a growing body of evidence demonstrating how the composition of and changes in the ECM can modulate glucose metabolism[5,6]. The data also offer a potential explanation for the aberrant glucose regulation reported in metabolically dysfunctional fatty breast tissue of people who are obese, in which the connective tissue, called the stroma, is often stiff[7]. Park and colleagues' work is consistent with, and extends, another study that has linked cytoskeletal organization to glycolysis[8], as well as work showing how the application of forces between cells can increase cellular metabolism[9].

One of Park and co-workers' most compelling observations is that tension-mediated sequestration of TRIM21 might explain why tumours exhibit abnormally high levels of glycolysis[10]. The group's unbiased computational assessment showed high levels of PFKP in tissue from people who had lung cancer. The authors engineered their human lung cell line to express some of the cancer-promoting proteins that are frequently overexpressed in lung tumours. PFKP levels were consistently higher in these cells than in the normal lung cells, regardless of the substrate on which they were grown. By contrast, the enzyme's levels were variable in healthy lung tissue, and the authors' lung cell line showed varying PFKP levels in response to changes in substrate stiffness.

Tumours often undergo a desmoplastic response — an increase in production, remodelling and crosslinking of components in the ECM, causing a tissue-scarring process called fibrosis that stiffens the stroma. The desmoplastic response promotes tumour-cell growth, survival and invasion[11]. This might suggest that a stiff tumour ECM fosters tension-induced actin bundling, which acts to sequester TRIM21 and stabilize PFKP, thereby promoting unfettered glycolysis. If this hypothesis is correct, treatments that prevent fibrosis would be expected to normalize tumour-cell metabolism.

Unfortunately, such strategies have been disappointing clinically[12]. One explanation is that many tumour cells also show increased activity of the RhoGTPase and ROCK enzymes[13,14], which promote actin-filament assembly, or overexpress oncogenes (which encode cancer-promoting proteins such as Ras and EGFR) that cause elevated actin–myosin tension[13,14]. The cells therefore probably form stress fibres regardless of ECM conditions. Indeed, when Park and colleagues grew lung cancer cells carrying oncogene mutations on soft substrates, they observed that the cells not only maintained prominent actin stress fibres, but also had reduced TRIM21 expression, elevated PFKP levels and high rates of glycolysis.

Finally, the authors showed that the abnormally high glycolysis in their tumour cells could be normalized simply by increasing *TRIM21* gene expression. This finding argues that compounds that stimulate protein degradation, augment ligase activity or reduce actin-fibre assembly should similarly normalize tumour glycolysis and hence could be new antitumour therapies. Perhaps the same goal could be accomplished by reducing tumour-cell tension or treating patients with ROCK inhibitors that have already been developed for clinical use — at least in those tumours in which TRIM21 or similar E3 ligases are not mutated (mutations in several E3 ligases have been implicated in either tumour suppression or tumour progression[15]). Indeed, ROCK and EGFR inhibition can reverse the proliferative and invasive traits of 3D *in vitro* tumour structures called spheroids, and prevent the progression of various cancer types in animal models *in vivo*[13,14,16].

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Plant biology

Making sense of hydrogen peroxide signals

Christine H. Foyer

The discovery of a sensor that detects hydrogen peroxide at the surface of a cell provides insights into the mechanisms by which plant cells perceive and respond to environmental stress. **See p.577**

Chemically reactive, oxygen-containing molecules called reactive oxygen species (ROS) are central to cell function. Plant cells generate various ROS, including hydrogen peroxide (H_2O_2), which has a key role in cell signalling. It is produced in an extracellular space between the plasma membrane and cell wall called the apoplast, in response to a range of factors, including stressors, plant hormones such as abscisic acid, and physical or chemical changes outside the cell[1]. But whether and how this extracellular H_2O_2 (eH_2O_2) is sensed at the cell surface is unknown. On page 577, Wu *et al.*[2] identify the first known cell-surface H_2O_2 receptor in plants.

The apoplast and cell wall act as a dynamic interface between plant cells and the outside world, with all its threats, challenges and opportunities. Some eH_2O_2 moves from the apoplast into the cytoplasm through channel proteins called aquaporins[3]. However, unlike the cytoplasm, the apoplast contains relatively few molecules that counteract oxidation[1] — and so ROS, including H_2O_2, can survive for much longer in the apoplast than in the cytoplasm. This is a compelling reason to suspect that there is a sensor for eH_2O_2 in the apoplast.

Although little is known about the initial target of eH_2O_2, the consequences of its production are much better defined[4]. It is clear that eH_2O_2 triggers an influx of calcium ions (Ca^{2+}) into the cell, which then leads to the systemic transmission of signals between cells in waves, activating processes such as pathogen resistance or acclimation to stress across the entire plant[5]. In addition, eH_2O_2 signals regulate the polarized growth of pollen tubes and root hairs[6], and control the opening and closing of stomata[3] — pores on the outer layer of the leaf formed by two guard cells. Stomata enable the free passage of molecules such as

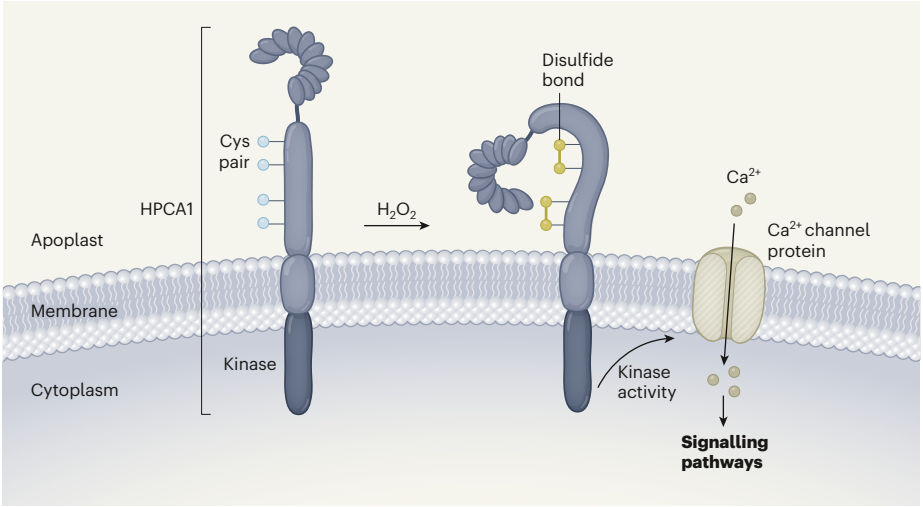

Figure 1 | The HPCA1 protein. Wu *et al.*[2] have identified the first extracellular sensor of hydrogen peroxide (H_2O_2) in plants, HPCA1. The protein has an intracellular kinase enzyme domain, and an extracellular domain that protrudes into the apoplast — the compartment between a plant cell's plasma membrane and the cell wall. HPCA1 has two special pairs of cysteine (Cys) amino-acid residues. The authors demonstrate that H_2O_2 oxidizes thiol groups (not shown) on these residues, forming sulfenic acid (SOH; not shown) and disulfide bonds. This oxidation triggers a conformational change and kinase activity, which, through unknown mechanisms, lead to the opening of calcium-ion (Ca^{2+}) channels and Ca^{2+} influx into the cell, triggering intrinsic and systemic signalling pathways.

carbon dioxide and oxygen into the plant when open, and can close to prevent water loss from the plant.

Wu *et al.* set out to identify cell-surface receptors for eH_2O_2 that trigger Ca^{2+} signalling, using a 'forward' genetic-screen approach. They treated seeds of the plant *Arabidopsis thaliana* with a chemical that induces DNA mutations, then screened the resulting plants to identify mutants that showed low Ca^{2+} influxes in response to H_2O_2. They named these mutant plants *hydrogen-peroxide-induced Ca^{2+} increases 1* (*hpca1*).

The authors then identified the HPCA1 protein. They report that HPCA1 is a membrane-spanning enzyme of a protein family known as leucine-rich repeat (LRR) receptor kinases. The group also showed that HPCA1 has two special pairs of cysteine (Cys) amino-acid residues in its extracellular domain. The thiol groups of Cys residues are known[7] to be a target for oxidation by H_2O_2. The authors demonstrate that the presence of eH_2O_2 leads to oxidation of the extracellular Cys residues of HPCA1 in guard cells. This modification activates HPCA1's intracellular kinase activity, triggering Ca^{2+}-channel activation and Ca^{2+} influx, followed by stomatal closure (Fig. 1).

In the absence of eH_2O_2, the *hpca1* seedlings showed no differences from wild-type seedlings. However, their guard cells were less sensitive to eH_2O_2 than were those of the wild-type seedlings, showing lower than wild-type levels of Ca^{2+} influx in response to eH_2O_2. HPCA1 is therefore required to convert the eH_2O_2 signal into a physiological response. Moreover, the abscisic acid-dependent production of eH_2O_2 by guard cells was defective in the *hpca1* mutants. Of note, the function of HPCA1 in eH_2O_2 signalling was not limited to guard cells, and the authors provided evidence that eH_2O_2 signalling helps to transmit environmental signals to the nucleus of various cell types to regulate gene expression.

Oxidation of Cys by H_2O_2 leads to the formation of a sulfenic acid (SOH), which is at the heart of reduction–oxidation (redox) signalling. Sulfenic acids are rather unstable intermediates that can be further oxidized to sulfinic (SO_2H) and sulfonic (SO_3H) acid, or can undergo 'exchange reactions' to form disulfide bonds. For HPCA1 to function properly as a receptor for eH_2O_2, the Cys oxidation process must be readily reversible, re-forming thiol residues that can be oxidized again. However, the factors that mediate reduction of the oxidized HPCA1 are unknown. One candidate is a membrane-bound electron-transport system, such as the one that reduces an oxidized form of the antioxidant molecule ascorbic acid in the apoplast[8]. Membrane-bound and apoplastic thioredoxin-like proteins are also putative candidates, given that thioredoxin is a well-characterized reducing agent for oxidized Cys residues of proteins.

"HPCA1 protein is required to convert the extracellular hydrogen peroxide signal into a physiological response."

Wu and colleagues have uncovered a receptor-kinase-mediated eH_2O_2 sensing mechanism that does not resemble any known eH_2O_2 receptors or sensors reported in other organisms. Nonetheless, HPCA1 might be part of a much wider portfolio of sensors used by plants to perceive and respond to environmental changes through ROS signals. The identification of such receptors has proved challenging, not least because likely candidates are members of very large protein families. Sophisticated screens, such as that used by Wu *et al.*, will be required to tease out the family members that have ROS sensing and signalling roles. Once these sensors have been identified, it should be relatively easy to manipulate their properties to produce model plants and crops that have, for example, increased or depressed sensitivity to environmental H_2O_2 signals, and so show altered tolerance to environmental threats.

Stomatal closure is not regulated just by H_2O_2; it is also a response to elevated atmospheric CO_2 levels[3,9]. It will be intriguing to see how proteins such as HPCA1 function in redox signalling networks that are likely to prepare plants for life in a future high-CO_2 world. High CO_2 levels can stimulate photosynthesis and depress photorespiration; changes in the photosynthesis:respiration ratio have a wide-ranging impact on cellular redox balance, because photorespiration generates a molecule of H_2O_2 in one organelle, the peroxisome, for every oxygen molecule assimilated in another organelle, the chloroplast, during photosynthesis. Perhaps other H_2O_2 sensors act together with HPCA1 to transmit organelle-specific redox messages to the nucleus, along with messages from the external face of the plasma membrane.

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