2- Fungal Nutrition and Cellular Biosynthesis

2.1 Chemical Requirements for Growth

Yeasts and fungi have relatively simple nutritional needs and most species would be able to survive quite well in aerobic conditions if supplied with glucose, ammonium salts, inorganic ions, and a few growth factors. Exceptions to this would include, for example, obligate symbionts such as the vesicular-arbuscular mycorrhizal (VAM) fungi which require growth of a plant partner for cultivation. Macronutrients, supplied at millimolar concentrations, consist of sources of carbon, nitrogen, oxygen, sulfur, phosphorus, potassium, and magnesium; and micronutrients, supplied at micromolar concentrations, consist of trace elements like calcium, copper, iron, manganese, and zinc and would be required for fungal cell growth (Table 1a&b).

Table 1a Elemental requirements of fungal cells

Element	Common sources	Cellular functions
Carbon	Sugars	Structural element of fungal cells in combination with hydrogen, oxygen, and nitrogen. Energy source
Hydrogen	Protons from acidic environments	Transmembrane proton motive force vital for fungal nutrition. Intracellular acidic pH (around 5–6) necessary for fungal metabolism
Oxygen	Air, O_2	Substrate for respiratory and other mixed-function oxidative enzymes. Essential for ergosterol and unsaturated fatty acid synthesis
Nitrogen	NH_4^+ salts, urea, amino acids	Structurally and functionally as organic amino nitrogen in proteins and enzymes

Phosphorus	Phosphates	Energy transduction, nucleic acid, and membrane structure
Potassium	K^+ salts	Ionic balance, enzyme activity
Magnesium	Mg^{2+} salts	Enzyme activity, cell and organelle structure
Sulfur	Sulfates, methionine	Sulfhydryl amino acids and vitamins
Calcium	Ca^{2+} salts	Possible second messenger in signal transduction
Copper	Cupric salts	Redox pigments
Iron	Ferric salts. Fe^{3+} is chelated by siderophores and released as Fe^{2+} within the cell	Heme-proteins, cytochromes
Manganese	Mn^{2+} salts	Enzyme activity
Zinc	Zn^{2+} salts	Enzyme activity
Nickel	Ni^{2+} salts	Urease activity
Molybdenum	$Na_2 MoO_4$	Nitrate metabolism, vitamin B12

Table 1b Metals required for fungal growth and metabolic functions

Metal ion	Concentration1	Main cellular functions supplied in growth medium
Macroelements		
K	2–4mM	Osmoregulation, enzyme activity
Mg	2–4mM	Enzyme activity, cell division
Microelements		
Mn	2–4 µM	Enzyme cofactor
Ca	<1 µM	Second messenger, yeast flocculation
Cu	1.5 µM	Redox pigments
Fe	1–3 µM	Heme-proteins, cytochromes
Zn	4–8 µM	Enzyme activity, protein structure
Ni	~10 µM	Urease activity
Mo	1.5 µM	Nitrate metabolism, vitamin B12
Co	0.1 µM	Cobalamin, coenzymes

- Carbon: fungi need fixed forms of organic compounds for their carbon and energy supply (Table 2). [**Supplementary**]

- Nitrogen: fungi are nondiazotrophic (cannot fix nitrogen) and need to be supplied with nitrogenous compounds, either in inorganic form such as ammonium salts, or in organic form such as amino acids. Nitrite can be utilize as a source of N.

  (Nitrite $\xrightarrow{\text{Nitrate reductase then Nitrite reductase}}$ Ammonia)

- Oxygen: most fungi are aerobes and are often described as being microaerophilic (preferring an oxygen tension below that of normal atmospheric). Different fungal species respond to oxygen availability in diverse ways (Table 2).

Table 2 Yeast and fungal metabolism based on responses to oxygen availability

Mode of energy metabolism	Examples	Comments
Obligate fermentative	Yeasts: *Candida pintolopesii* (*Saccharomyces telluris*)	Naturally occurring respiratory- deficient yeasts. Only ferment, even in presence of oxygen
	Fungi: facultative and obligate anerobes	No oxygen requirement for these fungi. Two categories exist with respect to the effects of air: facultative anerobes (e.g. Aqualinderella and Blastocladia) and obligate anerobes (e.g. Neocallimastix)
Facultatively fermentative		
Crabtree-positive	*Saccharomyces cerevisiae*	Such yeasts predominantly ferment high sugar-containing media in the presence of oxygen

Crabtree-negative	*Candida utilis*	Such yeasts do not form ethanol under aerobic conditions and cannot grow anaerobically
Nonfermentative	Yeasts: *Rhodotorula rubra*	Such yeasts do not produce ethanol, in either the presence or absence of oxygen
	Fungi: *Phycomyces*	Oxygen is essential for such (obligately oxidative) fungi
Obligate aerobes	*Gaemannomyces graminis* (the take-all fungus)	Growth of these is markedly reduced if oxygen partial pressure falls below normal atmospheric

- Fungal growth factors: are organic compounds occasionally needed in very low concentrations for specific enzymatic or structural roles, but not as energy. These include vitamins (e.g. thiamine, biotin), purines, pyrimidines, nucleosides, nucleotides, amino acids, fatty acids, and sterols. For fungi to have a growth factor requirement, this indicates that cells cannot synthesize the particular factor, resulting in the reduction of growth without its provision in culture media. Some fungi (e.g. *Aspergillus niger*, *Penicillium chrysogenum*) have very simple nutritional needs and are able to synthesize their own growth factors from glucose.

2.2 Fungal Cultivation Media

Fungal nutritional requirements are important not only for successful cultivation in the laboratory but also for the optimization for industrial purposes.

- **In the laboratory**, it is easy to grow yeasts and fungi on complex culture media such as malt extract or potato-dextrose agar or broth, which are both carbon rich and in the acidic pH range. Mushrooms are cultivated on various solid-substrates depending on their provincial availability. Therefore, *Agaricus bisporus* (common button mushroom) is grown in the United Kingdom, United States, and France on wheat-straw; the padi-straw mushroom (*Volvariella volvacea*) is grown in South-east Asia on damp rice- straw and in Hong Kong on cotton waste; and in Japan, the shiitake mushroom (*Lentinus edodes*) is cultivated on fresh oak logs.

- **In industry**, either use natural media such as malt wort for brewing or molasses for baker's yeast production. For other processes, semi-defined medium are used like corn steep liquor, molasses or malt broth with additional nutrients and growth factors may be necessary. For example, for penicillin production by *Penicillium* spp. the following may constitute a suitable fermentation medium – sucrose (3 g/L), corn steep liquor (100 g/L), $KH_2 PO_4$ (1g/L), $(NH4)_2 SO_4$ (12g/L), $CaCl_2 .2H_2O$ (0.06g/L), phenoxyacetic acid (5.7 g/L); whereas other industrial processes such as the growth of *Fusarium graminarium* for the production of Quorn™ mycoprotein require culture on a completely defined medium.

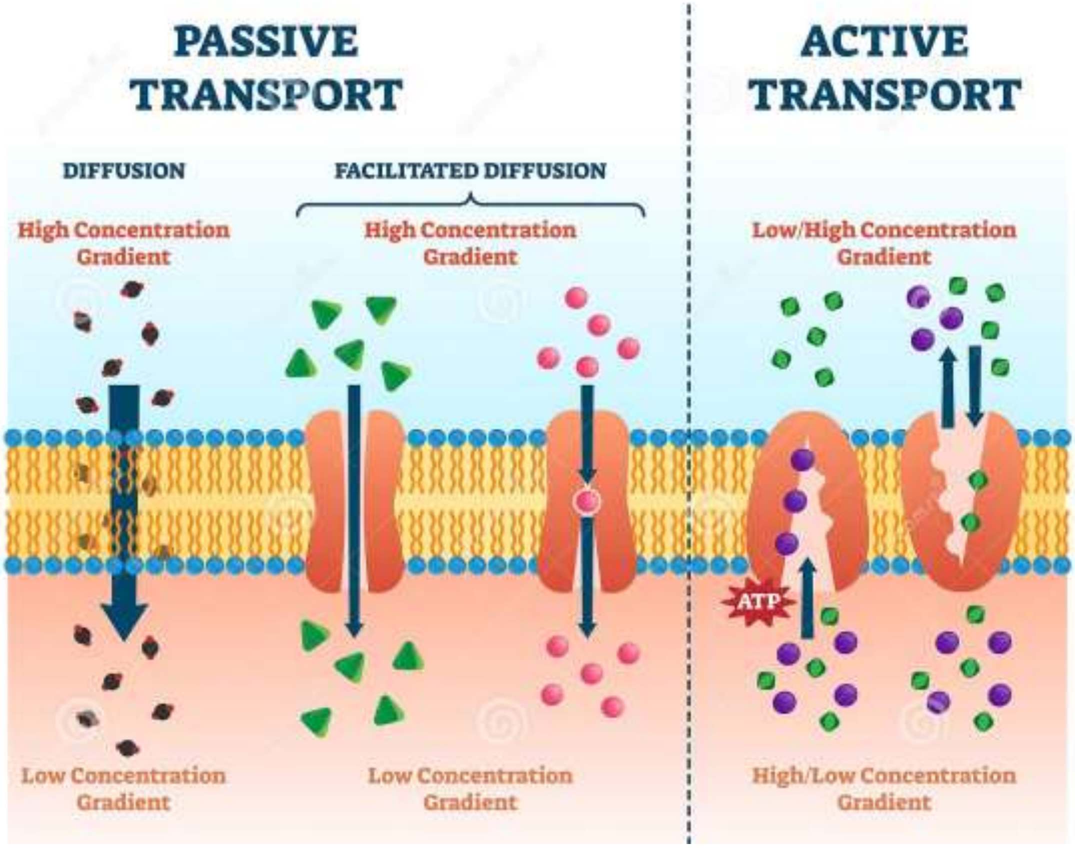
2.3 Nutrient Uptake and Assimilation

In fungal cell, there are several cellular envelope barriers to nutrient uptake, namely the capsule, the cell wall, the periplasm and the cell membrane.

- Fungal cell walls are not a freely porous structures, fungal cell walls are relatively porous/permeable to molecules up to an average molecular mass of around 300 Dalton (Da; atomic mass unit), and will generally retain/keep molecules greater than around 700 Da. Typically, fungi absorb only small soluble nutrients such as monosaccharides and amino acids.
- Plasma membrane is the major selectively permeable barrier which control nutrient entry and metabolite exit from the fungal cell. Membrane transport mechanisms are important in

fungal physiology since they govern the rates at which cells metabolize, grow, and divide. Fungi possess different modes of passive and active uptake at the plasma membrane: diffusion, facilitated diffusion, and active transport (Table 3).

- Active transport of nutrients such as sugars, amino acids, nitrate, ammonium, sulfate, and phosphate in filamentous fungi involves spatial separation of the ion pumps mostly behind the apex, whereas the symport proteins are active close to the tip. Thus, nutrient uptake occurs at the hyphal tip as it continuously drives into fresh resource, and the mitochondria localized behind the apex supply ATP to support the ion pump and generate proton motive force.


PASSIVE TRANSPORT
ACTIVE TRANSPORT
DIFFUSION
FACILITATED DIFFUSION
High Concentration Gradient
High Concentration Gradient
Low/High Concentration Gradient
ATP
Low Concentration Gradient
Low Concentration Gradient
High/Low Concentration Gradient

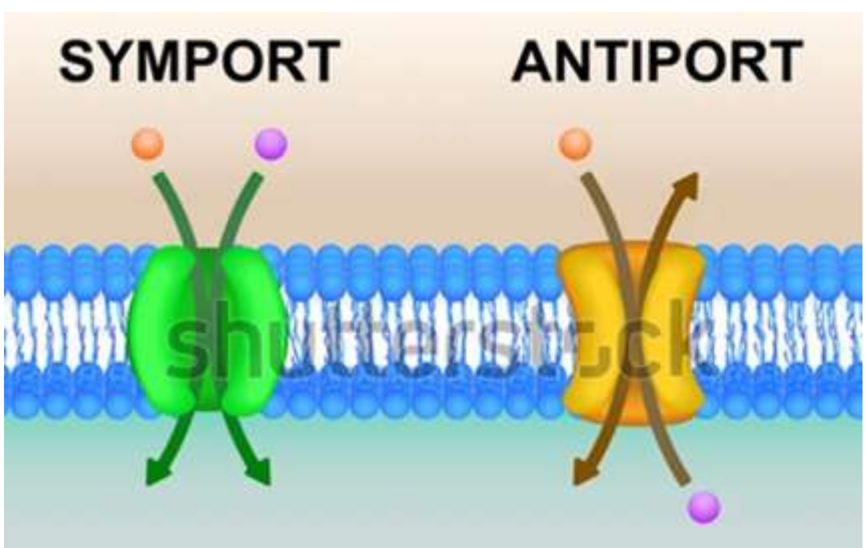

SYMPORT
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Table 3 Difference between Active and Passive Transport

	Active Transport	Passive Transport
1	Requires cellular energy ATP.	does not require cellular energy.
2	Flows from lower concentrated areas to the higher concentrated areas	flows from the higher concentrated areas to the lower concentrated areas
3	Involved transporting all molecules including complex sugars, proteins, large cells, ions, etc. (highly selective)	Involved transporting soluble molecules includes water, oxygen, carbon dioxide, monosaccharides, lipids (non-selective)
4	Involve in the transportation of different molecules in the cell.	Involve in maintaining the equilibrium level in the cell.
5	It Occurs in one direction.	It Occurs in bidirectional.
6	It is Affected by temperature.	It is not affected by temperature.
7	This procedure reduces or stops as the level of oxygen content is reduced.	This procedure is not affected by the oxygen content.
8	In active transport Metabolic inhibitors stop the active transport.	In passive transport Metabolic inhibitors do not influence passive transport.
9	Example: Endocytosis, exocytosis, and sodium-potassium pump	Example: Osmosis, diffusion, and the facilitated diffusion

2.4 Overview of Fungal Biosynthetic Pathways

Anabolic pathways are energy-consuming, reductive processes which lead to the biosynthesis of new cellular material and are mediated by dehydrogenase enzymes which predominantly use reduced NADP+ as the redox cofactor (Figure 1&2). NADPH is generated by the hexose monophosphate pathway (or Warburg– Dickens pathway) which accompanies glycolysis. In S. cerevisiae, up to 20% of total glucose may be degraded via the hexose monophosphate pathway. This pathway generates cytosolic NADPH (following the dehydrogenation of glucose 6-phosphate using glucose 6-phosphate dehydrogenase and NADP+ as hydrogen acceptor) for biosynthetic reactions, leading to the production of fatty acids, amino acids, sugar alcohols, structural and storage polysaccharides, and secondary metabolites. Besides generating NADPH, the hexose monophosphate pathway also produces ribose sugars for the synthesis of nucleic acids, RNA, and DNA and for nucleotide coenzymes, NAD, NADP, FAD, and FMN. This is summarized as follows:

$$\text{Glucose 6 phosphate} + 2NADP \longrightarrow \text{Ribulose 5-phosphate} + CO_2 + NADPH + 2H^+$$

and complete oxidation of glucose 6-phosphate would result in:

$$\text{Glucose 6 phosphate} + 12NADP \longrightarrow 6CO_2 + 12NADPH + 12H^+ + Pi$$

Figure 1 Anabolic pathways

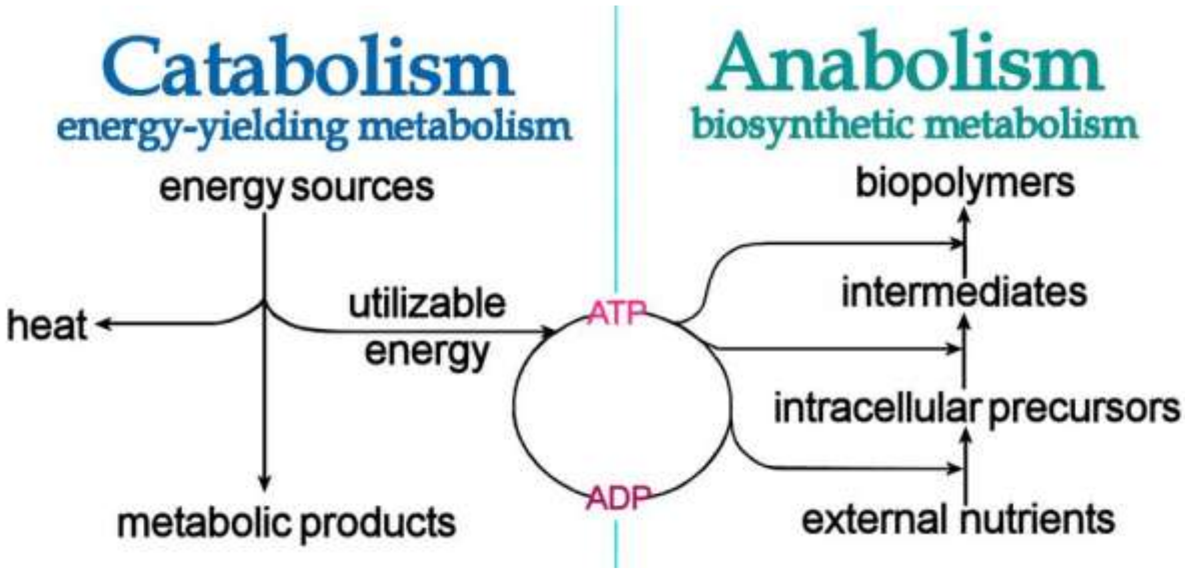
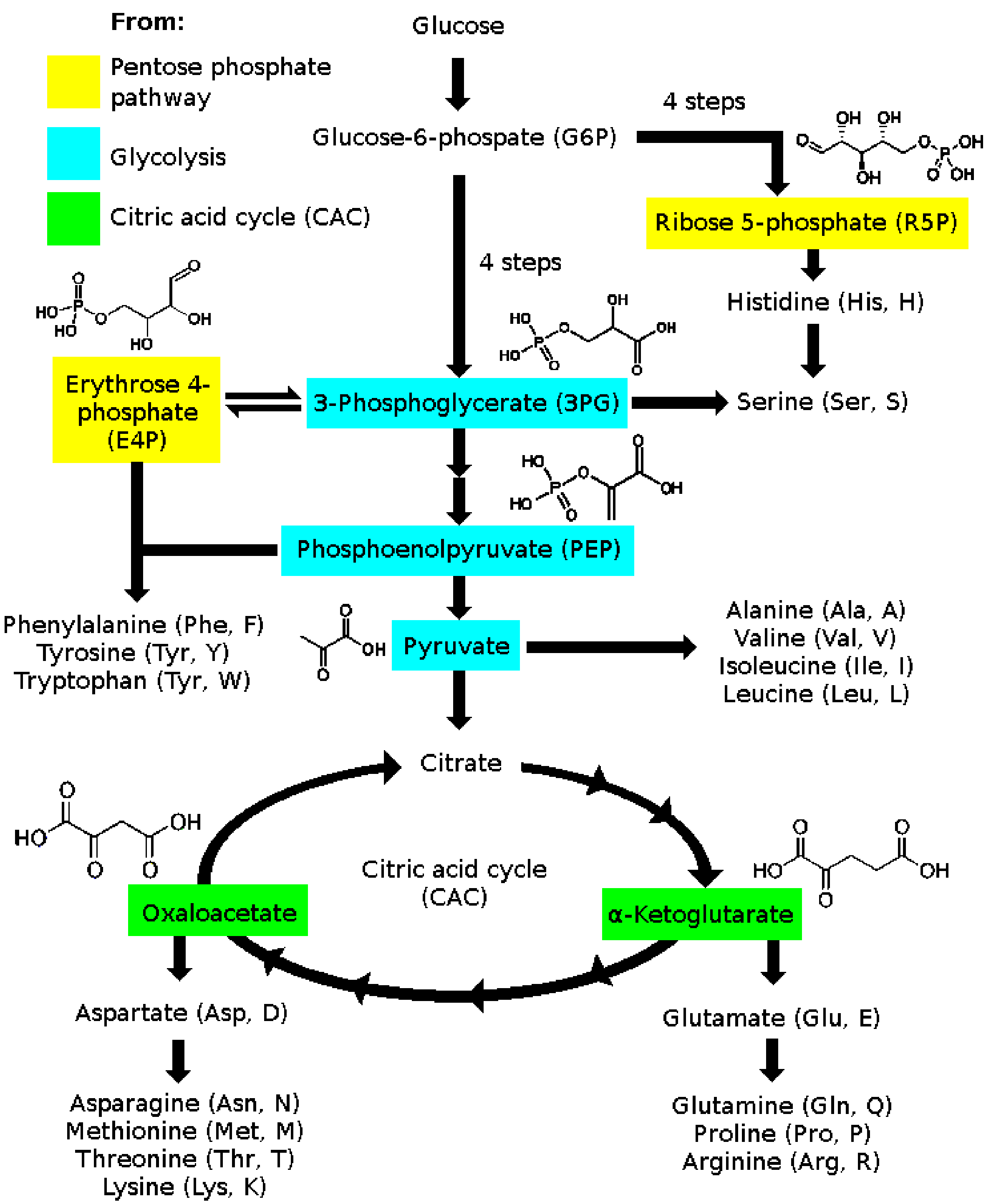
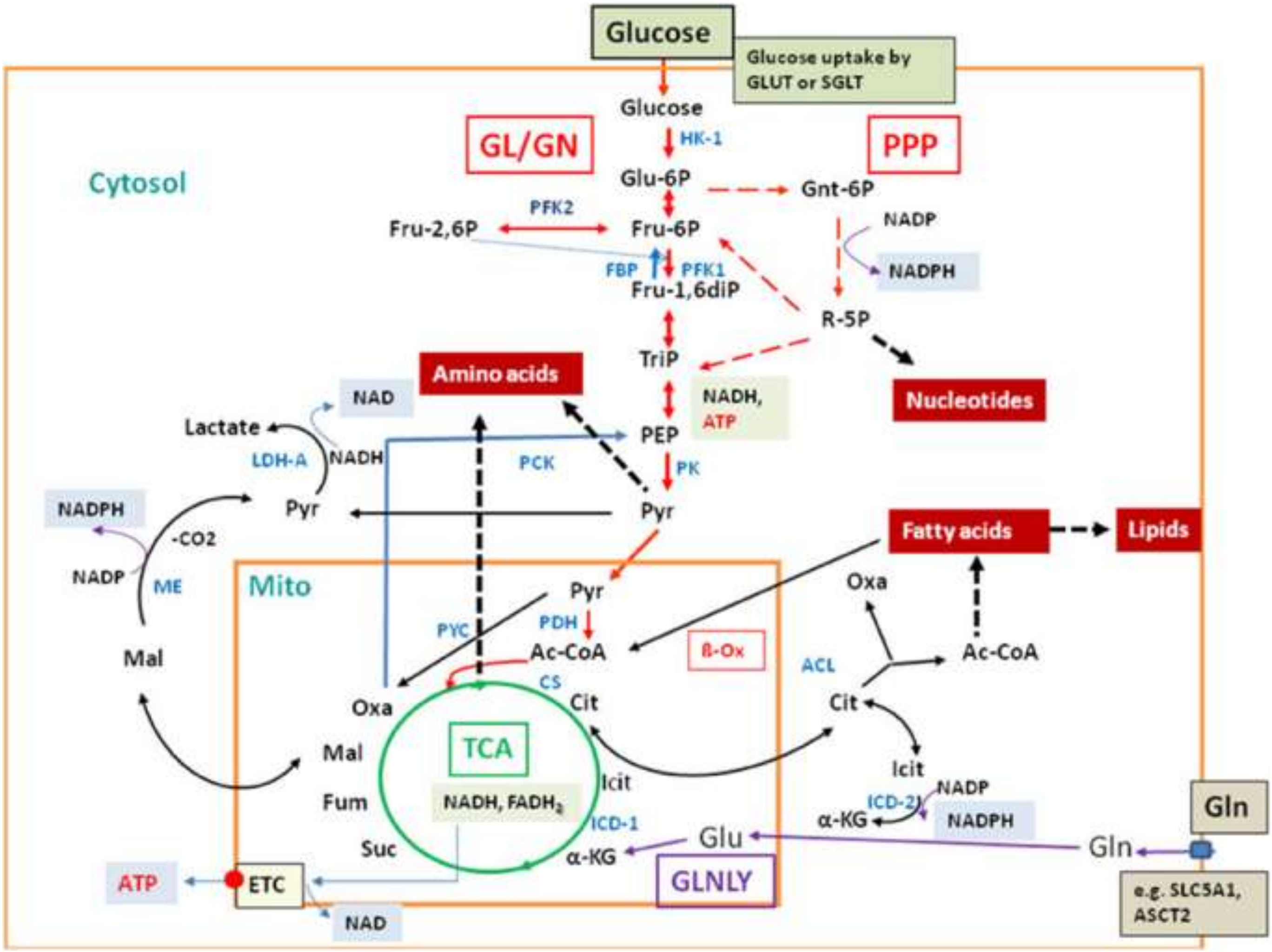


Figure 2 Anabolic pathways





2.5 Fungal Cell Wall Growth (NEXT LECTURE)