Lecture 11 - *Intracellular Dynamics*

**Background Reading: Cell Division**

*Proteins within cells can be precisely localized*
- Cells are not bags of random stuff
- Subcellular organization can be critical for proper physiological function
- Sometimes, protein movements can also be critical for function

- *E. coli*’s Nose
- Chromosome Localization
- MinD Oscillation
- Cck Oscillation in *Caulobacter*
- Actin-like Cytoskeleton in Rod-Shaped Bacteria
- Replication “factory” in *Caulobacter*

**How do microbes respond morphologically to their environment**
- Cells may change shape and function
- Cells may behave differently
- Different cell types may be appropriate under different conditions
- These processes will be regulated in a fashion similar to metabolic responses

- Processes already discussed
- Flagellar biosynthesis in *Salmonella*
- *Caulobacter*: Obligate asymmetry
- Spatial Adaptation in *Pseudomonas*
- Swarming and sporulation in *Myxococcus*

**E. coli’s Nose**

**Bags of proteins**
- Many people view prokaryotic cells as bags of proteins, where all the components are mixed up randomly within the cytoplasm
- For many years, the only distinction made in cellular localization of proteins was “cytoplasmic” vs. “membrane”
- As detailed below, this is clearly insufficient for many proteins, whose activity depends upon its specific address *within* the cytoplasm
- DNA is found in a highly organized nucleoid, rather than randomly unwound in the cytoplasm
- Consider the *E. coli* chromosome, 4,850,000 base pairs, or 4850 kb in length.
- A DNA helix of 10 bp is 0.4 nM in length
- So, 1000 bp (1 kb) is 40 nM in length
- So, 4850 kb is 194000 nM in length, or 194 μM.
- That would form a circle with a diameter of almost 62 μM in diameter, or 60 times the length of the cell
Clearly, there must be organization of this molecule in the cytoplasm. Replication likely proceeds through a specially positioned replisome (the “factory” model of DNA replication). In many cases, this level of organization is as important to cellular physiology as the broad localization of a protein in the cytoplasm or periplasm, or within one of the cell membranes.

**Where are the MCP’s?**
- MCP’s are the methyl-accepting chemotaxis proteins that sense attractants and repellants.
- Although they can work anywhere in the cell, they are preferentially localized at one pole of the enteric bacterial cell.
- This localization requires interactions with the cytoplasmic CheA and CheW proteins.
- The group of MCP’s has been referred to as *E. coli*’s “nose.”
- The description of the nose opened up the field of subcellular localization in prokaryotes.

**Chromosome Localization**

**Cells divide by binary fission**
- When most prokaryotes divide, a mother cells undergoes binary fission to yield two daughter cells.
- The chromosomes are apportioned equally between the two daughter cells.
  - Bacteria do not maintain a single, haploid chromosome as many people think.
  - First of all, many Bacteria have multiple chromosomes.
  - Moreover, each chromosome is typically in the process of replication when it is segregated into daughter cells.
- How does the cell ensure that each daughter cell receives a single copy of each chromosome?
- How do low copy-number plasmids (like F, or a P1 prophage) segregate into each daughter cell to avoid stochastic loss?

**Chromosome localization**
- We know that DNA is not randomly distributed in the cytoplasm.
  - Rather, it is found in a condensed structure call the nucleoid.
- The chromosome itself is not randomly distributed throughout the nucleoid.
  - One can perform a thermodynamic calculation of how much energy would be required to reverse the entropic consequences of distributing both daughter chromosomes throughout a cytoplasm, and then separating them prior to cell division; it is many times the energy budget of the cell.
  - Rather, newly replicated chromosomes must be apportioned unequally in the cytoplasm, so that they are each in the region of cytoplasm destined to become one of the daughter cells.

**Following a region of the chromosome**
- Regions of the chromosome can be followed visually using a LacI-GFP fusion protein.
  - GFP is the Green Fluorescent Protein, derived from jellyfish. It emits green light when excited.
  - GFP was fused to the *E. coli* LacI repressor, making a LacI derivative that glows green.
  - Recall that LacI binds the lacO operator.
  - One can place arrays of lacO sites at specific locations around the chromosome.
  - Binding of the LacI-GFP protein to these sites is visualized as a green dot within the cell.
Where are the origin and terminus of replication?

− Using the LacI-GFP system described above, one can follow lacO arrays placed either close to the origin of replication, or close to the terminus of replication
− This has been done in several organisms, including *Escherichia coli* and *Bacillus subtilis*
− Freshly divided cells have 2 origins of replication and 1 terminus
  − Recall that it takes longer for a cell to replicate its DNA than to divide; cells are essentially born “pregnant,” with their chromosome half-replicated already
− If we consider a cell’s length are normalized to a 0-100 scale (so we can compare differently sized cells), the two origins are found roughly at positions 25 and 75
− Moreover, the single terminus is found between them, at about position 50
  − This is dead center of the cell, and the two origins are about halfway between the terminus and the edge of the cell
− As the cell grows longer, two more origins are created; they all move to positions 12, 38, 62, & 88
  − We interpret longer cells as those further along in the cell cycle, where more DNA has been replicated
− When replication finishes, the two termini migrate to positions 25 and 75
  − Notice that these positions will be the center of the new daughter cells
  − Moreover, the two termini wind up between their two respective origins
− The terminus of replication is the last region to dwell at the cell midline when the septum is built;
  − DNA is often trapped in the septum and must be “pumped” to the proper daughter cell.
  − This occurs using the FtsK DNA translocase, which loads onto DNA and moves unidirectionally towards the terminus of replication. This protein is homologous to the DNA pumps which move DNA into the forespore during sporulation.

Other DNA elements

− The single F plasmid within enteric bacteria is found localized at position 50, the cell midpoint
− The episomal prophage for bacteriophage P1 is also found at position 50
− Prior to cell division, these DNA elements replicate and move to positions 25 and 75

FtsZ Localization

− One of the first proteins to be localized in bacteria (in 1991) was FtsZ, a homologue of tubulin
− FtsZ forms a ring around the medial plane prior to cell division, dead center of the cell
  − The 3-dimensional distribution of the FtsZ was reconstructed using Fluorescent *In Situ* Hybridization (FISH), de-convolution microscopy and other advanced imaging techniques
− Late in the cell cycle, FtsZ relocates in less than 1 minute from its present-medial position to future-medial positions in the upcoming daughter cells (where the two replication termini are)
− The localization of these proteins, and the localization of the chromosome, strongly indicates that the cell has a way of mapping out the interior of the cell

Replication “Factory” within Caulobacter

Replicating the nucleoid

− In *Caulobacter*, recall that there is not nested cell division (that is, one round of replication is not initiated until the previous round is completed)
− That makes this organism ideal to study test the “factory model” of DNA replication, whereby the DNA replication machinery is thought to be localized to a single location.
In *E. coli*, it is thought that the replication machinery is localized in the center of the cell, and the action of DNA replication pushes the DNA in opposite directions, facilitating separation of the nucleoids.

In *Caulobacter*, the replication factory was visualized with GFP-tagged proteins, and the origin and terminus of the chromosome could be followed using our friend the LacI::GFP fusion protein binding to a *lacO* array.

In *Caulobacter*, the origin is initially located at the stalk pole of the cell prior to replication.

When a second origin is made, it migrates to the opposite pole (where the flagellum will be).

The DNA replication machinery is initially localized at the stalk pole and the migrates to the center of the cell during replication.

When replication finished, the centrally-located replication machine dissipates into the cytoplasm to reform at the stalk pole.

**MinD Oscillation**

**Preventing Minicells**

- MinC is an *E. coli* protein that prevents cell division at the poles of cells (recall a cell pole used to be a medial plate!)
- MinC associates with MinD; MinD is unusual in that is oscillates in location between each pole of the cell
- This oscillation occurs with a duty cycle of 20-30 **seconds**
- It carries MinC, which inhibits FtsZ-ring formation at the poles
- If either MinC or MinD is mutated, then FtsZ forms divisional septa at the poles, and chromosome-less “minicells” are produced

**Localizing FtsZ via MinE**

- The localization of the bacterial chromosome requires the action of MinE
- MinE also appeared to localize to a ring at the midpoint of the cell; as we see below, this is not strictly true. MinE is not always found at the midpoint of the cell
- This localization requires the action of the oscillating MinC/D protein complex
- MinE is a bifunctional protein; the N-terminus interacts with MinD while the C-terminus antagonizes MinC, preventing it from interfering with FtsZ-ring formation at the midpoint of the cell
- MinE allows for FtsZ ring formation at the midpoint and appropriate cell division

**The MinE Sweeper**

- Upon closer inspection, it appears that MinE oscillates along with MinD, but in a region much closer to the cell midpoint.
- A model has been proposed to explain colocalization of the MinE ring with the center-edge of the MinD distribution, as well as explain the protein-protein interactions.
- Consider the following cycle of events:
  - MinD forms a lattice around the cytoplasmic membrane with a focus at one pole, extending towards the cell center. MinD carries MinC, preventing FtsZ from forming a division ring at this pole.
  - MinE binds to the free edge of the MinD lattice near the cell center forming a ring
  - MinE then dismantles the MinC/D lattice, migrating (as it does so) towards the cell pole
  - The free MinC/D protein complex diffuse to the opposite pole and reassemble the lattice there
– Consistent with this model, fts mutants that form filaments have multiple “zones” of MinC/D MinE oscillation

**How it all works**
– Over time, MinD carries MinC to be at the poles with highest frequency. So, the poles are the area least likely to have FtsZ begin a new septum.
– The area with the lowest concentration of FtsZ is the cell midpoint; as a result, septa are most likely to form new the cell midpoint, thus dividing the cell cleanly in half
– The alternation of MinD/C between poles is not universal. Some bacteria (like Bacillus) show static patterns, where MinDC are constantly bound near the poles. This is thought to be related to the eventual process of polar septation that initiates sporulation.

**Effect of cell size**
– Immediately after cell division, the length of each rod-shaped daughter cell is far shorter than their parent; therefore, the MinD lattice covers the mid-point of the cell and prevents FtsZ ring formation
– As the cell grows, the center of the cell is left “uncovered;” more accurately, this is the location with the least amount of MinD occupancy, thus allowing FtsZ ring formation

**Age of the poles**
– The two poles of the cell are not identical; while one is fresh, having been created by the most recent septum, the other is necessarily older.
– In “young” cells, both poles are recent; “older” cells have one pole that was formed many generations ago
– Elegant experiments in micro-fluidic devices have shown that “older” cells have lower growth rates, losing competitive ability by about 1% per generation
– Therefore, bacterial cells “die” after a pole reaches about 100 generations in age.
– This necessarily leads to some heterogeneity in bacterial populations in terms of gene expression, as well as growth abilities.

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**Cck Oscillation in Caulobacter**

**Cell cycle cues**
– Unlike the formation of flagellae in *Salmonella*, the flagellated swarmer cell of *Caulobacter* is formed every single generation
– Instead of stress cues integrating to induce flagellum biosynthesis, cell cycle cues are used
  – This is an area of intense investigation that is somewhat beyond the scope of this course

**More Protein Movement**
– Cell cycle control of development in *Caulobacter*, like other bacteria, involves the deployment of proteins both in time and in space
– The CckA kinase plays a critical role in the coupling of cell cycle control (when to replicate and divide) with differentiation
– The CckA protein obtains information from the poles of the cells (where stalks and flagellea are located) and relays it to the replication control apparatus
– Consistent with this role, the CckA protein oscillates in cell location on a 10-20 minute cycle
  – This period is 10-15% of the cell cycle, so this is not itself a cell-cycle directed event
− CckA oscillates between an amorphous distribution (throughout the cell) and polar localization

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**Actin-like cytoskeleton in Rod-Shaped Bacteria**

**Adoption of cell shape**
− From the earliest examinations of *Infusoria* and *Animacules*, Bacteria have been classified by shape: cocci (spheres), bacilli (rods) and spirilli (spirals)
− But we see that many rod-shaped bacteria (like *E. coli*) become sphere-shaped when entering stationary phase
− This shape-change, as well a shape differences among bacterial lineages, raises the question as to how cell shape is controlled
− Recall that cell shape is very important in controlling rates of nutrient uptake

**Actin-like proteins in *Bacillus subtilis***
− Two proteins in *Bacillus subtilis*, MreB and Mbl, belong to the actin superfamily of proteins; these proteins include non-structure proteins like hexokinase, so that weak similarity certainty does not obligate these proteins to serve any structural role
− When MreB was depleted from cells (which express the mreBCD operon from a xylose-induced promoter), cells became round and larger before eventually lysing
− When the mbl gene was mutated, cells became twisted at irregular angles, although they did grow
− The MreB protein has been localized to the inside of the cytoplasmic membrane at the center of the cell, where is appears to form a small coil (not a circle). The Mbl protein appears to be distributed in a figure-8 pattern around the inside of the cell membrane.
− These two proteins are thought to cooperate in maintaining the rod-shaped character of the *Bacillus* cell, which each exerting pressure along the short and long axes of the cell, respectively.

**Crystal structure of MreB**
− The three-dimensional structure of the *Thermatoga maritima* MreB protein has been determined by x-ray crystallography (structures are often made of proteins encoded by organisms dwelling at high temperature since they lack extraneous cytoplasmic loops destabilize these proteins at high temperature, and often disrupt the formation of crystals; as well).
− Electron microscopy of the MreB filaments from *Thermatoga maritima* show ordered arrays of proteins forming long chains, much like actin
− The Xray-structure shows that the protein very much looks like actin, and the spacing between the monomers in the crystal is identical to the spacing of the monomers in the filaments observed in the electron microscope
− These data suggest that MreB (and by analogy Mbl) contribute to an actin-like cytoskeleton within bacterial cells to allow them to maintain proper shape.
− This skeleton has been proposed by some to be a superstructure along which DNA translocates when DNA is apportioned into daughter cells.

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**Processes already discussed**

**What is differentiation?**
− Differentiation is a catch phrase for the generation of an alternate cell types
− It is used predominately in the eukaryotic literature to describe developmental pathways
The production of morphologically distinct cell types is not as uncommon in bacteria as you may think.

**Stationary phase**
- As discussed earlier, many bacteria can enter a transient quiescent phase where they persist, but do not reproduce.
- This has been termed stationary phase since cell numbers do not increase.
- However, we know that cell undergoes dramatic morphological changes to allow it to continue to live under nutrient starvation conditions.

**Flagellar biosynthesis**
- We’ve talked about chemotaxis and how bacteria respond to spatial gradients and swim to better food sources.
- Flagellated cells are different from non-flagellated cells by virtue of the complex machinery comprising the flagellum, motor, and chemotactic apparatus.
- The motile cell is different in many ways, not just in the appearance of a flagellum; among other things, pili synthesized for substrate adherence are not produced.

**Heterocyst formation**
- *Anabaena* formed heterocysts under nitrogen starvation.
- Heterocysts expressed genes and performed functions quite distinct from vegetative cells.
- There were morphological changes in the cell that corresponded to this physiological shift.
- Therefore, we can consider the heterocysts to be a terminally differentiated cell, which can no longer return to an undifferentiated state.

**Sporulation**
- *Bacillus* forms two specific cell types in response to environmental stress.
- The Forespore differentiates to form a long-lasting, resistant spore.
- The Mother Cell is a specific cell type that performs functions not seen in other cell types.
- Both the Forespore and the Mother Cell are distinct from vegetatively growing *Bacillus*.
- The Mother cell is also a terminally differentiated cell type.
- The Forespore is a distinct cell type, but it is not terminally differentiated; it can germinate to form a vegetative cell.

**Regulation vs. Differentiation**
- It may seem difficult to drawn the line between regulation and differentiation.
- This is because a spectrum of cellular responses can characterize such transitions:
  - When *E. coli* grows on lactose, proteins are made that were not present earlier.
  - When growing anaerobically, large numbers of proteins are either produced or avoided; this is a larger scale change.
  - In these cases, one could argue that the cell’s fate has not changed.
- Yet what about flagellar biosynthesis, the morphology of the cell has changed and the strategy for food assimilation has been altered. Is this a differentiation? Or is this a transient response?
- Similarly, is the formation of the Forespore significantly different from the formation of a flagellated cell?
− In some circles, differentiation is reserved for a terminal state: the cell always remains as that cell type. This is useful for thinking about multicellular organisms, but oversimplifies the process for primarily single-celled organisms.
− If this is the definition used, then only three processes we will discuss have this characteristic:
  − Heterocyst formation in *Anaebaena*
  − Mother cell formation in *Bacillus*
  − Stalked cell formation in *Caulobacter*
− Yet this distinction leads to odd, nonsensical demarcations:
  − If heterocysts could recover and grow, this would no longer be a differentiation?
  − Mother cells differentiate, but Forespores do not?
  − The Swarmer → Stalked cell transition is a differentiation, but Stalked → Swarmer is not?
− Therefore, we will consider these processes as representative of a spectrum of cellular response, which illustrate approaches to adaptation, sensing, and signaling.

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Flagellar biosynthesis in *Salmonella*

**Flagellae**
− Enteric bacteria have 5 - 15 flagellae per cell, typically 8
− Their synthesis requires 2% of the cell’s energy budget
− Flagellae are turned by molecular motors to move the bacteria cell
− When flagella turn clockwise (viewed from the outside), the cell “tumbles” randomly
− When flagellae turn counterclockwise, they form a bundle, causing the cell to “run”
− Without external signals, enteric bacteria switch motor directions every 1 - 2 seconds
− The chemotaxis system transduces signals from methyl-accepting chemotaxis proteins to the flagellae to allow the cell to swim toward attractants and away from repellents
− The CheA:CheAS proteins mediate signal transduction via phosphorylation of CheY
− The PTS system communicates via CheA to direct the cell to swim toward PTS sugars.
− But when food is tremendously abundant, there is no need to have flagellae, or to move in gradients
− Unflagellated cells make flagellae and the chemotaxis machinery in response to environmental stress
− At least the Crp protein (with the cAMP effector) is known to influence flagellar biosynthesis

**Signal cascade to make flagellae**
− Just like in *Bacillus* development, you can’t make a flagellum all at once
− First, the basal body and hook assembly needs to be made
− After they are completed, then the cell can add the flagellin to extend the structure, add motors, and make the chemotaxis machinery
− This sequence of events is mediated via a sigma cascade

**Overview of Flagellar synthesis**
− The flagellum is perhaps the most complicated subcellular structure built by bacterial cells
− Synthesis of flagellae and chemotaxis protein require the coordinated expression of more than 52 genes, or 1.5% of the *E. coli* genome
− These genes are found in four large clusters (see table)
− Very few genes (23 kb) separate regions 2 and 3 at minute 40 on the *Salmonella* chromosome
− Region 3 is interrupted between the *fliE* and *fliT* genes with 5 non-flagellar genes
− The flagellar genes are organized into 17 operons in *Salmonella*
Flagellar synthesis begin with a structure in the cytoplasmic membrane
- The structure is built through the outer membrane
- A hollow hook is added outside of the cell
- The filament is added last
- The motors and switch complex are assembled in the cytoplasm and in the cytoplasmic membrane
- The motors use proton translocation to turn the flagellum
- The switch binds CheY-P to control direction of rotation
- The “ring” structures anchor the flagellum to the cytoplasmic and outer membranes
- The “rod” transduces the rotational energy of the motors to the hook and filament

Overview of the regulation of flagellar operons
- The 17 operons are controlled in 3 phases of genes expression
  - These phases are denoted 1, 2, and 3 in the table on the left
  - Within each operon, the genes are tightly coupled, many times with ATGA overlapping start and stop codons
  - Transcription of all genes requires a specific sigma factor

Class 1 Genes
- The master switch and sole Class 1 operon is the \textit{flhDC} operon
- Transcription of the \textit{flhDC} operon is activated by Crp/cAMP
- The $\sigma^{70}$ holoenzyme may transcribe this operon
- The FlhDC proteins may act as a sigma factor, or as an activator
- Yet neither FlhC nor FlhD resembles a sigma factor in amino-acid sequence, but that does not rule out this activity (recall $\sigma^{54}$ doesn’t look like a sigma factor either)
- Without the FlhDC proteins, no other flagellar gene can be expressed

Class 2 Genes
- FlhDC is required to express Class 2 genes
- Class 2 genes encode proteins for the \textit{intracellular} flagellar structures
- Class 2 genes have a consensus -10 sequence of GCCGATAA
- This is quite distinct from the $\sigma^{70}$ consensus -10 sequence of TATAAT
- Class 2 genes have no consensus -35 sites
- These data suggest that a special sigma factor may allow transcription of Class 2 genes; FlhDC may act as this factor

The \textit{fliA} Class 2 gene is a sigma factor
- Among the Class 2 genes is \textit{fliA}, which encodes an alternative sigma factor, $\sigma^{28}$
- Since this \textit{is} a Class 2 gene, it cannot be the sigma responsible for Class 2 gene transcription
- Rather, $\sigma^{28}$ is required for transcription of Class 3 genes

Class 3 genes
- Class 3 genes have the same -10 consensus as Class 2 genes: GCCGATAA
- Class 3 genes have a consensus -35 site of TAAA; this is distinct from the $\sigma^{70}$ consensus -35 sequence of TTGACA
- Class 3 genes encode proteins for extracellular flagellar structures, the motor, and the chemotaxis apparatus
- Class 3 genes require only the $\sigma^{28}$ sigma factor for their expression, not FlhDC
If $fliA$ is expressed from a foreign promoter, $flhDC$ mutants do not affect expression of Class 3 genes.

The FliA sigma factor is regulated post-translationally by FlgM

- What prevents the $\sigma^{28}$ from initiating transcription of Class 3 genes as soon as it is made?
- This would be less than useful, since the flagellar rod and ring structure must be in place before the filament and motors are added (these are products of Class 3 genes).
- $\sigma^{28}$ is prevented from associating with the core RNA polymerase by the FlgM protein.
- FlgM binds specifically to $\sigma^{28}$, thus preventing its action.
- Therefore, FlgM is an anti-sigma factor.

When is FliA activated?

- If FlgM is bound to $\sigma^{28}$, when is $\sigma^{28}$ allowed to work and express Class 3 genes?
- FliA can only work when the entire flagellar substructure has been built.
- Mutations in any Class 2 gene that prevents proper flagellar synthesis prevent FliA from activating Class 3 genes.
- How can FlgM/FliA detect a finished structure?

The export pathway

- The filament and hook are assembled outside of the cell boundaries.
- How are the proteins exported to the outside of the cell to assemble these structures?
- Normal protein export pathways are not used.
- Rather, a special flagellar-specific export pathway is used.
- This pathway uses a hollow channel right through the middle of the flagellum.

Export of FlgM

- The intracellular portion of the flagellum is complete upon completion of the hook.
- At this point, the export pathway is fully functional.
- The FlgM protein is exported through the channel and out of the cell.
- The FliA protein can now act as a sigma factor to express Class 3 genes.
- Filament subunits ($FliC$ or $FlgB$) are exported through the hollow channel and extend the flagellar filament.
- Of course two types of filaments could be made: Phase I or Phase II.
- The phase is controlled by the site-specific hin inversion system discussed below.

Similarity to virulence systems

- Some bacteria use a similar process to regulate production of virulence factors.
- The flagellar export apparatus is a Type III secretion system, similar to those used by pathogenic bacteria to inject proteins into host cells.
- Some virulent bacteria are thought to excrete anti-sigma factors through Type III secretion systems in an analogous fashion to the excretion of the FlgM anti-sigma factor.
At this point, the freed sigma factor could express the virulence genes appropriate for that stage of infection

**Shutting down Class 3 genes**
- Notice that a Class 3 promoter transcribes the \( \text{flgM} \) gene once again
- This will likely lead to an increase in the anti-sigma factor once again
- When FlgM levels increase high enough, FliA will be sequestered and Class 3 gene expression will cease
- The signals for shutting down class 1 and 2 gene expression are not known

**Flagellar shearing**
- Inactivating the anti-sigma by export allows for an effective way of initiating expression of class III genes
- However, it is also an effective mechanisms for re-establishing expression of class III genes after they have shut down
- This may be important after flagellae are sheared off and need repair
- When enough flagellae are damaged, FlgM is exported through the flagella pore again, allowing \( \sigma^{28} \) to work in expressing flagellin

**Summary**
- Synthesis of flagellae requires three distinct steps
- The first step is the activation of the \( \text{flhDC} \) Class 1 master control operon
- The second step is the FlhDC-mediated activation of Class 2 genes; whose products build the basal structure
- The third step is the expression of Class 3 genes, whose products build the filament and motors
- Activation of Class 2 genes requires the Class 1 gene products
- Activation of Class 3 genes requires a Class 2 gene product (\( \sigma^{28} \)) and the export of another Class 2 gene product
- In this way, the sequence of flagellar synthesis can be controlled precisely

**Smelling, thinking and running in bacteria**
- Bacteria move using flagellae, long proteinaceous structures attached to both cell membranes and to the cell wall
- Motors attach to the flagellae causing them to rotate
- Clockwise (CW) rotation of flagellae causing tumbling
- Counterclockwise (CCW) rotation of flagellae causing running in a straight line
- Flagellae switch rotation in response to signal proteins
- Sensor proteins on the outside of the cell detect environmental conditions and control signal proteins
- Direction swimming is called chemotaxis
- Sensors are called methyl-accepting chemotaxis proteins (MCPs)
- Signal transduction will be covered in lecture 10

**Flagellae**
- Complex structures comprising a base unit, a hook, and a filament
- Measures 20 nm in diameter (eukaryotic flagella are 200 nm in diameter)
- Measures 5 - 10 μM in length
- Propels *E. coli* at 40 μM/sec, or 1.22 km/year
- Synthesis of the flagella takes 2% of the cell's energy budget
- Control of flagellum synthesis will be covered in lectures 19 & 20
- Moving increases energy consumption by 0.1% over baseline

**Inversion of the hin region**
- Inversion of the *fljAB* promoter is catalyzed by the product of the *hin* gene
- The *hin* gene located within the invertible fragment
- The Hin protein recognizes 14 bp perfect inverted repeats
- Recombination between the 14 bp repeats redirects the orientation of the *fljAB* promoter
- When the *fljAB* operon is expressed, the FlgA protein represses the *fliC* gene, and type II flagellin is made from the *fljB* gene
- When the *fljAB* operon is not expressed, the *fliC* gene makes type I flagellin

**Effects of Inversion of the hin region**
- Inversion occurs at a low frequency at all times
- A clonal population expressing type I flagellin will spawn sub-clones making type II flagellin
- A clonal population expressing type II flagellin will spawn sub-clones making type I flagellin

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**Caulobacter - Obligate asymmetry**

**The sedentary lifestyle**
- *Caulobacter* resides as a stationary, marine bacterium, attached to substrates via its stalk
- Yet dividing to make another stationary stalked cell is not a cogent strategy for dispersal
- Rather, *Caulobacter* divides asymmetrically to form a polar-flagellated swarmer cell
- This motile cell does not divide, but it does disperse to other locations
  - Here is a case, like stationary-phase *E. coli*, where the cell performs metabolism to live, but not to reproduce
  - This is an obligate differentiation, as the stalked cell divides only to form as swarmer cell

**A second differentiation**
- The swarmer cell cannot divide
- Rather, it differentiates into a stalked cell and takes up residence as a stationary cell
  - While can consider this to be a terminal differentiation, for the stalked cell will never return to its flagellated lifestyle, this term is reserved in the microbial literature to mean a differentiation leading to a mortal cell-type
- The flagellum is shed and a stalk grows at the pole of the cell previously occupied by a flagellum

**Cell cycle cues**
- Unlike the formation of flagellae in *Salmonella*, the flagellated swarmer cell of *Caulobacter* is formed every single generation
− Instead of stress cues integrating to induce flagellum biosynthesis, cell cycle cues are used

**Protein Movement**
− Cell cycle control of development in Caulobacter, like other bacteria, involves the deployment of proteins both in time and in space
− The CckA kinase plays a critical role in the coupling of cell cycle control (when to replicate and divide) with differentiation
− The CckA protein obtains information from the poles of the cells (where stalks and flagellea are located) and relays it to the replication control apparatus
− Consistent with this role, the CckA protein oscillates in cell location on a 10-20 minute cell
  − This period is 10-15% of the cell cycle, so this is not itself a cell-cycle directed event
  − CckA oscillates between an amorphous distribution (throughout the cell) and polar localization

**Other localized proteins**
− The first protein to be localized in bacteria was FtsZ, the tubulin homologue that forms a ring around the medial plane prior to cell division
  − Late in the cell cycle, FtsZ relocates in less than 1 minute from its present-medial position to future-medial positions in the upcoming daughter cells
− MinD is an *E. coli* protein that prevents cell division at the poles of cells (recall a cell pole used to be a medial plate!)
  − MinD does localize to the poles, but not uniformly
  − MinD oscillates between the two poles with a frequency of 20-30 sec
− The chromosome itself is not randomly distributed throughout the cytoplasm
  − One can perform a thermodynamic calculation of how much energy would be required to reverse the entropic consequences of distributing both daughter chromosomes throughout a cytoplasm, and then separating them prior to cell division; it is many times the energy budget of the cell
  − Rather, specific portions of the cell are clearly localized within the cytoplasm.

**Spatial Adaptation in Pseudomonas**

**Life in a test tube**
− Not exactly a good model for microbial growth under any natural environment
  − There is constant aeration, which is not seen in any water column
  − There is no spatial structure
  − There are no nutrient gradients
  − This is no real oxygen gradient (due to the shaking)
  − There is little effect of gravity, since growth is very short-term
− Therefore, it’s not surprising all cells coming out of a test tube look similar and behave similarly

**Undisturbed Cultures**
− Liquid broth cultures, but left standing
− Gradients may be established
− Some cells still do well in the broth phase
− But others colonize the air/water interface, forming a biofilm
− And others colonize the bottom of the container, where oxygen is low (it is cut off by the biofilm on top) but nutrients accumulate
**Genetic changes and the Wrinkly Spreader**
- The smooth morph is characteristic of broth-grown cells
- Many other colony morphologies are observed for cells grown in standing culture
  - Wrinkly spreaders look wrinkly and typically colonize the surface of the broth
  - Fuzzy spreaders look fuzzy and typically colonize the bottom of the flask
- The Wrinkly Spreaders are the best studied: why do they look wrinkly? What changed?
  - They look dry and wrinkly on a plate
    - This is because of a polysaccharide they over-produce and to a surfactant they excrete
    - These factors help them stick to each other and not fall away from the surface
  - Oxygen concentrations are highest at the surface, so the \( \Delta G \) of aerobic respiration is highest, allowing maximum ATP production from poor substrates
  - The cost is the exo-polysaccharide that must be produced
  - Wrinkly spreaders could do well in natural environments such as these
    - The mutations required to generate them likely reflect the many generations the laboratory strain has been propagated in shaking test tubes.

**Swarming and sporulation in *Myxococcus***

**Social sporulation in *Bacillus***
- Last lecture, we discussed sporulation in Bacillus as a single-cell process
- However, we know that wild-type (non-laboratory) strains of *Bacillus* sporulate together to form complex structures
  - These structures form from a *Bacillus* biofilm
  - Aerial projections allow for greater spore dispersal
  - Most sporulation occurs in the tips of these structures, as shown by the expression of \( \text{lacZ} \) fusions made to sporulation-specific genes
    - These structures are reminiscent of aerial exospore structures formed by Streptomycetes and fruiting bodies formed by Myxobacteria.
  - The Myxobacteria are sufficiently strange to warrant further discussion.

**Group hunters**
- The Myxobacteria are a group of predatory bacteria which attack other cells
- They move in groups, called swarms, to enable effective digestion of their prey
- They excrete exoenzymes - enzymes that work outside of the cell - to digest the cell walls and cell membranes of their prey
- After lysis of their prey cells, the swarms of Myxobacteria consume their cytoplasmic contents
- Much of the genetics of Myxobacteria have been investigated in *Myxococcus xanthus*

**Gliding motility**
- To move on solid surfaces as swarms, Myxobacteria cannot use flagellae, which are the little propellers that work well in liquids
- Rather, they employ a mechanism called gliding motility to enable motility across surfaces
- No one really knows how gliding motility works; a polysaccharide “slime” trail is produced and is required to enable the cells to move, but the mechanisms by which movement is accomplished is not really known
- Several models have been proposed, including:
− Floor-polisher model, where rotating appendages propel the cell
− Pili-propulsion, where pili can pull the cell in 1 direction
− Social motility in *Myxococcus* appears to require pili, whereas adventurous motility does not, suggesting that at least two different mechanisms are at work.

**Social swarming**
− Myxobacteria exhibit social motility; that is, they know how to move together
− This is accomplished by the production of pheromones that are used in a quorum sensing mechanism
− The gradient of the auto-attractant can be sensed by a chemotaxis-derived mechanism
− Movement away from the group is detect through the drop in concentration of the auto-attractant, causing reversal of motion
− This is being investigated as a good model system for cell-cell communication

**Sporulation**
− When times are tough, Myxobacteria sporulate
− But since they hunt in packs, a single germinating cell has little chance of survival on its own
− For this reason, Myxobacteria sporulate to form elaborate fruiting bodies
− By virtue of their cell-cell communication, Myxobacteria time their cell cycles and enter sporulation phase simultaneously
− They do not form endospores like *Bacillus*; rather, Myxobacteria alter their vegetative cell to become smaller and more stress-resistant
− They are more transparent to light as spores and thus can be distinguished visually from vegetative cells

**Fruiting body formation**
− The fruiting body itself can be spectacular in design, made up of thousands of individual cells
− As the developmental program progressed, mature spores migrate toward the protected center of the fruiting body
− This structure ensures greater protection against noxious agents, as well as a collection vessel for multiple spores
− The fruiting body allows for large groups of Myxobacteria to sporulate in unison. This is thought to be advantageous because (theoretically) multiple spores would be transported and deposited at a single location, allowing multiple cells to germinate simultaneously.
− Multiple cell germination would be highly advantageous for Myxobacteria since they require the secretion of exoenzymes from many cells to digest their prey species.