e.g. Chen and Hoffman 1995; Pollack 2001). Figure 5 shows an example using Ca^{2+} on a synthetic gel; the change in volume is abrupt and reversible. Phase transition can increase the ion permeability of the gel 1,000-fold; it can shift solutes, increase the freedom of motion of water molecules and propel ions; some gels can oscillate in volume (Yoshida et al. 1999), and others act mechanically to propel a gel strip along another gel in response to an electric field (Pollack 2001; Pollack and Reitz 2001). Many of these properties are similar to the known capabilities of cells. Some conductive gels can oscillate their internal current when exposed to a constant current. Oscillations in plant cell electrical potential are not uncommonly reported (e.g. Shabala et al. 1997). An important corollary is that if gels retain their shape and the cytoplasm is largely a gel, what then is the real function of the plasma membrane? Clearly, it is not in traditional view as a bag to hold the contents in.

The threshold character and abruptness of phase change indicates the underlying mechanism relies on the cooperative behaviour amongst the constituent molecules. Two mechanisms of phase change can be envisaged. The first possibility is that described by Pollack (2001). Once a few non-covalent linkages in the gel polymer structure are unpicked, all the additional linkages rapidly collapse; the structure unzips as it were and collapses into a more stable state when a stimulus threshold is crossed. The originating factor here is surely noise in molecular structure and the low entropy structure that provides the energy for phase transition. This mechanism for gel phase transition argues that local structural change in a few linkages induces an electron cloud shift in a component polymer that then in turn induces and propagates an electron cloud shift along the whole molecule and then to other molecules. Alternatively, quantum coherence might explain the process. Figure 4d shows an example diagrammatically in which Ca^{2+} unpicks structured water by cross-linking polypeptide chains.

The second possibility is that described by Bray and Duke (2004) as Conformational Spread. They report the evidence that from a number of systems, for example, actin filaments and others, conformational changes can propagate through extended lattices of protein molecules. All these phenomena show high cooperativity (*narrow range of stimulus change between threshold and full response*). In the case of an actin gel, for example, the binding of gelsolin can solubilise actin filaments, changing gel characteristics. Binding of a single gelsolin molecule at one end propagates a conformational change along the whole actin molecule, and that may be sufficient to disrupt the structured water between adjacent molecules, thus breaking the actin gel structure. Conformational spread would continue disrupting the whole filamentous gel. Cofilin may work in similar fashion reducing filaments to monomers by conformational spread. Again, molecular noise would allow some gelsolin molecules to attach and initiate the process.

Perhaps, equally significant are the subtle environmental shifts that initiate transition cooperativity in synthetic gels. These are slight changes in pH, temperature, chemicals/biochemicals, salts, solvents and electrical and mechanical stimuli (Pollack 2001). This list is remarkably similar to summaries I constructed of environmental changes that induced bud and seed dormancy breakage, induced adventitious root formation, abscission or cell division (Trewavas 1992). Does cytoplasmic gel phase transition initiate these aspects of plant development? If conformational change enables critical proteins to now contact each other, might this not be sufficient to initiate new changes in development?

5.7 The Role of Ca^{2+} in Structured-Water Disorganisation and Signalling

Changes in cytoplasmic Ca^{2+} accompany many if not all signalling processes in plants. There are substantial amounts of Ca^{2+} in the cytoplasm in a bound form and these are probably several orders of magnitude higher than the 'free', resting Ca^{2+} detectable by fluorescence ratio imaging or aequorin (Gilroy and Trewavas 2001). Bound cytoplasmic Ca^{2+} may be involved in non-covalent, cross-linking of different protein molecules or of different regions of proteins. Such cross-linking, if present, will prevent the formation of structured water. The much larger, unbound but hydrated Ca^{2+} ion (compared to the hydrated K^+ ion) may also be mainly restricted to cellular regions free of structured water.

However, an increase in cytoplasmic Ca^{2+}, initiated by signalling, will act to initiate a phase transition in many areas of cytoplasmic gel. Actin gels illustrate the potential. Ca^{2+} is known to cause precipitation of actin, bundling of filamentous actin and initiate actin gel contraction in volume with concomitant expulsion of water (Bray 1992). The effect of Ca^{2+} is to disrupt the structured water around actin chains (Fig. 4d) and thus presumably to cross-link adjacent actin proteins through negatively charged side chains such as the carboxyl groups on aspartate and

glutamate residues. However, other amino acid residues may be involved. Urry (1971) has indicated that Ca^{2+} binding in two proteins and no doubt many others takes place in areas rich in glycine residues increasing the potential binding sites available and quotes sulfhydryl groups as potential binding sites too. There are probably many proteins able to bind Ca^{2+}. The structure of the gel must be in some sort of dynamic state enabling some Ca^{2+} ions to penetrate the gel structure to initiate cross-linking. Once started, the whole structured-water complex is cooperatively destabilised using the energy available from the low entropy structure of structured water.

5.8 *Is There a Role for K^+ in Phase Transition?*

The common view of Ca^{2+} signalling is that signals open relevant channels in either the vacuole membrane or in the plasma membrane allowing the flow of Ca^{2+} down its electrochemical gradient into the cytoplasm. However, an alternative is to release Ca^{2+} from its bound form in the cytoplasm itself. A detailed compartmental analysis using washout procedures of $^{45}Ca^{2+}$ indicated the identified cytoplasmic compartment as having about 2 mM Ca^{2+} (Smart and Trewavas 1984). Many hundreds of measurements in plant cells place free cytoplasmic Ca^{2+} as at least four orders of magnitude lower. There are proteins that bind very large numbers of Ca^{2+} ions and these might be an explanation of these contradictory observations.

If signalling initiates a local disorganisation of structured water, then bound potassium will be effectively solubilised and could displace Ca^{2+} from these weakly bound cytoplasmic sites. Any signal that initially increases free cytoplasmic K^+ will, in turn, transiently increase cytoplasmic Ca^{2+}. But the intervention of the vacuole should ensure the removal of excess free cytoplasmic K^+ and see situation rapidly returned to what it was before. Any excess Ca^{2+} remaining will be mopped up by the activation of Ca^{2+}-dependent ATPases and sequestered into cellular stores before a slow return to the initial state. The effects of phase transition will be temporary overall, but the likelihood is that the new gel structure that is reconstructed will be different because of the metabolic and phosphorylation events that have occurred during the transition and the new environmental circumstances that have been sensed.

In both cases described above, where transient Ca^{2+} elevations are observed and structured water disorganised, the cytoplasmic volume should transiently increase; although, the additional water might be taken up by the vacuole or expelled to the wall. Interactions between different kinds of proteins that were previously hindered by structured water can now occur more freely. For example, Ca^{2+}-dependent protein kinases might more easily contact and phosphorylate protein substrates increasing their negative charge and thus increasing Ca^{2+} binding sites. As substrates increase their negative charge, they in turn could be cross-linked by Ca^{2+} providing larger cytoplasmic areas free of structured water and enabling further downstream signalling processes to continue.

5.9 *Could Phase Transitions Be Communicated Through The Plasmodesmata?*

Plasmodesmata are regarded as organelles that provide for cytoplasmic continuity between adjacent cells (Oparka 2005). Plasmodesmata are concerned with the potential transport of signals during host-pathogen interactions, predation signals and aspects of development that require communication between cells. The structure is complex, involving ER and protein bodies, and each pore is lined with plasma membrane. Early measurements indicated that plasmodesmatal pores would only allow passage of molecules less than 1 kDa (Erwee and Goodwin 1983). But viruses can pass through plasmodesmata using a movement protein and can open the size exclusion limit to molecules larger than 10 kDa. This increase in size exclusion limit can be also obtained by treatment with azide or anaerobic stress, that is, conditions that damage oxidative respiration (Oparka 2005). Molecule size movement can therefore be controlled, is dependent on ATP and can permit protein movement between adjacent cells.

The presence of actin and some other associated proteins in plasmodesmata has been known for some time (White et al. 1994; Faulkner et al. 2009). The realisation that actin and other proteins might form a gel in the plasmodesmata and that phase transitions in gel structure might explain changes in size exclusion limits seems not to be generally appreciated. Gels will of course allow the movement of small molecules by rapid diffusion but structured water in the gel will seriously retard the movement of proteins and larger molecules. The only way that larger molecules could pass would be to dismember the gel structure and thus release the inhibition on movement posed by structured water.

Ding et al. (1996) used fluorescent dextrans of varying sizes to detect permeability between cells and observed that cytochalasin D and profilin both now permitted molecules as large as 20 kDa to pass through the plasmodesmata. Actin filaments are in a dynamic state and cytochalasin D and profilin will dismember them. Concomitantly, structured water will be disrupted and break apart the gel structure. Movement of proteins is thus enabled. Cytochalasin D and profilin will initiate an actin gel phase transition. Azide and anaerobic stress will inhibit cellular ATP production, and thus, both these treatments can be expected to impair the dynamics of actin polymerisation into filaments and ensure structured water and gel disruption. Phalloidin, on the other hand, stabilises actin gel structure by cross-linking actin filaments. Ding et al. (1996) reported that phalloidin counteracted the opening of plasmodesmatal pore size by cytochalasin D and profilin. Potentially then, viruses increase the size exclusion limit by disrupting the actin gel structure and causing the breakdown of structured water that inhibits their movement between cells.

Increases in cytoplasmic Ca^{2+} have been shown to shut the plasmodesmatal valve (Erwee and Goodwin 1983; Tucker 1990). The effect of Ca^{2+} on actin gels is to cause the formation of a plug (Bray 1992). As actin gels contract, the volume

diminishes, expelling some water. In the small plasmodesmatal pore, such phase transitions should either reduce its permeability or even completely plug it.

Could such changes in gel structure be communicated to adjacent cells? When phytochrome is activated by red light, transient increases in cytoplasmic Ca^{2+} have been observed (Shacklock et al. 1992). Nick et al. (1993) did indeed observe that red light effects were limited to individual cells or small clusters. Thus, the change in Ca^{2+} seems to be limited to the cell which senses the signal, by closing the size exclusion pore. The reason that Ca^{2+} shuts down the size exclusion limit is surely to ensure that further communication between cells must continue to operate through the wall as much of auxin movement is known to occur. Thus, the aim is temporary exclusion of movement of other soluble growth regulators. If under normal conditions the size exclusion limit is low and plasmodesmatal actin in the form of a gel, then there is the potential for gel phase transition induced by other signals to be communicated into adjacent cells dismembering local gel structure with consequences for transmission and influence beyond the responding cell.

6 Conclusions

Thresholds seem to be important elements in plant cell and tissue behaviour. Two ways have been suggested whereby threshold might be explained. The first of these is assumed to be positive feedback accompanied by noise in critical transcription factors. The second sees thresholds as developing from abrupt phase transitions in gels. These phase changes may be limited to micro-domains in the cytoplasm because one feature of Ca^{2+} signalling is its pronounced spatial character. The crucial issue here is that thresholds coupled with a probability of transition through the threshold provides for a simple way in which either a population of plants or tissues or cells from a plant exhibit a quantitative response to differing strengths of signals. More research on the threshold is now surely warranted.

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