Aminosugars occur widely in nature and are found in a great variety of compounds including structural polysaccharides, teichoic acid, lipopolysaccharides, glycolipids, mucoproteins and in a number of antibiotics. The aminosugars e.g. glucosamine, galactosamine, sialic acid (derivative of mannosamine) and muramic acid (derivative of glucosamine) are very common. Aminosugars of the natural products of interest generally contain their amino group as acetylated amino derivative. N-acetylglucosamine (GlcNAc) is formed in a reaction catalysed by an enzyme N-acetylase with acetyl CoA as acyl donor. However, later studies with N-acetylating enzymes of amino sugars in microbial and mammalian systems showed that Glucosamine-6-phosphate (GlcN-6-P) was the specific acceptor. Crude preparations from mammalian liver could acetylate both GlcN and GlcN-6-P in the presence of acetyl CoA. On purification, an enzyme fraction was obtained which could accept only GlcN-6-P as substrate whereas another fraction which acetylated GlcN (and also aromatic amines) did not act on GlcN-6-P. The enzyme preparation purified from Neurospora crassa however, acetylated both GlcN-6-P and GalN-6-P. N-Acetylase has been purified from the extract of sheep brain by Pattabiraman and Bachhawat.
The metabolism of hexosamines is intimately linked with the key glycolytic intermediate, fructose-6-phosphate\(^1\). Leloir and Cardini\(^4\) first reported that crude extract of *Neurospora crassa* converted hexose-P and glutamine to N-acetylglucosamine-6-phosphate, hexosamine nitrogen being derived from the amino group of glutamine. The biosynthesis of hexosamine-6-P has been studied in cell free systems from *Neurospora crassa*\(^1\), *Escherichia coli*\(^1\) and rat liver\(^1\). These studies revealed that each of these enzymes was specific for Fru-6-P whereas Glc-6-P was completely inactive as substrate for the purified preparations.

The first step in the utilization of glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) is the phosphorylation of these compounds in C-6 position of the hexose molecule. GlcN is phosphorylated by a nonspecific glucokinase\(^1\), whereas the phosphorylation of GlcNAc requires a specific kinase\(^1\). The presence of GlcNAc kinase has been shown in bacteria\(^2\), hog spleen\(^2\), human intestinal mucosa\(^2\) and recently in yeast\(^2\). Hog spleen enzyme has been purified 3500 fold and detailed kinetic properties have been reported\(^2\). The enzyme is inhibited by the end product of the pathway of GlcNAc metabolism, UDP-GlcNAc and also by the reaction product GlcNAc-6-P\(^2\). The inhibition of ADP is competitive with ATP whereas the
inhibition of GlcNAc-6-P and UDP-GlcNAc is noncompetitive.

In case of yeast, the enzyme kinase is inducible and continued presence of the inducer is necessary for its synthesis\(^2\). Kinases for galactosamine and N-acetylgalactosamine are present in mammalian tissue\(^19\) and the reaction products are 1-phosphate esters. N-acetylmannosamine kinase which converts the aminosugar to its 6-phosphate derivative has been purified from rat liver\(^25\) and glycerol grown Salmonella typhimurium\(^26\). This enzyme does not appear to be synthesized in bacteria if they are grown in the presence of glucose, however, if glucose is replaced by glycerol, this enzyme is synthesized\(^27\). However, this enzyme is absent in yeast even when it is grown in the presence of N-acetylmannosamine. Instead of the synthesis of specific kinase for N-acetylmannosamine, there is new synthesis of another enzyme, N-acetylmannosamine epimerase (M. Biswas, Ph.D. thesis). This enzyme converts N-acetylmannosamine to N-acetylglucosamine and hence the aminosugar is utilized through the N-acetylglucosamine pathway.

GlcNAc-6-P deacetylase and GlcN-6-P deaminase have been shown to play pivotal role in the utilization of exogenous aminosugar, as a source of carbon and/or nitrogen by Escherichia coli\(^28,29\). Mutants lacking the deaminase are unable to grow on GlcN or GlcNAc as sole source of carbon or nitrogen but can still incorporate
the aminosugar into mucopeptides. On the other hand, mutants devoid of deacetylase can utilize GlcN normally, but are unable to grow on GlcNAc or to incorporate it into mucopeptides. The genetic loci for these two enzymes on the *E. coli* K-12 genetic map have been determined and found to be closely linked. Although deaminase catalyses a reversible reaction, it does not function anabolically in *E. coli* as mutants lacking GlcN-6-P synthetase, lyse in the absence of exogenous aminosugar, even though they have normal deaminase levels. Furthermore, deaminase-loss mutants have no requirement for exogenous aminosugar. GlcNAc-6-P-deacetylase has also been detected in extracts of *Bacillus subtilis* and *Bifidobacterium bifidum* var. *pennsylvanicus* and in bovine parotid gland. Roseman has described a deacetylase which converts GlcNAc to GlcN. This enzyme has been found in a number of bacterial systems but not in mammalian tissue extracts. However, the presence of this enzyme has not yet been reported in yeast. GlcN-6-P deaminase has been purified from a variety of sources, including *E. coli*, *Proteus vulgaris* and pig kidney. Comb and Roseman have shown that GlcNAc-6-P stimulates the enzyme activity in both bacterial as well as mammalian systems. In our laboratory we have found that this enzyme is present in various yeasts and its level increases many fold when grown in presence of
N-acetylglucosamine (Chapter 4 of this thesis).

Interconversion of N-acetylmannosamine and N-acetylglucosamine occurs by the enzyme epimerase as reported by Ghosh and Roseman in animal tissues. Datta purified this enzyme 1500-fold from the extracts of hog kidney and it was demonstrated that ATP stimulates epimerase activity about 20-fold by an allosteric mechanism. Recently, we have been able to induce this enzyme in yeast Candida albicans by growing either on GlcNAc or ManNAc as sole carbon source (M. Biswas, Ph.D. thesis). Sommar and Ellis have described another type of epimerase i.e. UDP-GlcNAc-2'-epimerase from rat liver.

A crystalline phosphoglucomutase has been shown to catalyse the interconversion of GlcN-6-P and GlcN-1-P. A specific mutase in Neurospora crassa which acts on GlcNAc-6-P and GlcNAc-1-P has been also reported.

The enzymatic synthesis of UDP-GlcNAc which was first isolated by Cabib et al. from yeast is catalysed by a pyrophosphorylase that acts on GlcNAc-1-P in the presence of UTP as shown below:

\[
\text{UTP} + \text{GlcNAc-1-P} \rightarrow \text{UDP-GlcNAc} + \text{PPi}
\]

Roseman has reviewed some enzymatic reactions involving mannosamine and sialic acids. In short we can summarize the reactions involved in amino sugar metabolism as shown in Fig. 1.
Fig. 1: REGULATION OF AMINOSUGAR METABOLISM. FROM DATTA.²⁴
Candida albicans and Candidiasis

Candidiasis is a fungal disease which is caused by various species of yeast, *Candida albicans* being the most common causative organism. *Candida albicans* is a normal inhabitant of the mucous membrane of oral cavity, gastrointestinal tract or vagina in approximately 50% of the population. However, it is an opportunistic organism and on breakdown of host defences or a change in local environmental factors, it may become virulent and then be responsible for a wide variety of clinical diseases.

Most of the viscera are susceptible to involvement in systemic infections due to *Candida albicans* particularly the kidney, less frequently the heart, liver, central nervous system, lungs, spleen and the deeper tissues of the digestive tract and still less frequently the pancreas and thyroid. Amphotericin B and 5-fluorocytosine are the two most effective drugs in the treatment of candidiasis. But amphotericin B which alters the membrane permeability by interacting with membrane sterols is toxic towards certain mammalian tissues, such as erythrocytes and renal tubular cells. Therefore, toxicity is the major problem with therapy. Another polyene antibiotic, nystatin has some use in the treatment of superficial candidiasis. In case of 5-fluorocytosine therapy, fungal cells become resistant quite often towards this drug, rendering it ineffective.
At this stage one may emphasize the need to go into the detailed investigation of the metabolism of the causative organism under varied growth conditions. The host factors probably play an important role in transforming non virulent Candida albicans to virulent form. These factors may range from pH, $E_H$, carbon source in relation to the competition for it with other microbes, temperature in the local tissue to the hormonal and immunological disturbances.

An essential part of infection by any microorganism is survival at the site of deposition for sufficiently long time to allow multiplication and consequently penetration to the tissues. Our studies have revealed that GlcNAc can be utilized as a carbon source by Candida albicans and other pathogenic yeasts. Since aminosugars constitute a major portion of the constituents available for growth at the site of infection (mucous membranes), we have undertaken a programme to study the regulation of aminosugar metabolism in various yeasts.

**Regulation of gene expression**

Two major avenues have been followed in quest of the molecular events surrounding gene expression. One avenue involves monitoring the metabolism of collective mRNA, the other, assay of specific gene products. In eukaryotic systems, translation of mRNA into protein takes place in
the cytoplasm remote from the place of production, the nucleus. This confers certain amount of stability in terms of measurable rate of decay of eukaryotic mRNA unlike bacterial system. The presence of sequence of poly(A) at the 3'-OH end of eukaryotic mRNA (except that of histone) has provided a powerful tool for the study of biogenesis and metabolism of mRNA\textsuperscript{52-56}. In higher eukaryotes, half life of mRNA ranging from 3 hours to several days has been reported\textsuperscript{57-62}. In \textit{Candida albicans} the half life of mRNA is about 60 minutes, as determined by two methods, continuous labelling and pulse chase experiment\textsuperscript{63}. This value is in striking contrast to the 23 minutes half life for mRNA obtained by following the decay of polyribosomes at 36°C (non-permissive temperature) in a temperature sensitive mutant of \textit{Saccharomyces cerevisiae}\textsuperscript{64}. However, a detailed account of gene expression and its control ultimately requires a model systems having a specific well defined set of genes.

\textbf{Synthesis of inducible enzymes offers a useful tool in the study of gene expression.} Most of the results on prokaryotic gene expression have been elucidated due to characterization of simple inducible catabolic enzymes. On the other hand, absence of a suitable inducible enzyme system, is partly responsible for the slow progress in
understanding the complexity of eukaryotic gene expression. In yeast, the synthesis of GlcNAc kinase, the first enzyme in the pathway of GlcNAc metabolism can be induced by its substrate. It has been observed that for continuous synthesis of the enzyme, the presence of inducer is required. This enzyme is absent in the cells grown in other carbon sources. The synthesis of the enzyme can be blocked by inhibitors of RNA and protein synthesis and the process therefore appears to be due to de novo enzyme synthesis but other explanations are also tenable. Mechanism of enzyme induction of yeast may be similar to that in prokaryotes as at least in one case "operon" like genetic unit has been identified. Using genetic techniques, it has also been shown in Neurospora crassa that quinic acid catabolism is controlled by tightly linked cluster of four genes, the qa cluster. Three of these genes are the structural genes for the qa enzymes and fourth gene is a regulatory locus whose product controls the expression of the three structural genes. The evidence suggests that the regulatory protein encoded by qa-1 gene acts positively in conjunction with the inducer, quinic acid, to initiate enzyme synthesis.

In case of Saccharomyces cerevisiae, Cooper and Lawther have characterized the allophanate degradative
pathway whose enzymes are induced by the end product of the pathway, urea. Induction of the first enzyme of the pathway allophanate hydrolase begins immediately upon addition of inducer. Once induction has been initiated, removal of inducer does not result in immediate loss of synthetic capacity. They have demonstrated that the increase in allophanate hydrolase levels, after addition of inducer, results at least from two processes. First is accumulation of the potential to synthesize the enzyme. The process begins immediately after addition of inducer, is moderately temperature dependent, requires RNA synthesis and does not require protein synthesis. The second process is expression of accumulated potential. This can occur in the absence of inducer and RNA synthesis, is not apparently temperature dependent, but does require protein synthesis.

In higher organisms, the genetic material is present in the form of nucleoprotein complex, chromatin, composed of DNA, RNA and the various chromosomal proteins. The arrangement of these components in chromatin as well as the role of various components in transcription are not fully known. In prokaryotes, the structural genes are in the form of clusters. The process of information transfer takes place by synthesis of a single RNA chain (polycistronic) which carries information of all structural
genes of the operon and finally it is translated to different polypeptides. Main evidence for such a process comes from the isolation of different polar mutants in one operon. However, monocistronic mRNA appears to be a general characteristic of eukaryotic cells. Reticulocytes have monocistronic mRNA. Kuff and Roberts have shown that mouse tumor cells have monocistronic mRNA. Although polarity effect has been observed in case of Saccharomyces cerevisiae, a simple eukaryotic organism, Petersen and McLaughlin also reported that the majority of the mRNA is monocistronic. However, a class of high molecular weight proteins in yeast with a high turnover rate, is also evident from the recent studies in our laboratory (A. Bhattacharya, Ph.D. thesis, 1976). Kinetics of labelling of different molecular weight proteins reveal that these proteins rapidly decay to smaller polypeptides. It is very tempting to conclude that at least part of the information transfer in yeast takes place via synthesis of a different type of monocistronic message (monocistronic type II). Active proteins are generated by post-translational cleavage of larger polypeptide chain, a process similar to that observed in certain animal viruses.

In yeast, we have shown earlier that GlcNAc kinase is the first enzyme in the pathway of aminosugar metabolism.
Further in our laboratory, studies including the induction of other enzymes in the pathway and their functional interrelationship being carried out now, will be helpful in determining the transcriptional unit of *Candida albicans*, the pathogenic yeast.

**Mechanism of sugar transport in yeast**

Based on the concept that glucose is actively transported into the yeast cells, Rothstein\(^7\), proposed a mechanism of sugar transport. He reported that the yeast cells were impermeable to inorganic phosphate but there was concomitant decrease of Pi in the medium. Furthermore, he found that surface reactions showed properties typical of enzyme reactions. These observations led Rothstein to suggest that glucose was phosphorylated by ATP and hexokinase at the permeability barrier and the resulting sugar phosphate could then pass this barrier. ATP was supposed to be regenerated. This reaction would then serve to pick up the orthophosphate from the medium.

Transport associated phosphorylation model was later modified and corroborated by the work of Van Steveninck. In 1964, Van Steveninck and Boolij\(^7\) showed that in Baker's yeast, strain Delft I, the binding of uranyl ions (UO\(_2^{2+}\)) to polyphosphate at the outside of cell
completely inhibited glucose uptake, an indication that this polyphosphate must be involved in glucose transport. There was a decrease in Cobalt (Co) and Nickel (Ni)-binding capacity of cells during glucose uptake due to decrease in number of cation binding sites. One polyphosphate monomer disappeared with uptake of one molecule of glucose in iodoacetic acid (IAA)-poisoned cells. Based mainly on these observations, Van Steveninck and Booij theorized that in the uptake of glucose, a carrier-phosphate-glucose complex forms, the phosphate being donated by the polyphosphate in the membrane. Further reports from Van Steveninck's laboratory\textsuperscript{76,77} demonstrated that the uptake of sugars by yeast can occur by two distinct mechanisms.

The first mechanism, facilitated diffusion, functions in the uptake of sorbose or galactose in uninduced cells and glucose in IAA-poisoned cells of commercial baker's yeast. These uptakes, which have high Michaelis constant and low maximal rates, do not involve changes in Ni- and Co-binding and are not inhibited by Ni ions. They are inhibited by Uranyl ions (UO$_2^{2+}$) in relatively high concentrations.

In case of second mechanism, the uptake of glucose in unpoisoned cells and galactose in galactose-induced cells are accompanied by phosphorylation. These uptakes
are characterized by reduction of Ni- and Co-binding, partial inhibition by Ni ions, inhibition by UO$_2^+$ ions at low concentration, low Km and high Vm. Furthermore, Van Steveninck$^{78-81}$ demonstrated a transport associated phosphorylation of 2-deoxy-D-glucose (dGlc) in \textit{Sacch. cerevisiae} Hansen CBS 1172, glucose in CBS 1172 and "Koningagist", $\alpha$-methylglucoside in \textit{Sacch. cerevisiae} NCYC 240 and galactose in \textit{Sacch. cerevisiae} CBS 1172. Van Steveninck's hypothesis was supported by the following additional observations:

(i) During glucose transport in IAA poisoned cells, the low concentration of ATP could not account for the observed phosphorylation; the concentration of polyphosphate on the other hand could.

(ii) Pulse label experiments, revealed that the phosphorylated sugar was the precursor of the free intracellular sugar.

The implication of this hypothesis that polyphosphates are localized peripherally was supported by the studies of Wiemberg and Orton$^{82}$ and of Souzu$^{83}$. Kulaev$^{84}$ produced evidence of a similar localization of polyphosphate fraction in \textit{Neurospora crassa}. Deierkauf and Booj$^{85}$ provided data for a scheme in which the transfer of a phosphate monomer from polyphosphate to a carrier-phosphate-sugar complex, could proceed via the phosphatidyl glycerol phosphate-phosphatidyl glycerol system (Fig. 2).
Fig. 2: GENERALIZED HYPOTHESIS ON THE ROLE OF THE INTERACTION, PHOSPHATIDYL GLYCEROL PHOSPHATE (PGP) - PHOSPHATIDYL GLYCEROL (PG) IN ACTIVE TRANSPORT.

Results of Kulaev's experiments with *Neurospora crassa* strongly corroborated the transport-associated phosphorylation.

After the discovery that polyphosphatase was localized entirely on the periphery of the cells, i.e., in direct contact with the highest polymer fraction of the polyphosphate, Kulaev made a parallel study of the activity of polyphosphatase and the active transport of glucose into cells under various conditions of culturing. A strict correlation between the polyphosphatase activity and the initial rate of active transport of glucose was found. Both the systems were repressed when the organism grew on a glucose containing medium and derepressed after removal of glucose from the...
medium. Furthermore, the ratio between the initial rate of glucose transport and the polyphosphatase was close to one. This makes it likely that polyphosphatase participates directly in the active transport of glucose in *Neurospora crassa*. There is evidently a hydrolysis of one phosphoanhydride bond of polyphosphate for one molecule of absorbed sugar. Finally, he also proved that ATP was not necessary for the normal functioning of the system of active transport of glucose.

More recently, Meredith and Romano\(^87\) and Jaspers and Van Steveninck\(^88\) have shown that transport associated phosphorylation of 2-deoxy-D-glucose in case of baker's yeast and *Saccharomyces fragilis*.

However, picture of sugar transport is not as simple. There are a number of reports specially from Kotyk's laboratory\(^89\) and Eddy's group\(^90\) which contradict the phosphorylation hypothesis. Pulse labelling experiments of Kotyk and Michaljanicova\(^89\) have shown that glucose and galactose are transported to yeast in free form and phosphorylation takes place only subsequent to transport. Also, it appears from the work of Kuo and Cirillo\(^91,92\) that inducible galactose transport system of baker's yeast does not involve phosphorylation during transport. Working on *Saccharomyces cerevisiae* mutants deficient in
galactokinase\textsuperscript{91} or in UDP-galactose-6-P uridyl transferase\textsuperscript{92}, they have shown transport of free galactose by a facilitated diffusion system followed by intracellular phosphorylation by an inducible galactokinase.

Recently, Brocklehurst \textit{et al.}\textsuperscript{90} working on $\alpha$-methyl-glucoside ($\alpha$-MG) \textit{and} $\alpha$-thioethyl glucoside ($\alpha$-TEG) in yeast N.C.Y.C. 240 have got results in disagreement with Steveninck's findings\textsuperscript{80}. According to them $\alpha$-MG and $\alpha$-TEG are transported by symport mechanism involving absorption of protons alongwith the sugar. This type of disparity between the results of various people on the transport of the same sugar, has been generally attributed to the use of different yeast strains by different authors.

Van Steveninck\textsuperscript{93} has described the experiments on uptake of glucosamine in strain CBS 1172. The results indicated active transport of this aminosugar but without transport associated phosphorylation. Very recently Jaspers and Steveninck have got the same result in case of 2-deoxy-D-galactose transport in \textit{Saccharomyces fragilis}\textsuperscript{94}. Consequently it can be said beyond any doubt that at least some sugars are transported as free form. In this type of active transport, energy coupling takes place via ionic gradient. Eddy who studied the stoichiometry of proton absorption with specific carbohydrates in yeast, claimed that 2 proton equivalents were absorbed in the transport of
maltose in *Sacch.* _fractilis*\textsuperscript{95,96}. Misra and Hofer\textsuperscript{97} presented data, indicating that sugar transport in *Rhodotorula gracilis* used a proton gradient which resulted in considerable accumulation against a concentration gradient.

Komor et al.\textsuperscript{98} and Komor and Tanner\textsuperscript{99,100} proposed a model for sugar uptake in *Chlorella vulgaris* in which they observed up to 1500 fold accumulation due to proton motive force.

In short, there are at least three transport mechanisms for sugars in yeast: (i) Facilitated diffusion, (ii) Active transport with transport associated phosphorylation, and (iii) Active transport with energy coupling via an ionic gradient.

During our studies on aminosugar metabolism in yeast, we came across an interesting situation. We found that GlcNAc transport occurs in yeast devoid of a specific kinase, which is inducible only in *Candida albicans*. Therefore, as a part of our programme, we have undertaken a comparative study on GlcNAc transport in various yeasts. Furthermore, to our knowledge there is no single report so far on transport of N-acetylglucosamine in yeast. The results presented in Chapter 2 of this thesis strongly support the hypothesis of Van Steveninck.