"All bacterial genes are expressed constitutively"

True or False?

Adapting to environmental change

Know any examples of bacterial regulatory systems?

- lac operon

Aims and objectives

- **Main aims for lectures:**
  - Give an overview of how bacteria regulate gene expression
  - provide detailed examples of important regulatory systems
  - highlight how current genomic analysis techniques promote understanding of regulation in individual bacterial species.
- **Main Objectives for students:**
  - to understand the range of strategies used by bacteria to adapt to changing conditions
  - have a detailed knowledge of a set of examples of regulatory systems.
  - to be able to describe how genome analysis methods help elucidate regulatory mechanisms

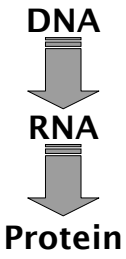
Adaptive responses in bacteria

- Why do bacteria control gene expression?
  - economy –RNA and Protein synthesis
  - selective disadvantage in certain environments
  - clashes in physiological processes
  - developmental pathways
- Define adaptive responses
  - the act of adapting to an environment with a changed set of enzymatic activities.
  - Includes generation of genetic diversity
- Other definitions:
  - regulon: set of genes under the control of a particular regulatory system
  - stimulon: set of genes regulated in response to the same stimulus.

Regulatory Strategies

- Need to modulate gene expression in order to respond to environmental changes.
- Many examples assoc. with virulence determinant expression

- Genomic
- transcriptional
- post-transcriptional
  - pre-translational
  - translational
  - post translational



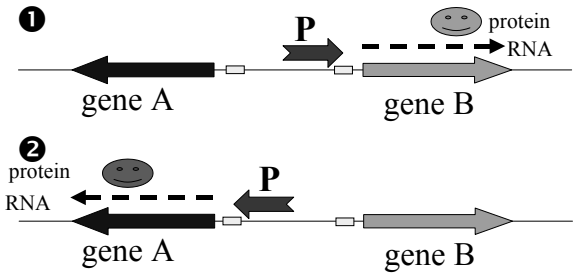
Adaption through the generation of genetic diversity

- Produce genetic diversity in population
- Selection of individuals where gene expression best fitted for environment
  - Unpredictive environmental changes
- Clonal expansion
- Genome plasticity
- Indiscriminate spontaneous mutation disadvantageous
- Genetic change that is:
  - Discriminate,
  - Reversible,
  - High frequency
  - Results in reversible change in gene expression
- Contingency loci

Genomic Changes

- Recombinational
  - genomic rearrangements **example2**
  - gene duplication
  - site-specific recombination (switching promoter elements) **example1**
- Slipped strand mispairing-based events **example3**
  - phase/antigenic variation

'Flipping' promoters



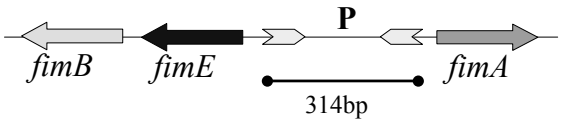
- sequence containing the promoter can invert realigning promoter with new ORF.

The type I pilus in *E. coli*.

A bit of background….

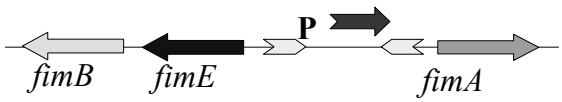
- Pili (US) or fimbriae (UK) –surface appendages associated with attachment to host (eukaryotic) cells
- Type I confers mannose-sensitive adherence
- Produced by *E. coli* and other Enterobacteriaceae (eg *Salmonella typhimurium*)
- expression of pili shows phase variation (ie the phase varies between ON and OFF).

The system…..



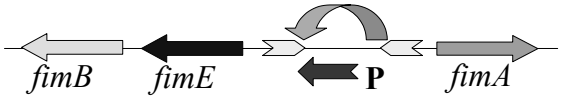
- The *fimA* gene encodes the pilus subunit. Without the FimA subunit there is no pili
- The promoter ("P") is flanked by two 9bp inverted repeats. This forms a 314bp segment
- *fimB* and *fimE* encode recombinases that are very similar

The ON phase: fimbriated



- Promoter element in one orientation.
- Promotes transcription of *fimA* gene
- Synthesis of FimA leads to piliated cell
- ON phase

The OFF phase: non-fimbriated



- Inversion of promoter segment between inverted repeats
- mediated by recombinases
- promotion of transcription of *fimB* and *fimE*
- no transcription of *fimA*
- cells non-piliated and in OFF phase.

Site-specific recombination of the 314bp segment

- The recombinases FimB and FimE are very similar at the amino acid level
- FimB is a segment orientation-independent recombinase
- FimE involved mainly in ON to OFF switch
- needs host accessory factors IHF (integration host factor) and H-NS (binds curved DNA)
  - involved in ensuring DNA around event in correct 3D arrangement

site-specific recombination systems

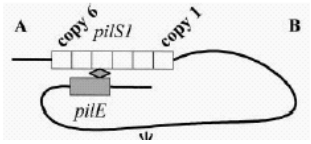
- FimB and FimE are similar to bacteriophage λ integration/excision system
- λ integration/excision system is best understood at molecular level -read up about this
  - look at bacterial cell physiology and growth phase and 'decision' for excision
  - involvement of host factors in bringing recombination sites together.

Gene conversion reactions

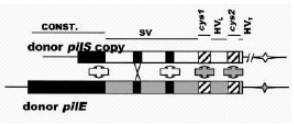
Gonococcal pilin antigen variation

- Avoidance of human host defences
- Maintain high infection rate in high risk population
- Pilus has high level of anitigenic variation
  - Regions of high variation and regions very conserved
- One expressed pilin gene: *pilE*
- Many unexpressed partial copies: *pilS*
- 1-6 copies of *pilS* in 4-6 loci around genome
- Silent copies rarely change despite change in *pilE* gene: Gene Conversion
  - RecA dependent & RecA independent recombination
  - 10-3 recombinants/generation
  - Frequency increases in stressful conditions
  - Requires two chromosomes

The proposed model: cross-over I



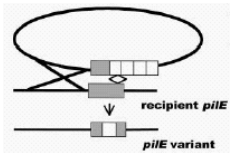
- Intrachromosomal recombination
- One of copies of *pilS* and *pilE*
- Forms episomal circular chromosomal derivative



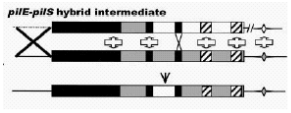
Howell-Adams & Seifert 2000

- Recombination at one conserved region
- Single cross over at one of several conserved regions
- RecA independent

The proposed model: cross-over II



- Second double cross over
- episomal *pilE variant* and chromosome 2
- Episomal variant lost
- pilE variant but no reciprocal pilS: specialised gene conversion

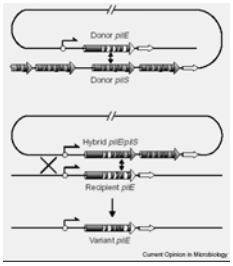


- Recombination between flanking region and conserved region
- One of copies of *pilS* and *pilE*
- "Sma/Cla" region required

Specialised gene conversion

Model predictions tested

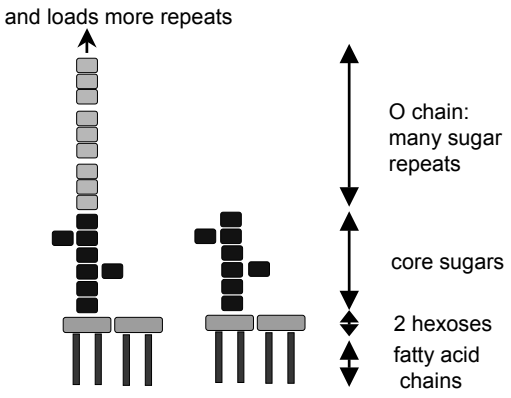
- Two crossing overs at conserved regions
  - Not require RecA
- Forms circular intermediate containing hybrid pilS/E sequence
  - Targeted to recipient pilE locus



- Other examples:
- *Neisseria menigitidis*
  - Cell surface function genes
- *Borrelia burgdorferi*
  - Surface proteins

Phase Variation in lipopolysaccharide biosynthesis in *Haemophilus influenzae*

- what is *Haemophilus influenzae*?
  - Gram negative bacterium that causes meningitis and otitis media
- what is LPS?
  - complex heterogeneous polysaccharide attached to lipid
  - essential in cell wall structure & integrity
  - endotoxic, inflammatory
  - avoid/resist host responses



LPS biosynthesis genes

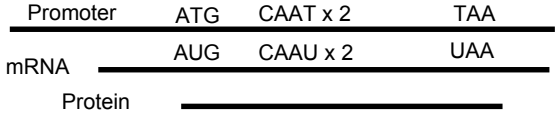
- genes involved in:
  - sugar synthesis,
  - addition to growing oligosaccharide chain
  - specific linkage positions.
- switching on/off expression of certain genes would affect:
  - which sugars added
  - where added
- different phenotype and different epitopes present
- contingency genes

LOS phase variation

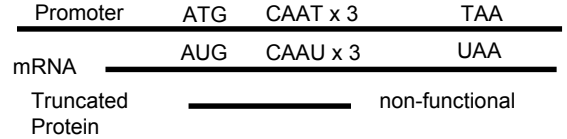
- translation of *lic* genes switched on and off at high frequency
- mechanism involves bringing open reading frame in and out of frame with start codons.
- multiple tandem repeats of 5'-CAAT-3'
- switching by variation in number of CAAT repeats
- probable mechanism of slipped strand mispairing
- transcript still made

Number of CAAT repeats affects protein expression

Two CAAT repeats



Three CAAT repeats



Frame is important

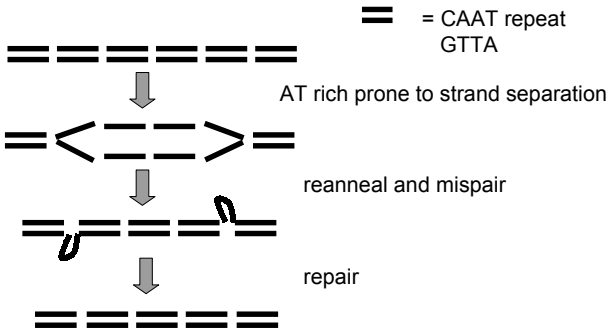
```
start    repeat        stop
ATG TAT CAA TTC TTG TAA ATA G


start    repeatx2
ATG TAT CAA TCA ATT CTT GTA AAT AGnnnn


start    repeatx3                      stop
ATG TAT CAA TCA ATC AAT TCT TGT AAA TAG
```

Actually its more complicated…

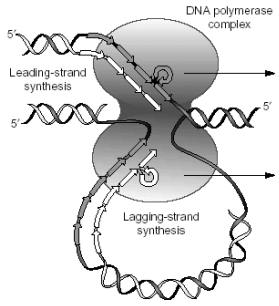
- The *lic* genes have more than one ATG start codons
- *lic1* locus has 29-31 CAAT repeats
- *lic2* locus has 15-17 repeats
- extra levels of regulation

Nº. repeats	ATG used	expression
$(CAAT)_{29}$	none	none
$(CAAT)_{30}$	Nº 3	low
$(CAAT)_{31}$	Nº 1 & 2	high

Mechanisms



Short repeat instability

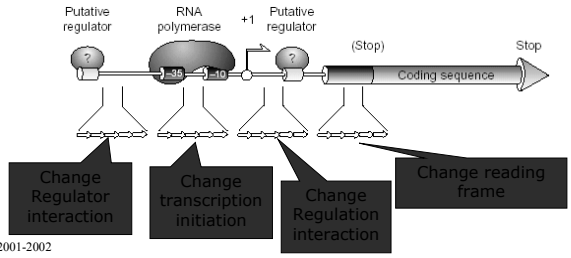


Contraction of repeat when loop forms on template strand in leading strand synthesis

Extension of repeat when DNA pol slips back and forms loop on synthesised strand in lagging strand synthesis

CAAT repeats

- The CAAT repeat number changes due to slipped strand mispairing
- follow expression by coupling to reporter gene –for example LacZ
- RecA-independent

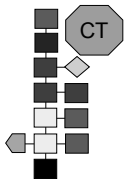


Homopolymeric tracts

- Several examples of homopolymeric runs of G/C causing variation in expression
- number of G bp changes as a result of slipped strand mispairing

- Examples:
  - *Campylobacter jejuni*
  - *Helicobacter jejuni*
  - *Neisseria meningitidis*

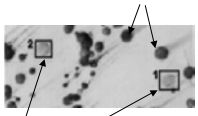
Tracts and *Campylobacter* LPS biosynthesis

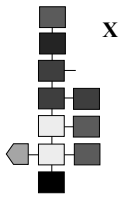


8

aa**g ggg ggg g**ta tgg gta gat ctt gat
K G G V W V D L D

Normal length protein





X CT

9

aa**g ggg ggg gg**t atg ggt aga tct tga t
K G G G M G R S ***

Shortened non-functional protein

Is there control of phase variation?

- Stochastic processes to generate genetic diversity?
- Control frequency and modulate adaptability
- Site-specific inversion systems:
  - In Hin recombinase system:
    - FIS enhancer element between recombination sites: increase in log phase
  - FimB/E system
    - Off to on influenced by temp (>37ºC)
    - Host DNA binding and bending: HNS and IHF
- Gene conversion:
  - *B. burgdorferi*: *in vivo* but not *in vitro*
- SSM:
  - elements of mismatch repair systems missing
  - Tract length
  - Transcription

Summary so far…

- Changing gene expression
- genomic re-arrangements
- 3 examples
  - recombination of genomic segments
  - gene conversion
  - repeats
- population based
- selection driven

- Transcriptional: next topic
- Post-transcriptional