

Figure 10.0 Sunbeams



Guard cell basics - 1

- Guard cells regulate the dimensions of stomata;
- By regulating stomatal dimensions guard cells regulate rates of transpiration and photosynthesis

Figure 36.1 An overview of transport in whole plants (Layer 4)

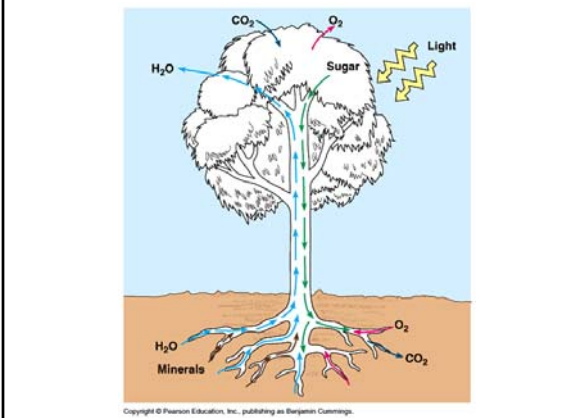
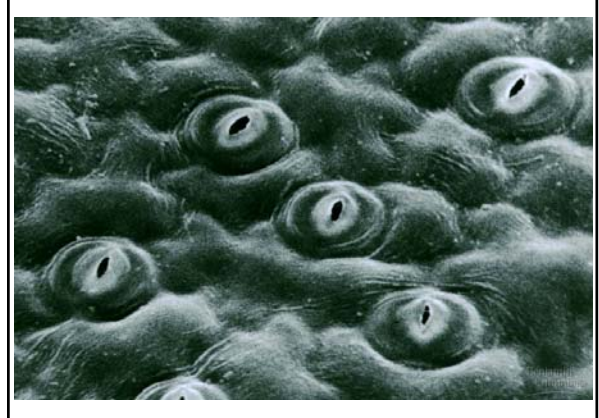
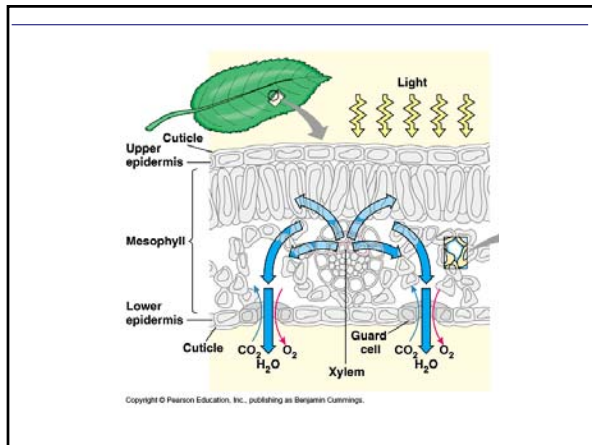
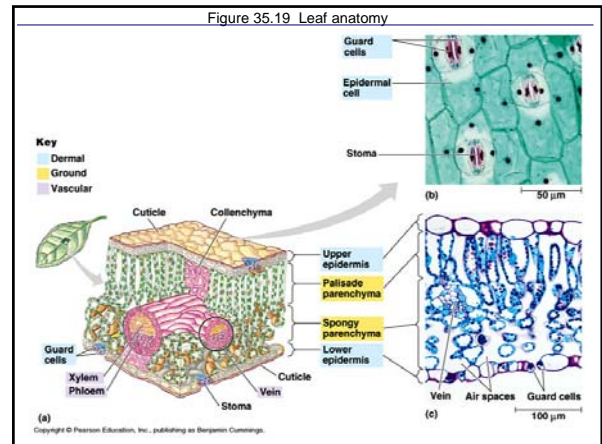


Figure 36.12x Stomata on the underside of a leaf





Guard cell basics - 2

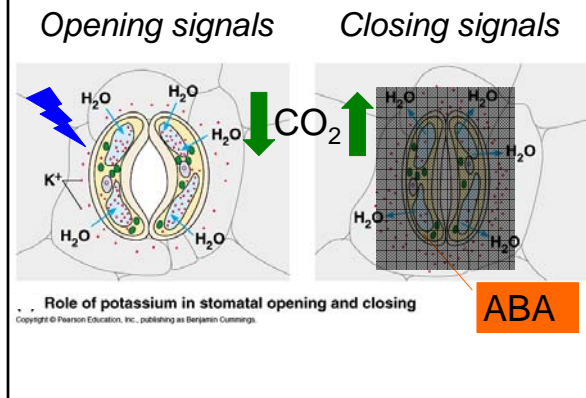
- Guard cells are “motor” cells = they move;
- Guard cells change shape through changes in their turgor pressure;
- Changes in turgor pressure = changes in shape = changes in stomatal dimensions



Guard cell basics - 3

- Guard cells change shape in response to a variety of environmental signals;
- Signals include:
 - light (intensity and color)
 - intercellular leaf CO₂ concentrations
 - plant hormones (e.g., abscisic acid; ABA)
 - humidity
 - pollutants like ozone

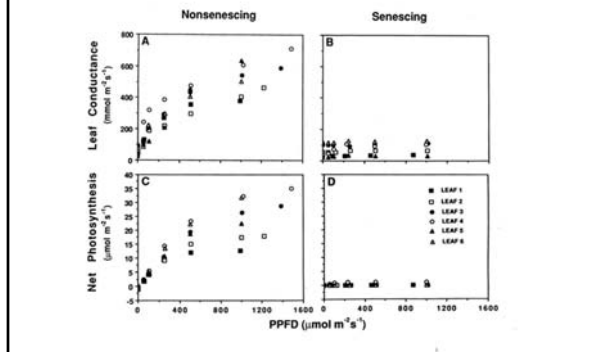
Figure 36.13b The mechanism of stomatal opening and closing



Stomata open in response to light.

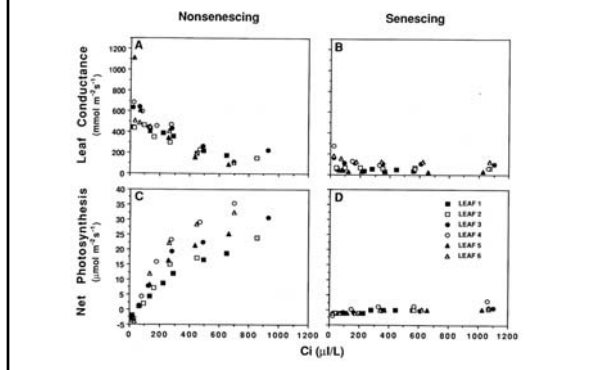
- Opening is asymptotically proportional to light intensity
- Guard cells are particularly “sensitive” to blue light

Increasing light levels increases stomatal opening.



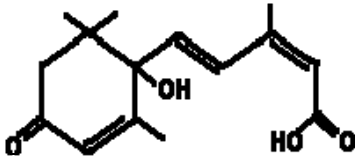
Stomata open in response to decreased intercellular concentrations of carbon dioxide in the leaf = c_i caused by photosynthesis.

Decreasing c_i induces stomatal opening.



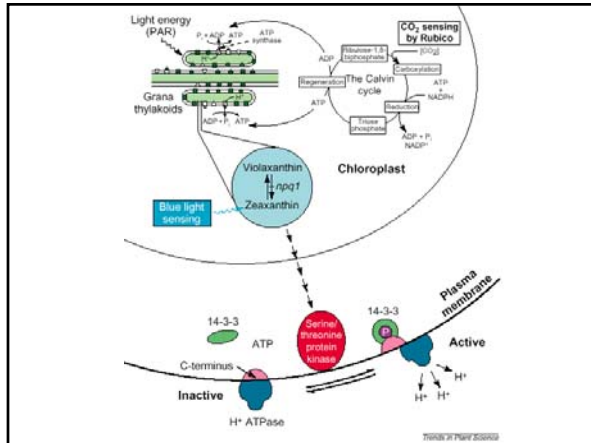
Stomata close in response to increased intercellular concentrations of carbon dioxide in the leaf = c_i caused by decreases in photosynthesis.

Stomata close when treated with ABA.



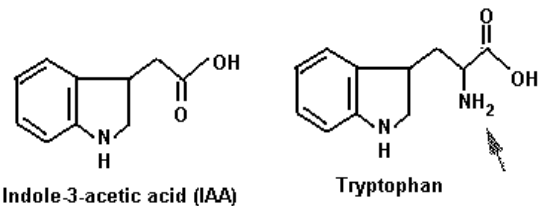
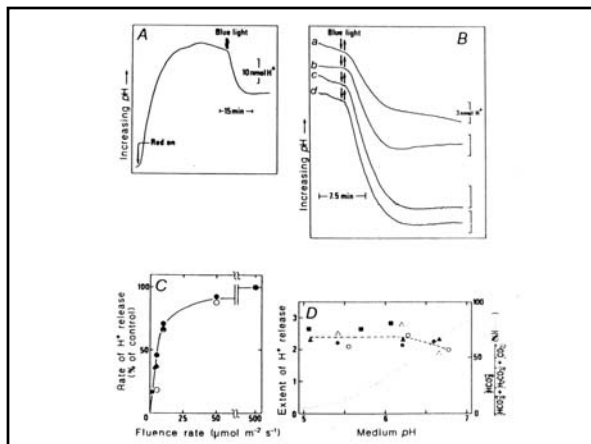
Guard cell signaling

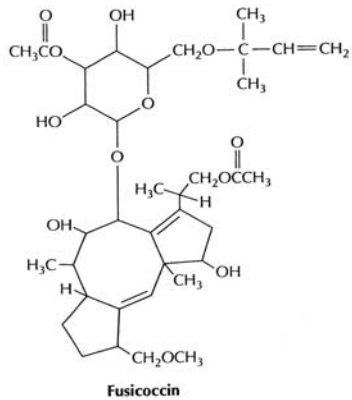
- Guard cells transduce signals through signal transduction pathways;
- All opening signals activate a chemi-osmotic mechanism of stomatal opening;
- All opening signals initially activate a plasma membrane proton-translocating ATPase = an ATP-fueled proton pump



Proton pump characteristics

- The pump is *activated* by:
 - low intensities of blue light (over a red light background);
 - reduced CO₂ concentrations (measured under red light);
 - the plant hormone, auxin;
 - the fungal toxin, fusicoccin
- The pump is *inhibited* by:
 - calcium ions



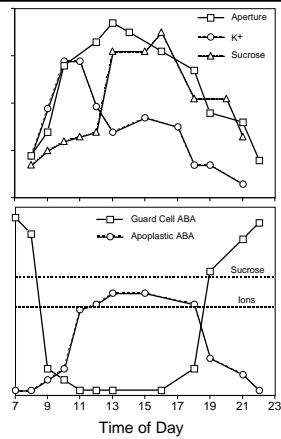


Main events in stomatal opening

- Activation of the proton pump;
- “*Transient hyperpolarization*” of the plasma membrane = inside becomes even more negative than outside temporarily;
- Hyperpolarization changes “*membrane electric potential*” (voltage);
- *Inward-rectifying, voltage-gated* potassium ion channels open;
- Potassium ions enter cell along “*electrochemical gradient*”;
- Water enters cell by osmosis because water goes where solutes go;
- Water entry increases turgor pressure = cell bowing

Other events in stomatal opening

- During potassium ion uptake guard cells also accumulate malate ion and chloride ion;
- Malate synthesis is catalyzed by PEP carboxylase;
- Chloride ion enters through inward-rectifying, voltage-gated chloride ion channels;
- Outward-rectifying potassium ion and anion channels for malate and chloride ions close during stomatal opening;
- Sucrose replaces potassium ions at mid-day by an unknown mechanism.



Pump and channel activity can be measured by patch clamping.



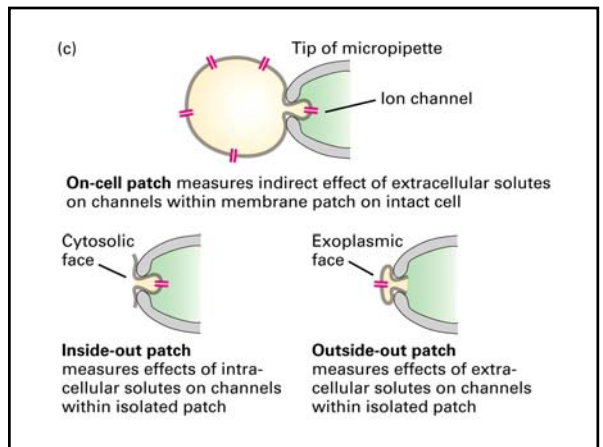
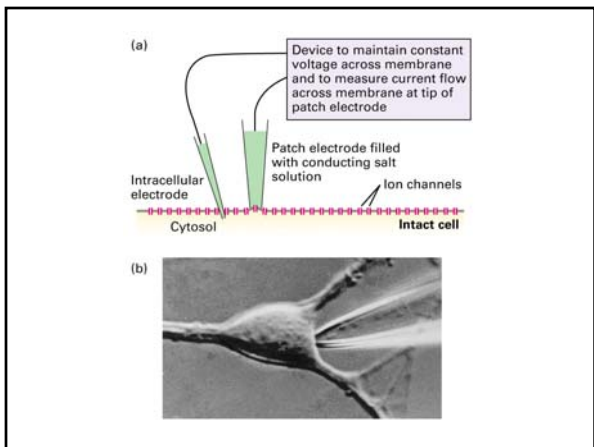
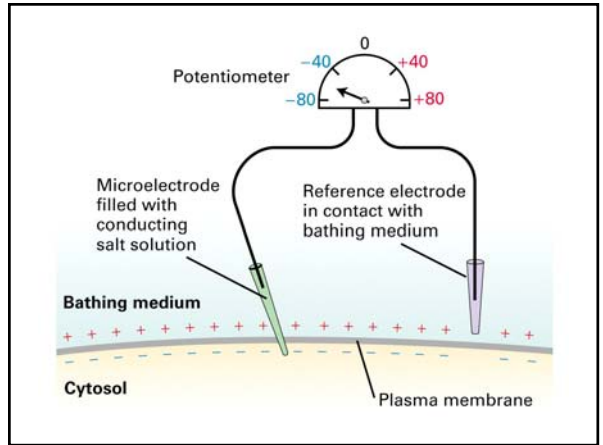
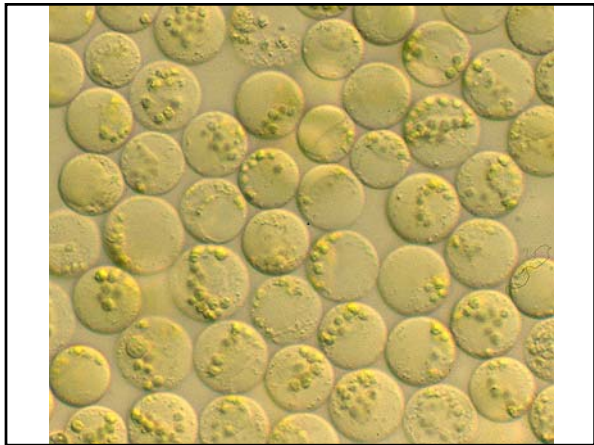
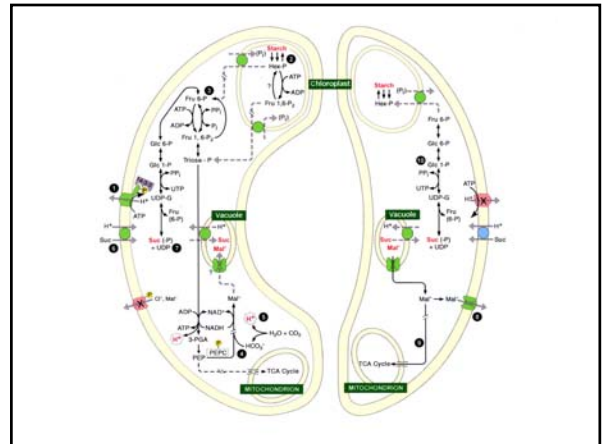
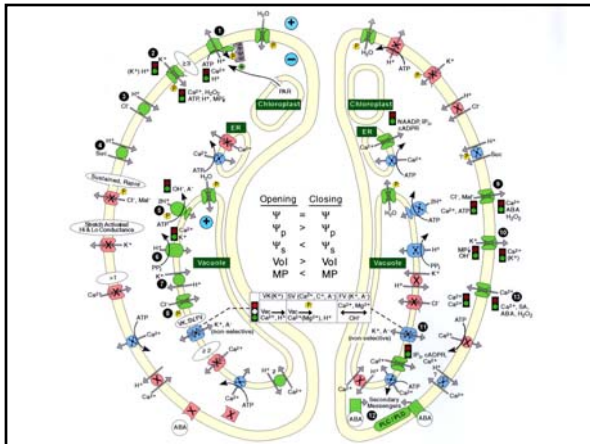
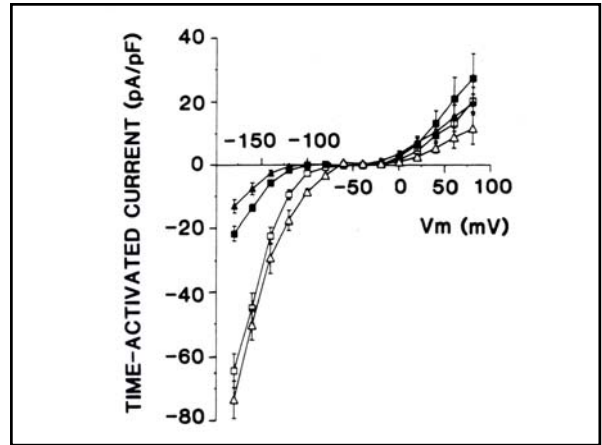
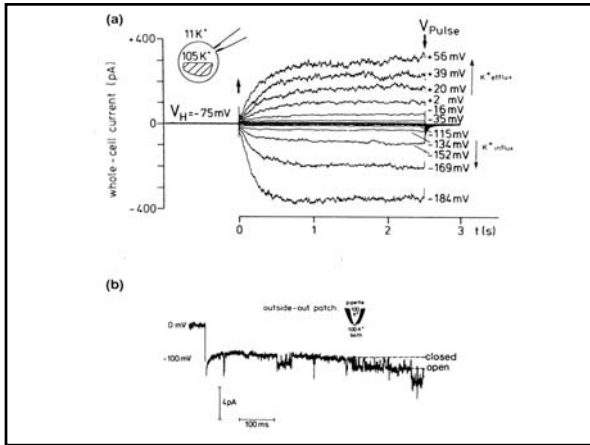
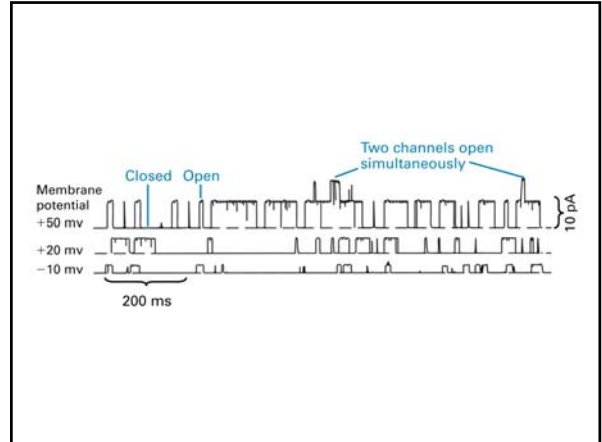
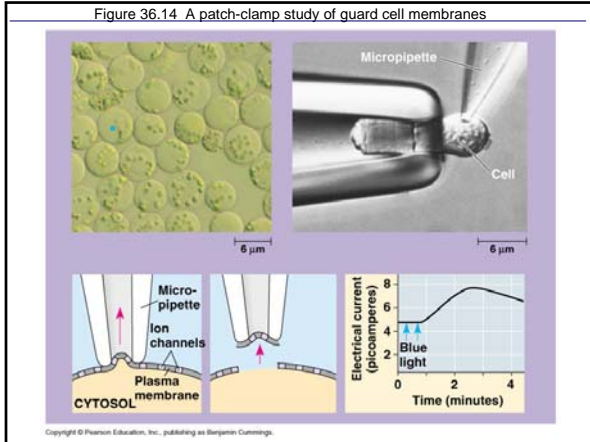
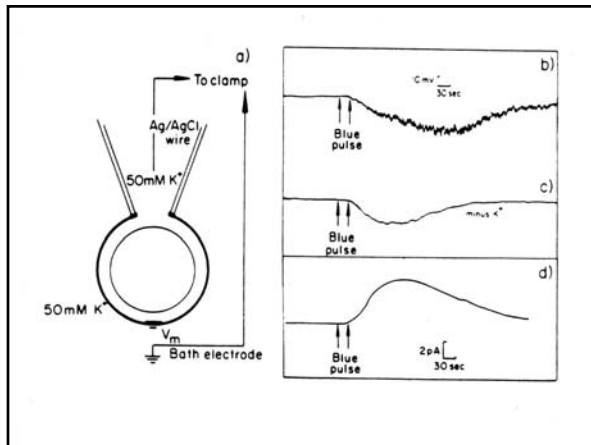
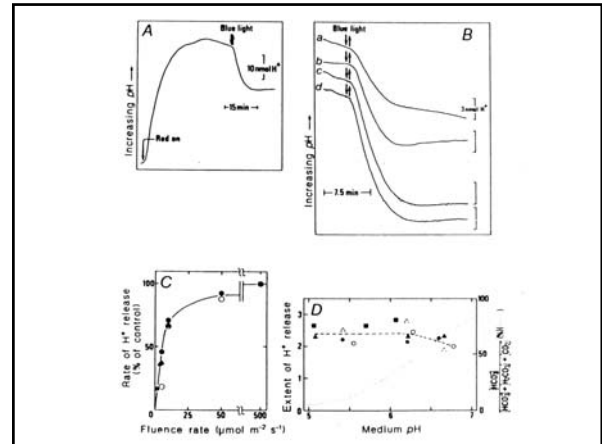


Figure 36.14 A patch-clamp study of guard cell membranes

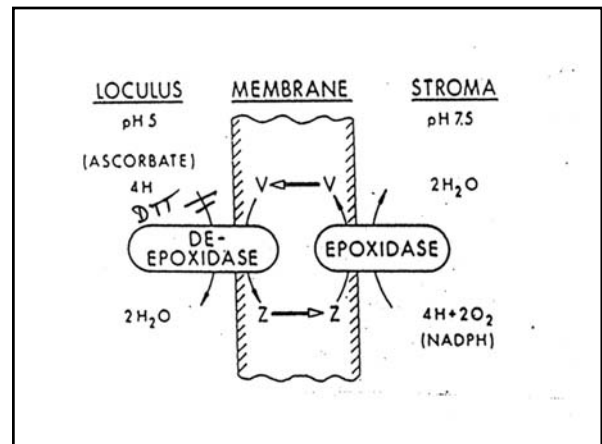
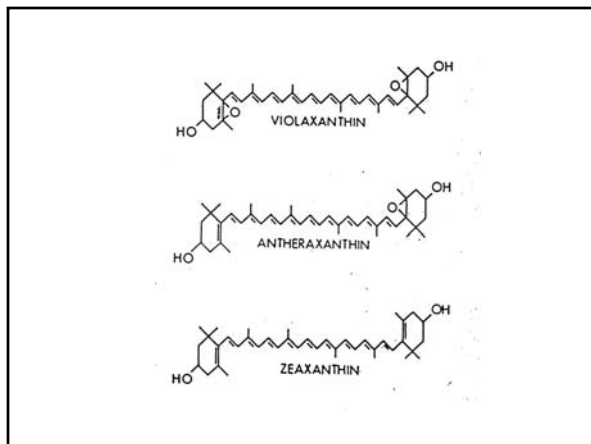


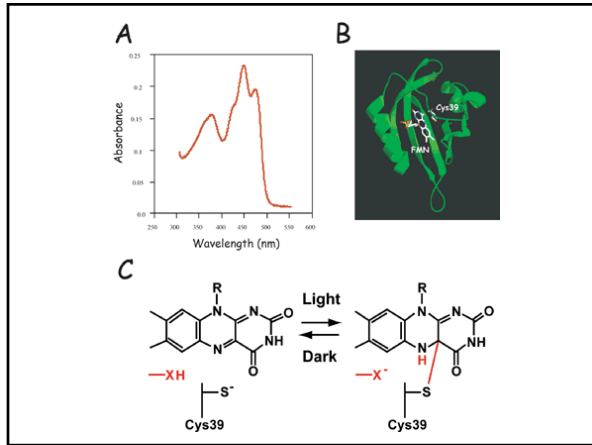
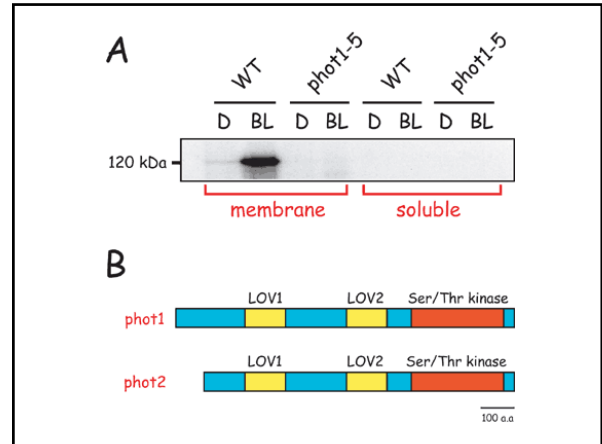
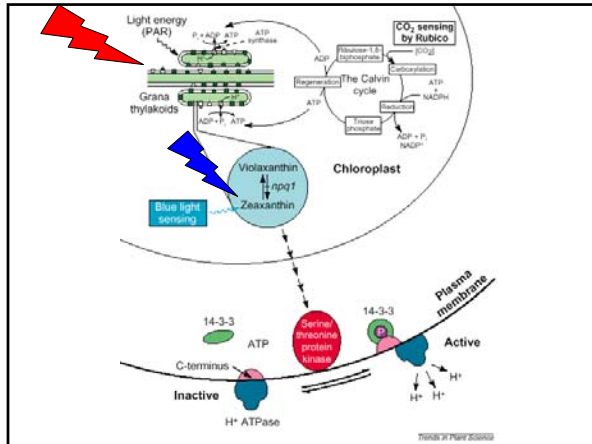
Blue light activates the proton pump and inward-rectifying potassium ion channels.



Two blue light photoreceptors have been proposed:

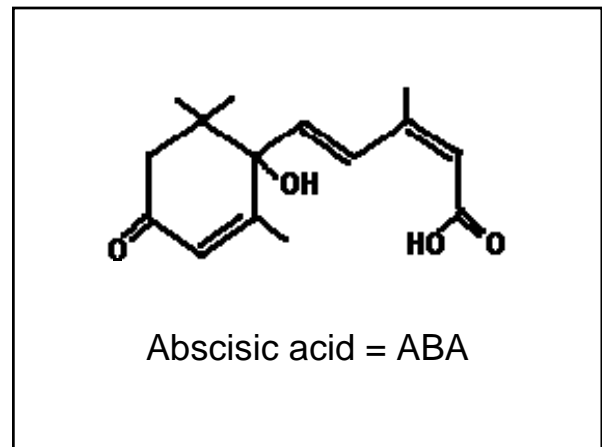
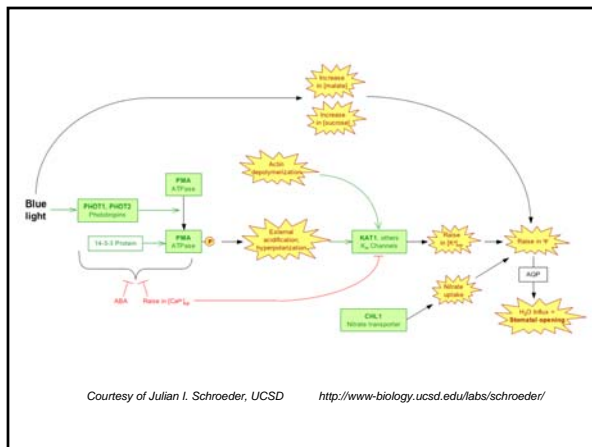
- 1) Zeaxanthin (E. Zeiger, UCLA)
- 2) Phototropin (K. Shimazaki, Kyushu Univ.)





Other players in the blue light signal transduction pathway:

- Calmodulin
- A serine/threonine kinase
- A 14-3-3 protein that binds to the proton pump to prevent its dephosphorylation = inactivation
- *NOTE: similar components have been identified for opening induced by low CO₂*



How does ABA cause stomata to close/prevent stomatal opening?

- There are multiple mechanisms
- Mechanisms may be calcium-dependent or calcium-independent
- Calcium-dependent mechanisms are best understood
- ABA causes massive efflux of potassium ions and anions (malate and chloride) from guard cells

ABA-induced stomatal closure: one scenario

- ABA binds to a G-protein-coupled receptor
- External Ca^{2+} is admitted through channels and/or IP_3 is produced and internal Ca^{2+} is released to the cytoplasm from internal stores; proton pump activity slows
- The plasma membrane depolarizes to voltage that opens “fast” and then “slow” anion efflux channels
- Cytosolic pH increases
- When pH and membrane voltage reach the right values, K^+ efflux channels open; influx channels close

A G Protein-Coupled Receptor Is a Plasma Membrane Receptor for the Plant Hormone Abscisic Acid

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The plant hormone abscisic acid (ABA) regulates many physiological and developmental processes in plants. The mechanism of ABA perception at the cell surface is not understood. Here, we report that a G protein-coupled receptor genetically and physically interacts with the G protein α subunit GPA2 to mediate at least one ABA response in *Arabidopsis*. Overexpressing this receptor results in an ABA-hyperresponsive phenotype. This receptor binds ABA with high affinity at physiological concentration with expected kinetics and stereospecificity. The binding of ABA to the receptor leads to the dissociation of the receptor-GPA2 complex in yeast. Our results demonstrate that this G protein-coupled receptor is a plasma membrane ABA receptor.

Abscisic acid (ABA) is an important hormone that mediates many aspects of plant growth and development, particularly in response to the environmental stresses (1-3). Several components involved in the ABA signaling pathway have been identified (4). Two recent reports have shown that the nuclear RNA binding protein flowering time control protein (FTC) (5) and the chloroplast protein Mg-chelatase H subunit (Mg) are ABA receptors (6).

In contrast, several earlier experiments had suggested that extracellular potassium is critical for ABA to induce its functions (7-9). Thus, other ABA receptors, especially plasma membrane-localized receptors, may be the major players for perceiving extracellular ABA and mediating the classic ABA signaling response.

Ligand-mediated signaling through G protein-coupled receptors (GPCRs) is a common mechanism for the conserved signal perception at the plasma membrane in eukaryotic organisms (10). The GPCR-mediated signaling pathway plays a central role in vital processes such as vision, taste, and olfaction in animals (11). However, the higher plant *Arabidopsis thaliana* has only one canonical GPCR (GPA1) (12). In addition, one Gβ subunit and two Gγ subunits (12-16). The significance of these subunits in plant systems is poorly understood; only one (17).

Arabidopsis putative GPCR protein (GPCR1) has been characterized in plants (17,20), and no ligand has been defined for any plant GPCR.

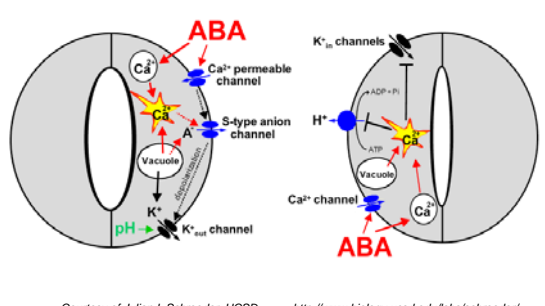
To identify previously uncharacterized GPCR proteins in *Arabidopsis*, we randomly screened the *Arabidopsis* genome and found a gene (GCR2, GenBank accession code At1g2920) encoding a putative GPCR. Transmembrane structure prediction suggests that GCR2 is a membrane protein with seven transmembrane helices (Fig. S1, A and B). The subsequent cellular localization analysis confirmed its plasma membrane localization in the transgenic plant root (Fig. S1C). GCR2-yellow fluorescent protein (YFP) is detected in the membrane fraction isolated from the GCR2-YFP transgenic plant. Similar to GCR1 (17), GCR2 is closely associated with the membrane fraction (Fig. S1E). Furthermore, even after washing with detergent or a higher pH buffer, GCR2 is retained with the membrane fraction, suggesting that GCR2 is an integral membrane protein (Fig. S1D).

The nature of the GPCR is its ability to interact with G proteins to form a complex, to mediate the physical interaction between GCR2 and Gα, we used four different approaches to detect their interaction. We first used surface plasmon resonance spectroscopy to investigate the interaction between GCR2 and GPA1. For this purpose, we expressed and purified recombinant GCR2 and GPA1 proteins in bacteria (Fig. S2). This *in vitro* assay clearly indicated that GPA1 is capable of binding to GCR2, whereas no binding activity was detected between GPA1 and bovine serum albumin (BSA) (Fig. S3, A and B). The dissociation binding constant (K_d) for GCR2 and GPA1 is 2.1×10^{-7} M (Fig. S3C).

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ABA mediates stomatal closing



Courtesy of Julian I. Schroeder, UCSD

<http://www.biology.ucsd.edu/labs/schroeder/>

Other players in ABA-induced stomatal closure

- Nitric oxide (NO)
- Hydrogen peroxide
- Sphingosine-1-phosphate
- ABA-dependent protein kinase (AAPK; involved in calcium-independent pathway?)
- Actin
- Additional receptor(s), possibly intracellular?

