Cells are the building blocks of various structures and arrays of proteins and lipids, within and around the plasma membrane, that are essential for the proper functioning of both these molecules and the plasma membrane. In the recruitement, multimerization and assembly of specific membrane proteins and lipids, one of the critical processes is the regulation of the movement of these molecules. Protein are not free floating in a sea of excessive lipids; in other words, the cells have various means by which they control the mobility and specific assembly of membrane proteins. Lipids on the other hand, are capable of executing two types of motional modes-intermolecular movements (rotational, segmental and polar head group movements exhibited by the molecule) and intramolecular movements (rotational and translational motions as a whole with repect to its neighbours). In biological membranes the transbilayer motions of lipids have been found to result in large concentration gradients of the various lipid components on the either monolayer. The apparent stability of such gradients suggests that they must have some functional implications. Investigations reveal that the asymmetric disposition of membrane lipids play an important role in several biological phenomenon viz., membrane fusion, endocytosis, exocytosis, regulation of membrane protein activity, modulation of surface potential, clearance of aged and diseased cells from the circulation.

Association between the cytoskeleton and cellular membranes, both within the cell and at points of cell contact, plays a central role in determining various cellular functions and may play as an organizer of molecules in the plasma membrane. It is becoming clear that the non-homogenous distribution and assembly of proteins in the plasma membrane

are, in part, regulated through the membrane associated portions of the cytoskeleton, that is, the membrane skeleton. As a consequence, more attention is being directed toward understanding membrane-cytoskeleton interactions and their roles in regulating the architecture of the plasma membrane.

Due to the relative simplicity of the red blood cell, most of the initial investigations on membrane organization have been conducted using this cell as the model. The symmetric transbilayer distribution of the phopsholipids in blood cells is widely accepted as paradigm for the plasma membrane of a variety of cell types. However, inspite of number of studies, the translocase has eluded precise identification. Moreover, it has been difficult to ascertain whether the membrane skeleton has any crucial role to play in maintaining the aysmmetric lipid distributions. Due to the non-specificity in the biochemical methods of investigations, it becomes essential to carryout further studies using mutants that have genetically defined modifications in the cytoskeleton proteins and the putative translocase. Due to the ease of propagation and the ability to introduce defined mutations, microorganisms such as bacteria, viruses and yeasts have been used extenisvely in correlating protein structure and biological functions. Of these, the yeasts stand out in having a phospholipid distribution pattern that is typical of higher eukaryotes. In addition, the yeasts also possess a membrane skeleton that is lacking in both bacteria and viruses. Hence they might serve as a better model in studying lipid asymmetry. For this work we chose two conditional lethal temperature sensitive mutants of *Saccharomyce cerevisiae*, mutated in the actin gene ACT1, thus having a weakened cytoskeleton and

their phenotype differs from the wildtype both at their permissive temperature (23°C) and restricitive temperatures (37°C).

Role of cytoskeleton in regulating the plasma membrane functional activities

Transporters constitute a major portion of proteins present in the plasma membrane and therefore, can easily depict the functional status of the plasma membrane. To start with, we measured transport of amino acids (arginine and methionine) and ions (proton and calcium) using radiolabelled L[-^{14}C]-arginine, L[-^{14}C]-methionine and $^{45}Ca^{++}$. Both mutants showed marked differences in arginine transport compared to parent strain while no difference were observed in other transports. Further, to confirm that this defect is because of alterations in actin cytoskeleton only, we isolated and purified arginine-binding protein known to be released while washing the cells and thus affecting arginine transport. No differences were observed in the release of arginine-binding protein from all the strains. Now it became interesting to check whether or not gene for arginine CAN1 and actin ACT1 are linked to each other. Seggregation analysis was carried out by back crossing the mutant strain with the wildtype. This time, we did not see any linkage pattern based on tetrad analysis due to which we could not lead to a firm conclusion that actin cytoskeleton disruption is the sole reason for the defective arginine transport.

Role of cytoskeleton in regulating the plasma membrane organization and dynamics

We analyzed the plasma membrane structure and dynamics from these strains by measuring phospholipid distribution, aminophospholipid asymmetry and membrane phospholipid anisotropy. Membrane anisotropy study was done by fluorescence polarization using DPH and TMA-DPH as the probes. Since very little information is

available regarding the presence of aminophopsholipid translocase in yeast plasma membrane, initial experiments conducted were aimed at attributing properties of speculated aminophopsholipid translocase in maintaining aminophospholipids transbilayer distribution in the yeast plasma membrane. To examine whether this process is influenced by the actin cytoskeleton, we have studied the PtdEtn translocation in S. cerevisiae cells having defined substitution mutations in actin (Pro32-to-Leu and Thr58-to-Ala) and also after treating these cells with some microfilament or microtubule disrupting agents. The microfilaments were disrupted by treating the cells with various cytochalasins. The PtdEtn translocation was studied by measuring the external PtdEtn levels, using fluorescamine as the external membrane probe, in the ATP-depleted, ATP-depleted and repleted, and N-ethylmaleimide-treated cells. Results from these studies showed that the transbilayer phosphatidylethanolamine (PtdEtn) movements in the *Saccharomyces cerevisiae* plasma membrane are regulated by an ATP-dependent , protein-mediated process(es). The PtdEtn translocation was defective in one actin mutant but not in the other one. Similarly, the translocation became abnormal in the cytochalasin-treated cells but not in the cells that received treatment with microtubule-disrupting agents, like colchicine or benomyl. **These results have been interpreted to suggest that actin cytoskeleton is involved in regulating the aminophospholipid translocase activity in the yeast cell plasma membrane.**