Chapter 10 Lecture Notes: Microbial Metabolism – The Use of Energy in Biosynthesis

I. Principles governing biosynthesis
   A. Construct larger macromolecules from smaller subunits to conserve genetic storage capacity, raw materials, and energy
      1. inorganic molecules \(\rightarrow\) simple precursor molecules \(\rightarrow\) monomers \(\rightarrow\) macromolecules
      2. Fig. 10-1
   B. In bacteria: almost all of the building blocks/monomers are synthesized from only 12 precursor metabolites
      1. glucose 6-phosphate
      2. fructose 6-phosphate
      3. ribose 5-phosphate
      4. erythrose 4-phosphate
      5. triose phosphate
      6. 3-phosphoglycerate
      7. phosphoenol pyruvate (PEP)
      8. pyruvate
      9. acetyl CoA
      10. a-ketoglutarate
      11. succinyl CoA
      12. oxaloacetate
   C. Biosynthesis requires energy
   D. Use of amphibolic pathways (Fig. 10-2)
      1. have reactions go forward and backward with one enzyme
      2. one step should be catalyzed by a nonreversible enzyme in prokaryotes
      3. anabolic pathways have a nonreversible step at end for regulatory purposes
      4. compartmentalization of anabolic and catabolic pathways in eukaryotes
      5. different cofactors in anabolic and catabolic pathways
   E. Self-assembly for supramolecular structures
F. Elucidation of pathways
   1. in vitro analysis
   2. radiolabeled precursors (add radiolabeled precursors to cells and ask in what end product does radioactivity end up)
   3. biosynthetic mutant analysis
      a) analysis of intermediates that accumulate in the mutants
      b) analysis of addition of putative intermediates to mutants (either by cross feeding or by adding chemicals)
      c) If a mutant is blocked in a metabolic pathway at some point, it can not make any of the intermediates in the pathway that are after the block.
         - If you supply the mutant with an intermediate compound that is made before the block, there will be no effect (no final product).
         - If you supply the mutant with an intermediate compound that is made after the block, the mutant can now make the end product
         - Compound made late in the pathway will support the growth of more mutants.
         - Mutants late in the pathway can not be restored with as many compounds as mutant that are early in the pathway.

Example from Beadle and Tatum in the 1940’s: Irradiated *Neurospora* (haploid fungus) to induce mutagenesis. They then screened for arginine auxotrophs and found three that contained mutations that mapped to different locations on the chromosome (arg-1 arg-2 arg-3).

Each of the mutants would grow when media was supplemented with arginine. Other compounds similar to arginine could also support growth:

<table>
<thead>
<tr>
<th></th>
<th>ornithine</th>
<th>citrulline</th>
<th>arginine</th>
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<tr>
<td>wt</td>
<td>+</td>
<td>+</td>
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<tr>
<td>arg-1</td>
<td>+</td>
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<td>arg-2</td>
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<td>arg-3</td>
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Based on this data Beadle and Tatum proposed the following enzymatic pathway for the production of arginine:

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Enzyme X   Enzyme Y   Enzyme Z
Precursor   → ornithine   → citrulline   → arginine
arg1        (arg2)      (arg3)
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II. Incorporation of carbon into precursor molecules
   A. Why? Carbon based life!!!!!
   B. Sources of carbon can be CO$_2$ (autotrophic) or organic molecules (heterotrophic)
   C. Autotrophic fixation of CO$_2$ as sole mechanism via Calvin cycle (AKA Calvin-Benson cycle or reductive pentose phosphate cycle)
      1. Who? autotrophs
      2. Location
         a) chloroplast stroma (eukaryotic microbial autotrophs)
         b) carboxysomes – inclusion bodies that contain RUBISCO enzyme that is required for fixation (cyanobacteria, nitrifying bacteria, thiobacilli)
      3. Mechanism (Fig. 10-4)
         a) Carboxylation phase (Fig. 10-3)
            (1) addition of CO$_2$ to ribulose 1,5 bisphosphate
            (2) catalyzed by ribulose-1,5-bisphosphate carboxylase
            (3) yields 2 3-phosphoglycerate
         b) Reduction phase: yields 2 glyceraldehyde-3-P
         c) Regeneration phase:
            (1) several transketose and transaldolase reactions that regenerate ribulose 1,5 bis-phosphate
            (2) several intermediates that can be used in biosynthesis
               (a) fructose 6-P
               (b) erythrose 4-P
               (c) ribose 5-P
   D. Autotrophic fixation of CO$_2$ as sole mechanism via acetyl CoA pathway
      1. Who? methanogens, acetogenic bacteria, sulfate reducing bacteria
      2. Location: cytoplasm
      3. Mechanism (see attached)
         a) Reduction of CO$_2$ to CH$_4$
         b) Transfer of methyl group to CO to form acetyl group
         c) Transfer of acetyl group to CoA to form acetyl CoA
         d) Reductive carboxylation of acetyl CoA to pyruvate
   E. Autotrophic fixation of CO$_2$ as sole mechanism via reductive TCA cycle
      1. Who? green photosynthetic bacterium Chlorobium; Hydrogenbacter, arecheons Sulfolobus, Thermoproteus
      2. Location: Cytoplasm
      3. Mechanism (see attached)
         a) PEP + CO$_2$ $\rightarrow$ oxaloacetate
         b) feed oxaloacetate into reversed TCA
F. Incorporation of reduced organic compounds
   1. Who? heterotrophs
   2. Enormous variety of carbon sources = many mechanisms of incorporation, many of which are “energy” pathways such as glycolysis, TCA, pentose phosphate (Fig. 10-17)
   3. Need for anaeplerosis
      a) Because C skeletons are removed from intermediates in energy generating pathways in heterotrophs, the intermediates must be replenished (anaeplerosis)
      b) Mechanisms
         (1) “CO2 fixation”
         (2) glyoxalate cycle – modified TCA cycle which bypasses decarboxylation steps allowing acetyl CoA to be incorporated into oxaloacetate and other intermediates (Fig. 10-20)

III. Incorporation of nitrogen into primary molecules glutamate and glutamine
   A. Why? Component of protein, nucleic acid, coenzymes
   B. Initial incorporation into organic material is directly from NH3
   C. Sources of nitrogen
      1. NH3
         a) Initial reactions:
            (1) a-ketoglutamate + NH3 + NADH + H+ \(\rightarrow\) glutamate + NAD + H2O via glutamate dehydrogenase (GDH) (Fig. 10-10)
            (2) glutamine and glutamate synthetases (GS) (Fig. 10-12)
            (3) GS has higher affinity for ammonia than GDH but uses ATP
         b) Secondary reactions
            (1) transamination of amino groups from above amino acids to other C skeletons
      2. nitrate via assimilatory nitrate reduction (Fig. 10-13)
         NO3– + \(\rightarrow\) NO2– + \(\rightarrow\) NH2OH \(\rightarrow\) NH3 \(\rightarrow\) to #1
      3. atmospheric nitrogen N2 via nitrogen fixation (Fig. 10-14,16)
         a) N2 \(\rightarrow\) NH3 \(\rightarrow\) to #1 via nitrogenase
         b) very important for restoring ammonia to soil for plant growth
         c) only bacteria do this
            (1) some free-living bacteria
            (2) bacteria associated with plants (Rhizobium)
            (3) cyanobacteria
IV. Incorporation of sulfur into primary molecule cysteine
   A. Why? Component of cysteine, methionine, coenzyme A, and biotin
   B. Initial incorporation into organic material is directly from \( \text{H}_2\text{S} \)
   C. Sources
      1. \( \text{H}_2\text{S} \) (unstable in aerobic environment because of oxidation)
         a) Initial reaction:
            
            \[
            \text{H}_2\text{S} \xrightarrow{\text{O-acetyl serine sulphydrylase}} \text{O-acetyl serine} \rightarrow \text{acetyl} \rightarrow \text{cysteine}
            \]
         b) Secondary reaction: incorporation of S from cysteine into other S containing compounds
      2. Sulfate (only low amounts in soil) which is reduced to \( \text{H}_2\text{S} \) via assimilatory sulfate reduction → to 1 (Fig. 10-9)
      3. Exogenous cysteine and methionine (main method)

V. Incorporation of phosphate into ATP
   A. Why? Component of nucleic acids, proteins, phospholipids, ATP, NADP
   B. Sources
      1. From inorganic phosphate incorporation into ATP via
         a) photophosphorylation
         b) oxidative phosphorylation
         c) substrate level phosphorylation
      2. From organic phosphate esters that are cleaved by phosphatases to liberate inorganic phosphate

VI. Synthesis of sugars (constituent parts of polysaccharides, nucleic acids) from precursor molecules
   A. Gluconeogenesis = synthesis of glucose from noncarbohydrate precursors via a reversal of glycolysis, except for three steps; figure 10-5
   B. Synthesis of other sugars via rearrangements of glucose and fructose
      fructose 6-P ↔ mannose 6-P
   C. Nucleoside diphosphate route
      Several sugars are synthesized while attached to a nucleoside diphosphate
      1. uridine diphosphate glucose (UDPG – Fig. 10-6): synthesis of galactose and glucuronic acid (Fig. 10-7) and cell wall synthesis
      2. ADPG for starch synthesis
   D. Pentose phosphate pathway
VII. Synthesis of amino acids (constituent parts of proteins): from precursor molecules
   A. Note that the primary route for incorporation of inorganic N is via glutamate and glutamine from ammonia (see above) → transamination of amino groups from glutamate and glutamine to other precursor C skeletons yields other amino acids
   B. Precursor C skeletons for the 6 biosynthetic classes of amino acids
      1. pyruvate
      2. oxaloacetate: Fig 10-18
      3. a-ketoglutarate
      4. 3-phosphoglycerate
      5. PEP + erythrose-4-P: Fig. 10-19
      6. 5-phosphoribosyl-1-pyrophosphate + ATP

VIII. Synthesis of purines, pyrimidines, and nucleotides (constituent parts of DNA, RNA, ATP, cofactors) from precursors
   A. Definitions
      1. nitrogenous bases
         a) purine (adenine, guanine)
         b) pyrimidine (cytosine, thymine, uracil)
      2. nucleoside (base + sugar)
         adenosine, guanosine, cytidine, thymidine, uridine
      3. nucleotide (base + sugar + phosphate) i.e. adenosine monophosphate (AMP), etc
   B. Purine nucleotide biosynthesis:
      1. The purine ring is constructed by sequential addition of atoms to the ribose 5-phosphate derivative 5-phosphoribosyl-pyrophosphate (PRPP) using C and N from a variety of sources to generate inosinic acid monophosphate (IMP)
      2. IMP is then converted to either AMP or GMP: Fig. 10-22
      3. AMP + ATP → 2 ADP via adenylate kinase
      4. ATP via substrate-level or oxidative phosphorylation
      5. GMP → GDP via nucleoside monophosphate kinase
      6. GDP → GTP via nucleoside diphosphate kinase
   C. Pyrimidine biosynthesis (Fig. 10-23)
      1. Construction of pyrimidine ring from aspartate, ammonia, and carbon dioxide
      2. Addition of PRPP and decarboxylation to generate UMP
      3. UMP → UDP via nucleoside monophosphate kinase
      4. UDP → UTP via nucleoside diphosphate kinase
      5. UTP + glutamine → CTP via CTP synthetase
   D. Reduction of ribonucleotides to deoxyribonucleotides
      1. For all but dTTP
         a) reductive dehydration of XDP catalyzed by ribonucleoside diphosphate reductase (see attached)
         b) reductive dehydration of XTP catalyzed by ribonucleoside diphosphate reductase (rarer)
      2. For dTTP: UMP → dUMP → dTMP → dTTP (Fig. 10-24)
IX. Synthesis of fatty acids (constituent parts of lipids)

A. Saturated fatty acids (Fig 10.25)
   1. Initial transfer of acetyl group from acetyl CoA to ACP
   2. Transfer of malonyl group from malonyl CoA to another ACP
   3. Condensation reaction releases CO₂
   4. NADPH dependent reduction
   5. Dehydration
   6. NADPH dependent reduction
   7. Repeat 2-6 with condensation of next malonyl-ACP to growing fatty acid chain-ACP

B. Unsaturated fatty acids
   1. Aerobic pathway – oxidation with O₂ and also uses NADPH
   2. Anaerobic pathway – dehydration of hydroxy fatty acid during synthesis

C. Triglycerols (Fig. 10-26)
   1. Dihydroxyacetone phosphate is reduced to glycerol 3-P
   2. Esterification with 2 fatty acids to give phosphatic acid
   3. Hydrolysis of the phosphate group
   4. Attachment of the third fatty acid

D. Phospholipid synthesis (Fig. 10-26)
   1. From phosphatic acid → addition of CTP
   2. Addition of serine and release of CMP

X. Peptidoglycan biosynthesis
A. Structure review
B. Very complex process
C. Carrier molecules
   1. uridine diphosphate
   2. bactoprenol (Fig. 10-27) carries peptidoglycan components through membrane
D. Synthesis
   1. UDP derivative of NAM and NAG are synthesized in the cytoplasm
   2. Amino acids are added to UDP-NAM to form pentapeptide chains
   3. Transfer of NAM-pentapeptide to bactoprenol phosphate at membrane surface
   4. UDP-NAG adds NAG to NAM-pentapeptide to form peptidoglycan repeating unit
   5. Transport of unit across the membrane
   6. Attachment of unit to growing end of peptidoglycan chain
   7. Bactoprenol recycles to cytoplasm
   8. Transpeptidation (cross links between peptidoglycan chains) amino group from DAP (or glycine from peptide interbridge) in position 3 attacks the carbonyl carbon in peptide bond holding 2 alanines together on another strand → terminal alanine is released
E. Patterns of cell wall formation (Fig. 10-30)