AIM AND SCOPE

The molecular basis of gene expression, whereby organisms develop inherited characteristics, is of great importance in understanding the regulatory circuits in biological systems. In prokaryotes, sufficient information is available to explain the expression of genes\(^1\). But in eukaryotes, the fine mechanism of control is yet to be unfolded. It is not possible to extend the knowledge of prokaryotes to unravel the organization and functional aspects of eukaryotic genes, because components of nuclear material are much different and complicated in the latter case.

It is difficult to have an insight of the phenomenon at the molecular level using higher organisms as test material. The complexities in the higher organisms due to larger genome does not permit such a probe. However, yeast, a lower eukaryote, provides an appropriate experimental system. Since, it has a simple physiology and comparatively smaller genome, genetic analysis can be applied critically. In our study, we have chosen \textit{Candida albicans}, a pathogenic yeast, which causes \textit{Candidiasis} in man\(^2\). The molecular basis of this study will help us to understand the pathogenicity of this organism.
In our laboratory, we have reported that only pathogenic yeasts, such as Candida albicans can utilize N-acetylglucosamine as the sole carbon source. This yeast grows on the surface of mucous membranes which are rich in amino sugars. Systemic infections caused by this organism occur in kidney, heart, liver, lungs and spleen. It has been found to be a normal inhabitant of oral cavity, gastro-intestinal tract and vagina. Polyene antibiotics such as nystatin and amphotericin B are used against superficial infections. But due to the development of resistance towards the drug and undesirable side effects on mammalian tissues, these are not advisable to use. The aim of the present study is to understand the molecular basis of pathogenicity which will help in finding better therapeutic agents.

It has been found that a set of four enzymes are induced in Candida albicans in the presence of GlcNAc. These enzymes viz., permease, kinase, deacetylase and deaminase constitute the aminosugar catabolic pathway, which convert GlcNAc into fructose-6-phosphate. A study of the factors controlling enzyme induction in this yeast provides a system for elucidating the mechanism of transcriptional and translational control.
However, it is not clear whether there is any polarity effect in *Candida albicans*. Since *Candida albicans* is a lower eukaryote, the nature of mRNA is not certain. Like prokaryotes, it can be polycistronic or as in higher eukaryotes it can be monocistronic. Findings in our lab suggest that an intermediate type of message as reported in animal virus is sprobably present in this system.

*Candida albicans*, a dimorphic yeast can also serve as a model system to study the biochemical basis of morphogenesis. This yeast exists in two well defined forms: yeast and mycelium. A knowledge of the correlation between biochemical and morphological steps that occur during germination of the yeast form is essential for understanding the phenomenon. The intermediate morphological step in the process of germination involves germ tube formation. Although the process appears simple, it is evident that at the molecular level, complex biochemical changes accompany the sequence of morphological events. Since defect in germination occurs due to blockage in the biochemical pathway, study of defective strains would help to identify critical enzymes necessary for orderly progression of germination.
Germination in *Candida albicans* is particularly suitable for studying the regulation of gene expression. Developmentally regulated changes in both protein and mRNA synthesis occur during the transition from yeast form to germ tube stage⁹. One of the major problems is to determine the nature of the mechanisms that control the activation and expression of genes that are necessary for orderly progression of development. One way of studying the regulation of expression is to recognize those genes whose activations are regulated during the morphogenesis of this yeast.

A study of the mechanisms which turn on and off the synthesis of specific mRNA can best be accomplished with cloned genes. In addition, the sequence of developmentally regulated genes can be established to determine whether there are any structural features that can be related to their control. We decided to purify GlcNAc kinase, the first enzyme of the pathway to raise antibody against it. As a part of the programme, we plan to isolate inducible genes by screening *Candida albicans* genomic library using cDNA probes. These genes would be used to isolate mRNA which will be subsequently translated in *vitro*. Kinase specific antibody will be utilized to identify the translation products. Furthermore, mRNA at
different stages of germination can be used to work out the mechanism of morphogenesis at the molecular level. It can specifically be used for sequence determination of regulatory regions, and identification of regulatory molecules during germination.
Aminosugars are a class of compound that play an important role in the physiology of organisms. They are ubiquitous in nature. Their distribution ranges from the outer skeleton of prokaryotes to connective tissues of mammals. In a variety of natural macromolecules, major portion of the carbohydrate part is composed to aminosugars. Some antibiotics such as streptidine, desosamine, mycaminose, rhodasamine, erythromycin and carbamycin also have aminosugars as their components.

Some of the physiological properties of a molecule are determined by the part that contains aminosugars. For example, in case of mucin viscosity is determined by its glycan part. It is important in protecting and lubricating the mucous membranes of the digestive tract. Aminosugars have important role in determination of blood groups and other immunological properties of erythrocyte surface. It is possibly involved in membrane-membrane interactions also.

Several kinds of aminosugars are known to exist in the living organisms. The best known and possibly
The most important are N-acetylglucosamine, N-acetylmannosamine and N-acetylgalactosamine. They are derivatives of glucose, mannose and galactose respectively, the second carbon of which is aminocylated. As structural material, they occur in the outer shell of crustaceans, scale of insects, capsule of bacteria and different tissues of animals and plants. Chitin, colominic acid, hyaluronic acid, dermatan sulfate, heparin, teichoic acid and murein are some of the natural forms of aminosugars. In many other cases such as lipopolysaccharides and glycoproteins, aminosugars constitute only the carbohydrate part of the molecule. But some for instance chitin, a constituent of crustacean shell and scale of insects, are homopolymers of aminosugars. On the other hand, peptidoglycans, the building blocks of bacterial capsule are made of repeating aminosugar disaccharide. Many compounds such as heparin, hyaluronic acid and dermatan sulfate are found in microorganisms and connective tissues of animals and have aglycan units in addition to aminosugar-containing glycan part.

Secreted proteins are another group of aminosugar-containing compounds. There are a number of
evidences that these proteins and other glycoproteins have a carbohydrate core which is made of GlcNAc and mannose. Here, the protein and carbohydrate moieties are linked by an asparagine-GlcNAc linkage. But in mucoproteins, the linkage is between GlcNAc and serine.

The synthesis of all aminosugar containing compounds proceeds by repetitive addition of aminosugar units to a primer molecule. In most cases, lipid and UDP act as aminosugar carrier. In glycoprotein synthesis, oligosaccharide core is first assembled on a lipid, which then transfers it to a protein molecule. The function of the lipid carrier in these cases is to enable the hydrophilic oligosaccharide to traverse the endoplasmic reticular membrane. The participation of oligosaccharide-lipid in glycosylation of endogenous membrane protein of oviduct, matbeans and certain secretary proteins is firmly established. It is suggested that the elongation of saccharide can occur only while it is linked to the carrier lipid, and once it is transferred to protein, no further addition can occur. The transfer of carbohydrate residue from the lipid bound saccharide has also been observed in cell free systems. In the majority of other cases, UDP acts as a carrier. It transfers the sugar unit to a
primer in a stepwise fashion. In fact, it acts as carrier also in the formation of aminosugar-lipid complex. This has been demonstrated in the enzyme preparations from several animal and plant sources.

Aminosugars As the Source of Energy

GlcNAc and ManNAc besides being a part of structural molecules, also act as the source of energy in biological systems. The pathway for their utilization as energy source has been elucidated only in the recent past. Transport of GlcNAc inside the cell is essential before it can be utilized. This is done by a membrane bound permease system. Other enzymes that are involved in the process are kinase, deacetylase and deaminase. These result in sequential phosphorylation and removal of acetyl and amino group from the substrate. In addition to these four enzymes that are directly involved in the process, other enzymes such as epimerase and mutase are also present. They play an important role in the interconversion of GlcNAc, ManNAc and GalNAc. The pathway is represented in the Fig. 1.

The pathway is essentially the same in all organisms. However, there is a minor variation in the distribution of enzymes in different systems. GlcNAc
Fig. 1: AMINOSUGAR METABOLISM IN YEAST.
kinase is constitutive in E. coli. However, in animal systems all enzymes of the pathway are constitutive. We have shown in Candida albicans, a pathogenic yeast that they are inducible. All enzymes of the pathway are induced in the presence of a single inducer: GlcNAc or ManNAc. Unlike other inducible systems, induction of these enzymes in Candida albicans requires continuous presence of the inducer. When inducer is depleted from medium either by metabolic conversion or specific removal, there is, disappearance of enzyme activities. The $T_\text{1/2}$ of these enzymes is approximately 2.5 hr. On the other hand, enzymes of other inducible systems from prokaryotes are comparatively stable even in the absence of inducer.

In the inducible GlcNAc catabolic pathway, two different sets of enzymes are available. While kinase and permease are absent in the non-inducing conditions, deaminase and deacetylase have been found in measurable quantities in such conditions. However, in both cases level of enzymes increases many folds after addition of inducer to the medium. An inducible GlcNAc binding protein has also been
observed in *Candida albicans*. Many of its properties have been found to be common with permease. In *Candida albicans*, it has been reported that the synthesis of RNA and proteins are obligatory for the induction of all these enzymes.

Many other inducible systems are known in different organisms. But advances in our knowledge in the subject have been almost exclusively on the observations in bacteria and yeast. Concentration of studies in microorganisms had been mainly because the magnitude of induction is large in these systems. Inducible systems that have been studied most intensively are: utilization of lactose and galactose in *E. coli*, arabinose in *S. typhimurium* and *E. coli*. Inducible allophanate, \(\beta\)-galactosidase and galactose catabolising enzymes in yeast, penicill\(\text{\`e}\)ase in *Staphylococcus aureus* and quinic acid catabolic pathway in *N. crassa* are some other well known systems.

Frequently, a compound induces several enzymes which form a part of its catabolic pathway. But compounds inducing only one enzyme have also been reported. The quantum of induction varies with system and organism. The extent to which a given organism responds to the presence of an inducer is genetically determined.
Increases in enzyme content ranging from 2-1000 folds may be observed on induction in different strains. The genetic heritage of the cell thus determines not only the nature but also the magnitude of the response of the inducer.

**N-Acetylglucosamine Catabolism**

The pathway for chitin synthesis and GlcNAc breakdown for energy follows the same course upto a certain stage. The fate of intermediate products depends on the internal constitution of the cell. For example, at cell division and germ tube formation, a part of aminosugar pool is utilized for the synthesis of chitin\textsuperscript{32}. But at other stages of cell cycle, there is no chitin synthesis. The phosphorylation of substrate is first and common step in both anabolism and catabolism of GlcNAc. ATP in the presence of Mg\textsuperscript{2+} and a specific kinase acts as phosphate group donor to the primary alcoholic group of GlcNAc molecule. Kinases which phosphorylate other aminosugars have also been reported in different systems. In general, unacetylated sugars are phosphorylated by nonspecific kinases\textsuperscript{33,34}, but specific kinases are needed for phosphorylation of acetylated sugars\textsuperscript{35}. 
GlcNAc kinase has been reported in different systems and has been purified from *E. coli* 36, *Streptococcus pyogenes* 37, hog spleen 19 and human gastric muscosa 38. The enzyme is constitutively present in all these systems. Enzyme from different sources vary in their substrate specificity. They have been found to differ also in pH optima, nucleotide specificity and stability. In hog spleen, Datta 39 has found it to be under allosteric control.

ManNAc kinase, a related enzyme has been detected in glycerol grown *S. typhimurium* 40,41. It has been found also in rat liver. But interestingly, this enzyme is absent in aminosugar metabolising yeasts. Instead, another enzyme, ManNAc epimerase is present 22. Induction of this enzyme which catalyzes the interconversion of ManNAc and GlcNAc, occurs either by GlcNAc or ManNAc. This possibly spares yeast from its need for kinase.

It has been observed in our lab that nonpathogenic yeasts such as *S. cerevisiae* can not utilize GlcNAc as the source of carbon 3. *S. cerevisiae* 3059 which occasionally becomes pathogenic, however, is an exception. In this strain, three enzymes of GlcNAc
catabolic pathway are present but GlcNAc kinase is not detectable. It appears that the phosphorylation function in this organism is done by a membrane bound system. It is supported by the studies of Wiemberg et al. and Souzu.

GlcNAc-6-P, the product of kinase reaction, serves as substrate for deacetylase and isomerase. This can also be formed by acetylation of glcosaminine-6-phosphate (Gm-6-P). Utilisation of GlcNAc-6-P depends on equilibrium constants of different reactions. When GlcNAc acts as the source of energy, its phosphate derivative is acted upon by deacetylase and the reaction is directed towards catabolism. At the time of cell division, a part of GlcNAc-6-P pool is utilized anabolically for chitin synthesis, GlcNAc-6-P deacetylase has been detected in the extracts of E. coli, E. subtilis, Bifidobacterium bifidum and bovine parotid gland. Deaminase, the next enzyme of the sequence, acts on GlcN-6-P. This enzyme has been found to be stimulated by its substrate in both microbial and mammalian systems. It has been reported to be semiconstitutive in Candida albicans. Unlike, other inducible forms of enzymes, inducible
and constitutive deaminases are almost identical in their properties. They appear to be doing the same function in vivo but are probably under different controls. It is a low molecular weight enzyme (mol. wt. 17,500) and is exceptionally stable. Das and Datta have reported that specificity of these enzymes are stringent in all respects. Deaminase has also been purified from \textit{E. coli}, \textit{Proteus vulgaris}, pig kidney and \textit{Candida albicans}. Studies in \textit{E. coli} mutants suggest that the genetic loci for deacetylase and deaminase are closely linked.

\textit{Candida albicans} spheroplasts induce enzymes of GlcNAc catabolic pathway in the same way as does the natural yeast. The presence of inducer is essential for induction in this also. Though most of the properties of inducible system are similar to the natural yeast, some enzyme has been found to leak out during the process.

\textbf{Catabolite Repression/Inactivation to Control Enzyme Activity}

The expression of genetic information is regulated during ontogeny and differentiation of an organisms. For proper adjustment with the environment,
responsiveness to extrinsic signals is essential. In the absence of nutrients, for instance, an organism must avoid synthesis of 'unnecessary' enzymes. This would help it to use the available nutrients to its maximum advantage.

As organisms evolved, more sophisticated mechanisms have developed to secure their survival in complex environment. Basically two types of regulations, positive and negative are present in different life forms. In the first case, there is an increase in the expression of genetic information in the presence of regulatory molecules but in the second it decreases. In few instances double negative control mechanism is present. In such cases, the net effect is like positive control.

At present, the molecular details of regulation are best understood in microorganisms, which lack the complexities of hormonal and nervous control that exists in higher animals. The options that are available for the regulation of enzyme activity are—the change in absolute quantity, alteration in the pool size of reactants and the alteration in the catalytic activity of the enzymes.
The absolute quantity of a given enzyme present in a cell at a particular time is determined by the rate of its synthesis ($K_S$) and the rate of its degradation ($K_d$).

$$\begin{align*}
\text{Enzyme} & \quad \text{Amino Acids} \\
K_d & \quad \quad K_s
\end{align*}$$

These two processes are distinct in all forms of life. They are catalyzed by entirely different sets of enzymes and thus their regulation is readily achieved inside the cell.

The presence of a substance may signal the cell for the synthesis of some enzymes (induction) or for the suppression of the synthesis of others (repression). The catabolite repression is a similar phenomenon. It refers to the ability of a product or intermediate in a sequence of reactions to repress the synthesis of some or all catabolic enzymes concerned. It was first noted in E. coli grown on carbon source other than glucose\textsuperscript{57}. In such a situation, the synthesis of catabolic enzymes was repressed in the presence of glucose and other oxidizable nutrients. This phenomenon has been observed in many other microbial systems including bacteria and growing yeasts. But interestingly, we have shown that there is no repression of GlcNAc
catabolic enzymes in *Candida albicans* \(^{58}\). Unlike other systems, glucose, glycerol and succinic acid could not repress the induction of these enzymes in this yeast.

Apart from control of enzyme activity by an induction and repression process, there is a regular turnover of enzymes in living systems. Under different conditions, the turnover rate of enzymes is different. From our laboratory, it has been shown by Biswas *et al.* \(^{20}\) that the rate of turnover of kinase in *Candida albicans* in non-growing conditions is 2.6 hr, whereas in growing conditions, it is 1 hr. This differential rate of turnover of enzymes has been reported from other systems also \(^{59}\).

Besides turnover, other mechanisms that result in a decrease in the enzyme activity are modification and inactivation. In the first case, loss of enzyme activity is reversible and it can be restored when the enzyme attains the original state. For example, glutamine synthetase in *E. coli* does not loose all its activity during adenylation. Its activity varies with adenylation and the ratio of \(\text{Mg}^{2+}\) and \(\text{Mn}^{2+}\) in the cell. Thus, the completely adenylated glutamine synthetase has no activity with \(\text{Mn}^{2+}\) but maximum activity with \(\text{Mg}^{2+}\).
whereas the fully adenylated enzyme is inactive with Mg$^{2+}$ while with Mn$^{2+}$ it achieves about one fourth of maximal activity. The intermediate levels of enzyme activities are attained both by lower level of adenylation and variation in Mg$^{2+}$ and Mn$^{2+}$ ratio in the cell. Many other enzymes such as glycogen phosphorylase, phosphorylase b kinase, glycogen synthetase, pyruvate dehydrogenase and hexose biphosphate aldolase have also been reported to be regulated by this mechanism.

Enzyme inactivation is an irreversible process. Its use to control enzyme activity is possibly dependent on the metabolic state of an organism. However, there is some restriction on situations where enzyme inactivation can act as the mode of control. This is because once inactivation occurs, there is a need for de novo synthesis of enzymes. In the actively growing microbial cells there is some protein turnover but enzyme activity remains constant. However, during metabolic transitions, enzymes have been found to be depleted. In such situations, the regulation of enzyme activity occurs by inactivation mechanism. This type of control has been reported whenever there is a
nutritional shift. This has also been found during different stages of microbial growth and morphogenesis.

In a majority of situations, more than one such control mechanisms operate simultaneously. In yeast cell, for example, glucose addition causes not only "catabolite repression" but also "catabolite inactivation" a process that causes decrease in specific activity of enzymes. The rate of decrease in the case of the latter is faster than can be explained by catabolite repression and dilution by newly synthesized proteins.

The presence of catabolite inactivation in addition to or in place of other mechanisms is of some advantage. In many situations, inactivating enzymes involved in the process are subject to allosteric control involving covalent modifications. In such cases, when the regulatory metabolite instead of directly acting, acts through other enzymes, the sensitivity of mechanism is multiplied many folds. A repression of 'unnecessary' enzyme is inadequate when cells are not actively synthesizing proteins. In these conditions, a more effective mechanism like inactivation is necessary for the organism. Besides blocking wasteful reactions,
Degradative inactivation also provides amino acids for the synthesis of new enzymes and catabolites for metabolic energy.

A number of biochemical reactions are reversible. This is because, there are two separate enzymes catalysing the forward and the backward reaction. The cell utilizes these two different sets of enzymes to impose a fine regulation on this type of reactions. Such controls are important, otherwise these opposing reactions would couple and will lead to a futile cycle. For instance, there will be cycle involving a net ATPase reaction, when cells are transferred from glucose medium (glycolytic mode of growth) to an acetate medium (gluconeogenic mode of growth). When such transitions occur, both glycolysis and gluconeogenesis would face a difficult barrier at these points. To avoid this situation, the cell inactivates gluconeogenic enzymes viz. phosphoenol pyruvate kinase, fructose biphosphatase and cytoplasmic malate dehydrogenase. A similar observation has been made during anaerobic growth of R. gelatinosa. In this system, citrate lyase has been found to be inactivated during citrate synthesis, to avoid opposing reactions at the same time. Another well documented inactivation occurs in S. cerevisiae. In the presence of arginine, the bio-
synthetic enzyme-ornithine transcarbamylase is depleted to avoid futile cycle between arginine synthesis and its catabolism.

In many cases, catabolite inactivation is also involved in the regulation of branched biosynthetic pathways. Removal of critical enzymes by inactivation avoids some undesirable metabolic pathways. For example, nutritional shift from glucose to acetate in *E. coli*, requires isocitrate utilization by glyoxalate cycle instead of tricarboxylic acid cycle (TCA). Isocitrate dehydrogenase is inactivated to effect this change. Similar inactivation of amino acids and nucleotide synthesizing enzymes has been observed in the stationary phase of Bacillus sp. In *S. cerevisiae*, it has been found that the ability to ferment galactose is lost in 3 h in the presence of glucose. Mertern and Holzer have reported that in galactose utilization system, this is effected by a decrease in enzyme affinity for the substrate. Though *V*\(_{\text{max}}\) remains constant, *K*\(_{\text{m}}\) of galactose uptake system increases by about 3 folds. In many bacteria and yeast, some metabolites like glucose and fructose are utilized preferentially. To effect this change,
regulation by catabolite inactivation has been observed in most of these cases. Ironically, different sugars have been found to differ in their potentialities to inactivate enzymes. Glucose, fructose and mannose have been found to be most effective whereas the effectiveness of galactose and 2-deoxyglucose is of a lesser degree. Some like glycerol, xylulose and gluconate are totally ineffective. Glutamate dehydrogenase and the enzymes of nitrogen fixation system have also been reported to be inactivated in yeast and fungi. It possibly spares metabolic energy by switching off the reactions.

Mechanisms involved in the inactivation process is not yet clear. Diverse types of situations in which it operates, demands different methods for inactivation. It is probable that the cell employs more than one method for this function. Some of the methods that have been explored, include modification of enzyme by binding or dissociation of low molecular weight compounds, cleavage of peptide bonds of the enzyme and proteolysis. Production and
binding of cyanide to nitrate reductase on addition of \( \text{NH}_4^+ \) is well established\(^7\). Threonine dehydratase from \textit{E. coli} has been found to be inactivated by the binding of pyruvate\(^7\),\(^5\). In many other cases, phosphorylation or dephosphorylation of enzyme has been found to be the cause of inactivation.

Entian\(^7\) has found a \textit{S. cerevisiae} mutant that is deficient in carbon catabolite inactivation. It appears that one of the hexokinase isoenzymes is altered in this mutant and it possibly initiates the enzyme inactivation by causing conformational change.

Malate dehydrogenase, a gluconeogenic enzyme, is present in both cytoplasm and mitochondria. It has been found that only cytoplasmic MDH (cMDH) is under control of catabolite inactivation\(^7\). This catabolite inactivation is believed to occur by proteolysis of the enzyme. These are many other instances where inactivation occurs by proteolysis. cMDH for example can be inactivated by heat, glycer-aldehyde-3-phosphate and changes in nutritional conditions. Neef \textit{et al.}\(^7\) have developed antibody against MDH and have found that it reacts with both
cytoplasmic and mitochondrial MDH. Ag-Ab complex is formed both by heat inactivated and glyceraldehyde-3-phosphate inactivated enzyme in vitro. But catabolite inactivated enzyme could not be detected by this antiserum. An additional evidence of proteolysis has been found in case of this enzyme. When cycloheximide, an inhibitor of protein synthesis is added, the enzyme is not reactivated in the presence of acetate. Contrary to the general belief, studies with proteinase B mutant suggest that this enzyme is not involved in the process of inactivation. In S. cerevisiae, 6-8 different types of proteinases are known to exist. Of these 5 are known to be present in yeast vacuoles. Due to this compartmentalization of enzyme, it appears that the specificity of catabolite inactivation is at the level of transport in vacuoles. But such mechanisms cannot account for inactivation in B. subtilis where no such subcellular organization exists. In cases of Fr-1,6-diphosphatase, PEP, carboxykinase and aspartate transcarbamylase, mutation studies argue against the involvement of proteinases as the primary step of inactivation. The involvement of ATP is also suspected in many cases but its significance is not clear.

In yeast, two mechanisms operate at the same time to control the activity of fructose-1,6-biphos-
phatase. About 60% of enzyme that is rapidly inactivating can be reactivated by restoring original conditions. Since cycloheximide could not inhibit the process, it appears that covalent interconversion is involved. This postulation has also been supported by immunological studies. However, a part of fructose-1,6-bisphosphatase inactivation is cycloheximide sensitive. It occurs at a later stage and certainly involves a more complicated mechanism.

Holzer has reported that some effector molecule binds to the enzyme and renders them more vulnerable to proteolysis. In the light of present information, this generalization does not hold good. Instead, it seems to be a function of the genetic make up of the cell and the nature of inactivating molecule. The view is supported by the observations in *S. cerevisiae* M which has faster rate of inactivation of cytoplasmic MDH than the wild type strain\(^7\).

**Dimorphism and Pathogenicity in Candida albicans**

The change in structure and composition is a common occurrence in fungi. By manipulating the construction, fungal cell assumes a variety of well
defined shapes. Some of the conditions that necessitate these changes are vegetative growth, substrate colonization, reproduction, dispersal and penetration. In many organisms as _N. crassa_\textsuperscript{81}, _Histoplasma capsulatum_\textsuperscript{82}, _Candida albicans_\textsuperscript{83} etc., two different forms are simultaneously found in the vegetative state. The regulation of interchangeability of two forms is not clear. It appears that factors responsible for the transformation differ with the organism.

In general, changes that are brought about during the transformation are of two kinds: ultrastructural and biochemical. Much work has been done on the structural aspect that accompany the change, but the biochemical mechanism involved in the process is not clear.

One of the simplest morphogenetic phenomenon in fungi is budding of cells. A detailed study of the process has been done by many workers. Evidences suggest that the emergence of a bud is mainly determined by hydrodynamic flow\textsuperscript{84}. The site of the bud formation is mostly a point of maximum curvature\textsuperscript{85}. But the new budding site never coincides with the earlier site. Development of spindle plaques have
been observed during this process. Many microtubules radiate out from these spindle plaques. Some of these move into the nucleus whereas others enter the area of incipient bud. Possibly, this is a part of the apparatus that specifies the budding site and also helps the nucleus to migrate into the bud. An increased number of vesicles appear to accompany this structural transformation in the initial stages\(^86,87\). They originate from the endoplasmic reticulum and possibly carry some information about the budding site.

The morphogenetic phenomena, in general, are cell wall transformation and so a change in its composition is strongly argued. Studies support that a quantitative change in the composition of cell wall components, enzymes and macromolecules occurs in the process. Chitin, mannan and glucan being the major components of the wall, seems to be directly related with the process.

In fungi, a new chitin synthesis precedes the bud formation. In *Candida albicans*, an increase of about five folds in chitin has been observed in mycelial form as compared to yeast form\(^88\). This is brought about by change in the activities of regulatory enzymes of chitin.
biosynthetic pathway. About 4-fold increase in the activities of aminotransferase and chitin synthetase has been found in the germ tubes of this organism\textsuperscript{32}. A higher level of chitin synthetase is also found during budding. Normally, chitin synthetase is present in the cell wall in an inactive form. It has been proposed that proteinase B is involved in the process of activation\textsuperscript{80}. But this enzyme has not yet been detected in \textit{Candida albicans}. In \textit{vitro} studies with the proenzyme of \textit{Candida albicans} suggest that a proteolytic cleavage is necessary for its activation\textsuperscript{89}. According to Saheki \textit{et al.}\textsuperscript{90} proteinase A hydrolyzes a proteinase B inhibitor and therefore activates inhibitor B inhibited proteinase B. A possible signal for this cascade might be a local decrease in pH at the site of chitin synthesis\textsuperscript{91}. This would induce proteinase A by partial dissociation of inactive proteinase-inhibitor complex. Two different kinds of chitin synthetases have been found in mycelia of \textit{Candida albicans}\textsuperscript{32}. These two enzymes might have some role in the transformation of this yeast. Though chitin is an important component of the cell wall, polyoxin-D, an inhibitor of its synthesis, does not inhibit yeast mycelial transition in \textit{Candida albicans}\textsuperscript{32}. 


This is possibly due to the lack of access of the inhibitor to the site of chitin synthesis.

Mannan concentration in the cell wall also changes during the transformation in various dimorphic fungi. But there are conflicting reports about its variation. For instance, in *Mucor rouxii*, 5-6 folds increase in mannan has been found in yeast form whereas in *H. capsulatum* the reverse is true. In *S. cerevisiae* there are two cellular forms: ellipsoidal and spheroidal. In both these forms, there is no difference in mannan concentration. In *N. crassa* a change in the ratio of mannan and glucan has been observed during transition from branched to colonial form. Since the distribution of the different forms of mannan has not been studied, it is difficult to ascribe any significance to these variations.

In *Candida albicans*, an increase in dry weight with simultaneous increase in RNA and protein synthesis has been observed. Inhibitors of RNA and protein synthesis are also potent inhibitor of germ tube formation. This suggest that a new synthesis of these macromolecules is essential for morphogenesis. Interestingly, no change in DNA concentration has been found
during the initial stages of this process. But after 4 hr of germ tube formation in *Candida albicans*, synthesis of DNA shoots up. It is interesting that in the budding cycle, DNA synthesis accompanies cell growth, whereas in mycelial development it is initially switched off. DNA synthesis at the later stage is possibly needed for the division of nucleus.

There are many inhibitors that inhibit yeast-mycelial transition. These include sulphydryl regent reagents such as iodoacetamide and uncouplers like 2,4-DNP. Since inhibitors have many sites of action, the most important phenomenon in this transition is not clear. But it is certain that it is a complex process and involves many simultaneous developments.

*Candida albicans* occurs both in normal and diseased human beings. When pathogenic, it causes a disease called Candidiasis. In most cases, this yeast does not show any consistent morphological pattern during its parasitic growth. Gresham and Whittle and Rogers have proposed that the morphological pattern observed during candidiasis is determined by host factors. Louria et al., however, could not find any correlation of pathogenicity with yeast and mycelial forms.
It is an extraordinarily versatile organism and can be primary as well as secondary cause of Candidiasis. Some of the precipitating causes and diseases induced by \textit{Candida} are listed in the Table 1. In many cases such as skin infections, thrush and most of systemic infections, it is induced by medical procedures. These follow the use of steroids, broad spectrum antibiotics, immunosuppressive drugs, catherization and certain operations. Chronic mucocutaneous candidiasis, however, is a result of congenital deficiencies in the immune system or iron absorption.

In our laboratory, we started investigating the mechanism of pathogenicity in this organism. Since, mucous membranes of human body are the main site of infection, a relationship between the membrane environment and its disease causing ability is possible. Work is in progress to find the factors that influence the virulence in this organisms. Simultaneously, we are trying to explore the gene expression in two phases of its growth. It would enable us to pinpoint events responsible for the transformation and also help to understand the molecular basis of Candidiasis.
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<tr>
<th>Clinical type</th>
<th>Predisposing factor</th>
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<td>Oral thrush (babies)</td>
<td>Birth infection from mother</td>
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<td>Oral thrush (adults)</td>
<td>Immunosuppressive drugs</td>
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<td>Vulvo vaginal thrush</td>
<td>Pregnancy, pill, sexual partner, diabetes</td>
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<td>Chronic paranychia (nailfold infection)</td>
<td>Wet work of house wives</td>
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<td>Systemic Candidiasis (various organs)</td>
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<td>Bronchial carcinoma, bronchiectasis</td>
</tr>
<tr>
<td>Keratomycosis (Corneal infection)</td>
<td>Trauma, steroids</td>
</tr>
</tbody>
</table>