* Previously – glycolysis, gluconeogenesis, PPP, Krebs cycle
* A whole bunch of aa generated from Krebs Cycle – only making small amount of ATPs
* We need ATPs
* Oxidative phosphorylation – what happens to the oxygen that we breathe in, what does it react with
* Respiratory states and control
* How mitochondria manage all these things
* Mitochondrial diseases – include autisms – brains cannot generate enough oxygen
* We don’t want oxygen to be free in the cells – O2 reactive
* Look at objectives – make sure you understand before exams
* Make 10^21 molecules of ATP per second – equivalent to your own weight of ATPs – 1 glucose produces 4 ATPs in glycolysis, 2 in CAC – so 6 ATPs
* Make 10 mols of NAD+ per moles of glucose – harness energy from the carriers
* Electrons pumped out and get back into the etc and generate ATPs
* Complex II in ETC is part of CAC – need to think of them all as complexes
* Only 3 chemical reactions – NAD+ to NADH, FADH2 to FAD, and ADP to ATP in oxidative phosphorylation
* OxPhos – 1 integral membrane complex (V) coupled to ETC
* What led to observation of ATP synthesis – in 197, need intact mitochondria membrane for these whole things to occur – mt membrane is impermeable to ions – need to transport using proteins – charged so cannot transfer through bilipid – energy generated is called proton motive force protons from inside to outside mitochondira
  + Need things to make holes in mitochondria – destroy gradients and stop ATP synthesis
  + 2 main ways – make mutation to potential proteins – if mutation stops things from working – OR use drugs to inhibit and stop effect – so the protein is doing the job we’re interested in
* Phosphate-to-oxygen ratio
  + Number of ATP…
  + When transfer electrons from NADH to O2 – some come from FADH2
  + Not a direct connection between them
* Through study using inhibitors, can work out that the electron ratios change – no more 1.5 or 2.5
  + Introduce drugs, ratio changes – know how much it changes – eg. from 2.5 to 1.5, know that the drug inhibited Complex I – coupling site is complex I
  + Only 3 coupling sites – complex II inhibited won’t change the equation
  + But if complex II inhibited – nth to pass to III – will change the pathway too
* Another way of figuring how ATP synthesis works
  + Uncoupler valinomycin – toxic – forms lipid-soluble complex – reduces the chemiosmotic gradient – transfer K+ out – change the charge across the membrane
  + So we need charges for ATP synthesis to occur
* Isolate mitochondria from potatoes
  + No gradient change so no oxidisable substrates
  + If resupspend…valinomysin will transport K+ outside of the membrane – get ATP synthesis occurring without it being inside the cell – as gradient is generated
  + So oxidative phosphorylation is the charge gradient in the membrane
* Peter Mitchell – free energy of electron is conserved…- outside of the mitochondria means intermembrane space in this lecture
* Chemiosmotic coupling
  + Using energy from NADH and moving electrons
  + Each time NADH goes to NAD+, proton gets pushed out
  + From glycolysis and Krebs cycle, 4 protons pumped out from I, 4 from III, 2 from IV – other direction from V
  + Only I, III, and IV pump out protons
  + As protons are pumped out, electrons that move out from I to IV, need to react with something, electrons end up with water
    - E- are so reactive that they just need to react
    - Oxygen comes in and reacti with water
  + Gradients – positive on outside – negative inside – biochemically, any opening anywhere protons will rush back in to balance
  + Atpase – those protons pumped out come back in though ATPase – all effors of I to IV are to pump out protons and bring them back in through ATPase
* How to know it occurs
  + If make holes in membranes or transfer things not through ATPase – no ATP is generated
  + DNP brings that in
  + There is a charge and also pH gradient
  + Pump out a lot of protons – so protons outside lower pH – more acidic outside – more basic inside
  + If uncouple the oxidative phosphorylation pathway simply by making holes or artificially transport protons inside – no ATPs produce
* Respiratory control
  + Respiratory control of ADP and P
  + Once introduce ADP and extra P, the ADP synthase join these 2 together
  + If without those 2, oxygen gets used up in a very slow rate
* Effects of inhibitors
  + Oligomycin – ATP synthase inhibitor – inhibit synthesis of ATP
  + Inhibit complex V or add DNP – even add ADP, amount of ATP will not go up – because uncouple destroys the protein gradients
    - Electrons still used up, oxygen still reacts but not generating ATP
* Complex V
  + ATP synthase
  + C-ring – 10 subunits – 8 in high eukaryotes – protons are pumped in
  + F0 subunit
  + Stalk that holds the molecules together
  + F1 region that moves
  + The binding-change model of ATP synthase
  + F1 has beta and alpha subunit – shape changes depending on whether or not they are bound to ATP or ADP – protein conformation changes – use movement that is amplified – move in circle form – moves molecules for each ATP that is generated
  + ADP binds to open form of conformation between alpha and beta – molecule moves – changes shape – have reaction – get tight forms – ATP get releases when it goes to open form – repeat in cycles
  + No need to remember all steps – just remember that the conformation change occurs that result in physical change that results in movement – movement is the one that generates ATP – need mechanical movement to happen
  + As moving it, it joins ADP and P to generate ATP
  + Movement comes from proton gradient
  + Model is based on X-ray crystal structure
* Rotating counterclockwise is energetically favourable because no repulsive interactions
* Main point – the idea of conformational change – quaternary structure – why certain enzymes work better on certain substrates – what is happening at the interface
* Atpase put inorganic P to ADP through movement of protons gradient
* Bound actin filament that is fluorescently label to the stalk – see the rotation of the molecule – always counterclockwise
* Summary
  + Rotinon in rat poison – target complex I
* Control of oxidative mechanism
  + Kind of like electricity – generated as being used – not being stored
  + Need to be controlled well
  + Low cell charge – high NADH – need greater cyt c o activity – more respiration
  + Can control amount of substrates coming from the Krebs cycle
* Transport through mitochondria membranes
  + Can’t transport stuff across the inner mt membrane – cuz will uncouple the system – need transport system – antiport and symport
  + Outer mt membrane is freely permeable
* Transport reducing equivalents
  + Thru membranes via certain proteins
  + G3P can transfer FADH2 outside membrane
  + Oxaloacetate and malate can control amount of…[recording cut off]