OPINION

Signal processing and transduction in plant cells: the end of the beginning?

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Plants have a very different lifestyle to animals, and one might expect that unique molecules and processes would underpin plant-cell signal transduction. But, with a few notable exceptions, the list is remarkably familiar and could have been constructed from animal studies. Wherein, then, does lifestyle specificity emerge?

Plants and animals have solved the problems of being multicellular in different ways. Eukaryotic photosynthesis evolved some 2,000 million years ago in the oceans. The ubiquity of light over the surface of the globe is thought to have been responsible for a major evolutionary decision by the primordial plant eukaryotic cell; to remain sessile and, as a consequence, to tolerate inevitable predation[1,2]. When plants invaded the land, they found the supply and distribution of water, minerals and light much more variable than in the oceans. Among the primary advances made on land were an elaboration of tip branching and the evolution of a differentiated modular structure. The module elements — leaves, buds (dormant meristems), flowers, abscission zones and branch roots — are reiterated many times during development, as are their signal-transduction capacities. Such modularity ensures that predation and environmental damage are minimized because some modules usually survive to regenerate the individual.

In general, tissue and cell functional specialization is minimized in plants to limit fatal predatory damage. However, some distribution of functions among different cells is required; for example, the specialized cellular structure of the plant vascular system precludes its direct role in photosynthesis. But most plant cells can sense nearly all the signals to which the individual plant responds. Owing to their very different physical environments, however, there are some differences between signals perceived by the root compared with the shoot. How, then, can a plant cell process these myriad signals through to an appropriate response? Here we emphasize the structural and spatial characteristics of plant signal transduction, and conclude that organization emerges from the interrelationships of specific components.

Exploitation of growth resources

The growing shoot can accurately perceive gradients of light, and reflected light from leaves is used to detect the position of neighbours[3]. A three-dimensional image is constructed by the shoot, and growth (and leaf angle) is redirected if necessary to optimize light capture. Each shoot cell acts like an individual ommatidium of the insect eye. Below ground, recent observations have shown[4] that plants prefer patchily distributed minerals in the soil. Remarkably, the plant can sense the volume of the patch, maximize growth when an optimal volume is sensed and perceive the steepness of the gradient across the patch boundary. How these soil variables are perceived is not understood[4]. But single plant cells can sense very slight gradients of many environmental factors (BOX 1).

Perception of these important plant resources takes place within the context of an environment that changes from minute to minute. At least 17 environmental variables are sensed (FIG. 1), and each can modify the response to the others[5]. A complex array of external information is therefore either summed or integrated, whereas between other groups of variables, synergistic interactions are common[6–8].

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Box 1 | Single cells can sense fine gradients

The classic example of fine sensing is the zygote of the marine alga *Fucus*. This single cell can respond to remarkably slight gradients in temperature, osmotic pressure, light, pH, minerals (K^+, Ca^{2+}), solution flow, electrical fields, other chemicals, gases and probably gravity, and direct the orientation of growth accordingly many hours later[69]. These gradients usually have a narrow time window in which they are sensed by the single cell, although in a population of zygotes this window is stochastically distributed around a mean value of several hours. By contrast, the sperm entry site can be remembered for at least a day and be used to specify the direction of growth if no other cues have been detected[70]. A similar remarkable sensitivity to signal gradients is shown by single-celled euglenoids, which can sense their own cytoplasmic weight and modify swimming activity[71].

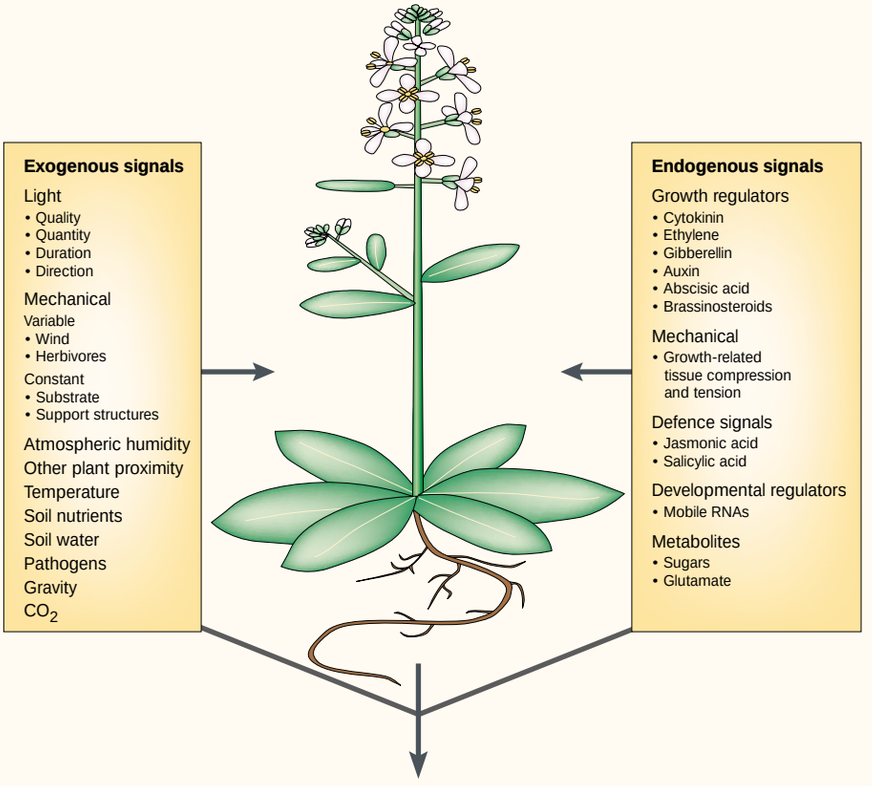

Figure 1 | **A wide range of disparate external and internal signals is monitored by plants and used to compute appropriate developmental responses.** The molecular elements of the plant sensory apparatus and signal-transduction systems can integrate these signals and reach a finely balanced decision as to how to grow and develop to most successfully survive and exploit the environment. As plant responses are generally irreversible growth responses, these signalling systems must compute each developmental decision with extreme care.

Decisions about exploitation of basic nutrient resources can be made by plants before any nutritional benefit is derived. Dodder, a parasitic plant, can sense the level of circulating nutrients when it first touches a putative host[9,10]. Within one hour, it 'decides' whether it is worth initiating a developmental programme, which involves shoot-coiling around the host and the formation of haustoria several days later. Rejection of the putative host is frequent. Once haustoria penetrate the host vascular system, nutrients are gained and used for growth. Remarkably, the number of coils of the parasite around the host stem reflects with some accuracy the nutrients in the host and the likely subsequent return in growth resources. What is required of plant-cell signal-transduction studies, then, is to account for the capacity for 'intelligent' decision-making; computation of the right choice between close alternatives.

To exploit patchily distributed environmental resources, dormant meristems can be activated, and individual growing meristems on a single plant often show striking degrees of independence in growth and signal response. A network of growing and branching meristems is constructed, and this efficiently mines local light, minerals and water. The overall organization of signal sensing–response and interactions between the growing regions is thus democratically arranged, with no overarching, controlling tissue like a nervous system. Competition between growing points is common. However, the resultant architecture of the plant is invariably highly functional, indicating that these sensing and control systems must also be highly coordinated.

Internal signals: more complexity

A plethora of internal signals circulate around a vascular system in which flow rate can vary from minute to minute. These signals include growth regulators, ions, wall fragments, sugars, water and amino acids, all of which can modify development[11]. The degree of fine vascular branching can be limited and many cells can be 10–20 cells away from direct contact. So these active agents often arrive at individual growing cells in a polarized manner and might be perceived as gradients across them. Plant cells are permanently joined through a contiguous wall. Because the cytoplasm contains a turgor pressure of about eight atmospheres, compression and tension gradients are common. Nodal points of tension/compression can be expected to elicit changes in development[12]. Such mechanical signals can act as specific morphogens[13] and can contribute to the polarized nature of growth and development. Proteins that alter the mechanical characteristics of the cell wall, such as the recently described expansins[14,15], might therefore act as plant-specific developmental regulators. Mechanical sensing by higher plants is extremely sensitive, and only slight movement or touch is necessary to induce immediate responses in cytoplasmic calcium[16].

The developmental production of successive modules can also programme changes in signal sensing. During cereal root development, for instance, the lateral roots grow horizontally at first, only later assuming a characteristic vertical direction[17]. Successive roots become progressively more vertical with respect to gravity, leading to a network of roots that efficiently mines the local soil around the stem.

Phenotypic plasticity

Because plants usually have little choice over their immediate growth environment, an ability to modify development to cope with an environment of enormous variability is believed to increase fitness. Phenotypic plasticity — that is, the capability of a single genotype to generate many phenotypes — is a pronounced and unusual characteristic of plant development[1]. It is also a crucial feature of plant-cell signal transduction. Specific phenotypic adaptations in morphology, physiology, anatomy, development, reproductive timing, breeding systems and offspring developmental patterns have all often been observed[18]. Enormous variability in module numbers is common. One view is that a direct coupling of signal transduction to gene expression regulates plasticity. However, the mechanism might not be straightforward and epigenetic processes or even cell individuality, as we indicate later, might be crucial to the response.

Some aspects of development and morphology are strongly resistant to environmental variation. Numerous complex feedback controls must therefore be operative, but detection of these is clearly in its infancy[19,20]. Furthermore, redundancy in control elements will help strengthen reliability in the face of environmental disruption. Redundancy was an early control feature introduced into computer design to ensure reliable performance. Polyploidy seems to have had an important

role in genome evolution in angiosperms. The common presence of several copies of genes (and gene products) — and thus potential redundancy in the plant genome — might be a reaction to the complexity of the environment as plants perceive it.

The importance of individuality

The term individuality is used to describe situations in which morphologically similar cells, tissues or plants show non-similar or unique responses to signals. Commonly, individuality can be identified in situations in which development is 'all-or-none'[21]. The cell or tissue does, or does not, respond to the inducing signal; flowering, abscission, germination, bud break and root formation are good examples. In these cases, an increasing strength of stimulus (light photoperiods, growth regulator concentrations) leads to a response from more of the population. The dose–response curve therefore represents the different sensitivities of the individuals of the population to the stimulus[22].

Cells of the stomatal complex[23], aleurone[24,25] and pericycle[26,27] have all been observed as heterogeneous populations in a single tissue. When the concentration of a modifying stimulus such as auxin, gibberellin or abscisin is increased, progressively more cells respond (FIG. 2). Each cell therefore has its own sensing threshold and, when this is exceeded, a response is initiated. However, there is also variation in the lag period before individual cells respond, and in the duration of the response[23,26]. Individuality in regulation of the *lac* operon in bacterial populations was observed many years ago[28]. Partial expression of the *lac* operon represented simply the numbers of bacteria that had made the transition.

Explanation of such individuality probably lies in certain stochastic processes during plant-cell development. The cytoplasm of a mature plant cell is little more than a few picolitres in volume and contains about 20% protein. Depending on the cell type and the signalling pathway, the numbers of molecules in each cell concerned with signal transduction and the control of gene expression are estimated to range from single figures to under a thousand[29]. Predictions of cellular properties are usually based on the assumption that the cytoplasm is a homogeneous, relatively dilute solution, containing statistically large numbers of molecules; cellular kinetics are assumed to rely on concentration and equilibrium constants to determine interactions. In neither case is this true for the cell.

How accurately, for example, can a cell control the amount or behaviour of regulatory proteins or transcription factors that number fewer than ten to a hundred? Can we comprehend regulation when only a few dozen molecules are involved, and in cases where stochastic or chaotic events could be crucial in determining the outcome? How will environmental variation during cell or tissue specification modify the partition or synthesis of such small numbers of proteins at crucial cell cycles? Even when dealing with several thousand protein molecules, are cellular processes sufficiently accurate to ensure an identical number of copies between different cells? And there are at least 10,000 cellular proteins.

It is at crucial stages of cell specification that individuality emerges. The adult plant originates with the first division of the zygote. Many tissues in plants originate from just one or a few cells. And particular cells within tissues such as leaf guard cells certainly originate from single cells. Variations in the small number of crucial transcription factors at any of these stages will ensure the individuality that is subsequently observed.

Such epigenetic 'noise' could be consid-

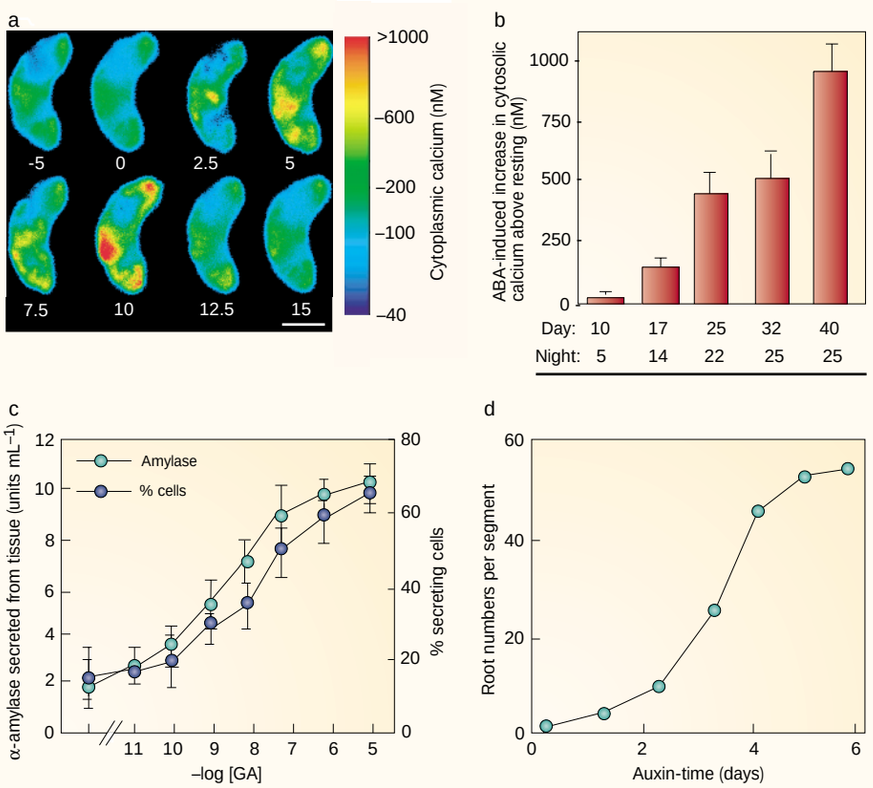

Figure 2 | **Plant cell signalling systems show characteristics of cell-to-cell individuality, threshold phenomena and environmental entrainment. a** | Cytosolic Ca^{2+} changes in stomatal guard cells of *Arabidopsis thaliana* treated with abscisic acid (ABA). Ca^{2+} levels were monitored by confocal ratio imaging of the Ca^{2+}-sensitive ratiometric dye Indo-1 dextran microinjected into the cells. Note the spatial and temporal variability in the Ca^{2+} increase induced by ABA within a single cell. Calcium levels are pseudocolour-coded according to the inset scale. Numbers reflect time after addition of 20-mM ABA. Scale bar represents 5 μm (S.G., unpublished data). **b** | The precise kinetics of plant signalling systems can be environmentally determined. The maximal ABA-induced Ca^{2+} increase in guard cells of *Commelina communis* depends on the previous growth conditions of the plant. The lower the growth temperature, the less the guard cells seem to use a Ca^{2+}-dependent ABA signalling system. (Data redrawn from REF 83.) **c** | Dose–response curve of α-amylase secretion from barley aleurone induced in response to gibberellin (GA) shows threshold phenomena for plant-cell responses. The aleurone is a secretory tissue that responds to the growth regulator gibberellin by producing hydrolases as part of the reserve mobilization system that supports cereal grain germination. Note the dose response of the whole tissue reflects the recruitment of more cells with higher threshold for activation to the secreting population as the gibberellin levels are increased[24,25]. **d** | Root segments (*Haplopappus ravenii*) were exposed to auxin (5×10^{-7} M) for the numbers of days shown (auxin-time) and then placed in auxin-free media for the remainder of the 6-day total incubation period when lateral roots were counted. As roots are formed by division from root pericycle cells, these data (redrawn from REF. 27) indicate substantive time variation in sensitivity to auxin. The first derivative of these data produces a bell-shaped curve indicating stochastic variation in time sensitivity to auxin among a supposedly uniform tissue[27].

Box 2 | Biological advantages to individuality

Individuality allows phenotypically plastic responses to the environment. Plants can adjust the numbers of branch roots to best fit the prevailing circumstances[72]. Variation in individual aleurone cells allows amylase production in the germinating cereal seedling to be adjusted according to variable germination circumstances[24–26]. Trees can optimize the number of leaves to a reduced water supply simply by abscising the excess[73]. In the case of leaf guard cells, sub-populations more sensitive than others to light, abscisic acid, water deficit, humidity or carbon dioxide, for example, allow the leaf to optimize water relations[26] by using different combinations of the sub-populations of guard cells.

Individuality in signal transduction also allows plants to deal more effectively with herbivores and disease. The same herbivore signal causes different induced responses in different plants of the same species and in different tissues of the same individual[74]. Every leaf can assume a different phenotype owing to the expression of resistance genes. And even though each step might be transcriptionally regulated, the net effect of the induced response might seem random, without detailed knowledge of the position, age, history and chemical environment of the affected tissue. This moving target, n-phenotype strategy[74] is a crucial example of plant individuality and plasticity.

ered irrelevant, a biological nuisance, but plants probably engineer such variation because it allows a graded response from the population of plants, tissues or cells and thus increases fitness (BOX 2). Clearly, individuality forms a basis for phenotypic plasticity in terms of numbers of roots, flowers or leaves. But during tissue specification, the crucial transcription factor numbers might instead ensure the production of a small population of mother cells with different potentialities. According to environmental conditions at the time, one or other mother cell could be cloned to produce the tissue most relevant to prevailing circumstances.

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Individuality in calcium signalling

Changes in cytosolic Ca^{2+} are recognized as ubiquitous regulators of cell function and provide some of the clearest indications of the individual behaviour of plant signalling molecules when viewed at the single-cell level. Calcium responses induced by the same signal are rarely identical between any two plant cells of the same type[22,30,31]. Such individuality probably results from the low numbers of channels and receptors involved in Ca^{2+} entry. This cell-specific behaviour suggests that the cell orchestrates the essential Ca^{2+} kinetics of the signal necessary to elicit the appropriate response, rather than the reverse[32].

However, a further unusual property of plant signalling systems is that signals usually induce the synthesis of the proteins that are involved in mediating the response. One obvious example is that increases in the levels of Ca^{2+} induce the synthesis of calmodulin, but many others (phospholipases, calmodulin-like domain protein kinases, mitogen-activated protein kinases, resting levels of second messengers and so on) have been recorded[33–35]. One explanation is the construction of new signal-transduction equipment in each cell, designed to take account of the new circumstances after the first set of signals. As such, these changes in expression would represent a cellular 'memory' of the environmental history of the cell, perhaps providing a molecular explanation for how a plant can incorporate its growth history into its future developmental decisions. In addition, a form of cellular learning[34] takes place because increased information flux through cytosolic Ca^{2+} should result. An alternative is that more cells might be slowly recruited into a signalling mode, so the answer lies in explanations from individuality. Whatever the functional basis for this signal-induced synthesis of signalling proteins, it seems an unusual and widespread feature of plant signalling systems worthy of further investigation.

Principles of perception

Occupied receptors are usually considered the start of any signal-transduction network, and our knowledge of plant receptors has advanced considerably over the past 5–10 years. Small families of receptors for red and blue light, ethylene and brassinosteroids have been isolated[32,36–38] (FIG. 3). Sugars, which can act as internal plant morphogens, might be sensed by hexokinase[39]. Receptor-like kinases are prominent in the *Arabidopsis thaliana* genome[40] and, with their putative ligands, they are thought to mediate processes such as incompatible pollen–stigma interactions[41] and the maintenance of meristem structure[19].

Phytochrome, the red/far-red-light sensor, has some characteristics of a two-component-like phosphorylation system similar to those in bacteria, although it acts as a serine/threonine rather than histidine kinase[5,25]. The blue-light sensors cryptochrome and NPH (non-phototropic), which use flavins or pterins as chromophores, might couple into redox systems[42,43]. Ethylene is one of five main growth regulators, and its receptors (such as Eth-1) have been characterized as histidine kinases similar to bacterial two-component signalling systems[44]. A candidate cytokinin receptor has also recently been identified as a histidine-kinase-like protein[45]. A close relationship between auxin transport and perception has been predicted, and this may be clarified now that candidates for auxin receptors and auxin-transport proteins have emerged[46,47]. Despite such successes, however, we still lack specific candidate receptors for the growth regulators gibberellin and abscisin. But structure–activity relationships indicate that all of these growth substances might be sensed through proteinaceous receptors. Nearly all the receptors shown in FIG. 3 are located in the plasma membrane. The exceptions — for example, phytochrome and cryptochrome — must be fixed to some specific spatial cellular domain because cells can sense gradients of light. Sites near the plasma membrane, fixed perhaps to an attached cytoskeleton, have been suggested[32].

The plasma membrane as a computer

Perception of signals is, however, more complex than the limited families of receptors indicated above might suggest. For example, in the case of light, not only can red and blue light be easily distinguished, but plant cells can assess the total quantity of light received, the direction from which the light comes, the intensity during exposure, the time (minutes to many hours) that light was available, and the temporal order in which red or blue light has been perceived[48,49,50]. It has been speculated that an unknown group of PAS/kinase proteins revealed by the *Arabidopsis* genome initiative[51] could be a new class of photoreceptors. However, it is likely that many of these complex light-perception events are done through interactions between the small number of receptors already identified.

The timing, direction and quantity characteristics are almost certainly shared in the perception of growth regulators, nitrate, water, gravity, temperature and mechanical signals. Whatever the receptors for these latter signals, transduction mechanisms have to account for a complexity of perception not easily explained by single classes of receptor. Furthermore, as indicated earlier, it is necessary to account for an ability to integrate many signals and to compute 'intelligent' decisions.

Although there are many sites within the cell where signal integration and processing can occur, such as organelles, cytosol, cytoskeleton and endomembranes, the unusual properties of the plant-cell plasma membrane make it a prime candidate for the location of the 'cellular computer'. Probably 500–1,000 proteins and enzymes at very high density are embedded or attached to the plasma membrane[11,52]. These include receptors (FIG. 3), protein kinases[53,54], ion channels[55], microfilament anchorage and signal-transduction proteins involved in second-messenger production[32]. Scaffold proteins on the cytoplasmic face of the membrane similar to ankyrin are probably also present because ankyrin-binding regions in some plant proteins have been identified. The domain on the cytoplasmic surface is likely to be hydrophobic, with limited numbers of water molecules encouraging protein–protein interactions. Early electron microscope studies indicated that complexes of proteins were present and that the whole membrane was enormously differentiated[56]. Limited identification of these complexes has been made, although some are probably transient and formed only after signalling has commenced (BOX 3).

Current views on plasma membrane behaviour suggest a fluid mosaic structure[52]. But these models are often derived from motile animal cells and there are reasons (such as polarized cell growth and tissue morphology, sensing the direction and gradients of incoming signals, lack of cell mobility) that indicate that many functions in plant cells might be fixed rather than mobile. Even if large protein complexes are effectively free to move, diffusion will be extremely slow. The wall provides an obvious anchor for proteins, particularly for those that straddle the membrane[52].

The plasma membrane is under turgor pressure and is compressed against the wall. Movement of proteins will be hindered by wall constituents and thus membrane fluidity will be reduced by the pressure. Changes in turgor (for example, from hypo-osmotic shock) or bending of the cell will concomitantly alter the conformation of structurally attached proteins by stretching or otherwise deforming the bilayer. Such treatments result in immediate transients in cytosolic Ca^{2+} (REFS 57,58). The implication is that channel proteins are either directly or indirectly anchored to the wall as the Ca^{2+} involved enters from outside the cell. Changes in wall–membrane protein interactions could provide the rapid channel gating observed under these conditions.

The large numbers of protein kinases and phosphatases found in cells present serious problems for fluid mosaic models. At least 1,000 protein kinases are present in the *Arabidopsis* genome[51] and the density at membrane surfaces is probably very high. For any signal to navigate, with fidelity, through the forest of protein kinases and phosphatases, requires severe spatial constraints on plasma membrane protein kinases to ensure specific modification of protein substrates[32]. Some kinases might be permanently tethered to scaffolds constructed around the plasma membrane and the associated cytoskeleton, but others might transiently

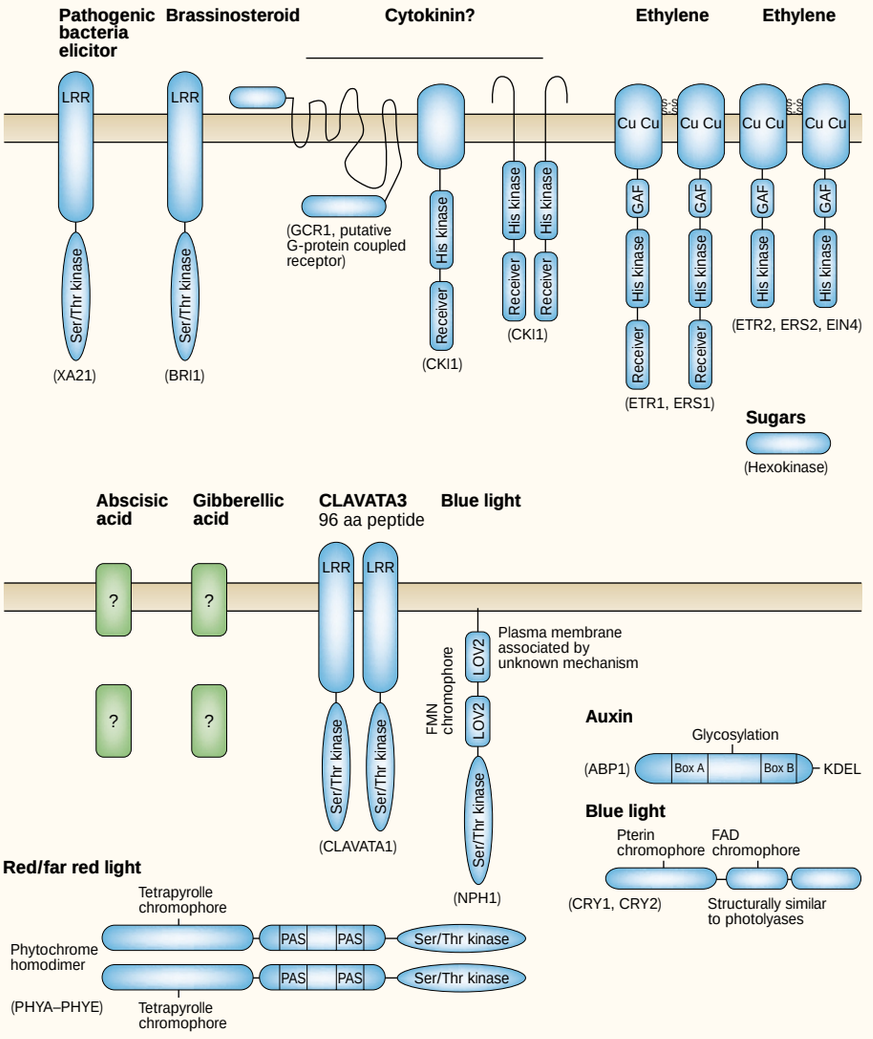

Figure 3 | **The domain structures of several known plant receptor proteins, putative receptors or components of putative perception complexes, and their respective ligands.** His kinase, histidine protein kinase domain; KDEL, endoplasmic-reticulum retention sequence; LOV, light/oxidation/voltage sensor-like protein domain; LRR, leucine-rich-repeat motif; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; PAS, carboxy-terminal structural PAS repeat domain; Receiver, domain homologous to bacterial two-component signalling system receiver proteins; Ser/Thr kinase, serine/threonine protein kinase domain; ? unknown protein. For more detailed discussion of the structure and function of these receptors and putative receptors see: phytochromes (PHYA–E)[38]; cryptochromes (CRY1,2)[42]; auxin (ABP1)[47]; phototropin (NPH1)[43]; hexokinase[39]; LRR protein kinases as receptors for brassinosteroids (BRI1) and pathogens (XA21)[84]; ethylene (ETR1)[44]; cytokinin (CRE1, CKI1, GCR1)[45,85]; CLAVATA1[86] (also see the review by Steven E. Clark on page 276 of this issue); and references therein.

Box 3 | Stable and transient protein complexes

There are over 1,000 protein kinases in plant cells and, for a signal to navigate correctly its way through this morass of transduction proteins, spatial location is essential. Stable connections of protein kinases to the plasma membrane involve farnesylation, myristoylation or prenylation; less permanent ones involve autophosphorylation, phosphorylation of scaffold proteins, or localized high concentrations of activating second messengers. Simple, calcium-induced transduction complexes — such as prenylated calmodulin targeted to the plasma membrane[67], or a membrane-associated calmodulin-like domain protein kinase (CDPK) regulating a membrane transporter[54] — are probably dwarfed by semi-permanent structures such as those described in animal cells by caveolae and rafts[75,76]. These structures (transducons[11]) are nucleated around particular membrane lipids or even scaffold-like proteins, and contain many plant-cell transduction proteins that are involved in phosphoinositide production and phospholipid modification[77,78], Ca^{2+}-signalling proteins, calmodulin, kinases, water channels, nitric oxide synthase, anchorage proteins and some enzymes. The constituents of these transducons are dynamic, moving in and out of the complex after signalling.

Although researchers have barely begun to define these structures in plants, the Cop9 signalosome (an eight-subunit complex regulating de-etiolation, and controlled by phosphorylation[79]) is a clear case for a stable plant-transduction complex. Three kinds of less stable signalling complex — but nonetheless associated with the plasma membrane — have also been reported. And many more can be expected.

Complexes can form around pleckstrin homology (PH) domains[80] in plant cells, and about ten genes in the *Arabidopsis thaliana* genome contain a PH-like domain, including protein kinases[81]. The PH domain binds to phospholipids, and aggregation is usually initiated by phosphorylation or autophosphorylation resulting from receptor occupation. The aggregate can ensure substrate activation or phosphorylation leading to the initiation of, for example, mitogen-activated protein kinase (MAPK) cascades[35]. A second set of less stable complexes has been reported to form with ClAVATA, Rop GTPase, other regulatory proteins and MAPKs[40]. Finally, the 14-3-3 proteins are represented in plant cells by a family of about ten genes. Usually such proteins cross-link others after phosphorylation, and CDPK has been reported to activate 14-3-3 proteins, which are probably involved in controlling ATPase activity within the plasma membrane[82].

connect with their substrates only after specific binding sites have been exposed.

Electrical properties

Although few plants use action potentials for communication, in those that do, the enzymatic and electrical properties of the plasma membrane allow summation, integration and computation of electrical properties; just as they do in nerve cells[5,59,60]. Like many other aspects of plant life, action potentials in plants are slow compared with those in animals. But even slower again, and lasting minutes, are the very pronounced transient falls in membrane potential that might be regarded as a kind of pseudo action potential. Many plant-cell signals induce these changes in membrane potential; auxin, gravity, abscisic acid, blue light and red light are excellent examples[5,50]. These electrical changes are unlikely to be used for cell–cell communication. But the electrical changes will be as profound on the properties of the plasma membrane as those of the genuine action potential itself. Furthermore, a change in membrane potential should allow signal integration as observed for action potentials.

Much electrophysiological information indicates the presence of voltage-gated channels in the plant plasma membrane[61]. As the membrane potential changes, the channel proteins undergo conformational changes that promote opening (or closing) and subsequent altered ion flux. However, there is no reason to think that channels are the only proteins to undergo electrically dependent structural change. On the cytoplasmic face of the plasma membrane, higher rates of ion flux will radically alter the ionic strength, particularly near the mouth of channels; the availability of water will be changed, modifying protein–protein interaction and protein complex status; electrical changes will modify the three-dimensional conformation of many proteins, exposing groups for phosphorylation/phosphatase action and alterations in surface charge might even alter membrane lipid mobilities.

We propose that one important result will be to modify receptor phosphorylation and diversify receptor behaviour. Phosphorylation alters both conformation and function and can generate proteins with different activities according to sites and numbers of phosphorylated amino acids. A simple feedback loop is closed in which perception modifies subsequent perception. The structure of many protein complexes and channels might be altered for extended periods by phosphorylation. Mechanisms for timing of the signal exposure, estimates of the quantity of signal arriving and for long-term modifications of plasma membrane function can therefore easily be constructed. In cases that there are no obvious receptor proteins (such as for water or nitrate), transporting proteins themselves (in this case, the water channels or nitrate transporters) might provide the necessary basis for perception[11,62].

The significance of a change in membrane potential itself to signalling is supported by older observations, which showed that many organic chemicals, thiol (SH)-group reagents and respiratory inhibitors can break seed and bud dormancy, or induce root formation for

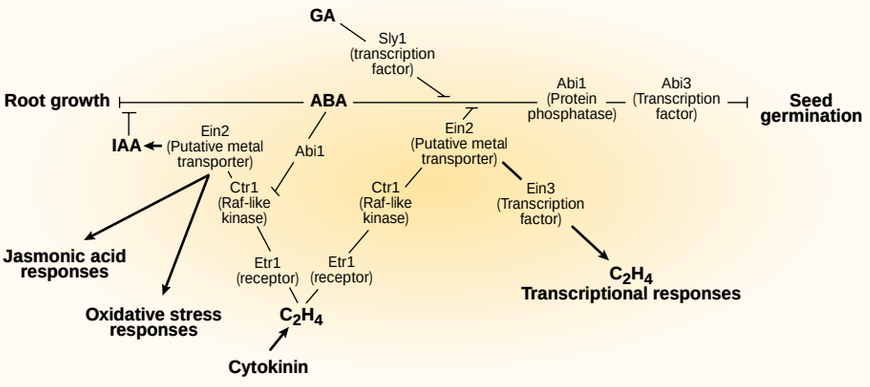

Figure 4 | **Plants show extensive cross-talk and interactions between signalling systems.** Recent genetic analysis of the physiological responses of mutants of *Arabidopsis thaliana* has uncovered possible molecular elements of a complex interacting network of control allowing growth regulators, such as auxin (IAA), cytokinin, ethylene (C_2H_4), abscisic acid (ABA) and gibberellin (GA), to interact in the regulation of root growth, stress and defence responses (such as oxidative stress and jasmonic-acid responses), and seed germination[87–89]. Despite its complexity, this is a simplified view of the true regulatory interactions that occur in the plant cell.

example. The common property linking these chemicals is a demonstrated change in membrane potential[2,63,64].

Signal computation with attitude

If the plasma membrane is highly structured and activation of channels is localized, then a means to coordinate the behaviour of the membrane becomes necessary. Changes in Ca^{2+} fluxes across this membrane provide one example of how such coordination could be effected. The basis of Ca^{2+} signalling is the separation of different concentrations across a membrane, energized by Ca^{2+} ATPases. Upon signalling, channels open in the requisite membrane, allowing Ca^{2+} to move down its electrochemical potential gradient. Cytosolic Ca^{2+} has three properties that make it ideal for plasma membrane coordination. Calcium is not a mobile ion in the cytoplasm[65]; Ca^{2+} signals move as waves that are thought to be constrained to the cytoplasmic surface of the membrane[33], and these waves are usually initiated at specific cellular sites[57].

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When plant cells are subjected to several signals, they seem able to access different sources of cytosolic Ca^{2+}, producing a different response to each signal[57]. Single-cell imaging of Ca^{2+} changes in response to different signals confirms these observations[30]. A highly structured arrangement of channels, Ca^{2+} stores and wave direction is implied. These waves might have complex fractal-like forms. Only certain discrete spatial regions of the plasma membrane may be activated by Ca^{2+} elevation[57,66] (FIG. 2). By varying the combinations of plasma membrane regions that are activated, considerable potential for the computation of signals emerges. Several important Ca^{2+}-binding proteins, such as calmodulin[66,67] and CDPK[53,54], are attached to the plasma membrane.

Evidence that structural rearrangements of the plasma membrane result from signalling can be deduced from an experimental separation of signals from the associated Ca^{2+} transients and physiological effects. Plant cells given a hyperosmotic shock[58] or exposed to red light[68] will normally express some transient increase in cytosolic Ca^{2+}. However, if the signal is imposed in the absence of extracellular Ca^{2+}, no Ca^{2+} transient is observed. The physiological response and the Ca^{2+} transient are delayed until Ca^{2+} is added back to the cells, when both progress normally. Some 'excited' state is induced by the initial signal; this lasts 20 minutes with hyperosmotic shock and up to 4 hours with red light.

Future directions

Fundamentally, life is organization. The cell is a product of the special properties that emerge from the complex interactions and spatial structures between the many thousands of molecules and enzymes of which it is composed. The same panoply of building blocks can be used in transduction between both plants and animals but, by changing their relationships and interactions, different properties will emerge. The plasma membrane (perhaps more so in a plant cell than others) acts as a relatively permanent structure on which many kinds of transduction structure can be made. This might represent part of the answer to the question posed in the Preface. Emphases on spatial relationships and cross-talk[8] between signalling pathways seems to be crucial.

The completion of the sequencing of various plant genomes will provide us with a phenomenally rich array of candidate regulators of plant-cell function. The direction now must be to define which molecules interact with each other and where these interactions occur *in vivo*. Emerging technologies, such as green fluorescent protein fusions, live cell imaging of fluorescence resonance energy transfer and fluorescence lifetime imaging[31], are beginning to approach these questions in the only setting where these interactions can really be determined — the intact, functioning cell. By this means, we will slowly unravel the network of connections (FIG. 4) that provides unity to cellular and plant activities and that is undoubtedly present. The particular properties of the living cell are shared in some way or another through every constituent molecule, forming a highly integrated regulatory network. Equally, the environmental context, whether from outside the plant or from within, contributes to shaping how information is processed by each cell. In trying to understand signal transduction, we are doing no more than trying to understand life itself.

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Links

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ENCYCLOPEDIA OF LIFE SCIENCES Plant growth factors and receptors | Plant plasma membrane

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