

## Lysosome and vacuolar membrane

In earlier lecture we have studied about plasma membrane. However some cell organelles have depending on the function which they perform have modified membranes. We will study the membrane of a lysosome and vacuoles in detail in this lecture.

### Lysosomes:

Lysosomes are central, acidic and membrane bound organelles that contain hydrolase enzyme for the breakdown of all types of biological polymers- proteins, nucleic acids, carbohydrates and lipids. They are mostly found in animal cells, while in yeast and plants, it acts as lytic vacuoles. It is enclosed by membrane known as lysosomal membrane that maintains the digestive enzyme at pH 4.5. Figure 1 shows the structure of lysosome.

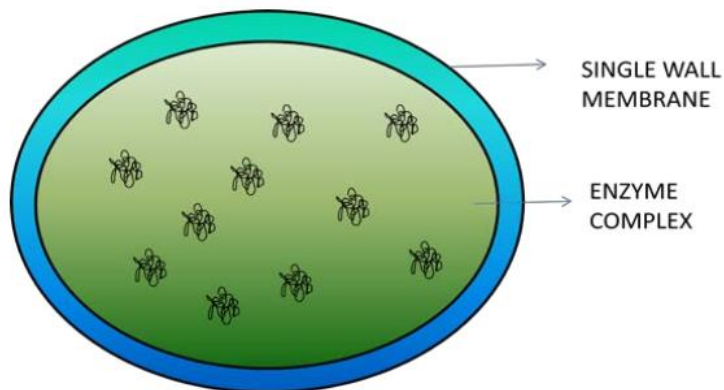


Figure 1: Lysosome

### Functions of lysosomes:

- Maintains pH by pumping protons from cytosol across the membrane via proton pumps and chloride ion channels.
- Protects the cytosol and rest of the cells from degradative enzymes within the lysosome.
- Acts as digestive system of the cell, serving both to degrade material taken up from the outside of the cell and to digest obsolete components of cell itself.

## Intracellular analysis

- Sequestration of lysosomal enzymes.
- Mediation of fusion events between lysosomes and other organelles.
- Transport of degradation products to the cytoplasm

**Lysosomal Membrane:** To perform its function with efficacy the lysosomal membrane needs some additional features in its membrane. It is slightly thicker than that of the plasma membrane. It contains substantial amounts of carbohydrate component, particularly sialic acid. In fact, most lysosomal membrane proteins are highly glycosylated, which may help protect them from the lysosomal proteases in the lumen. The lysosomal membrane has another unique property of fusing with other membranes of the cell. This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration. Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. Drugs like cortisone, hydrocortisone and others tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue. The entire process of digestion is carried out within the lysosome. Most lysosomal enzymes act in an acid medium. Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates  $H^+$  inside the organelle. Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

### **Lysosomal membrane composition:**

The V-class  $H^+$  ATPase pump is generally present in lysosomal membrane. This class of ATPase pump only transports  $H^+$  ions. Its main function is to acidify the lumen of the organelles. The proton gradient between the lysosomal lumen (pH  $\approx 4.5-5.0$ ) and the cytosol (pH  $\approx 7.0$ ) depends on ATP production by the cell.

These V-class proton pumps contain two domains: a cytosolic hydrophilic domain ( $V_1$ ) and a transmembrane domain ( $V_0$ ) with multiple subunits in each domain. Binding and hydrolysis of ATP by the B subunits in  $V_1$  provides the energy for pumping of  $H^+$  ions

through the proton-conducting channel formed by the c and a subunits in  $V_0$ . These V-class proton pumps are not phosphorylated and dephosphorylated during proton transport. Figure 2 depicts a V-class proton pump.

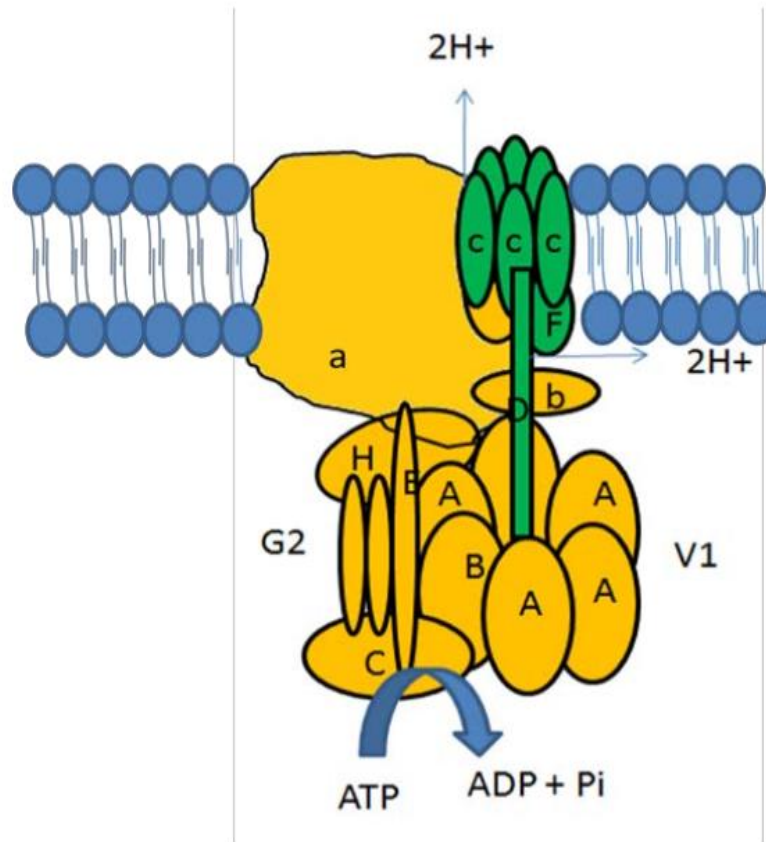
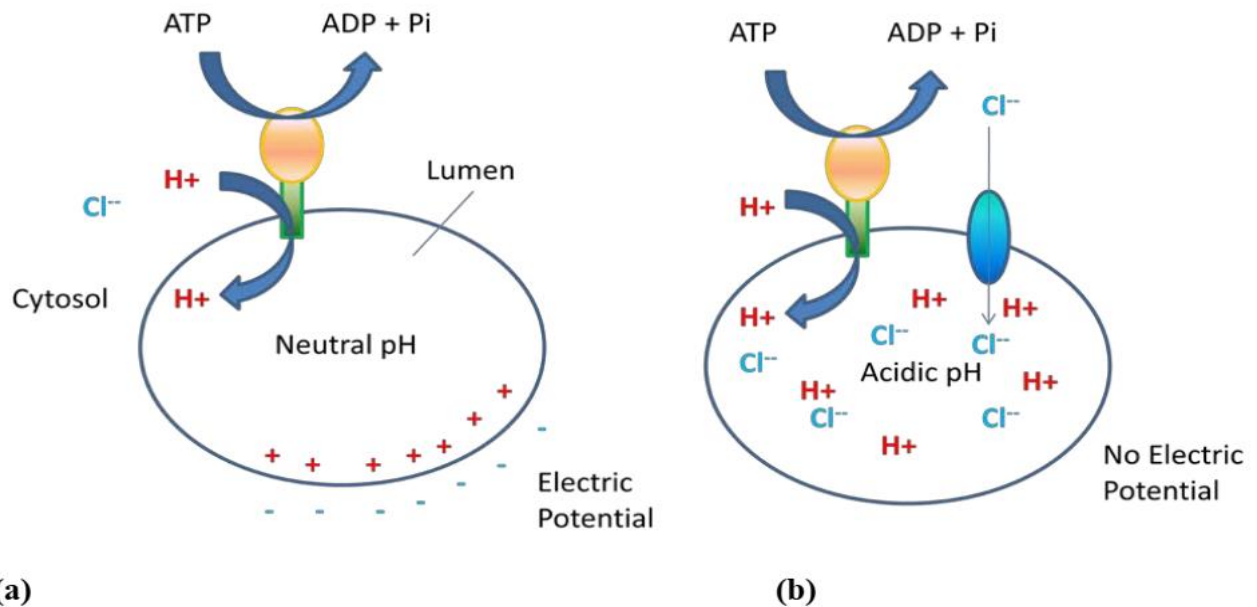


Figure 2: V-class proton pump

These protons cannot acidify by themselves because a net movement of electric charge occurs. Only a few protons build up positive  $H^+$  ions on exoplasmic face (inside) and for each  $H^+$  pumped across, a negative ion will be left behind on cytosolic face, building negative charged ions. These oppositely charged ions attract each other on opposite faces of the membrane, generating a charge separation, or electric potential, across the membrane. If more protons are pumped, the excess positive ions on exoplasmic face repels other  $H^+$  ions and prevents pumping of extra proton long before a significant transmembrane  $H^+$  concentration gradient had been established .



**Figure 3: Effect of proton pumping by V-class ion pumps on H<sup>+</sup> concentration gradients and electric potential gradients across cellular membranes. (a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, but no significant change occurs in the intraluminal pH. (b) If the organelle membrane also contains Cl<sup>-</sup> channels, anions passively follow the pumped protons, resulting in an accumulation of H<sup>+</sup> ions (low luminal pH) but no electric potential across the membrane.**

**Lysosomal membrane proteins:**

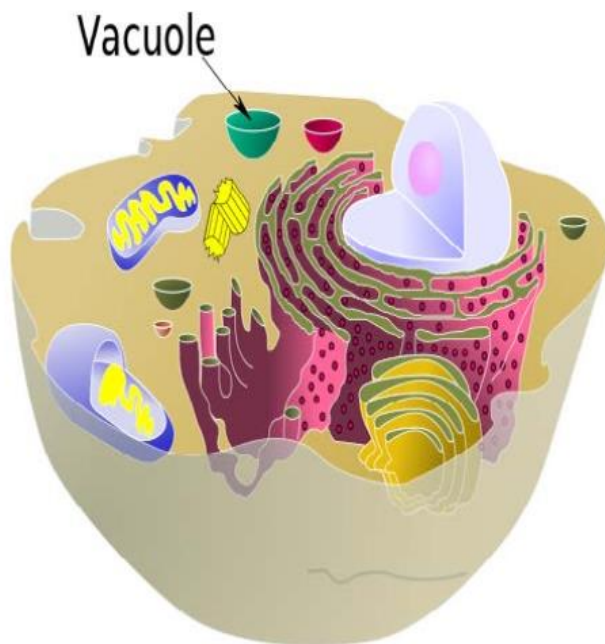
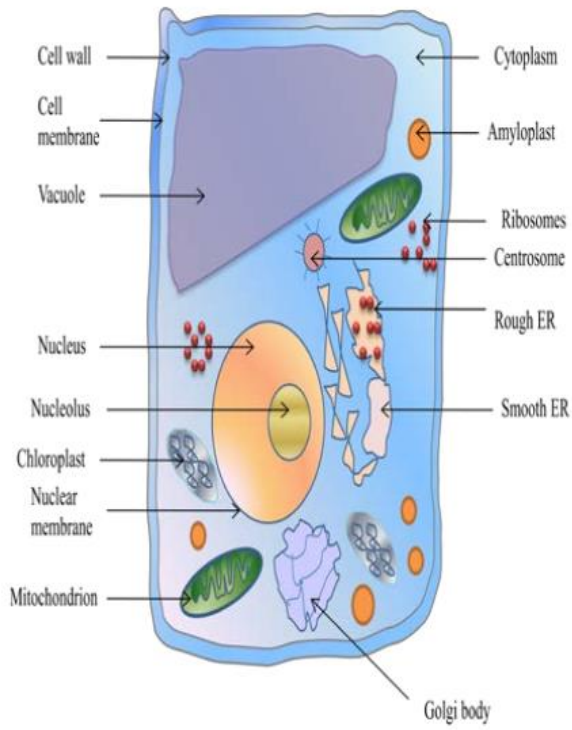
Lysosomes are formed by the fusion of transport vesicles budded from Golgi network with endosomes, which contain molecules taken up at the cell surface. And its membrane proteins are usually highly glycosylated proteins decorating the luminal surface of lysosomal membranes. They are most often known as lysosomal associated membrane proteins (LAMP). LAMP-1, LAMP-2 and LAMP-2 are the most abundant components of this membrane. And mainly involved in transport of newly synthesized hydrolases to the lysosome (lysosomal integral membrane protein 2 (LAMP2)) and across the lysosomal membrane (the V-type H<sup>+</sup>-ATPase complex and chloride channel protein 7 (CLC7)).

**Vacuolar membrane:**

Vacuoles are the membrane bound sac within the cytoplasm which are filled with water containing organic and inorganic molecules including enzymes and mostly present in plants, fungi and some animals. This vacuole slowly develops as the cell matures by

## Intracellular analysis

fusion of smaller vacuoles (vesicles) derived from the endoplasmic reticulum and Golgi apparatus.



**Figure 4: Plant cell structure**

**Figure 5: Animal cell structure**

**Function of vacuoles:**

- Acts as storage organelles and contains water and small molecules. Stores salts, minerals, nutrients, proteins, pigments, helps in plant growth, and plays an important structural role for the plant.
- Maintains internal hydrostatic pressure or turgor pressure within the cell
- Maintains an acidic internal pH
- Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole. Also maintains turgor pressure against the cell wall. Because of osmosis, water diffuses into the vacuole, and exerting pressure on the cell wall. And water loss leads to shrinkage of the cell. Hence turgor pressure needs to be maintained. Turgor pressure also dictates the rigidity of the cell and is associated with the difference between the osmotic pressure inside and outside of the cell.
- In seeds, stored proteins needed for germination are kept in protein bodies, which are modified vacuole.
- Regulating the movements of ions around the cell.
- Transports proton from cytosol to vacuole and hence stabilizes cytoplasmic pH making the vacuolar interior most acidic by creating a proton motive force which in turn used for the transport of nutrients into and out of the cell and allows degradative enzymes to act.
- Vacuoles also often store the pigments that give certain flowers their colors, which aid them in the attraction of bees and other pollinators, but also can release molecules that are poisonous, odoriferous, or unpalatable to various insects and animals, thus discouraging them from consuming the plant.

**Plant vacuoles:**

Most of the plant cell contains large, single central vacuoles and can occupy at least 30% to 80% of the cell. Generally vacuole is surrounded by membrane known as tonoplast, or vacuolar membrane. It separates the vacuolar contents from cell's cytoplasm and an

important and highly integrated component of the plant internal membrane network (endomembrane) system. The vacuole solution (also known as cell sap) differs markedly from that of the surrounding cytoplasm.

**Vacuolar membrane:**

The V-class H<sup>+</sup> ATPase pump is present in vacuolar membrane. More details of V-class H<sup>+</sup> ATPase pump is described earlier (Figure 2 and Figure 3).

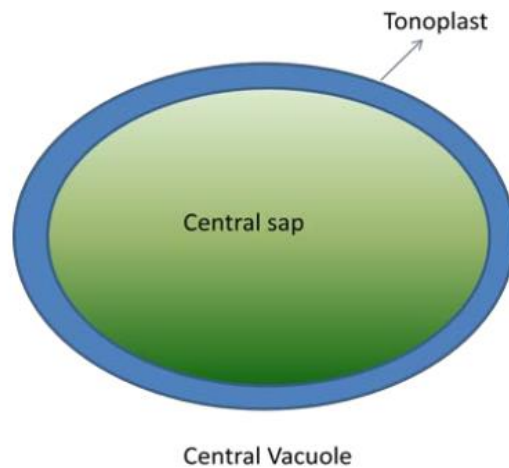


Figure 6: Plant cell vacuole

**Other transport proteins present in vacuolar membrane:**

**1. Proton Pump:**

Proton pumps play a central role in the function of the tonoplast by generating a transmembrane H<sup>+</sup> electrochemical gradient which can be utilized to drive the transport of solutes. The tonoplast contains different proton pumps, an ATPase and a PPase. V-ATPases (vacuolar-type) are present on different membranes of eukaryotic cells and is constituted of 13 subunits whereas tonoplast PPase is also an integral entity of the tonoplast and consists of one 80 kDa protein.

**2. Aquaporins:**

Major intrinsic membrane proteins (MIPs), are very small hydrophobic proteins abundantly present in membranes. But these MIPs form water channels. Later  $\alpha$ -TIP (tonoplast intrinsic protein) which is a member of MIPs was described and found

abundantly. Another major membrane protein of the central vacuole is the  $\gamma$ -TIP (observed in radish). Both TIPs have been shown to act as water channels.  $\alpha$ -TIP is associated with the storage vacuole while the  $\gamma$ -TIP is localized on the lytic vacuole. Interestingly,  $\alpha$ -TIP has to be phosphorylated in order to exhibit water channel activity.

### **3. ABC transporters:**

Another class of transporters are ABC type transporters, which are directly energized by MgATP and do not depend on the electrochemical force. Their substrates are organic anions formed by conjugation, e.g. to glutathione.

### **Examples of solute transport across vacuolar membrane in plant cells:**

#### **Transport of products of primary metabolites:**

The various types of Primary metabolites could be:

**1. Carbohydrates:** Sucrose uptake occurs by facilitated diffusion in leaf vacuoles. Later it was also observed that active transport of sucrose takes place for vacuoles isolated from sugar cane cell cultures, which accumulates sucrose at concentrations comparable to those in the stalk tissue and tomato fruit vacuoles. Furthermore, it was also found that sucrose transport was stimulated by MgATP and to occur via a sucrose/H<sup>+</sup> antiport in red beet. Larger carbohydrates such as stachyose, which is present in large quantities in *Stachys sieboldi*, may also be accumulated in the vacuole by proton antiport mechanisms. Many sugar alcohols also found in plants accumulate within the vacuoles. Transport of sorbitol across the tonoplast appears to be ATP-dependent in case of immature apple fruit tissue. Transport experiments suggest that mannitol crosses the tonoplast by facilitated diffusion.

#### **2. Amino acid:**

The first amino acid transport system was observed in barley plants. These are carriers or channels which are modulated by free ATP (but not by MgATP) which induces inward as well as outward fluxes of all amino acids tested.

### **3. Organic acids:**

With context to organic acids, malate transport across the vacuolar membrane has been studied most intensively. This is due to the central role of malate in plant metabolism. The uptake of malate is mainly governed by the electrical component of the electrochemical potential generated by the proton pumps. This channel also mediates uptake of succinate, fumarate, and oxaloacetate. The malate channel is not affected by cytosolic  $\text{Ca}^{2+}$  or ATP and it is a 32 kDa subunit protein. Citrate crosses the tonoplast using the same transporter as malate.

### **4. Inorganic anions:**

The  $\text{H}^+$  pumps generate a positive potential inside the vacuole, which is the driving force for anion movements. Anion-dependent dissipation of a proton-pump generated by anions revealed that  $\text{NO}_3^-$  permeates more rapidly than  $\text{Cl}^-$  and  $\text{SO}_4^-$  whereas  $\text{HPO}_4^{2-}$  crossed the tonoplast considerably slowly.

#### **Chloride:**

An ATP-dependent  $\text{Cl}^-$  uptake was studied in barley mesophyll vacuoles. Later a vacuolar  $\text{Cl}^-$  channel (VCL) was identified in *Vicia faba* guard cells which is activated by a calcium dependent protein kinase (CDPK) in the presence of ATP and  $\text{Ca}^{2+}$  and, to a weaker extent (22%), by protein kinase A. The VCL channel was activated at physiological potentials enabling  $\text{Cl}^-$  uptake into vacuoles.

#### **Nitrate:**

Amongst the anions it exhibits the highest permeability through the vacuolar membrane. It was concluded in one of the experiment that a membrane potential driven nitrate transporter, a  $\text{NO}_3^-/\text{H}^+$  antiporter is present on the tonoplast.

#### **Sulphate:**

Using tonoplast vesicles, it has been shown that  $\text{SO}_4^{2-}$  and  $\text{HPO}_4^{2-}$  anions cross the tonoplast slowly as compared to  $\text{NO}_3^-$  or  $\text{Cl}^-$ . It has been found that  $\text{SO}_4^{2-}$  uptake is stimulated by  $\text{Mg}^+$ -ATP.

**Phosphate:**

Pi starvation leads to an efflux of Pi from the vacuole. It has been shown that Pi concentrations in the cytosol are maintained at a constant level in *Acer pseudoplatanus* cells using  $^{31}\text{P}$  NMR.

**5. Inorganic cation:**

The membrane potential of the cytosol with respect to the vacuole is negative (20–40 mV). This implies that cations are excluded from the vacuole unless transport is coupled to an energy-dependent uptake mechanism.

**Potassium:**

Several channels exhibiting potassium permeability have been described. The first channel demonstrated for vacuolar membrane was called SV (slow activating vacuolar) channel. This channel is a slow activated channel and is associated with  $\text{Ca}^{2+}$  and calmodulin-induced  $\text{K}^+$  and  $\text{Ca}^{2+}$  fluxes. These channels have been reported for the permeability of  $\text{Na}^+$  if  $\text{Ca}^{2+}$  concentrations are increased by a signal. Secondly, FV (fast vacuolar) channel activates instantaneously in response to voltage changes. These channels may allow the release of  $\text{K}^+$  at low  $\text{Ca}^{2+}$  concentrations. Thirdly, the vacuolar  $\text{K}^+$  (VK) channel is activated instantaneously but it can be distinguished from the FV channel. It is voltage independent and fully activated at low cytosolic pH.

A  $\text{K}^+/\text{H}^+$  antiport mechanism has been also reported for tonoplast enriched fractions from zucchini, *Brassica napus* hypocotyls, and *Atriplex*.

**Sodium:**

$\text{Na}^+$  accumulation is accompanied by vacuolar alkalinization in barley roots. This was established by using NMR spectroscopy.

**Calcium:**

Calcium plays a central role in signal transduction and higher concentrations are observed in apoplast and within the vacuole. An energized, highly specific calcium uptake by the vacuole is, therefore, a prerequisite for maintaining a low cytosolic calcium concentration. P-type Ca-ATPases have been identified at the plasma membrane, the ER, and the vacuolar membrane. A  $\text{Ca}^{2+}$  pump called a  $\text{Ca}^{2+}/\text{H}^+$  antiporter has been demonstrated in vacuolar membrane fractions. This antiporter exhibits a far lower affinity

than the  $\text{Ca}^{2+}$ -ATPase. Also, a vacuolar voltage gated  $\text{Ca}^{2+}$  channel (VVCa) has been reported which is activated on membrane hyperpolarization.

**Magnesium:**

The presence of a  $\text{Mg}^{2+}/\text{H}^{+}$  antiporter has been described for the vacuole-like luteoids of *Hevea brasiliensis* and tonoplast vesicles isolated from maize roots.

**Heavy metals:**

Plants need some heavy metals such as  $\text{Cu}^{2+}$  or  $\text{Zn}^{2+}$  as micronutrients. Therefore, they need to be transported and a large portion of the heavy metals absorbed by the cell is usually concentrated within the vacuole. A vacuolar  $\text{Cd}^{2+}/\text{H}^{+}$  antiport activity has been demonstrated. However, it is known that plants form chelates with heavy metals by synthesizing phytochelatins (PCs), and these PCs can be transported into vacuoles of *Schizosaccharomyces* as apoPC or as PC-Cd complexes by ABC transporters. Vacuoles of higher plants are also known to transport phytochelatins.

**Transport of products of the secondary metabolites:** Involvement of secondary energized transporters and directly energized, ABC-type transporters

Plants synthesize an huge number of secondary metabolites and many of these have been found to be exclusively localized in the vacuole. The electrochemical gradient established by the two vacuolar proton pumps is used by the secondary energized transporters as a source of energy. It was demonstrated that the  $\Delta\text{pH}$  was essential for the uptake of a number of phenolics, such as esculin, *o*-coumaric acid glucoside, apigenin- 7-(6-*O*-malonyl) glucoside, and anthocyanins from carrot. Recently it became evident that in addition to transporters depending on the proton motive force, directly energized transporters are also present on the vacuolar membrane. The first demonstration for a directly activated transport of solutes into the vacuole was provided for glutathione conjugates. Flavonoid glucuronides, a secondary plant compounds in rye vacuoles are transported by directly energized transport processes. Furthermore, studies with lucifer yellow, a sulfonated compound also indicates that sulfonated and sulfated secondary compounds cross the tonoplast by direct energization.

