Exam 2 Study Guide

E. coli Catabolism and cell cycle

1. Blackwood’s stuff
	1. Describe three stages of catabolism, including inputs and outputs of each and how they are involved in production of common substrates or energy extraction. Which of these are taken up from or excreted to the environment?
	2. How do catabolic pathways differ depending on substrates taken up by *E. coli*?
	3. What are alternatives to aerobic respiration that *E. coli* can use? What are the changes to inputs, outputs, and energy yields? In what environments would these be advantageous for *E. coli*?
	4. Which pathways within catabolism produce intermediates that can be used for biosynthesis? What process in chemoorganoheterotrophs competes with biosynthesis for these metabolic intermediates?
	5. What processes are fermentative bacteria unable to do unless they use ATP to make a H+ ion gradient?
	6. How does *E.coli* adjust catabolic processes to maximize energy gained?
2. Key terminology
	1. **Anabolism =** the synthesis of complex molecules in living organisms from simpler ones together with the storage of energy; constructive metabolism
	2. **Catabolism =** the breakdown of complex molecules in living organisms for form simpler ones, together with the release of energy; destructive metabolism
3. Bacterial cell cycle
	1. Key terminology
		1. **Replisome =** a group of proteins needed for DNA synthesis assembled at the origin
		2. **MreB =** cytoskeletal protein that is similar to eukaryotic actin that is impt in producing rod-shaped cells and may be involved in bacteria chromosome separation; a bacterial cytoskeleton filament that directs the peptidoglycan machinery to the right part of the cell
		3. **Septation =** the process of forming a cross wall btwn 2 daughter cells
	2. Biological significance: synthesis of peptidoglycan during cell cycle is target of antibiotics to treat bacterial infections
	3. 2 pathways during the bacterial cell cycle (act simultaneously)
		1. *Pathway 1:* replicates and partitions the DNA into progeny cells; overlaps w 2 bc septation starts while DNA replication is just getting done
		2. *Pathway 2:* carries out cytokinesis
	4. Chromosome replication and partitioning
		1. *2 hypotheses on how chromosomes are segregated among daughter cells:* (1): pushed by the replisome (i.e. replication forks) or ribosomes (2) pulled apart by MreB (cells w mutations in gene encoding for MreB don’t segregate their chromosomes properly)
	5. Cytokinesis
		1. *Septation: (5 steps)*
			1. Peptidoglycan deposited only in the middle of the cell
			2. Invagination of the cell wall leads to 2 cells
			3. Accumulation of MreB in the center of the cell—for more peptidoglycan deposition in the center
			4. Formation of the Z- ring
				1. One of the primary proteins = FtsZ (repeating protein subunit)
				2. Z-ring constricts the cell through the net loss of FtsZ subunits
				3. Assembly of Z ring (made of FtsZ)\*\* must be formed for subsequent steps to occur

**Z-ring =** cytoskeletal protein network that plays a major role in invagination of plasma membrane

**FtsZ =** cytoskeletal protein homologous to eukaryotic tubulin; forms the Z ring by polymerizing to form filaments to create meshwork that makes up the Z ring

**MinCDE =** system that limits Z-ring formation to the center of the cell (3 proteins that oscillate from 1 cell end to other to create high MinC concentrations at the poles to prevent formation of Z-ring)

If mutated then a weird shape can arise

**Divisome =** division machinery after the Z ring is formed that links the Z ring to the membrane

* + - 1. Constriction of cell and septum formation
	1. Process of cell cycle in E. coli
		1. Initiation of cell cycle via increase in mass of e. coli cell $\rightarrow $ accumulation of DnaA protein that initiates DNA replication $\rightarrow $ origin of replication migrates to center of cell $\rightarrow $ proteins that make up replisome assemble $\rightarrow $ DNA replication proceeds and newly made chromosomes move toward poles and the cell increases in length $\rightarrow $ threshold length is reached and septum formation is triggered $\rightarrow $ cytokineses occurs $\rightarrow $ 2 daughter cells are made each with 1 chromosome each
1. Bacterial cell growth and determination of cell shape
	1. Key terminology
		1. (**turgor pressure =** the force pushing against cell wall determined by osmolarity of the cytoplasmic contents)
	2. Cell shape dependent on function of cell wall
		* 1. Cell wall constrains turgor pressure exerted by cytoplasm
			2. Peptidoglycan is responsible for protecting cell from lysis
			3. *Problem:* strength of peptidoglycan in cell wall has to be maintained as new peptidoglycan subunits are added
			4. *Solution:* as e. coli grows it makes cuts into the peptidoglycan via 2 enzymes
				1. **Bactoprenol =** protein carrier for NAM-NAG dimers
				2. **Autolysins =** cut peptidoglycan and insert NAM-NAG dimers
	3. Peptidoglycan synthesis accomplished by 3 things
		1. **Penicillin-binding proteins = PBPs =** link strands of peptidoglycan together and catalyze controlled degradation so that new units can be inserted during cell growth
		2. **Autolysins =** the PBPs that degrade peptidoglycan
		3. **Bactoprenol =** lipid soluble transporter that takes the NAG-NAM pentapeptide building block across plasma membrane and releases it into the periplasmic space so they can be inserted into peptidoglycan strand
	4. Cell shapes and how they form

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| --- | --- |
| Cell shape  | How its formed |
| Coccus  | * New peptidoglycan only formed at central septum during growth
* When daughter cells separate each has 1 new and 1 old hemisphere
* FtsZ localization determines site of cell wall growth by recruiting PBPs
 |
| Rod-shaped | * Prior to cell division they elongate
* MreB proteins polymerize and create patches of filaments along cytoplasmic face of plasma membrane; functions like a scaffold inside cytoplasm where cell wall synthesizing machinery is made
* FtsZ ring forms at midcell
* If MreB is depleted they assume spherical shape
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| Comma-shaped  | * Produce crescentin (homologous to intermediate filaments) that slows insertion of peptidoglycan resulting in asymmetrical cell wall
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1. Organism types
	1. Electron sources for reducing power

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| --- | --- | --- |
| Electron source | Organism type | How they get it |
| Carbon | Autotroph | * Use carbon dioxide
* Use principal biosynthetic carbon source
 |
| Carbon | Heterotrophs | * Reduced, preformed, organic molecules from other organisms
 |
| Energy | Phototrophs | * Light
 |
| Energy | Chemotrophs | * Oxidation of organic or inorganic compounds
 |
| Electron | Lithotrophs | * Reduced inorganic molecules
 |
| Electron | Organotrophs | * Organic molecules
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* 1. Microorganism nutritional types

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| --- | --- | --- | --- | --- | --- |
| Nutritional type of microorganism | Carbon source | Energy source | Electron source | Electron Acceptor | Ex.  |
| Photolithoautotroph | CO2 | Light | Inorganic electron donor |  | * Purple and green sulfur bacteria
* Cyanobacteria
* Diatoms
 |
| Photoorganoheterotroph | Organic carbon | Light | Organic electron donor |  | * Purple nonsulfur bacteria
* Green nonsulfur bacteria
 |
| Chemolithoautotroph | CO2 | Inorganic chemicals | Inorganic electron donor |  | * Sulfur-oxidizing bacteria
* Hydrogen oxidizing bacteria
* Methanogens
* Nitrifying bacteria
* Iron oxidizing bacteria
 |
| Chemolithoheterotroph | Organic carbon | Inorganic chemicals | Inorganic electron donor |  | * Some sulfur oxidizing bacteria (e.g. Beggiatoa)
 |
| Chemoorganoheterotroph—bacteria, all fungi, all protozoa, all animals, all pathogens | Organic carbon | Organic chemical (often the same as the carbon source) | Organic electron donor (often same as C source) | Variable (e.g. Oxygen or nitrate) | * Non-photosynthetic microbes
* Pathogens
* Fungi
* Protists and archaea
 |

* 1. Electron energy
		1. *Main idea:* the farther apart on the redox scale the substrates are the more energy is released and the more they can capture; facultative anaerobes show the most growth due to oxygen being the best electron acceptor at the top and it is growing on an organic compound at the opposite end of the scale
	2. Chemoorganotrophic fueling processes
		1. *Main idea:* Chemoorganotrophs oxidize organic energy sources and release electrons to be accepted by NAD+ and FAD—electron carriers
			1. When the reduced electrons carriers (now NADH and FADH2) donate electrons to ETC, we get respiration
		2. *2 broad and general options for fueling processes*
			1. ***Respiration*** = metabolic process that used the ETC to pass electrons through it to a final electron acceptor, generating a proton gradient used to synthesize ATP from ADP and Pi (2 types)
				1. **Aerobic respiration =** respiration in which the final electron acceptor is oxygen; process that can completely catabolize a reduced organic energy source into CO2 using glycolytic pathways and TCA cycle

*ATP yield during aerobic respiration: 2 factors affecting yield of ATP*

PMF made by electron transport is used for other functions aside from ATP synthesis

For each molecule of glucose that is degraded there are a lot of precursor metabolites which means microbe needs to decide if its needed for anabolism or if it can go on with catabolism

* + - * 1. **Anaerobic respiration =** respiration in which the final electron acceptor is a diff oxidized molecule
			1. ***Fermentation* =** metabolic process that uses an electron acceptor that is endogenous (within the cell) and doesn’t involve an ETC; endogenous electron acceptor usually is an intermediate of catabolic pathway to degrade and oxidize energy source; good in an anoxic-protein rich evmt; occurs when microbes don’t have ETCs or they don’t make ETC components under anaerobic conditions
				1. *When we use it:* No oxygen or nitrate (for e. coli) and thus no final electron acceptors
				2. *Problem:* facultative anaerobes are in an evmt without either oxygen or terminal electron acceptor they use for anaerobic respiration but NADH made from Embden-Meyerhof Pathway still needs to be oxidized back to NAD+ to keep glycolysis going without ETC.

*Solution:* microbes slow/stop pyruvate dehydrogenase activity and use pyruvate/derivatives as electron acceptor for NADH re-oxidation

* + - * 1. *4 main ideas about microbial fermentation*

NADH is oxidized to NAD+

O2 isn’t needed

Electron acceptor is either pyruvate or pyruvate derivative

ETC can’t operate which reduces ATP yield per glucose

* + - * 1. *How ATP is made:* Substrate is only partially oxidized and ATP is formed via substrate-level phosphorylation

**Substrate level phosphorylation =** a process in which a phosphate is transferred to ADP from high energy molecule

Microbes use ATP synthase in reverse direction to drive PMF (i.e. ATP synthase pumps protons out of cell and fuels transport when releasing energy when ATP is hydrolyzed)

* + - * 1. *Pathways*: named after major acid/alcohol produced (e.g. lactic acid fermentation)

*Lactic acid fermentation*: pyruvate is reduced to lactate (2 groups of lactic acid fermenters)

**Homolactic fermenters =** use the Embden-Meyerhof pathway and directly reduce almost all pyruvate to lactate via lactate dehydrogenase

**Heterolactic fermenters =** form big amounts of products other than lactate; can also mad ethanol and CO2

*Alcohol fermentation:* ferment sugars into ethanol and carbon dioxide; pyruvate decarboxylated to acetaldehyde. Reduced to ethanol via alcohol dehydrogenase with NADH as electron donor

*Mixed acid fermentation:* metabolize pyruvate to many diff products using diff pathways at the same time

* + - * 1. *Products of fermentation*

|  |  |  |
| --- | --- | --- |
| Fermentation Product | Acid growth (pH 6) | Alkaline growth (pH 8) |
| Ethanol | 50 | 50 |
| Formic acid | 2 | 86 |
| Acetic acid | 36 | 39 |
| Lactic acid | 80 | 70 |
| Succinic acid | 11 | 15 |
| Carbon dioxide | 88 | 2 |
| Hydrogen gas | 75 | 0.5 |

1. Catabolism
	1. Main stages and events

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| Stage | Main Events |
| **Stage 1**—Depolymerization – **figure 11.21** | * polymers are broken down into monomers (bc polymers can’t be taken up but monomers can)
* breakdown provides simplified range of compounds to feed into metabolism
* performed by extracellular enzymes that are secreted outside of the cell
* polysaccharides are degraded via hydrolysis
* disaccharides are degraded by hydrolysis or phosphorylysis
* no phagocytosis in bacteria/archaea/fungi
* after digestion monomers enter cytosol and oxidative breakdown starts
 |
| **Stage 2**—for glucose (e.g. bc something is growing on lactose and it gets broken down into glucose and galactose and we proceed in stage 2) – **figure 11.5** | * glycolysis occurs; **glycolysis = Embden-Meyerhof Pathway =** the chain of rxns that splits each molecule of glucose into 2 smaller molecules of pyruvate
* Glucose + 2ADP + 2Pi + 2NAD+ $\rightarrow $ 2 pyruvate + 2 ATP + 2 NADH + 2 H+
* in the cytosol
* glycolysis is generating ATP and NADH (2 activated carriers)—ATP is made by substrate-level phosphorylation
* pyruvate is transported from the cytosol into the mitochondrial matrix (**mitochondrial matrix =** the mitochondria’s internal compartment)
* pyruvate is converted into carbon dioxide and acetyl CoA in matrix
* acetyl CoA is made by oxidative breakdown of fatty acids in matrix as well
 |
| **Alternative Stage 2**—if environment is changed or if something is fed to e. coli that is weird | * **Pentose phosphate pathway**: can be used simultaneously with Embden-Meyerhof pathway
* Purpose is to pull out intermediates and use for biosynthesis (aromatic AAs/nucleic acids) or can complete glycolysis
* Produces NADPH to be used in biosynthesis
* Glucose + 12NAD+ + 7H2O + ATP $\rightarrow $ 6CO2 += 12NADPH + 12H+ + ADP + Pi
 |
| **Stage 3—TCA cycle – figure 11.8** | * occurs in cytoplasmic matrix of bacteria
* complete or partial system is common in bacteria
* fully oxidizes pyruvate $\rightarrow $ 3CO2 (starts by releasing CO2 and forming acetyl CoA)
* 2 cycles needed per glucose
* **citric acid cycle =** series of reactions in which the acetyl group is oxidized to carbon dioxide and makes large amounts of NADH
* yields 4 NADH, 1 FADH2, and 1 GTP per cycle of pyruvate
* 8 NADH, 2 FADH2, 2 GTP per flucose
* high energy electrons from NADH that was made during the citric acid cycle are passed along the electron-transport chain (**electron transport chain =** a series of enzymes within the mitochondrial inner membrane where the energy released by their transfer drives oxidative phosphorylation that makes ATP and consumes O2)
* the most ATP is made in this stage
 |
| **Stage 3—Respiration – figure 11.1, figure 11.13** | * ETC in membrane creates proton gradient
* E. coli electron transport chains (2 diff branches):
* *Low O2:* bd (top) branch—stationary phase (when used), higher affinity, lower efficiency, 2 H+
* E. coli uses this when oxygen is low and nitrate is absent
* *High O2:* bo (bottom) branch—lag phase (when used), lower affinity, higher efficiency, 4 H+ ; more efficient than bd branch, gets more energy
* When air is present e. coli will use aerobic respiration with the bo branch
* Mitochondria transport 10 H+ per NADH + H+ oxidized
* Efficiency of the ETC can be determined by the final electron acceptor (e.g. oxygen is the best because it has the highest value on the electron energy chart)
 |

* 1. Denitrification = dissimilatory nitrate reduction = form of anaerobic respiration that leads to the production of gaseous compounds; cannot construct N-containing molecules (i.e. AA and nucleotides) thus these products are not incorporated into the cell
		1. *When used:* Stage 3 of catabolism in anaerobic respiration
		2. *4 key enzymes*
			1. **nitrate reductase = Nar =** catalyzes reduction of NO3- to NO2-
			2. **nitrite reductase = Nir =** reduces nitrite to NO (nitric oxide)
			3. **nitric oxide reductase = Nor =** catalyzes formation of nitrous oxide N2O from NO
			4. **nitrous oxide reductase = Nos =** catalyzes formation of N2 from N2O
1. Glycolytic pathways = from glucose to pyruvate
	1. 3 metabolic pathways to catabolize glucose to pyruvate so microorganisms can harvest energy

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| Pathway | Description |
| **Embden-Meyerhof Pathway** = the most common pathway for microbesGlucose + 2ADP + 2Pi + 2NAD+ $\rightarrow $ 2 pyruvate + 2ATP  | * Functions in the presence or absence of oxygen
* Provides precursor metabolites, NADH and ATP
* Occur in cytoplasm
* Divided into 2 parts
* *6-carbon phase: “investment phase”:* 2 ATP are used to phosphorylate glucose twice
* 2 ATP used
* 1 NADH made used during aerobic respiration
* 2 ATP formed (net ATP = 0)
* *3-carbon phase:* “*energy-conserving phase”:* 2 molecules of glyceraldehyde 3-phosphate are catabolized to pyruvate; NADH and ATP are made
* NADH formed when glyceraldehyde 3-phosphate is oxidized with NAD+ as electron acceptor
* **Substrate-level phosphorylation = (ex)** when ADP phosphorylation is coupled w exergonic hydrolysis of high-energy molecule to have a higher phosphate transfer potential than ATP
* 4 ATP made (2/glucose)
* 4 NADH made (2/glucose)
* 1 glucose – 2 ATP + 2 NADH + 4 ATP + 2 pyruvate)
 |
| **Entner-Doudoroff pathway =** pathway used by soil bacteria and some gran neg bacteria to transform glucose to pyruvate | * yields pyruvate and glyceraldehyde 3-phosphate instead of 2 molecules of glyceraldehyde 3-phosphate
* microbes that use it have enzymes that function in second phase of Embden-Meyerhof pathway to catabolize glyceraldehyde 3-phosphate to 2nd pyruvate
* 1 ATP, 1 NADH, and 1 NADPH
 |
| **Pentose phosphate pathway =** pathway that turns glucose to pyruvate that is used as the same time as the Entner-Doudoroff pathway and Embden Meyerhof pathway | * can work with or without oxygen
* provides reducing power and precursor metabolites
* *important oxidation:* glucose 6-phosphate to 6-phosphogluconate and then 6-phosphogluconate to ribulose 5-phosphate and CO2 (NADPH is made here)
* *2 important enzymes:*
* transketolase: catalyzes transfer of 2-carbon groups
* transaldolase: transfers a 3-carbon group
* intermediate result: 3 glucose 6-phosphate + 6NADP+ + 3H2O $\rightarrow $ 2 fructose 6-phosphate + glyceraldehyde 3-phosphate + 3CO2 + 6NADPH + 6H+
* 3 options
* F6P can be changed back to G6P
* Glyc3P can be converted to pyruvate by enzymes of the Embden-Meyerhof Pathway
* 2Glyc3Ps can combine to form F16biP that is converted back to G6P
* AKA, these intermediates can be fed into the EMP with 2 results
* Continued degradation to pyruvate
* Regeneration of G6P via gluconeogensis
* final results: 6-phosphate + 12 NADP+ + 7H2O $\rightarrow $ 6CO2 + 12NADPH +12H+ + Pi
* *2 reasons why its impt*
* NADPH made is an electron source for reduction of molecules during biosynthesis
* 2 precursor metabolites mad
* *erythrose 4-phosphate-* synthesizes AAs and B6
* *ribose 5-phosphate-* component of nucleic acids
 |

1. Tricarboxylic Acid Cycle = TCA Cycle
	1. Purpose: to continue catabolic processes after aerobic respiration
	2. What’s happening: oxidizing pyruvate to 3 CO2s
	3. End result per pyruvate: 2 CO2 molecules, 3 NADH molecules, 1 FADH2 molecule, and 1 ATP/GTP for each
	4. Biological advantage: energy conservation due to many NADH and FADH2 molecules made
	5. Major players:
		1. **Pyruvate dehydrogenase complex =** multi-enzyme system that oxidizes and cleaves pyruvate to from 1 CO2 and 1 acetyl-CoA
			1. **Acetyl-coenzyme A =** energy rich 2 carbon molecule; energy rich bc hydrolysis of bond linking acetic acid to coenzyme A has large negative change in free energy
				1. Carbohydrates and FAs and AAs can be converted to acetyl CoA
	6. Process:

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| Step | What’s Happening |
| 1 | * Enzyme removed proton from methyl group on acetyl CoA and a bond to carbonyl carbon is made
 |
| 2 | * Isomerization occurs in which water is removed then re-added and moves the hydroxyl group from 1 carbon to its neighbor
 |
| 3 | * The carbon with the hydroxyl group is converted to carbonyl group
* Intermediate is unstable and loses CO2
 |
| 4 | * Oxidation is catalyzed to make NADH, CO2, and thioester bond to CoA
 |
| 5 | * Phosphate molecule from soln displace CoA
* Phosphate is passed to GDP to make GTP
 |
| 6 | * FAD accepts 2 H atoms
 |
| 7 | * Water is added to fumarate and places hydroxyl group next to carbonyl carbon
 |
| 8 | * Carbon carrying hydroxyl group is converted to carbonyl group and oxaloacetate needed for step 1 is re-made
 |

1. Electron Transport Chain (ETC) – only during respiration
	1. Key terminology
		1. **Electron transport chain** = mitochondrial system that is made of a series of electron carriers that operate together to transfer electrons from donors (NADH and FADH2) into O2; electrons flow through carriers from those w negative reduction potentials to those with more positive potentials until the electrons combine with O2 or other endogenous electron acceptor
	2. Location in bacteria and archaea: plasma membrane; pump them from plasma membrane into periplasmic space
		1. *Example: E. coli*
			1. *2 branches ETC branches that can operate under diff Oxygen levels:*
				1. *Cytochrome bo branch =* used when oxygen is available
				2. *Cytochrome bd branch =* used when oxygen isn’t readily available; less efficient than bo bc it puts fewer protons in periplasmic space
	3. Efficiency of the ETC depends on 2 things
		1. Specific cytochromes and other electron carriers in the ETC
		2. Terminal electron acceptor
	4. Lower ATP yield due to 2 things
		1. alternate electron acceptors having less positive reduction potentials than O2
		2. less energy being available to make ATP
	5. Electron acceptors

|  |  |  |  |
| --- | --- | --- | --- |
| Aerobic/anaerobic respiration | Electron acceptor | Reduced product(s) | Ex |
| Aerobic | O2 | H2O | All aerobes |
| Anaerobic | NO3- | NO2- | Enteric bacteria |
|  | NO3- | NO2-, N2O, N2 | Pseudomonas, Bacillus, and Paracoccus |
|  | SO42- | H2S |  |
|  | CO2 | CH4 | Methanogens |
|  | CO2 | Acetate | Acetogens |
|  | S0 | H2S |  |
|  | Fe3+ | Fe2+ |  |

1. Oxidative phosphorylation
	1. Key terminology
		1. **Oxidative Phosphorylation** = the process by which ATP is synthesized as the result of electron driven by the oxidation of a chemical energy source; yields 7x as much ATP than substrate-level phosphorylation
		2. **Proton motive force = PTF =** the combined chemical and electrical potential differences used to drive protons across the membrane; created bc the cytoplasm in bacteria and archaea is more alkaline and negative than periplasmic space
		3. **ATP synthase =** enzyme that catalyzes the use of PMF for ATP synthesis; located on inner surface of plasma membrane
	2. PMF used for 3 things
		1. to perform work when protons flow back across the membrane, down the concentration/charge gradients, and into cytoplasm
		2. to move nutrients into cell
		3. rotate flagellar motor
2. When bacteria isn’t growing on glucose—catabolism of other organic molecules
	1. Carbohydrates
		1. *Catabolic pathways for glucose/fructose/mannose/galactose*
			1. Glucose, fructose, and mannose are phosphorylated via ATP and EMP is used
			2. Galactose needs to be converted to UDP-gal via phosphorylation and then converted to G6P
		2. *Disaccharides:* cleaved to monosaccharides by 2 mechanisms
			1. Directly hydrolyzed (i.e. maltose, sucrose, lactose)
			2. Phosphorolysis = phosphate attack on bond between 2 sugars (i.e. maltose, cellobiose, sucrose)
		3. *Polysaccharides:* cleaved via hydrolysis *and* phosphorolysis
			1. Ex: starch and glycogen are hydrolyzed via amylases to glucose/maltose
	2. Lipid catabolism
		1. Triglycerides hydrolyzed to glycerol and fatty acids via lipases
			1. Glycerol is phosphorylated and oxidized to dihydroxyacetone phosphate (intermediate in embden-meyerhof)
		2. **Beta-oxidation pathway =** pathway that oxidizes lipids after they’re linked to coenzyme A to shorten the fatty acids by 2 carbons with each turn of the cycle
			1. 2 carbons released as acetyl-CoA that is given to TCA cycle or used for biosynthesis
	3. AA catabolism
		1. *What uses proteins:* pathogenic/food spoilage/soil microbes use proteins as source of carbon and energy
		2. *3 general things to be done*
		3. Proteins are hydrolyzed by proteases (**Proteases =** hydrolyze proteins to AAs that are transported into cell)
			1. Enzymes are secreted in the periplasmic space and extracellular space
		4. Deamination step removes the Nitrogen (**Deamination =** first step in AA catabolism that removes the amino group from the amino acid; accomplished via transamination)
			1. Organic acid that results is converted to pyruvate/acetyla-CoA/a TCA intermediate
			2. Excess N can be excreted as ammonium ion

E. coli Anabolism

1. Blackwood’s LO
	1. What is the typical redox state of N in proteins and other N-containing macromolecules?
	2. What are the variety of ways that *E. coli* can obtain N for biosynthesis? Under what environmental conditions would each method be the most efficient?
	3. Describe the process of growth of the cell wall and septation.
	4. Why is there a theta structure formed due to activity of DNA polymerase in bacteria but not eukaryotes?
	5. What form of energy drives flagellum rotation? Toxin secretion? Uptake of something in the environment (there are 4)?
	6. How is the flagellum basal body like a Type III secretion system?
	7. What structures in *E. coli* are related to virulence factors related to adhesion? Avoidance of the immune system? Toxin production? What about structures related to taking up resources from the environment? Protection from environmental stress?
	8. Which secretion systems are Sec-dependent? What do they rely on the Sec system for?
	9. What is unusual about Type IV secretion systems? Type V secretion systems?
	10. What is an advantage of group translocation? What about solute binding proteins?
	11. How does *E.coli* adjust anabolic processes to save energy?
2. Precursor metabolites
	1. Significance: essential to anabolism bc they give rise to all other molecules
	2. 2 important things
		1. all of precursor metabolites are intermediates of the glycolytic pathways and the TCA cycle
		2. most are used for synthesis of AAs and nucleotides
	3. Key terminology
		1. **Precursor metabolites =** carbon skeletons used as the starting substrates for the synthesis of monomers and other building blocks needed to make macromolecules; they lack functional moieties. intermediates of core catabolic reactions (i.e. central metabolic pathways)
		2. **Anapleurotic =** a reversible reaction the microbe can engage in that allows it to have other key intermediates
	4. Examples
		1. Taking glutamate from alpha-ketoglutarate and making AAs out of it
		2. Acetyl-CoA getting broken down into lipids or AAs
		3. Making other carbohydrates from G6P and FGP
		4. Making AAs from pyruvate (the carbon is removed as CO2)
	5. Examples involving organism types
		1. If E. coli is growing on an AA and *not glucose*, it still needs to build its slime layer and peptidoglycan. Thus, it will reverse the rxn and make another intermediate (Anapleurotic)
		2. If chemoorganotroph uses glucose as its energy, electron, and C source it makes the precursor metabolites as it makes ATP and reducing power
		3. If chemoorganotroph is using an AA as its source of energy, electron, and C source they they will go through deamination steps
		4. If heterotrophs are growing on something other than glucose it will convert the C source into 1 or more intermediates of the central metabolic pathways and then they can make the precursor metabolites
3. Biosynthesis
	1. Key terminology
		1. **Recycling principle in physiology =** microbe will not spend energy to make something if its already in the evmt
	2. 6 principles of biosynthesis
		1. Large molecules are made from small molecules to save genetic storage capacity
		2. Enzymes do double duty (i.e. amphibolic)
		3. Some enzymes in amphibolic pathways function in 1 direction only to allow independent regulation of each metabolism type
		4. Anabolism consumes energy bc cells connect anabolism rxns to ATP breakdown to propel the rxn fwd
		5. Catabolism and anabolism can be physically separated via cell compartmentalization to allow simultaneous and independent operation
		6. Catabolism and anabolism use different cofactors
			1. *catabolic oxidations* made NADH (substrate for ET)
			2. *anabolic pathways* NADPH is the donor
4. Nutrient uptake mechanisms
	1. Key terminology
		1. Porins = portals into periplasmic space
	2. Problem: E. coli has a hard time getting nutrients in bc it has 2 membranes (outer and plasma).
	3. Solution: The outer membrane thus has receptor proteins that are linked to plasma membrane transporters for certain substrates in the evmt
	4. Mechanisms of nutrient uptake across plasma membrane

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| --- | --- |
| Mechanism | Description |
| Passive diffusion | * Doesn’t require any expenditure of energy
* Occurs when….
* Concentration gradient high to low thus substrate is going with it
* Membrane is permeable (due to porin)
* Small uncharged molecule moving through
 |
| Facilitated diffusion | * No energy expenditure, but a protein facilitates movement
 |
| Active transport – **figure 3.12, 3.13, 3.14** | * Transport of a substance that requires energy
* *Symport* = something else is moving in the same direction along its concentration gradient thus spending energy to move the other molecule across its concentration gradient in the same direction
* *Antiport* = 1 molecule is moving with its concentration gradient spending energy to get the other molecule against its concentration gradient in the opposite direction (can utilize PMF)
* *ABC transport = ATP Binding cassette =* have a solute binding protein that has been secreted and binds to a compound that binds to the transporter protein embedded in membrane. Hydrolysis of ATP on the nucleotide binding domain opens up the transporter and allows molecule to come into cytoplasmic matrix
* *PTS system =* series of proteins are being phosphorylated that eventually phosphorylates mannitol-1P or Glucose-6P; efficient bc it phosphorylates the glucose directly to set it up for Embden-Meyerhof pathway
 |

1. Nutrients
	1. Iron
		1. Needs soluble protein carriers
		2. ***Siderophores =*** secreted to scavenge Fe that protect it from uptake by other cells and keep it from getting oxidized
			1. *Importance:* contributes to the microbe’s virulence by allowing them to grow in inhospitable evmts due to low levels
			2. *Barrier to overcome:* iron starvation
			3. ExPEC strains often have this to allow them to colonize in many tissues
	2. Nitrogen and Sulfur
		1. *Main ideas: (1)* E. coli incorporates inorganic N and S to make AAs from precursor metabolites. (2) inorganic nutrients (NO3-, NH4+, SO42-, PO42-) need to be incorporated into organic compounds first because they are so oxidized
		2. *Incorporation mechanisms*

|  |  |  |
| --- | --- | --- |
| Nutrient being incorporated | Mechanism | What’s happening |
| Ammonia incorporation – easier bc ammonia is more reduced | Reductive amination pathway | * Done by glutamate dehydrogenase (GDH) when ammonia concentration is high
* Glutamate is formed from alpha-ketoglutarate (from TCA cycle)
* After the glutamate has been made the alpha-amino group is transferred by transaminases to other C skeletons
* Efficient
 |
|  | Glutamine synthetase-glutamate synthase system (GS-GOGAT) | * Works well when there are low amounts of ammonium present
* Less efficient
* 2 enzymes involved
* *Glutamine synthetase:* makes glutamine from glutamate
* *Glutamate synthase:* transfers the amide N to alpha-ketoglutarate to make a new glutamate molecule
 |
| Nitrogen incorporation | Assimilatory nitrate reduction | * Nitrate is incorporated into the cell material and doesn’t partake in energy conservation
* Occurs in cytoplasm
* Nitrate reductase involved in reducing nitrite
* Nitrite is then reduced to ammonia through 2 electron additions
 |
| Sulfur incorporation | Assimilatory sulfur reduction | * Makes cysteine and methionine and coenzymes
* Sulfur obtained from 2 things
* Cysteine and methionine (cysteine will be used if both sulfate and cysteine are present)
* Sulfate (which needs to be reduced to H2S to be incorporated to compounds)
 |

1. Synthesis of carbohydrates
	1. Key terminology
		1. **Gluconeogenesis =** the synthesis of glucose from non-carbohydrate precursors
	2. Synthesis of monosaccharides and polysaccharides
		1. *Main idea:* Gluconeogenesis makes fructose-6P and glucose 6-P. after they’ve been formed other common sugars can be made (e.g. mannose from F6P)
2. Synthesis of peptidoglycan
	1. Key terminology
		1. **Peptidoglycan =** long, complex molecule made of long polysaccharide chains made of NAM and NAG
	2. What’s happening: bacterium needs to add new peptidoglycan to its cell wall while maintaining wall shape and integrity high osmotic pressure so peptidoglycan is degraded just enough via autolysins to provide acceptor ends for incorporation of new peptidoglycan units. Adjacent polysaccharide chains are cross-linked by bonds made between pentapeptides
	3. 2 carriers involved
		1. *uridine diphosphate*: function in the cytoplasmic rxns
		2. *bactoprenol phosphate*: functions in the plasma membrane rxns
	4. Significance: peptidoglycan is target for antimicrobial agents bc its super impt to bacterium cell wall structure and function. Inhibition of any stage of making it weakens cell wall and can lead to lysis
3. Filament synthesis
	1. Location: cytoplasm via self-assembly
	2. Process: basal body forms hollow tube through 3 membranes. Flagellin subunits are secreted through the basal body and pop out at end and polymerize
4. Secretion systems
	1. Key terminology
		1. **Sec system (secretory system)** = common system found throughout bacteria and eukaryotes that are used to move compounds across the plasma membrane; translocates unfolded proteins post/cotranslationally
		2. **Sec-independent =** needs no aid from a separate system to move things across
		3. **Sec-dependent =** need assistance to secrete across the plasma membrane
	2. Sec-dependent protein secretion
		1. *4 major players*
			1. Preprotein: formed in cytoplasm and includes signal peptide at the amino-terminus
			2. Chaperones (SecB): prevent preprotein folding
			3. Signal peptidase: cleaves it off after secretion
			4. Protein: folds after the removal of the signal peptide
	3. Secretion systems in gram negative cells
		1. *Main idea:* can have more than 1 copy of a secretion system in the cell (i.e. diff proteins can have diff forms of the same secretion system)

|  |  |  |
| --- | --- | --- |
| System Type | Sec-dependent/independent | What it’s secreting |
| Type I system (EHEC and ExPEC) | Sec-independent  | * Hemolysin
* Can secrete other solubles (e.g. AAs or proteins)
* Toxin, proteases, lipases secretion
* Spans the plasma membrane, periplasmic space *and* outer membrane
 |
| Type III system | Sec-independent | * Flagellin (e.g. basal body of the flagella is a Type III)
* Injects virulence factors directly onto host cells (toxin secretion)
 |
| Type II | Sec-dependent | * Degradative enzymes (e.g. beta-galactosidase, proteases)
 |
| Type V  | Sec-dependent | * Autotransporters (once they’re in periplasmic space a part of the protein itself punctures hole in the outer membrane and puts itself through)
* Toxins for EPEC and EHEC
 |
| Type IV | Sec-independent | * Transfer DNA from donor bacterium to recipient (i.e. conjugation)
 |

E. coli gene expression

1. Blackwood’s LO
	1. Describe aspects of bacterial genome organization and transcription that are possible because of no nuclear membrane
	2. Explain the difference between these different ways to describe operons: positive/negative control and inducible/repressible.
	3. Describe how investment of energy in production of a protein is regulated at each of these steps:
		1. Before RNA polymerase binds to DNA (multiple mechanisms)
			1. Positive control
			2. Negative control
		2. After RNA polymerase binds to DNA but before transcription is done
			1. Attenuation
		3. After transcription has completed (multiple mechanisms)
			1. Have whole mRNA molecule that’s been transcribed so what happens to stop protein from being made
			2. Riboswitch – binds metabolite between the metabolite and mRNA and stops ribosome from binding
			3. Anti-sense RNAs – can block the binding site
	4. Describe mechanisms of transcriptional control for *lac* operon in response to environmental lactose and glucose concentrations. Include responses to the environment and the molecular mechanisms by which responses are accomplished.
	5. Describe mechanisms of transcriptional control for *trp* operon in response to environmental tryptophan concentration and growth rate (or protein synthesis rate). Include responses to the environment and the molecular mechanisms by which responses are accomplished.
	6. What information do you need to know to determine if an operon is inducible or repressible? What about if it is under positive or negative control?
	7. Make predictions about transcriptional control of operons for other metabolic pathways in *E. coli* that we discussed as advantageous only under certain environmental conditions (e.g., electron transport chains, nitrogen uptake systems, catabolism of polymers).
	8. Why are repressor proteins and activator proteins constitutively expressed?
	9. When repressor proteins involved in *trp* and *lac* operons are made, how do they differ?
	10. What rates are being compared during attenuation?
2. Bacterial gene structure
	1. Key terminology
		1. **Gene =** basic unit of genetic information
		2. **Cistron =** a segment of DNA that encodes a single polypeptide
		3. **Promoter =** site located at the start of a gene that is the binding site for RNA polymerase and functions strictly to orient the RNA polymerase
		4. **Leader =** the initially transcribed portion of the gene that doesn’t code for AAs so it’s transcribed into mRNA but not translated into AAs
3. Control of gene expression in bacteria
	1. Key terminology
		1. **Diauxic growth =** pattern of growth that characterizes sequential use of glucose and lactose substrates
		2. **Catabolite repression =** glucose represses genes that are used in the breakdown of lactose
	2. Transcriptional control purpose: a lot of microbes are in changing evmts and gene products aren’t needed all the time so if they weren’t regulated it would be energetically wasteful
	3. Catabolism
		1. 2 reasons why genes involved in catabolism are expressed
			1. relevant substrate is present in evmt
				1. if it is, relevant enzymes need to be made
				2. if it isn’t make enzyme to break the worse substrate down
			2. better substrate isn’t present in evmt
	4. Anabolism
		1. 1 reason why genes involved in anabolism are expressed
			1. relevant structural compound isn’t present in evmt
	5. Glucose
		1. *Main idea:* if glucose is present, the cell uses it over the other carbohydrates; E. coli doesn’t get energy from breaking down lactose but needs to do it so it can get to glucose and galactose
		2. *Bacterial growth:* exponential growth as glucose is used
	6. Bacterial gene expression vs. eukaryotic gene expression
		1. Bacteria have 1 RNA polymerase, eukaryotes have 3