LECTURE 15 KF: RESISTING OXIDATIVE STRESS

Oxidation = gain O2, lose H or lose electrons. An oxidant causes oxidation and is itself reduced (gains electrons or loses O).

Sources of oxidants:
Host metabolism/competing bacteria

Phagocytosis (deliberate host defence)

- ROS are made by phagocytes – enzymes MPO (myeloperoxidase) and NADPH oxidase
- MPO is made in the lysosome and is a heme-containing protein that catalyses production of hypochlorous acid (HOCl) from H2O2 and Cl.
- NADPH oxidase – produces an O2 radical from NADPH, can be further converted to H2O2.

Byproducts of own reactions (internal oxidants).

- This is NEVER intentional in bacteria as they have limited O2 tolerance.
- ROS (O2-/H2O2) are produced as *accidental* byproducts of AEROBIC metabolism when an O2 molecule accidentally oxidises redox enzymes (dehydrogenases).
- Redox enzymes should transfer electrons (as hydride anions, H-) from organic substrates to electron acceptor substrates (NAD+/NADP+ or a flavin coenzyme FAD). Reduced flavins MUST pass on their electrons in order to be recycled. We want them to transfer electrons onto secondary redox molecules e.g. iron-sulfur (Fe-S) clusters.
- However, O2 may react with reduced Flavin (FADH2) BEFORE it has passes on its electrons, and the electron instead transfers to O2 to create superoxide (O2-). This can occur with NADH, not just FADH2.
- Oxygen-limiting environments may be advantageous, as too much O2 drives the reaction to be faster, and the above process is more likely to accidentally occur.
- Example situation: the succinate dehydrogenase reaction
    - In the Krebs cycle, succinate is converted to fumarate via succinate dehydrogenase, which reduces FAD to FADH2.
    - FADH2 and its half-life (how long it sticks around for) is important. If there is lots of O2 present, will form superoxide (FADH-O2-).
    - FADH-O2- can be used in two pathways:
        - Can spin flip to form **H2O2** and FAD (more common with NADH dehydrogenase)
        - Can form an **O2 radical** and FAD (more common with **succinate dehydrogenase** and fumarate dehydrogenase)

Anti-oxidant (defence proteins):

- Excess iron drives oxidative stress – bacteria and host need chelators to regulate iron, such as sequestration proteins (lactoferrin) and siderophores (microbial sequestering proteins). These protect from ox damage and allow us to obtain trace elements.
- But when oxidants are produced, can mop them up using ANTIOXIDANTS - disease/damage results due to an imbalance of O/AO. Expression of these increases in response to oxidative stress.

Key antioxidant mechanisms:
<u>OxyR regulatory system (NOT an AO, just interacts with AOs):</u>

- A two-component system, rapidly activated in response to H2O2.
- Component 1: sensing protein
    - Has two cysteines = 199 and 208 = in free thiol form.
    - In the presence of too much H2O2, the cysteines get <u>oxidised</u> to SOH, forming an intramolecular disulphide bond between 199 and 208.

- Component 2: regulatory protein
    - Switched on when the disulphide bond forms and binds to promoter regions for several antioxidant-encoding genes:
        - KatG = catalase (deals with H2O2)
        - AhpCF
        - GorA = glutathione reductase
        - Grx1 = salvage mechanism/feedback to keep OxyR switch on and avoid AO waste. Reduces the disulphide bond of OxyR to recover the inactive form
- Regulatory proteins (TFs) bind to promoter regions of OTHER genes and activate/repress them.

Key antioxidants:

- The first two barriers against endogenous (bacterial metabolism)/exogenous (host) oxidants are SOD and catalase.

SOD = superoxide dismutase → deals with superoxide (O2-)

- Often encode 2 types in case accidentally mutate one, and pathogenic bacteria make LOTS of SOD copies with lots of cofactors
- Superoxide is made as a byproduct of metabolic reactions
- Reiterates the Fenton reaction (?)
- Converts superoxide to H2O2 (then allows catalsae to take over)
- Several bacterial cofactors = Fe-SOD (sodB), Mn-SOD (sodM), Ni-Sod and Cu-Zn-SOD. We need many of these to ensure redundancy, in case in an environment where we lack an element, and to allow for variation in cell location (some speicalised to periplasm/cytoplasm, eetc.)

Alkylhydroperoxide reductase C (AhpC):

- Constitutively expressed in bacteria. We have 405 of this for redundancy.
- Instead of catalysing a reaction like SOD (superoxide to H2O2), these proteins mop up excess oxidant by binding it to themselves, as in OxyR.
- Have an active site cysteine Cys 46 (SP) that is 'catalytic'/'peroxidatic' - becomes oxidised (thus CAUSING reduction of the oxidant) and forms an **inter-**molecular disulphide bond to Cys 165 (SR) - a 'resolving' cysteine on a SECOND AhpC molecule. Byproduct water.
- Therefore each AhpC has 2 x Cys – one catalytic, one resolving – but do not bond each other (unlike in OxyR).
- AhpC is then salvaged (reduced) by the actions of AhpF flavoprotein – a disulphide reductase that contains an iron centre, using NADPH.
- Careful – *excess* ROS may over-oxidate the peroxidatic Cys to Cys-SOH/SO2H/SO3H – which may not be recoverable by AhpF ☹

LECTURE 15 SAQs:

1. **Define oxidation, reduction, oxidant, reductant**
2. **What are the sources of oxidants that bacteria face?**
3. **Is bacteria ROS production ever intentional? How does it usually occur (give an example of the Krebs cycle reaction, and the Fe-S cluster reaction)? In what conditions?**
4. **What biomolecules can oxidants damage? What part of the proteins is targeted? How do these two protein parts change when oxidised? Try to COMPARE them.**
5. **What are the first two barriers against endogenous and exogenous oxidants**
6. **List all of the antioxidant mechanisms (regulators, AO proteins, recyclers/helpers) – what do they deal with (an oxidised protein or an oxidant)? How do they regenerate?**
7. **How is DNA damaged, and how may it be repaired?**
8. **What is the iron conundrum? What is a siderophore? How is iron acquisition regulated?**