

# Week 1 – History of microbiology

11 October 2021 10:06

Sl.No.	Type of Exam(s)	Marks
1	Internal	10
2	Midsem	30
3	Endsem	10
	Total	50

**Total Number of classes 16**

Sl.No.	Dates	No of classes
1	October 11, 18, 25	3
2	November 01, 08, 15, 22, 29	5

Internal = 2 quizzes x 5 marks

## Historically important events

- Robert Hooke - First to visualise microbes under microscope, named them "cells"
- Antony Van Leeuwenhoek - First to observe living microbes
- Francesco Redi - Disprove theory of abiogenesis/ spontaneous generation
- Louis Pasteur - Swan neck flask experiment
- Oliver Holmes: Showed transmission of sepsis via touch
- MJ Berkley - Demonstrated that by great potato blight was caused by fungus
- Edward Jenner- First vaccine
- Robert Koch - Germ theory of disease (the microbe should found in diseased individuals not in healthy, microbes should isolated and grown in pure cultures, cultured microbes should cause disease in healthy individuals, reisolated microbes from inoculated individuals should be identical to original microbes)
- Emil von Behring- passive immunisation
- Walter Hesse - Used agar
- Richard Petri - Developed petri dish
- Christian Gram - Gram staining
- Ferdinand Cohn - Discovery of endospores
- John Tyndall - Sterilisation
- Alexander Flemming - Discovery of penicillin
- Selman Walksman - Discovery of tetracycline and streptomycin
- Dimitri Ivanovski - Discovery of tobacco mosaic virus

# Week 2 –Microbial taxonomy

18 October 2021 10:10

Q: How to determine how many microbes are there in a given sample?

And: Performs 1/10 serial dilutions and check for growth. When no more growth is observed, the total dilution factor tells us about the number of organisms. For example: If 39 colonies are observed in the  $10^{-4}$  dilution, then the total number of microbes in the original sample is  $3.9 \times 10^5$  cfu/ml (colony forming units). CfU/ml is a measure to estimate the number of viable cells in a sample. Colony count between 30-300 is a good plate count.

OpenCFUL: Open source software program to count colonies

NICE: Matlab program

What kind of organisms come under microbes? Protozoans, Fungi, Algae, Yeast, Helminth, Bacteria, Viruses

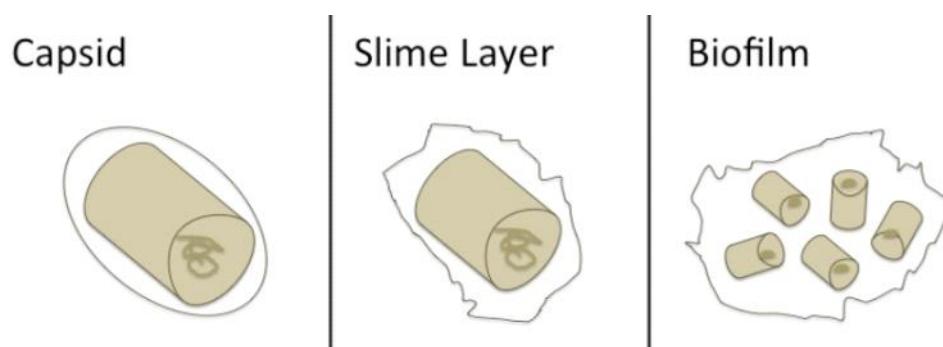
Microbiota(def): The term for collective bacteria and other microbes in a host

## 1. Macroscopic observations of colony morphology

- Size
- Shape
- Colour/pigmentation
- Texture
- Height (elevation)
- Edge (or margins)

## 2. Capsules

- The bacterial capsule is a large structure common to many bacteria. It is a polysaccharide layer that lies outside the cell envelope, and is thus deemed part of the outer envelope of a bacterial cell. It is a well-organized layer, not easily washed off, and it can be the cause of various diseases.
- The capsule—which can be found in both gram negative and gram-positive bacteria—is different from the second lipid membrane – bacterial outer membrane, which contains lipopolysaccharides and lipoproteins and is found only in gram-negative bacteria



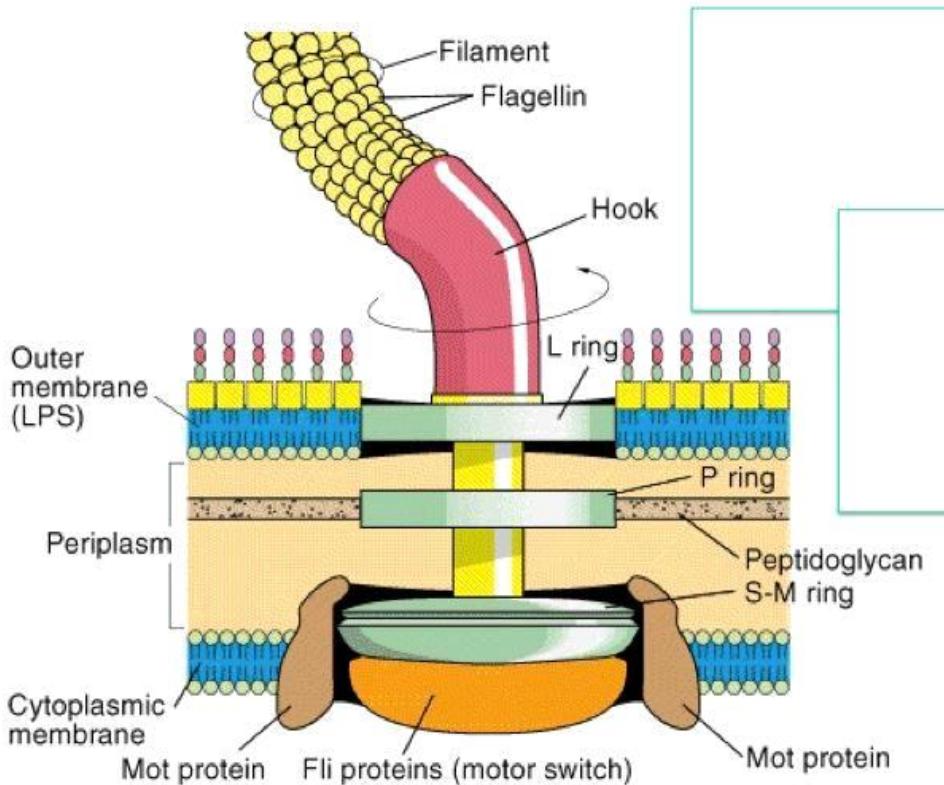
- Function: Protects from phagocytosis, protects from desiccation

## 1. Flagella

- The flagellar motor is composed of a rotor ring complex and multiple transmembrane stator units and converts the ion flux through an ion channel of each stator unit into the mechanical work required for motor rotation.
- Three parts: the basal body as a rotary motor, the hook as a universal joint and the filament as a molecular screw in common  
The flagellar motor is composed of a rotor and multiple stator units. Each stator unit (MoT proteins) acts as a transmembrane ion channel to conduct cations such as protons or sodium ions
- The axial structure of the bacterial flagellum is commonly a helical assembly composed

of 11 protofilaments and is divided into at least three structural parts: the rod, the hook and the filament from the proximal to the distal end. The rod is straight and rigid against bending and twisting and acts as a drive shaft. The hook is supercoiled and flexible against bending and acts as a universal joint to smoothly transmit torque produced by the motor to the filament. The filament is also supercoiled but stiff against bending

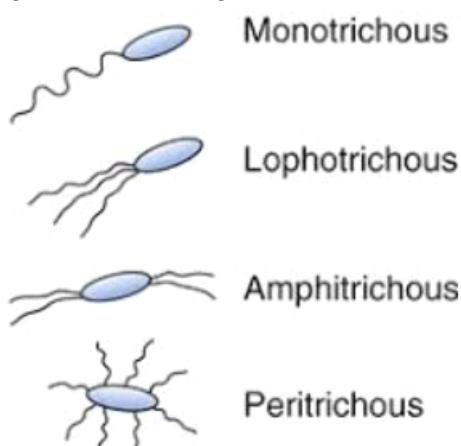
- Flagella filament is made of protein flagellin



- There are four rings in the gram negative bacteria(L,P,MS, C rings) , two in gram positive (MS,C)
- the flagella of spirochetes reside within the periplasmic space and not only act as a cytoskeleton to determine the helicity of the cell body, but also rotate or undulate the helical cell body for propulsion

(Nakamura S, Minamino T. Flagella-Driven Motility of Bacteria. Biomolecules. 2019; 9(7):279. <https://doi.org/10.3390/biom9070279>)

- Organization of flagella



#### 4. Motility Assay

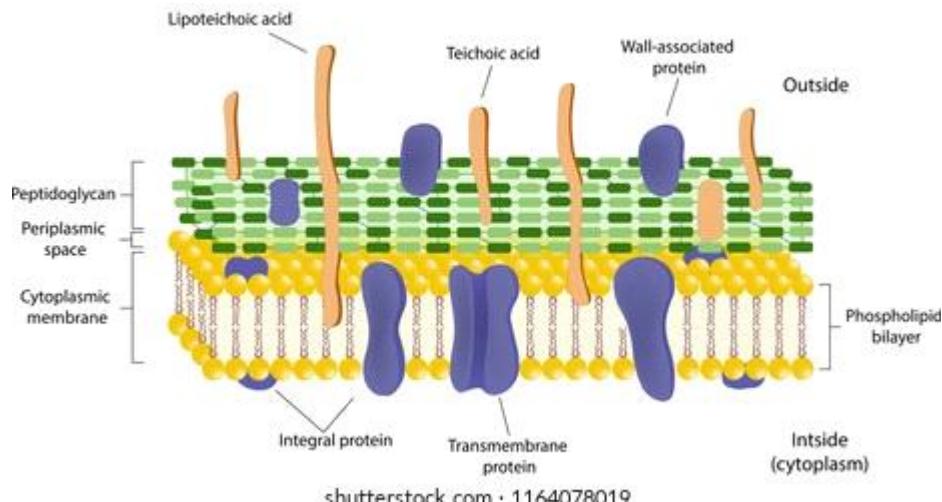
- Can be done with a fresh culture and manual observation under microscope
- Test tube method: inoculate in semi solid medium through a stab line. Motile bacteria will migrate out and make the surrounding media look turbid

#### 5. Gram Staining

- Bacterial cell wall: Their cell walls are made of peptidoglycans. Peptidoglycans are made of a structural heteropolymer of N-acetyl muramic acid and N-acetyl glucosamine. D amino acids are also found in prokaryotic cell walls.

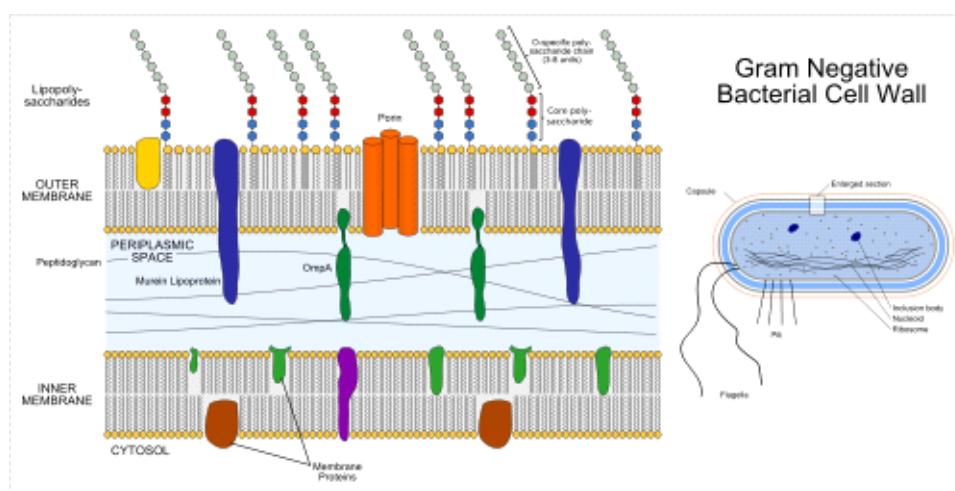
#### 6. For gram positive bacteria, the peptidoglycan layer is thick, with no external lipid layer on top of it. Teichoic acids are unique to gram positive bacteria. Lipoteichoic acids go all the way down to the cell membrane. They help in adhesion. Gram positive bacteria retain crystal violet stain.

##### Gram-positive bacteria

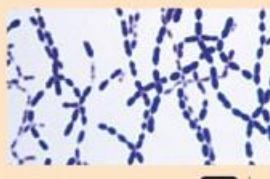


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#### 7. Gram negative bacteria have a very thin peptidoglycan layer (single layered), which has a lipid layer external to it. They contain lipopolysaccharides (LPS). LPS are also termed endotoxins as they trigger immune responses. LPS binds to CDH4/TLR . Based on their location, they can be classified into K antigens (capsule), O antigens and H antigens. Porins can be found in gram negative bacteria. Periplasmic space is also present (which is absent in positive bacteria).

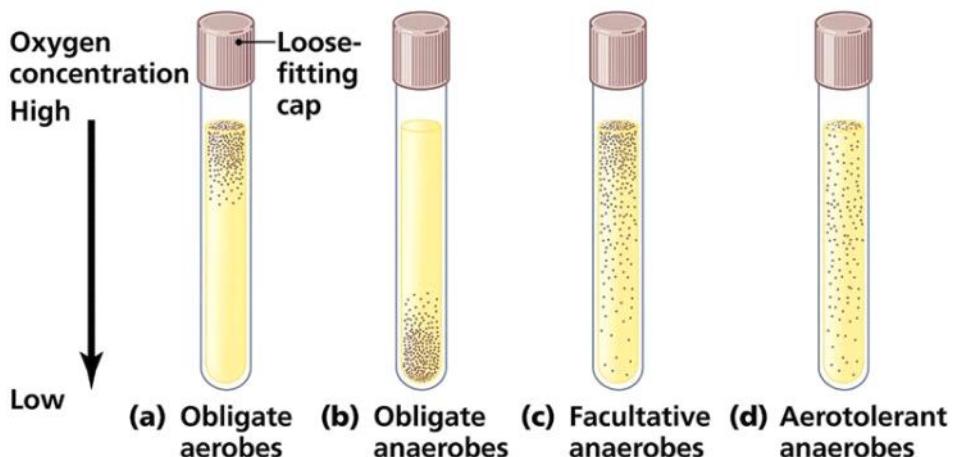


- Peptidoglycan is a heteropolymer of NAM and NAG joined by beta 1->4 glycosidic bond. Cross linked polymer with peptides
- Certain bacteria cannot be classified via gram staining

Characteristic	Gram-Positive	Gram-Negative
	 LM 4 μm	 LM 4 μm
Gram Reaction	Retain crystal violet dye and stain blue or purple	Can be decolorized to accept counterstain (safranin) and stain pink or red
Peptidoglycan Layer	Thick (multilayered)	Thin (single-layered)
Teichoic Acids	Present in many	Absent
Periplasmic Space	Absent	Present
Outer Membrane	Absent	Present
Lipopolysaccharide (LPS) Content	Virtually none	High
Lipid and Lipoprotein Content	Low (acid-fast bacteria have lipids linked to peptidoglycan)	High (because of presence of outer membrane)
Flagellar Structure	2 rings in basal body	4 rings in basal body
Toxins Produced	Exotoxins	Endotoxins and exotoxins
Resistance to Physical Disruption	High	Low
Cell Wall Disruption by Lysozyme	High	Low (requires pretreatment to destabilize outer membrane)
Susceptibility to Penicillin and Sulfonamide	High	Low

## 8. Aerobic and anaerobic test

- Microbes can be classified on basis of their respiration by growing them in sodium thioglycolate culture (which consumes oxygen)



- Obligate aerobes: Need oxygen, so gather at the top
- Obligate anaerobes: Gather at the bottom, poisoned by oxygen
- Facultative anaerobes: Can respire aerobically or anaerobically but prefer aerobic respiration due to higher energy output
- Aerotolerant: Survive without oxygen but are not poisoned by it

## 9. Catalase test

- Catalase converts hydrogen peroxide into water and oxygen. Formation of bubbles indicated presence of catalase (release of O<sub>2</sub>)

## 10. Hemolysis test

- Some bacteria produce exoenzymes capable of lysing RBCs and haemoglobin. Bacteria are grown in agar + 5% sheep blood.
- Beta hemolysis: Complete breakdown of both RBC and haemoglobin, leaving a clear region on the plate
- Alpha hemolysis: Partial break down of RBCs, leaving a greenish colour. Colour due to

biliverdin, by product of haemoglobin breakdown. Caused by hydrogen peroxide production.

- Gamma hemolysis: No hemolytic activity

#### 11. Coagulase test

- Coagulase converts fibrinogen (soluble) to fibrin (insoluble) and is found in some bacteria.

#### 12. Acid Fastness

- The property of certain cells to resists decolourisation by treatment with acid/ ethanol.  
Eg: *Mycobacterium* (responsible for tuberculosis). Acid-fastness due to mycolic acid in cell walls.

#### 13. Ziehl-Neelsen stain technique to test for acid-fastness

- Heat the fixed smear of bacteria (makes lipid membrane more fluid and allows for penetration of stain)
- Wash with acid/alcohol solution (decolourisation test)
- Stain with counter stain. Non-acid fast bacteria take up counter stain.

#### 14. Capsule Stain - to visualise capsule

- Combination of india ink (stains background) and crystal violet (stains bacterial cells). Capsule is visible as a halo.

#### 15. Citrate utilisation test

- To see if microbes have the ability to utilise citrate as a carbon source. Culture media contains citrate and ammonium phosphate.
- Upon metabolism of citrate, ammonia is released from the salts, pH increases and is visible as a colour change ( colour change from green to blue)
- Eg: Positive bacteria - *Salmonella*, *Citrobacter*
- Negative bacteria - *Escherichia*, *Shigella*

## PART 2

*Gevers, D., Cohan, F., Lawrence, J. et al. Re-evaluating prokaryotic species. Nat Rev Microbiol 3, 733–739 (2005). <https://doi.org/10.1038/nrmicro1236>*

### Microbial Species Concept

No general consensus on definition of species for prokaryotes.

At present, a prokaryotic species is defined as “a category that circumscribes a (preferably) genetically coherent group of individual isolates/strains sharing a high degree of similarity in(many) independent features, comparatively tested under highly standardised conditions”

Practically, a prokaryotic species is considered to be a group of strains (including the type strain) that are characterized by a certain degree of phenotypic consistency, showing 70% of DNA–DNA binding and over 97% of 16S ribosomal RNA (rRNA) gene-sequence identity.

Polyphasic approach to taxonomy: combining phenotypes and genotypes.  
This includes phenotypic data (for example, the results of biochemical tests, fatty-acid composition), genotypic data (for example, DNA fingerprint data) and phylogenetic information (for example, rRNA gene sequences).

Phenetic approach: relying on phenotypic features for taxonomy. Not useful as taxa can often be polyphyletic. Includes morphology, cell type, nutrition type, staining properties, metabolism. Does not give information on evolutionary histories.

## DNA-DNA Hybridisation

DNA–DNA hybridization is a molecular biology technique that measures the degree of genetic similarity between pools of DNA sequences. It is usually used to determine the genetic distance between two organisms and has been used extensively in phylogeny and taxonomy.

The DNA of one organism is labelled, then mixed with the unlabelled DNA to be compared against. The mixture is incubated to allow DNA strands to dissociate and then cooled to form renewed hybrid double-stranded DNA. Hybridized sequences with a high degree of similarity will bind more firmly, and require more energy to separate them: i.e. they separate when heated at a higher temperature than dissimilar sequences, a process known as "DNA melting".

Disadvantages: Time consuming, not suitable for rapid identification, not applicable for prokaryotes that cannot be cultured, a sample cannot be analysed against a database (technique only allows pairwise comparison)

Threshold: >70% DDH for same species

## rRNA sequencing

It allows for classification based on universal trait, comparison can be done against a database. Fast and easy. Useful phylogenetic marker or assigning to families. Usually done for 16srRNA gene for bacteria. Existence of universal primers due to existence of conserved domains.

Method lacks resolution when it comes to taxonomy due to slow rate of evolution. Merely having high (>97%) sequence similarity does not imply that they are same species (other criteria not met).

## Multi locus Sequence Typing

rRNA sequences do not evolve fast enough for high resolution of species. MLST involves sequencing multiple loci to buffer against distortions caused by recombination. Usually 6-7 housekeeping genes ubiquitous to the taxa. Can be used to different members of same genus.

Other methods: GC content (if two isolates differ in their GC concentration by more than 10% they likely are different species). Serotyping - on the basis of reaction with particular antibodies. Phage detection-

# Week 3 – NGS and culture techniques

01 November 2021 14:46

## Bacterial metagenomics

(Thomas T, Gilbert J, Meyer F. Metagenomics - a guide from sampling to data analysis. *Microb Inform Exp.* 2012;2(1):3. Published 2012 Feb 9. doi:10.1186/2042-5783-2-3)

- The primers for the amplification step is designed for the conserved regions that are ubiquitous.
- Performing analysis of isolated from different sources can be done together if the isolates were amplified using different primers with unique identification markers.

## Next Generation Sequencing

- Whole genome sequencing can be done by breaking up genome via RE or sonication
- First generation
- Second generation:
- Third generation: allows for long reads. Nanopore protein

## Illumina

- Steps involved in Illumina sequencing:
  - Generation of library by fragmented the DNA
  - End repair and addition of dA
  - Ligation with adaptor on both ends. Adaptor contains the binding site for primers, the index (barcodes), and the region complementary to oligos on the flow cell
  - Add to flow cell membrane which has complementary sequences to this adaptor
  - Perform bridge amplification to generate the cluster. Each DNA sequence in the original library generates its own cluster. Wash away reverse strands
  - Add cyclic reversible primers, excite with light and capture the emission.
  - Wash away dNTPs and remove the blocking group
  - Repeat until complete
  - Now repeat for sequencing the index sequence of the cluster on both adaptors
  - Repeat with reverse strands

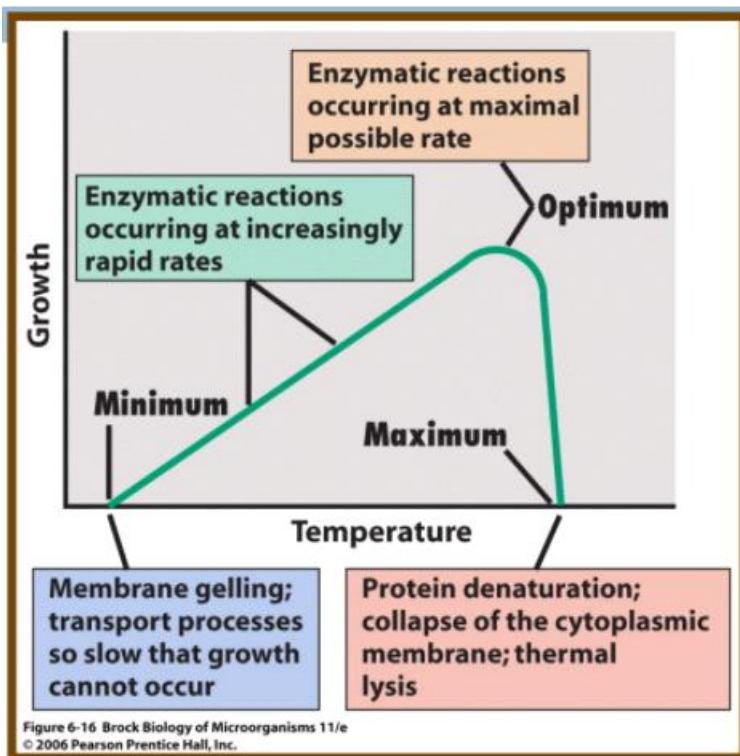
## 454 Sequencing System

- Prepare adapter ligated ssDNA library
- Emulsion PCR for clonal amplification: micelle droplets containing dNTPs, polymerase,
- Load beads and enzymes onto plate
- Perform 454 sequencing

## Nanopore

- Advantages: sequencer size, read length

<b>Defined media:</b> precise amounts of purified chemicals	<b>Complex (non-synthetic) media:</b> high nutritious substances where the quantities of certain components are unknown
<b>Selective media:</b> selects for growth of target organism and inhibit unwanted microbes Eg: <b>Mannitol salt agar:</b> for <i>S. aureus</i>	<b>Differential media:</b> allows for differentiation of organisms based on some properties. Shows up as visible changes in colour/bubbles/precipitation Eg: <b>Spirillum Blue Agar:</b> Dark blue colour around colonies that hydrolyse fats, <b>blood agar</b> for hemolysis, mannitol salt agar



- Acidophiles: 1-4 pH (*Helicobacter pylori*)
- Alkalophiles: 8.3-11 (*Vibrio cholera*)
- Most grow in 6-8 pH
- Psychrophiles (0-20), mesophiles (20-45), thermophiles (50-80), hyperthermophiles (80+)

Liquid media	Water based, do not solidify	LB broth, methylene blue milk, thioglycolate
Semi-solid media	Clot-like consistency	Sulfide indole motility (H <sub>2</sub> S, indole, motility) rest
Solid media	Useful for isolating/culturing	Gelatin/ agar(liquefiable) Cooked meat media, potato slices (non-liquefiable)

- Enrichment media: used to encourage growth of a particular microbe. Eg: mannitol salt, blood agar.
- Pour plate: pour media + culture and colonies grow inside solidified media  
Spread plate: spread culture on solid media, colonies only grow on surface

# Week 4 - Prions

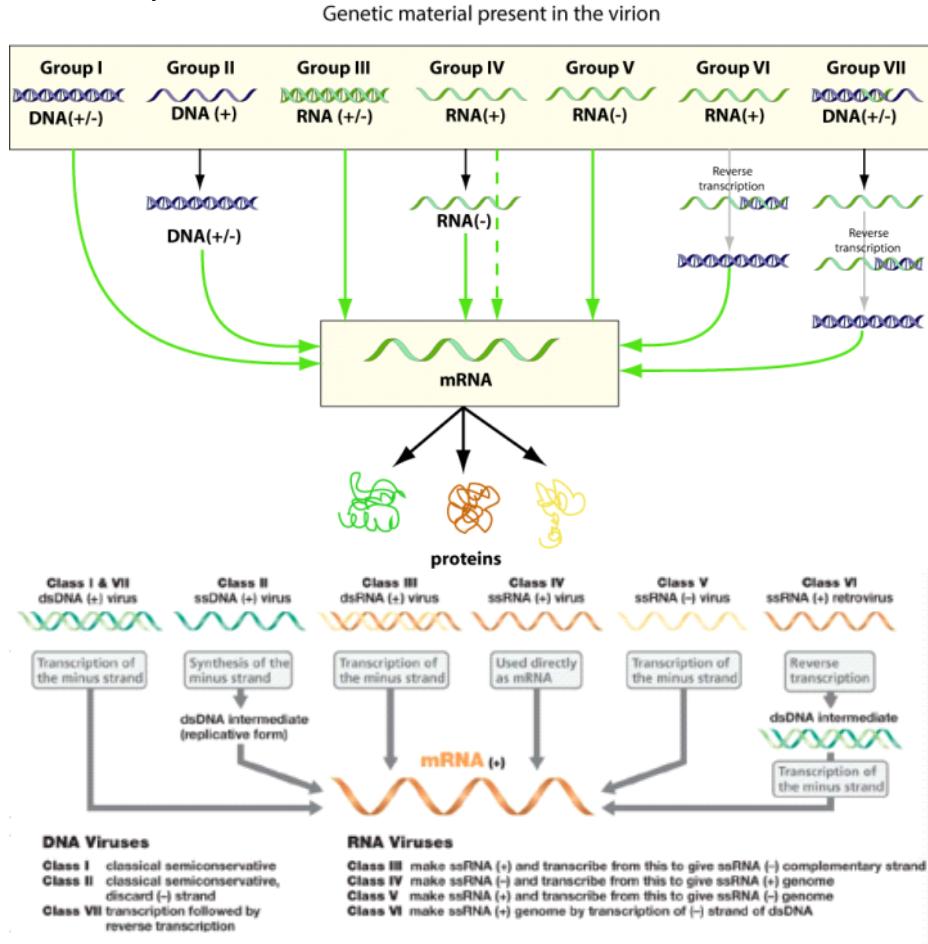
27 November 2021 17:09

- Def: Prion: A type of protein that can trigger normal proteins to misfold. Eg of prion disease, Creutzfeldt Jacob Syndrome, Bovine spongiform encephalopathy (mad cow disease), scrapie. PD, AZ, ALS also have possible prion based pathology but not for PrP<sub>c</sub> protein.
- They are capable of transmission and infection
- Resistant to proteases, high temperature or standard disinfection procedures. Not degraded by proteasome.
- Prions induce formation amyloid aggregates. PrP<sub>Sc</sub> amyloid aggregates as proto-fibrils and fibrils capable of replication. Ends break off and participate in seeding.
- Prions exist in 2 forms: PrP<sub>c</sub> and PrP<sub>Sc</sub>. Disease is caused by conformational conversion of PrP<sub>c</sub> to PrP<sub>Sc</sub>, the pathogenic form which itself catalyzes further conversion of PrP<sub>c</sub>. Conversion increases % of beta sheet and PrP<sub>Sc</sub> has protease resistant core. (*Wei Chen et al, Structural and Dynamic Properties of the Human Prion Protein, Biophysical Journal, 2014*)
- PrP<sub>c</sub> is a normal cell-surface glycoprotein that highly conserved among different species. It plays in Cu uptake in post synaptic nerve cells, protects from oxidative stress.
- Causes of prion diseases:
  - Sporadic: rapid and random misfolding
  - Familial: mutations of PrP<sub>c</sub> that make it susceptible to misfolding.
  - Transmissible: From one individual to another. Eg: Transmissible spongiform encephalopathy (TSE). Neurodegenerative disease.
- Potential therapy: drugs to disrupt replication cycle, vaccine against epitopes of PrP<sub>Sc</sub>, etc.

# Week 5 - Viruses

27 November 2021 18:14

- General structure: **genetic material**, **capsid** and **envelope** (may not be present) and envelope proteins (may or may not present).
- Some viruses have segmented or fragmented genetic material
- Baltimore system of classification



- (+) RNA (non-coding): Directly translated
- (-) RNA (coding): Needs RDRpol to form (-) strand that is transcribed

## Viral pathogenesis

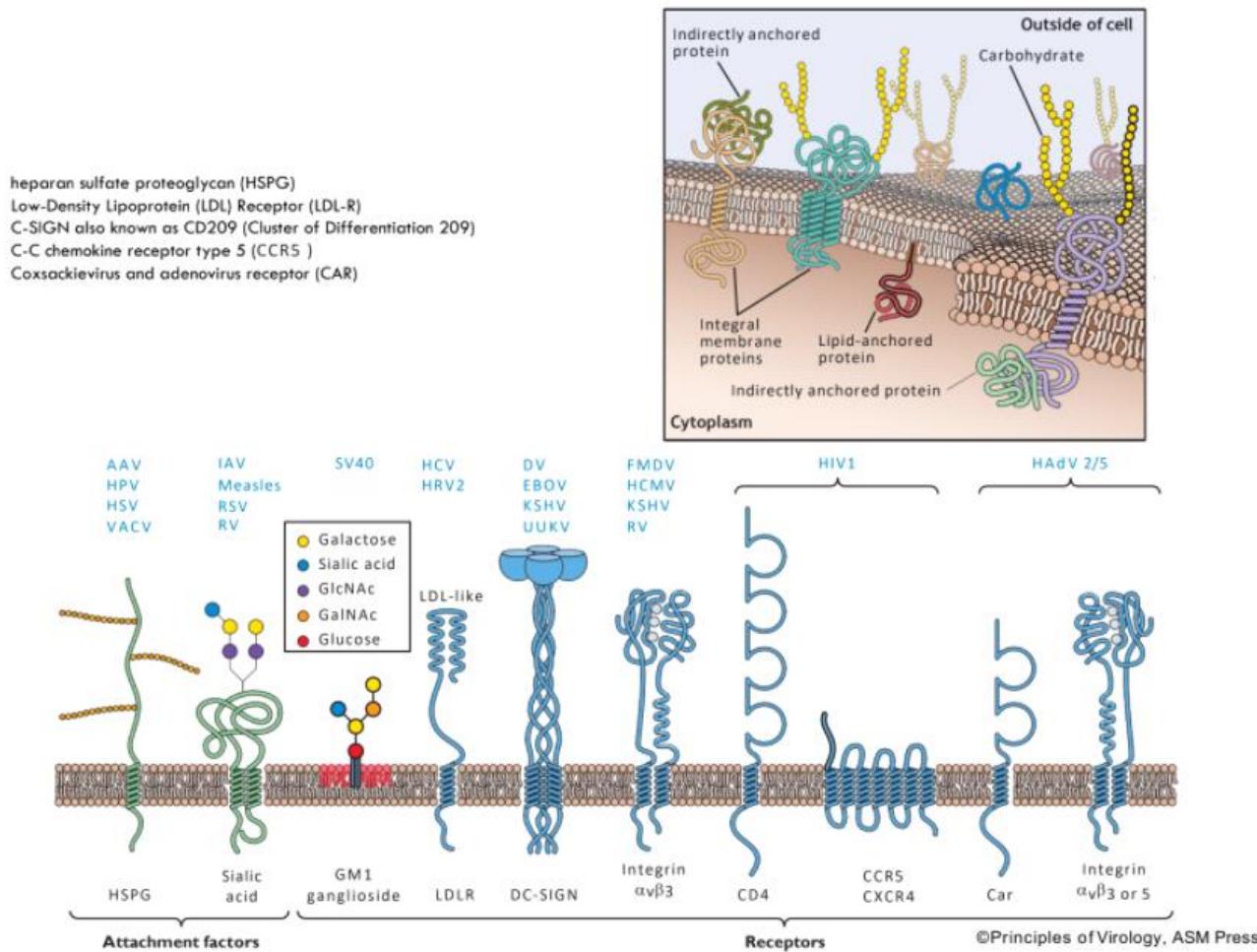
- Many infections are subclinical. Pathogenesis is when viral infection leads to disease.
- Forms of chronic infection: Silent subclinical. Long incubation period, reactivation to cause acute disease,
- Factors in viral pathogenesis: entry into host, course of infection, cell/tissue damage and tropism, host immune response, viral clearance or persistence
- Mucus contains **defensins, lysozyme and other antibiotics**
- Bronchiolitis:** RSV (respiratory syncytial virus), influenza virus, parainfluenza, adenovirus
- Croup** (lower respiratory infection): RSV, para, influenza virus
- Upper respiratory infection:** rhinovirus, coronavirus, RSV
- Tonsilitis: EBV, Adenovirus

## Viral entry into cells

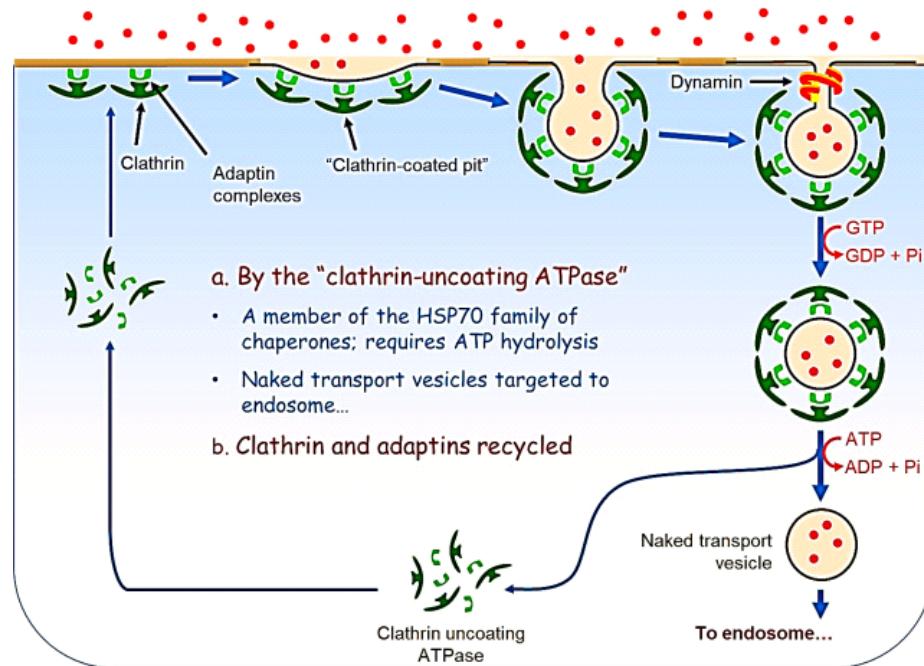
- Virus-receptor binding is highly specific for species and cell type. **Viral receptors can be protein, carbohydrate or lipid**
- Co-receptor: in some viruses they bind to two receptors to enter cells and

- Attachment factors: Simply allow for virus to attach to the cell vs viral receptors which allow for entry into cells

(Jolly C.L., Sattentau Q.J. (2006) Attachment Factors. In: Pöhlmann S., Simmons G. (eds) Viral Entry into Host Cells. Advances in Experimental Medicine and Biology, vol 790. Springer, New York, NY. [https://doi.org/10.1007/978-1-4614-7651-1\\_1](https://doi.org/10.1007/978-1-4614-7651-1_1))



- Receptor mediated endocytosis (clathrin-mediated endocytosis): Eg: adenovirus



- Adaptins and clathrin form a coated pit containing the ligand and dynamin (GTPase)

**pinches the membrane to form a vesicle**

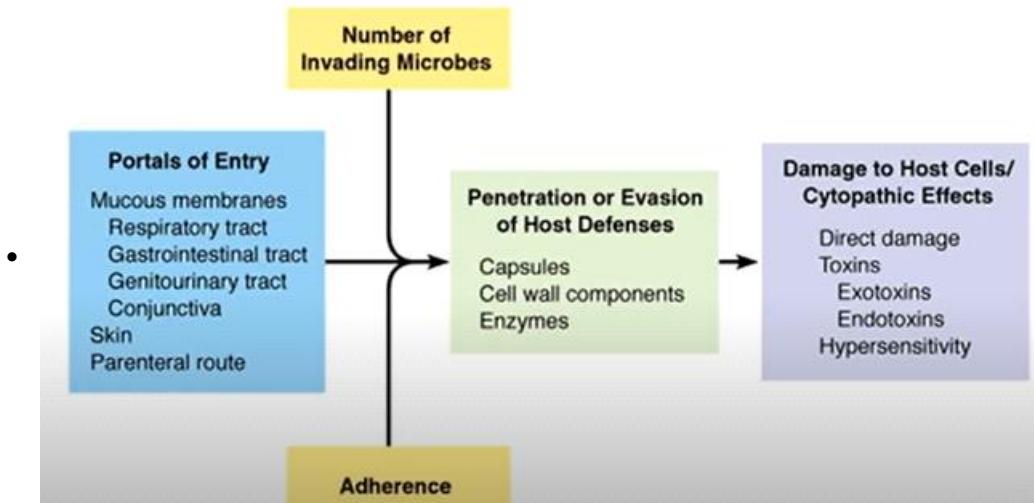
- Low pH triggers escape of viral particle from the endosome
- Fusion with cell membrane is another method (HIV)
- Caveolae are omega-shaped invaginations of the plasma membrane with a diameter of 50-100 nm.

# Week 6 - Microbial pathogenesis

04 December 2021 11:56

Pathogenicity: the ability to produce disease	Virulence: the degree of pathogenicity, or disease producing power
Primary infection: infection that develops in an otherwise healthy individual	Secondary infection: infection that develops in someone already infected with another pathogen
Clinical infection: observable or detectable symptoms	Subclinical infection: no or few symptoms
Mechanical animal vector: physical transmission by the animal but pathogen does not grow or incubate inside the vector. Eg: pathogen sticks to the body of fly	Biological animal vectors: incubation or part of life cycle completed in the vector. Eg: malaria

- Main portals of entry: skin, mucosal membranes, parenteral



- Unbroken skin is impenetrable to most microbes but some can gain access by hair follicles and sweat glands
- Parenteral route:** microbes are deposited into tissue below the skin or mucosal membranes (Eg: through cuts, lesions)
- LD50:** lethal dose that kills 50% of inoculated test subjects
- ID50:** Dose that will cause a demonstrable infection in 50% of inoculated subjects
- Adhesins:** virulence factors that allow bacteria to attach to host cells. Can be present on glycocalyx, fimbriae, viral capsids.

F proteins	on the ends of lipoteichoic acids of <i>S. pyogenes</i> . F proteins bind to fibronectin.
fimH	gene codes for mannose binding residues on the fimbriae of <i>E. coli</i>
M proteins	of <i>S. pyogenes</i> binds to serum factor H and helps evade phagocytosis and opsonization

- Antigens on *Salmonella typhimurium*: Somatic (O antigens): LPS and Flagellar (H antigens): highly immunogenic
- Mycobacterium: gram positive and acid fast (generally)
- Atypical TB: Mycobacterium avium complex infection are caused by *M. avium* and *M. intracellulare*. No known drugs.

# Week 7 - Microbial pathogenesis

13 December 2021 11:28

Gram negative spiral bacteria	Gastroenteritis	<i>Campylobacter jejuni</i>
Gram negative spirochetes	Syphilis	<i>Treponema pallidum</i>
	Lyme disease	<i>Borrelia burgdorferi</i>
	Legionnaire's disease (lower respiratory tract infection)	<i>Legionella pneumophila</i>
Gram negative aerobic rods	Pertussis	<i>Bordetella pertussis</i>
	Opportunistic pathogen	<i>Pseudomonas aeruginosa</i>
Gram negative facultative rods	Cholera	<i>Vibrio Cholerae</i>
	Flesh eating disease	<i>Vibrio vulnificus</i>
	Enteric bacteria	<i>E Coli, Salmonella, Shigella</i>
Gram negative anaerobic bacteria	Gum disease	<i>Fusobacterium</i>
Gram negative cocci	Gonorrhoeae	<i>Neisseria gonorrhoeae</i>
	Meningitis	<i>Neisseria meningitidis</i>
	Chlamydia	<i>Chlamydia trachomatis</i>
	Rocky Mountain spotted fever	<i>Rickettsia</i>
Mycoplasma (no cell wall so you cannot treat with penicillin)	Pneumonia	
Gram positive cocci	MRSA (skin infection)	<i>Staphylococcus aureus</i>
		<i>Streptococcus pyogenes</i>
Gram positive rod	Tetanus (spoon shaped due to endospore)	<i>Clostridium tetani</i>
	gangrene	<i>Clostridium perfringens</i>
	Anthrax (zoonotic source)	<i>Bacillus anthracis</i>
	Diphtheria	<i>Corynebacterium diphtheriae (pleioformic)</i>

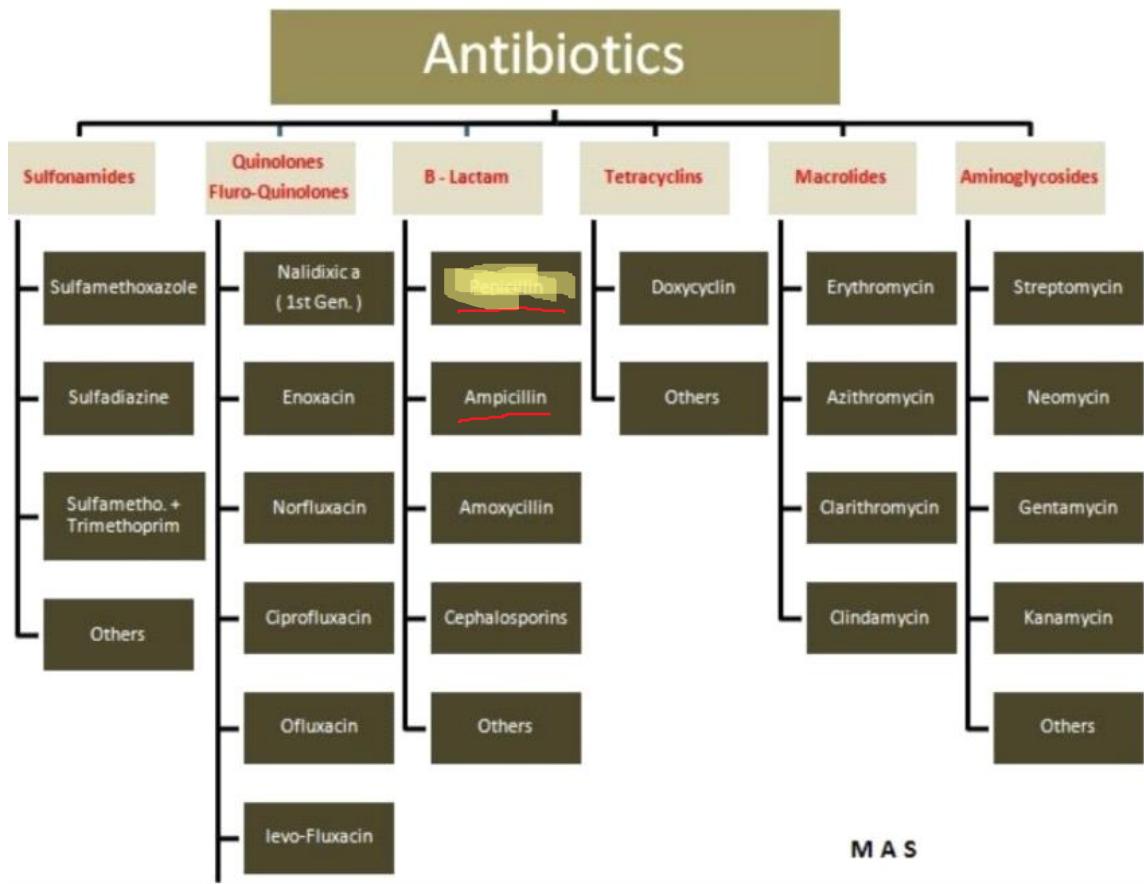
## Mycobacterium

- Mycolic acids: alpha branched lipids in cell walls. Contribute to virulence by preventing attack from cationic proteins and lysozyme, complement deposition, allows survival inside macrophages
- It is an intracellular pathogen that enters via alveoli and are taken up by macrophages
- **Granuloma:** pathological hallmark of TB. Composed of immune cells to contain pathogen. Allows TB to remain dormant for years in this state.
- **Diagnosis of TB:** acid fast staining, lung X-ray, intradermal injection of tuberculin (TB antigen) to check for swelling after 48 hrs

# Week 8 - Antibiotics

13 December 2021 17:22

- Bactericidal: kill bacteria. Needed where immune response is not enough.  
Eg: endocarditis, meningitis, osteomyelitis. Disadvantage: rapid bacterial lysis may cause overwhelming inflammatory response
- Bacteriostatic: inhibit growth but host immune cells needed for eradication



- 3 broad mechanisms: disrupt bacterial cell wall, block translation, inhibit DNA replication

Class	Mechanism	Example
Beta lactams	Inhibition of cell wall synthesis (beta lactam prevents cross linking of peptidoglycans by inhibiting transpeptidase(penicillin binding proteins))	Penicillin, Cephalosporin, Ampicillin, carbapenem, monobactams
Aminoglycosides	Inhibition of protein synthesis by irreversible binding to 30S subunit	Streptomycin, neomycin, gentamycin, kanamycin
Macrolides	Inhibition of protein synthesis by binding to 50S subunit	Erythromycin, azithromycin
Tetracyclines	Inhibition of protein synthesis by preventing attachment of tRNA (attachment of 30S)	Doxycyclin
Sulphonamides	Antimetabolite drugs	
Quinolones	Binding to DNA gyrase	

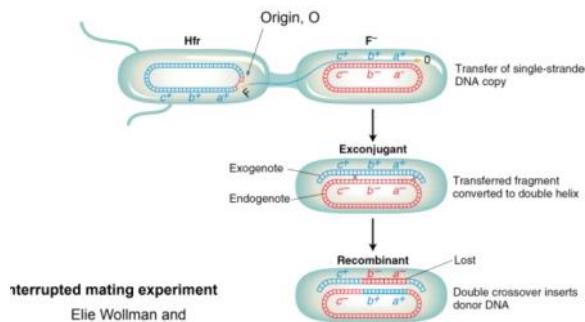
- Chloramphenicol: inhibition of cell wall synthesis

# Week 9 - Bacterial Genetics

02 February 2022 12:00

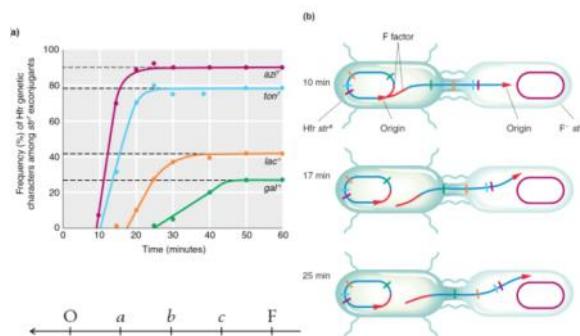
Source: Chapter on bacterial genetics, Introduction to Genetic Analysis by Anthony Griffith

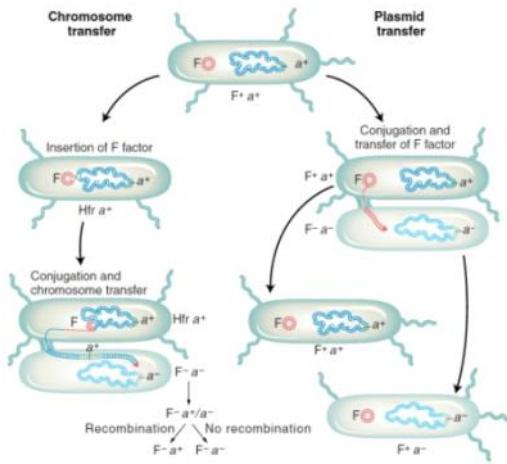
1. Griffith's experiment
  - Smooth (S) strain of Streptococcus pneumoniae is lethal while rough (R) strain is not lethal.
  - Discovery of a 'transforming principle'
2. Avery, MacLeod and MacLeod experiment
  - This 'transforming principle' is DNA
3. Horizontal gene transfer can happen in three ways
  - **Transformation**: picking up free DNA from the environment
  - **Transduction**: transfer of DNA by viruses
  - **Conjugation**: transfer via a pilus
4. Lederberg and Tatum experiment
  - Incubating two strains auxotrophic for different markers produces bacteria that grows in absence of all compounds
  - **Showed that physical contact between bacteria is needed for this transfer of genes** (U-tube experiment)
5. Hfr strains
  - When the F factor integrates with the genome, and Hfr bacteria is generated



- The recombination allows for insertion of genes from donor bacterium into recipient's genome
- Whatever the F factor is attached to will be transferred during conjugation. F factor contains origin of transfer

Hfr *azr<sup>r</sup> ton<sup>r</sup> lac<sup>+</sup> gal<sup>+</sup> str<sup>s</sup>* × F- *azr<sup>s</sup> ton<sup>s</sup> lac<sup>-</sup> gal<sup>-</sup> str<sup>r</sup>*

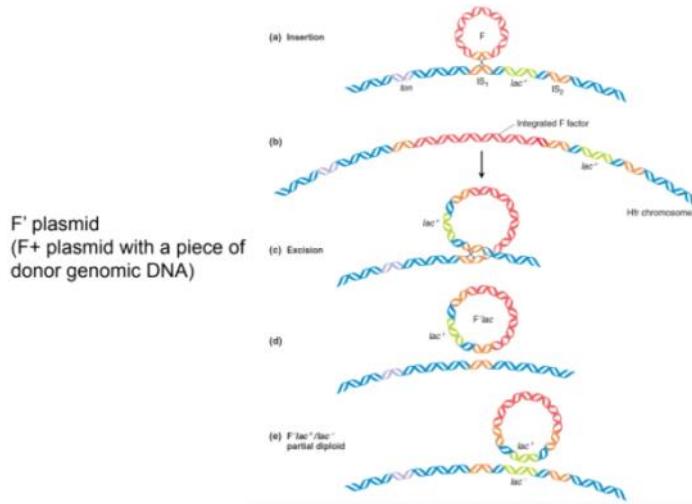




**Figure 5-14 Conjugation summary.** Summary of the various events that take place in the conjugational cycle of *E. coli*.

#### 6. F' plasmids

- The **F factor can integrate into the genome** at certain **insertion sequences (IS)** via recombination
- The integrated plasmid can also leave the genome by recombination between flanking insertion sequences. In such cases, the **new plasmid contains certain adjacent genomic material** (incorrect excision)



#### 7. R plasmids

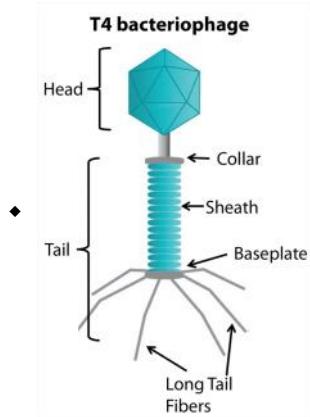
- Contain **genes for resistance** against many agents (antibiotics, metal ions, UV, phages).
- May contain **genes from many different sources and species**
- Eg: pk 214

#### 8. Transformation

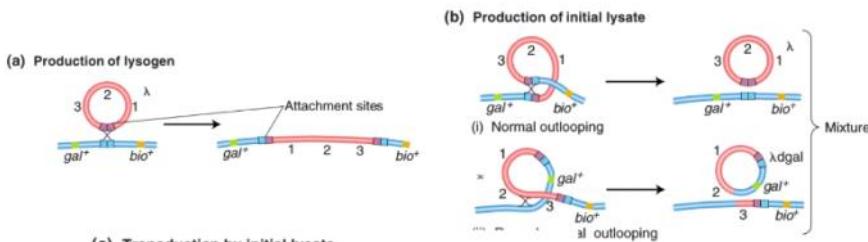
- Naturally competent bacteria:** *B. subtilis*, *N. gonorrhoeae*, *H. influenzae*
- Non-competent bacteria:** *E. coli*
- If DNA is circular, it may combine with the genomic DNA. If it is a plasmid, it is maintained as the extrachromosomal plasmid
- Cotransformation of linked genes:** Genes located together are often transferred as a single unit to recipient cells. Genes closer together will be more likely to be transferred together and both phenotypes can be seen in the recipient cell. Cotransformation can be used for gene mapping

#### 9. Transduction

- Mediated by a bacteriophage



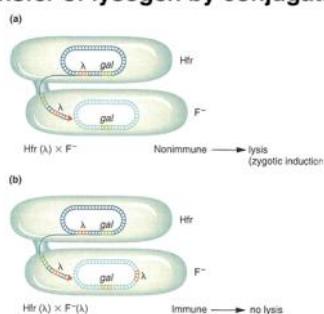
- The phage 'injects' the genetic material into the host bacterium
- Lysogenic cycle:** inserted genetic material is called **prophage** and is **transferred vertically**. **Presence of prophage restricts infection by other phages of same type**.
- Choice between lysogeny and lytic cycle depends on environmental factors, colony dynamics, cell development.
- Phage infections can be seen as a zone of clearance in a bacterial lawn.
- Temperate phages:** capable of lysogeny and lytic cycle
- During packaging of phage DNA in particles, bacterial genes may also be included.
- Generalized transduction:** Random pieces of DNA of bacterial genome are packaged into the head by erroneous viral packaging mechanisms, or via recombination into the prophage. It is a rare event. Generalized phages: **Salmonella P22 and E. coli P1**
- Specialized transduction:** The genes flanking the prophage are **included by improper excision**. Eg: **Lambda phage**



#### 10. Zygotic induction

- In the **donor cell**, a **repressor protein** encoded by the prophage (viral DNA) **keeps the viral genes turned off so that virus is not produced**. When **DNA is transferred to the recipient cell** by conjugation, the viral genes in the transferred DNA are immediately turned on because the recipient cell lacks the repressor. As a result, many virus are made in the recipient cell, and lysis eventually occurs to release the new virus.

#### Transfer of lysogen by conjugation



#### 11. Chromosomal mapping by phage crosses

- cross of T2 phages originally studied by Alfred Hershey

Hershey's cross were  $h^- r^+ \times h^+ r^-$

$h^-$ : can infect two different *E. coli* strains (which we can call strains 1 and 2)

$h^+$ : can infect only strain 1

$r^-$ : rapidly lyses cells, thereby producing large plaques

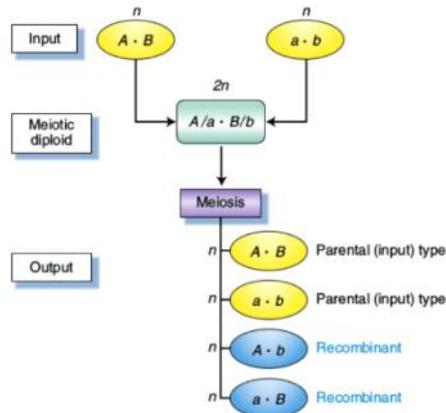
$r^+$ : slowly lyses cells, producing small plaques

$$\text{Recombination frequency} = (h+r+) + (h-r-) / \text{total number of plaques}$$

$h+r+$  and  $h-r-$  are recombinants

## 12. Eukaryotic mapping

- Detection of cross overs in haploids or in gametes

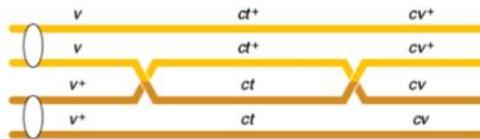


**Figure 4-6 Meiotic recombination.** Recombinants are those products of meiosis with allele combinations different from those of the haploid cells that formed the meiotic diploid.

- Detection of recombination in diploids

Take heterozygous P1, obtain gametes as above, cross with test cross (recessive at both loci).

## 13. Interference and double cross over



**Figure 4-12 Example of a double crossover involving two chromatids.** Notice that a double crossover produces double recombinant chromatids that have the parental allele combinations at the outer loci. The position of the centromere cannot be determined from the data. It has been added for completeness.

Interference is quantified by first calculating a term called the coefficient of coincidence (c.o.c.), which is the ratio of observed to expected double recombinants. Interference (*I*) is defined as  $1 - \text{c.o.c.}$  Hence

$$I = 1 - \frac{\text{observed frequency, or}}{\text{expected frequency, or}} \frac{\text{number, of double recombinants}}{\text{number, of double recombinants}}$$

Expected frequency of double recombinants =  $(\text{RF of } v \text{ and } ct * \text{RF of } ct \text{ and } vc) * \text{total number}$

RF can be obtained from map distances

Here the double recombinants are *v* . *Ct* . *Cv<sup>+</sup>* and *v<sup>+</sup>*. *Ct<sup>+</sup>*. *Cv*

## 14. F factor

### Structure [edit]

The most common functional segments constituting F factors are [9]:

- OriT (Origin of Transfer): The sequence which marks the starting point of conjugative transfer.
- OriV (Origin of Vegetative Replication): The sequence starting with which the plasmid-DNA will be replicated in the recipient cell.
- tra-region (transfer genes): Genes coding the F-Pilus and DNA transfer process.
- IS (Insertion Elements) composed of one copy of IS2, two copies of IS3, and one copy of IS1000: so-called "selfish genes" (sequence fragments which can integrate copies of themselves at different locations). [10]

Some F plasmid genes and their Function:

- traA: F-pilin, Major subunit of the F-pilus.

### Relation to the genome [edit]

The episome that harbors the F factor can exist as an independent plasmid or integrate into the bacterial cell's genome. There are several names for the possible states:

- Hfr bacteria possess the entire F episome integrated into the bacterial genome.
- F<sup>+</sup> bacteria possess F factor as a plasmid independent of the bacterial genome. The F plasmid contains only F factor DNA and no DNA from the bacterial genome.
- F' (F-prime) bacteria are formed by incorrect excision from the chromosome, resulting in F plasmid carrying bacterial sequences that are next to where the F episome has been inserted.
- F<sup>-</sup> bacteria do not contain F factor and act as the recipients.

# Week 11- Transposons

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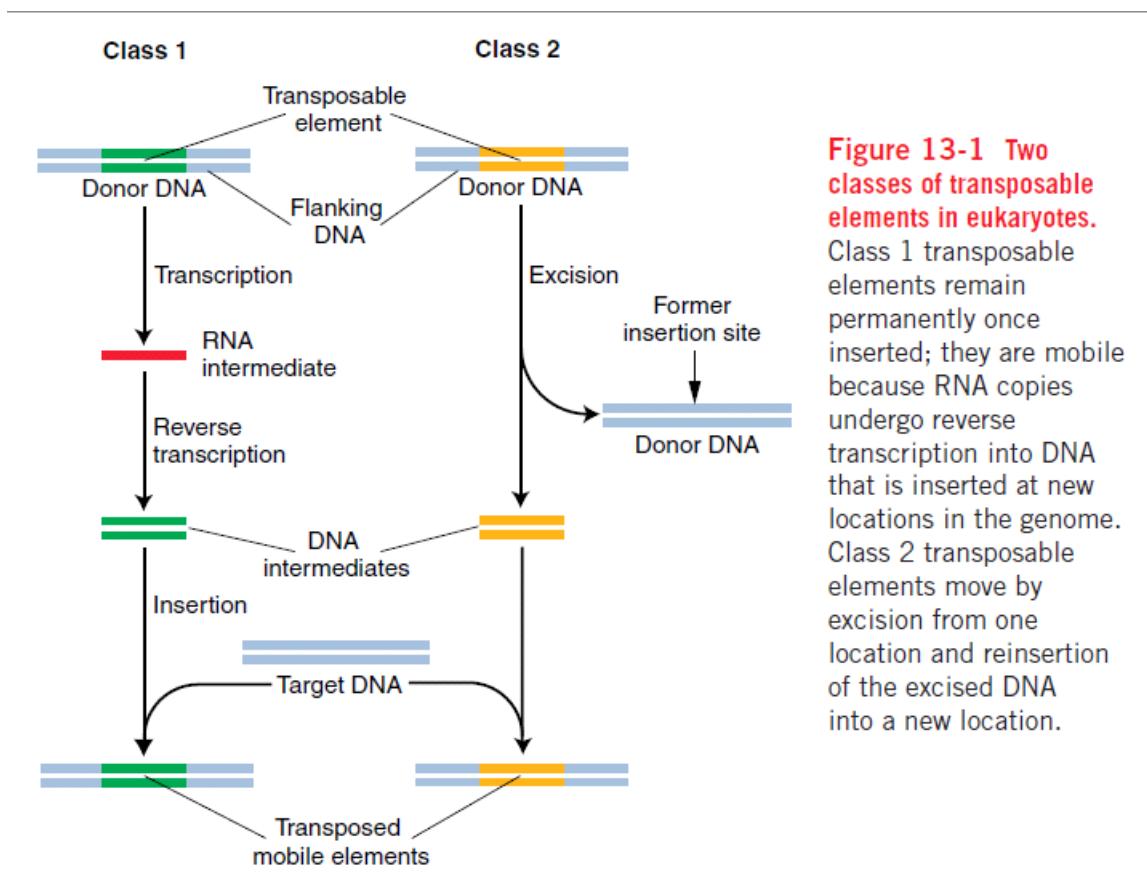
Source: Chapter 13, Introduction to Genetic Analysis by Anthony Griffiths

## 1. Introduction

- 50% of human genome is made of transposable elements
- Epigenetic mechanisms used to regulate proliferation

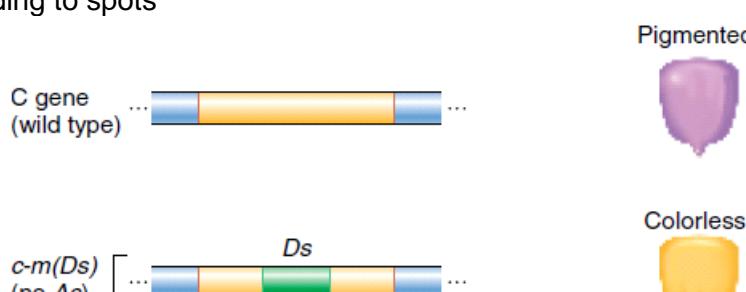
## 2. Class 1 and 2

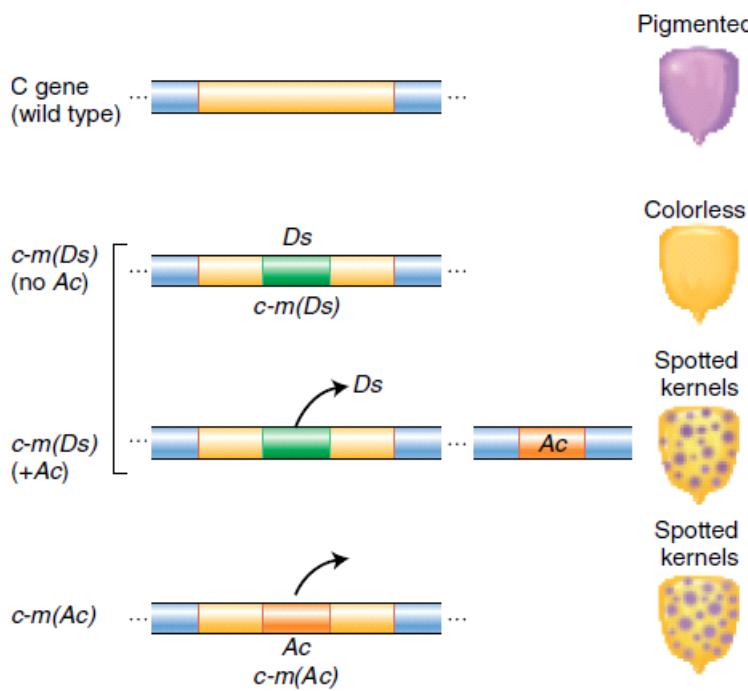
Class 1 elements (RNA elements)	Class 2 elements (DNA elements)
Mediated by RNA intermediate	No RNA intermediate
Higher copy number	Lower copy number
Fixed position once it is inserted	Can be excised and moved to another location (and hence reversal of mutations caused by insertion)



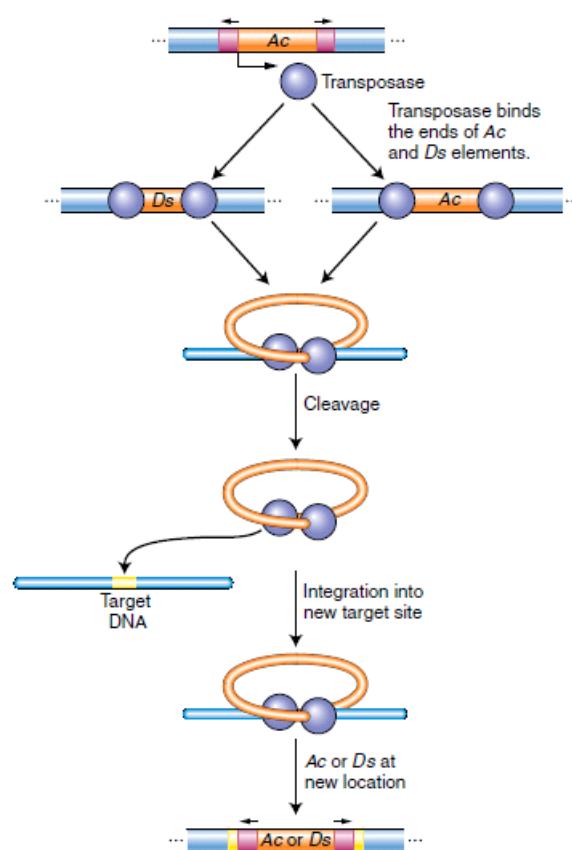
## 3. Discovery

- Discovery of class 2 elements by McClintock in maize
- Insertion and excision from coding regions of genes responsible for colour in kernels leading to spots





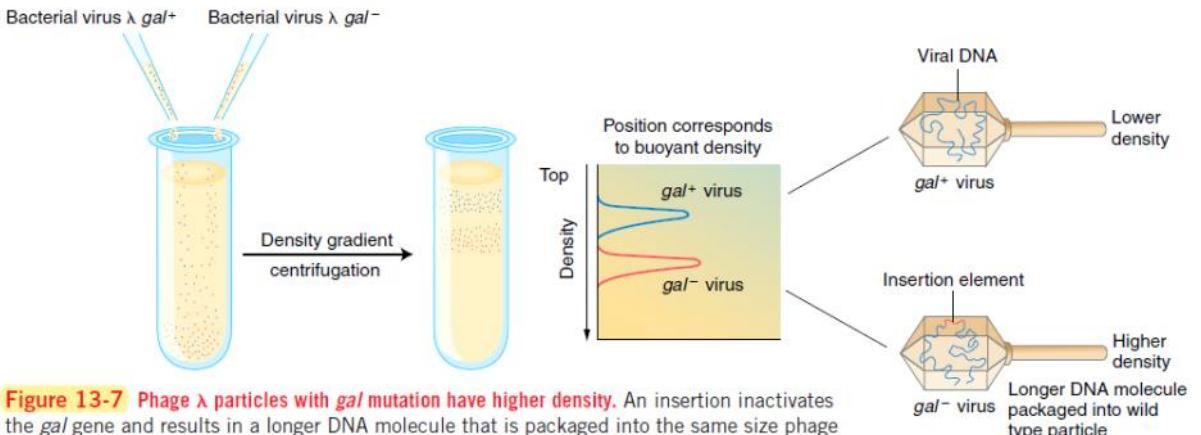
**Figure 13-4** Summary of the main effects of transposable elements in corn. *Ac* and *Ds* are used as examples, acting on the *C* gene controlling pigment.



**Figure 13-21** Action of the *Ac* element in maize. The *Ac* element encodes a transposase that binds its own ends or those of a *Ds* element, excising the element, cleaving the target site, and allowing the element to insert elsewhere in the genome.

- **Ds** is the nonautonomous element that can only move if **Ac** is present
- **Ac** present alone can also show mobility and spotted phenotype and does not require other elements to move (autonomous)

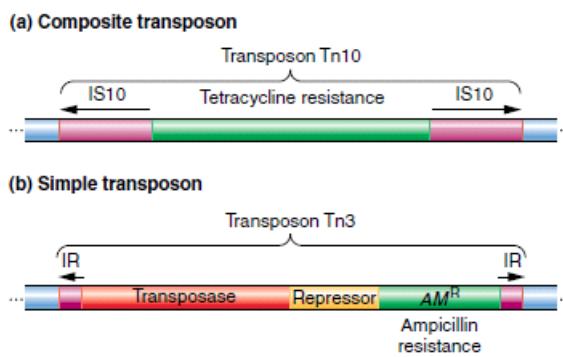
- Nonautonomous elements require autonomous elements for mobility in the genome
4. Transposable elements in prokaryotes
- Insertion sequences (IS): bacterial DNA mobile elements.** They can block the expression of many genes in the same operon. These are class 2 DNA transposons
  - Phages with gal- inactivated by insertion of IS were longer than gal+ DNA



**Figure 13-7 Phage  $\lambda$  particles with  $gal$  mutation have higher density.** An insertion inactivates the  $gal$  gene and results in a longer DNA molecule that is packaged into the same size phage particle as wild type. [From S. M. Cohen and J. A. Shapiro, "Transposable Genetic Elements." Copyright 1980 by Scientific American, Inc. All rights reserved.]

- Many different insertion mutations are caused by small set of insertion sequences
- Hybridization expt confirmed that same IS was responsible for  $gal$  inactivation
- IS elements are few hundred bp long
- They only encode a **transposase required for mobility** and **start and end with inverted repeats.**
- Since IS regions are identical, they become sites for recombination
- IS sites that contain extra genes (Eg: antibiotic resistance) can be classified as composite or simple

Composite Transposon	Simple Transposon
One of the two flanking IS encode the transposase	IRs do not encode the transposase but simple transposon carry their own transposase
IR sequences are longer	IR sequences are shorter

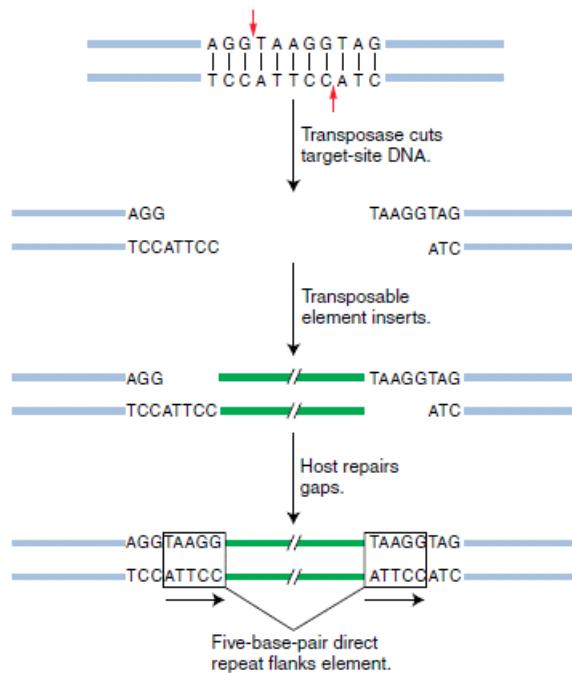


**Figure 13-9 Structural features of composite and simple transposons.** (a) Tn10, an example of a composite transposon. The IS elements are inserted in opposite orientation and form inverted repeats (IRs). (b) Tn3, an example of a simple transposon. Short inverted repeats contain no transposase. Instead, simple transposons encode their own transposase. The repressor is a protein that regulates the transposase gene.

- Transposons can jump between plasmid to bacteria

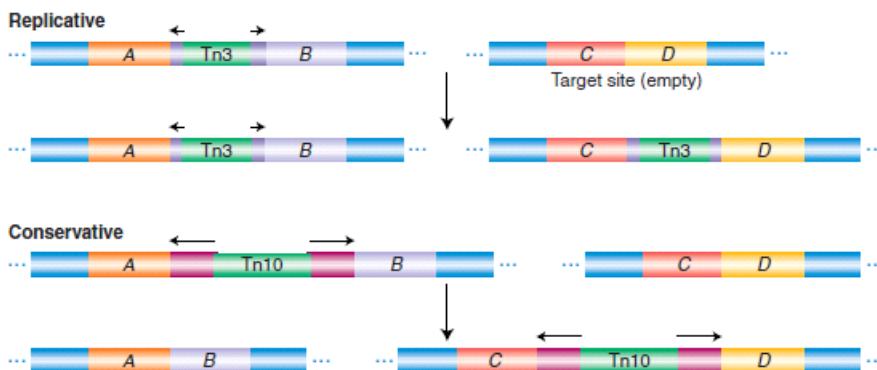
## 5. Mechanism of transposition

- Generation of overhangs at target DNA site
- Insertion of transposon
- Target site duplication



**Figure 13-11 Duplication of a short sequence of DNA at the insertion site.** The recipient DNA is cleaved at staggered sites (a 5-bp staggered cut is shown), leading to the production of two copies of the five-base-pair sequence flanking the inserted element.

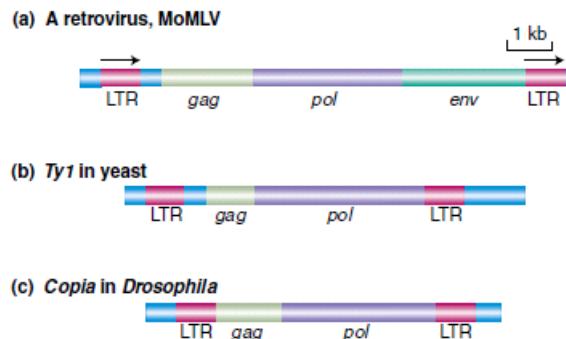
- Transposition can be replicative or conservative



**Figure 13-12 Two general modes of mobile-element transposition.** See text for details. [Adapted with permission from *Nature Reviews: Genetics* 1, no. 2, p. 138, Figure 3, November 2000, "Mobile Elements and the Human Genome," E. T. Luning Prak and H. H. Kazazian, Jr. Copyright 2000 by Macmillan Magazines Ltd.]

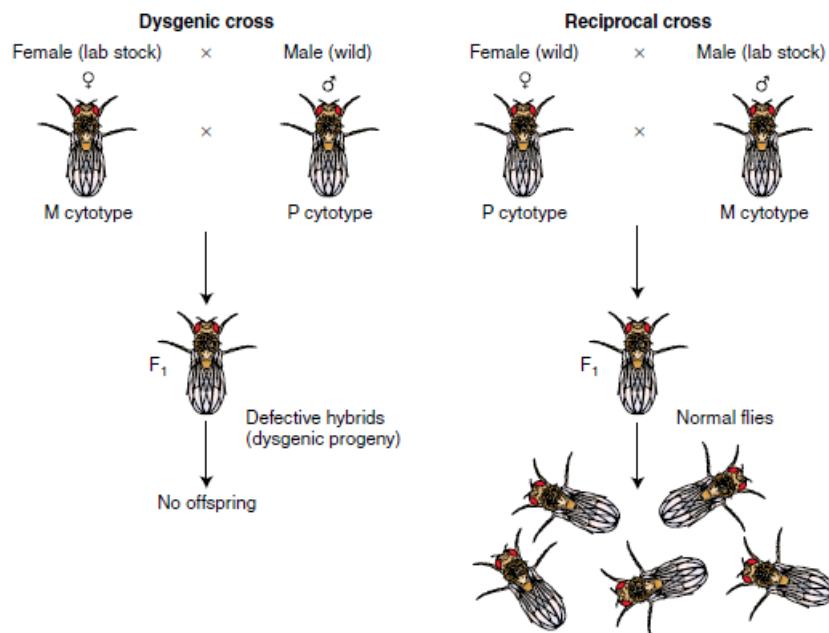
## 6. Class 1 retrotransposons

- Discovery in yeast mutants that has a reversible unstable His<sup>+</sup>/- phenotype. Mutants contained large DNA insert.
- These inserts resembled retrovirus in structure. Gag: enzyme for RNA maturation, pol: reverse transcriptase

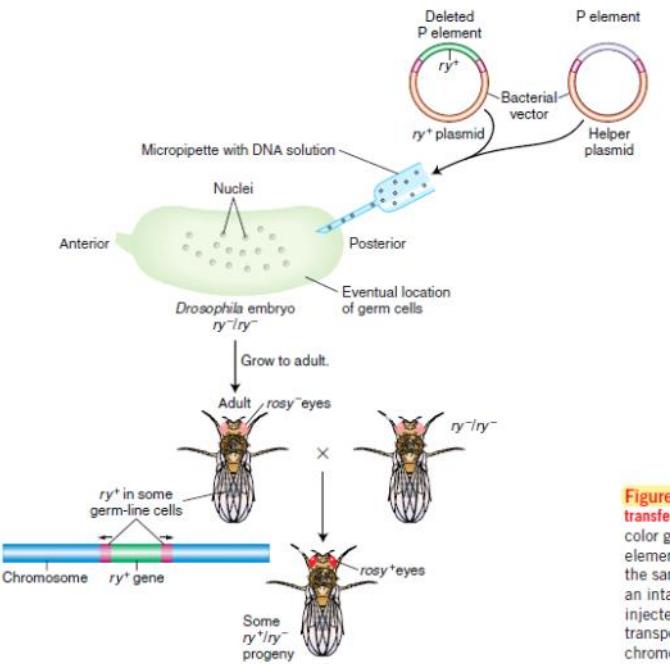


- These are retrotransposons that use an RNA intermediate to move and encode reverse transcriptase
- Retrotransposons are only found in eukaryotes
- Eg: LINEs and SINES

7. Class 2 DNA transposons
  - Found in bacteria, and were the transposons found by McClintock
8. P elements of drosophila
  - Discovered during experiments on hybrid dysgenesis (high mutation rate, sterility)
  - Dysgenic mutations were unstable and would revert
  - Mutations due to insertion of transposons called P elements which were absent in M strain
  - P elements resemble bacteria transposons and encodes a transposase gene and encode a repressor
  - In M female x P male, the zygotic nucleus has P elements in an repressor-free environment and wreak havoc
  - P elements only mobilize in germ cells



9. P elements for gene transfer
  - Transfer of genes into the germ lines
  - Recipient is homozygous recessive for the phenotype
  - The P element containing ry+ replicated and inserts into the genome of germ line with the help of helper plasmid with intact P element
  - F2 offspring have ry+ phenotype



**Figure 13-22 P-element-mediated gene transfer in *Drosophila*.** The *rosy<sup>+</sup>* (*ry<sup>+</sup>*) eye-color gene is engineered into a deleted *P* element carried on a bacterial vector. At the same time, a helper plasmid bearing an intact *P* element is used. Both are injected into an *ry<sup>-</sup>* embryo, where *ry<sup>+</sup>* transposes with the *P* element into the chromosomes of the germ-line cells.

Q. How do humans survive with such a large number of transposons?

Three major reasons. First, most of the transposable elements' sequences are mutant and no longer capable of transposition. Second, the transposition of the few active elements in the genome is usually prevented by host regulatory mechanisms. Finally, the vast majority of transposable-element sequences in the human genome are in noncoding DNA including telomeres, centromeres, intergenic DNA, and introns

Types of transposable elements in the human genome

Element	Transposition	Structure	Length	Copy number	Fraction of genome
LINEs	Autonomous	ORF1      ORF2 ( <i>pol</i> ) AAA	1–5 kb	20,000–40,000	21%
SINEs	Nonautonomous	AAA	100–300 bp	1,500,000	13%
DNA transposons	Autonomous	←      transposase      → ←      →	2–3 kb 80–3000 bp	300,000	3%
	Nonautonomous				

**Figure 13-23 The general classes of transposable elements found in the human genome.**

# Week 12-Quorum sensing and chemotaxis

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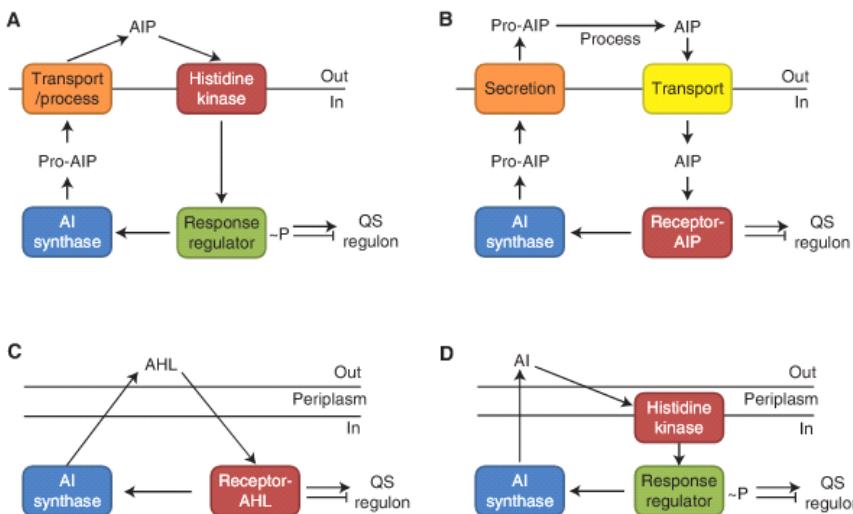
## 1. Quorum sensing

- Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density.
- Quorum sensing bacteria produce and release chemical signal molecules called autoinducers that increase in concentration as a function of cell density. The detection of a minimal threshold stimulatory concentration of an autoinducer leads to an alteration in gene expression.
- Affects symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation.
- Gram-negative bacteria use acylated homoserine lactones as autoinducers
- Gram-positive bacteria use processed oligo-peptides to communicate

## 2. Three properties

- Members of the community produce the Ais
- AIs are detected by receptors
- AIs induce the production of genes as well as the AI itself, leading to a positive feedback loop

## 3. General schematic



**Figure 1.** Canonical bacterial quorum-sensing (QS) circuits. Autoinducing peptide (AIP) QS in Gram-positive bacteria by (A) two-component signaling, or (B) an AIP-binding transcription factor. Small molecule QS in Gram-negative bacteria by (C) a LuxI/LuxR-type system, or (D) two-component signaling.

Source: Cold Spring Harb Perspect Med-2012-Rutherford-Quorum sensing

## 1. Gram positive bacteria

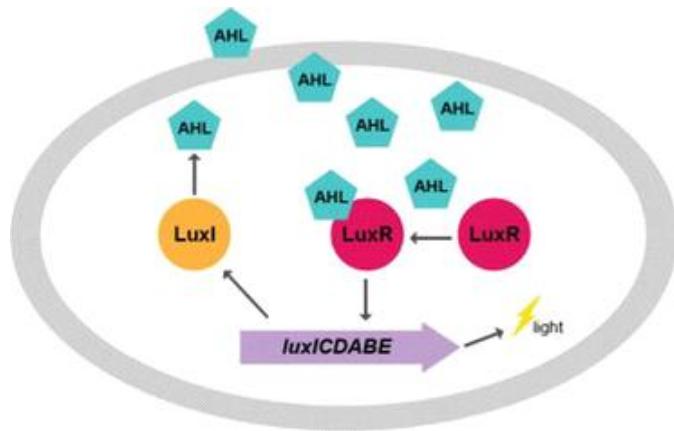
- Use small peptide AI
- Peptides need transporters/ secretors which also convert from proAI to AI
- They bind to histidine kinases which phosphorylate downstream regulators that turn on QS genes

## 2. Gram negative bacteria

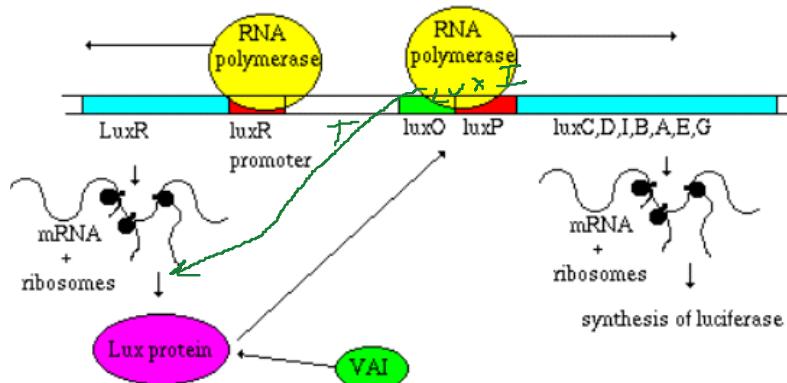
- Use acyl-homoserine lactones (AHLs) or S-adenosyl methionine derived molecules
- Different bacterium have different side chains for AHLs

## 3. QS in *Vibrio fischeri* (LuxI/LuxR system)

- AI: N-3-oxo-hexanoyl L homoserine lactone
- Colonizes Hawaiian bobtail squid



### The Lux Operon



There exists a species of squid that swims around at the top of the ocean at night, skimming for food. Unfortunately, to any predator below, this squid appears as a very dark object moving against the very bright background of the moon. To solve this problem, the squid has evolved a **light organ** in which it cultures a very pure, very dense population of a bacteria called *Vibrio fischeri*. This bacteria produces a substance called **luciferase**, which glows with the same intensity as the moon, rendering the squid invisible to predators from the depths of the ocean.

When *Vibrio fischeri* is not in the squid's light organ, it does not need to be making luciferase, since glowing will not help it or anything else. On the other hand, when inside the light organ, it is to the bacteria's advantage to glow, because then the squid will not get eaten and will feed it, away from competition from any other kinds of bacteria. So how can the bacteria know that it is in a light organ in order to turn on synthesis of luciferase?

The answer lies in the concept of **quorum sensing**. When the density of bacteria is very high, the bacteria know that they must be inside a light organ instead of floating around in the ocean. Each bacterium is continuously secreting a unique small molecule called **VAI** (*Vibrio fischeri* autoinducer) that can diffuse readily through the cell membrane. Thus, there is a declining concentration of the small molecule in a growing circumference around the bacterium. When there are many bacteria around, the local concentration of the small molecule will be very high.

The genes for making luciferase are contained in the lux operon. A DNA binding site (luxO) near the lux promoter (luxP) binds a protein called luxR. This protein somehow calls RNA polymerase over when it is bound to the DNA, thus increasing transcription of the DNA and making mre polymerase. **Thus, luxR is a transcriptional activator of the lux operon.** When the local concentration of VAI is very high, it binds to luxR, enabling it to bind to the operator and turn on transcription. On the other hand, if VAI is low, luxR is in a conformation such that it cannot bind to the operator, and not very much luciferase can be made. In this fashion, the bacteria only make **luciferase** when there are lots of other bacteria around. LuxR is consistently transcribed at a low level so that there is always some around to affect regulation.

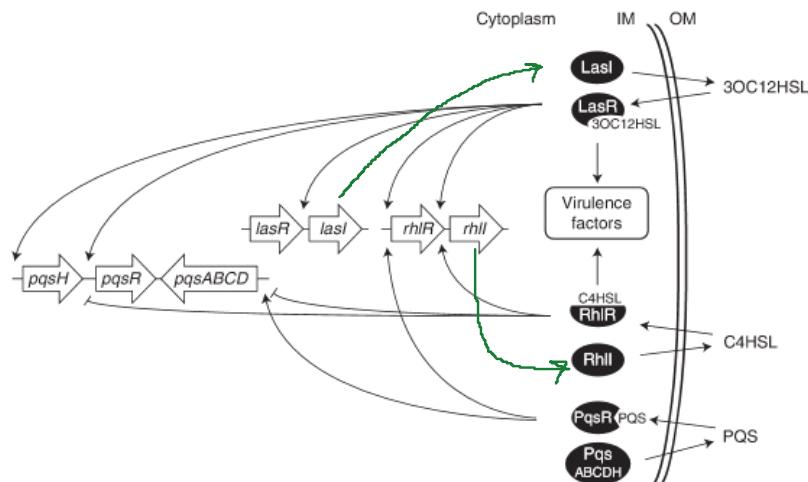
It is important to note that LuxI is the gene that encodes for the enzyme that synthesizes VAI. When a bacterium undergoes the transition from not making luciferase to making luciferase, it needs to have the autoinducer around in order to promote binding of LuxR to the operator. But before the operon is turned on, how can LuxI be made so that there is a continuous level of autoinducer being made? The answer is that operons, in general, are never completely turned off. There is always some **basal level** of transcription going on, but, for example in this case, the uninduced LuxR protein still has a minimal affinity for the DNA binding site so that some DNA can be transcribed to make enough LuxI so that autoinducer is continuously made.

- LuxR is constitutively produced and binds to AHL
- LuxA, luxB - luciferases
- LuxI- AI synthase

[Source](#)

7. QS in *pseudomonas aeruginosa* (LasI/LasR)

- Uses QS circuit to regulate expression of virulence factors and biofilm formation
- Has 3 circuits: two LuxI/LuxR homologues (LasI/LasR and RhI/RhI) which act in series and one non-LuxI/LuxR system (pqsABCDH/pqsR)

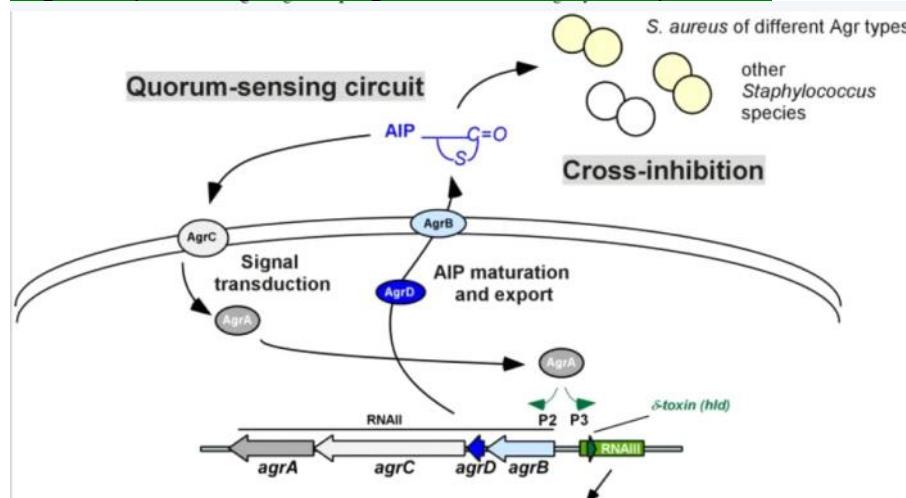


**Figure 4.** *P. aeruginosa* QS circuits. The three AI synthases, LasI, RhI, and PqsABCDH, produce the AIs 3OC12HSL, C4HSL, and PQS, respectively. The AIs are detected by the cytoplasmic transcription factors LasR, RhIR, and PqsR, respectively. Each transcription factor regulates expression of its corresponding AI synthase as well as additional targets as indicated by the arrows.

[Source](#)

8. QS in *Staphylococcus aureus* (Agr)

- AI are short peptides 5-17 aa long
- The **accessory gene regulator (agr)** circuit is a prototype for QS in gram positive bacterium
- Agr locus has **two divergent transcriptional units- RNAII and RNAIII** with P2 and P3 promoter
- RNAII: agrA, agrB, agrC, agrD
  - agrD: precursor to AI
  - agrB: transmembrane transporter and modifies the AI precursor to activate it
  - agrC: histidine kinase sensor that phosphorylates agrA
  - agrA: response regulator of agrC, binds to P1 and P2 promoter



[Source](#)

9. Hybrid QS circuit in *Vibrio harveyi*

- Marine bacterium that is free living
- Uses QS for bioluminescence
- QS circuit is hybrid between gram positive and gram negative bacteria

10. Chemotaxis in *e. coli*

### 3.1 Escherichia coli

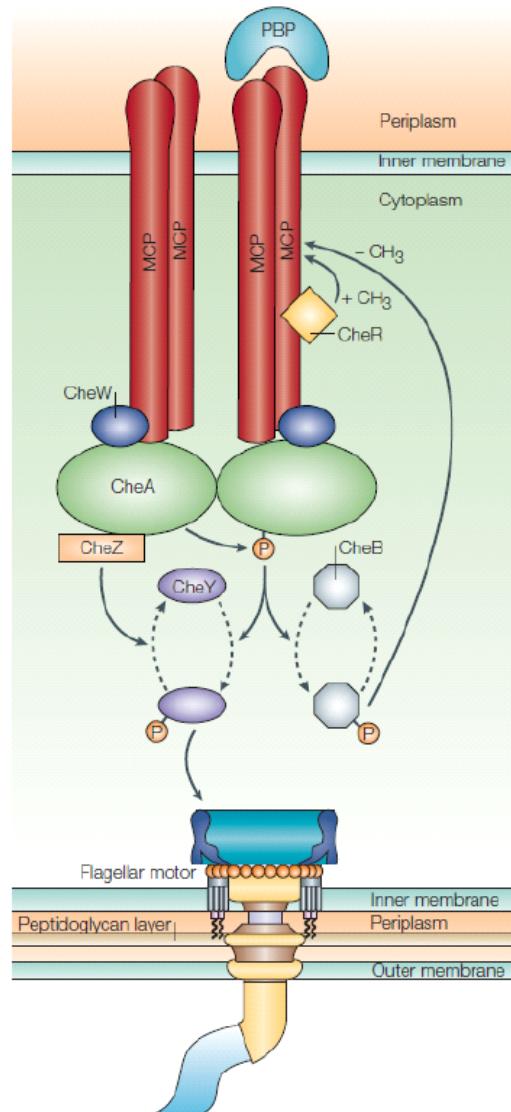
*E. coli* is a gram-negative bacteria that is present in the soil and animal gastrointestinal tracts. Being a model organism, the signalling pathways and mechanisms associated with chemotaxis in *E. coli* are well characterized. The flagella of this bacterium are responsible for its motility, and the arrangement of its flagella is peritrichous [15]. When the flagellar motors rotate counterclockwise, the flagella form a bundle that propels the cell forward in a straight line. When some, or all flagellar motors rotate clockwise, the flagella unbundle and the cell tumbles in place. In the absence of chemical gradients, the movement of *E. coli* is modelled as a random walk, reflecting the combination of straight runs and tumbles it performs. When a chemical gradient is present, the random walk is biased such that it results in a net propagation in the direction of, or against, the chemical gradient [9]. On average, bacterial cells change direction every second by tumbling. A higher tumbling frequency causes the cell to steer away from its current direction while a lower frequency enables the cell to maintain longer straight runs in current direction.

The sensitivity of the chemoreceptors of bacteria is remarkable— they have the ability to detect changes as small as a few molecules in the volume of the cell [19]. As bacterial cell sizes tend to be a few micron, they rely on temporal sensing of chemical gradients rather than measuring absolute concentrations along their lengths [12]. The receptors can be thought of as possessing ‘memory’ wherein they sense chemical concentrations relative to a previous time point.

In *E. coli*, chemical signal perception is performed by transmembrane methyl accepting proteins (MCPs). The proteins downstream to the MCPs are a system of chemotaxis proteins (Che proteins). CheA is a dimeric, cytosolic histidine protein kinase, which is associated with the MCPs via an adaptor CheW. Two response regulators, CheY and CheB bind with CheA. CheY is a single-domain protein that binds to flagellar motor proteins to induce clockwise rotation. CheB is a methylesterase that plays a role in the adaptation of the MCPs. A decreasing concentration of an attractant decreases ligand binding to the MCPs, resulting in the trans-autophosphorylation of histidine residues of the monomers of CheA. CheY is phosphorylated to CheY-P, which acts as the effector molecule by reversing the direction of rotation of the flagellar motors. The cell now tumbles at a greater frequency,

resulting in direction change. The CheY-P signal is terminated by phosphatase CheZ. In addition to phosphorylating CheY, CheA also phosphorylates CheB. CheB-P exhibits increased methylesterase activity and demethylates the MCPs in order to adapt the receptors. Demethylated MCPs show decreased ability to induce autophosphorylation in CheA, which returns the cell to its pre-stimulus state. The cell can now respond to further increments to decrements in the chemical concentrations. The opposite occurs when attractant concentrations increase. Autophosphorylation of CheA is inhibited, CheY-P concentrations decrease, the rate of reversal of flagella decreases, the cell tumbles less frequently and tends to move in a straight line. CheB activity is also reduced, allowing the constitutive methyltransferase CheR to methylate the MCPs. Methylated MCPs show increased ability to stimulate autophosphorylation in CheA, allowing further elevations in attractant concentration to induce the pathway all over again [20].

*E. coli* has five chemoreceptors  $T_{ar}$  (aspartate and maltose chemoreceptor),  $T_{sr}$  (serine, leucine chemoreceptor),  $T_{rg}$  (ribose and galactose chemoreceptor),  $T_{ap}$  (dipeptide chemoreceptor), and  $Aer$  (oxygen). The cytoplasmic domains of these receptors are highly conserved, even across bacterial species, while their periplasmic domains are variable and adapted to the specific target ligands.



# Week 13 -Staining and bacterial organelles

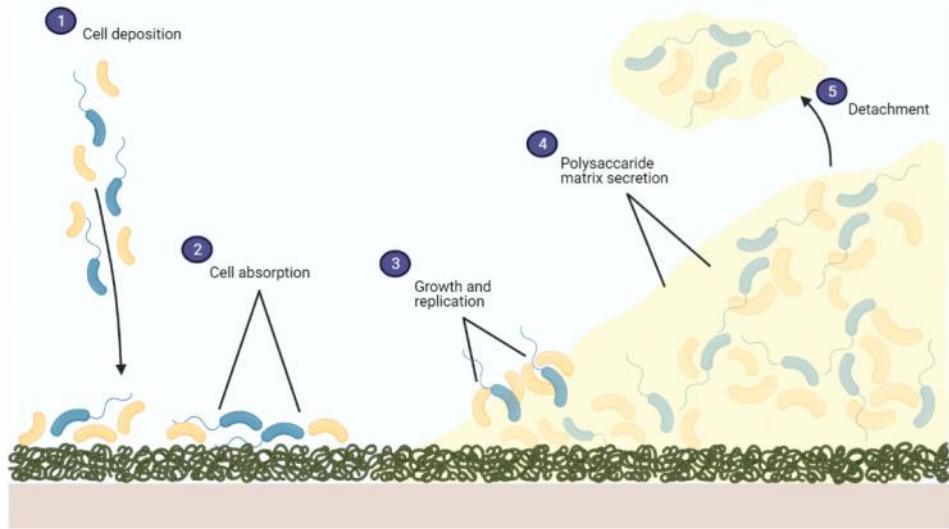
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1. Min system
  - The **min system** is used by *E.coli* to **properly orient the septum** prior to cell division
  - Places the FtsZ (Z) ring in the centre
2. Z ring
  - FtsZ is a protein encoded by the **ftsZ gene** that **assembles into a ring** at the future site of bacterial cell division.
  - FtsZ is a prokaryotic homologue of the eukaryotic protein tubulin
3. Types of staining
  - **Simple staining:** used for the visualization of morphology. Can be positive or negative staining
  - **Differential staining:** using **two contrasting stains separated by a decolourizing agent**. Can be for **identification** (gram stain or acid-fast stain) or for **visualization of specialized structure** (capsule stain, spore stain)
4. Simple stains
  - Dyes in an aqueous or alcoholic solution
  - Bacteria are **slightly negatively charged at 7 pH**
  - Basic dyes stain the bacteria: **methylene blue**, toluidine blue, thionine, **carbol fuchsin**, **crystal violet**
  - Acidic dyes stain the background: **Nigrosine**, Picric acid, **Eosin**, Acid fuschin, **India ink**
5. **Endospore staining-** Schaefffer-Fulton method or Bartholomew and Mittwer's method
  - Application of **malachite green as the primary stain**. It is **alkaline and stains the spore**
  - **Mordant:** a process or chemical that helps fix a dye. Here heat is the mordant
  - Application of decolourizer: water
  - Application of **safranin, the counter stain** which stains the rest of the bacterium
  - Mostly seen in gram positive bacteria
6. **Ziehl Neelsen** stain for acid fastness-
  - Used for bacterium that cannot undergo gram staining
  - **Primary dye: carbolfuchsin**
  - **Counter stain: methylene blue**
  - Acid fastness is useful for mycobacterium which are acid fast due to presence of mycolic acids
7. Leifson flagella stain
  - Leifson flagella stain method uses **tannic acid and a dye**.
  - When bacterial flagella absorbs this tannic acid and a dye, dye forms a colloidal precipitate as a result the flagella is colorized and as well as increase in diameter, thus amenable to viewing by light microscopy.
8. Capsule staining
  - **Carbofuscin (basic stain and stains bacteria) and nigrosin (acidic stain, stains background)** is used
  - Capsule is visible as a halo
9. Mesosome
  - A membranous organelle and are involved in nitrification and convert ammonium to nitrite or nitrate
10. Capsule/slime layer

- Anti-serum treatment can allow to see the capsule
- They are generally polysaccharides but sometimes have proteins
- *Bacillus anthracis* has many proteins in the capsule
- Capsules are different for different bacterium and strains
- Serotype: classifications of microbes on the basis of surface antigens
- Capsules can affect the serotypes. The virulence is different among different capsular serotypes
- Capsules help by preventing phagocytosis
- Pneumococcal polysaccharide is alternating glucopyranose and glucuronic acid with alternating beta-1,3 and beta-1,4 bonds
- Slime polysaccharide in E.coli forms the M antigen or K antigen (in different serotypes of E.coli)
- Acetobacter xylinum synthesizes cellulose in its capsule. This has been bioengineered for various methods as a biomaterial
- Capsule is not essential for survival but contributes to survival and virulence

## 11. Biofilms

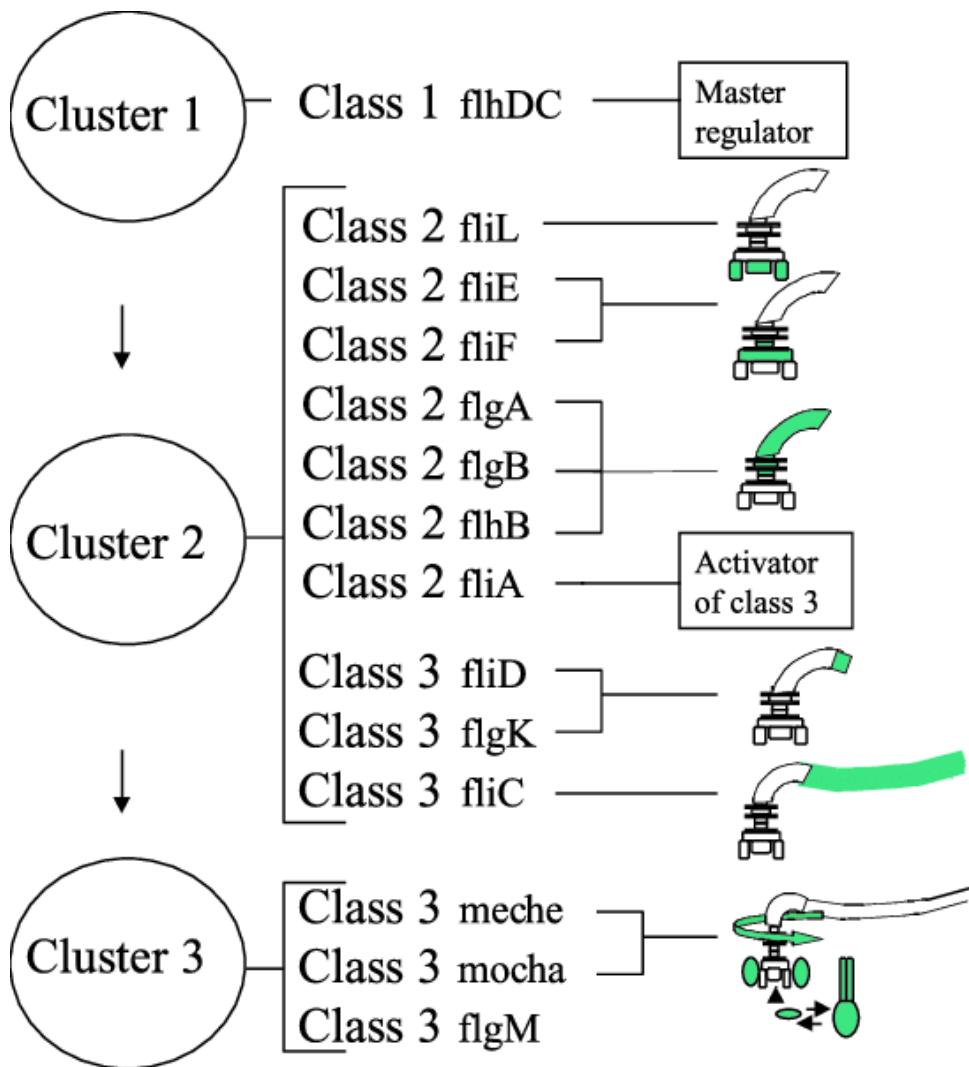
- An extracellular matrix (exopolysaccharide) where a bacterial community lives
- *Bacillus subtilis* is a model organism to study biofilm formation
- Sequential steps of attachment of free living bacteria, differentiation into matrix producing cells, biofilm maturation and sporulation and finally spore/free motile bacteria dispersal



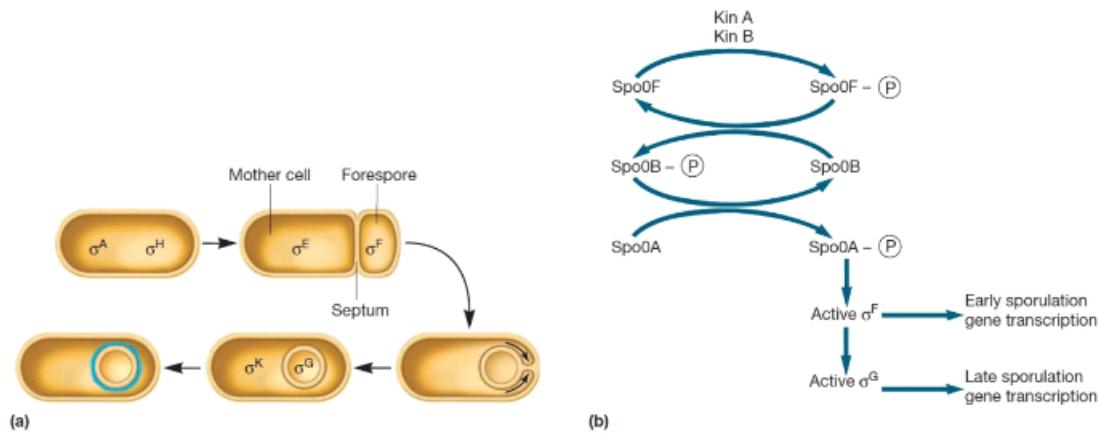
- The biofilm bacteria can share nutrients and are sheltered from harmful factors in the environment, such as desiccation, antibiotics, and a host body's immune system
- *sirR* and *SinR* regulate the process
- *EpsA operon* is upstream of the functional genes for biofilm formation
- Biofilms can form on plants. Plants release malic acid, a chemoattractant for *B. subtilis* which forms the biofilm and protects from pathogenic bacterium
- D amino acids and polyamines cause the maturation of the biofilm and is the signal to leave the biofilm

## 12. Operon cascade for flagellar assembly

- Three different classes of operons and genes that ensure the sequential assembly of the flagella
- Checkpoint based mechanism
- Cluster 1- Master regulator
- Cluster 2- rings, rod, hook, flagella
- Cluster 3- MCP and Che proteins



13. Gliding mobility involved
  - **Bacteria glide on surfaces using pili**
  - Eg: cyanobacteria
14. Pili
15. Only found in **gram negative bacteria**
  - **F pili** are involved in conjugation
  - **Some pili are involved in adhesion**
  - Can also aid in **twitching motility** (jerky translocation across solid surface)
15. Sporulation in *B. subtilis*



**Figure 12.21 Genetic Regulation of Sporulation in *Bacillus subtilis*.** (a) The initiation of sporulation is governed in part by the activities of two spatially separated sigma factors.  $\sigma^F$  is located in the forespore, while  $\sigma^E$  is confined to the mother cell. These sigma factors direct the initiation of transcription of genes whose products are needed for early events in sporulation. Later,  $\sigma^G$  and  $\sigma^K$  are localized to the developing endospore and mother cell, respectively. They control the expression of genes whose products are involved in the later steps of sporulation. (b) The activation of  $\sigma^F$  is accomplished through a phosphorelay system that is triggered by the activation of the sensor kinase protein KinA. When KinA senses starvation, it autophosphorylates a specific histidine residue. The phosphoryl group is then passed in relay fashion from Spo0F to Spo0B and finally to Spo0A. See text for details.

# Week 14 -Cyanobacteria and Fungi

27 February 2022 09:53

1. Archaea
  - They are extremophile
  - The first divergence of prokaryotes from archaea is by thermophilic bacteria which are chemolithotrophs and use inorganic compounds as source of energy. Eg: Aquificae and Thermotoga are gram negative rods with a functional glycolytic
2. Deinococci
  - Resistance to high doses of radiation
  - They're not are gram positive by have an outer membrane like gram negative bacteria
3. Gram negative photosynthetic bacteria
  - Purple bacteria
  - Green bacteria
  - Cyanobacteria
4. Photosynthesis in bacteria
  - Cyanobacteria have chlorophyll a and PS I and II
  - Water is the source of electrons in cyanobacteria
  - H<sub>2</sub>S, S are electron donors in green and purple bacteria and do not release oxygen
  - Green and purple bacteria use bacteriochlorophyll
  - Cyanobacteria can be oxygenic or anoxygenic
5. Carbohydrate metabolism in cyanobacteria
  - No full citric acid cycle (lacking AKG dehydrogenase)
  - Have PPP
6. Accessory pigment
  - Phycocyanin and phycoerythrin are different pigments
  - Chromatic adaption: changing the relative amounts of pigments to adapt to different light conditions
7. Motility
  - Usage of gas vacuoles to position themselves at optimum depth for required illumination (form of phototaxis)
  - Some use gliding motility
8. Shape
  - Unicellular
  - Colonies
  - Filamentous (eg; anabaena): branched or straight
9. Structure
  - Pigments are located in thylakoid like structures
  - Phycobilisomes: particles embedded in thylakoids contains phycocyanin and phycoerythrin
  - Carboxysome: perform fixation by RuBisCO
10. Cellular differentiation in some cyanobacteria
  - Vegetative cells: forming the mature filaments
  - Akinetes: dormant cells for survival. Thick cell walls, glycogen stores, nitrogen stores as cyanophycin
  - Heterocyst: carries out nitrogen fixation. They have nitrogenase which is sensitive to oxygen and not compatible with oxygenic photosynthesis. They have special membrane (glycolipid and polysaccharide) to act as a gas barrier. There is exchange of carbohydrates and glutamine between vegetative cells and heterocysts.

- **Hormogonia cells:** Small motile filaments lacking akinetes, heterocysts, and used for dispersion. Used to establish colonization of plants for symbiosis

11. Filament structure

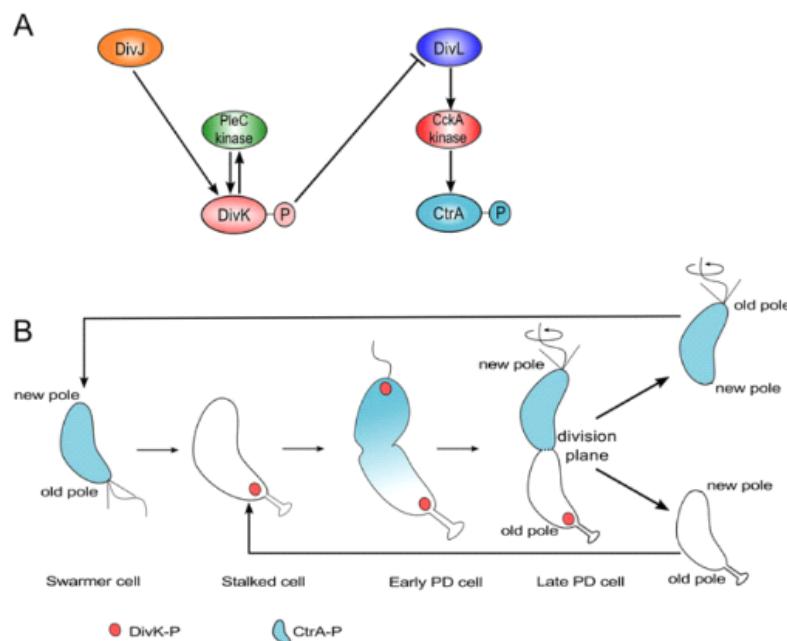
- **The periplasmic space is continuous**
- Cell-cell junction molecules present
- Cytoplasm is discontinuous

12. Nitrogen stress and heterocyst formation

- Nitrogen deficiency manifests as low levels of glutamine and increased levels of AKG. This causes **NtcA** to induce expression of genes for heterocysts formation
- There is a release of **PatS** so that adjacent cells are inhibited from undergoing differentiation

13. Differentiation in *Caulobacter crescentus*

- **Swarmer cells:** motile, dispersal, does not replicate
  - **Stalked cells:** reproductive, capable of division and DNA replication, non-motile and bound to a substrate via a stalk
- 



- Many proteins involved in cytokinesis are involved in stalk production
- A crossband separates cell body cytoplasm from the stalk cytoplasm
- Phosphate limitation enhances stalk formation
- The peptidoglycan layer in the stalk is different from the rest of the body

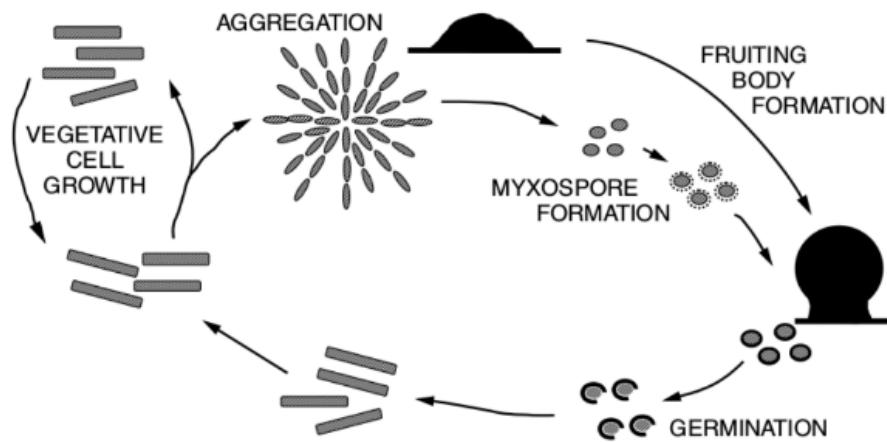
14. Cell division in *C. crescentus*

- The transition from swarmer cell to stalked cell is necessary for cell division
- **CtrA-P inhibits cell division** and is present in the swarmer cells
- During differentiation, **DivL localizes to the new pole** where it phosphorylates CtrA to CtrA-P such that cell division is inhibited and a CtrA-P gradient is established
- DivK is present at new pole but is phosphorylated only at the old pole

15. *Myxococcus xanthus* life cycle

- *Myxococcus xanthus* is a **gram-negative**, rod-shaped species of myxobacteria that exhibits various forms of self-organizing behaviour in response to environmental cues
- In **response to starvation** they develop into **fruiting bodies** and **release myxospores** once food is available
- The synchronized emergence of spores enables cooperative feeding
- **Wolf packs:** cells move together and there is high concentration of extracellular

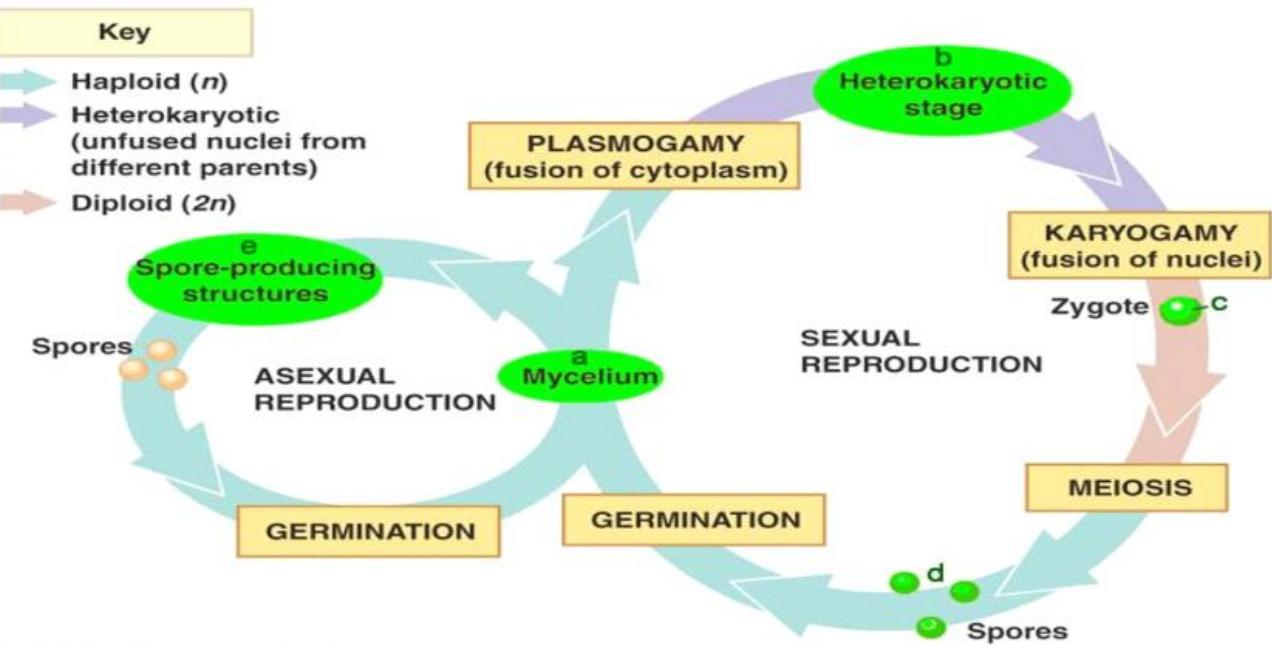
digestive enzymes to enable predation



- Conditions leading to differentiation: nutrient limitation, presence of solid surface and high cell density
- MrpC/FruA pathway for differentiation

FruA	master regulator and is a committed step for sporulation
relA	(p)ppGpp synthase. Induced by amino acid depletion
(p)ppGpp (guanosine pentaphosphate)	intracellular communicator. It is produced by relA (synthase) and induces stress response
SocE	negative regulator of relA

16. Importance of fungi
  - Decomposers
  - Mutualistic symbionts (mycorrhizae)
  - Parasitic/ pathogens
  - Economic use: food, synthesis of chemicals, cheese
17. Heterotrophic
  - Secrete exoenzymes to digest food
18. Fungal cell walls
  - Chitin: polymer of N acetyl glucosamine
  - Oomycetes (exception): cellulose wall, no longer classified as fungi
19. Unicellular
  - Eg: yeast
20. Multicellular
  - Consist of multicellular hyphae
  - Mycelium: mass of hyphae (body of fungus)
  - Septate hyphae: hyphae with septum
  - Coenocytic hyphae: hyphae without septum
  - Haustoria: rootlike structure that grows into or around another structure to absorb water or nutrients. Important for parasitic plants
21. Life cycle of fungus

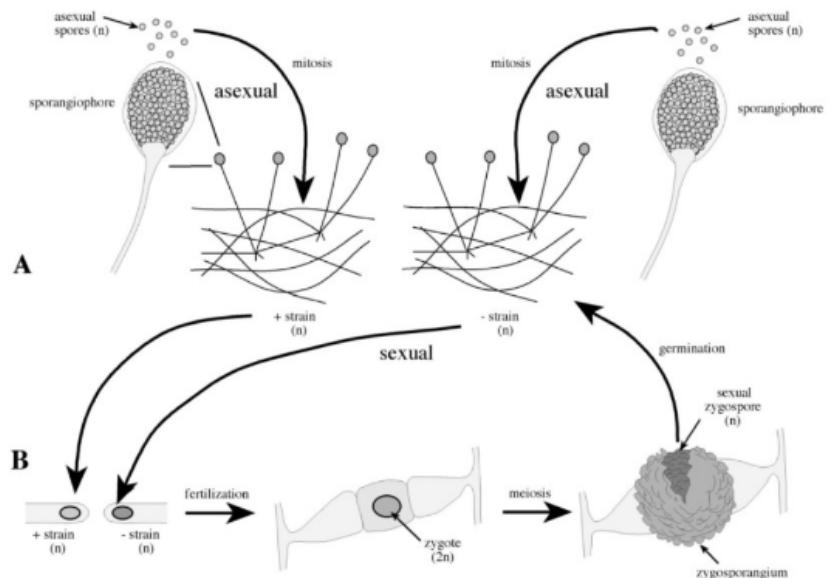


- Fungus mycelium is haploid
- Most fungi are haplontic
- Heterothallic species: Have 'sexes' that reside in individuals and one of each strain needed for mating. Two loci: MAT A and MAT a (mating type locus)
- Homothallic: capable of sexual reproduction with a single organism

## 22. Fungal classification

Chytrids	Aquatic with flagellated cells Alternation of generation Motile spores
Zygomycetes Eg: rhizopus	Non-motile reproductive spores Haplontic life cycle Vegetative cells ( $n$ ) as hyphae zygosporangiophores which release ( $n$ ) asexual spores
Glomeromycetes	Form symbiotic mycorrhizae Coenocytic hyphae
Ascomycetes eg: yeast, morels	Spores internal in ascus (sac) Septate hyphae Hyphae dikaryotic
Basidiomycetes eg: button mushroom	Spores are free and external Dikaryotic ( $n+n$ ) (basidocarp) phase dominant

Life cycle of zygomycetes



# Week 15-Applied microbiology

28 February 2022 15:59

## 1. Applications of microbiology

Amylase, proteases	Spot removal
Streptokinase	Breakdown of blood clots
RE, ligases, polymerases	Cloning
Amino acids, vitamins	Supplements
Sorbic acid	Food preservative
Ethanol, methane	Fuels
Antimicrobials	Treatment of infections
Insulin, human growth factor	Medicine
Bt toxin	Insecticide

## 2. Genetic manipulations of microbes

- Mutagenesis
- Protoplast fusion (somatic fusion)
- Insertions
- Heterologous gene expression
- Protein evolution: forced, adaptive mutation and in vivo evolution

## 3. Genetic engineering systems in bacteria

Localized SOS mutagenesis (DNA repair by SOS genes is repair prone and leads to mutagenesis)	Base substitutions and frameshifts
Adapted frameshifting	Frameshifting
Transposable elements	
DNA uptake (transformation)	

## 4. Preservation of microorganisms

- Periodic transfer to new media
- Mineral oil slant: covering media with sterile mineral oil for storage
- Refrigeration in minimal media, distilled water
- Drying to remove water in sterile soil/ filter paper/ gelatin drops
- Freeze drying along with cryoprotective agent
- Ultrafreezing in liquid nitrogen

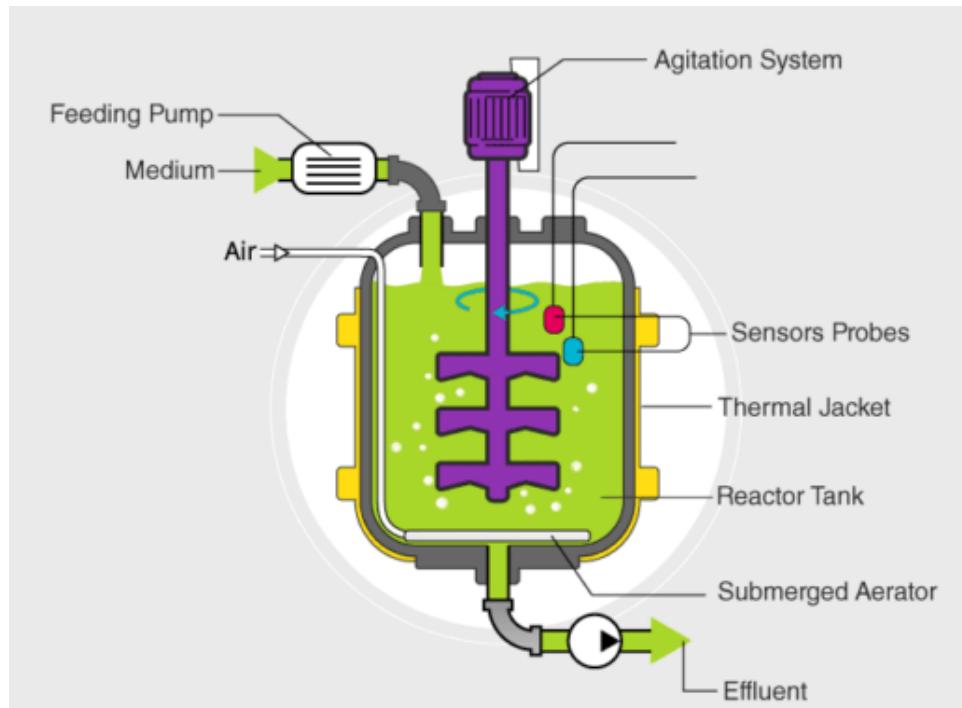
## 5. Industrial growth media components

- Carbon: molasses, whey, grains
- Nitrogen: soybean meal, ammonium salts, nitrates
- Vitamins: crude plant or animal products
- Antifoam agents (foam is caused due to aeration of the medium and the nutrient media can cause cell entrapment, cell damage, clogging of vents): alcohols, silicones, esters

## 6. Bioreactors

- A bioreactor refers to any manufactured device or system that supports a biologically active environment
- The environmental conditions inside the bioreactor, such as temperature, nutrient concentrations, pH, and dissolved gases (especially oxygen for aerobic fermentations) affect the growth and productivity of the organisms
- In an aerobic process, optimal oxygen transfer is sometimes the rate limiting step

- Oxygen transfer is usually helped by agitation, which is also needed to mix nutrients and to keep the fermentation homogeneous.
- Fouling: accumulation of unwanted substances on walls of reactors
- Non-newtonian broth- due to accumulation/aggregation of filamentous microbes. Generally unwanted



- Sampling devices allow to take samples of cultures for testing

## 7. Different methods of mass culture

Lift tube fermenter	Air-lift fermenter uses air injected from the bottom to aerate and mix the media
Solid state fermenter	growth of microbes on a solid support in the absence of free flowing fluid
Fixed bed reactor	microbes on fixed support as fluid flows or it
Fluidized bed reactor	Microbe looks are immobilized on surfaces of particles suspended in gas or liquid stream
Dialysis culture	products diffuse from culture into an adjacent buffer separated by a membrane and medium diffuses into the culture
Continuous culture	Medium continuously in and out of the reactor
Fed batch	one or more nutrients (substrates) are fed (supplied) to the bioreactor during cultivation and in which the product(s) remain in the bioreactor until the end of the run

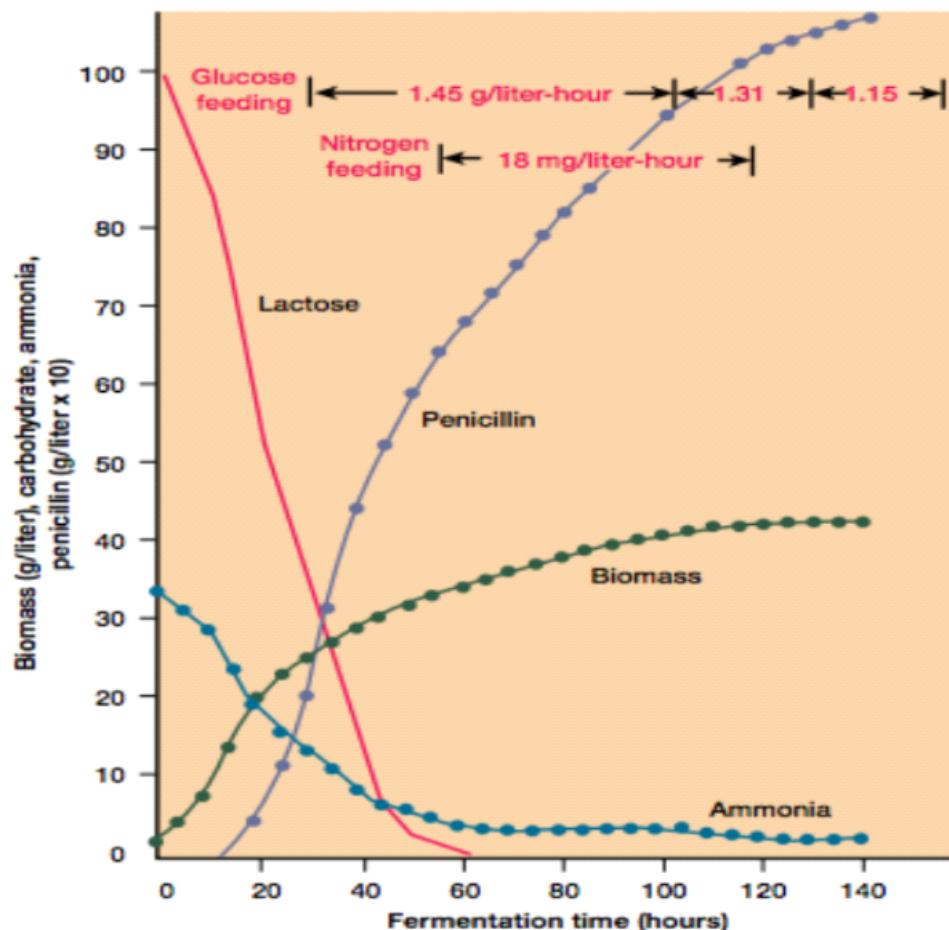
## 8. Batch vs continuous reactors

Batch	Continuous
Good for slurries and products that tend to foul	Able to cope with higher reactant concentration and better heat transfer capacities
Economical for slow reactions	Have higher mixing rates, with output rates that can be varied
Good for secondary metabolites as microbe population is allowed to saturate	Good for primary metabolites as microbes are continuously in growth phase

Primary metabolite	Secondary metabolite
9. Production coincides with growth	Production occurs after the population saturates in the media

10. Production kinetics

- First C and N are given in plenty to reach required biomass
- Limitation of C/N to induce stress response and hence production of secondary metabolite



**Figure 42.10 Penicillin Fermentation Involves Precise Control of Nutrients.** The synthesis of penicillin begins when nitrogen from ammonia becomes limiting. After most of the lactose (a slowly catabolized disaccharide) has been degraded, glucose (a rapidly used monosaccharide) is added along with a low level of nitrogen. This stimulates maximum transformation of the carbon sources to penicillin.

11. Glutamic acid

- Sodium glutamate is flavouring agent
- Glutamic acid extracted from *Corynebacterium glutamicum*
- Source of glutamic acid: AKG
- During initial growth period glyoxylate bypass is used and carbon flux is directed for energy production
- After growth, carbon flux is directed to AKG and glutamate synthesis

12. Other organic acids

Acetic acid	Acetobacter
Citric acid	<i>Aspergillus niger</i>
Gluconic acid	<i>Aspergillus niger</i>

- Mn and Fe limitation - use of ion exchange resin and sequesters away Fe and Mn
- Low level of biotin and added fatty acid derivatives increases membrane permeability and improve yield of glutamic acid

13. Biopolymers

Dextrans	blood expanders and absorbents
Erwinia	in paints
Cellulose	Acetobacter xylinum
Cyclodextrins	cholesterol removal from eggs and butter
Xanthan	Xanthomonas

14. Recombinant protein expression

- Involves gene cloning, expression and protein purification
- Human gene (exons and introns) --> mRNA --> mRNA splicing --> reverse transcriptase to generate cDNA --> convert ss cDNA to ds DNA
- Cloning can be ligation independent, restriction enzyme based, gateway, recombination based
- pBAD is tightly regulated

15. Protein folding tags

- GST tag: GST folds rapidly into a stable and highly soluble protein upon translation, inclusion of the GST tag often promotes greater expression
- GFP reporter
- Maltose-binding protein (MBP): is one of the most popular fusion partners being used for producing recombinant proteins in bacterial cells which increases solubility

16. Protein purification

- 6x His tag
- Protein G
- Immobilized metal affinity chromatography (IMAC)

17. Problems with protein purification

- Poor lysis
- Recombination proteins may fail to bind to affinity columns
- Bacterial proteins copurify (use size exclusion chromatography to overcome this problem)

18. Bioconversion

- Microbes as biocatalysts for reactions: hydroxylation, keto addition, saturation
- Advantages: stereospecific, milder reaction conditions

# Quick review: genes and factors

01 March 2022 15:27

NtcA	Induced formation of heterocysts in cyanobacteria during nitrogen stress
PatS	Intercellular molecule that suppresses formation of heterocysts in adjacent cells
CtrA-P	Inhibits cells division
DivL	Activates CtrA by phosphorylating it
DivK-P	Inhibits DivL
relA	Synthase. Synthesizes pentaphosphate guanosine which induces fruiting body formation in <i>M. xanthus</i>
SocE	Negative regulator of relA and maintains vegetative state
FruA	First committed step in fruiting body formation
Slr, sinR	Biofilm production
Spo0F, spo0B spo0A	Form a phosphorylation cascade to initiate production of sigma factors that are useful for endospore gene related transcription
kinA kinB	Phosphorylation of spo0F kinA senses starvation
Sigma F sigma G	Transcription factors for endospore
LuxR	Transcriptional activator of lux promoter. Activated by AHL
LuxI	AHL
CheA	Histidine kinase
MCP	Methyl accepting proteins
CheY	When phosphorylated, it binds to flagella to cause CW and hence makes the cell go in straight runs
CheB	When phosphorylated it is a methylesterase that reduced activity of MCP
CheZ	Phosphotase that terminates CheY
CheR	Constitutive methyltransferase that increases acitivity of MCP

Staining method	Primary stain	Counter stain
Gram staining	Crystal violet	Safranin
Endospore staining	Malachite green	Safranin
Liefson flagellar	Tannic acid + dye	
Capsule	Carbol fuchsin	Nigrosine
Ziehl Neelson	Carbol fuchsin	Methylene blue

# Cold Spring Harb Perspect Med-2012-Rutherford- Quorum sensing

22 February 2022 09:01



## Bacterial Quorum Sensing: Its Role in Virulence and Possibilities for Its Control

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Quorum sensing is a process of cell–cell communication that allows bacteria to share information about cell density and adjust gene expression accordingly. This process enables bacteria to express energetically expensive processes as a collective only when the impact of those processes on the environment or on a host will be maximized. Among the many traits controlled by quorum sensing is the expression of virulence factors by pathogenic bacteria. Here we review the quorum-sensing circuits of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Vibrio cholerae*. We outline these canonical quorum-sensing mechanisms and how each uniquely controls virulence factor production. Additionally, we examine recent efforts to inhibit quorum sensing in these pathogens with the goal of designing novel antimicrobial therapeutics.

Quorum sensing (QS) is a bacterial cell–cell communication process that involves the production, detection, and response to extracellular signaling molecules called autoinducers (AIs). AIs accumulate in the environment as the bacterial population density increases, and bacteria monitor this information to track changes in their cell numbers and collectively alter gene expression. QS controls genes that direct activities that are beneficial when performed by groups of bacteria acting in synchrony. Processes controlled by QS include bioluminescence, sporulation, competence, antibiotic production, biofilm formation, and virulence factor secretion (reviewed in Novick and Geisinger 2008; Ng and Bassler 2009; Williams and Camastra 2009).

Despite differences in regulatory components and molecular mechanisms, all known QS systems depend on three basic principles. First, the members of the community produce AIs, which are the signaling molecules. At low cell density (LCD), AIs diffuse away, and, therefore, are present at concentrations below the threshold required for detection. At high cell density (HCD), the cumulative production of AIs leads to a local high concentration, enabling detection and response (Kaplan and Greenberg 1985). Second, AIs are detected by receptors that exist in the cytoplasm or in the membrane. Third, in addition to activating expression of genes necessary for cooperative behaviors, detection of AIs results in activation of AI production (Novick et al. 1995; Seed et al. 1995). This

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S.T. Rutherford and B.L. Bassler

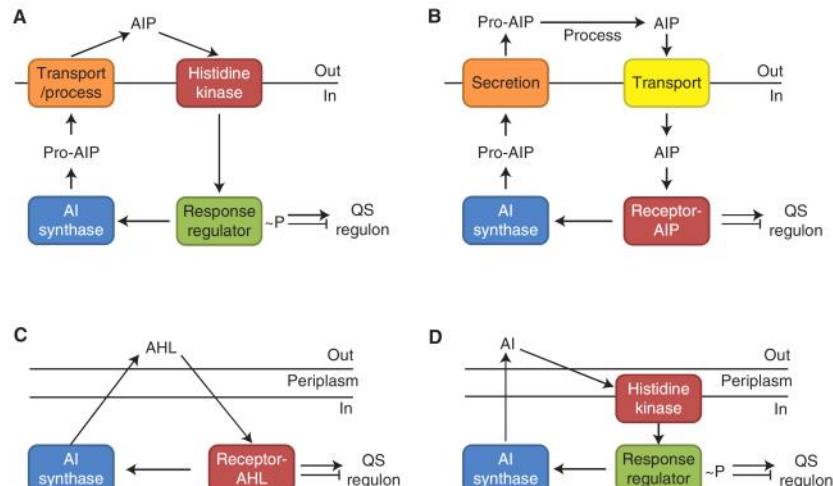
feed-forward autoinduction loop presumably promotes synchrony in the population.

Gram-positive and Gram-negative bacteria use different types of QS systems (Fig. 1 shows the four paradigmatic QS wiring diagrams). Gram-positive bacteria use peptides, called autoinducing peptides (AIPs), as signaling molecules. Once produced in the cell, AIPs are processed and secreted. When the extracellular concentration of the AIP is high, which occurs at HCD, it binds to a cognate membrane-bound two-component histidine kinase receptor. Usually, binding activates the receptor's kinase activity, it autophosphorylates, and passes phosphate to a cognate cytoplasmic response regulator. The phosphorylated response regulator activates transcription of the genes in the QS regulon (Fig. 1A). In some cases of Gram-positive bacterial QS, AIPs are transported back into the cell cytoplasm where they interact with transcription factors to modulate the transcription factor's activity and, in turn, modulate gene expression changes (Fig. 1B).

Gram-negative bacteria communicate using small molecules as AIs. These are either acyl-

homoserine lactones (AHLs) or other molecules whose production depends on S-adenosylmethionine (SAM) as a substrate (Wei et al. 2011). AIs are produced in the cell and freely diffuse across the inner and outer membranes. When the concentration of AIs is sufficiently high, which occurs at HCD, they bind cytoplasmic receptors that are transcription factors. The AI-bound receptors regulate expression of the genes in the QS regulon (Fig. 1C). In some cases of Gram-negative bacterial QS, AIs are detected by two-component histidine kinase receptors that function analogously to those described in the preceding paragraph for Gram-positive bacteria (Fig. 1D).

Dozens of clinically-relevant bacteria use QS to regulate the collective production of virulence factors. Here, we highlight the QS systems of four human pathogens that exemplify the diversity of QS systems. First, we outline how *Staphylococcus aureus* uses the paradigmatic Agr system to regulate adhesion and production of virulence factors. We also discuss the PlcR/PapR system that controls virulence factor production in the Gram-positive bacterium *Bacillus cereus*.



**Figure 1.** Canonical bacterial quorum-sensing (QS) circuits. Autoinducing peptide (AIP) QS in Gram-positive bacteria by (A) two-component signaling, or (B) an AIP-binding transcription factor. Small molecule QS in Gram-negative bacteria by (C) a LuxI/LuxR-type system, or (D) two-component signaling.

## Quorum Sensing and Virulence

Next, we describe the canonical Gram-negative LuxI/LuxR QS circuit and how *Pseudomonas aeruginosa* exploits two such circuits arranged in tandem to control virulence factor production and biofilm formation. Finally, we discuss QS in *Vibrio cholerae*, a Gram-negative bacterium that uses two parallel two-component QS systems to control virulence factor production and biofilm formation. Importantly, in all of these cases, the QS circuits are tailored to promote the specific disease. *P. aeruginosa* and *S. aureus* cause persistent diseases while *V. cholerae* and *B. cereus* cause acute infections. We also outline efforts to develop inhibitors of these QS systems to be deployed as novel antimicrobials.

### QS CONTROL OF VIRULENCE FACTORS IN GRAM-POSITIVE BACTERIA

#### Two-Component QS in Gram-Positive Bacteria

QS in Gram-positive bacteria relies on principles common to all QS circuits: production, detection, and response to AIs. In many Gram-positive bacteria, the AIs are oligopeptide AIPs that are detected by membrane-bound two-component signal transduction systems (Fig. 1A) (Havarstein et al. 1995; Ji et al. 1995; Solomon et al. 1996).

The AIPs are encoded as precursors (pro-AIPs) and are diverse in sequence and structure (Havarstein et al. 1995; Otto et al. 1998; Lazazzera 2001; Nakayama et al. 2001; Kalkum et al. 2003; Okada et al. 2005; Thoendel et al. 2011). Because the cell membrane is impermeable to peptides, specialized transporters are required to secrete AIPs. The AIP transporters also process the pro-AIPs. The final processed AIPs range in size from 5 to 17 amino acids, can be posttranslationally modified, and can be linear or cyclized (Magnuson et al. 1994; Havarstein et al. 1995; Mayville et al. 1999; Okada et al. 2005; Bouillaut et al. 2008). Extracellular AIPs are detected via membrane-bound two-component sensor kinases (Hoch and Silhavy 1995; Inouye and Dutta 2003; Simon et al. 2007). The sensor kinases autophosphorylate at conserved histi-

dines when bound by the AIP. The phosphoryl group is passed from the histidine to a conserved aspartate on a cognate cytoplasmic response-regulator protein, and the phosphorylated response regulator controls expression of QS-target genes. In these Gram-positive QS circuits, the pro-AIP, transporter, histidine kinase receptor, and response regulator are typically encoded in an operon (Ji et al. 1995; Peterson et al. 2000). Expression of this operon is activated by the phosphorylated response regulator, resulting in an autoinducing feed-forward loop that synchronizes the QS response.

Some examples of Gram-positive QS behaviors are competence in *Streptococcus pneumoniae* and *Bacillus subtilis* and sporulation in *B. subtilis* (Kleerebezem et al. 1997). QS controls virulence factor production in Gram-positive human pathogens including *S. aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Clostridium perfringens* (Autret et al. 2003; Podbielski and Kreikemeyer 2004; Ohtani et al. 2009; Riedel et al. 2009; Thoendel et al. 2011). The most well-studied system in this group of pathogens is the *S. aureus* Agr system (reviewed extensively in Thoendel et al. 2011).

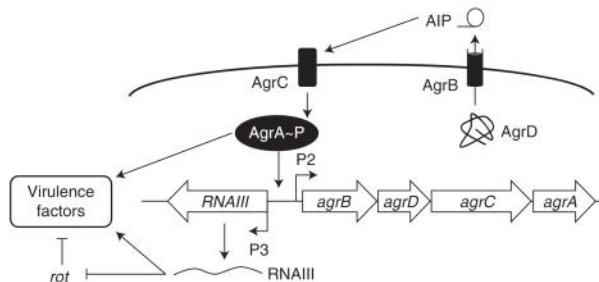
#### *S. aureus* Quorum Sensing

*S. aureus* is found among the normal human skin flora. If the epithelial barrier is compromised, *S. aureus* can cause minor skin infections. These infections can lead to pneumonia, bacteremia, and sepsis (Lowy 1998; Massey et al. 2006). *S. aureus* is the leading cause of hospital-related infections in the United States. Its ability to cause disease depends on expression of an array of adhesion molecules, toxins, and compounds that affect the immune system. QS regulates expression of genes encoding these virulence factors.

*S. aureus* uses a canonical Gram-positive two-component QS system encoded by the *agr* locus (Fig. 2). The P2 promoter drives expression of a transcript (RNAII), which encodes the four components of the QS system (Novick et al. 1995). *agrD* encodes the pro-AIP, which is processed to the final AIP and secreted by the transmembrane transporter protein AgrB (Ji et al.



S.T. Rutherford and B.L. Bassler



**Figure 2.** *S. aureus* Agr QS circuit. The autoinducing peptide (AIP) is synthesized as a precursor from *agrD*. The AIP transporter AgrB processes the precursor to the mature AIP and transports it out of the cell. AIPs are detected by a two-component signal transduction pathway. AgrC is the membrane-bound histidine kinase and AgrA is the response regulator. Phosphorylated AgrA activates the P2 and P3 promoters encoding the *agr* operon (called RNAII and the RNAIII regulatory RNA, respectively. RNAIII posttranscriptionally activates virulence factor production and represses expression of *rot*, the repressor of toxins, leading to further derepression of virulence factors.

1995; Saenz et al. 2000; Thoendel and Horswill 2009, 2010; Thoendel et al. 2011). Processing involves truncating the 45–47 residue pro-AIP to a 7–9 residues peptide, coupled with cyclization of a five-membered peptide ring via a thio-lactone bond between a central cysteine residue and the carboxyl terminus (Table 1). When the AIP accumulates, it binds the membrane-bound histidine kinase AgrC, which autophosphorylates at a conserved histidine and transfers the phosphate group to an aspartate on the response regulator AgrA (Lina et al. 1998). Phosphorylated AgrA binds upstream of the P2 promoter to autoinduce the *agr* operon (Novick et al. 1995).

In addition to activating the P2 promoter, phosphorylated AgrA activates the divergently encoded P3 promoter. The P3 promoter controls expression of RNAIII (Novick et al. 1993). The 5' region of RNAIII harbors the *hld* gene, which encodes the virulence factor δ-hemolysin (Janzon and Arvidson 1990). A more prominent role for RNAIII is as a regulatory RNA (Novick et al. 1993). RNAIII has the dual-function of activating production of α-toxin and repressing expression of *rot*, fibronectin binding proteins A and B, protein A, coagulase, and other surface proteins (Morfeldt et al. 1995; Dunman et al. 2001). Repression of *rot*, which encodes a repressor of toxins, leads to de-repres-

sion of additional toxins, proteases, lipases, enterotoxins, superantigens, and urease (Said-Salim et al. 2003; Geisinger et al. 2006). The net result of this QS regulatory cascade is down-regulation of surface virulence factors (such as protein A), and up-regulation of secreted virulence factors (such as α-toxin). Most of the effects of QS on regulation of virulence in *S. aureus* are mediated through direct and indirect regulation by RNAIII, however, phosphorylated AgrA also directly activates at least two additional virulence genes encoding phenol-soluble modulines (Queck et al. 2008).

Another key component of the *S. aureus* virulence program is biofilm development. In *S. aureus*, the *agr* system inhibits biofilm formation (Vuong et al. 2000; Boles and Horswill 2008). One interpretation of this finding is that establishing a biofilm community at LCD allows *S. aureus* time to grow to HCD and, at that point, it is optimally poised to secrete virulence factors (Yarwood et al. 2004; Boles and Horswill 2008). To facilitate its dispersal, *S. aureus* terminates biofilm production and decreases surface proteins and adhesions at HCD. This behavior is analogous to the strategy used by *V. cholerae* (see below).

Numerous additional regulatory factors converge at the P2 and P3 promoters, which add



Quorum Sensing and Virulence

**Table 1.** *S. aureus* AIPs and inhibitors

	AIP	Truncations	Substitutions	Hybrids
AIP-I				
AIP-II				
AIP-III				
AIP-IV				

Inhibitors 11, 13, 15, 16, and 17 are from (Lyon et al. 2002); inhibitor 12 is from (Lyon et al. 2000); and inhibitor 14 is from (McDowell et al. 2001).



layers of regulation to the *S. aureus* QS circuitry, presumably ensuring that the QS program is expressed under the most ideal situations. There is evidence for regulation by ArlRS, CcpA, CodY, Rsr,  $\sigma^B$ , SarA, SarR, SarT, SarU, SarX, SarZ, Rsr, and SrrA/SrrB (Cheung and Projan 1994; Schmidt et al. 2001; Yarwood et al. 2001; Manna and Cheung 2003, 2006a,b; Liang et al. 2005; Seidl et al. 2006; Majerczyk et al. 2008; Lauderdale et al. 2009; Tamber and Cheung 2009; Tamber et al. 2010). One of the interesting themes common to several of these accessory *agr* regulators is that they provide *S. aureus* the ability to respond to extracellular environmental signals, in addition to AIPs, using the QS circuitry. For example, following extracellular stress, the alternative sigma factor  $\sigma^B$  interacts with core RNAP to direct transcription of surface proteins and pigment production, and inhibit expression of secreted toxins and proteases. RNAIII levels also increase when the  $\sigma^B$  gene is deleted (Lauderdale et al. 2009). No consensus sequence for  $\sigma^B$  exists at the P2 or P3 promoters, so it is likely that  $\sigma^B$  regulates some as-

yet-unknown regulator of *agr*.  $\sigma^B$  regulation presumably ensures that *S. aureus* does not undergo QS, which is energetically costly, under conditions when the bacteria must dedicate resources to alleviating stress. Another example involves CodY, which responds to isoleucine limitation in *S. aureus*. Deletion of *codY* increases RNAII and RNAIII expression (Majerczyk et al. 2008; Pohl et al. 2009). CodY binding sites are located in the *agrC* gene (Majerczyk et al. 2010). Thus, CodY allows *S. aureus* to delay its QS response until it exists in a nutrient-rich environment. Finally, the SrrA/SrrB two-component system, which regulates gene expression in response to anaerobic environments, also controls the P2 and P3 promoter region via SrrB binding (Yarwood et al. 2001). Overexpression of SrrA/SrrB decreases virulence, likely due, at least in part, to inhibition of *agr* expression. Again, this regulation is thought to ensure that QS is not undertaken in a less than ideal environment.

A final interesting aspect of the *S. aureus* QS system is cross-competition among AIP specificity types. Hypervariability exists in the *agrD*

S.T. Rutherford and B.L. Bassler

gene and a portion of the *agrB* gene (Dufour et al. 2002). This variability leads to production of one of four different types of *S. aureus* AIPs depending on the strain (Table 1). The identity of the AIP allows typing of *S. aureus* into four specificity groups (I–IV) (Ji et al. 1997; Jarraud et al. 2000). A corresponding hypervariability exists in the portion of the *agrC* gene encoding the sensing domain of the AIP receptor (Dufour et al. 2002). Thus, each specific AIP is detected by a coevolved cognate AgrC sensor. The presence of a noncognate AIP results in inhibition of QS, highlighting the specific nature of the AIP-receptor interactions. Specificity is proposed to be determined sterically and binding of the incorrect AIP-type can lead to stabilization of an inhibitory confirmation of AgrC, thus halting cell-cell signaling (Mayville et al. 1999; Lyon et al. 2002; Geisinger et al. 2009). The biological consequence of this mechanism is that the first strain to establish its QS cascade in the host is the one that causes the infection (Fleming et al. 2006).

#### *S. aureus* QS as a Therapeutic Target

The prevalence of *S. aureus* infections coupled with the emergence of methicillin-resistant *S. aureus* (MRSA) highlight the importance of studying and controlling this pathogen (Brumfitt and Hamilton-Miller 1989). The rationale for targeting the *S. aureus* QS system is that *agr* mutants are defective in virulence. The principal target considered to date is the receptor AgrC. Because the AIPs naturally cross-inhibit, analyses of their variations coupled with synthetic chemistry have provided a mechanistic understanding of AIP antagonism. By varying the length (Table 2, peptides 11–13) or amino acid sequence (Table 2, peptides 14) of the AIP or by combining substitutions and truncations to make hybrid AIPs (Table 2, peptides 15–17), universal inhibitors capable of out-competing all four AIP types have been designed (Lyon et al. 2000, 2002; McDowell et al. 2001).

The AIPs themselves have also been targeted for inhibition by inactivation through interaction with specific antibodies. The AIPs are poor antigens, however, monoclonal antibodies aga-

inst AIPs coupled to carriers have been raised and are promising (Park et al. 2007). For example, screening of a library of monoclonal antibodies identified one antibody that reduced RNAIII and toxin production. This antibody acts as a highly-specific QS quencher.

One of the concerns with targeting the *S. aureus* QS system is the trade-off between virulence factor production and biofilm formation (Otto 2004). Inhibition of *agr* causes *S. aureus* to become more adherent because of increased biofilm formation. This facet of the circuit can lead to persistent colonization by *S. aureus*, which is of utmost concern for medically-implanted devices. Although there are reports that deletion of *agr* does not enhance biofilm formation under all conditions (Shenkman et al. 2001), targeting *agr* will likely become one part of a combination therapy for combating *S. aureus* and MRSA.

#### QS by Peptide-Binding Transcription Factors in Gram-Positive Bacteria

Some Gram-positive bacteria use a QS system in which AIPs, following release, are imported back into the cell and detected by cytoplasmic transcription factors (Fig. 1B). In these systems, the pro-AIP is secreted and processed into the mature AIP by extracellular proteases. Following transport back into the cell, the AIP binds to and alters the activity of a transcription factor (Slamti and Lereclus 2002).

Some examples of Gram-positive systems in which AIPs are imported include sporulation, competence, and enzyme production in *B. subtilis* (Pottathil and Lazazzera 2003) and plasmid transfer in *E. faecalis* (Dunny 2007). Additionally, AIP-bound transcription factors control virulence factor production in the *Bacillus cereus* group (Slamti and Lereclus 2002; Bouillaut et al. 2008) and we describe this system here (see review by Rocha-Estrada et al. 2010).

#### *B. cereus* Quorum Sensing

The *B. cereus* group of Gram-positive bacteria consists of several closely-related bacteria relevant to human health including *B. cereus*, *B.*



## Quorum Sensing and Virulence

*anthracis*, and *B. thuringiensis*. *B. cereus* causes both intestinal and nonintestinal infections in humans and is most commonly associated with food poisoning (Bottone 2010). Its ability to cause acute diarrheal disease is caused by the production and secretion of a variety of hemolysins, phospholipases, and toxins (Bottone 2010).

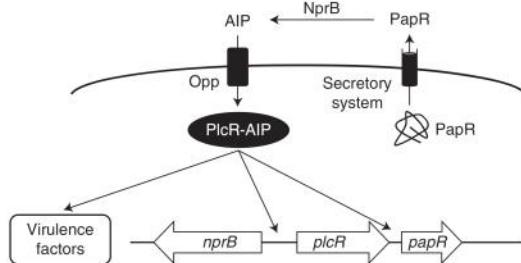
QS in *B. cereus* requires the transcription factor PlcR, which controls expression of most *B. cereus* virulence factors following binding to the intracellular AIP derived from the PapR protein (Fig. 3) (Slamti and Lereclus 2002). *papR* is encoded 70 basepairs downstream of *plcR*. PapR is 48 amino acids long and contains an amino-terminal signal peptide that targets it for the secretory pathway (Okstad et al. 1999). Once outside the cell, the PapR pro-AIP is processed by the secreted neutral protease B (NprB) to form the active AIP (Pomerantsev et al. 2009). *nprB* is encoded divergently from *plcR* and *nprB* expression is activated by AIP-bound PlcR (Okstad et al. 1999). NprB cleaves the pro-AIP PapR into peptides of 5, 7, 8, and 11 amino acids in length, all of which are derived from the carboxyl terminus of full-length PapR (Pomerantsev et al. 2009). Only the pentapeptide and heptapeptide activate PlcR activity, however, the heptapeptide causes maximal activation and is more prevalent in vivo (Bouilliaut et al. 2008; Pomerantsev et al. 2009).

Similar to the *S. aureus* Agr system (see above), there is sequence diversity in the PapR

AIPs that enables classification of members of the *B. cereus* group into four pherotypes. Specificity for the PlcR receptor stems from the identities of the first and fifth amino acids in the pentapeptides: LPFE(F/Y), VP(F/Y)E(F/Y), MPFEE, and LPFEH (Slamti and Lereclus 2005). There is low cross-reactivity with heterologous PlcRs, indicating that the AIP and its receptor (PlcR) coevolved (Slamti and Lereclus 2005). Another interesting aspect of the pherotypes is that some contain different species, and can therefore communicate across species, whereas in other cases, different isolates of the same species fall into different subgroups and are prevented from communicating with one another (Slamti and Lereclus 2005; Rocha-Estrada et al. 2010).

The processed PapR AIP is imported back into the cell by the oligopeptide permease system (Opp) (Fig. 3) (Gominet et al. 2001). Once inside the cell, the AIP binds to the transcription factor PlcR, and this causes conformational changes in the DNA-binding domain of PlcR, facilitates PlcR oligomerization, DNA binding, and regulation of transcription (Declerck et al. 2007).

When PlcR interacts with the PapR AIP and oligomerizes, it binds to "PlcR boxes" to regulate transcription of target genes (Agaisse et al. 1999). PlcR controls expression of 45 genes, many of which encode extracellular proteins including several enterotoxins, hemolysins, phos-



**Figure 3.** *B. cereus* QS circuit. The pro-AIP PapR is secreted and is then processed to the mature heptapeptide AIP by the extracellular protease NprB. The mature AIP is transported back into the cell by Opp. Intracellular AIP binds to the transcription factor PlcR and activates it. The PlcR–AIP complex regulates virulence factor production and activates expression of *papR*.

S.T. Rutherford and B.L. Bassler

pholipases, and proteases (Lereclus et al. 1996; Gohar et al. 2002, 2008). As is the case in all QS systems, AIP-bound PlcR also feedback activates expression of *papR* (Lereclus et al. 1996). In addition to its role in regulating extracellular virulence factors, AIP-bound PlcR has other regulatory targets. These include two-component systems, transport systems, and GGDEF-containing proteins (Gohar et al. 2008). Finally, deletion of *plcR* results in increased biofilm production (Hsueh et al. 2006). However, despite its important role in regulating virulence factor production, disruption of *plcR* does not fully eliminate virulence (Lereclus et al. 2000; Callegan et al. 2003) because several additional systems feed into the *B. cereus* QS circuit and virulence factor regulation. These additional sensory inputs include sporulation through SpoOA ~ P, nutritional state through CodY, motility through FlhA, and other two-component systems (Lereclus et al. 2000; Bouillaut et al. 2005; Brillard et al. 2008; Frenzel et al. 2012).

To date, no anti-PlcR/PapR QS molecules or screens for inhibitors of QS in *B. cereus* have been reported. Analogous to the *S. aureus* anti-QS strategies described above, the *B. cereus* AIPs could certainly be investigated for development into QS inhibitors. The NprB protease, the Opp transporter, and the PlcR receptor are other obvious candidates for exploration of whether or not they are vulnerable to small molecule inhibition.

### QS CONTROL OF VIRULENCE IN GRAM-NEGATIVE BACTERIA

#### LuxI/LuxR Quorum Sensing

Gram-negative bacteria typically use LuxI/LuxR-type QS systems homologous to the first described QS system from the bioluminescent marine symbiotic bacterium *Vibrio fischeri* (Fig. 1C) (Ruby 1996; Hastings and Greenberg 1999). In these systems, the LuxI homolog is an AI synthetase that catalyzes a reaction between SAM and an acyl carrier protein (ACP) to produce a freely diffusible acyl homoserine lactone (AHL) AI (Engebrecht and Silverman 1984; More et al. 1996; Schaefer et al. 1996; Ng and Bassler

2009). At high concentrations, AHL AIs bind to cognate cytoplasmic LuxR-like transcription factors. When not bound by AI, LuxR-type proteins are rapidly degraded, presumably to prevent bacteria from "short-circuiting" their QS systems. AI binding stabilizes the LuxR-type proteins, allowing them to fold, bind DNA, and activate transcription of target genes (Engebrecht et al. 1983; Engebrecht and Silverman 1984; Stevens et al. 1994; Zhu and Winans 1999, 2001). Typically, AHL-bound LuxR-type proteins also activate *luxI* expression, forming a feed-forward autoinduction loop that floods the vicinity with AI (Engebrecht et al. 1983; Fuqua and Winans 1994; Seed et al. 1995).

LuxI/LuxR homologs have been identified in more than 100 Gram-negative bacterial species (Case et al. 2008). AHLs produced by different bacteria possess different length side chains and side-chain decorations. Acyl chains ranging from C4 to C18 have been identified with modifications such as carbonyl and hydroxy moieties at the C3 position (Fuqua et al. 2001; Ng and Bassler 2009). This chemical diversity promotes intraspecies-specific bacterial cell-cell communication in that interactions with partner LuxR proteins are usually highly specific. The substrate binding pockets of the various LuxI homologs have correspondingly different sizes and shapes, ensuring accommodation of only a particular acyl-ACP for synthesis of a particular AI (Watson et al. 2002; Gould et al. 2004). Likewise, the AI-detecting LuxR homologs possess unique binding pockets that specifically accommodate particular AHL ligands (Vannini et al. 2002; Zhang et al. 2002; Yao et al. 2006; Bottomley et al. 2007; Chen et al. 2011).

Numerous Gram-negative pathogens control virulence factor production using LuxI/LuxR-type QS circuits. Some examples are LasI/LasR and RhlI/RhlR in *P. aeruginosa*, SmaI/SmaR in *Serratia marcescens*, VjbR (an orphan LuxR homolog) in *Brucella melitensis*, and CviI/CviR in *Chromobacterium violaceum* (Passador et al. 1993; Brink and Ohman 1995; McClean et al. 1997; Thomson et al. 2000; Weeks et al. 2010). We describe the *P. aeruginosa* systems as the canonical example here (reviewed in detail in Williams and Camara 2009).



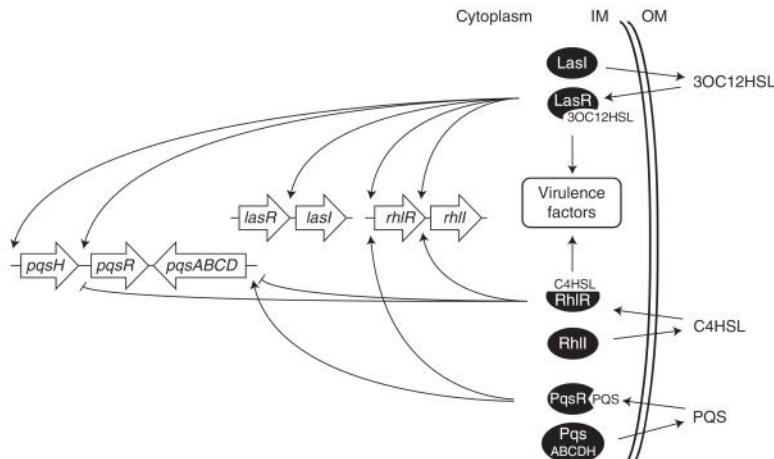
## Quorum Sensing and Virulence

### *P. aeruginosa* QS

*P. aeruginosa* is a ubiquitous Gram-negative bacterium that can cause both acute and chronic infections in humans (Chugani and Greenberg 2007; Mena and Gerba 2009). *P. aeruginosa* infection generally depends on the host having a compromised immune system. Typically, *P. aeruginosa* infections are found in the lungs of people with cystic fibrosis (Lyczak et al. 2002; Zemanick et al. 2011). This infection leads to declined pulmonary function and increased mortality. *P. aeruginosa* also causes acute infections in people with compromised epithelial barriers; a common trait among patients with severe burns or tracheal intubation and mechanical ventilation (Bielecki et al. 2008). In both scenarios, *P. aeruginosa* uses QS to collectively produce a suite of virulence factors that contribute to its disease-causing ability.

*P. aeruginosa* harbors three QS systems: two LuxI/LuxR-type QS circuits that function in series to control expression of virulence factors as well as a third, non-LuxI/LuxR-type system called the *Pseudomonas* quinolone signal (PQS)

system (Fig. 4). In the first circuit, the LuxI homolog LasI synthesizes 3-oxo-C12-homoserine lactone (3OC12HSL) (Table 2) (More et al. 1996; Val and Cronan 1998; Parsek et al. 1999; Gould et al. 2004; Bottomley et al. 2007). At HCD, this AI is detected by the cytoplasmic LuxR homolog LasR. The LasR–3OC12HSL complex activates transcription of target genes including those encoding virulence factors such as elastase, proteases, and exotoxin A (Gambello and Iglesias 1991; Gambello et al. 1993; Schuster et al. 2003, 2004). One of the LasR–3OC12HSL targets is *lasI*, which establishes an autoinducing feed-forward loop (Seed et al. 1995). Another target of regulation by LasR–3OC12HSL is a second LuxI homolog called *rhlI* (Latifi et al. 1996; Pesci et al. 1997). RhlI synthesizes a second AI, butanoyl homoserine lactone (C4HSL) (Table 2) (Ochsner et al. 1994; Pearson et al. 1995). At high concentrations, this AI binds to RhlR, a second LuxR homolog. RhlR–C4HSL activates target genes, including those encoding elastase, proteases, pyocyanin, and siderophores (Schuster et al. 2003; Schuster and Greenberg 2007). Among



**Figure 4.** *P. aeruginosa* QS circuits. The three AI synthases, LasI, RhlI, and PqsABCDH, produce the AIs 3OC12HSL, C4HSL, and PQS, respectively. The AIs are detected by the cytoplasmic transcription factors LasR, RhlR, and PqsR, respectively. Each transcription factor regulates expression of its corresponding AI synthase as well as additional targets as indicated by the arrows.

S.T. Rutherford and B.L. Bassler

**Table 2.** *P. aeruginosa* AIs and inhibitors

Receptor/target	Autoinducer	Antagonists	
LasR		(1) (2)	
			(3) (4)
		(5) (6)	
RhlR		(7) (8)	
PqsR		(9)	
QscR			(10)

The three AIs (and their cognate receptors) are 3OC12HSL (LasR), C4HSL (RhlR), and PQS (PqsR). Inhibitors 1 and 2 are modified from 3OC12HSL by McInnis and Blackwell (2011); inhibitor 3 is a furanone described in Hentzer et al. (2002) and Wu et al. (2004); inhibitor 4 is patulin described in Rasmussen et al. (2005); inhibitors 5 and 6 are from a chemical library described in Muh et al. (2006b); inhibitor 7 is a triphenyl derivative from Muh et al. (2006a); inhibitor 8 is C10-acylcyclopentylamine from Ishida et al. (2007); inhibitor 9 is solenopsin from Park et al. (2008); and compound 10 is propanoyl homoserine lactone from Mattmann et al. (2008).



## Quorum Sensing and Virulence

its targets is *rhlI*, leading to autoinduction of this second QS circuit.

The crystal structure of the ligand binding domain of LasR bound to 3OC12HSL has been solved, revealing interactions between the receptor protein and the AI (Bottomeley et al. 2007). Structures of other LuxR-type QS regulators, TraR, SdiR, and CviR, have also been solved enabling comparative analysis (Vannini et al. 2002; Zhang et al. 2002; Yao et al. 2006; Chen et al. 2011). In all cases, the common lactone head group of each AHL is bound by conserved residues in the binding pocket, whereas the residues forming the pockets for the different acyl tails are diverse. Comparing the LasR binding pocket for 3OC12HSL with the TraR binding pocket for 3OC8HSL shows different sizes and hydrophobic characteristics in the channels that accommodate the different acyl chains. These structures coupled with biochemical data indicate that binding of ligand to the receptor stabilizes folding of the hydrophobic core of the protein. Once folded properly, LasR (and presumably other LuxR-type receptors) can dimerize, bind DNA, and activate transcription (Zhu and Winans 2001; Kiratisin et al. 2002; Schuster et al. 2004; Urbanowski et al. 2004).

*P. aeruginosa* uses an additional non-LuxI/LuxR QS system to control virulence factor gene expression. PQS, 2-heptyl-3-hydroxy-4-quino-lone, is produced by PqsA, PqsB, PqsC, PqsD, and PqsH and is detected by the regulator PqsR (also called MvfR) (Fig. 4 and Table 2). Expression of *pqsH* and *pqsR* is activated by LasR–3OC12HSL, whereas RhIIR–C4HSL represses *pqsABCD* and *pqsR* (Gallagher et al. 2002; Deziel et al. 2004; Xiao et al. 2006b). PqsR–PQS autoinduces PQS synthesis and further activates *rhlI* and *rhlR* expression (Xiao et al. 2006a; Diggle et al. 2007). Thus, the PQS circuit is intimately tied to the LasI/LasR and RhIIR/RhlR QS systems and, therefore, also influences virulence factor production.

*P. aeruginosa* QS-activated virulence factors include elastase, proteases, pyocyanin, lectin, swarming motility, rhamnolipids, and toxins. Although distinct regulons for LasR–3OC12HSL and RhIIR–C4HSL have been reported, it is apparent that there is extensive overlap be-

tween the regulons (Whiteley et al. 1999; Schuster et al. 2003, 2004; Wagner et al. 2004; Schuster and Greenberg 2007). Thus, most genes originally thought to be targeted only by LasR or only by RhIIR can in fact be activated by either one. For example, a  $\Delta$ lasR mutant, which is defective for *rhlI* induction, expresses virulence factors originally reported to be LasR-dependent (Dekimpe and Deziel 2009). Apparently, low level *rhlI* and *rhlR* expression promotes the eventual accumulation of C4HSL and autoinduction of the RhIIR/RhlR system. Accordingly, activation of virulence factors is delayed in the  $\Delta$ lasR strain. These findings are of medical relevance because many clinical isolates of *P. aeruginosa* possess mutations in *lasR* (Smith et al. 2006).

Another QS-controlled activity in *P. aeruginosa* is biofilm formation. Although regulation of biofilm formation in *P. aeruginosa* largely depends on additional environmental signals, QS regulation of rhamnolipids, swarming motility, and siderophores also contribute to *P. aeruginosa* biofilm formation (Ochsner et al. 1994; Deziel et al. 2003; De Kievit 2008; Patriquin et al. 2008; Rahman et al. 2010).

Up to 10% of the *P. aeruginosa* genome is controlled by QS (Schuster and Greenberg 2006), thus it is not surprising that there are additional levels of regulation impinging on the QS circuits. Vfr, AlgR, PhoR/B, RpoN, RpoS, DksA, RelA, GidA, QscR, RsmY, RsmZ, RsmA, PrrF, PhrS, VqsM, VqsR, and RsaL have all been reported to feed information into the *P. aeruginosa* QS circuit (Albus et al. 1997; de Kievit et al. 1999; Branny et al. 2001; Pessi et al. 2001; van Delden et al. 2001; Heurlier et al. 2003; Ledgham et al. 2003; Medina et al. 2003; Hogardt et al. 2004; Juhas et al. 2004; Dong et al. 2005; Jensen et al. 2006; Morici et al. 2007; Schuster and Greenberg 2007; Gupta et al. 2009; Sonnleitner et al. 2010). These accessory regulators of *P. aeruginosa* QS presumably fine-tune the network so that virulence factor production occurs with optimal precision. Indeed, when AIs are added to exponentially growing *P. aeruginosa*, they do not induce expression of the entire QS regulon (Schuster and Greenberg 2007). Only when cells are exposed to stimuli



S.T. Rutherford and B.L. Bassler

associated with stationary phase as well as AIs is the complete set of QS targets launched. Although there are several fascinating regulatory events, here we highlight only two posttranscriptional regulation mechanisms.

### *QscR*

*QscR* is an orphan LuxR homolog that does not have a partner LuxI homolog. *QscR* can, however, bind to the AI produced by *LasI*, 3OC12HSL (Lequette et al. 2006; Lintz et al. 2011; Oinuma and Greenberg 2011). Additionally, it forms mixed dimers with *LasR* and *RhlR*, rendering them inactive (Ledgham et al. 2003). Thus, *QscR* likely prevents aberrant QS responses before the cells reaching "a quorum."

### *sRNAs*

Small regulatory RNAs (sRNAs) in bacteria act by basepairing with mRNA targets to repress or activate gene expression (reviewed in Gottesman 2004; Papenfort and Vogel 2010; Storz et al. 2011). In *P. aeruginosa*, the sRNAs *PrrF1*, *PrrF2*, and *PhrS* regulate the PQS system in response to limiting iron (*PrrF1* and *PrrF2*) or limiting oxygen (*PhrS*) (Wilderman et al. 2004; Sonnleitner et al. 2010; Sonnleitner and Haas 2011). *PhrS* stimulates translation of an open reading frame in the 5' end of the *pqsR* mRNA and this action activates *pqsR* translation by altering the secondary structure surrounding the *pqrR* RBS (Sonnleitner et al. 2010). *PrrF1* and *PrrF2* repress expression of genes encoding enzymes that degrade a precursor of PQS (anthranilate), thus allowing increased PQS under low iron conditions (Oglesby et al. 2008; Oglesby-Sherrouse and Vasil 2010; Sonnleitner and Haas 2011). Two other sRNAs involved in *P. aeruginosa* QS regulation are *RsmY* and *RsmZ*, sRNAs that are activated by the *GacS/GacA* two-component system in response to limiting magnesium and other unknown signals (Kay et al. 2006; Mulcahy and Lewenza 2011; O'Callaghan et al. 2011). *RsmY* and *RsmZ* repress the transcription factor *RsmA*, which, in turn, represses production of both 3OC12HSL and C4HSL (Pessi et al. 2001; Kay et al. 2006).

### *P. aeruginosa* QS as a Therapeutic Target

The *P. aeruginosa* QS circuits make attractive targets for novel antimicrobials because QS controls virulence factor production and no homologs to known QS components exist in humans. This is especially critical in the treatment of persistent infections in cystic fibrosis patients given the resistance of many *P. aeruginosa* isolates to available antibiotics. Small molecule inhibitors of *P. aeruginosa* QS have been extensively reviewed (Mattmann and Blackwell 2010). Here we highlight a few recent advances.

Because *LasR* sits at the top of the *P. aeruginosa* QS cascade, identifying *LasR* inhibitors has been a major focus. Competitive inhibitors have been reported that contain modifications to the native 3OC12HSL ligand (Geske et al. 2005, 2007, 2008; Mattmann and Blackwell 2010; McInnis and Blackwell 2011). In this realm, alterations to both the head group and the acyl tail have led to molecules that out-compete 3OC12HSL for binding to *LasR* (Table 2, compounds 1 and 2). Importantly, some of these modifications, such as substitution of the lactone ring for a thiolactone ring (McInnis and Blackwell 2011), are useful because of their increased stability under biological conditions. Second, natural products have been isolated that inhibit QS by antagonizing *LasR* (Hentzer et al. 2002; Wu et al. 2004; Rasmussen et al. 2005; Ren et al. 2005; Kim et al. 2008). These inhibitors include furanones and patulin (Table 2, compounds 3 and 4, respectively), which have been further modified to increase their efficacy. Finally, high-throughput screens of small molecule libraries have revealed additional scaffolds for the design of inhibitors (Muh et al. 2006a,b; Borlee et al. 2010) (for example, Table 2, compounds 5–7). A potential complication in targeting *LasR* is that, as mentioned, some clinical *P. aeruginosa* isolates possess defective *LasR* proteins (Smith et al. 2006). Nonetheless, establishment of the initial infection is known to be *LasI/LasR*-dependent, suggesting that, at a minimum, *LasR* inhibitors could be used as prophylactics.

Other factors involved in *P. aeruginosa* QS have also been targeted for drug discovery. For



## Quorum Sensing and Virulence

example, inhibitors of LasR have been successfully modified to act as competitive inhibitors of RhlR and in some cases these molecules have proven to be potent inhibitors of both LasR and RhlR (Table 2, compound 8) (Smith et al. 2003; Glansdorp et al. 2004; Ishida et al. 2007). Natural products have been isolated that inhibit RhlR (Table 2, compound 9) (Park et al. 2008).

A final approach is to target regulators that affect both the LasI/LasR and RhlI/RhlR QS systems. For example, a small molecule library was screened for agonists and antagonists of QscR, which, as described, influences both the LasI/LasR and RhlI/RhlR systems (Lee et al. 2006; Mattmann et al. 2008, 2011; Amara et al. 2009; Liu et al. 2010). By agonizing QscR, it could be possible to diminish the overall QS response and thus prevent or delay expression of virulence factors. Interestingly, some non-natural AHLs that target QscR also inhibit LasR, raising the intriguing possibility of a compound that can act broadly to target all of the *P. aeruginosa* QS systems (Table 2, compound 10) (Mattmann and Blackwell 2010).

### *V. cholerae* Quorum Sensing

*V. cholerae* is the causative agent of the disease cholera (Faruque et al. 1998). This diarrheal disease is endemic in underdeveloped regions and has epidemic and pandemic potential, especially when clean water supplies are compromised. The principal symptom of the disease, profuse watery diarrhea, can lead to dehydration and death if not properly treated. This symptom is caused by an enterotoxin called cholera toxin, expression of which is controlled by QS (Jobling and Holmes 1997; Zhu et al. 2002).

*V. cholerae*, a Gram-negative bacterium, produces and responds to two AIs using two parallel QS circuits. One AI is (S)-3-hydroxytridecan-4-one (CAI-1), which is synthesized by the CqsA enzyme using SAM and decanoyl-coenzyme A as substrates (Table 3) (Higgins et al. 2007; Ng et al. 2011; Wei et al. 2011). Homologs of CqsA have been identified in all vibrio species consistent with a proposed role for CAI-1 as an intergenus communication molecule, allowing communication among other vibrios harboring

*cqsA* (Miller et al. 2002; Henke and Bassler 2004; Higgins et al. 2007). The second AI, AI-2, is synthesized by LuxS. LuxS converts the SAM cycle intermediate S-ribosylhomocysteine to 4,5-dihydroxy-2,3-penanediol (DPD) and homocysteine. DPD spontaneously converts into AI-2 (Table 3) (Surette et al. 1999; Schauder et al. 2001; Chen et al. 2002). Homologs of *luxS* exist in hundreds of Gram-negative and Gram-positive bacteria, consistent with a role for AI-2 in interspecies communication that allows other *luxS*-encoding bacteria in a particular environment to contribute to the overall cell-density information (Federle and Bassler 2003; Xavier and Bassler 2005). Thus, using two different AIs presumably allows *V. cholerae* to detect both the number of other vibrios and the total number of bacteria in the environment.

*V. cholerae* detects CAI-1 and AI-2 using two parallel membrane-bound two-component receptors (Fig. 5). CqsS detects CAI-1 and LuxPQ detects AI-2 (Bassler et al. 1994; Neiditch et al. 2005, 2006; Ng et al. 2010, 2011). In the absence of ligand, at LCD, both of these receptors function as kinases. Upon autophosphorylation, CqsS and LuxPQ transfer phosphate to the shared response regulator LuxO via the phosphotransfer protein LuxU (Bassler et al. 1994; Freeman and Bassler 1999a,b; Lilley and Bassler 2000). Phosphorylated LuxO activates expression of genes encoding four homologous quorum-regulatory sRNAs, called Qrr1-4 (Lenz et al. 2004). Transcription of the *qrr* sRNA genes is directed by the alternative sigma factor  $\sigma^{54}$ . To date, the four *qrr* promoters and the *luxO* promoter itself are the only known targets for regulation by LuxO.

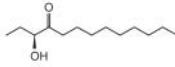
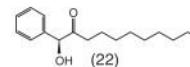
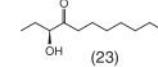
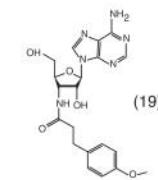
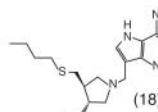
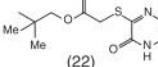
At LCD, the Qrr sRNAs are expressed and regulate target mRNAs by base-pairing. The principal targets of the Qrr RNAs are the mRNAs encoding the master QS regulators HapR and AphA (Lenz et al. 2004; Rutherford et al. 2011). However, the Qrr sRNAs have opposite effects on these two mRNA targets. The Qrr sRNAs base-pair with the *hapR* mRNA overlapping the RBS, prevent ribosome binding, and facilitate degradation of *hapR* mRNA (Lenz et al. 2004; Tu and Bassler 2007). The Qrr sRNAs activate production of *aphA* by binding to the

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13

S.T. Rutherford and B.L. Bassler

**Table 3.** *V. cholerae* AIs and inhibitors

Receptor/target	Autoinducer	Antagonists
CqsS		 
LuxPQ		
MTAN		
HapR		 
LuxO		

The autoinducers are CAI-1 and AI-2. Inhibitor 18 is a MTAN transition state analog from Schramm et al. (2008); inhibitor 19 is a modified nucleoside from Brackman et al. (2009); inhibitors 20 and 21 are cinnamaldehydes described in Niu et al. (2006); Brackman et al. (2008, 2011); compounds 22 (phenyl-CAI-1) and 23 (C8-CAI-1) are from Ng et al. (2010); and compound 24 a 6-thio-5-azauracil derivative from Ng et al. (2012).

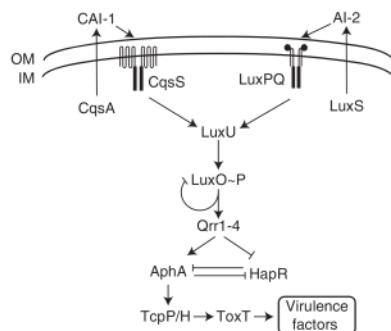


untranslated region of the *aphA* mRNA and inducing an alternative secondary structure, which reveals the RBS to allow translation (Rutherford et al. 2011; Shao and Bassler 2012). Thus, at LCD, the Qrr sRNAs prevent *hapR* expression and activate *aphA* expression so that HapR levels are low and AphA levels are high.

When CAI-1 and AI-2 accumulate at HCD, they bind CqsS and LuxPQ, respectively, which switches the receptors to phosphatases (Neiditch et al. 2006; Ng et al. 2010). It is not known

how AI-2 impinges on in the LuxPQ kinase/phosphatase reaction but CAI-1 functions by inhibiting CqsS autophosphorylation (Wei et al. 2012). CAI-1 has no effect on the phosphotransfer step or the CqsS phosphatase activity (Wei et al. 2012). Once bound by AIs, LuxPQ and CqsS de-phosphorylate LuxO, leading to cessation of *qrr* expression, and the freeing of the *hapR* mRNA for translation. In the absence of Qrr sRNAs, in contrast, the *aphA* mRNA assumes a conformation refractory to

## Quorum Sensing and Virulence



**Figure 5.** *V. cholerae* QS circuit. *V. cholerae* detects CAI-1 (produced by CqsA) and AI-2 (produced by LuxS) via two two-component histidine kinases, CqsS and LuxPQ, respectively. In the absence of AIs at LCD, the receptors function as kinases resulting in phosphorylation of LuxO, via LuxU, and activation of *qrr* gene expression. The *qrr* sRNAs activate *aphA* and repress *hapR* expression. AphA represses *hapR* and activates *tcpPH*. *TcpPH* activates *toxT* expression and *ToxT* activates expression of the major virulence factors. At HCD, AIs bind the receptors switching them to phosphatases resulting in dephosphorylation of LuxO, cessation of *qrr* gene expression, and increased *hapR* expression. HapR represses *aphA* to shut down virulence factor production.

ribosome binding. The two master QS regulators are thus produced in reciprocal gradients with AphA highest at LCD and HapR highest at HCD. This dichotomy is reinforced by mutual repression whereby AphA represses *hapR* expression and HapR represses *aphA* expression (Kovacikova and Skorupski 2002; Lin et al. 2007; Pompeani et al. 2008; Rutherford et al. 2011).

Interestingly, *V. cholerae* expresses its suite of virulence factors at LCD (Jobling and Holmes 1997; Zhu et al. 2002). At LCD, AphA, a winged-helix transcription factor, works together with a LysR-type transcription factor, AphB, to activate transcription of *tcpPH* (Kovacikova and Skorupski 1999, 2001; Skorupski and Taylor 1999). *TcpP* is a trans-membrane DNA binding protein, the function of which is enhanced by *TcpH* (Carroll et al. 1997; Beck et al. 2004; Matson et al. 2007). *TcpPH* activates expression of

*toxT*. *ToxT* is a transcription factor that activates expression of the genes encoding cholera toxin and the toxin co-regulated pilus, which are the major *V. cholerae* virulence factors (Fig. 5) (Higgins et al. 1992; Withey and DiRita 2006; Matson et al. 2007). Expression of *toxT* is also activated by *ToxRS*, which is homologous to *TcpPH* (Peterson and Mekalanos 1988; Skorupski and Taylor 1997; Matson et al. 2007).

Along with other regulators, QS also controls biofilm formation in *V. cholerae* (Hammer and Bassler 2003; Zhu and Mekalanos 2003). Biofilm formation is activated at LCD and repressed at HCD. HapR, expressed at HCD, plays a major role in this aspect of the program. First, HapR directly represses genes encoding components of the biofilm factory (Yildiz et al. 2004). Second, HapR represses expression of two transcription factors that activate biofilm formation genes, *VpsR* and *VpsT* (Beyhan et al. 2007; Waters et al. 2008; Shikuma et al. 2009; Srivastava et al. 2011). Third, HapR represses expression of genes required for the synthesis of the second messenger cyclic-di-GMP (Lim et al. 2006, 2007; Waters et al. 2008). Cyclic-di-GMP is sensed through the transcription factor *VpsT* to activate biofilm formation (Kraleva et al. 2010). This LCD biofilm lifestyle allows *V. cholerae* to remain attached to host tissue when virulence factors are expressed. At HCD, QS repression of biofilms and virulence factor production facilitates dispersal of *V. cholerae* back into the environment. This strategy allows *V. cholerae* to maximally compete for nutrients in the host at LCD and maximize its ability to exit and spread to other hosts once it reaches HCD (Nadell and Bassler 2011). Because a high infective dose is required, the host serves as the incubator, and it appears that when the titer is high enough to ensure infection of a new host, the population is dispersed.

### *V. cholerae* QS Components as Targets for Therapeutics

The *V. cholerae* QS circuit has several possible targets for the discovery or design of novel antimicrobial agents. Some interesting approaches have targeted pathways involved in the synthesis

S.T. Rutherford and B.L. Bassler

of the AIs. In the SAM cycle, MTAN, which is found only in bacteria, is a hydrolase that turns over methylthioadenosine to maintain the homeostatic SAM pool. This step is critical for the synthesis of both CAI-1 and AI-2 (Schauder et al. 2001; Wei et al. 2011). A synthetic inhibitor of MTAN blocks QS in *V. cholerae* without affecting growth (Table 3, compound 18) (Schramm et al. 2008). This MTAN inhibitor blocks AI-2 production in enterohemorrhagic *Escherichia coli* as well. In a screen for nucleoside analogs able to disrupt AI-2 QS, another molecule was identified that disrupts AI-2-based QS without affecting growth in *V. harveyi*, a close relative of *V. cholerae* (Table 3, compound 19) (Brackman et al. 2009). This compound likely targets the signaling pathway for the AI-2 receptor, LuxPQ, which is present in numerous vibrio species including *V. cholerae* (Fig. 5).

Cinnamaldehyde is a natural product that inhibits QS in vibrios (Niu et al. 2006). Cinnamaldehyde and derivatives are proposed to target HapR and its homologs (Table 3, compounds 20 and 21), thus inhibiting virulence factor production and biofilm formation without dramatically affecting growth (Brackman et al. 2008, 2011). These compounds increase survival of model host nematodes infected with different vibrios leading to the interesting possibility that HapR can be regulated by a cell permeable ligand.

Several synthetic ligands of the receptor CqsS have been identified. Using these molecules has been instrumental in determining the mechanism of signal recognition and discrimination in *V. cholerae* QS. For example, CqsS does not respond to CAI-1 molecules with bulky head groups (Table 3, compound 22) or shortened tail groups (Table 3, compound 23) (Ng et al. 2010). Indeed, such molecules are CqsS antagonists. Owing to the fact that *V. cholerae* expresses virulence factors at LCD in the absence of AIs, CqsS represents an interesting target because compounds that prematurely trigger QS (i.e., potent CqsS agonists) could prevent virulence factor expression.

Finally, in addition to the receptors and the transcription factor HapR, intermediate components in the QS circuit are targets for ther-

apeutics. For example, a high-throughput chemical screen led to the identification of a LuxO antagonist (Ng et al. 2012). The molecule and potent analogs are all 6-thio-5-azauracil derivatives (Table 3, compound 24, for example). This family of molecules uncompetitively bind the preformed LuxO–ATP complex and inhibit ATP hydrolysis, which prevents transcription activation. When inhibited, LuxO is not able to activate expression of the *qrr* sRNA genes, thus locking *V. cholerae* into HCD mode and preventing expression of virulence factors (Fig. 5). Because LuxO exists in all vibrio QS circuits, this family of molecules has broad spectrum anti-virulence activity in marine vibrios (Ng et al. 2012).

#### CONCLUDING REMARKS

QS is a vital regulatory mechanism used by many bacteria to control collective traits that allow bacteria to exploit particular niches. For example, QS enables access of symbionts to nutrient-rich environments in hosts (Ruby 1996). Bacterial populations use QS to control biofilm formation, which provides members of the population superior access nutrients and thus enables them to out-compete non-biofilm-producing neighbors (Nadell and Bassler 2011). Finally, as discussed in the above examples, bacteria that make their living by exploiting eukaryotic hosts have coupled production of the virulence factors necessary for a pathogenic lifestyle to their ability to detect changes in cell population density.

Four trends emerge when considering the examples described above. First, it is clear that QS gene regulation is the result of regulatory networks layered onto the basic AI production and detection apparatuses. Because of the global genetic programs controlled by QS, these additional regulatory mechanisms likely ensure that altered gene expression occurs only under precisely-defined conditions. Alternatively, given that QS regulates global gene expression patterns, perhaps other regulatory systems have co-opted QS systems to control additional products in response to particular environmental conditions. Second, it is interesting that in the



## Quorum Sensing and Virulence

cases described here, as well as in other cases, QS is linked to biofilm production. Although the mechanism and timing of production differ among these species, in each case, QS plays a role in dictating when this process occurs. Members of biofilm communities most certainly benefit from the ability to communicate and, likewise, bacterial communication is probably more robust when the cells are in the intimate associations provided by biofilms. Third, there appears to be a prominent role for regulation involving sRNAs. QS controls expression of hundreds of genes and a commitment to a lifestyle that could be detrimental to the individual. One of the general benefits of regulation by sRNAs is the rapidity with which regulation can occur because translation and protein folding are not required to produce the regulator. Thus, it is possible that QS systems rely heavily on sRNAs to quickly adjust global gene expression programs as bacteria transition between disparate niches. Finally, small molecules analogous to those involved in QS are increasingly becoming recognized as key modulators of pathogenic behaviors and important players in biofilm formation in bacteria. Thus, strategies aimed at manipulating small molecule control of bacterial behaviors are now viewed as especially promising. Presumably, therapies that affect bacterial behavior will not be as prone to resistance as are the targets of traditional antibiotics that result in outright killing of bacteria or inhibition of their growth. Thus, therapeutics that interfere with small molecule-controlled pathways could have longer functional shelf lives than second and third generation antibiotics.

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S.T. Rutherford and B.L. Bassler

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19



S.T. Rutherford and B.L. Bassler

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21



S.T. Rutherford and B.L. Bassler

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## Quorum Sensing and Virulence

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S.T. Rutherford and B.L. Bassler

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## Bacterial Quorum Sensing: Its Role in Virulence and Possibilities for Its Control

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