

*Biology of the Cell***How Do the Cells of a Growing Plant Know in Which Direction to Elongate?**

Sometimes questions that seem simple can be devilishly difficult to answer. Imagine, for example, that you are holding a green blade of grass in your hand. The grass blade has been actively growing, its cells dividing and then stretching and elongating as the blade lengthens. Did you ever wonder how the individual cells within the blade of grass know in what direction to grow?

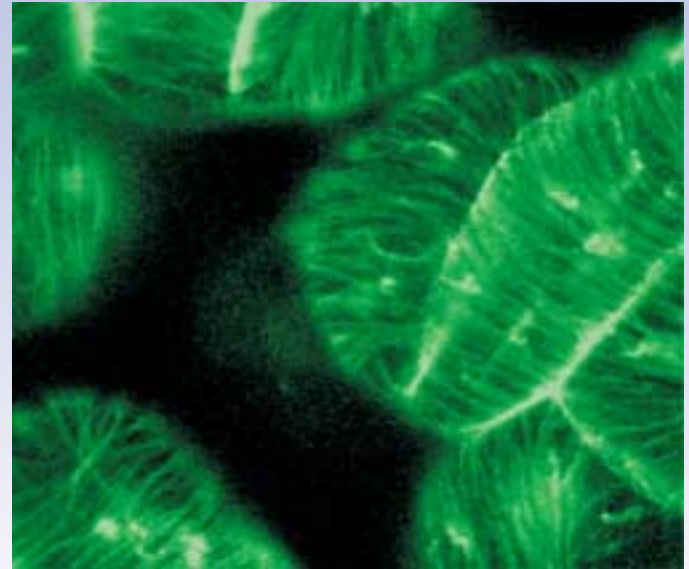
To answer this deceptively simple question, we will first need to provide answers to several others. Like Sherlock Holmes following a trail of clues, we must approach the answer we seek in stages.

Question One. First, we need to ask how a blade of grass is able to grow at all. Plant cells are very different from animal cells in one key respect: every plant cell is encased within a tough cell wall made of cellulose and other tough building materials. This wall provides structural strength and protection to the plant cell, just as armor plate does for a battle tank. But battle tanks can't stretch into longer shapes! How is a plant cell able to elongate?

It works like this. A growing cell first performs a little chemistry to make its wall slightly acidic. The acidity activates enzymes that attack the cell wall from the inside, rearranging cellulose cross-links until the wall loses its rigidity. The cell wall is now able to stretch. The cell then sucks in water, creating pressure. Like blowing up a long balloon, the now-stretchable cell elongates.

Question Two. In a growing plant organ, like the blade of grass, each growing cell balloons out lengthwise. Stating this more formally, a botanist would say the cell elongates parallel to the axis along which the blade of grass is extending. This observation leads to the second question we must answer: How does an individual plant cell control the direction in which it elongates?

It works like this. Before the stretchable cell balloons out, tiny microfibrils of cellulose are laid down along its inside surface. On a per weight basis, these tiny fibrils have the tensile strength of steel! Arrays of these cellulose microfibrils are



Seeing cortical microtubules. Cortical microtubules in epidermal cells of a fava bean are tagged with a fluorescent protein so that their ordered array can be seen.

organized in bands perpendicular to the axis of elongation, like steel belts. These tough bands reinforce the plant cell wall laterally, so that when the cell sucks in water, there is only one way for the cell to expand—lengthwise, along the axis.

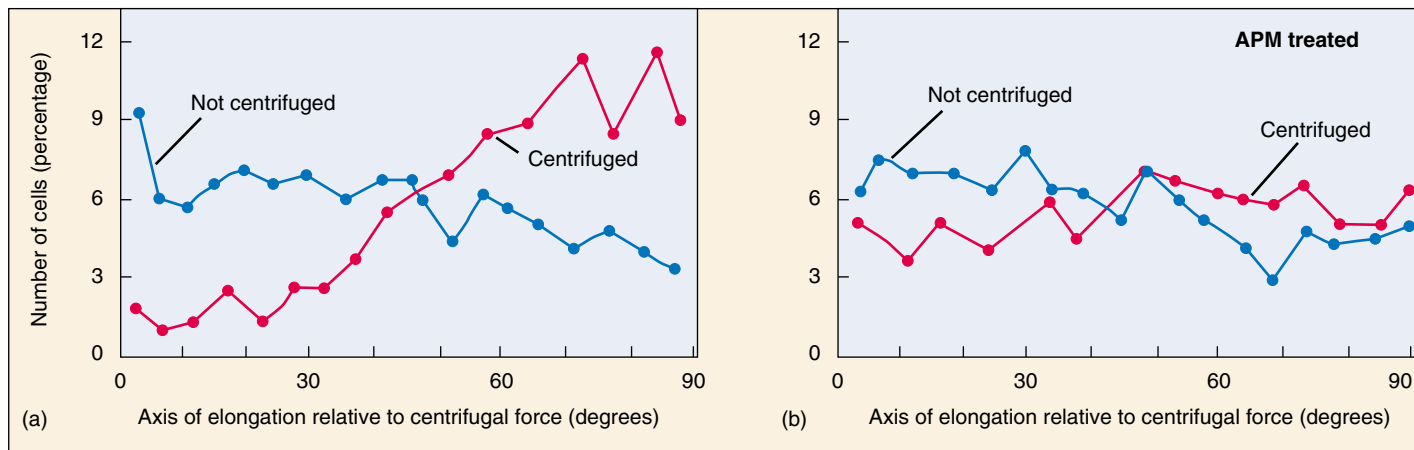
Question Three. Now we're getting somewhere. How are the newly made cellulose microfibrils laid down so that they are oriented correctly, perpendicular to the axis of elongation?

It works like this. The complicated enzymic machine that makes the cellulose microfibrils is guided by special guiderails that run like railroad tracks along the interior surface. The enzyme complex travels along these guiderails, laying down microfibrils as it goes. The guiderails are constructed of chainlike protein molecules called microtubules, assembled into overlapping arrays. Botanists call these arrays of microtubules associated with the interior of the cell surface "cortical microtubules."

Question Four. But we have only traded one puzzle for another. How are the cortical microtubules positioned correctly, perpendicular to the axis of elongation?

It works like this. In newly made cells, the microtubule assemblies are already present, but are not organized. They simply lie about in random disarray. As the cell prepares to elongate by lessening the rigidity of its cell wall, the microtubule assemblies become organized into the orderly transverse arrays we call cortical microtubules.

Question Five. Finally, we arrive at the question we had initially set out to answer. How are microtubule assemblies aligned properly? What sort of signal directs them to orient perpendicular to the axis of elongation? THAT is the question we need to answer.



Effects of centrifugation on cell elongation. (a) Protoplasts (plant cells without cell walls) that were centrifuged showed preferential elongation in a direction approximately perpendicular to the direction of the force. (b) Protoplasts exposed to APM, a microtubule disrupting chemical, exhibited random cell elongation with or without centrifugation.

The Experiment

This question has been addressed experimentally in a simple and direct way in the laboratory of Richard Cyr at Pennsylvania State University. Rigid plant cells conduct mechanical force well from one cell to another, and Carol Wymer (then a graduate student in the Cyr lab) suspected some sort of mechanical force is the signal guiding cortical microtubule alignment.

Wymer set out to test this hypothesis using centrifugation. If cortical microtubules are obtaining their positional information from an applied force, then their alignment should be affected by centrifugal force, and should be impossible if the integrity of the cell wall (which is supposedly transmitting the mechanical force) is perturbed with chemicals that prevent cell wall formation.

Wymer, along with others in the Cyr lab, started out with cells that were not elongated. She isolated protoplasts (cells without walls) from the tobacco plant, *Nicotiana tabacum*, by exposing the plant cells to enzymes that break down the cell wall, creating a spherical plant cell. If allowed to grow in culture, these protoplasts will eventually re-form their cell walls.

In order to examine the effects of directional force on the elongation patterns of plant cells, Wymer and co-workers exposed the tobacco protoplasts to a directional force generated by a centrifuge. Prior experiments had determined that centrifugation at the low speeds used in these experiments does not disrupt the integrity or shape of the protoplasts. The protoplasts were immobilized for centrifugation by embedding them in an agar medium supported in a mold. The embedded protoplasts were spun in a centrifuge at 450 rpm for 15 minutes. Following centrifugation, the embedded cells were cultured for 72 hours, allowing for cell elongation to occur.

Following centrifugation, fluorescently tagged microtubule antibody was applied to the protoplasts, which were then examined with immunofluorescence microscopy for microtubule orientation.

To confirm the involvement of microtubules as sensors of directional force in cell elongation, some protoplasts were incubated prior to centrifugation with a chemical herbicide, APM, which disrupts microtubules.

The Results

The biophysical force of centrifugation had significant effects on the pattern of elongation in the protoplasts following the 72-hour culturing period. The microtubules were randomly arranged in protoplasts that were not centrifuged but were more ordered in protoplasts that had been centrifuged. The microtubules in these cells were oriented parallel to the direction of the force, in a direction approximately perpendicular to the axis of elongation (graph *a* above). These results support the hypothesis that plant cell growth responds to an external biophysical force.

It is true that plant cells are not usually exposed to the type of mechanical force generated by centrifugation but this manipulation demonstrates how a physical force can affect cell growth, assumably by influencing the orientation of cortical microtubules. These could be small, transient biophysical forces acting at the subcellular level.

In preparations exposed to the microtubule disrupting chemical amiprofos-methyl (APM), directed elongation was blocked (graph *b* above). This suggests that reorientation of microtubules is indeed necessary to direct the elongation axis of the plant.

Taken together, these experiments support the hypothesis that the microtubule reorientation that directs cell elongation may be oriented by a mechanical force. Just what the natural force might be is an open question, providing an opportunity for lots of interesting future experiments that are being pursued in the Cyr lab.



To explore this experiment further, go to the Virtual Lab at www.mhhe.com/raven6/vlab2.mhtml