
José Márquez

Surviving drought

We've all felt it: a quickening of the heart and a slight shortness of breath as you walk into an exam room. Most of us recognise that the hormone adrenaline is responsible for this reaction, but we're not unique in responding to stress with a release of hormones. Plants do this too – but unlike you and I, they don't have the option to flee; rooted to the spot, they can only stay and fight it out. To do this, plants release the hormone abscisic acid (ABA), which coordinates their response to stresses such as drought, extreme temperature and high salt levels.

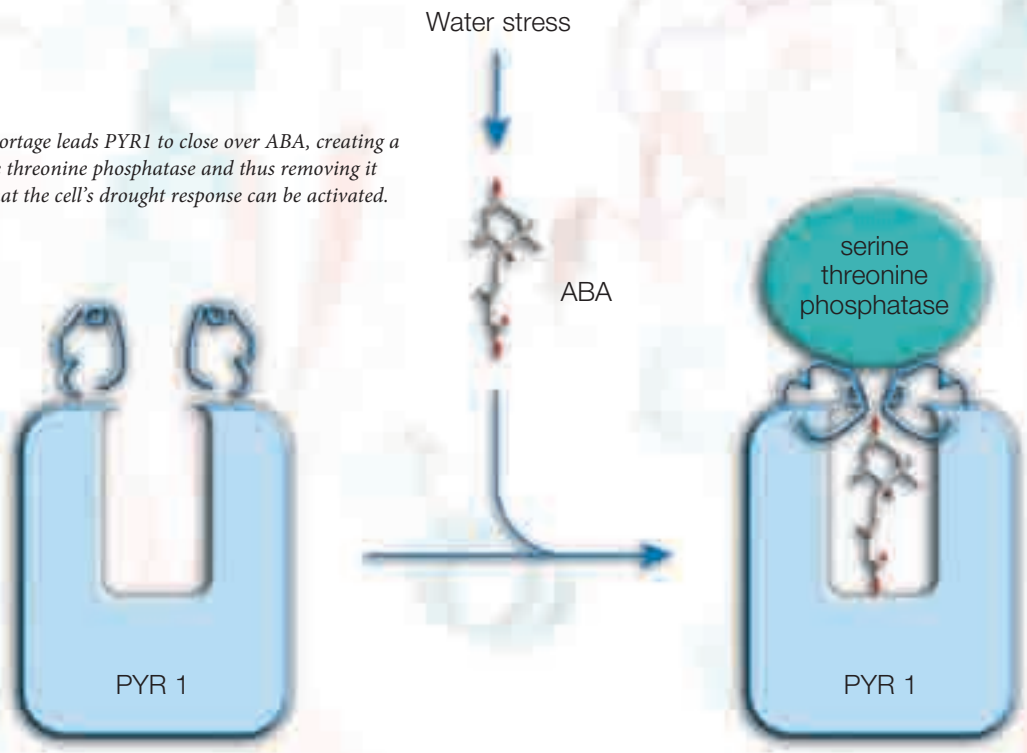
ABA acts as a chemical courier, relaying messages from one cell to another. Cells respond to the hormone if they possess a receptor, which, once bound to the hormone, signals to the cell to go on the offensive. For plants, this means closing the tiny holes in their leaves to avoid water loss, diverting resources to their roots to increase water uptake and switching on the production of proteins that protect cells from dehydration.

Understanding how plants transmit these water-saving measures to their roots and leaves could help farmers ward off the effects of drought in crops, and has prompted many scientists to search for the receptor that binds ABA. But identifying this receptor has proved unusually challenging.

Since 2006, several proteins have been suggested but, because of conflicting findings, their exact role remained controversial. Then in 2009, two groups independently homed in on yet another potential ABA receptor , PYR1, and a family of proteins that bound ABA was identified, although it was still not clear how these proteins interacted with the hormone. Given the struggles of the past three years, some plant biologists reserved final judgment on whether the hunt for the ABA receptor was over until they could visualise the receptor physically interacting with the hormone. This is where plant biologist Pedro Luis Rodriguez from the Universidad Politecnica de Valencia in Spain stepped in, approaching structural biologist José Márquez from EMBL Grenoble with a question: would it be possible to obtain the structure of the ABA receptor?

"For such a contentious field, knowing the structure is the final proof, because you not only see the receptors but you also see how they bind the hormone," explains José, who, together with his team, examines protein structures to understand how they transmit messages. "Because of our interest in signalling, we were very keen to collaborate on this project."

But José and Pedro were not alone in finding this an interesting puzzle to solve. Four other groups – two in Asia and two in the US – simultaneously set out to define the structure of the receptor. And so the race began. The first step for José's group was to produce vast quantities of the ABA receptor in bacteria before adding the protein to a cocktail of chemicals to try to induce it to form a crystal. José explains that the proteins usually fall to the bottom of the flask in an amorphous blob, but occasionally they stack together in a regular fashion. This is what they were searching for — crystals.

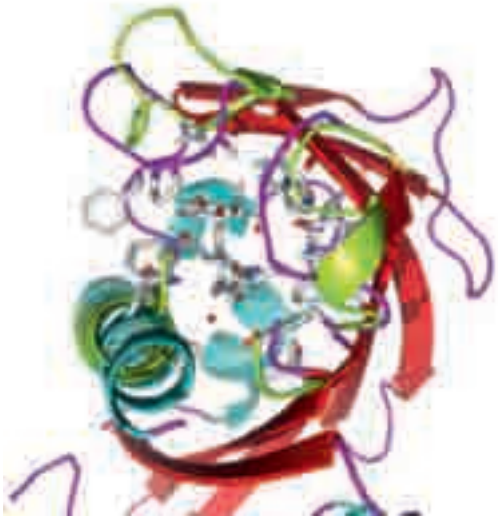


The stress of water shortage leads PYR1 to close over ABA, creating a docking site for serine threonine phosphatase and thus removing it from circulation so that the cell's drought response can be activated.

To increase the likelihood of crystal formation, they set up hundreds of experiments, each one differing slightly in the chemicals added. "Growing crystals can take days, weeks, or even months," says José, adding that each flask needs to be checked every few days. "So as you can see, with all these samples you would quickly run into problems."

Fortunately, José's high-throughput crystallisation facility was on hand to help solve the problem. Using robots to dispense and regularly check close to 3000 samples, it was only a matter of weeks before the researchers found a crystal. "This was exceptionally fast and shows the power of this type of collaboration," says Pedro. Another advantage of automating the crystal-forming process is that less of the protein is wasted because of the accuracy with which the robots can dispense the samples. "They can dispense one tenth of a millionth of a litre – a quantity that humans would find impossible to repeatedly pipette," explains Pedro. By reducing protein waste, the researchers could set up more experiments and so were more likely to secure the perfect cocktail of chemicals in which to grow the crystal.

The structure of PYR1 (coloured ribbons) in its open, unbound state (light green loops) and how it folds around ABA (white rods) when it binds to this hormone (turquoise and purple loops).



With the crystal in hand, José's group used the powerful X-ray beams of the European Synchrotron Radiation Facility in Grenoble to determine the structure of the crystalline ABA receptor (for an overview of this technique, see page 56). Ahead of the other international teams, and in collaboration

After 15 days of drought, an Arabidopsis thaliana *plant will normally be withered and dry (far left), unless it has been genetically engineered to enhance its response to ABA (centre left, centre right and right).*

with Adam Round from EMBL Grenoble who is involved in the Partnership for Structural Biology, they revealed that the ABA receptor acts in pairs: two copies of the protein bind together, each side creating a pocket into which ABA slots. Guarding the entrance to these pockets, they found long flexible loops, which close like a lid over the hormone trapped inside.

This closing mechanism led to an unexpected discovery. José's group realised that the 'closing lid' could create a docking site for an enzyme called serine threonine phosphatase, which is known for having the capacity to suppress the stress-signalling pathway. But when locked in place on the docking site, it can no longer prevent the series of signals cascading through the plant that prepare it for the stress of losing water. "The revelation came when we superimposed the receptor bound to ABA with the unbound form, and saw the difference in the loops," says José. He thinks that when the enzyme binds at the docking site, this inactivates it and prevents it from circulating freely. This then allows another enzyme, a protein kinase, to activate transcription factors that turn on the stress-response genes.

José hopes that this finding could help alleviate crop damage in drought-prone areas. If scientists find a molecule that mimics ABA – which is too expensive to manufacture in large quantities – they could simulate the stress response in plants to help protect them against water loss. The new molecule could be sprayed over crops before a period of dry weather, stimulating them to save water for the harsh times to come.

This is something that José's group has already started to do using their structural models. José explains: "We're trying to fit millions of different molecules into the receptor's pocket in the hope of getting one to fit."

If this works, it could have far-reaching effects for all of us, because most of our food is grown in semi-arid regions such as North Africa and South Asia, which suffer recursive droughts. These regions see huge quantities of food go to waste because of damage caused by unusually dry periods within the growing season. Reducing this waste would particularly benefit people in developing countries who typically sustain themselves on cheaper, more traditional crop varieties that are less drought-resistant. Who'd have thought stress could be a good thing?

Santiago J, Dupeux F, Round A, Antoni R, Park S-Y, Jamin M, Cutler S, Rodriguez P, Márquez J (2009) The abscisic acid receptor PYR1 in complex with abscisic acid. *Nature* **462**: 665-668