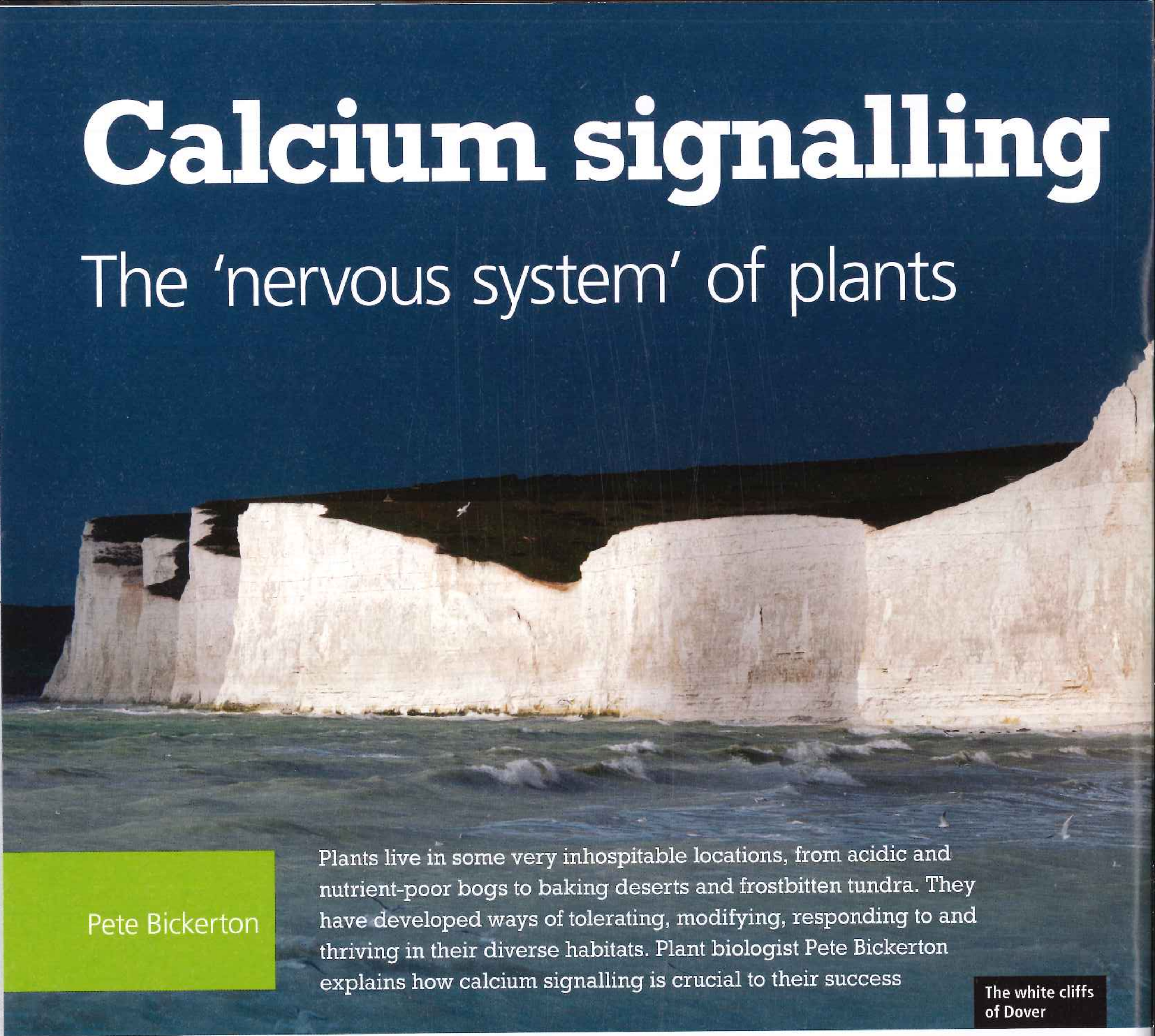
Calcium signalling

The 'nervous system' of plants



Pete Bickerton

Plants live in some very inhospitable locations, from acidic and nutrient-poor bogs to baking deserts and frostbitten tundra. They have developed ways of tolerating, modifying, responding to and thriving in their diverse habitats. Plant biologist Pete Bickerton explains how calcium signalling is crucial to their success

The white cliffs of Dover

AQA: 3.2.3 Transport across cell membranes; 3.6.1.1 Taxes and kineses; 3.6.2.1 Depolarisation of membranes; 3.6.3 Role of calcium ions in muscle contraction

Edexcel A: 2.2 to 2.4 Movement across cell membranes; 7.2 Role of calcium ions in muscle contraction

Edexcel B: 4.2 Cell transport mechanisms; 9.5(i) Resting potential

OCR A: 2.1.5(d) Movement of molecules across membranes; 5.1.1(b) Communication between cells by cell signalling; 5.1.3(c) Resting potential; 5.1.5(l) Role of calcium in muscle contraction

OCR B: 2.1.1(l) The movement of molecules across plasma membranes; 4.1.2(l) Role of calcium ions in muscle contraction; 5.2.1(d) Resting potential

WJEC Eduqas: AS Unit 1, 3 Cell membranes and transport; A2 Unit 4 Option B 1(c) Muscle contraction

Key words

- Ca^{2+} signal
- Ca^{2+} channel
- Ca^{2+} sensor
- Depolarisation
- Flagella
- Channelrhodopsin

Unlike most animals, most plants don't move very far. Some plants can change the position of their leaves relative to the sun, thus maximising photosynthesis, but otherwise have to put up with what life throws at them from the very place that they germinate.

When animals get too warm, they can seek shade. When under attack, animals can run away, or at least immediately fight back. Plants don't have this luxury, yet some are able to stay in the same place, for thousands of years, responding to all the stresses that go with being quite literally fixed to the ground.

Much like animals and all other life forms, plants can sense their environment. Plants can detect wind, touch, heat, cold, salt and **osmotic stress**, and all of the wavelengths of light from ultraviolet to far red. They can detect when they are under attack from pests or pathogens, or when a **symbiotic** fungus or bacterium works together with the roots to collect nutrient ions such as nitrate, sulfate and phosphate.

They don't have a nervous system so how do they do this? One way, which is universal to all eukaryotes, is calcium signalling.

Calcium: the universal cell signal

Calcium is the fifth most abundant element in the Earth's crust and is vital to the survival of all cellular life. In the form of calcium ions (Ca^{2+}), it helps form mammalian bones and teeth and the shells of snails. The white cliffs of Dover are made almost entirely of the calcium carbonate shells of dead algae known as coccolithophores, which are now more famously recognised as chalk. Not only is Ca^{2+} an important constituent of cell walls and membranes in plant cells, it is a highly versatile **signalling molecule**.

One reason that Ca^{2+} is so useful is that it is toxic to cells at high concentrations. Since the dawn of life and through billions of years of adaptation to a high Ca^{2+} environment, cells strictly regulate the concentration of Ca^{2+} in their cytoplasm. This regulation is so tight that the resting concentration of Ca^{2+} in the cytoplasm of plant cells is a tiny figure — about 10^{-7} mol dm^{-3} — around 10 000 times lower than in the watery **apoplast** that fills the space between plant cells.

Ca^{2+} homeostasis involves protein channels and transporters that are found either in the cell surface membrane or in the membranes of organelles, including the vacuole, endoplasmic reticulum, chloroplast and nucleus. Ca^{2+} channels allow Ca^{2+} to enter a cell along a concentration gradient, while transporters actively remove Ca^{2+} from the cytoplasm using energy (ATP) and protons (H^+) — either pumping Ca^{2+} out entirely or storing Ca^{2+} in organelles.

The very mechanisms that evolved in the process of guarding cells from high concentrations of Ca^{2+} seem to have prompted the almost universal adoption of Ca^{2+} as a central signalling molecule involved in a huge variety of biochemical responses throughout the eukaryotes.

Nature's Morse code

The Ca^{2+} channels in the cell surface membrane (see Box 1) take on a range of forms, some being activated by changes in voltage while others are activated by such things as stretching ('mechanosensitive' ion channels) — from wind or by

Coloured scanning electron micrograph of coccolithophores (×8500) touch. Once a Ca^{2+} channel has been activated, Ca^{2+} diffuses into the cytoplasm.

A combination of Ca^{2+} channels, Ca^{2+} transporters and Ca^{2+} binding proteins then alters the amount of Ca^{2+} diffusing into the cell depending on the environmental stimulus, meaning that each stimulus causes a specific Ca^{2+} response.

This works rather like Morse code. For each different stimulus — cold, heat, light, touch, wounding, wind, saltiness, heavy metals or disease — plants respond by increasing the intracellular concentration of Ca^{2+} in a specific pattern. In some cases, the Ca^{2+} oscillates continuously in response to stimuli (the concentration increases and decreases repeatedly over time, like a radio wave). In other cases there is a swift, sharp spike, and in others Ca^{2+} is continuously elevated. Often, calcium ions move like a wave through cells and tissues. To draw a mental picture, on a graph the signals tend to resemble a trace on a heart monitor (see Box 2). Each pattern, with its unique shape (frequency and amplitude), holds the information that plants can use to produce the correct response.

As well as the proteins that move Ca^{2+} into and out of the cytoplasm, there are 'sensor' proteins that bind to Ca^{2+} (such as calmodulin and calcium-binding protein). These proteins are responsible for

Terms explained

Apoplast The non-living space between plant cells, which has an important role in transporting water from roots to xylem vessels.

Depolarisation Normally, cell membranes are charged due to the presence of ions (such as Na^+ and K^+) on either side. Most cells keep a negative charge inside the cell, which becomes depolarised when many ions move into the cell (the charge becomes less negative). The opposite of this is repolarisation and together these make up the 'action potential' in nerve cells. Depolarisation is a common signalling mechanism in many cells.

Osmotic stress When a cell is exposed to extreme saline conditions, water moves out of the cell quickly by osmosis towards the saltier solution, causing a phenomenon known as osmotic stress.

Signalling molecule Cells use biochemical signals to relay information inside and between them. These signals can be proteins, lipids, ions, or other molecules, and usually act as a ligand that binds to receptors either inside cells or outside on the cell surface membrane.

Symbiotic Symbiosis is the phenomenon by which two different organisms live together, often benefiting one or both of the organisms involved. One such example is the fungi that live in and among plant roots, providing nutrients, water and protection from disease.

Box 1 Ca^{2+} homeostasis in a plant cell

Calcium (Ca^{2+}) enters the cytoplasm along a concentration gradient via Ca^{2+} channels (CC in Figure 1.1). Ca^{2+} is actively pumped out of cells using energy (ATP) via Ca^{2+} pumps (CP). Ca^{2+} can also exit the cytoplasm in exchange for other ions: sodium (Na^+) via Na^+/Ca^{2+} exchangers (NCX) and protons (H^+) via Ca^{2+}/H^+ exchangers (CAX). Ca^{2+} can enter the cytoplasm from the external environment or from internal stores in organelles.

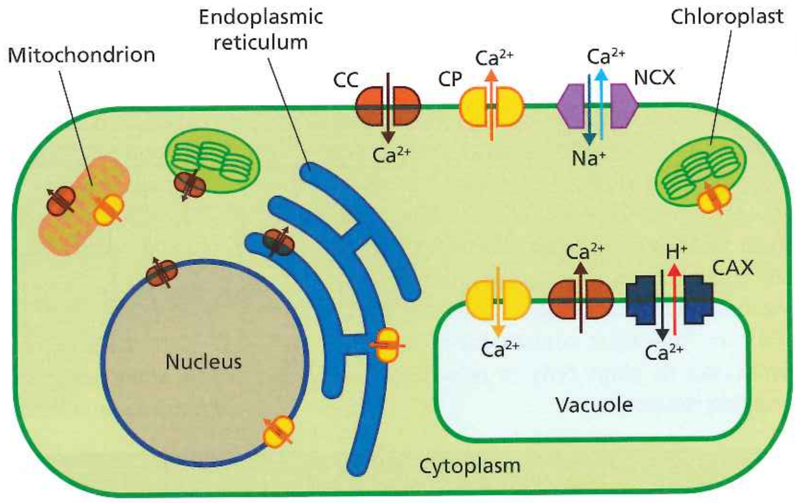


Figure 1.1

Box 2 A typical Ca^{2+} signalling pathway

A stress such as cold, a pathogen or the hormone abscisic acid (ABA) causes specific increases in Ca^{2+} known as a Ca^{2+} signal. This signal can be detected by sensors, e.g. calcineurin B-like protein (CBL), calmodulin (CaM), Ca^{2+}-dependent protein kinases (CDPK), calmodulin-like proteins (CML) and Ca^{2+}-independent protein kinases (CIPK) — see Figure 2.1. These proteins relay the signal by activating other proteins, e.g. transporters at the cell membrane, or by increasing or decreasing gene expression by either directly binding to DNA or via transcription factors (TF). Protein kinases (CDPK, CIPK) target downstream proteins by supplying a phosphate group (P), which can cause enzymes to change their structure and become active (i.e. reveal an active site).

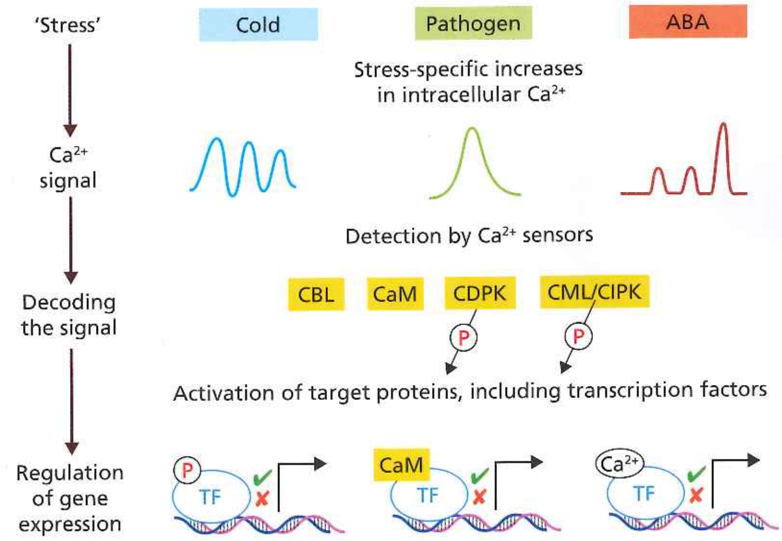


Figure 2.1

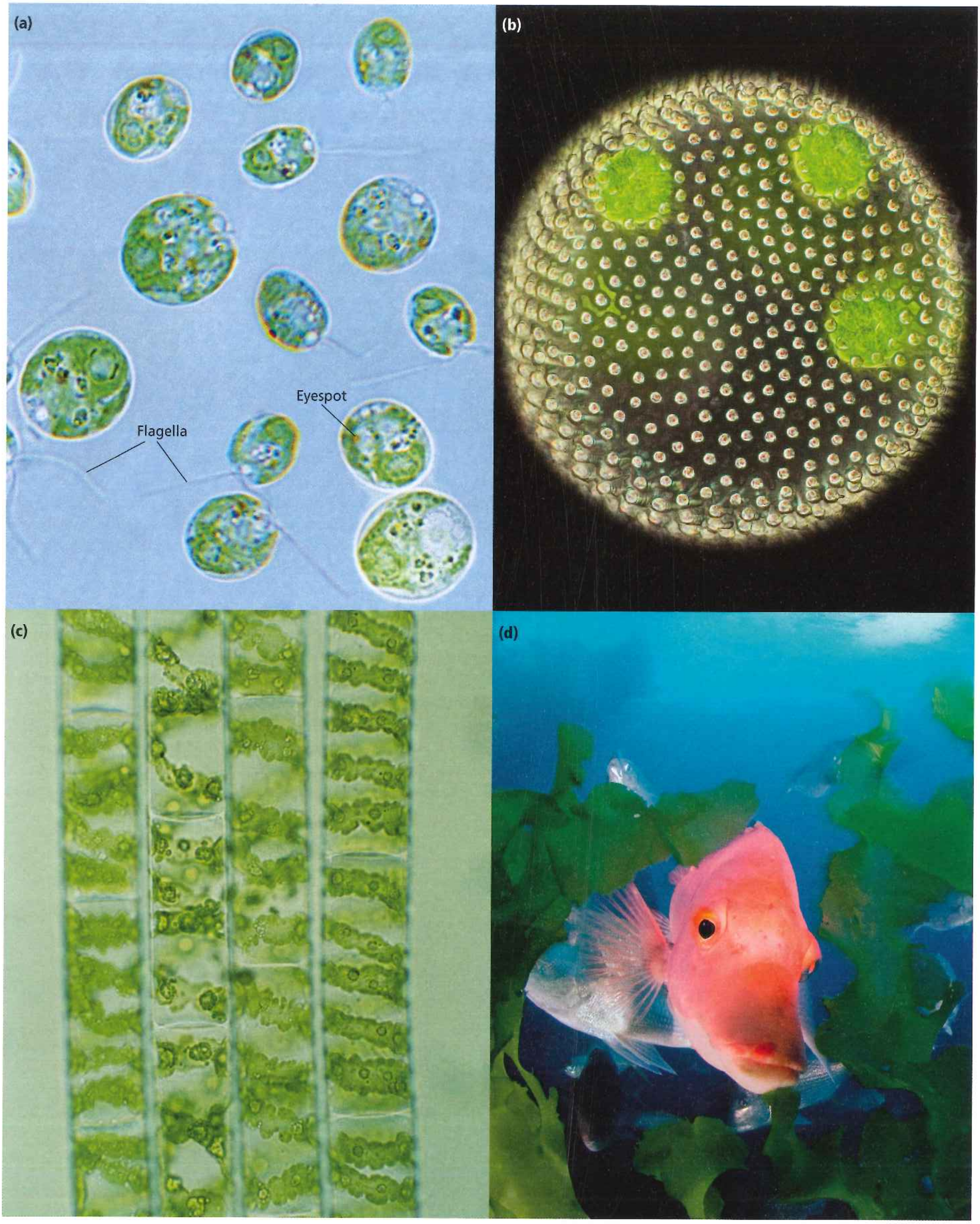


Figure 1 A diversity of green algae. **(a)** Light micrograph of *Chlamydomonas* cells (×1500). **(b)** Darkfield light micrograph of *Volvox*, a motile form which has hundreds of *Chlamydomonas*-like cells bound into a transparent matrix. **(c)** Light micrograph of *Spirogyra*, a multicellular non-motile freshwater alga with characteristic spiral chloroplasts (×350). **(d)** Fish swimming among multicellular non-motile, marine *Ulva*

Box 3 Phototaxis in green algae

(a) A *Chlamydomonas* cell swims towards the light. When the light direction changes (b), activation of channelrhodopsin in the eyespot (red) causes depolarisation of the cell. A subsequent increase in calcium (Ca^{2+}) in the flagella causes one to move independently of the other, so that the cell quickly changes direction to swim once more towards the source of the light (c).

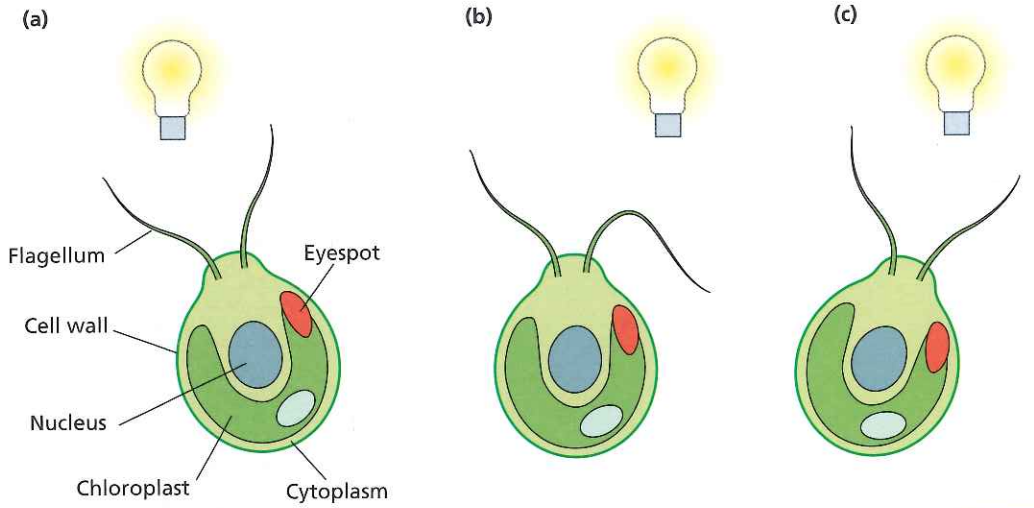


Figure 3.1

relaying the signal to the correct enzyme or DNA-binding transcription factor. Eventually, depending on the amounts of Ca^{2+} and the proteins that each individual Ca^{2+} pattern activates, specific genes and other regions in DNA can be turned on or turned off, leading to a response.

Calcium signalling in action

One example of how calcium signalling can cause a response comes from green algae (see Figure 1 and centrespread), some of which are not limited to one position but can swim, thanks to their flagella. *Chlamydomonas* has two flagella that pull the cell through the water in a manner similar to someone doing breaststroke while simultaneously rotating their body.

Green algae have chloroplasts with which they harvest the sun's energy to make sugar. They all therefore need sunlight and if you put a container of motile green algae in a dark chamber they will swim towards a light source turned on at one side. If you then change the light source to the opposite side of the chamber, they will turn around and swim the other way (a process known as phototaxis).

First, we have to understand the important parts of a motile green alga such as *Chlamydomonas*. One is a red area called an eyespot. This eyespot contains proteins that are remarkably like those in the human eye, called channelrhodopsin (an ancient ancestor of what is now found in rods in the human retina). The channelrhodopsin is directly activated by light, so when a particular wavelength of light reaches the eyespot, the protein changes shape, allowing various ions, including Ca^{2+}, sodium (Na^+), potassium (K^+) and protons (H^+), to rush into the algal cell. This influx of ions causes the algal cell to **depolarise** (a loss of a difference in charge on either side of the cell surface membrane, much like in a human nerve cell). This then activates voltage-gated Ca^{2+} channels in the flagella. Of the two flagella, only one continues to rotate, which has the same result as paddling a kayak on only one side — the alga turns on the spot (see Box 3). Polarisation is restored, both flagella resume beating and the cell swims towards the light.

A million responses

The responses that calcium signalling allows could form many more articles, and more is being discovered about the impressively intricate system that plants use to interpret signals from the environment. The fact is that plants manage to process a wealth of information in conditions where many environmental factors are being signalled all at the same time. This makes understanding the whole process tricky outside of the tightly controlled environment of a laboratory setting. However, what is certain is that plants are well equipped to withstand what the world throws at them, even those that are rooted to the spot.

Dr Pete Bickerton is the scientific communications and outreach manager at the Earlham Institute: www.earlham.ac.uk

Further viewing

Watch *Chlamydomonas* swimming and changing direction here: **www.youtube.com/watch?v=l1LocoqXQf4**

Watch *Volvox* swimming here: **www.youtube.com/watch?v=kaUJULOYbhY**

Key points

- Calcium ions (Ca^{2+}) form a universal signal in eukaryotes in response to a large number of environmental stimuli.
- Ca^{2+} signals are specific for each type of stimulus and lead to different responses, sometimes almost instant, often via changes in gene expression.
- Ca^{2+} channels and transporter proteins in plant cell surface and organelle membranes shape the Ca^{2+} signals, moving Ca^{2+} into and out of the cytoplasm in a tightly regulated way.