3. Transport can be active or passive.

•**Passive** transport is movement **down** an electrochemical gradient**.**

•**Active** transport is movement **against** an electrochemical gradient.

What is an electrochemical gradient?

How is it formed?

Passive and active transport of ions result in electric potential difference across membranes.

•Movement of an uncharged mol Is dependent on conc. gradient alone.

•Movement of an ion depends on the electric gradient and the conc. gradient.

•Diffusion potential-

Pump potential-

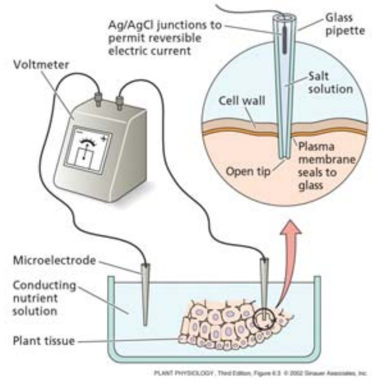
How do you know if an ion is moving uphill or downhill?

Nernst Eq

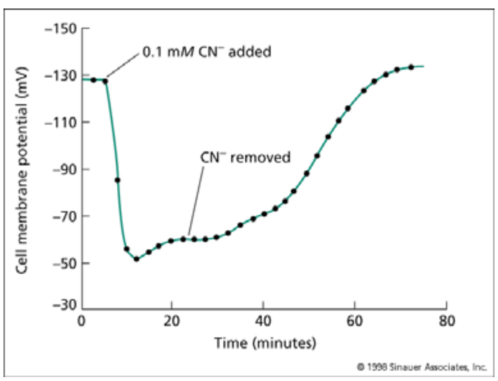
What is the driving force for uphill movement?

A) ATP ; b) H+ gradient

F 6-3 Taiz. Microelectrodes are used to measure membrane potentials across cell membrane



6-5. Pump potential and diffusion potential.



How can we determine whether an ion moves in or out by active or passive transport?

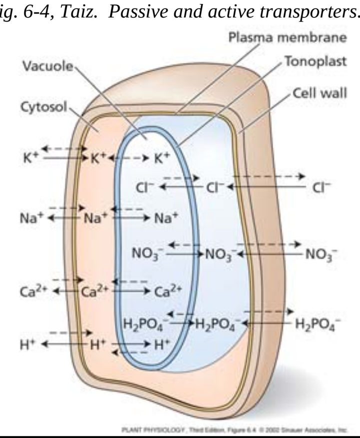
Nernst equation states that at equilibrium the difference in concentration of an ion between two compartments is balanced by the voltage difference. Thus it can predict the ion conc at equilibrium at a certain ΔE.

Very useful to predict active or passive transport of an ion.

Tab 6-1, Taiz . **Using the Nernst equation to predict ion conc. at equilibrium when the Cell electrical potential, $\Delta\psi$ = -110 mV**

Ext Conc.	Ion	Internal concentration (mM) observed	Internal concentration (mM) Nernst (Predicted)
1 mM	K^+	75 mM	74
1 mM	Na^+	8 mM	74
1 mM	Ca^{2+}	2 mM	5,000
0.2 mM	Mg^{2+}	3	1,340
2 mM	NO_3^-	5 mM	0.02
1	Cl^-	10 mM	0.01
1	$H_2PO_4^-$	21	0.01

Fig. 6-4, Taiz. Passive and active transporters.



Summary: In general

Cation uptake: passive

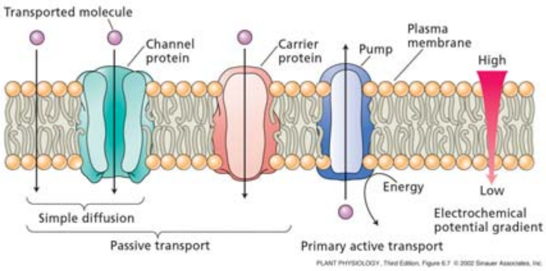
Cation efflux: active

Anion uptake: active

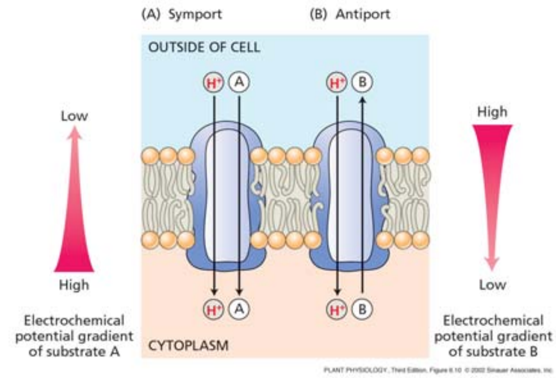
Anion release: passive

6-7. Proteins catalyze transport.

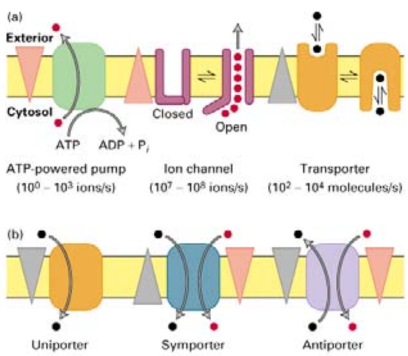
Channel, carrier pump



6-10. Secondary active transport



15-3 Lodish. Three types of transport proteins



Outline: Major Transport Proteins in Plants

1. **Primary Pumps**:
H^+-pumping ATPases are the major ion pumps in plants
Ca-pumps. Cu, Zn. Few ions moved by pumps.

2. **Secondary active transport**: H^+-coupled cotransport
Energy from H+ gradient is used to drive uphill movement of other nutrients. E.g. ions, sugars, amino-acids,

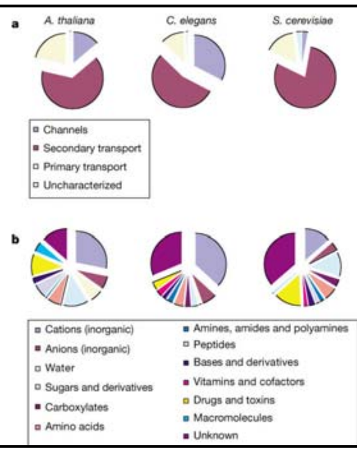
3. **Channels** allow rapid, passive transport of ions and metabolites.

4. **Water Channels** or AQUAPORINS in membranes that conduct large volumes of water rapidly.

5. **ABC transporters** pump organic molecules. E.g. auxins, Cd-X

Genome of Arabidopsis was completely sequenced in 2000. ~ 30 k genes

~5% of genes encode transporters. E.g.
Channels
Secondary transport
Primary transport



TAGI 2000. Nature 408, 796

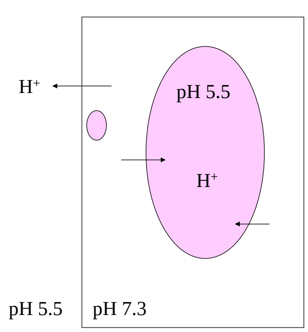
H^+ pumps generate a proton electrochemical gradient

3 H^+ pumps:

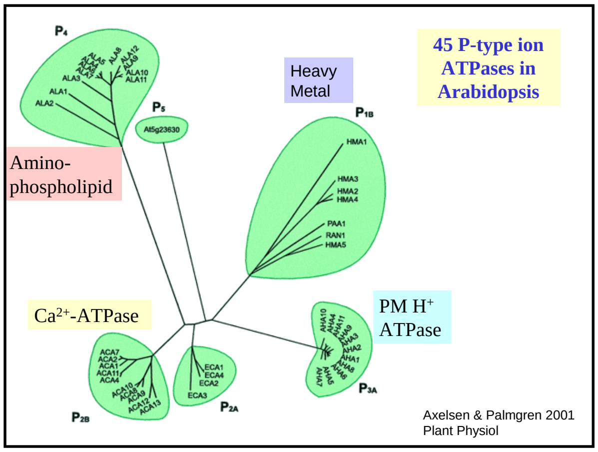
PM H^+-ATPase

Vac H^+-ATPase

Vac H^+-PPase



Why are H^+ pumps important?



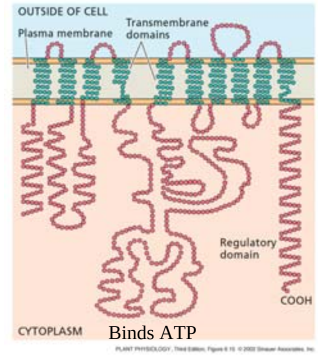
6-17. Taiz. PM-H+-ATPase acidify the cell exterior

10 Transmembrane

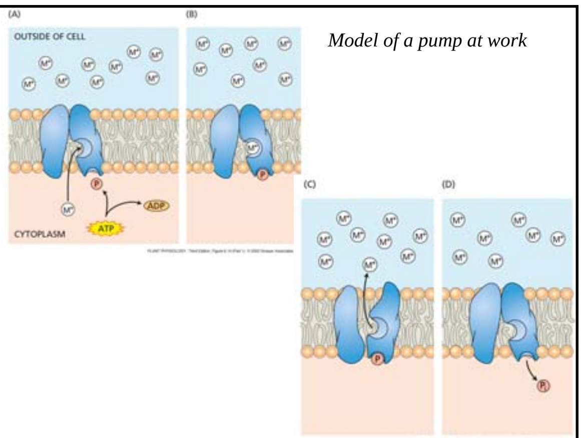
100 kDa

Arabidopsis: 12 genes

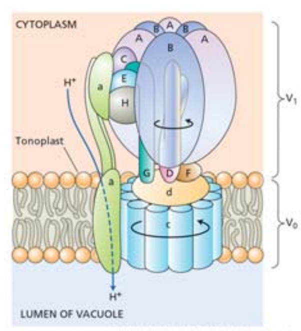
Activity is Regulated by hormone, light, pathogen

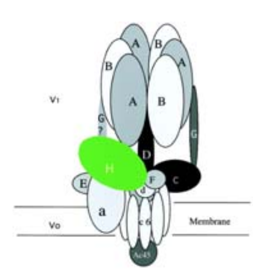


Model of a pump at work



6-16 Taiz. Vacuolar H+-ATPase acidify endomembrane compartments: vacuole, Golgi, vesicles.

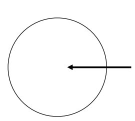




From Wilkens et al. 1999. JBC; Sagerman et al 2001. PNAS

Evidence for H^+ pumps

- Cell- vac pH is acidic
- Isolated membrane vesicles- ATP generate pH gradient and electrical gradient
- Purify protein & reconstitute activity in liposomes
- ----------------
- Clone gene
- Express & show transport in yeast

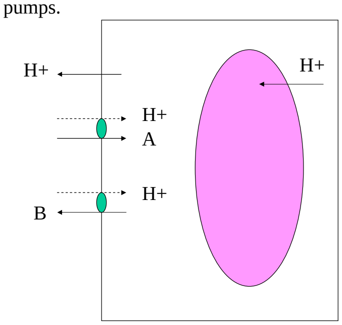


Expt. Evidence for H^+ pumps.

Importance of H^+ pumps.

Symport

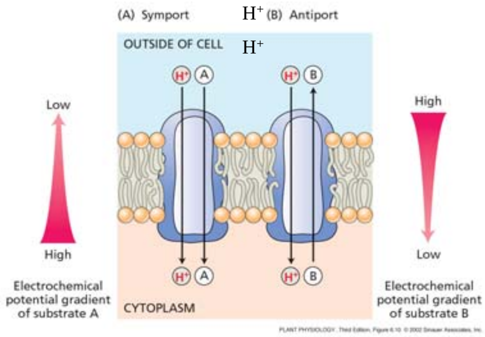
Antiport



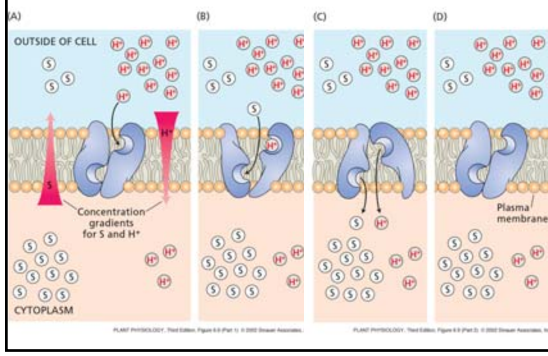
How will uptake of A affect the membrane potential?

pH gradient?

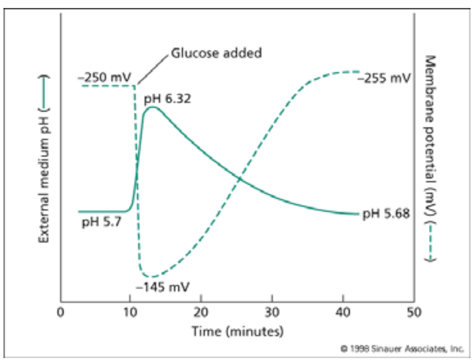
Active transport of many ions and metabolites is dependent on Secondary active transport: H^+-coupled cotransport (Fig. 6-11)



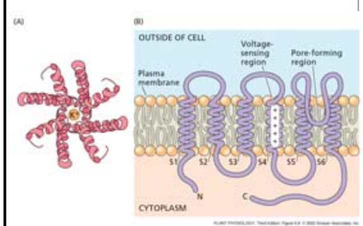
Model of an antiporter

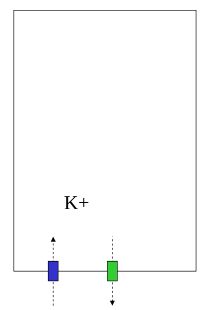


Taiz. Evidence that Glucose uptake depends on H^+-coupled glucose symport



Channels allow rapid passive diffusion of ions.
Fig. 6-15. K^+ enters cells via a gated channel.





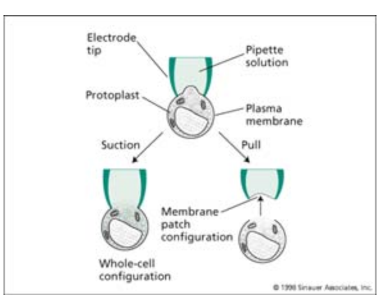
Channels are gated by

-voltage,
-Ligands: Ca, hormone, ATP, Glutamate

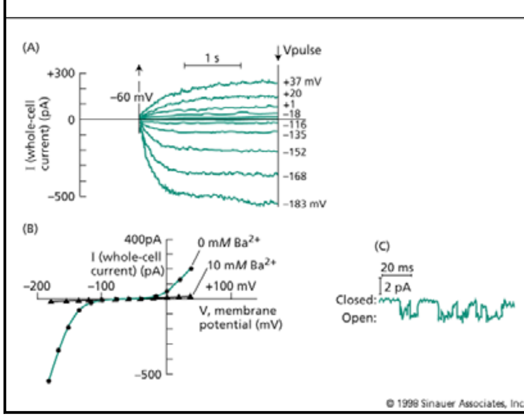
Hyperpolarization-activated

Depolarization-act

Web topic. Box 6-1 Taiz. Measuring ion fluxes via channels with patch clamp method. Measures current (movement of a + charge)



Voltage-gated current. Evidence for K+ conductance



Aquaporin: water channel

Proteins that conduct fast diffusion of water down its water potential gradient.

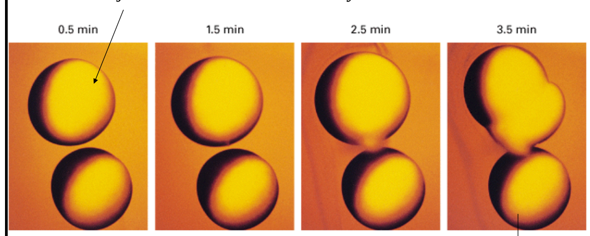
Experimental evidence.

How do you prove this?
1. *Express a candidate gene in an animal cell.*
2. *Put cell in dilute solution.*
3. *Measure swelling……. Until boom!*

Cells control their osmotic conc and cell vol.

15-32. Evidence for a water channel protein (aquaporin)

Microinject CHIP 28 mRNA into oocyte



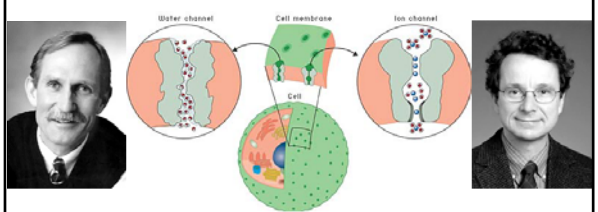
Transfer cell from 0.2 M --> 0.035 M

control

Control oocyte has low permeability to water.

Nobel Laureates in Chemistry 2003

"for discoveries concerning channels in cell membranes"



Agre

"for the discovery of water channels"

McKinnon

"for structural and mechanistic studies of ion channels"

Putting all the info. together

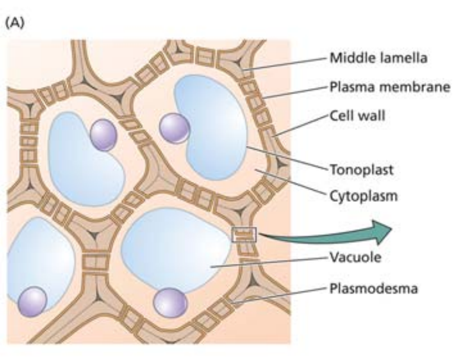
How is a nutrient transported into roots?

How do guard cells open and close?

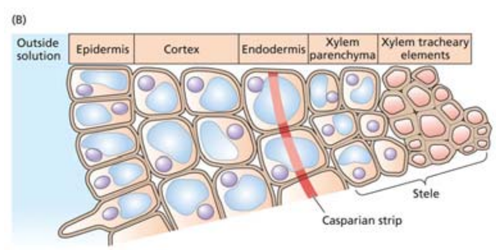
How does a mineral nutrient move from root to leaf?

a. Movement into root.
   Consider the mode of transport at each stage.
   Passive, active, and type of transport protein?
b. Up the xylem in the stem to the leaf
c. Movement into mesophyll.

Solutes move through apoplast and symplast



Ion transport into roots

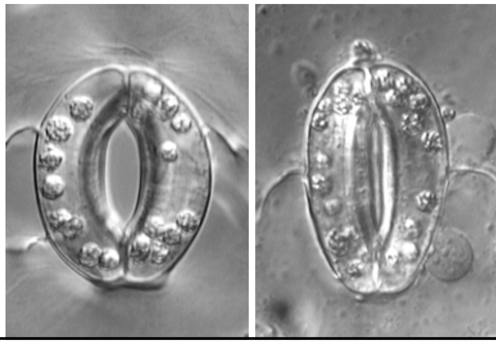


Xylem loading by xylem parenchyma cells.

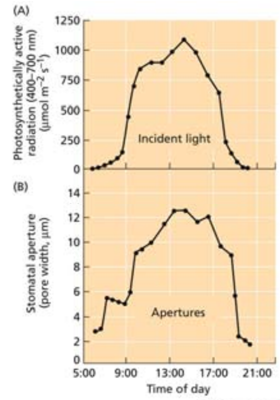
18-8. Stomata. Open and closed state

What controls opening? How? Increase in turgor pressure

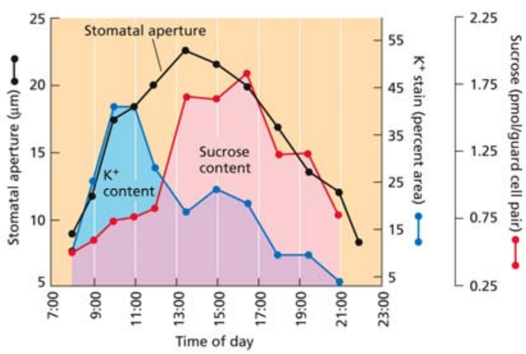
What controls closing?



18.9 Stomatal opening tracks photosynthetic active radiation at the leaf surface



18.16 Daily course of changes in stomatal aperture

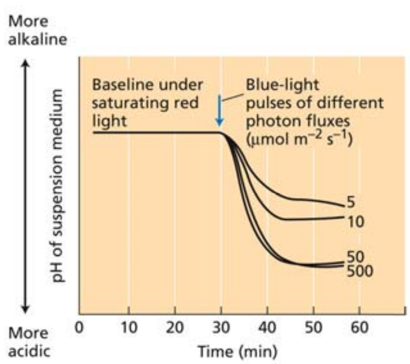


Light stimulates stomatal opening

How?

Draw on board

18.13 Acidification of a suspension medium of guard cell protoplasts of *V. faba*

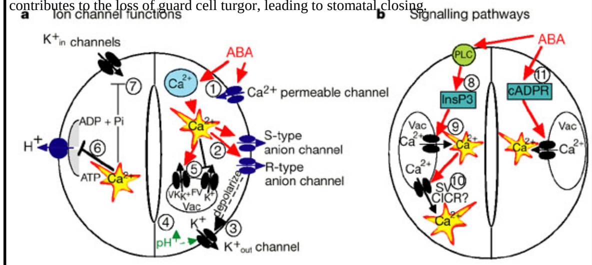


- Membrane potential hyperpolarize.
- K+ conc. Increase 100 mM → 400 mM
- Anions: Cl- and malate increase
- Water potential drops, water enters
- Guard cells increase their turgor pressure
- Stomata open.

Closure

- How?

Closing stomatal aperture: the long-term efflux of both anions and K+ from guard cells contributes to the loss of guard cell turgor, leading to stomatal closing.



From Schroeder and Allen. 2001. Nature

Lab. - guard cell movement

1. *Test effect of light versus dark on stomatal aperture*
2. *Determine which ion or solute is needed for stomatal opening: KCl, choline Cl, or mannitol*
3. *Determine the effect of a stress hormone, ABA, on stomatal aperture.*

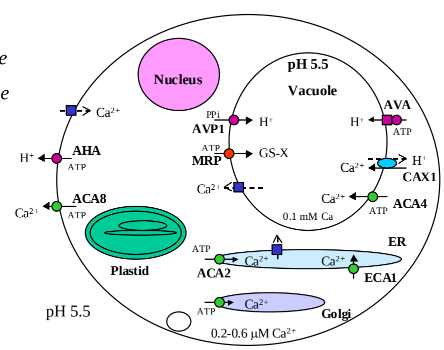
Ion Pumps in Arabidopsis

3 H+ pumps:

PM H+-ATPase

Vac H+-ATPase

Vac H+-PPase



energy from proton gradient is used for secondary active transport.