INTRODUCTION AND REVIEW OF LITERATURE
INTRODUCTION

In eukaryotes, mitogen activated protein kinase (MAPK) cascades constitute very important signal transduction pathways that are conduits of various extracellular stimuli arising out of fluctuations in the environment or cell-cell communications. These cascades control various aspects of cellular physiology such as cell proliferation, differentiation and adaptive response to changes in the extracellular environment. A typical MAPK cascade is composed of three conserved families of protein kinases; the MAPK (also known as ERK i.e. extracellular signal regulated kinase); a MAPK activator: MAPK kinase (MAPKK, MEK, MKK, extracellular signal regulated kinase kinase); and a MEK activator: MAPK kinase kinase (MAPKKK, MEK kinase [MEKK], extracellular signal regulated kinase kinase kinase) (Robinson and Cobb, 1997; Banuett, 1998). These kinases act in succession to form a cascade that ultimately phosphorylates both cytoplasmic and nuclear targets (Martinez-Pastor et al., 1996; Raitt et al., 2000). In mammals pathways involving MAPKs are divided into three sub families p42-p44 (ERK) MAPKs; and p46-p54 JNKs and p38s based on the degree of homology, biological activity and phosphorylation motif (Xia et al., 1995). ERK pathways mostly regulate growth and differentiation whereas JNK and p38 pathways are required for stress signaling. These enzymes are strongly induced in response to stress signals such as heat shock, UV radiation, cytokines and metabolic inhibitors. MAPK pathways seem to be essentially conserved from lower eukaryotes such as yeast to higher organisms, though their numbers and functional complexities vary. In fact studies done in yeast, particularly *Saccharomyces cerevisiae*, a single cell, genetically amenable eukaryote has been an excellent model system and have contributed to a great extent to our understanding of these pathways at the molecular level.

Extensive genetic and biochemical analysis aided by complete genome sequencing has revealed the presence of five functionally distinct MAPK modules in yeast *S. cerevisiae* (Gustin et al., 1998) (Fig 1.1). Among these pathways, one is required for spore wall assembly and normally not present in growing cells, whereas other four are
Fig. 1.1 Mitogen activated protein kinase (MAPK) signaling pathways in the yeast *S. cerevisiae*  
In yeast five MAPK modules regulate mating, filamentation, high-osmolarity responses, cell wall remodeling and sporulation. Core module of MAPK pathways is composed of three kinases (MAPKKK, MAPKK, MAPK) those are sequentially activated by phosphorylation.
present in growing cells. They are responsible for mating, filamentation, cell wall integrity and growth under high-osmolar conditions (reviewed by Herskowitz et al., 1995; Madhani and Fink, 1998). When exposed to high osmolar medium, yeast cells respond by synthesizing and accumulating glycerol as compatible solute inside the cell to counterbalance the external osmotic pressure (Mager and Varella, 1993). This is achieved by activation of the MAPK pathway called as HOG (High osmolarity glycerol) pathway (Brewester et al., 1993). MAPK of this pathway is Hog1p, which is activated by Pbs2p. This pathway is closely related to mammalian p38 pathway that is activated by different stress conditions including osmotic stress. Thus it appeared that many principles of osmoadaptation are conserved among eukaryotes.

HOG pathway seems to be a prototype of stress activated protein kinase pathway and is ubiquitous among yeast and fungi. Homologous pathway in Schizosaccharomyces pombe is called as Sty1 pathway. This pathway has been shown to be activated by different types of stress such as osmotic shock, heat shock, nutrient limitation and oxidative stress (Degols et al., 1996; Samejima et al., 1997). On the contrary in S. cerevisiae HOG pathway is known to be specifically stimulated by osmotic shock (Schuller et al., 1994; Tamas et al., 2000). Most of our knowledge pertaining to stress activated MAPK pathways is based on the studies carried out in these two model yeast species. Both these organisms are considered as moderately osmotolerant yeast. In comparison to these Debaryomyces hansenii is considered a highly halotolerant, osmotolerant yeast. Previous work in our laboratory, suggested that Hog1 homologue is also present in this organism. However, detailed information about other components of the pathway is lacking. Such studies will definitely help to unravel molecular mechanism of high osmotolerance exhibited by this yeast.
REVIEW OF LITERATURE

In the following sections, an effort have been made to present an overview of MAPK pathways in yeast particularly HOG (High Osmolarity Glycerol Response) pathway that orchestrate response to extracellular osmolarity. The HOG pathway was originally discovered in *S. cerevisiae* and most of our present-day knowledge has emanated from the work carried out in this species. Therefore, large part of the discussion concerning the mechanism of HOG pathway activation, signal transmission and its physiological responses is related to this species. Furthermore, an outline to explain the regulation and specificity of this pathway are presented.

1.1 MAP kinase pathways in the yeast *S. cerevisiae*

Despite their placid appearance, cells of *S. cerevisiae* possess rapidly responding, highly efficient signaling pathways. The ease of genetic analysis in yeast, especially *S. cerevisiae* provided great opportunity to obtain insights into complex signal transduction pathways of higher eukaryotes. In the yeast *S. cerevisiae*, five MAPK modules have been identified so far, they regulate: mating, filamentation, high-osmolarity responses, cell wall remodeling, and sporulation (reviewed by Herskowitz *et al.*, 1995; Madhani and Fink, 1998).

1.1.1 Mating/ Pheromone response pathway

Mating pathway is one of the best-defined MAPK pathway in yeast (Blumer and Johnson, 1994; Errede and Levin, 1993; Schultz *et al.*, 1995). Yeast exists as haploid and diploid cells. Haploid cells have two sexual phenotypes: a or α mating types. Strains of opposite mating type can mate and form a diploid i.e. controlled by the sexual pheromone (a-factor and α-factor) that bind to their respective receptors. The a cells expresses the
receptor for $\alpha$ factor and it is encoded by $STE2$ gene. Similarly in $\alpha$ cells, $STE3$ encodes receptor for $\alpha$ factor. Both the receptors have characteristic seven putative membrane spanning domains of G protein linked receptor family. The signal is transduced by heterotrimeric G protein consisting of $\alpha$, $\beta$, $\gamma$ subunits encoded by $SGAI/GPA1$ gene; $STE4$ gene; $STE18$ gene. Pheromone binding to the receptor then activates downstream protein Ste20p (Cairns et al., 1992; Leberer et al., 1992) and these in turn stimulate the Ste11p, Ste7p and Fus3p MAPK cascade. The specificity in this pathway is maintained by Ste5p that act as a scaffold protein. It interacts with Ste11p (MEKK), Ste7p (MEK), Fus3p (MAPK) (Choi et al., 1994; Inoue et al., 1995; Kranz et al., 1994). Activation of MAPK Fus3p regulates the activity of transcription factors required for the expression of components of the mating pathway itself and genes necessary for cell cycle arrest and cell fusion. Fus3p phosphorylates transcription factor Ste12p that mediates the induction of pheromone response genes such as $FAR1$ that inactivates Cdc28p/Clnp kinase complex (Cln1p and Cln2p) and thus inhibits cell growth (Elion et al., 1993; Peter et al., 1993). Homologous pathway has also been studied in $S. pombe$ (Errede et al., 1993).

1.1.2 Pseudohyphal pathway of diploids and invasive growth of haploids

In $S. cerevisiae$, upon nitrogen starvation, diploid yeast cells undergo filamentous growth (Gimeno et al., 1992, a, b). The pseudohyphal state of cells is characterized by changes in bud site selection from bipolar to unipolar, cell elongation and invasive growth (Mosch et al., 1996). Mother and the daughter cells remains attached and produce filaments composed of a linear chain of elongated cells called pseudohypha. The mother yeast cells produce colonies on the surface of an agar plate, whereas the pseudohypha invades the agar (Gimeno and Fink, 1994; Gimeno et al., 1992, a, b). Haploid cells can also invade the agar and grow beneath the surface. A MAPK cascade consisting of Ste11p (MKKK), Ste7p (MKK) and Kss1p (MAPK) transduce the signal leading to invasive growth. The kinase activity of Kss1 is required for filamentous growth both in haploid and diploid cells (Cook et al., 1997; Madhani et al., 1997). Active Kss1p
phosphorylates and activates Ste12p, leading to activation of genes under the control of promoters containing filamentation and invasion response elements (Madhani et al., 1997). MAPK Fus3p functions as a repressor of invasive growth i.e., employed to suppress invasive growth in haploid cells and facilitate their entry into the mating pathway. This is possible as the inhibitory activity of Fus3p on invasive growth is higher than the stimulatory activity of Kss1p (Bardwell et al., 1996; Elion et al., 1993). Thus the balance of these kinase activities, both regulated by MAPKK Ste7p plays a role in choosing between invasive response and mating in haploid cells.

1.1.3 Cell Integrity pathway/PKC pathway

In yeast cell wall composition changes under variety of conditions such as during cell division, pheromone response, changes in temperature and osmolarity of the medium. One of the MAPK pathway plays the major role in maintaining cell wall integrity under these conditions. The MAPK module is composed of MAPKKK, Bcklp; a pair of redundant MAPKKs, Mkk1p/Mkk2p and MAPK, Slt2p (Irie et al., 1993). The MAPK of this pathway Slt2p is also called as Mpk1p (Torres et al., 1991). This cascade is controlled by Pkc1p (MKKKK), the only Protein Kinase C homolog present in yeast S. cerevisiae. Rho1p binds to and is required for the activity of Pkc1p in-vivo (Nonaka et al., 1995). Pkc1p is activated in Rho1p dependent manner. Rho1p is a small GTP binding protein of the Rho subfamily of Ras related proteins and is required for cell growth (Kamada et al., 1996; Levin et al., 1990). Further downstream, Pkc1p can directly phosphorylate and activate MAPKKK Bck1p (Lee et al., 1993). Downstream substrates of the MAPK Slt2p are transcriptionally regulated proteins Rlm1p, Swi4p and Swi6p (together form the transcription factor SBF) and are required for the normal expression of the G1 cyclin genes CLN1, CLN2, PCL1 and PCL2 (reviewed by Gustin et al., 1998). PKC1p and Rho1p are the points where other pathways forming network with cell integrity pathway diverge or converge. Thus, this pathway has interconnections with the calcineurin pathway (Garret-Engele et al., 1995), HOG pathway (Davenport et al., 1995)
and Cdc28p in cell cycle phosphatidylinositol pathway (Yoshida et al., 1994; Levin et al., 1990).

In the fission yeast *S. pombe* it was found that Pmk1p (MAPK) is a part of a MAPK pathway involved in maintenance of cell wall integrity. However Pmk1p may not be a downstream target of PKC in *S. pombe* but function in coordination with PKC to regulate cell integrity (Toda et al., 1991; Zaitsevskaya-Carter and Cooper, 1997). The upstream components of this pathway in *S. pombe*, remains to be identified.

1.1.4 *Spore wall assembly pathway*

In diploid yeast cells, upon nutritional starvation, spore formation takes place. Sporulation is a process that involves meiosis and leads to packaging of haploid nuclei into spores (Mitchell, 1994). Cell withdraws from normal cell cycle and there are sequential changes in gene expression to undergo meiosis I and II, followed by assembly of a spore wall around each of the nuclei. The final differentiated spore wall consists of four layers. A MAPK Smk1p has been shown to be involved in the assembly of spore wall (Krisak et al., 1994). However upstream MEKK and MEK of this pathway has not been identified. However two other proteins Sps1p (Friesen et al., 1994) and Cak1p (Wagner et al., 1997) have been shown to be a part of this pathway. Both Sps1p and smk1p mutants display similar phenotypes: they proceed normally through meiosis but then are defective in spore wall assembly (Herskowitz, 1995; Krisak et al., 1994).

1.1.5 *High Osmolarity Glycerol (HOG) Response Pathway*

Exposure of living cells to high osmolarity results in an array of changes those can be distinguished into different stages:

i. There is rapid efflux of water which leads to loss of turgor and hence shrinkage of cells (Hohmann, 1997). Intracellularly it results in recruitment of water from vacuole into cytoplasm, thus compensating for sudden increase in concentration. In case of severe
stress cytoskeleton collapses leading to depolarization of actin patches. These immediate effects are caused by physico-mechanical forces operating under these conditions.

ii). The primary response to hyperosmotic stress consists of several molecular events in order to elicit protection, repair and recovery from damage. First step is temporary growth arrest (Belli et al., 2001; Alexander et al., 2001) that serve as a strategic re-setting mechanism required for proper adjustment to new growth conditions. Secondly, the glycerol channel Fps1p closes to regain turgor. Thirdly, the high osmolarity glycerol (HOG) mitogen activated protein (MAP) kinase pathway is triggered (Gustin et al., 1998).

iii). Sustained adaptive events sets gene expression to a new steady state (Rep et al., 2000). Intracellular glycerol accumulates by the combined effects of diminished efflux and enhanced synthesis. This ultimately leads to intake of water and swelling of cell to a size which remains smaller than before the osmotic challenge. When the critical size is attained growth is resumed and cell continues to divide. Cytoskeleton is repaired and displays actin repolarization.

Osmoadaptation mechanisms received much wider scientific interest with the discovery that MAPK cascade is involved in this process. A breakthrough in our understanding of the yeast osmoreponse came with the identification components of HOG pathway in S. cerevisiae (Brewester et al., 1993; Maeda et al., 1994) (Fig 1.2).

Thus the HOG pathway in S. cerevisiae plays an important and specialized role in adaptation to stress. This pathway obtains signal from two upstream branches. One branch is the two component signaling system comprising Snl1, Ypd1, and Ssk1 (Maeda et al., 1994; Ota and Varshavsky, 1993; Posas et al., 1996). Ssk1p activates the functionally redundant (MAPKKKs) Ssk2p/Ssk22p, which then activates MAP Kinase Kinase Pbs2p, which in turn dually phosphorylates and activates MAP kinase Hog1p. A second branch of HOG1 is activated by the osmosensor Sho1p (Maeda et al., 1995), which signals to (MAPKK) Ste11p, Pbs2p and finally to Hog1p (O'Rourke and Herskowitz, 1998; Posas and Saito; 1997). Studies of the HOG pathway have indeed
Fig. 1.2 Schematic representation of HOG MAPK Pathway
In response to hyperosmolarity HOG pathway is activated through two upstream branches. One pathway is activated through two component signal transduction system and the other receives signal through membrane protein Sho1p. Hog1p is activated by phosphorylation and modulates its target proteins both in nucleus and in cell cytoplasm. Adopted from review by Mager and Siderius, 2002 with few modifications.
contributed to our understanding of MAPK pathways by providing important insights into activation mechanisms of MAPK pathways.

1.2 Structural components of HOG pathway

1.2.1 Osmosensors of HOG pathway

Adaptation to high osmolarity is an elaborate physiological response. In an organism, to elicit such a response, it depends on cell's ability to monitor fluctuations through osmosensor in external osmolarity and initiation of HOG pathway activation for osmoadaptation. In HOG pathway each upstream branch is regulated by a different osmosensor. In the SLN1 branch, a transmembrane protein Sln1p serves as an osmosensor. In other branch, Sho1p appears to be involved in osmosensing.

1.2.1.1 Sln1p

It is a histidine kinase closely resembles the prokaryotic two-component system composed of Sln1p, Ypd1p and SSk1p. Sln1p is a protein of 1,220 amino acids. It is composed of a sensor molecule and a response regulator molecule. It has four distinct regions, N-terminal region with two transmembrane domains separated by a loop, linker region, histidine kinase domain and a receiver domain. The architecture of Sln1p is unorthodox as it contains both a histidine kinase domain and the receiver domain within the same molecule hence Sln1p is in itself a complete two component system. Sln1p is the only sensor histidine kinase in S. cerevisiae, so far no histidine kinase has been reported in animals (Stock et al., 2000). It shows structural similarity to EnvZ osmosensor of Escherichia coli except that EnvZ lacks the C-terminal receiver domain (Pratt and Silhavy, 1995). Sln1p catalyzes the transfer of phosphate from ATP to histidine residue in histidine kinase domain which is then transferred to the conserved asparagine residue in the receiver domain of another Sln1p. Recent evidences suggest
that Sln1p monitor turgor pressure to sense changes in osmolarity of the external media. Exposure to high osmolarity results in loss of turgor pressure which inactivates Sln1p histidine kinase activity, followed by induction of HOG1 MAPK pathway (Reiser et al., 2003). This type of histidine kinase is also present in other fungi such as Candida albicans has three, and one each have been reported for Aspergillus nidulans and Aspergillus fumigatus (reviewed by Hohmann, 2002).

1.2.1.2 Sho1p

It is a protein of 367 amino acids. It consists of four transmembrane domains at N-terminus, linker domain and SH3 domain (Posas and Saito, 1997; Pretorius, 2000). It is not clear at present how Sho1p could be functioning as an osmosensor. It has been shown that membrane targeting of cytoplasmic domain of Sho1p either through the transmembrane segments or by myristoylation is necessary and sufficient for its role in osmotic stress. Sho1p interacts with Pbs2p through its SH3 domain and pull it to the cell surface. It was found that upon targeting Pbs2 directly to the plasma membrane, Hog1 phosphorylation is induced upon hyperosmotic shock without any binding to Sho1p (Raitt et al., 2000). Thus it appears that Sho1p may not be functioning as true osmosensor rather acting as facilitator of signaling modular assembly in this branch. Sho1p is localized to the bud in G1-S phase cells and was particulary enriched in the plasma membrane of the emerging bud, mating projections and also to the vacuoles (Raitt et al., 2000). Sho1p participates in both HOG pathway and Pseudohyphal pathway. Functional homologs of Sho1p have been isolated from the yeasts Candida utilis and Kluyveromyces lactis by complementation of the S. cerevisiae sho1 mutant (Siderus and Mager, 1997).

1.2.2 MAPK kinase kinases of HOG pathway

The first kinase of the three-component activation module is a MAPK kinase kinase (MKKK). Specific MKKK are known to be activated either by phosphorylation by a MAPK kinase kinase kinase (MKKKK) or by interaction with a small GTP-binding
protein of the Ras or Rho family. Other potential modes of activation include oligomerization and subcellular localization (Siow et al., 1997). It was found that usually in a cell MKKKs out number MAP Kinases. MKKK are able to mix and match with different MKK-MAPK combinations and are responsible for cross talk between pathways. The large number of MKKK allows for diversity of inputs from numerous stimuli to feed into specific MAPK pathways. In *S. cerevisiae* three MAPKKS i.e. Ste11p, Ssk2p and Ssk22p participates in this pathway.

1.2.2.1 MAPK Kinase Kinases Ssk2, Ssk22

Ssk2p and Ssk22p are the redundant MEKK of SLN1 branch. Upon osmotic shock Ssk2p is activated by Ssk1p. Two hybrid analysis and coimmunoprecipitation demonstrated that Ssk1p activation takes place in two steps, binding of Ssk1p to the N-terminal, regulatory domain of Ssk2p and autophosphorylation of Ssk2p (Maeda et al., 1995; Posas and Saito, 1998). In vitro study showed that the MAPKK Pbs2p was a direct substrate for phosphorylated Ssk2p and this confirmed the place of Ssk2p upstream of Pbs2p. Ssk2p, Ssk22p (MAPKKs) sequence comparison revealed that both are similar with (69 %) identity at C-terminal ends and (47 %) at their N-terminus (Posas et al., 1998). Ssk2p, Ssk22p are activated only in response to osmolarity stress. This specificity is provided by the presence of docking domain at the N-terminus of Pbs2p (Tatebayashi et al., 2003).

1.2.2.2 MAPK Kinase Kinase Ste11

MEKK Ste11p is a signaling device that participates in three functionally distinct MAPK cascades in yeast. Ste11p was identified as a third MEKK of HOG pathway (Posas and Saito, 1997). Ste11p is required for *in vivo* hyperosmotic stress induced phosphorylation of Pbs2p in a strain lacking the MEKKS Ssk2p and Ssk22p. Expression of Ste11p, lacking its N-terminus (Ste11pΔN) is hyperactive and leads to cell death. Deletion of either of downstream Pbs2p of HOG pathway or Ste7p of filamentation-
invasion pathway does not suppress the lethality of Ste11pΔN, whereas simultaneous deletion of both MEKs, Ste7p and Pbs2p, completely suppresses the lethality (Posas and Saito, 1997). The ability of Ste11p to function in separate pathways require stable interactions with pathway-specific proteins. In pheromone response pathway it interacts with pathway specific scaffold protein Ste5p. In HOG pathway MAPKK Pbs2p serves as a scaffold protein interacting with Ste11p (Posas and Saito, 1997) and Sho1p (Maeda et al., 1995).

1.2.3 MAPK Kinase Pbs2p

In HOG pathway, the MAPKK is encoded by PBS2 gene. It was originally isolated in a screen designed to isolate polymyxin B resistance genes and hence its name PBS2 (Polymyxin B Sensitivity). Subsequently it was isolated as a component of osmoregulatory pathway in S. cerevisiae. It was found that strains with HOG1 and PBS2 deletion make less glycerol and were unable to grow on high osmolarity (Brewester et al., 1993). The PBS2 gene is located on chromosome X and code for a protein of 710 amino acids. Gene disruption experiments and Southern blot analysis proved single copy existence in the yeast genome and is not essential for viability under normal conditions (Boguslawski, 1992; Boguslawski and Polazzi, 1987). MAPK Kinases are the dual specificity kinases which when activated (Ser514 and Thr518 in case of Pbs2) by MKKKs in turn phosphorylate a Thr-X-Tyr motif in the activation loop of MAPK (Gartner et al., 1992). MAPKKs are least numerous in the MAPK module, they are the point of convergence of multiple MAPKKKs but should maintain high specificity for MAPKs to generate an appropriate output signal (Dhanasekaran and Reddy, 1998). Pbs2p integrates signal from two different osmosensors. It is phosphorylated by three MAPKKKs Ssk2p/Ssk22p and Ste11p and its auto-phosphorylation requires Lys389. Thus, Pbs2p plays a very complex role as it receives signals from both the upstream branches and allows the HOG pathway to operate over a wide range of sensitivity.
Pbs2p also acts as a scaffold protein, interacting with Ste11p, Sho1p and Hog1p. Pbs2p is distinctive from other scaffolds as it simultaneously acts as a MAPKK of the pathway and also as a scaffold protein. It interacts with various components of SHO1 pathway and also with MAPKKK of alternate pathway. Specialized domains with in Pbs2p can serve to facilitate productive interactions between upstream and down stream components of the cascade. Pbs2p was identified as relevant target for Sho1p interaction by studying mutant with Ser-Pro substitution at position 96 in pro-rich sequence (KPLPPLPVA). Additionally two-hybrid analysis concluded that SH3 domain of Sho1p binds to the Pbs2p proline rich motif (Maeda et al., 1995) and Pbs2p anchors to Ste11 and prevents undue activation of Ste7p in highly osmolar medium (Posas and Saito, 1997).

In addition to the role of PBS2 in osmoregulation, studies have suggested that PBS2 is involved in diverse physiological processes. As indicated earlier this gene was first isolated in a screen targeted to identify genes having role in polymyxin B sensitivity. Polymyxin B is a complex antibiotic that destroys the cell membrane structure (Storm et al., 1977). Overexpression of this gene provides Polymyxin B resistance, whereas its deletion results in polymyxin B sensitivity (Boguslawski, 1992; Boguslawski and Polazzi, 1987). Thus it appears that PBS2 helps in maintaining the integrity of cell membrane. Recently a unique pbs2 mutant allele was isolated that was capable of conferring resistance to calcofluor but sustained full growth at 1.4 M NaCl, (Garcia Rodriguez et al., 2000). It was suggested that Hog1p dependant osmo-adaptation and calcofluor resistance are two physiologically different phenomenon. It was demonstrated that in these mutants there was no Hog1 phosphorylation in response to calcofluor and the glycerol accumulation due to calcofluor treatment was significantly different from that observed after salt treatment. PBS2 gene along with PTC1 was also implicated in cell wall assembly as both were involved in regulation of cell wall β1,6 glucan assembly (Jiang et al., 1995; Lai et al., 1997).
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PBS2 also plays an important role in regulation of carnitine uptake in *S. cerevisiae*. Carnitine is a small and highly polar zwitterionic compound that carries long chain fatty acids across the mitochondrial membrane. *AGP2* is a carnitine transporter which is essential for carnitine uptake from the medium into the cell. PBS2 when constitutively expressed was found to inhibit carnitine uptake and reduced the level of *AGP2* mRNA expression (Van Roermund *et al.*, 1999; Lee *et al.*, 2002). PBS2 was also found to participate in copper resistance of the *las21* mutants. Las21 contains a heavy metal associated motif at its N-terminus (Toh-e and Oguchi, 2001).

1.2.4 MAPK Hoglp

MAPKs are the final kinase in the MAPK cascade and phosphorylates substrates on serine and threonine residues. All of the MAPK family members elicit cellular response by activating or repressing specific transcription factors allowing the expression of the required set of genes (Reviewed by Widmann *et al.*, 1999). The function of MAP kinases is regulated at multiple levels: phosphorylation by activating kinases, nuclear transport and retention, and interaction with regulatory proteins and substrates.

*HOGL* is a MAPK of High osmolarity glycerol pathway. *HOGL* was isolated along with *PBS2* as a osmosensitive mutant (Brewester *et al.*, 1993). *HOGL* and *PBS2* deleted cells are unable to grow in the medium containing 0.5 M NaCl or equivalent. These mutants acquire an unusual morphology mating projections or pseudohyphae (Brewester *et al.*, 1993; Brewester and Gustin, 1994) as a result of inappropriate activation of pheromone response pathway and pseudohyphal development pathway. These mutants also accumulate lower level of glycerol as compared to wild type under osmotic stress (Albertyn *et al.*, 1994; Brewester *et al.*, 1993). Since *HOGL* behaved in the same way as *PBS2* they both were confined to the same pathway. It was confirmed by *PBS2* dependant phosphorylation of *HOGL* on Thr174 and Tyr176 that Hoglp is a downstream substrate for Pbs2p (Brewester *et al.*, 1993). Since then Hoglp phosphorylation is used as a sensitive measure for HOG pathway activation. Recently, it
was reported that upon phosphorylation by Pbs2p, Hog1p translocates into the nucleus (Ferrigno et al., 1998; Gorner et al., 1998; Reiser et al., 1999). Nuclear Hog1p mediates regulation, both repression and activation of gene expression for osmoadaptation (Reiser et al., 1999). Low level of Hog1p activation was also found in response to heat stress. Heat stress activation does not result in nuclear accumulation of Hog1p and consequently does not alter gene expression (Winkler et al., 2002).

1.3 Transduction of signals through two branches of HOG pathway

Presence of two branches suggested redundant functions of the branches. Later observations suggested that two branches possess different sensitivities and thus allow the cell to respond over a wide range of osmolarity changes (Maeda et al., 1995; O’Rourke and Herskowitz, 2004).

1.3.1 SHO1 branch

In signal transduction via this route a series of upstream components are involved. Activation of Hog1 by this branch involves recruitment of osmo signaling complex to the plasma membrane specifically at the places of growth (Raitt et al., 2000; Van Drogen et al., 2002). It was found that small GTPase, Cdc42p is located to the places where new cell wall material is deposited (reviewed by Chant, 1999; Erickson and Cerione, 2001; Johnson, 1999). Cdc42p binds to Ste20p which phosphorylates Ste11p and leads to the release of the inhibitory N-terminal domain of Ste11p from its catalytic C-terminal domain (Van Drogen et al., 2001; Raitt et al., 2000). Protein interaction studies in agreement with two-hybrid analysis showed that Ste50p, Sho1p, Ste11p and Pbs2p are also components of this complex (Raitt et al., 2000; Reiser et al., 2000). Ste50p binds with Ste11p in the N-terminal domain but not with Pbs2p or Sho1p. The Ste50p-Ste11p interaction is constitutive and is not affected by environmental conditions (O’Rourke and Herskowitz, 1998; Posas et al., 1998).
From all these studies it was evident that signaling complex consists of Cdc42p, Ste50p, Ste20p, Ste11p and Pbs2p, whereas Hog1p is not a part of this complex. The successful execution of signaling program, i.e. phosphorylation and activation of Pbs2p leads to dissociation of the complex. Activated Pbs2p results in phosphorylation of Hog1p (Maeda et al., 1995).

1.3.2 SLN1 branch

This branch of HOG pathway contains a phosphorelay system of three components: Sln1p, Ypd1p, and Ssk1p (Posas et al., 1996), that regulates the activation of two MEKKs Ssk2p and Ssk22p (Maeda et al., 1995). The Sln1p histidine kinase is activated by hypo-osmolarity (cell swelling) and inhibited by hyperosmolarity (cell shrinking). Upon exposure of cells to low osmolarity, Sln1p constantly autophosphorylates itself on His576. This phosphate is then transferred to Asp144, within the receiver domain of Sln1p of the same molecule or between different molecule. Subsequently, the phosphate group is transferred to His 64 on Ypd1p and further to Asp554 on Ssk1p (Janiak-Spens et al., 2000). This phosphorylation of Ssk1p is predicted to inhibit its ability to activate the MEKKs, Ssk2p and Ssk22p. Upon osmotic shock, Ssk2p becomes phosphorylated and it was established that under hyperosmotic stress the inactive Sln1 histidine kinase allows the unphosphorylated Ssk1p to modulate the autophosphorylation reaction of Ssk2p (Deak et al., 1997). Autophosphorylation and activation of Ssk2p in the unstimulated cells appear to be blocked by inhibitory domain at N-terminus. The Ssk1p binds close to this N-terminal, regulatory domain of Ssk2p and relieves the intramolecular inhibition of Ssk2p kinase activity. The MAPKK Pbs2p has been shown to be a direct substrate for phosphorylated Ssk2p in vitro (Posas et al., 1998). Sln1p and Ypd1p also functions as negative regulators of HOG pathway as their deletion is lethal to the cells due to overactivation of HOG pathway (Maeda et al., 1994). Overexpression of either SSK2ΔN or SSK22ΔN is also lethal to host cells and this lethality was suppressed by deletion mutations in either the PBS2 or HOG1 (Maeda et al.,
1995). Thus this branch in its own way is quite distinct from SHO1 branch. Studies suggest that Slnlp is more sensitive than the sensor of the SHO1 branch. In Δsho1Δssk22 mutant, Hoglp phosphorylation is apparent with even 100 mM NaCl (Maeda et al., 1995). Subcellular localization of components has not been reported and it was speculated that the Sho1 branch mainly monitors osmotic changes during cell growth and expansion, while the Slnlp mainly senses osmotic changes in the environment (Hohmann, 2002).

1.4 Targets of HOG pathway

In *S. cerevisiae*, an increase in external osmolarity induces expression of many genes. Genes activated in response to Hoglp activation includes: glycerol-3-phosphate dehydrogenase (*GPD1*), aldehyde dehydrogenase gene (*ALD2*), catalase (*CTT1*), and a small heat shock protein Hsp12p. Few of these genes are also activated by other stresses such as heat stress and have a DNA sequence element called as STRE in their promoter. The transcription factors mediating the expression of stress response genes have also been characterized. A pair of factors Msn2p and Msn4p that bind to stress response elements were identified. They are considered to mediate general stress response and are activated by phosphorylation upon stress by Protein kinase A. But it was found that Hoglp can target them as well (Martinez-Pastor et al., 1996). Two other factors, Hotlp and Msnlp appear to be specifically activated during osmotic stress (Rep et al., 1999). Hotlp affected glycerol biosynthesis genes are *GPD1* and *GPP2*. *STL1* is also a Hotlp target, which appeared in global expression analysis as the strongly osmostress induced gene (Gasch et al., 2000; Causton, 2001). Hotlp is a nuclear protein and Hoglp and Hotlp forms complex with *GPD1*, *STL1*, *HSP12*, and *CTT1* promoters and it follows kinetics of Hoglp phosphorylation and Hoglp nuclear accumulation. Msnlp is a closest homolog of Hotlp. Msnlp is also known to effect the expression of Gpdlp, Gpp2p, Cttlp and Stllp (Rep et al., 1999).
Although Hotlp as well Msnlp factors mediate the increased expression of target genes, derepression mechanisms are also involved. Skolp is a transcriptional repressor protein that belongs to family of bZIP transcription factors containing leucine zipper for dimerization and binds to cAMP response element (CRE)-like sequence on the promoter (De Cesare and Sassone-Corsi, 2000). Upon osmotic stress, activated Hoglp MAPK binds to Skol and results in inactivation of Skolp repressor by rapid phosphorylation and disruption of its association with Ssn6 and Tuplp proteins. Ssn6p and Tuplp are general co-repressors which form a complex with sequence specific proteins such as Skolp/Acr1p and represses expression of target genes under normal growth conditions (Proft and Serrano, 2001). Skolp/Acr1p represses ENAI, GRE2 and HALI expression under normal growth conditions and is activated upon hyperosmotic challenge in Hoglp dependant manner. These genes are known to play an important role in maintaining ion homeostasis that confers salt tolerance when overexpressed in yeast cells (Proft and Serrano, 2001).

Besides nuclear targets, Hoglp also activate cytoplasmic proteins. Recently, Rck2p a protein kinase was identified as cytoplasmic substrate of Hoglp in two-hybrid screening (Bisland-Marchesan et al., 2000). Hoglp phosphorylation resulted in Rck2p activation. It was found that overexpression of Rck2p could suppress the osmosensitive phenotype of HOG pathway mutants. Thus, Rck2p controls subset of responses induced by the MAPK upon osmotic stress.

1.5 Negative regulation of the HOG pathway

Constitutive activation of HOG pathway is lethal even under hyperosmolarity (Jacoby et al., 1997; Maeda et al., 1994) thus down regulation of HOG pathway is a vital function of cell. Regulation of MAPKs by phosphatases play a key role e.g. mutation of Drosophila puckered gene encoding a protein tyrosine phosphatase which inactivates C-jun N-terminal kinase results in defects in dorsal closure during morphogenesis (Martin
Blanco et al., 1998). There are three types of phosphatases that inactivate MAPKs in yeast and in vertebrates. Dual-specificity phosphatases are capable of dephosphorylating both Phospho-Thr and Phospho-Tyr; PTPs are specific for Phospho Tyr phosphatases and PP2Cs, are Ser-Thr phosphatases. Two PTPs, Ptp2 and Ptp3 inactivate Hog1p. Ptp2 may have a greater effect on Hog1p as compared to Ptp3 (Jacoby et al., 1997). This could be attributed to the fact that Ptp2 is nuclear and so is activated Hog1p, while Ptp3 is located both in the cytoplasm and the nucleus. HOG pathway is required for the induction of Ptp2 and Ptp3 transcripts in response to osmotic stress, indicating that activation of the HOG pathway triggers a negative feedback loop to inactivate the pathway. In addition to dephosphorylating Hog1p the PTPs modulates Hog1p subcellular localization by binding Hog1p (Mattison and Ota., 2000). PTPs, Ptp2 and Ptp3 can also prevent inappropriate activation of Hog1p. It was shown by Winkler et al. (2002) that hyperactive Mkk1-386 (MAPKK of cell integrity pathway) activates Hog1 only in the absence of ptp2Δ ptp3Δ.

Apart from PTPs, activated Hog1p is also regulated by PP2C family of phosphatases (dephosphorylating threonine residue in Hog1p) such as Ptc1, Ptc2 and Ptc3 (Mapes et al., 2002). PTC1 was identified as a gene whose deletion along with ptp2 but not ptp1 caused synthetic growth defect to the cell (Maeda et al., 1993). Ptc1 is evenly distributed both in the nucleus and in the cytoplasm irrespective of the osmotic stress. These phosphatases can also phosphorylate and inhibit upstream components of HOG pathway e.g. SLNI deletion is suppressed by overexpression of PTC1 or PTC3 (Maeda et al., 1995).

1.6 Intracellular localization of HOG pathway components

One of the important questions in signal transduction pathway is that how the osmosensor recruited at the cell surface can result in transcriptional activation of stress responsive genes in the nucleus. The spatial organization of components within the cell is an important determinant of relay of signal. Subcellular localization of HOG pathway
components has been analyzed recently by GFP-fusion constructs. These experiments indicated that Ste11p and Pbs2p are localized invariably in the cytoplasm of both unstressed cells and stressed cells (Ferrigno et al., 1998). However, polar localization of Pbs2p was evident in stress induced mutant form of Pbs2p (K389M). Pbs2p (K389M) was transiently (5-15 min) localized either at the growing bud tip or at the mother-bud neck region in Sho1p dependant manner which also involves Ste20p and Ste50p (Raitt et al., 2000).

The localization of Hog1p MAPK is remarkably controlled by stress and phosphorylation. In unstressed cells Hog1p is distributed throughout the cytoplasm. Upon exposure to salt stress Hog1p is redistributed to the nucleus, which is dependant on Pbs1p mediated Hog1p phosphorylation of Thr 174 and Tyr 176. Nuclear import of Hog1p is mediated through some proteins such as a small GTP-binding protein Ran-Gsp1 and nuclear import receptor NMD5. It was shown that cells with Ran-GSP1 deletion were unable to translocate Hog1p into the nucleus. As Hog1p is transiently localized into the nucleus therefore export mechanism also exists. It was found that Hog1p exclusion is mediated through importin β homolog, XPO1-CRM1 (Ferrigno et al.; 1998).

The localization of Hog1p is also modulated by protein-protein interactions. The nuclear retention of Hog1p depends upon the nuclear substrates acting downstream of Hog1p. The zinc finger transcription factors Msn2p and Msn4p tether Hog1p with in the nucleus (Reiser et al., 1999). In contrast, the period of nuclear accumulation was shortened in the msn2, msn4 mutant and in hotl, msn1 mutants, indicating that interaction of these transcription factors with Hog1p could be an important factor that determines the duration of nuclear residence of Hog1p (Rep et al., 1999). Further the tyrosine phosphatases that dephosphorylate Hog1p, Ptp2p and Ptp3p also regulate Hog1p localization (Mattison and Ota, 2000).

Besides Hog1p, subcellular redistribution of transcriptional regulatory proteins that control osmotic stress-responsive gene expression has also been observed. The Sko1p repressor is usually nuclear but under high osmolarity it is excluded to the
cytoplasm (Pascual-Ahuir et al., 2001). Msn2p and Msn4p are cytoplasmic, they translocate to the nucleus to regulate gene expression under stress (Gomer et al., 1998). The transcription factor Hot1p is a nuclear protein and its distribution is not modulated by osmotic stress (Rep et al., 1999).

1.7 Maintenance of signal specificity in MAPK pathways

1.7.1 Role of Scaffolds

MAPK cascades have such an intricate cross links that unraveling the mechanisms behind the organization of correct repertoire of enzymes and substrate recognition has become an emerging issue of signal transduction pathways. Studies have indicated that formation of signaling complexes and presence of docking domains are important. Signaling complexes may result from the physical interaction between components of particular signaling pathways or by the assembly of signaling molecules on anchor, adaptor or scaffold proteins that localize their binding partners to specific subcellular compartments or to their substrates (Fig. 1.3). In mammals JIP1 was identified as a scaffold protein that interacts with multiple components of the JNK signaling pathways. (Whitmarsh, 1998). MPl is another member of MAPK scaffold complex in mammals. The MPl scaffold complex appears to facilitate activation of ERK1 by MKK1 (Schaeffer et al., 1998).

1.7.1.1 Scaffold proteins in Yeast

Ste5p of pheromone response pathway in S. cerevisiae was the first scaffold protein discovered. In HOG pathway, the MAPKK Pbs2p has been shown to function as scaffold. Thus it appears that both accessory molecules and components of MAPK cascade can serve as scaffolds. Ste5p selectively associates with MKKK Ste11p; the M KK Ste7p, and the MAP kinases Fus3p or Kss1p and apparently functions by facilitating the formation of an oligomeric protein complexes (Choi et al., 1994; Marcus
Fig. 1.3 MAPK scaffold complexes

(A) In the pheromone-mating pathway in S. cerevisiae, scaffold protein Ste5p coordinates the MAPK cascade. (B) Pbs2p acts as a scaffold in the High Osmolarity Glycerol response pathway in yeast. The MAPKK protein interacts with the transducer Sho1p, Ste11p and the MAPK Hog1p and coordinates the components of an osmoregulatory MAPK signaling module. (C) JIP1 was identified as a scaffold protein for stress regulated JNK pathway in mammals. Adopted from a review by Whitmarsh and Davis, 1998 with few modifications.
et al., 1994; Printen and Sprague Jr, 1994). Ste5p increases the specificity of the kinase cascade by tethering components in close proximity and blocking inappropriate interactions with other pathways such as it does not allow Ste11p to activate Hog1p in response to pheromones. It is also responsible for the preferential channeling of mating signals into Fus3p and of invasive signals into Kss1p in the filamentation pathway.

Pbs2p has a scaffold role in SHO1 branch of HOG pathway and associates with Pbs2p activator Ste11p, MAPK Hog1p and a putative osmosensor Sho1p (Posas and Saito, 1997). The genetic analysis of mutation in proline rich region of Pbs2p (binding motif for SH3 domain) and interaction studies (two hybrid and co-precipitation assays) revealed that SH3 domain of Sho1p interact with Pbs2p (Maeda et al., 1995; Reiser et al., 2000). Ste11p is a signaling device that is used in three functionally distinct MAPK cascades therefore to maintain specificity of Ste11p activation in different pathways requires stable associations with pathway specific proteins. Scaffold proteins also helps to bring together proteins that do not interact or interact very weakly. For example Ste11p shows no or very week interaction with the MEK Ste7p in the two-hybrid system (Choi et al., 1994; Marcus et al., 1994) and Ste5p binds both these proteins. In the same way interaction between Sho1p and Ste11p is undetectable (Posas and Saito, 1997) and Pbs2p binds both proteins (Maeda et al., 1995; Posas and Saito, 1998). Besides increasing the efficiency of signal propagation these scaffold proteins also serve as an adaptor for kinase cascade activation by linking kinases to receptor sensor molecules e.g. Ste5p links Ste11p to Gβγ, Pbs2p links Ste11p to Sho1p for activation of respective pathways. Thus, these interactions between sensors and scaffold can be important in determining which kinase cascade will be activated by specific stimulus (Harris et al., 2001).

1.7.2 Role of Docking domains

Recently it became evident that MAPKs utilize docking domains to facilitate specific interactions with their activators, inhibitors and substrates (Sharrocks et al., 2000; Tanoue et al., 2000; Enslen and Davis, 2001). These docking domains recruit the
kinases to the correct substrates and enhance their fidelity and efficiency of action. The docking interaction is different from transient enzyme substrate interaction through the active center. It was indicated that MAPK docking site of MEKs has been structurally and functionally conserved from yeast to humans. In *S. cerevisiae* the region responsible for Ste7p-Fus3p interaction was localized to the N-terminal non-catalytic domain of Ste7p (Bardwell *et al.*, 2001). Although noncatalytic domains are less conserved as compared to catalytic domains but the docking motif found in Ste7p was also found in mammalian MAPKKs (Bardwell and Thorner, 1996). Characteristic feature of MAPK docking site motif are a cluster of at least two basic residues (Lys, Arg and rarely His) separated by a spacer of 2-6 residues from a hydrophobic-X-hydrophobic sequence where the hydrophobic residues are long chain aliphatics (Leu, Ileu, sometimes Val). Furthermore, site-directed mutagenesis and interaction studies demonstrated that both basic and hydrophobic residues are involved in specific recognition, binding and phosphorylation of their specific MAPKs (Bardwell *et al.*, 2001; Enslen *et al.*, 2000; Tanoue *et al.*, 2000; Xu *et al.*, 2001). As docking domains are positively charged clusters so it was assumed that their substrates must be composed of a cluster of negatively charged amino acids. In a family of mammalian MAPKs-ERK, p38 and JNK, conserved domains located at the C-terminal portion were identified, those were used for docking of their activators. Surprisingly these domains were also utilized by MAPK phosphatases and their substrates (MAPKAPKs).

Thus, docking domain interactions involves charged amino acids and increase the efficiency and specificity towards their substrates. Apart from this they underlie the molecular basis for the sequential and specific activation and inactivation of MAPKs. Further, docking domain interactions also function to determine the subcellular localization of MAPKs and MAPK interacting proteins e.g. the N-terminal portion of MEK1 is the ERK docking domain, and MEK1 can function as a cytoplasmic anchor for ERK2 (Fukuda *et al.*, 1997). These docking domains are also viewed as potential targets for drug development (Bardwell *et al.*, 2001; Tanoue *et al.*, 2000; Duesbery *et al.*, 1998)
1.8 Osmoadaptation mechanisms in different yeast species

MAPK pathways regulating osmoadaptation have been studied in other species of yeast. In fission yeast, \textit{S. pombe} Sty1 MAPK pathway regulates the osmotic stress. Studies in this pathway have led to some important findings, which clearly distinguishes it from the HOG pathway of \textit{S. cerevisiae}. Sty pathway is also activated by oxidative and heat shock stresses (Degols \textit{et al.}, 1996; Samejima \textit{et al.}, 1997), nutrient limitation, UV light and anisomycin (a protein synthesis inhibitor) (Shiozaki and Russel, 1996; Shiozaki \textit{et al} 1997). Sty1 pathway consists of the MAPK sty1, which is activated by phosphorylation on the Thr171 and Tyr173 residues by the MAPKK Wist (Warbrick and Fantes 1991; Millar \textit{et al.}, 1995; Shiozaki and Russel 1995). Wist obtains signal from redundant MAPKKKs Wis4 (also called as Wak1) and Win1 (Shieh \textit{et al.}, 1997a) which is in turn activated by Mcs4 which has amino acid sequence similarity to Ssk1 (Shieh \textit{et al.}, 1997a). Two sensors histidine kinases (Mak2 and Mak3) are known to monitor oxidative stress (Buck \textit{et al.}, 2001) but osmosensor for this pathway is not known. Sty1 is 82% identical to Hog1p. Cells lacking either Sty1 or Wis1 are considerably elongated at cell division (Warbrick and Fantes 1991; Millar \textit{et al.}, 1992, 1995). Sty1 pathway also links stress signaling with control of sexual differentiation (Yamamoto, 1996). Hence, the Sty1 pathway not only regulates stress response but also integrates this response with two processes fundamental to all eukaryotes; control of mitosis and initiation of meiosis. Transcriptional response of this pathway is mediated by three transcription factors: Atf1 (Takeda \textit{et al.}, 1995), a bZIP factor closely related to mammalian ATF2; Pap1 (Toda \textit{et al.}, 1992), which is closely related to budding yeast Yap1p; and Prr1p (Ohmiya \textit{et al.}, 1999) a response regulator transcription factor related to budding.

Studies in pathogenic yeast \textit{Candida albicans} revealed the presence of functional homologues (\textit{SLNJ}, \textit{YPDI}, \textit{SSK1} and \textit{HOG1}) of HOG pathway in the organism. The \textit{C. albicans hog1} mutant displays sensitivity to high osmolarity and diminished glycerol accumulation, as well as morphological alterations, cell wall defects, and diminished
virulence (Alonso-Monge et al., 1999). SHO1 homologs have also been reported from *Candida utilis* and *Kluyveromyces lactis* (Siderius et al., 2000). Furthermore MAPK homologues of *HOG1* have been isolated from the osmotolerant yeast *Zygosaccharomyces rouxii* (Iwaki et al., 1999), *Horatea werneckii* (Turk and Plemenitas, 2002) and *D. hansenii* (Bansal and Mondal, 2000).

### 1.9 *Debaryomyces hansenii*- a xerotolerant, halotolerant yeast

Yeast are ubiquitous unicellular fungi and amongst them those growing at reduced water activity are also wide spread. The natural habitat of xerotolerant yeasts include floral nectaries and are commonly associated with bees and honey. Here they are exposed to a highly variable environment with respect to the availability and quality of nutrients, temperature, pH, radiation, access to oxygen and especially water activity (Hohmann, 1997). They are of commercial importance in food industry since they cause spoilage of wine, syrups and conserves, fruit juices, dessert wines, dried fruits, molasses and malt extract. They are also used in the preparation of various fermented foods including soy sauce and misopaste. The most prevalent yeasts in these natural habitats belong to the genera of *Zygosaccharomyces, Debaryomyces, Hansenula, Pichia* and *Torulopsis* (Blomberg and Adler, 1992). The physiological basis of high osmotolerance in these species is not clearly known although several studies have been made primarily in *D. hansenii* and *Z. rouxii*. *D. hansenii* was originally isolated from marine environments and is considered to be a highly osmotolerant and halotolerant yeast. It has been found that it can grow in medium containing upto 4.0 M NaCl, whereas, *S. cerevisiae*, the most extensively studied yeast, is unable to grow in the medium having NaCl beyond 1.7 M.

The ability of xerotolerant yeasts to grow in high solute concentrations also appears to be based primarily on the intracellular accumulation of compatible solute. The intracellular levels of polyols in *D. hansenii* are markedly enhanced by high salinity, the
dominant solute being glycerol in the log phase and arabitol in the stationary-phase cells (Adler and Gustafsson, 1980). One interesting feature has been observed in *D. hansenii* that these strains do not differ much from *S. cerevisiae* in their ability to synthesize glycerol under osmotic stress but these are capable of retaining more glycerol under osmotic stress compared to *S. cerevisiae* (Edgley and Brown 1983; Brown 1978). Regulation of glycerol permeability of the membrane is important, so that the metabolic energy expanded in the synthesis of glycerol is not wasted by the leakage from the cell.

Another important distinction between *D. hansenii* and *S. cerevisiae* is in ion homeostasis. In *D. hansenii* Na⁺ is excluded and K⁺ is accumulated, so that the internal K⁺:Na⁺ ratio is much higher than that of the media. In this respect, *D. hansenii* has been found more efficient than *S. cerevisiae* (Norkrans and Kylin, 1969). It appears that high osmotolerance and halotolerance in *D. hansenii* results from the interplay of several physiological traits that has been acquired through evolution. Therefore, study of this species of yeast can provide better understanding of the molecular mechanism of the osmotolerance and halotolerance.

**SCOPE OF THE PRESENT STUDY**

Maintenance of water homeostasis is pivotal for survival and growth of all forms of life. Being a free-living, single cell eukaryote, yeast cells are exposed to sudden changes in the osmolarity of their surrounding environment. These tiny eukaryotes have been evolved with complex molecular mechanisms to overcome such fluctuations in their environments. In recent past, osmoadaptation in yeast has drawn considerable interest among the researchers as their aspirations to understand the mechanism of stress tolerance in simpler system and to apply these knowledge to higher eukaryotic organism have been intense. Exposure of yeast cells to high osmolar medium evoke a multitude of physiological responses, which renders cells to overcome the stress and adapt to the reduced water activity of their environment and the consequent decrease in turgor.
pressure. Enhanced synthesis and/or accumulation of glycerol and to some extent other polyols as compatible solutes have been observed to be the major feature of yeast osmoregulation. This creates an intracellular environment that is conducive for enzyme function and growth under water stress.

In *S. cerevisiae*, a signal transduction pathway involving members of mitogen activated protein kinase (MAPK) family have been shown to be responsible for adaptation to high osmolarity. This pathway is called high-osmolarity glycerol (HOG) response pathway. The MAP kinase of this pathway is Hog1p, which is activated by a MAP kinase kinase Pbs2p. It holds an essential position in osmoadaptation of the yeast *S. cerevisiae* as it integrates multiple upstream signals. Besides functioning as a MAPKKK, Pbs2p also acts as a scaffold protein in this pathway. HOG pathway seems to be a prototype of stress activated protein kinase pathway and is closely related to mammalian p38 pathway that is activated by different stress conditions including osmotic stress. Thus, it appeared that many principles of osmoadaptation are conserved among eukaryotes. Since the discovery of HOG pathway, it has received tremendous scientific interest. Most of our understanding relating to this pathway is primarily from two model yeast *S. cerevisiae* and *S. pombe*. In comparison to these two yeast, *D. hansenii* displays remarkable halotolerance and osmotolerance as it can grow in presence of as high as 4 M NaCl. However very little is known about the osmosensing signal transduction pathway in this yeast. Identification and characterization of the components of this pathway is therefore an important step towards unraveling molecular mechanism of high osmotolerance exhibited by this yeast.

Thus, the present study is undertaken with following objectives:

1. Molecular cloning of PBS2 homologue from *D. hansenii*
2. Nucleotide sequencing and characterization of the gene
3. Phenotypic complementation of pbs2 mutation by the cloned gene
4. Structure function analysis of PBS2 homologue
   a). To elucidate the role of C-terminus

5. Dynamics of HOG pathway activation in *D. hansenii*