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ENZYMES OF AMMONIA ASSIMILATION IN FUNGI: AN OVERVIEW

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Abstract

Nitrogen is a major element found in many of the simple compounds and nearly all of the complex macromolecules of living cells. Nitrogen can be obtained either from organic source or inorganic source but ultimately it is converted into ammonia and glutamate. Ammonia is the preferred source of nitrogen. The assimilation of ammonia into glutamate and glutamine plays a central role in the nitrogen metabolism of all organisms. Glutamate dehydrogenase (GDH), Glutamate synthase (GOGAT), and glutamine synthetase (GS) are the key enzymes involved in ammonia assimilation. The specific steps in these pathways vary with the organism, but in virtually all cells glutamate (85%) and glutamine (15%) serve as the nitrogen donors for biosynthetic reactions. In fungi lot of work has been carried out on these enzymes from lower fungi to the higher ones and there are differences in the role played by theses enzymes. Thus knowledge of the formation of glutamate and glutamine from various nitrogen sources is crucial to our understanding of cell growth. In this review an overall view of the elements present in ammonia assimilation especially in fungi will be carried out along with recent developments and concepts.

Keywords: Ammonia assimilation, Fungi, Glutamate dehydrogenase, Glutamate synthase, Glutamine synthetase

Introduction

In all biological systems the assimilation of nitrogen into macromolecules is essential for growth. Nitrogen is a major element found in many of the simple compounds and nearly all of the complex macromolecules of living cells. Nitrogen can be obtained either from organic source or inorganic source but ultimately it is converted into ammonia and glutamate. Ammonia is the preferred source of nitrogen. The assimilation of ammonia into glutamate and glutamine plays a central role in the nitrogen metabolism of all organisms. The metabolic pathways of nitrogen metabolism can be divided into two classes: the assimilatory pathways necessary for the utilization of nitrogen from compounds available in the medium, and the biosynthetic pathways leading to the production of the nitrogen-containing compounds of the cell. The specific steps in these pathways vary with the organism, but in virtually all cells glutamate (85%) and glutamine (15%) serve as the nitrogen donors for biosynthetic reactions. Thus knowledge of the formation of glutamate and glutamine from various nitrogen sources is crucial to our understanding of cell growth.

In this review an overall view of the elements present in ammonia assimilation especially in fungi will be carried out along with recent developments and concepts. Much of the information about nitrogen metabolism in fungi is from *Saccharomyces cerevisiae*, *Neurospora crassa*, and *Agaricus bisporous*, where as there is lack of much information in other species of fungi so in this review the emphasis will be to include all those fungi, but this review is limited to fungi nitrogen metabolism and deals primarily with experiments conducted in relation to ammonia assimilation.

During the last few years much experiments on the genetic regulation of ammonia assilimilation has been carried out especially in *S.cerevisiae*, *N. crassa, Aspergillus nidulans*, the nitrogen catabolite genes have been extensively studied, and a new role of TOR (target of rapamycin) kinases has been purposed.

Enzymes involved in ammonia assimilation

The enzymes which are mainly involved in ammonia assimilation are Glutamate dehydrogenase (GDH; E.C.1.4.1.X), Glutamine synthetase (GS; E.C.6.3.1.2) and Glutamate synthase (GOGAT; E.C.1.4.1.13). Based on cofactor specificities three different types of GDH are known which are Nicotinamide Adenine Dinuleotide-specific (NAD-GDH; E.C 1.4.1.2), Nicotinamide Adenine Dinuleotide Phosphate (NADP)-specific (NADP-GDH; E.C.1. 4. 1. 4) Or non-specific (NADP/NAD E.C 1.4.1.3) (i.e. can function with either coenzyme which is found only in animals).

The role of Glutamate dehydrogenase (GDH)

GDH plays a strategic role in the metabolic pathway of all living organism's connecting carbon and

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nitrogen metabolism.GDH (E.C.1.4.1.X) catalyzes the reductive amination of 2-oxoglutarate by ammonia in a reversible reaction utilizing either NADPH or NADH as cofactors. In certain fungi the main pathway of ammonia assimilation into glutamate involves the catalytic activity of NADPH-dependent GDH [27, 83] The NADP-GDH is hexamers with subunit molecular weight of 50 kDa, where as the NAD-GDH of fungi and-yeast is tetramers with much larger subunit size 115 kDa. The NAD-dependent GDH appear to serve a catabolic function, while the enzyme utilizing NADPH serve primarily for the biosynthesis of glutamate. The Km of GDH for ammonia is high (above 1mM) compared to GS, so it functions under high concentration of ammonia only.

GDH has been studied in a wide range of organisms from bacteria to mammals including humans. All higher fungi (deuteromycetes, ascomycetes and basidiomycetes) were found to have two distinct enzymes: one NAD-dependent and one NADP-dependent, whereas most of lower fungi (myxomycetes and phycomycetes) have only the NAD-dependent enzyme [55]. Among the lower fungi, oomycetes and hypochytridiomycetes have an unusual NAD-dependent GDH, which is allosterically regulated by NADP and is kinetically similar to the NADP dependent GDH of higher fungi.

Genetics of GDH formation

From the study of *in vivo* regulation of GDH and the isolation of mutants- deficient in NAD - or NADP – dependent GDH in higher fungi indicates that the NAD - dependent GDH functions primarily as a catabolic enzyme in the direction of the oxidative deamination of glutamate and the NADP– dependent GDH functions primarily as a biosynthetic enzyme in the direction of reductive amination of α-ketoglutarate. Studies of mutant strains of *A.nidulans* and *S. cerevisiae* showed that NAD-GDH mainly generates ammonia from glutamate, serving a catabolic function. Mutants of *A. nidulans* lacking NADP-GDH activity grow more poorly than wild-type strains on ammonium as a sole nitrogen source [57]. The leaky growth of these mutants is indicative of an alternative pathway of ammonium assimilation and glutamate biosynthesis [57]. *Schizosaccharomyces pombe* mutants lacking either NADPH-GDH or GOGAT are still able to grow on ammonium as sole nitrogen source. Complete lack of growth on ammonium as sole nitrogen source is seen only in double mutants lacking both NADPH-GDH and GOGAT [69]. The yeast *S. cerevisiae* synthesizes glutamate through the action of either NADP-glutamate dehydrogenase (NADP-GDH), encoded by *GDH1* (under conditions of ammonia excess), or through the combined action of GS and GOGAT, encoded by *GLN1* and *GLT1* (under conditions of ammonia limitation) [7].Triple mutants impaired in *GDH1*, *GLT1*, and *GDH3* of *S .cerevisiae* are strict glutamate auxotrophs, indicating that *GDH3* plays a significant physiological role, providing glutamate when *GDH1* and *GLT1* are impaired . This appears to be the first example of a microorganism possessing three pathways for glutamate biosynthesis [7].

Physiology of GDH production

The lower fungi have only one GDH enzyme, which is responsible for both ammonia assimilation and the deamination of glutamate. In these organisms there is a necessity for the enzyme to evolve complex regulatory properties. Possession of both a biosynthetic NADP-linked GDH and a catabolic NAD-linked enzyme by higher fungi renders it unnecessary for these enzymes to have complex regulatory properties. As the NAD and NADP dependent forms of the GDH seem to function differently and catalyze opposing reactions, they may be subject to some form of concurrent regulation. Another complication, which makes their study difficult that these enzymes appear to be controlled by the nitrogen and the carbon circuits.

Direct ammonia assimilation into glutamate via the catalytic action of NADPH-GDH was demonstrated in the food yeast *Candida utilis* by $^{15}NH_3$ tracer experiments and quantitative analysis of metabolic fluxes [83]. High activity of NADP– GDH is found in *Neurospora* and *Aspergillus* when wild–type cells are grown with a limited amount of inorganic nitrogen source such as ammonia or nitrate. Increase amount of inorganic nitrogen compounds repress NADP-GDH but cause an increase in NAD-GDH. A rich carbon source (such as glucose) leads to an increase in NADP-GDH but represses NAD-GDH, which suggests that the enzyme is controlled in large part by catabolite repression.

The NAD–GDH of the yeast, *S. cerevisiae* and *C. utilis* are both regulated by a phosphorylation – dephosphorylation system, which are catalyzes by protein kinase and phosphoprotein phosphatase. In *S. cerevisiae* the conversion of active NAD-dependant glutamate dehydrogenase to inactive form is regulated by the phosphorylation of the enzyme by both cAMP (cyclic Adenosine Mono Phosphate)-dependent and cAMP-independent protein kinase. In *C. utilis*, phosphorylation of the active NAD-GDH promoted by the starvation of glutamate, converts it in less active form. The NAD-linked enzyme present in the spores of *P. blakesleeanus* were activated by AMP and inactivated by ATP (Adenosine Tri Phosphate). In *Mucor racemosus,* it was found that the addition of glucose to the medium resulted in the repression of the NAD-dependent enzyme. From this it can be said that the utilization of glucose as carbon source lessens the need for enzymes of amino acid catabolism and such enzymes are commonly regulated by carbon catabolite repression.

A threefold nitrogen catabolite repression of the NAD-dependent enzyme was found in mycelial cells in *Mucor* upon the addition of NH_4Cl (ammonium chloride) to the defined medium containing glucose and amino acids. The NAD-GDH from *Laccaria bicolor* was moderately inhibited by ATP and ADP, while the NADP-dependent enzyme was strongly inhibited by ATP, but not affected by ADP, suggesting that both GDHs might be inversely regulated by these nucleotides. AMP exhibited a similar inhibitory effect on both NAD and NADP-GDHs in *L. bicolor*.

In the yeast *S. cerevisiae* two NADP dependent GDHs encoded by GDH1 and GDH3 catalyze the synthesis of glutamate from ammonium and α-ketoglutarate. *S. cerevisiae* has amino acid biosynthetic pathways such that it can use ammonium as sole nitrogen source. *S. cerevisiae* is able to grow using a variety of carbon sources under fermentative and respiratory conditions. Therefore it is necessary to see which specific mechanism allows α- keto glutarate utilization for glutamate biosynthesis without impairing the integrity of the TCA (Tri Carboxylic Acid) cycle as an energy providing system. In absence of activators ammonia inhibited the reductive amination reaction of Pythium GDH considerably.

In *Neurospora* the two enzymes NAD-GDH and NADP-GDH have been shown to be concurrently regulated by a repression derepression type of mechanism, in the presence of glutamate or its nitrogenous precursors (urea, ammonia, alanine, aspartate, etc) the NADP GDH is repressed and the NAD-GDH is simultaneously derepressed. The NAD-GDH activity was significantly inhibited by p-chloromercuribenzoate (PCMB) 10^{-3}M, Ethylene Diamine Tetra Acetic Acid (EDTA) (10^{-3}M) and glutaric acid $3x10^{-2}$M while the nucleotides guanosine tri phosphate (GTP), guanosine mono phosphate (GMP) and inosine mono phosphate (IMP) at a concentration of 10^{-3}M completely inhibited the activity.

In *Fusarium* EDTA is a potent inhibitor of NADP-GDH while NAD- GDH is less susceptible. NADP- GDH is also very sensitive to pCMBA while NAD -GDH is not inhibited to the same extent, the sensitivity to this reagent means that - SH groups are necessary for enzyme activity. Glutaric acid, the decarboxylic acid analog of glutamic acid inhibits the NAD -GDH and NADP by competing with the substrate for the active site. $^{13}NH_3$ tracer studies indicate that the GS-GOGAT pathway is the major route of ammonium assimilation in *C. albicans* and also in nitrogen-starved cultures of *S. cerevisiae* and *Candida tropicalis* [38, 39].

In *N. crassa* it has been suggested that the actual agent *in vivo* for the regulation of the two enzymes is ammonia (NH_4^+). Induction of the NAD- GDH and repression of NADP-GDH is proportional to NH_4^+ in the medium while the concentration of glutamate in the cells increases to a maximum at an external NH_4^+ of 0.1g/100ml. Increase of NH_4^+ beyond this level gives further induction of NAD –GDH. This is expected if ammonia rather than glutamate serves as a regulator of the two enzymes. In addition NH_4^+at high levels as well as glutamate can repress the NADP- GDH enzyme and it was deduced that the presence of both the substances is not essential for regulation. However ammonia disproportionately represses the NADP-GDH only at high concentrations this decrease could result from general ammonia toxicity. Citrate, pyruvate and succinate do not exert a significant effect on NAD -GDH although pyruvate and citrate do induce NADP-GDH. Additional studies have indicated that some balance between the internal amino acids and glucose metabolites controls the regulation of the two GDHs.

Miscellaneous molds

In *A. nidulans*, the activity of NADP-GDH is greatly decreased when the mold is grown on L-glutamate or on high levels of either NH_4^+ or urea and it has been suggested that glutamate alone determines the rate of synthesis of NADP-GDH other nitrogen sources might express their effects only on glutamate levels in the mycelium. Regulation of GDH synthesis in higher fungi containing both the NAD specific and the NADP specific enzymes has also been studied in *Aspergillus niger, Fusarium oxysporum Coprinus lagopus* and *Schizophyllum commune*.

In *A.niger* and *F.oxysporum* the levels of the two dehydrogenases are related to the age of the cultures and the organisms containing NH_4^+have high levels of NADP-GDH and low amounts of NAD-GDH during early stages of growth. Maximal specific activity of the NAD -GDH is obtained after two days of growth at which time the NADP-GDH level is much lower than during early growth. In *C.lagopus* the two GDHs do not appear to be under the direct regulation by either glutamate or NH_4^+. The results support the view that products of glucose metabolism repress the synthesis of the NAD-GDH and depress or induces that of the NADP-GDH and evidence is obtained that this regulator is α-ketoglutarate. It is concluded that more than one molecule is involved in the complete system of regulation. The NADP- GDH in *S.commune* is depressed during vegetative growth of mycelium on glucose containing media with NH_4^+ as sole nitrogen source and increased when glutamate is the nitrogen source.

Regulation of GDH by protein kinases

In *Saccharomyces* yeast the conversion of NAD-dependent glutamate dehydrogenase from the active enzyme form to the inactive enzyme form is regulated through the phosphorylation of the enzyme by both cAMP -dependent and cAMP -independent protein kinases. Hemmings indicated that the phosphorylation of NAD-dependent GDH from *C.utilis* was promoted by

glutamate starvation and was completely reversible. He found a phospho protein phosphatase which dephosphorylated proteins, phosphorylated by cAMP - dependent protein kinase and reactivated the phosphorylated form of NAD-dependent GDH, but it was not clear what kind of protein kinase could phosphorylate this enzyme *in vivo*.Phosphorylation of GDH is accompanied by an alteration of the properties of the enzyme .The regulation of the NAD-dependent glutamate dehydrogenase by phosphorylation offers yeast an effective means of glutamate catabolism and in turn the size of intracellular pool of the amino acid.

The role of Glutamate synthase (GOGAT)

Glutamate synthase is a multicomponent iron sulpher flavoprotein belonging to the class of N-terminal nucleophile amidotransferases.This enzyme catalyses the reductive transfer of the amide group of L-glutamine to 2-oxoglutrate providing two molecules of L-glutamate [73]. The reductive equivalent is provided by NADH, NADPH or reduced ferredoxin.In recent years x-ray structures of the ferredoxin-dependent glutamate synthase and of the α- subunit of the NADPH-dependent glutamate synthase have become available. Prior to 1970 it was generally assumed that ammonia was assimilated by direct assimilation of 2-oxoglutrate to produce L-glutamate in a single NAD (P) H-linked reaction catalyzed by the enzyme GDH. The pioneering work of Tempest and co-workers [86] demonstrated the existence of a glutamine synthetase/glutamate synthase pathway on low ammonia content. Glutamate synthase (encoded by GltS gene) is a ubiquitious enzyme in nature: it has been detected in prokaryotes, archaea and eukaryotes. However the enzyme is not found in higher eukaryotic systems such as *Homo sapiens*.

On the basis of primary structures and known biochemical properties three different classes of glutamate synthase are distinguished

1. NADPH-glutamate synthase : mostly found in bacteria is specific for NADPH and comprises of two subunits with a large α subunit of about 150 kDa and a smaller β subunit of about 50kDa that form an active protomer containing two flavins cofactors and three feLS clusters.
2. Ferredoxin –dependent glutamate synthase: Mostly found in plants and cyanobacteria, which is composed of only one subunit of about 150kDa, similar to the αsubunit of NADPH-GltS and contains one flavin and one or two, FeIS clusters.
3. NADH-glutamate synthase: Mainly found in fungi, lower animals and non-green tissuses like seeds and roots of plants [73]. The enzyme is composed of a single subunit of about 200 kDa, which is derived from a fusion of the two subunits of NADPH-GltS.

Physiology of GOGAT production

For many years it was thought that bacteria and higher plants assimilate ammonia into glutamate via the GDH pathway, as in certain fungi and yeasts. However, in bacteria it became clear in 1970 that an alternative pathway of ammonia assimilation involving GS and a GOGAT must be operating when ammonia is present in the growth medium at low levels [69]. Thus, nitrogen-starvation leads to derepression and activation of GS (with a high affinity for ammonia) and derepression of GOGAT and repression of GDH (with a relatively low affinity for ammonia).High ammonia availability leads to repression and deactivation of GS and induction of GDH [69].

In contrast to *Candida utilis* [83] analysis of ^{15}N-ammonium assimilation in actively growing mycelium of *Agaricus bisporus* indicates participation of the GS-GOGAT pathway, and no participation of NADP-GDH [8].. $^{13}NH_3$ tracer studies indicate that the GS-GOGAT pathway is the major route of ammonium assimilation in *Candida albicans* and also in nitrogen-starved cultures of *S. cerevisiae* and *Candida tropicalis* [38, 39]. GOGAT is essential for growth under nitrogen starvation, when GS (with its low Km) is used for ammonia assimilation.

The role of glutamine synthetase (GS)

Glutamine synthetase catalyzes the ATP-dependent production of glutamine from ammonia and glutamate's enzyme is an important in the ammonia assimilation system. It produces glutamine, which is essential amino acid, use as an amino donor for synthesis of other compounds. GS consists of 12 identical subunits of 50 kDa (molecular weight of 600 KD). The enzyme has low Km for ammonia compared to GDH so it functions at low ammonia concentrations.

Physiology of GS production

The activity of GS is also controlled by reversible covalent modification the attachment of an AMP unit by a phosphodiester bond to the hydroxyl group of a specific tyrosine residue in each subunit. This adenylylated enzyme is less active and more susceptible to cumulative feedback inhibition than is the deadenylylated form. The covalently attached AMP unit is removed from the adenylylated enzyme by phosphorolysis. The attachment of an AMP unit is the final step in an enzymatic cascade that is initiated several steps back by reactants and immediate products in glutamine synthesis.

The adenylation and phosphorolysis reactions are catalyzed by the same enzyme, adenylyl transferase. Sequence analysis indicates that this adenylyl transferase comprises two homologous halves,

suggesting that one half catalyzes the adenylation reaction and the other half the phospholytic de-adenylation reaction. The specificity of adenylyl transferase is controlled by a regulatory protein (designated P or P_{II}), a trimeric protein that can exist in two forms, P_A and P_D. The complex of P_A and adenylyl transferase catalyzes the attachment of an AMP unit to GS which reduces its activity. Conversely, the complex of P_D and adenylyl transferase removes AMP from the adenylylated enzyme.

This brings us to another level of reversible covalent modification. P_A is converted into P_D by the attachment of uridine monophosphate to a specific tyrosine residue. This reaction, which is catalyzed by uridylyl transferase, is stimulated by ATP and -ketoglutarate, whereas it is inhibited by glutamine. In turn, the UMP units on P_D are removed by hydrolysis, a reaction promoted by glutamine and inhibited by -ketoglutarate. These opposing catalytic activities are present on a single polypeptide chain, homologous to adenylyl transferase, and are controlled so that the enzyme does not simultaneously catalyze uridylylation and hydrolysis.

GS in *N. crassa* is unusually contains two non-identical polypeptides. When *N. crassa* is grown exponentially on ammonium excess, ammonium is fixed by a glutamate dehydrogenase and an octameric GS enzyme .The synthesis of this GS polypeptide (beta) is regulated by the nitrogen source present in the medium; high on glutamate, intermediate on ammonium, and low on glutamine [54].However, when *N. crassa* is grown in fed-batch ammonium-limited cultures a different polypeptide of GS (alpha), arranged as a tetramer, is synthesized [22].. The tetrameric alpha GS is proposed to function with glutamate synthase in the assimilation of low ammonium concentrations [69].

Mutants of the yeast *S. cerevisiae* have been isolated which fail to derepress glutamine synthetase upon glutamine limitation. The mutations define a single nuclear gene, *GLN3*. The elevated NAD-GDH activity normally found in glutamate-grown cells is not found in *gln3* mutants [63]. Glutamine limitation of *gln1* structural mutants has the opposite effect, causing elevated levels of NAD-GDH even in the presence of ammonia [63]. A regulatory circuit that responds to glutamine availability through the *GLN3* product has been proposed propose that production of GS in *S.cerevisiae* is controlled by three regulatory systems. One system responds to glutamine levels and depends on the positively acting *GLN3* product. The second system is general amino acid control, which couples derepression of a variety of biosynthetic enzymes to starvation for many single amino acids. This system operates through the positive regulatory element *GCN4*. A third system responds to purine limitation [63].

In *S.cerevisiae* GS is modulated by nitrogen repression and by two distinct inactivation processes .Addition of glutamine to exponentially grown yeast leads to rapid enzyme inactivation that is reversed by removing glutamine from the growth medium . In the food yeast *Candia utilis* GS is subject to cumulative feedback inhibition by end-products of glutamine metabolism *in vitro*, but this regulation was not demonstrable *in vivo* by direct measurements of the rate of glutamine synthesis [80]. It is regulated by glutamine-mediated repression and reversible deactivation involving dissociation of active octomers into deactive tetramers [24, 81]. Sims and coworkers demonstrated a rapid inactivation of GS in *Candida utilis* on the addition of ammonia to glutamate-grown cultures. An increase in glutamine and a decrease in 2-oxoglutarate is implicated in this control. High glutamine concentrations promote the "relaxation" of the native 15.4 S enzyme into a 14.2 S octamer which dissociates reversibly into two 8.7 S tetramers. PEP promotes relaxation and formation of enzyme tetramers. NAD^+, NADPH and ATP cause dissociation of tetramers into monomers. Glutamate and Mg^{2+} prevent dissociation and promote reassociation of tetramers [81].Whereas 2-oxoglutarate can prevent dissociation of octamers it cannot promote reassociation the tetramers of GS have the same transferase activity as octamers, but have reduced synthetase activity. In the presence of 2-oxoglutarate and glutamate the enzyme can maintain its structural integrity under conditions which would otherwise lead to dissociation [81].

Genetics of GS production

Aspergillus nidulans mutants disrupted in the *gltA* encoding GOGAT were found to be dispensable for growth on ammonium in the presence of NADP-GDH. However, a strain carrying the *gltA* inactivation together with an NADP-GDH structural gene mutation (*gdhA*) was unable to grow on ammonium or on nitrogen sources metabolized via ammonium [57]. *Schizosaccharomyces pombe* mutants lacking either NADPH-GDH or GOGAT are still able to grow on ammonium as sole nitrogen source .Complete lack of growth on ammonium as sole N source is seen only in double mutants lacking both NADPH-GDH and GOGAT [69].

The yeast *S.cerevisiae* synthesizes glutamate through the action of either NADP-GDH, encoded by *GDH1* (under conditions of ammonia excess), or through the combined action of GS and GOGAT, encoded by *GLN1* and *GLT1* (under conditions of ammonia limitation. Dynamic modeling indicates that the GS-GOGAT pathway plays a more important physiological role in yeast than is generally assumed. However, a double mutant of *S. cerevisiae* lacking NADP-GDH and GOGAT activities was able to grow on

ammonium as the sole nitrogen source and thus to synthesize glutamate through a third pathway [7].

Genetic Regulation of ammonia assimilation

When provided with a mixture of nitrogen sources in the growth medium, the organism shows a preference for the utilization of particular nitrogen sources. Preferred nitrogen sources such as glutamine are used first, and then no preferred nitrogen sources such as proline are used only after the preferred nitrogen sources have been depleted. The regulatory pathways that govern this hierarchy are collectively known as nitrogen regulation. In fungi, much of our knowledge of regulation of ammonia assimilation has been mainly from. *S.cerevisiae*, *A. nidulans* and *N. crassa.*

However, not all nitrogen sources support growth equally well. Growth on good nitrogen sources yields relatively higher growth rates than on poor nitrogen sources. Good nitrogen sources are ammonia, glutamine and asparagine whereas proline and urea are qualified as poor nitrogen sources. The organism selects the best nitrogen sources by a mechanism called nitrogen catabolite repression. Nitrogen regulation is the mechanism designed to prevent or reduce the unnecessary divergence of the cells' synthetic capacity to the formation of enzymes and permeases for the utilization of compounds that are non-preferred sources of glutamate and glutamine when a preferred nitrogen source is available.

The elements of nitrogen catabolite repression (NCR)

H. Holzer and his coworkers made the initial observations leading to the concept of nitrogen regulation in *S. cerevisiae*. They showed that the intracellular levels of the NAD^+-linked glutamate dehydrogenase and of glutamine synthetase were much lower in cells grown with ammonia, glutamine, or asparagine than in those grown with glutamate or aspartate as sources of nitrogen.

Amino acid permeases

The amino acid permeases are integral membrane proteins with 12 predicted transmembrane domains which are delivered by the secretory pathway to the plasma membrane which they function to take up amino acids for protein synthesis and for use as source of nitrogen [1,72,]. Grenson, Hou and Crabeel reported the discovery of a general amino acid permease (GAP) present in cells grown with proline, but not in those grown with ammonia as a source of nitrogen [33].General amino acid permease which catalyzes the transport of basic and neutral amino acids, but most probably not that of proline.

The general amino acid permease (Gap1p)appears to be constitutive, and its activity is inhibited when ammonium ions are added to the culture medium.Gap1p is a high-capacity permease that can transport all naturally occurring amino acids .Agp1p is also a general amino acid permease of *S.cerevisiae* which transports most uncharged amino acids encode 19aminoacid permeases .Bap2p, Bap3p, Dip5p, Tat2p, Put4p, Can1p, Cyp1p, Alp1p are some of the permeases found in *S.cerevisiae* [72].The delivery to the plasma membrane of the general amino acid permease, Gap1p, is regulated by the quality of the nitrogen source in the growth medium. Importantly, amino acids have the capacity to signal Gap1p sorting to the vacuole regardless of whether they can be used as a source of nitrogen. Gap1p sorting is not directly influenced by the TOR (target of rapamycin*)* pathway. Amino acids are a signal for sorting Gap1p to the vacuole and imply that the nitrogen-regulated Gap1p sorting machinery responds to amino acid-like compounds rather than to the overall nutritional status associated with growth on a particular nitrogen source [23].

Activation of permeases

When only non preferred source of nitrogen is present the permeases are activated by dephosphorylation. General amino acid is designated as GAP1 and its transcription is positively regulated by the GATA-type transcription factors Gln3p and Gat1p/Nil1p and negatively regulated by the cytoplasmic factor Ure2p, so that *GAP1* is expressed on non preferred nitrogen sources but repressed on preferred nitrogen sources. The GATT-binding family of transcription factors constitutes of DNA-binding proteins whose members both bind a consensus HGATAR motify and contain the class IV zinc finger motify. Most of the proteins described to date include one or two zinc fingers fitting the consensus $CX_2CX_{17-18}CX_2C$ followed by a basic region [42].The quality of the nitrogen source also regulates the intracellular sorting of Gap1p. During growth on the poor nitrogen sources urea, proline, or ammonia, Gap1p is sorted to the plasma membrane and its activity at the plasma membrane is high.

The delivery to the plasma membrane of the general amino acid permease, Gap1p, of *S.cerevisiae* is regulated by the quality of the nitrogen source in the growth medium [23].It is now known that the transcription factors Gln3p and Nil1p of the GATA family play a determinant role in expression of genes that are subject to nitrogen catabolite repression .Gan1p is required for full expression of *GLN1*, *GDH2* and also other nitrogen utilization genes, including *GAP1*, *PUT4*, *MEP2* and *GDH1* [84].

In *A. nidulans* and *N. crassa*, a global transcription factor AREA (NIT2) is responsible for mediating nitrogen metabolite repression. In the absence of the primary nitrogen sources ammonium and glutamine,

this transcription factor, which is a member of GATA type zinc finger proteins, facilitates the expression of more than 100 structural genes involved in nitrogen metabolism. AREA activity is modulated by at least three different mechanisms. One acts at the level of *areA* transcript degradation when sufficient nitrogen sources are available another acts post translational by directly binding a second regulatory protein, e.g. NMR (nitrogen metabolic regulation) to NIT2/AREA under the same conditions. Beside NMR, other regulatory proteins such as TamA may also interact with AREA and thus influence transcription levels of genes involved in nitrogen metabolism. A third factor influencing AREA activity seems to be glutamine. Analysis of glutamine synthetase-defective (*glnA*) mutants of *A. nidulans* revealed that glutamine, and not glutamine synthetase is the key effector of nitrogen metabolite repression.

Evolution of ammonia assimilating enzymes

More than forty species of lower fungi, Myxomycetes and phycomycetes, were found to possess only an NAD-linked glutamate dehydrogenase. The higher fungi, Deuteromycetes, Ascomycetes and Basidiomycetes seem to produce two distinct forms of the enzyme one NAD-linked and other NADP-linked. Among the lower fungi, oomycetes and hypochytridiomycetes have an unusual NAD-dependent GDH, which can represent a transitional form .This type of NAD-dependent GDH is allosterically regulated by NADP and is kinetically similar to the NADP-dependent GDH of higher fungi.

The unique distribution of these two coenzyme specific forms makes it necessary to understand the mechanisms of enzyme regulation of the glutamate dehydrogenases are operative in these fungi. The NAD-linked enzyme of the Phycomycetes can be divided into three classes on the basis of their regulatory properties. All the Chytridiales and Mucorales possess unregulated forms of glutamate dehydrogenase they form the type I enzyme. Type II enzyme is found in members of Blastocladiales, a large aquatic group and in *Absidia*, a genus of the Mucorales. These type II enzymes have a complex multivalent mode of regulation. Divalent metal ions such as Ca^{2+} and Mn^{2+} activate reductive amination reaction but inhibit the oxidative deamination reaction. The type III enzymes were found only in the Oomycetes and Hypochytridiomycetes these are the enzymes which use NAD+ as a substrate in catalysis, only interacting with NADP+ when it functions as an allosteric modulator. When Oomycetes and Hypochytridiomycetes are grown in the presence of glucose or sucrose and limited amounts of amino acids, their glutamate dehydrogenase production is repressed.

Conclusion

Ammonia assimilation is crucial for the survival of all the living organisms. Glutamate dehydrogenase (GDH), Glutamate synthase (GOGAT), and glutamine synthetase (GS) are the key enzymes involved in ammonia assimilation. GDH has evolved from NAD specific in lower fungi to NADP specific in higher ones. The NAD-dependent GDH appear to serve a catabolic function, while the enzyme utilizing NADPH serve primarily for the biosynthesis of glutamate. GDH functions under high concentration of ammonia were as GS under lower ammonia. There is a third GDH3 enzymes that has been identified in *S .cerevisiae* this appears to be the first example of a microorganism possessing three pathways for glutamate biosynthesis. These enzymes appear to be controlled by the nitrogen and the carbon circuits, catabolite repression and regulated by phosphorylation – dephosphorylation mechanisms. Three different classes of glutamate synthase are distinguished Ferredoxin –dependent, NADPH and NADH dependent. GOGAT is essential for growth under nitrogen starvation, when GS (with its low Km) is used for ammonia assimilation. The amino acid permeases function to take up amino acids for protein synthesis and for use as source of nitrogen. When only non preferred source of nitrogen is present the permeases are activated by dephosphorylation. In *A. nidulans* and *N. crassa*, a global transcription factor AREA (NIT2) is responsible for mediating nitrogen metabolite repression. Thus it is evident that lower fungi have a different control and regulation mechanism when compared to higher fungi and different cofactors play very important role in ammonia assimilation under varied conditions.

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