Stomatal Physiology

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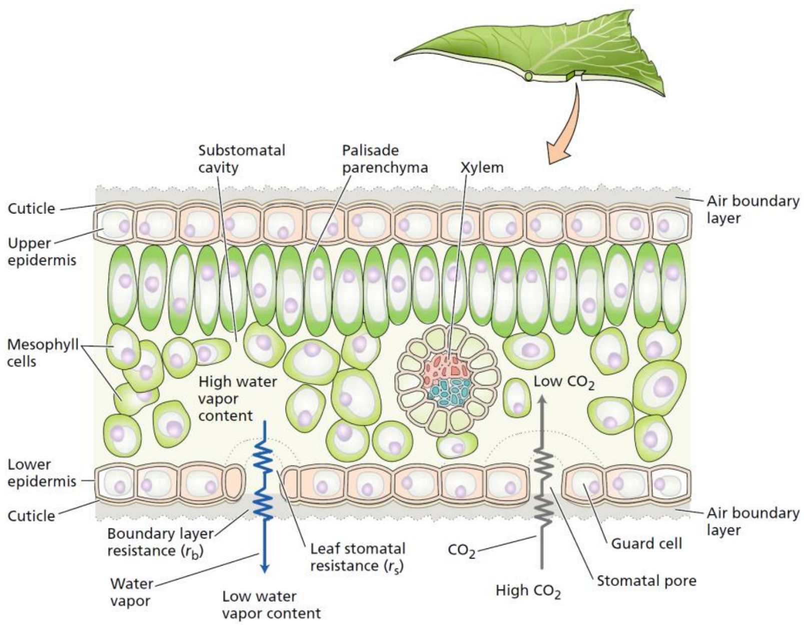
WATER MOVEMENT FROM THE LEAF TO THE ATMOSPHERE

- Diffusion is the primary means of movement of the water out of the leaf.
- The waxy cuticle that covers the leaf surface is a very effective barrier to water movement.
- Only about 5% of the water lost from leaves escapes through the cuticle.
- Diffusion of water vapor through the tiny pores of the stomatal apparatus: Most abundant on the lower surface of the leaf.
- Water is pulled from the xylem into the cell walls of the mesophyll, where it evaporates into the air spaces of the leaf.
- The water vapor then exits the leaf through the stomatal pore.
- Water moves along this pathway predominantly by diffusion, so this water movement is controlled by the ***concentration gradient*** of water vapor.



Water Vapor Diffuses Quickly in Air

- How long would it take for a water molecule to diffuse from the cell wall surfaces inside the leaf to the outside atmosphere?
- The distance through which a water molecule must diffuse from the site of evaporation inside the leaf to the outside air is approximately 1 mm (10^{-3} m).
- The diffusion coefficient of water in air is 2.4 × 10–5 $m^2\ s^{-1}$
- The average time needed for a water molecule to escape the leaf is approximately 0.042 s.
- Diffusion is adequate to move water vapor through the gas phase of the leaf.
- Transpiration from the leaf depends on two major factors:
  - The difference in water vapor concentration between the leaf air spaces and the external air and
  - The diffusional resistance (r) of this pathway.

Water Loss Is Also Regulated by the Pathway Resistances

- The second important factor: The diffusional resistance of the transpiration pathway.
- This consists of two varying components:
  - The resistance due to the layer of unstirred air next to the leaf surface through which water vapor must diffuse to reach the turbulent air of the atmosphere: The leaf **boundary layer resistance** (**rb**).
  - The resistance associated with diffusion through the stomatal pore, the leaf **stomatal resistance** (**rs**).
- The thickness of the boundary layer: Is determined by wind speed.
- When the air surrounding the leaf is very still:
  - The unstirred air on the leaf is the primary deterrent to water vapor loss.
  - Increases in stomatal apertures under such conditions have little effect on transpiration rate.
- When wind velocity is high:
  - The moving air reduces the thickness of the boundary layer at the leaf surface, reducing the resistance of this layer.
  - The stomatal resistance will largely control water loss from the leaf.

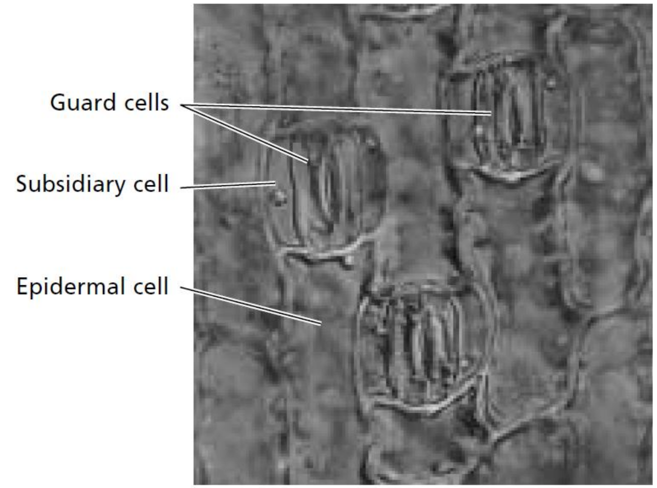
Water Loss Is Also Regulated by the Pathway Resistances

- Anatomical and morphological aspects of the leaf can influence the thickness of the boundary layer.
  - Hairs on the surface of leaves: serve as microscopic windbreaks.
  - Some plants have sunken stomata: provide a sheltered region outside the stomatal pore.
  - The size and shape of leaves also influence the way the wind sweeps across the leaf surface.
- These and other factors may influence the boundary layer, they are not characteristics that can be altered on an hour-to-hour or even day-to-day basis.

- For short-term regulation, control of stomatal apertures by the guard cells plays a crucial role in the regulation of leaf transpiration.

The Guard Cells

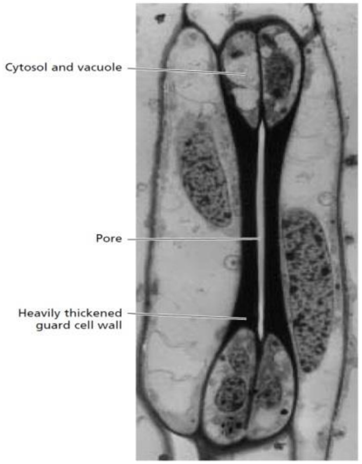
- When water is abundant, The *temporal* regulation of stomatal apertures—
  - Open during the day:
    - The demand for CO_2 inside the leaf is large.
    - The stomatal pores are wide open.
    - The stomatal resistance decreases to CO_2 diffusion.
    - Water loss by transpiration is substantial under these conditions.
    - Since the water supply is plentiful: The plant takes water for photosynthesis, which are essential for growth and reproduction.
  - Closed at night:
    - No photosynthesis
    - No demand for CO_2 inside the leaf,
    - Stomatal apertures are kept small
    - Prevents the unnecessary loss of water.

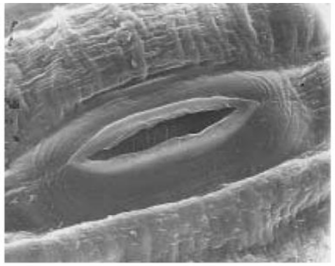


- When soil water is less abundant,
  - On a sunny morning:
    - The stomata will open less or even remain closed.
    - The plant avoids dehydration.
    - The values for (cwv(leaf) – cwv(air)) and for rb are not under the biological control.
    - But, stomatal resistance (rs) can be regulated by opening and closing of the stomatal pore.
    - This biological control is exerted by a pair of specialized epidermal cells, the ***guard cells***, which surround the stomatal pore.

The Cell Walls of Guard Cells Have Specialized Features

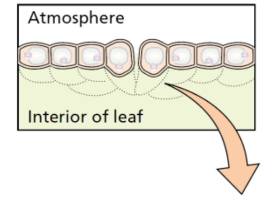
- Guard cells can be found:
  - In leaves of all vascular plants,
  - Organs from more primitive plants, such as the liverworts and the mosses.
- Morphological diversity, but we can distinguish two main types:
  - One: is typical of grasses and a few other monocots, such as palms;
    - In grasses : Guard cells have a characteristic dumbbell shape, with bulbous ends.
    - The pore proper is a long slit located between the two "handles" of the dumbbells.
    - These guard cells are always flanked by a pair of differentiated epidermal cells called **subsidiary cells.**
    - Subsidiary cells help the guard cells control the stomatal pores.
    - The guard cells, subsidiary cells, and pore are collectively called the **stomatal complex**.
  - The other is found in all dicots, in many monocots, and in mosses, ferns, and gymnosperms.
    - Kidney-shaped guard cells with an elliptical pore at its center.
    - Subsidiary cells are not uncommon in species with kidney-shaped stomata,
    - Subsidiary cells may be absent: The guard cells are surrounded by ordinary epidermal cells.



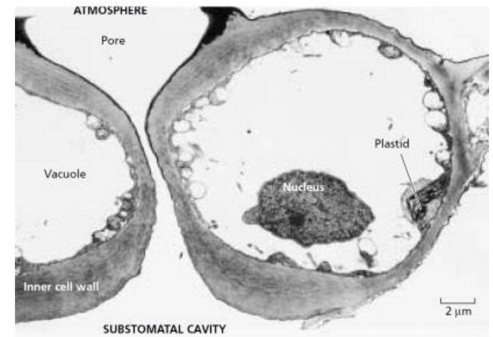

Stomatal complexes of the sedge, *Carex* (differential interference contrast light microscopy)

Scanning electron micrographs of onion epidermis.

Guard Cells Have Specialized Features……

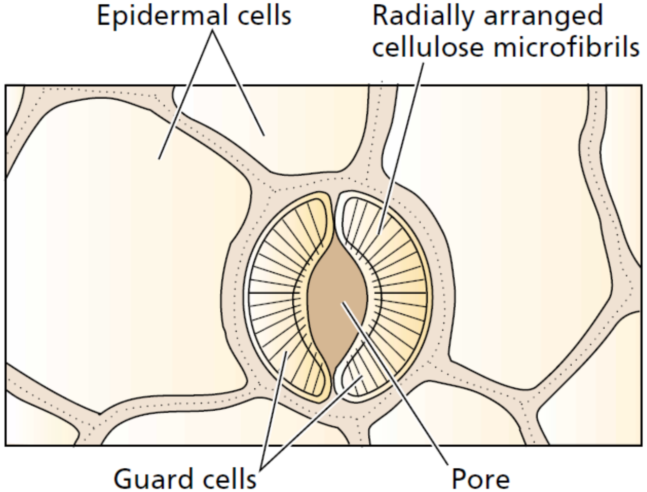


- A distinctive feature of the guard cells: The specialized structure of their walls.
- Portions of these walls are substantially thickened (up to 5 μm) across, in contrast to the 1 to 2 μm typical of epidermal cells.
- In kidney-shaped guard cells
  - A differential thickening pattern.
  - Thick inner and outer (lateral) walls,
  - Thickened ventral (pore) wall.
  - A thin dorsal wall (the wall in contact with epidermal cells)
- The portions of the wall that face the atmosphere extend into well-developed ledges, which form the pore proper.
- The alignment of **cellulose microfibrils**, plays an essential role in the opening and closing of the stomatal pore.
- In ordinary cells having a cylindrical shape:
  - Cellulose microfibrils are oriented transversely to the long axis of the cell.
  - The cell expands in the direction of its long axis.

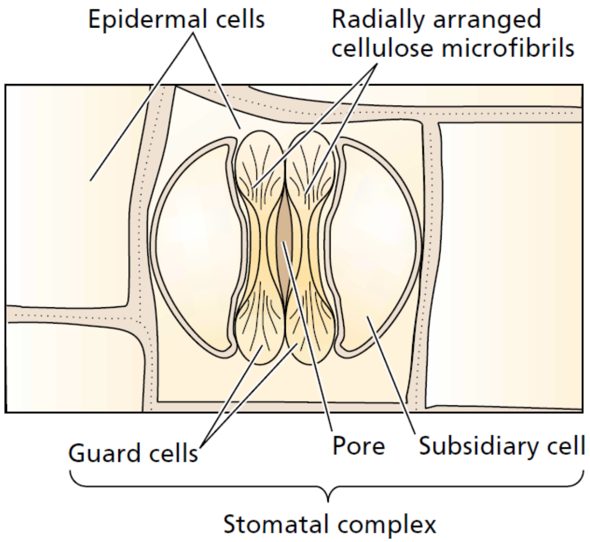


Electron micrograph showing a pair of guard cells from the dicot *Nicotiana tabacum* (tobacco).

- In guard cells the microfibril organization is different.
  - Cells have cellulose microfibrils fanning out radially from the pore.
  - The guard cells curve outward during stomatal opening.
- In grasses, the dumbbell-shaped guard cells: Looks like beams with inflatable ends.
  - The bulbous ends of the cells increase in volume and swell,
  - The beams are separated from each other and the slit between them widens.



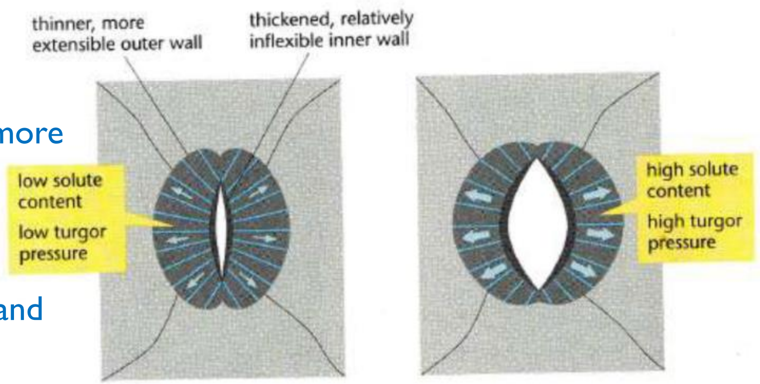
The radial alignment of the cellulose microfibrils in guard cells and epidermal cells of (A) a kidneyshaped stoma



A grasslike stoma

Opening and closing of a stoma

- The stoma is closed when the guard cells have a low turgor pressure.
- When turgor pressure increases, due to the net influx of solutes followed by water, the guard cells swell.
- The positioning of cellulose microfibrils and the differential thickening of the walls cause the cells to bulge outward, opening the stoma.
- Osmotic diffusion of water into guard cells:
  - When osmotic potential and water potential decreases that is becomes more negative related to the surrounding epidermal cells.
  - The osmotic potential and water potential decreases in guard cells when accumulation of osmotically active substances occur in guard cells.
  - Osmotic diffusion of water occurs from the surrounding epidermal cells and mesophyll cells into the guard cells increasing the turgidity of guard cells.
  - This results in the swelling of the guard cells full.
  - The guard cells increase is length and their adjacent thickened surfaces stretches stomata open.
- When osmotic potential and the water potential increases:
  - Due to the depletion of osmetically active substances relative to the surrounding cells (epidermal and mesophyll cells) water is released from the guard cells to the epidermal and mesophyll cells by diffusion
  - The guard cells become flaccid and
  - Closing of stomata occurs.



- Increase in osmotic potential and water potential (due to accumulation osmotically active metabolism)

↓

- Osmotic diffusion od water (IN) from surrounding epidermal cells and mesophyll cells to guard cells.

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- Increase in turgor pressure of guard cells

↓

- Guard cells become turgid

↓

- Guard cells swell

↓

- Increase in length ans adjacent thickening of surface: Stomata pore open.

↓

- Opening of stomata

An Increase in Guard Cell Turgor Pressure Opens the Stomata

- Guard cells function as ***multisensory hydraulic valves***.
- Environmental factors are sensed by guard cells:
  - Light intensity and quality,
  - Temperature,
  - Relative humidity, and
  - Intracellular CO_2 concentrations
- If leaves kept in the dark are illuminated: An opening signal, triggering a series of responses that result in opening of the stomatal pore.
- The early aspects: Are ion uptake and other metabolic changes in the guard cells.
- Ion uptake and from biosynthesis of organic molecules: Decreases in osmotic/solute potential (Ψs) in the guard cells. (Solute potential / Osmotic potential: **Pressure** which needs to be applied to a solution to **prevent** the **inward flow** of **water** across a semipermeable membrane)
  - $\Psi w = \Psi s + \Psi p + \Psi g$ (Ψs= The effects of solutes, Ψp= The the effects of pressure, and Ψg= The the effects of gravity)
  - Ψg is negligible and omitted.
  - Equation can be simplified as follows: $\Psi w = \Psi s + \Psi p$
- As Ψs decreases, the water potential decreases and water consequently moves into the guard cells.
- As water enters the cell, turgor pressure increases.
- Because of the elastic properties of their walls: guard cells can reversibly increase their volume by 40 to 100%,
- Because of the differential thickening: Changes in cell volume lead to opening or closing of the stomatal pore.

The Transpiration Ratio Measures the Relationship between Water Loss and Carbon Gain

- The effectiveness of plants
  - In moderating water loss
  - Allowing sufficient CO_2 uptake for photosynthesis.
- **Transpiration ratio**: The ***amount of water transpired*** by the plant, divided by the ***amount of carbon dioxide assimilated*** by photosynthesis.
- C3 plants: About 500 molecules of water are lost for every molecule of CO_2 fixed by photosynthesis (500/1), giving a transpiration ratio of 500.
- The reciprocal of the transpiration ratio, called the ***water use efficiency.***
- Plants with a transpiration ratio of 500 have a water use efficiency of 1/500, or 0.002.
- The large ratio of H_2O efflux to CO_2 influx results from three factors:
  - The concentration gradient driving water loss is about 50 times larger than that driving the influx of CO_2: It is due to the low concentration of CO_2 in air (about 0.03%) and the relatively high concentration of water vapor within the leaf.
  - CO_2 diffuses about 1.6 times more slowly through air than water does (the CO_2 molecule is larger than H_2O and has a smaller diffusion coefficient).
  - CO_2 uptake must cross the plasma membrane, the cytoplasm, and the chloroplast envelope before it is assimilated in the chloroplast. These membranes add to the resistance of the CO_2 diffusion pathway.

- The C4 and CAM plants:
  - They utilize variations in the usual photosynthetic pathway for fixation of carbon dioxide.
  - Plants with C4 photosynthesis:
    - Transpire less water per molecule of CO_2 fixed; a typical transpiration ratio for C4 plants is about 250.
  - Desert adapted plants with CAM:
    - Have lower transpiration ratios; values of about 50 are not unusual.

Theories of Opening and Closing of Stomata

1) Photosynthesis in guard cells (Van Mohr,1956):

- Stomata in veins open in light or day time and closed in the dark or night. According to him, the guard cells chloroplast manufacture of osmotically active photosynthates in presence of carbon dioxide which increases the turgor pressure and produces opening of stomata.
- Criticism:
  - His hypothesis could not explain the role of CO_2 in stomatal opening and closing.
  - In the bright Sun sunlight high CO_2 concentration causes partial stomata closure.
  - On the other hand, it has been found that in guard cells chloroplasts are either totally or partially inactive for carrying out photosynthesis.

2) Starch sugar inter-conversion theory

- It is based on the effect of pH on starch phosphorylase enzymes.
- It irreversibly catalyzes the conversion of starch and inorganic phosphate to glucose 1 phosphate.
- During day time:
  - pH in the guard cells is high which favours the hydrolysis of starch insoluble to glucose 1 phosphate soluble by the action of enzyme
  - Hence the osmotic potential becomes lower in guard cells.
  - Consequently water enters the guard cells from the surrounding epidermal and mesophyll cells.
  - By diffusion the guard cells become turgid and Stomata open.
- In the Dark:
  - The reversal process occurs.
  - Glucose 1 phosphate is converted back to start in the guard cells
  - This increases the osmotic potential the guard cell, water gets released and guard cells become flaccid and Stomata close.

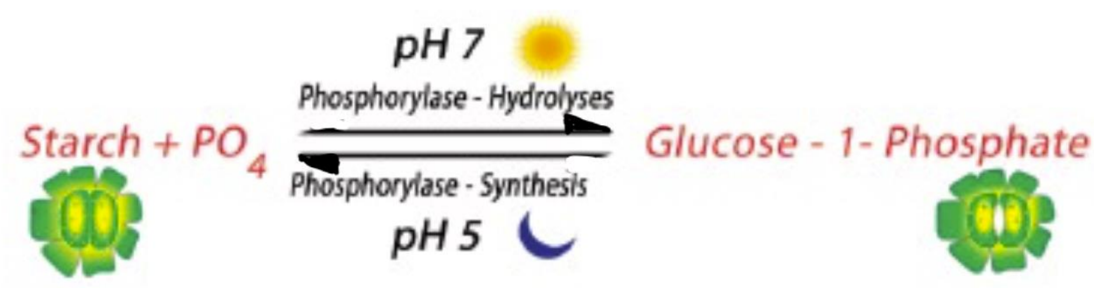
$$\text{Starch (insoluble)} + \text{pi} \underset{\text{Night (low pH)}}{\overset{\text{Light (high pH)}}{\rightleftharpoons}} \text{Glocose-1-P (soluble)}$$

3) Sayre (1920):

- Observed that the opening and closing of stomata depends upon change in pH of guard cells.
- Stomata open
  - At high pH during day time and become closed at low pH at night.
  - Utilization of CO_2 by photosynthesis during light period causes an increase in pH resulting in the conversion of starch to sugar.
  - Sugar increase in cell favours endosmosis and increases the turgor pressure which leads to opening of stomata.
- During night:
  - The accumulation of CO_2 in cells decrease the pH level resulting in the conversion of sugar to starch.
  - Starch decreases the turgor pressure of guard cell and stomata close.

4) **Hanes** (1940):

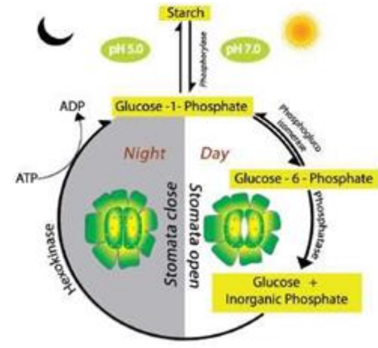
- The enzyme **phosphorylase** in guard cells supports the starch-sugar inte-rconversion theory.
- The enzyme *phosphorylase* hydrolyses starch into sugar and high pH followed by endosmosis and the opening of stomata.
- During light. The *vice versa* takes place.



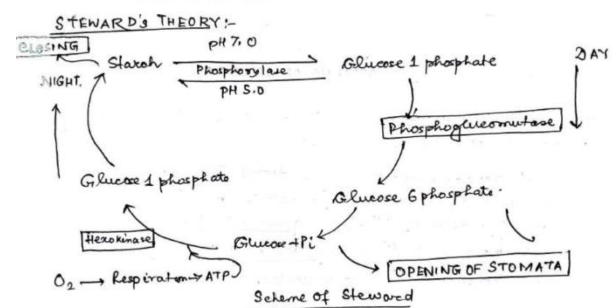
5) **Steward** (1964):

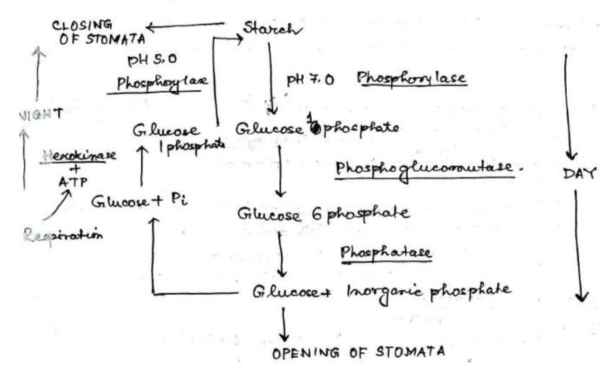
- He proposed a slightly modified scheme of starch-sugar interconversion theory.
- Glucose-1-phosphate is osmotically inactive.
- Removal of phosphate from Glucose- 1-phosphate converts to Glucose which is osmotically active.
- This increases the concentration of guard cell leading to opening of stomata.

- Steward modified his scheme
  - Glucose 1 phosphate should be father converted to glucose and inorganic phosphate for the opening of stomata.
  - Metabolic energy in the formation of ATP is needed for the closure of stomata when it comes from respiration.



Steward Scheme



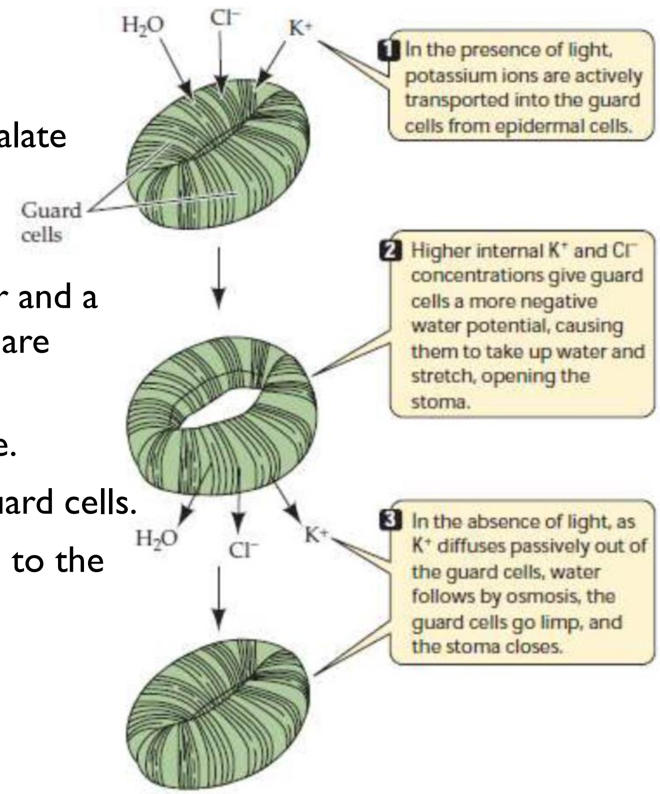


Objections to Starch-sugar interconversion theory

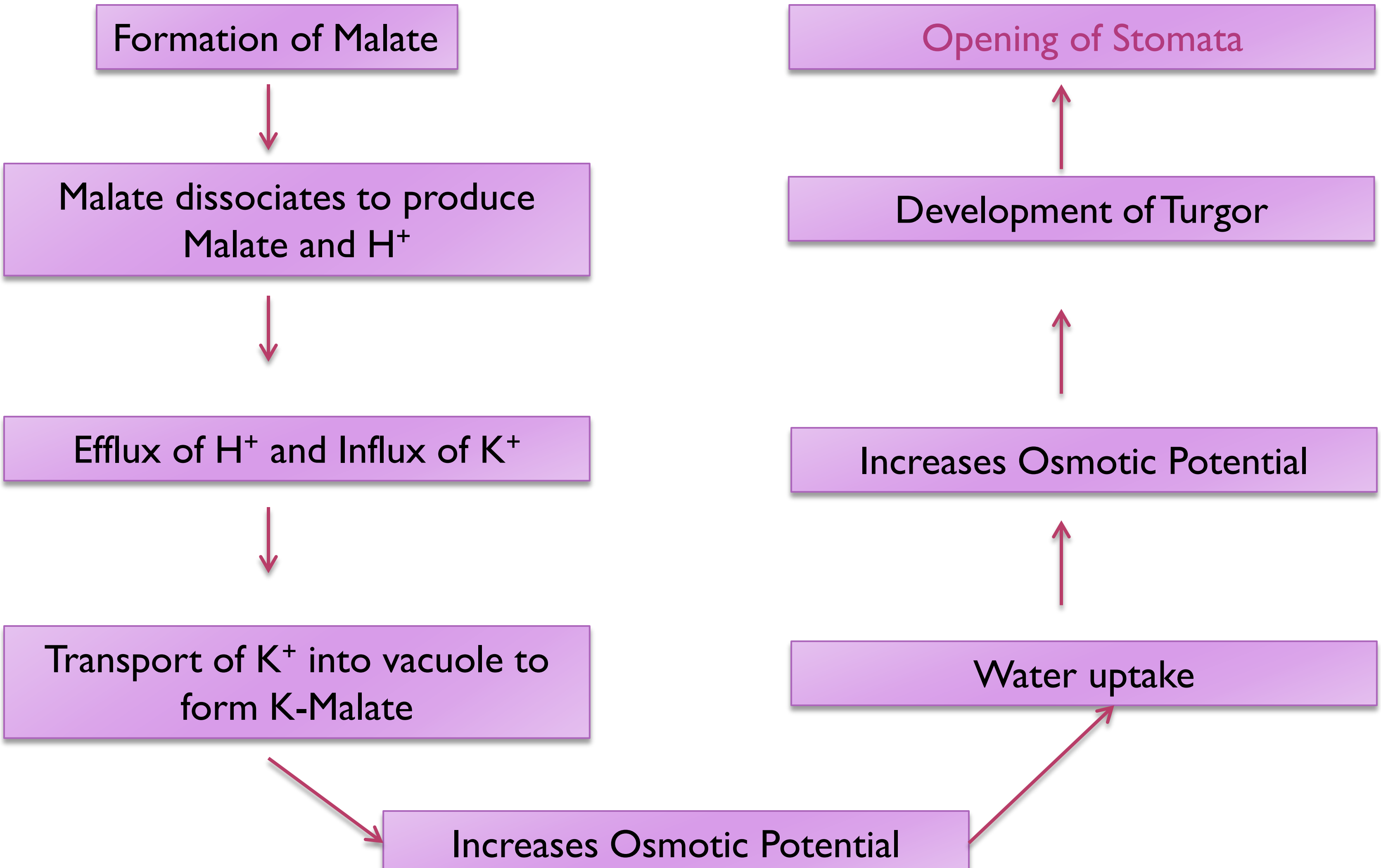
- In monocots, guard cell does not have starch.
- There is no evidence to show the presence of sugar at a time when starch disappears and stomata open.
- It fails to explain the drastic change in pH from 5 to 7 by change of CO_2.

ROLE OF POTTASIUM ION IN STOMATAL OPENING AND CLOSING

- Raschke (1975) elaborated role of K + ion in the opening and closing stomata.
  - Starch disappears during the day: Stomata is open
    - Starch is converted to malate by the action of PEP carboxylase and malate dehydrogenase.
    - Malic acid so formed is dissociated to form Malate and H^+.
    - This H^+ ions are pumped out resulting in a negatively charged interior and a increase in pH this results in synthesis of more malic acid as enzymes are activated.
    - To tolerate the negative charge or increase in pH K^+ influx takes place.
    - This exchange of H^+ and K^+ is followed by the entry of Cl^- into the guard cells.
    - K^+ forms potassium malate in the guard cells giving osmotic potential to the guard cells causing stomata opening.
  - In the dark:
    - The sequence of events leading to the closure of stomata.
    - The K^+ and Cl^- ions are transported into the guard cells.
    - Malate is decarboxylated to CO_2 and pyruvate
    - Pyruvate through the reversal of glycolysis is converted to sugar.
    - The sugar is converted to starch again.
    - As a result water moves out by Osmosis of the guard cells to lower the leading to closure of stomata.



A scanning electron micrograph of an open stoma formed by two sausage-shaped guard cells. (*b*) Potassium ion concentrations affect the water potential of the guard cells, controlling the opening and closing of stomata. Negatively charged ions accompanying K^+ maintain electrical balance and contribute to the changes in solute potential that open and close the stomata.


Formation of Malate
Malate dissociates to produce Malate and H^+
Efflux of H^+ and Influx of K^+
Transport of K^+ into vacuole to form K-Malate
Increases Osmotic Potential
Water uptake
Increases Osmotic Potential
Development of Turgor
Opening of Stomata

ROLE OF CO_2 OPENING AND CLOSING

- CO_2 concentration:
  - Stomata close: CO_2 free condition and CO_2 rich condition
  - Stomata open maximum: at the physiological CO_2
- CO_2 sensor of the guard cells: Malate concentration plays a role.
  - The balance between malate formation and malate removal from the vacuoles.
  - High malate concentration
    - Inhibits phosphoenol pyruvate carboxylase and
    - Inhibits its own synthesis.
  - High CO_2 concentration:
    - Acidification through Malate formation
    - Inhibit the formation of father malate.
  - Removal of CO_2
    - Deacidification of the guard cells
    - Stomatal closure

- The two enzymes play the key role
  - PEP carboxylase and
  - Malate dehydrogenase
- Zeaxanthin functions as carbon dioxide sensor in the guard cells.
  - Higher carbon dioxide causes a decrease in the guard cell zeaxanthin content
  - Stomatal closure.
- High concentration of carbon dioxide
  - The activation of anion channels in the plasma membrane:
  - Stimulate stomatal closing.
- Low concentrations of CO_2:
  - Inactivate the channels.
  - Induce stomatal opening.

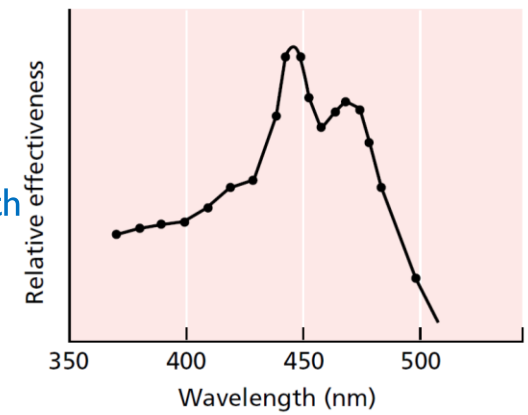
- CAM plants:
  - Reverse type of stomata opening takes place
  - Plants gets their higher water efficiency
  - During the decarboxylation phase (day time):
    - The internal CO_2 reaches a high concentration
    - High CO_2 concentration reflects in the changes in membrane permeability
    - The amplified acidification of the guard cell cytoplasm causes decrease in Proton Motive Force.
    - Stomatal closure.
  - During the carboxylation phase (night time)
    - Phytochrome regulate membrane potentials and Ion fluxes the plasma membrane
    - H^+ pump activated by the darkness in form of Pfr
    - K^+ then passively enters the guard cells and some Cl^- also transported
    - The complete charge of balance of K^+ is accompanied by the synthesis of malate.
    - Carbon dioxide is incorporated by carboxylation of phosphoenol pyruvate to oxaloacetate
    - OAA is then reduced to malate.
    - Malate accumulates and stored in the large vacuole of mesophyll cells.
    - Substantial amounts of malate among them will transported to guard cell vacuole through the plasmodesmata
    - Malic acid in the vacuole will create a requisite turgor pressure.
    - Open the stomatal aperture of CAM plants at night.

Blue-Light Responses: Stomatal Movements

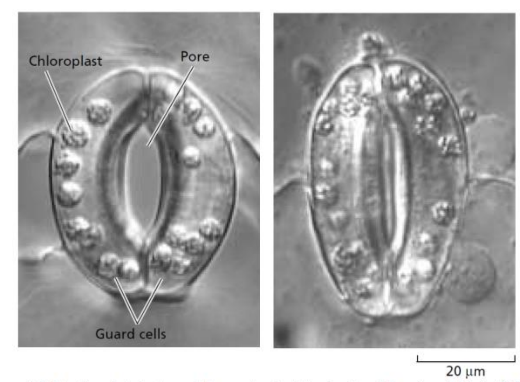
- House plants placed near a window have branches that grow toward the incoming light.
- This response, called ***phototropism***:
  - Plants alter their growth patterns in response to the direction of incident radiation.
  - It is an example of the use of light as an environmental signal.
- There are two major families of plant responses to light signals:
  - The phytochrome responses
  - The blue-light responses.
- There are numerous plant responses to blue light.
  - Phototropism.
  - Inhibition of hypocotyl elongation,
  - Stimulation of chlorophyll and carotenoid synthesis,
  - Activation of gene expression,
  - Stomatal movements
  - Phototaxis (the movement of motile unicellular organisms toward or away from light)
  - Enhancement of respiration, and anion uptake in algae.
  - Blue-light responses have been reported in higher plants, algae, ferns, fungi, and prokaryotes.

Blue Light Stimulates Stomatal Opening

- Bluelight responses on guard cells:
  - The stomatal response to blue light is rapid and reversible.
  - It is localized in a single cell type, the guard cell.
  - The perception of blue light: The signal transduction cascade that links with the opening of stomata.
- The stomatal response to light:
  - The osmo-regulatory mechanisms that drive stomatal movements
  - The role of a blue light–activated H+-ATPase in ion uptake by guard cells.
- In leaves of well-watered plants growing in natural environments:
  - Light is the dominant environmental signal controlling stomatal movements.
  - Stomata open as light levels reaching the leaf surface increase, and
  - Stomata close as they decrease.
  - In greenhouse-grown leaves of broad bean (*Vicia faba*), stomatal movements closely track incident solar radiation at the leaf surface



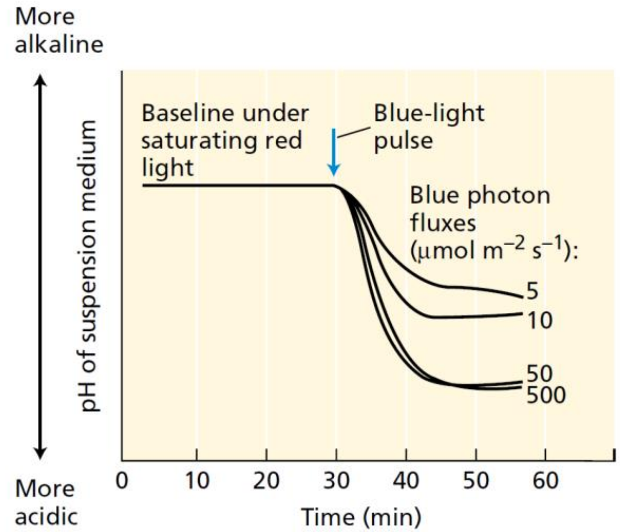
The action spectrum for blue light–stimulated stomatal opening (under a red-light background).



Light-stimulated stomatal opening in detached epidermis of *Vicia faba*.
Open: light-treated stoma   Closed: In the dark

Blue Light Activates a Proton Pump at the Guard Cell Plasma Membrane

- When guard cell protoplasts from broad bean (*Vicia faba*) are irradiated with blue light under background red-light illumination, the pH of the suspension medium becomes more acidic.
- This blue light–induced acidification is blocked by inhibitors that dissipate pH gradients
  - Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and
  - Inhibitors of the proton-pumping H+-ATPase (e.g. Vanadate)
- Acidification of a proton-pumping ATPase results from the activation by blue light that extrudes protons into the protoplast suspension medium and lowers its pH.
- In the intact leaf, this blue-light stimulation of proton pumping lowers the pH of the apoplastic space surrounding the guard cells.

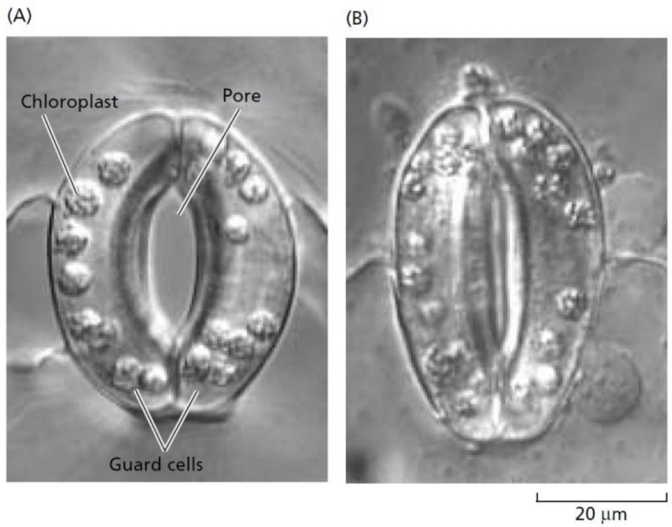


Acidification of a suspension medium of guard cell protoplasts of *Vicia faba* stimulated by a 30 s pulse of blue light.

Blue Light Regulates Osmotic Relations of Guard Cells

- Blue light modulates guard cell osmoregulation
  - Via its activation of proton pumping and
  - Via the stimulation of the synthesis of organic solutes.
- The guard cell turgor is regulated by osmotic changes resulting from starch–sugar inter-conversions.
- The changes in potassium concentrations in guard cells help in the guard cell osmoregulation by potassium and its counter ions.
- Potassium concentration in guard cells increases several fold when stomata open.
- These large concentration changes in the positively charged potassium ions are electrically balanced by the anions Cl^- and malate^{2-}.
- In species of the genus *Allium cepa*: K^+ ions are balanced solely by Cl^-.
- In most species: potassium fluxes are balanced by varying amounts of Cl^- and the organic anion malate^{2-}.

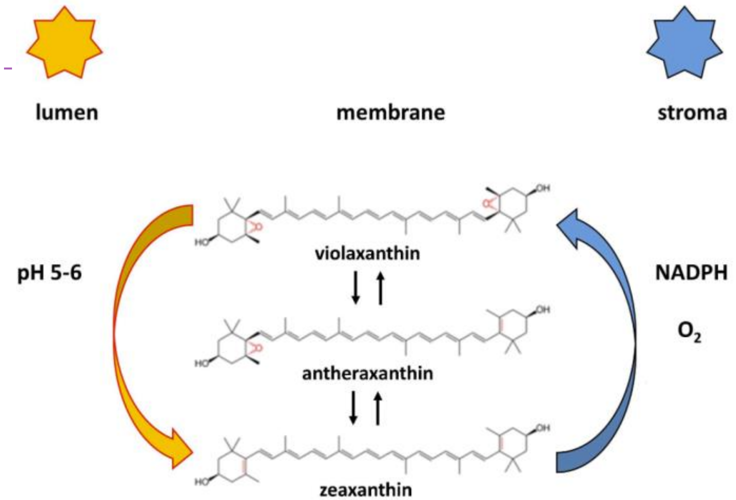
- Guard cell chloroplasts contain large starch grains.
- Their ***starch*** content
  - ***Decreases*** during stomatal opening and
  - ***Increases*** during closing.
- Starch, an insoluble, high-molecular-weight polymer of glucose, does not contribute to the cell's osmotic potential.
- The hydrolysis of starch into soluble sugars: Causes a ***decrease*** in the ***osmotic potential*** of guard cells.
- In the reverse process, starch synthesis decreases the sugar concentration, resulting in an increase of the cell's osmotic potential, which the starch–sugar is associated with stomatal closing.



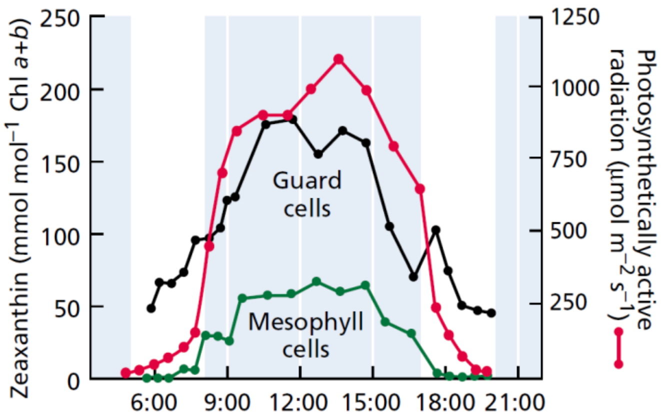
Light-stimulated stomatal opening in detached epidermis of *Vicia faba*.
A: Open, light-treated stoma, B: Closed in the dark-treated state

BLUE-LIGHT PHOTORECEPTORS

- The carotenoid zeaxanthin has been implicated as a photoreceptor in blue light–stimulated stomatal opening.
- Zeaxanthin is one of the three components of the xanthophyll cycle of chloroplasts, which protects photosynthetic pigments from excess excitation energy.



- In ***guard cells***, the changes in ***zeaxanthin content*** are distinctly different from the changes in mesophyll cells.
- The zeaxanthin content in guard cells:
  - Closely follows incident solar radiation at the leaf surface throughout the day.
  - It is nearly linearly proportional to incident photon fluxes in the early morning and late afternoon.

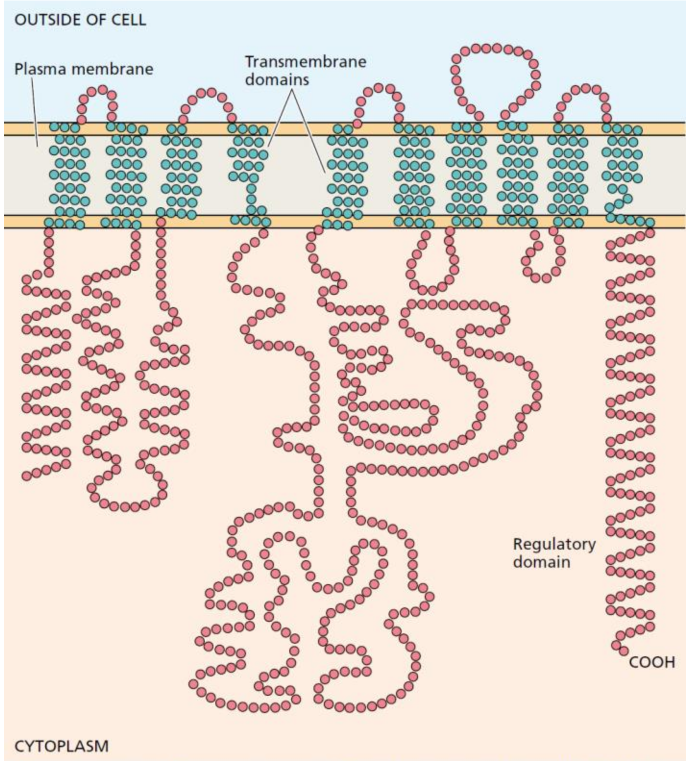


Daily course of photosynthetic active radiation reaching the leaf surface, and of zeaxanthin content of guard cells and mesophyll cells of *Vicia faba* leaves grown in a greenhouse. The white areas within the graph highlight the contrasting sensitivity of the xanthophyll cycle in mesophyll and guard cell chloroplasts under the low irradiances prevailing early and late in the day.

SIGNAL TRANSDUCTION

- Sensory transduction cascades:
  - The initial absorption of blue light by a chromophore and
  - The final expression of a blue-light response: stomatal opening.

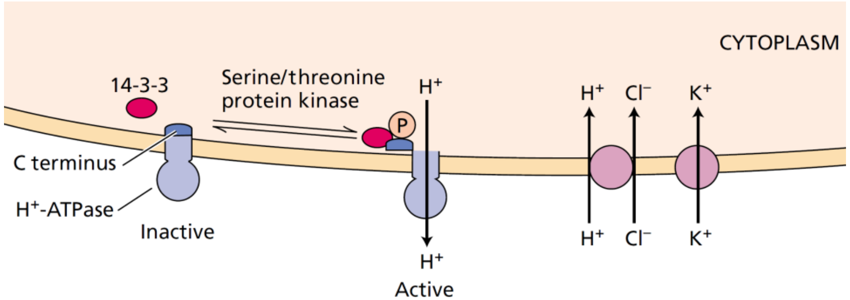
- Zeaxanthin isomerization start a cascade mediating Blue Light–Stimulated Stomatal Opening.
  - The C terminus of the H^+-ATPase has an auto-inhibitory domain
  - The auto-inhibitory domain of the C terminus lowers the activity of the enzyme by blocking its catalytic site.
  - If this auto-inhibitory domain is removed by a protease, the H+-ATPase becomes irreversibly activated.
  - ***Fusiccocin*** appears to activate the enzyme by moving the autoinhibitory domain away from the catalytic site.



Two-dimensional representation of the plasma membrane H+-ATPase. The H+-ATPase has 10 transmembrane segments. The regulatory domain is the autoinhibitory domain.

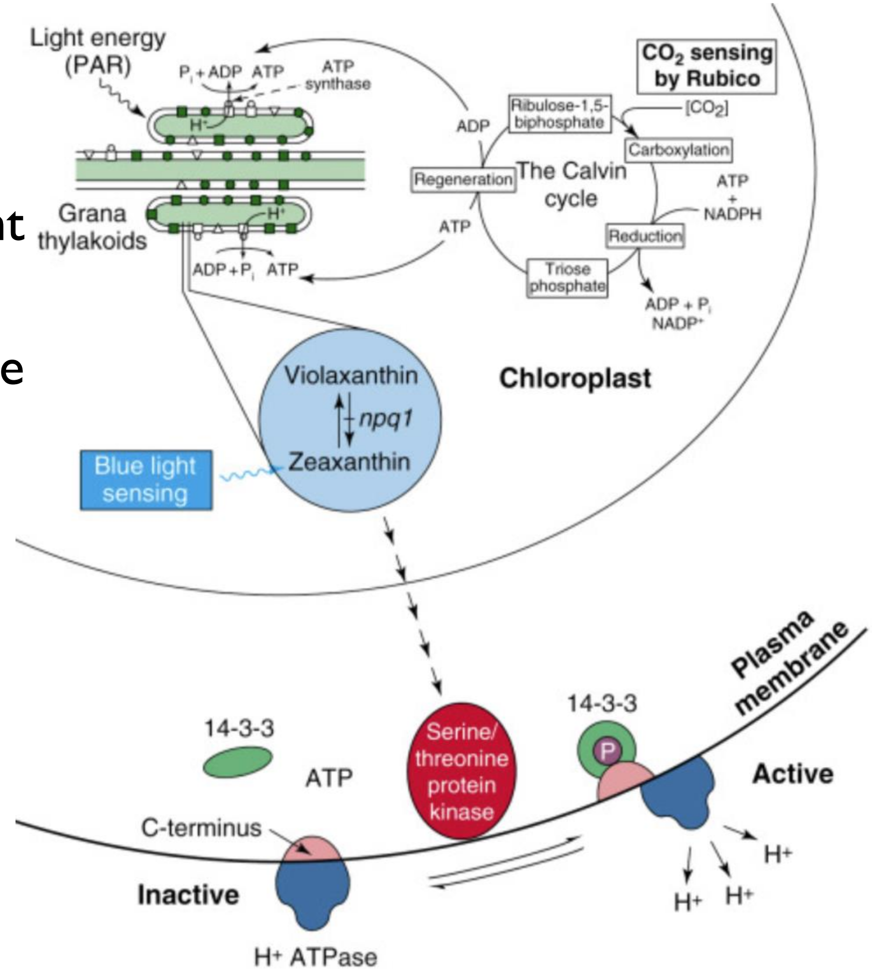
Activation of a serine/threonine protein kinase

- Blue light activates the H^+-ATPase.
- Activation involves the phosphorylation of serine and threonine residues of the C-terminal domain of the H^+-ATPase.
- Phosphorylation displace the auto-inhibitory domain of the C-terminal from the catalytic site of the enzyme.
- ***14-3-3 protein*** has been found to bind to the phosphorylated C terminus of the guard cell H^+-ATPase, but not the non-phosphorylated one.
- The 14-3-3 protein dissociates from the H+-ATPase upon dephosphorylation of the C-terminal domain.
- The electrochemical gradient generated by the proton pump drives ion uptake into the guard cells,
- This increases the turgor and turgor-mediated stomatal apertures.
- These steps define the major sensory transducing steps linking the activation of a serine/threonine protein kinase by blue light and blue light–stimulated stomatal opening



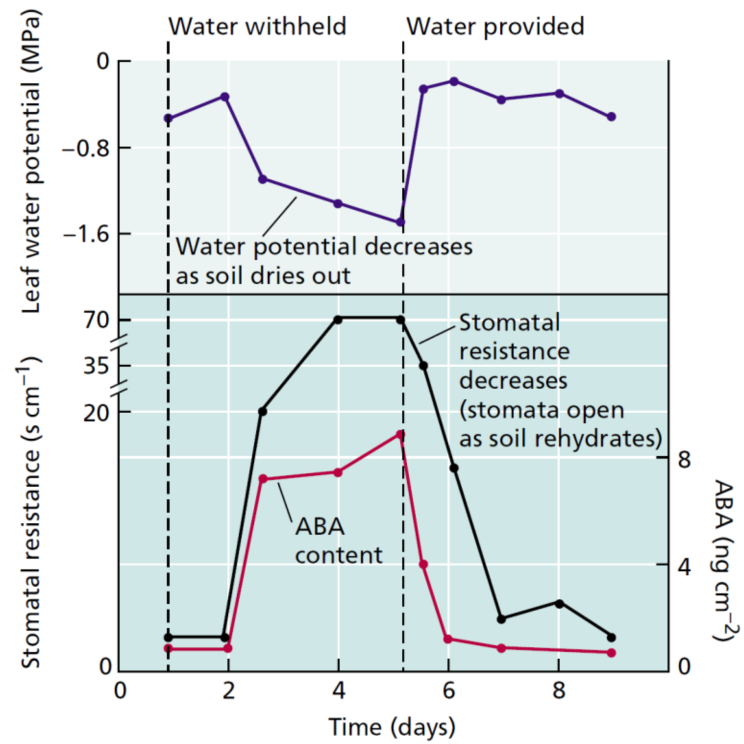
Excitation of zeaxanthin starts the sensory transduction cascade

- The excitation of zeaxanthin in the antenna bed of the guard cell chloroplast by blue light starts the sensory transduction cascade.
- It activates the serine/threonine kinase in the cytosol.
- Isomerization is the predominant photochemical reaction of carotenoids, so blue light would isomerize zeaxanthin and the conformational change would start the transducing cascade.



ABA Closes Stomata in Response to Water Stress

- ABA concentrations in leaves can increase up to 50 times under drought conditions.
- Stomatal closure: transpiration under water stress conditions
  - Redistribution or biosynthesis of ABA.
  - Accumulation in stressed leaves.
  - ABA synthesized in the roots and exported to the shoot: also cause stomatal closing.
- Mutants that lack the ability to produce ABA exhibit permanent wilting and are called *wilty* mutants (inability to close their stomata).
- Application of exogenous ABA to such mutants causes stomatal closure and a restoration of turgor pressure.



Changes in water potential, stomatal resistance (the inverse of stomatal conductance), and ABA content in maize in response to water stress. As the soil dried out, the water potential of the leaf decreased, and the ABA content and stomatal resistance increased. The process was reversed by rewatering.

CELLULAR AND MOLECULAR MODES OF ABA ACTION

- ABA is involved:
  - Short-term physiological effects: stomatal closure
  - Long-term developmental processes: changes in the pattern of gene expression.
- Signal transduction pathways are required for both the short-term and the long-term effects of ABA.
- Genetic studies: Many conserved signaling components regulate both short- and long-term responses.
- These indicate that they share common signaling mechanisms.
- Now we will describe what is known about the mechanism of ABA action at the cellular and molecular levels.

ABA Is Perceived Both Extra-cellularly and Intra-cellularly

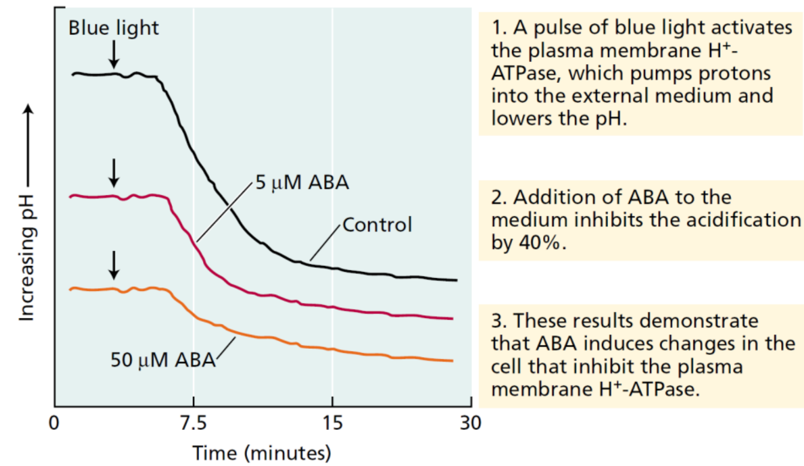
- Multiple perceptions:
  - ABA can act externally by binding to a receptor located on the outer surface of the plasma membrane or
  - The ABA must enter the cell to be effective.
- A receptor on the outer surface of the cell:
  - Microinjected ABA fails to alter stomatal opening in the spiderwort *Commelina*.
  - Impermeant ABA–protein conjugates have been shown to activate both ion channel activity and gene expression.
- An intracellular location for the ABA receptor:
  - Extracellular application of ABA, inhibit stomatal opening.
  - ABA supplied directly and continuously to the cytosol inhibited K^+ in channels, which are required for stomatal opening.
- Extracellular perception of ABA can prevent stomatal opening and regulate gene expression,
- Intracellular ABA can both induce stomatal closure and inhibit the K^+ in current required for opening.
- There appear to be both extracellular and intracellular ABA receptors.

ABA Increases Cytosolic Ca^{2+}, Raises Cytosolic pH, and Depolarizes the Membrane

- The first changes detected after exposure of guard cells to ABA are:
  - Transient membrane depolarization
  - It is caused by the net influx of positive charge, and
  - Transient increases in the cytosolic calcium concentration.
- ABA stimulates elevations in the concentration of cytosolic Ca^{2+} by:
  - Inducing both influx through plasma membrane channels and
  - Release of calcium into the cytosol from internal compartments (central vacuole).
- Stimulation of influx occurs via a pathway that uses **reactive oxygen species** (**ROS**):
  - Hydrogen peroxide (H_2O_2) or
  - Superoxide (O_2•–).
- They act as secondary messengers leading to plasma membrane channel activation:
  - Inositol 1,4,5-trisphosphate (IP3),
  - Cyclic ADP-ribose (cADPR), and
  - amplifying (calcium-induced) Ca^{2+} release.
- ABA stimulates nitric oxide (NO) synthesis in guard cells, which induces stomatal closure in a cADPR-dependent manner.
- ABA causes an alkalization of the cytosol (pH 7.67 to pH 7.94.)
- The increase in cytosolic pH activate the K^+ efflux channels on the plasma membrane.

ABA Activation of Slow Anion Channels Causes Long-Term Membrane Depolarization

- ABA activates slow anion channels in guard cells.
- The prolonged opening of these slow anion channels permits large quantities of Cl^- and malate^{2-} ions to escape from the cell, moving down their electrochemical gradients.
- The inside of the cell is negatively charged, thus pushing Cl– and malate^{2-} out of the cell, and the outside has lower Cl^- and malate^{2-} concentrations than the interior.
- The outward flow (of Cl^- and malate^{2-} ions) depolarizes the membrane, triggering the voltage-gated K^+ efflux channels to open.
- ABA inhibits blue light–stimulated proton pumping by guard cell protoplasts.
- 2-factors contribute to ABA inhibition of the plasma membrane
  1. Proton pump: an increase in the cytosolic Ca^{2+} concentration,
  2. Alkalinization of the cytosol.
- ABA prevents light-induced stomatal opening:
  - By inhibiting the inward K^+ channels
  - Inhibition of the inward K^+ channels is mediated by the ABA-induced increase in cytosolic calcium concentration.



ABA inhibition of blue light–stimulated proton pumping by guard cell protoplasts

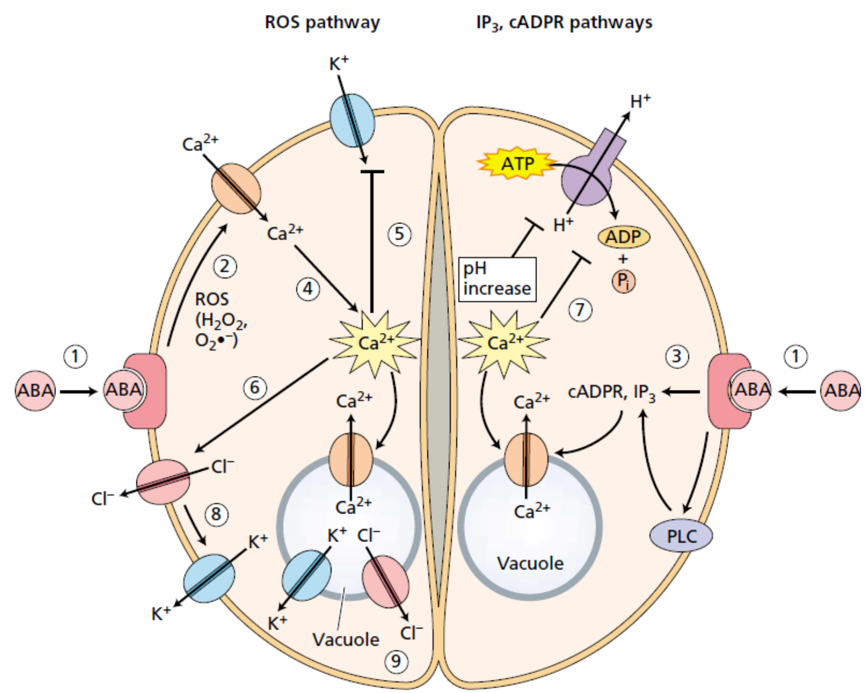
- Thus calcium and pH affect guard cell plasma membrane channels in two ways:
  1. They prevent stomatal opening by inhibiting inward K+ channels and plasma membrane proton pumps.
  2. They promote stomatal closing by activating outward anion channels, thus leading to activation of K+ efflux channels.

ABA Regulation of Gene Expression Is Mediated by Transcription Factors

- ABA causes changes in gene expression.
- Gene activation by ABA is mediated by transcription factors.
- Four main classes of regulatory sequences have been identified:
  - The maize VP1 (VIVIPAROUS-1) and
  - *Arabidopsis* ABI3 (ABA-INSENSITIVE3) genes, and the ABI4 and ABI5
- ***VP1/ ABI3***, and ***ABI4***: Are members of gene families found only in plants.
- ***ABI5*** is a member of the basic leucine zipper (bZIP) family: members are present in all eukaryotes

Model for ABA signaling in stomatal guard cells.

1. ABA binds to its receptors.
2. ABA-binding induces the formation of reactive oxygen species, which activate plasma membrane Ca^{2+} channels.
3. ABA increases the levels of cyclic ADP-ribose and IP3, which activate additional calcium channels on the tonoplast.
4. The influx of calcium initiates intracellular calcium oscillations and promotes the further release of calcium from vacuoles.
5. The rise in intracellular calcium blocks K^{+}_{in} channels.
6. The rise in intracellular calcium promotes the opening of Cl^{-}_{out} (anion) channels on the plasma membrane, causing membrane depolarization.
7. The plasma membrane proton pump is inhibited by the ABA-induced increase in cytostolic calcium and a rise in intracellular pH, further depolarizing the membrane.
8. Membrane depolarization activates K^+ out channels.
9. K^+ and anions to be released across the plasma membrane are first released from vacuoles into the cytosol.
10. The net effect is the loss of potassium and its anion (Cl^- or malate^{2-}) from the cell.



Simplified model for ABA signaling in stomatal guard cells. The net effect is the loss of potassium and its anion (Cl^- or malate^{2-}) from the cell. (R = receptor; ROS = reactive oxygen species; cADPR = cyclic ADP-ribose; G-protein = GTP-binding protein; PLC = phospholipiase C.)

Antitranspirants

- Definition:
  - Antitranspirants are substances applied to the plants for the purpose of reducing transpiration (water loss) without causing a significant effect on other plant processes, such as photosynthesis & growth.
- Use:
  - They have been used with some success in horticulture, especially in the ornamental industry.
- Antitranspirants may reduce transpiration in three different ways:
  - By reducing the absorption of solar energy and thereby reducing leaf temperatures and transpiration rates.
  - By forming thin transparent films which hinder the escape of water vapors from the leaves.
  - By promoting closure of stomata (by affecting the guard cells around the stomatal pore), thus decreasing the loss of water vapors from the leaf.

- They may be of four types
  - Stomatal closing type: They are metabolic inhibitors
    - They induce stomatal closing or decrease size and number of stomata which subsequently reduce the photosynthesis.
    - Phenyl Mercuric Acetate (PMA): When it is sprayed at very low concentrations, it results in a partial closure of stomata for 2 weeks.
    - Abscisic acid and
    - Aspirin.
  - Film coating type:
    - Plastic and waxy material which form a thin colourless film over the leaf surface and result in a physical barrier.
    - These form a colourless transparent film on the leaf surfaces, which is permeable to O_2 and CO_2 but not to water vapours.
    - These glossy films then reduce water loss on plants while at the same time allow them to breathe normally.
    - Silicon emulsions
    - Low viscosity waxes.
    - Colourless plastics.
  - Reflecting type: These are most commonly clay based and increase the reflection of light from leaf surface thus reducing leaf heating and water losses.
  - Growth retardant: These chemicals reduce shoot growth and increase root growth and thus enable the plants to resist drought. They may also induce stomatal closure.

- In agriculture: They are used reduce the crop productivity due to trnspiratory water loss.
  - Metabolic innovators:
    - Growth hormones ABA,
    - Herbicides,
    - Fungicides
    - Salicylic acid,
    - Phenyl Mercuric Acetate.
- According to the mode of action: two types:
  - Colourless plastic and low viscosity wax spray: Form a thin film on the surface preventing the transpiration and allowing the diffusion of carbon dioxide and O_2
  - Stomata Closing Chemicals: PMA, ABA: They retard the transpiration but do not hamper photosynthesis and respiration(not restricting gaseous exchange).
- Features: They should be
  - Non toxic
  - Non permanent damage to stomatal aperture
  - Specific effect and guard cells
  - Should be economic and
  - Easily available

- Use in the filed of agri-horticulture:
  - Used in high water demanding crops
  - Used during the transport of Fruit free seedlings (transpiration damage the young routes which cannot absorb water to compensate the loss).
  - They save several fruit from cracking by reducing water absorption by the fruit during rainfall.
  - Film coating anti transpirant are used to save ornamentals from desiccasion and delayed irrigation.
  - They are also used on plucked flowers to keep them fresh for a longer period.
  - Grass lawns are sprayed with antitranspirant to save a grasses from drying of when the lawn cannot be irrigated.