Amino Acid Biosynthesis

Obtaining the carbon skeleton

- Experiments with 14C glucose have shown that only **30%** of ingested glucose is released as CO2
  - See card
- However, the pathway is not **anapleurotic** - must be refilled
  - In animals, from **pyruvate** using **pyruvate carboxylase**
    - Requires ATP
  - For example, generation of **oxaloacetate**
  - In plants & bacteria, from **phosphoenolpyruvate** , using **PEP carboxylase**
  - This means that all organisms fix CO2. However, there is no **net gain**, since re-generating the 3C compound implies the loss of CO2

Obtaining reducing power

- **NADPH** acts as the source of reducing power [extra P ensures that it is not re-oxidised via respiratory chain]
  - However, how do non-photosynthetic organisms get it?
- Evidence for another pathway of carbohydrate metabolism apart from glycolysis
  - Identical samples of **pea roots** supplied with [1 - 14C]glucose and [6 - 14C]glucose
  - In the C-1 case, much more label ends up in CO2 than in the C-6 case
  - But should be the same
    - In glycolysis, the two identical triose phosphates produced eradicate the identity of the carbon
  - Seems there's another pathway that release C-1 but not C-6 as CO2
- Purpose of the Oxidative Pentose Phosphate Pathway
  - Provide NADPH for biosynthesis
  - Generate **pentose sugars** for nucleic acid synthesis
  - Act as a route for the **metabolic utilisation** of **pentose sugars**
- Two phases
  - Oxidative
    - **Glucose 6-phosphate** is **oxidised** to **ribulose 5-phosphate**
    - **2 NADPH** produced
    - The enzyme produced are **highly specific for NADP+**
  - Non-oxidative
    - **Ribulose 5-phosphate** converted into **glyceraldehyde 3-phosphate** and **fructose-6-phosphate**
    - Can be used in respiration again
  - This can be used to regulate all kinds of needs, since both stages are reversible
- Controlled to that effect
  - **Oxidative phase** - availability of NADP+ (intensified because NADPH also competes for active site)
  - **Non-oxidative phase** - availability of substrates
- Only minor flux through the pathway, even in growing cells
- **NOT** pathway for glucose oxidation! Doesn't do it completely and doesn't produce ATP!

Obtaining organic nitrogen

- Reducing inorganic nitrogen to NH3
  - Fixation of atmospheric nitrogen
    - Intro
      - Thermodynamically favourable, but very unstable intermediates
      - Higher organisms have lost this ability
      - Some **bacteria** still can - **several cyanobacteria** and the **rhizobium** species (lives symbiotically in roots of leguminous plants)
    - Process
      - **Nitrogenase complex** contains two enzymes
      - *Reductase*
        - 4Fe-4S cofactor
        - Obtains **high energy electrons** from **reduced ferrodoxin** (ultimately from NADPH)
      - *Nitrogenase*
        - MoFe protein
        - Uses those high energy electrons to reduce N2 to NH3
        - Very sensitive to inactivation by O2
        - In leguminous plants' root nodules, O2 is bound to **leghaemoglobin**
    - ATP
      - Used to transfer electrons from one part of the complex to the other
      - At least 2 per electron passed
      - **NOT** to make the process thermodynamically stable, but to overcome height of energy barriers
    - Biologically, not 100% efficient - some H2 produced
      - This means that we need 8 e- per N2
  - Reduction of nitrates in the soil
    - Nitrates (NO3-) very common in soil due to **nitrifying bacteria**
    - Plants and many bacteria reduce this in two steps
    - **Nitrate reductase**
      - Mini e-transfer chain of **FAD**, **cyt b557** and **Mo**
      - 2e- required per NO3- > NO2- (**nitrite**)
      - Electrons come from **NADH** or **NADPH** (depending on tissue)
    - **Nitrite reductase**
      - Contains **sirohaem** and **4Fe-4S centre**
      - 6e- required per NO3- > NH4+
      - Electrons come from **NAPDH** via **ferrodoxin**
- Incorporating NH3 into **glutamate**
  - Directly (**animals** and **fungi**)
    - **Reductive amination** of **ALPHA ketoglutarate** (**2 oxoglutarate**)
    - Done by **glutamate dehydrogenase**
    - Involves an intermediate **Schiff Base**
    - Requires 2 e-
      - Interestingly, in some species, enzyme can't distinguish between NADH and NADPH
    - Important because it determines the stereochemistry
    - See figure 1
  - First into glutamine (**plants** and **algae**)
    - First incorporated into **glutamine** by **glutamine synthase**
      - **Requires ATP** (takes part in reaction by creating an intermediate)
      - Very high affinity for NH4+
    - **Glutamate synthase** then able to reductively aminate alpha ketoglutarate using glutamine as the nitrogen donor
      - Requires NADPH
      - Generates **two** glutamates
    - Advantageous
      - Very high affinity for NH4+
      - Can be useful in low concentrations of ammonia
      - But requires ATP
      - [Effectively, ATP hydrolysis used to capture ammonia]
- Making other amino acids
  - **Transamination**
    - Reversible exchange of amino group between two keto acids
    - Catalysed by **aminotrasnferases**
    - Requires **pyridoxal P** (vitamin B6) cofactor
      - Contains an aldehyde group
      - This forms a **Schiff base** with the enzyme (lysine therein)
      - Enzyme then replaced by incoming AA
      - PLP catalyses transfer to new AA
    - Arginine residue in the enzyme ensures that protonation occurs in the right place to lead tot he correct stereochemistry
  - **Carbon skeleton alteration**
    - For example, proline and arginine
  - There are **9 essential amino acids** that humans aren't able to make

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