Glutamine and Glutamate as key entry points for NH$_4^+$

Amino acid catabolism

**Glutamine synthetase** enables toxic NH$_4^+$ to combine with glutamate to yield glutamine.

Transamination reactions collect the amino groups from many different amino acids in the form of L-glutamate.

**Glutamine synthetase** is found in ALL organisms.
Figure 18-8
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Glutamine and Glutamate as key entry points for NH$_4^+$

Bacteria and plants have *glutamate synthase*

$\alpha$-Ketoglutarate + glutamine + NADPH + H$^+$

catalyzed by *glutamate synthase*

2 glutamate + NADP$^+$
The large size (MW ca. 620 Kda) and the complex regulation patterns of Glutamine Synthetase (GS) stem from its central role in cellular nitrogen metabolism. It brings nitrogen into metabolism by condensing ammonia with glutamate, with the aid of ATP, to yield glutamine. GS is from S.typhimurium, has Mn$^{+2}$ bound, and is fully unadenylylated. Feedback Inhibition: Bacterial GS was previously shown to be inhibited by nine endproducts of glutamine metabolism. Each feedback inhibitor were proposed to have a separate site. However, x-ray data show: 1. AMP binds at the ATP substrate site. 2. The inhibiting amino acids Gly, Ala, and Ser bind at the Glu site. 3. Carbamyl-l-phosphate binds overlapping both the Glu and Pi sites. 4. The proximity of carbamyl- phosphate to the amino acid inhibitors hinders their binding to GS.
Regulation of Glutamine Synthetase in *Escherichia coli* - Cumulative Feedback Inhibition

More inhibitory to adenylylated form of GS
Cascade leading to adenylylation (inactivation) of glutamine synthetase.

GS is finely regulated by reversible inactivation involving a glutamate-dependent covalent attachment of an adenylyl group to a tyrosyl residue of each 12 subunits. This is catalyzed by an Adenylyltransferase (AT). It catalyses both the adenylation and denadenylation reactions. The adenylation to the 12 identical subunits does not have to be total and the activity is dependent upon the degree of adenylation. The partially adenylated GS is more sensitive to feedback inhibition than the unadenylated enzyme. The degree of adenylylation is dependent upon over 40 metabolites.

AT is a single peptide, 115kD. It is activated by ATP, glutamine and the PII regulatory protein. The activator of deadenylylation is αKG. PII regulatory protein can exist in two forms, uridylylate PII which stimulates deadenylylation and deuridylylated PII which stimulates adenylylation. This is catalyzed by Uridylyltransferase (UT).
Enzyme-catalyzed transaminations. In many aminotransferase reactions, α-ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP) as cofactor. Although the reaction is shown here in the direction of transfer of the amino group to α-ketoglutarate, it is readily reversible.

Figure 18-4
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Summary of amino acid catabolism

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Nitrogen-acquiring reactions in the synthesis of urea. The urea nitrogens are acquired in two reactions, each requiring ATP. (b) In the reaction catalyzed by argininosuccinate synthetase, the second nitrogen enters from aspartate. Activation of the ureido oxygen of citrulline in step 1 sets up the addition of aspartate in step 2.
In the presence of excess glutamate and acetyl-CoA there is the increased synthesis of N-acetylglutamate, which is the positive modulator of CPS 1 activity.

**Synthesis of N-acetylglutamate and its activation of carbamoyl phosphate synthetase I.**

**Figure 18-13**
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The 1/2 life of proteins are highly variable, dependent on: misfolding, oxidative damage, translational errors, and specific timing sequences i.e. PEST.

**N-end rule:** On average, a protein's half-life correlates with its N-terminal residue. Proteins with N-terminal Met, Ser, Ala, Thr, Val, or Gly have half lives greater than 20 hours. Proteins with N-terminal Phe, Leu, Asp, Lys, or Arg have half lives of 3 min or less. PEST proteins, rich in Pro, Glu, Ser and Thr, are more rapidly degraded than other proteins.

**Table 23.2 Dependence of the half-lives of cytoplasmic yeast proteins on the identity of their amino-terminal residues**

| Highly stabilizing residues ($t_{1/2} > 20$ hours) |
|---|---|---|---|---|
| Ala | Cys | Gly | Met |
| Pro | Ser | Thr | Val |

| Intrinsically destabilizing residues ($t_{1/2} = 2$ to 30 minutes) |
|---|---|---|---|---|
| Arg | His | Ile | Leu |
| Lys | Phe | Trp | Tyr |

| Destabilizing residues after chemical modification ($t_{1/2} = 3$ to 30 minutes) |
|---|---|---|---|
| Asn | Asp | Gln | Glu |

Ubiquitin is an 8.5 kDa protein, that tags proteins for destruction.

An isopeptide bond links the terminal carboxyl of ubiquitin to the e-amino group of a lysine residue of a "condemned" protein. The joining of ubiquitin to a condemned protein is ATP-dependent. Three enzymes are involved, designated E1, E2 and E3. Initially, the terminal carboxyl group of ubiquitin is joined in a thioester bond to a cysteine residue on Ubiquitin-Activating Enzyme (E1). This is the ATP-dependent step.

The ubiquitin is then transferred to a sulfhydryl group on a Ubiquitin-Conjugating Enzyme (E2). A Ubiquitin-Protein Ligase (E3) then promotes transfer of ubiquitin from E2 to the e-amino group of a lysine residue of a protein recognized by that E3, forming an isopeptide bond. There are many distinct Ubiquitin Ligases with differing substrate specificity. One E3 is responsible for the N-end rule. Some E3’s are specific for particular proteins. Some proteins (e.g., mitotic cyclins involved in regulation of the cell cycle) have a sequence called a destruction box that is recognized by a domain of the corresponding Ubiquitin Ligase.
Selective protein degradation occurs in the proteasome, a large protein complex located in the nucleus and cytosol of eukaryotic cells. The proteasome core complex, which has a sedimentation coefficient of 20S, contains 2 copies each of 14 different polypeptides:

- 7 a-type proteins form each of the two a rings, at the ends of the cylindrical structure.
- 7 b-type proteins form each of the two central b rings.

The 20S proteasome core complex encloses a cavity consisting of 3 compartments joined by narrow passageways. Protease activities are associated with three of the b subunits, each having different substrate specificity: One catalytic b subunit has a chymotrypsin-like activity with preference for tyrosine or phenylalanine at the P1 (peptide carbonyl) position. One has a trypsin-like activity with preference for arginine or lysine at the P1 position. One has a post-glutamyl activity with preference for glutamate or other acidic residue at the P1 position.
Methionine pathway, formation of SAM by the adenylation of methionine. SAM is formed by SAM synthetase.
A schematic representation of the methionine salvage and remethylation pathways showing the alternative routes for metabolism of 5'-methylthioadenosine (MTA) and S-adenosylhomocysteine (SAH) in mammalian (red) and bacterial pathways (blue). Those parts of the pathways that are in common are coloured grey.
Polyamine pathway
Catabolic pathways for alanine, glycine, serine, cysteine, tryptophan, and threonine. The fate of the indole group of tryptophan. Details of most of the reactions involving serine and glycine. The pathway for threonine degradation shown here accounts for only about a third of threonine catabolism. Several pathways for cysteine degradation lead to pyruvate. The sulfur of cysteine has several alternative fates. Carbon atoms here are color-coded as necessary to trace their fates.