A Brave New World

03 November 2014 11:03

Background

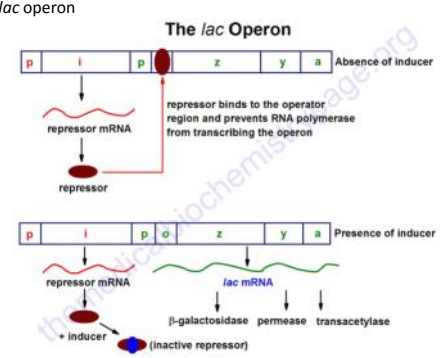
- Genomes are great at coding for functions, but not every function is needed
- Genes must be turned on or off

"What is true of E. Coli must also be true of an elephant" - Monod

Monod -

- Microbes prefer glucose
- A pause in bacterial growth occurs when all glucose has been consumed, before the bacteria can switch to consuming another sugar - **diauxic shift**
- The glucose consumption genes are always turned on, other sugars must be switched on
- Sugar presence regulates which genes are expressed
- Adaptive regulation of genes

E.g. *lac* operon



Repressor protein sits on operator, preventing expression. Inducer (lactose) binds to repressor, causing it to unbind, allowing expression of further genes/
If lactose is present, the enzymes to digest it are produced.
Glucose blocks lactose transport, default "off" position giving priority.

IPTG can act as a synthetic inducer, without being broken down. XGal is broken down to a blue pigment by lactases.

Plasmids

- Can be used to carry a DNA fragment, introduced by ligation
- Telling which bacteria carry a cloning plasmid is easy, because genes for antibiotic resistance can be placed in plasmid.
- Blue-white screening can be used to test for lactose intolerance and *lac* gene plasmid uptake
  - Only cells with plasmids grown on antibiotic treated agar
  - The plasmids carry the *lac* gene
  - The *lac* gene is broken if a DNA fragment is ligated into it, making - lactose intolerant - **white** colour
  - No fragment --> *lac* works --> XGal turns **blue**
  - White colonies contain the desired fragment

So we can swap genes between organisms, and even make them produce proteins.
But what if we could give organisms new function?
Or even make new organisms from scratch?

Synthetic Biology

- From scratch we can make:
  - New enzymes and genes
  - New metabolic pathways
  - New regulatory circuits
  - New biological materials
  - New biosensors
  - New genomes
  - New life forms?
- Synthetic DNA can be made to order
- Synthetic DNA fragments be linked up to create longer DNA molecules
- Entire virus or bacterium genomes can be made
- *Mycoplasma* genome reintroduced into cell in 2010
- Ancient viruses can be resurrected, e.g. from caribou faeces
- **Biosensors**
  - Arsenic
    - Arsenic toxicity affects 137 million people world wide
    - Quick and cheap testing is difficult.
    - New system:
      - *Asperigillus migar* is a fungus used in foodstuffs and is considered safe
      - Researchers measured which genes are turned on by arsenic exposure
      - They discovered one gene, which makes a protein pump to remove arsenic, is expressed 200x if arsenic is present
      - Green Fluorescent Protein was fused to the pump
      - Arsenic causes the fungus to glow green
  - Paper based Ebola test
    - Blood sample on strip
    - Colour change
      - Presence
      - Strain
    - Synthetic gene networks which turn on or off in the presence of Ebola
  - Diesel from *E. coli*
    - Fatty acids are synthesised
    - Modified by a synthetic alkane pathway to make diesel
    - Genes from plants, cyanobacteria, and bacteria stitched together to make the pathway and inserted into *E. coli*
- **Prospects**
  - Potential solutions to heath and envornment challenges
  - Limited by imagination and genetic diversity
  - Risks? Environmental/terrorism/health impacts
  - Ethics - should we be doing this?

Case Study - Ebola

Thursday, November 20, 2014 11:01 AM

- 2013/14 west Africa outbreak
- Guinea, mali, sierra Leone, Liberia
  - Over 5000 dead
- 50% fatality
- Biochemistry of Ebola virus
  - RNA genome
  - Lipid envelope
    - Buds from host
      - Takes part of membrane
    - Can't replicate alone
    - Glycoprotein spikes to attach to cells
    - Genome codes for
      - 7 structural proteins
      - 1 non-structural
    - High mutation rate
      - Rapid adaptation to humans
  - Once proteins have been produced by the cell they self-assemble and bud out, taking membrane
  - Focus has been on VP35 and VP24 proteins for vaccine
    - Looking for differences between virus and host

Drug and vaccine development

- VP35
  - Blocks interferon (immune system antiviral)
  - Block VP35 and the immune response can take effect
- VP24
  - Affects signal transport
  - Blocks STAT1 - interferon transporter
- Brincidofivor and favipiravir
  - Part of other treatments
  - Safe to use on humans
- Treatment strategies
  - Targeting before in host
    - Antibodies
  - Interfere with reproduction
    - Disrupts enzymes
    - Disrupt viral protein formation

Ebola and ethics

- Issues
  - Drug priorities
    - Very limited supplies
    - Not going to west Africans
      - White western doctors
  - Untested drugs
    - Does it work?
    - Has it been tested?
    - What other effects are there?
    - Rushed
  - Imposition of treatment on other countries

pH, Acids, and Bases

04 December 2014 14:16

- Biochemical reactions
  - Aqueous
  - Neutral, acid, basic
  - Buffers maintain pH

Reversible reaction

- Doesn't go to completion
- Equilibrium position depends on temperature/pressure
- Equilibrium constant
  - In the reaction:

$$aA + bB \rightleftharpoons cC + dD$$

$$K_c = \frac{[C]^c\,[D]^d}{[A]^a\,[B]^b}$$

[A] = concentration of A in $moldm^{-3}$
a = number of moles of A

Water

$K_w = [H^+][OH^-] = 1x10^{-14}$

Acid is a proton donor
Base is a proton acceptor

Strong acids dissociate to completion
$[H^+]=[HA]$
Strong bases use K_w to get $[H^+]$ from $[OH^-]$

$pH = -\log_{10}[H^+]$

Weak acids
Give fewer protons than the moles of acid

Buffers

- Aqueous solution
- Weak acid and conjugate base
- Weak base and conjugate acid

$CH_3COOH + H_2O <--> CH_3COO^- + H_3O^+$

- Without the buffer
  - Add OH^-
  - [OH] rises
  - pH rises
- With buffer
  - Add OH^-
  - $OH^- + CH_3COOH --> CH_3COO^- + H_2O$
  - Overall [OH] and pH maintained
- $OH^- + HA --> A^- + H_2O$
- In biological research
  - Enzymology
  - Blood
  - Industrial processes
  - Calibration of pH meters
  - Research