Module 1

Introduction to Microbial Physiology
Last time…
* Discussed the central dogma of molecular biology

* Look at, in detail, the process of replication, transcription and translation as a foundation of topic discussed later in this (and other) courses

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**Topics:**
Introduction to Microbial Physiology (Week 1)

Macromolecular Synthesis (Week 2)

**Structural Assembly (Week 3)**

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**Aims:**
* Outline the basics of post-translational changes that convert an amino acid sequence into a functional protein

* Examine the processes involved in localising cytoplasmically produced proteins to extracellular locations

* Examine the assembly of Gram-positive and Gram-negative cell walls

* Discuss the assembly of surface protrusions such as pili, fimbriae and flagella

* Introduce the process of environmental adaptation using chemotaxis as a model
**Topic 3: Structural Assembly**

Structure of Proteins

- A produced polypeptide is not necessarily a functional one.
- Many structural aspects affecting protein function:
  - Protein folding
  - Translocation / secretion
  - Subunit assembly
  - Post-translational modifications
1. Hydrophobic or nonpolar side groups

- Glycine (gly)
- L-Alanine (ala)
- L-Valine (val)
- L-Leucine (leu)
- L-Isoleucine (ile)
- L-Proline (pro)
- L-Phenylalanine (phe)
- L-Methionine (met)
- L-Tryptophan (trp)
- L-Cystine (cys)

2. Hydrophilic or polar side groups

- L-Serine (ser)
- L-Threonine (thr)
- L-Tyrosine (tyr)
- L-Asparagine (asn)
- L-Glutamine (gln)

3. Acidic side groups

- L-Aspartic acid (asp)
- L-Glutamic acid (glu)

4. Basic side groups

- L-Lysine (lys)
- L-Arginine (arg)
- L-Histidine (his)
• primary structure
  • the amino acid sequence of the protein
    • eg. MKFLDQISGGHDEQ

• secondary structure
  • localised interactions between individual amino acids
    • secondary structures are formed by the conformational entropy of the chain
    • usually H-bonds between amino acids
    • major secondary structures are \( \alpha \)-helices and \( \beta \)-sheets
      • \( \alpha \)-helices are formed by H-bonds between approx. every 4\textsuperscript{th} amino acid
• β-sheets (or pleated sheets) are formed between polypeptide chains that fold back on each other

• folded segments are called domains

• tertiary structure
- interactions between secondary structures to form the correct conformation of the polypeptide
- formed by the interaction of R-groups on specific amino acids
- not necessarily closely positioned in primary sequence
- disulfide bridges between sulfur-containing R-groups
- association between hydrophobic R-groups at the interior of the folded polypeptide
- hydrophilic R-groups located on the exterior of the folded polypeptide
  - weak interactions with polar R-groups or water

- quaternary structure
  - interaction between multiple folded polypeptides to give the final conformation of the functional protein
  - eg. core- and holo-enzyme forms of RNA polymerase

- Post-translational modifications
  - usually affect folding of the polypeptide
  - result in the biologically active form

- all newly synthesised bacterial proteins begin with formylmethionine
  - the formyl group is usually removed by deformylase
  - results in a normal methionine residue

- phosphorylation
  - addition of phosphate groups into specific amino acids in the elongating polypeptide chain
  - can occur at serine, tyrosine and threonine residues
  - done by ATP-dependent protein kinase
• methylation
  • substitution of a methyl group (CH$_3$) for a H atom on lysine residues

• addition of additional carboxyl groups to aspartate or glutamate residues

• conversion of proline to hydroxyproline

• disulfide bridge formation
  • can be interchain or intrachain
  • covalent bond
  • occurs between cysteine residues
  • formed enzymatically by oxidation of 2 sulfhydryl groups
  • may require chaperones

• other modifications as a result of translocation or secretion (see later)

• Protein-assisted folding
  • some proteins naturally assume their final conformation once translated
  • others require additional proteins to fold properly
    • require chaperones (or chaperonins)
    • chaperones are not part of the quaternary structure of the proteins they assist
  • other functions
    • some chaperonins prevent folding of the polypeptide until functionality is required
    • others prevent the denaturation of proteins under hostile environments
    • some aid in secretion (see next section)

• Protein secretion in *E. coli*
  • 20% of *E. coli* proteins reside in the periplasm

  • export to the periplasm is mediated by an amino terminal signal peptide
    • no consensus sequence
    • all structurally similar
    • 20-25 amino acids in length
• short sequence of positively charged residues (N domain) immediately following the initiating methionine
• long stretch of hydrophobic residues (H domain) follows the N domain
• C domain contains the cleavage site and follows the H domain
• signal peptide is cleaved during translocation by leader peptidase

• secretion can be either co-translational or post-translational
  • co-translational – as the message is being translated, the polypeptide is being secreted
  • post-translational – the message is completely translated prior to secretion

• protein export in *E. coli* requires chaperones
  • these proteins associate with the leader peptide and prevent the correct folding of the translating polypeptide

• Major chaperones involved in secretion
  • SecB, SecA, SecEGY

• Mechanism of translocation
  • SecA-polyp binds to SecEGY
  • ATP binds to SecA
  • SecA-polyp integrates into the inner membrane with part of the SecA-bound polypeptide protruding into the periplasmic space
  • the initial translocation event exposes the signal peptide which is cleaved by leader peptidase
  • ATP is hydrolysed
  • SecA releases part of the precursor
  • SecA is released from the membrane
  • the process repeats through rounds of ATP binding and hydrolysis
• Protein degradation
  • abnormal proteins
    • proteins with incorrect primary sequences
    • do not fold correctly
    • aggregate intracellularly
    • broken down by an ATP-dependent endoprotease
      • *lon* gene product
      • first broken down into small peptides
      • then di- and tri-peptides
      • finally, constituent amino acids
      • each degradation catalysed by Lon results in its autoinactivation until another suitable substrate is found

• normal proteins
  • some normal proteins are required to have a short half-life
  • many regulatory proteins need to be unstable in order to react quickly to changing conditions
  • instability can be brought about by…
    • primary sequence
    • conformations
    • chemical modification
Lipids

- general shape
  - polar head
  - hydrophobic hydrocarbon chain
    - saturated (with H atoms): no C=C
      - straight chain
    - unsaturated: has C=C
      - have kinks
      - less stable
Common fatty acids:

- \( C_{16} \) saturated (palmitic)

- \( C_{16} \) monounsaturated (palmitoleic)

- \( C_{18} \) saturated (stearic)

- \( C_{18} \) monounsaturated (oleic)

Simple lipids (triglycerides):
Fatty acids linked to glycerol by ester linkage

Complex lipid:
Phosphatidyl ethanolamine (a phospholipid)

Complex lipid:
Monogalactosyl diglyceride (a glycolipid)

FIGURE 2.7 Fatty acids, simple lipids (fats), and complex lipids. Simple lipids are formed by a dehydration reaction between fatty acids and glycerol to yield the ester linkage.
- simplest lipid: fatty acid
- triglycerides formed by the condensation of 3 fatty acids
  - polar head with 3 hydrophobic tails
- main lipid found in biological systems is the phospholipid
  - 2 hydrophobic hydrocarbon chains
  - phosphate polar head
  - formed by the addition of 2 fatty acids to glycerol-3-phosphate

- Lipids tend to form 3 structures
  - monolayer
    - when on an aqueous surface
  - micelles
    - formed in solution
    - polar heads on the outside
    - hydrophobic tails to the centre
  - bilayer
    - form under higher concentrations of lipids
    - forms the membranes of all cells
- Bacterial (and eukaryote) lipids versus Archaeal lipids
  - Bacteria: ester-links between tails and polar heads
  - Archaea: ether-links between tails and polar heads
Revision - Bacterial Cell Membrane Structures

Gram +

Peptidoglycan
Plasma membrane

Gram -

Outer membrane
Peptidoglycan
Periplasmic space
Plasma membrane
Synthesis of the Gram-positive Cell Wall
- contains thick layer of peptidoglycan
- comprises ~90% of the Gram-positive cell wall

peptidoglycan
- alternating sugar motif connected by inter-peptide bridges
  - N-acetylg glucosamine (NAG)
  - N-acetylmuramic acid
    - conversion product of N-acetylg glucosamine (NAM)
  - peptapeptide bridge
    - added to NAM
    - contains naturally occurring d-amino acids
      - biological systems usually use L- isomers
      - peptide bond is not formed by the ribosome
        - specific ligase that adds amino acids individually
  - the layer that interacts with the environment needs to be hydrophobic
• synthesis
  • 3-step process
    • Stage 1
      • occurs in the cytoplasm
      • NAG is converted to NAM
        • NAG to NAG:UDP (via UTP hydrolysis)
        • NAG:UDP to NAM:UDP (addition of PEP)
      • amino acids added to NAM:UDP
        • ala
        • glu
        • lys
        • ala-ala
    • Stage 2
      • occurs in the membrane
      • NAM-AELAA bind to a carrier lipid
        • undecaprenyl phosphate (C55)
      • NAG is bound to NAM-AELAA
- NAG-NAM-AELAA is released on the other side of the membrane

- Stage 3
  - occurs at the extracellular side of the membrane
  - individual peptidoglycan residues are polymerised into glycan chains
  - transpeptide bridges are formed
    - results in the release of the last ala residue

- outer wall is made up of thick many-layered peptidoglycan
  - as outer layers are lost, new inner layers are made to replace them
  - the loss of outer layers gradually pushes the new layers outward

- Teichoic acids
  - wall-bound acid characteristic of Gram-positive bacteria
  - can be either...
    - wall bound
      - wall teichoic acids
        - formed by the polymerisation of ribitol phosphate or glycerol phosphate
        - joined by a phosphodiester link
        - covalently linked to peptidoglycan through NAM residues
        - -OH group of ribitol or glycerol can be bound to sugars or amino acids allowing a wide variety of structures
        - provides antigenic specificity for individual strains
  
  - membrane-wall bound
    - lipoteichoic acids
      - generally 16-40 phosphodiester linked glycerolphosphate residues bound to a membrane anchor

- all teichoic acids scavenge divalent cations
  - ready supply at cell surface
Gram Positive Cell Envelope

- Lipoteichoic acid
- Peptidoglycan-teichoic acid

Cytoplasm
Synthesis of the Gram-negative Cell Wall

- peptidoglycan in Gram-negative bacteria is produced in an identical fashion to that in Gram-positive bacteria
  - NAG, NAM-pentapeptide are assembled in the cytoplasm
  - inserted into the inner membrane

- placement of proteins etc. in the outer membrane occurs via the continuity of the inner and outer membrane
  - Bayer junctions
A, Peptidoglycan Assembly in Gram-negative Bacteria. Cell wall precursors are assembled from the cytoplasm in the cytoplasmic membrane. They are translocated to the outside of the cytoplasmic membrane by CDP-carrier lipid (bactoprenol) and linked together to form nascent peptidoglycan by penicillin-binding proteins (PBP). The nascent peptidoglycan becomes incorporated into the cell wall layer by transpeptidation performed by other PBPs.

B, Outer Membrane Assembly in Gram-negative Bacteria. The Gram-negative bacterial outer membrane is assembled by three different mechanisms: (1) Phospholipids (LPS) are synthesized in the cytoplasmic membrane. (2) Lipopolysaccharide (LPS) (red) is assembled by enzymes in the cytoplasmic membrane and (3) bactoprenol, which translocates some of the growing polysaccharide chains to the outside of the membrane (as in peptidoglycan assembly). The Lipid A-core becomes attached to these translocated polysaccharide chains to form LPS. Proteins are synthesized on membrane-bound ribosomes and are secreted through the cytoplasmic membrane. The mechanism of movement of phospholipids, lipopolysaccharides and proteins from the cytoplasmic membrane to the outer membrane is not understood.
- Lipopolysaccharides
- LPS are important feature of Gram-negative cell walls
- consist of 3 units
  - Lipid A
    - membrane-bound part of the molecule
  - core polysaccharide
    - essentially the same between all Gram-negative bacteria
  - O-antigen
    - variable polysaccharide region
LPS synthesis occurs in the cell through 2 parallel processes:
- precursors for both processes are assembled in the inner membrane
- Lipid A serves as both a carrier as well as the primer for core polysaccharide addition
- O-antigen is synthesized on undecaprenyl phosphate
- transported to the outer surface
- O-antigen is added to Lipid A-core polysaccharide by a transfer enzyme
Flagella Assembly
- involves over 40 genes
- comprises a basal body, a hook and a tail
- basal body
  - consists of rings
    - 2 in $G^{+ve}$
    - 4 in $G^{-ve}$
  - rod that flagella attached to
- tail is made up of many flagellin sub-units
- assembly
  - bottom-up process
  - basal body assembled first
  - then hook
  - then filament
    - filament is made by exporting flagellin proteins through the central hollow core of the growing flagellum
    - self-assembly
  - structure of the flagellum allows it to spin like a propeller
  - basal body acts like a motor
Pili and Fimbriae

- less is known about their assembly
- pili
  - straight protein rods involved in conjugation (DNA transfer between cells)
  - comprised of pilin proteins
  - pilus tube is too small for pilin monomers to be added like flagellin
  - pilin proteins are synthesised in the cytoplasm
  - contranslationally translocated across the membrane
  - added to the base of the pilus
- fimbriae
  - often have proteins attached to the end
  - adhesins
  - involved in attachment
  - mechanism of assembly is unknown

Chemotaxis

- movement towards or away from chemical attractants or repellents
- signalled through receptors in the periplasmic or cytoplasmic membrane
- results in signals being sent to the flagellum
- two basic actions
  - runs
    - cell travels in a straight line
    - duration is usually 1-2 seconds
  - tumbles
    - cell randomly changes directions
    - duration is usually 0.1 seconds
Chemotaxis
- Major components
  - flagella
  - attractants
    - substances beneficial to the cell
  - repellents
    - substances harmful to the cell
  - methyl-accepting chemotaxis proteins (MCPs)
    - Tap: galactose-galactose-binding protein
    - Tar: aspartate
    - Tsr: serine
    - Trg: ribose-ribose binding protein
  - CheA
    - histidine kinase
  - CheW
    - involved in the autophosphorylation of CheA
  - CheR
    - methyl transferase
    - adds CH$_3$ groups to MCPs
  - CheB
    - methyl esterase
    - removes CH$_3$ groups from MCPs
  - CheZ
    - dephosphorylates CheY
  - CheY
    - interacts with flagella motor switch proteins FliG, FliN and FliM
• Cytoplasmic proteins CheA and CheW are associated with the membrane-bound MCPs

• Attractants / repellents bind to MCPs
• Attractant binds to MCP
  – CheA: sensor kinase
    – binding of attractant to MCP causes conformational change
    – this change (in association with CheW) results in the autophosphorylation of CheA
      – results in CheA-P
      – attractants bound to MCP decrease the phosphorylation rate
      – repellents bound to MCP increase the phosphorylation rate
• CheA-P levels effect 2 aspects of chemotaxis
  1. response of the cell to attractants and repellent by influencing the flagella movement
  2. adaptation to changing concentrations of attractants and repellents

• flagella movement
  – CheA-P phosphorylates CheY
  – results in CheY-P
  – CheY-P interacts with the flagella motor
    – induces clockwise flagella rotation
      ➢ tumble

• CheY
  – central regulator for chemotaxis by governing the direction of flagella rotation
  – CheY cannot interact with the motor
    – must be CheY-P
so far we have just described an on/off switch apparatus
bacteria must be able to adapt to changing concentrations of attractants and repellents – it responds to changes in concentration rather than absolute value
achieved by altering the sensitivity of MCPs for these chemicals
done through methylation
  - methylation of MCPs done by CheR
  - occurs continuously at a slow rate
  - 4 methylation sites on MCP
  - each methylation event decreases the sensitivity of the MCP for the attractant
    - up to 100-fold when completely methylated
    - requires increasing concentration of attractant to maintain current response
Adaptation
- must be able to switch between tumbles and runs
- the actions of CheY-P are disrupted by dephosphorylation of CheY-P with CheZ
  - allows the flagella to return to anticlockwise rotation
  - run
- cells must also be able to alter the sensitivity of MCPs
  - demethylation is also a function of CheA-P
  - results in the phosphorylation of CheB (to CheB-P)
    - CheB-P is a demethylase
    - removes the methyl groups from the MCP and alters their sensitivity accordingly
- by demethylating MCP, CheB acts as a feedback loop, resetting the MCP for excitation (ie. returning it to a sensitised state) after it has moved into a region of low attractant concentration.
• Chemotaxis for increasing attractant concentrations
  – attractant binds to MCP
  – as attractant increases, phosphorylation of CheA decreases
  – reduces the levels of CheY-P
  – flagella motor turns in an anticlockwise direction
  – cell continues to run

  o if levels of attractant remain high
  • levels of CheA-P (and subsequently CheY-P and CheB-P) remain low
  • cell swims smoothly
  • levels of methylation on MCPs increase
  • when fully methylated, MCPs no longer respond to attractant
    ➢ CheA-P levels increase
    ➢ CheY-P levels increase
    ⇒ initiates tumble
    ⇒ CheB-P levels increase
    ⇒ MCPs become demethylated and more sensitive to attractant

• The opposite is true for repellents
  – repellents increase phosphorylation rate
  – fully methylated MCPs respond best to repellents
• Learning Exercises

❖ revise lipids

❖ Gram-positive and Gram-negative cell walls (Topic 1)