微生物学
Microbiology

Lecture 9

2017.11.21

CHAPTER 12

Anabolism: The Use of Energy in Biosynthesis

OUTLINE

- Calvin cycle-CO_2 fixation
  ---building blocks-sugar
- Peptidoglycan synthesis
  ---polysaccharides
- Nitrogen fixed
  ---building blocks-Amino acid etc

Anabolism

- **the synthesis of complex organic molecules from simpler ones**
- **requires energy** (and reduction power) **from fueling reactions**
- **For growth or turnover**
- **Turnover更新: continual degradation and resynthesis再合成 of cellular constituents by nongrowing cells非生长的细胞**
- **metabolism is carefully regulated**
  - **for rate of turnover to be balanced by rate of biosynthesis**
  - **in response to organism's environment**

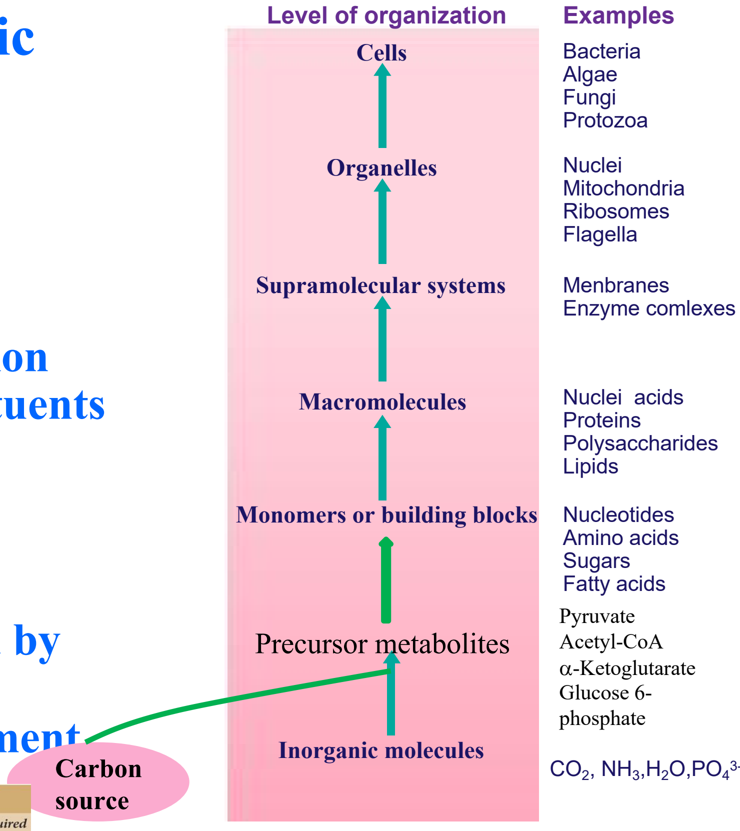


Table 11.1 Biosynthesis in *Escherichia coli*

Cell Constituent	*Number of Molecules per Cell*[a]	*Molecules Synthesized per Second*	*Molecules of ATP Required per Second for Synthesis*
DNA	1[b]	0.00083	60,000
RNA	15,000	12.5	75,000
Polysaccharides	39,000	32.5	65,000
Lipids	15,000,000	12,500.0	87,000
Proteins	1,700,000	1,400.0	2,120,000

From Bioenergetics by Albert Lehninger.

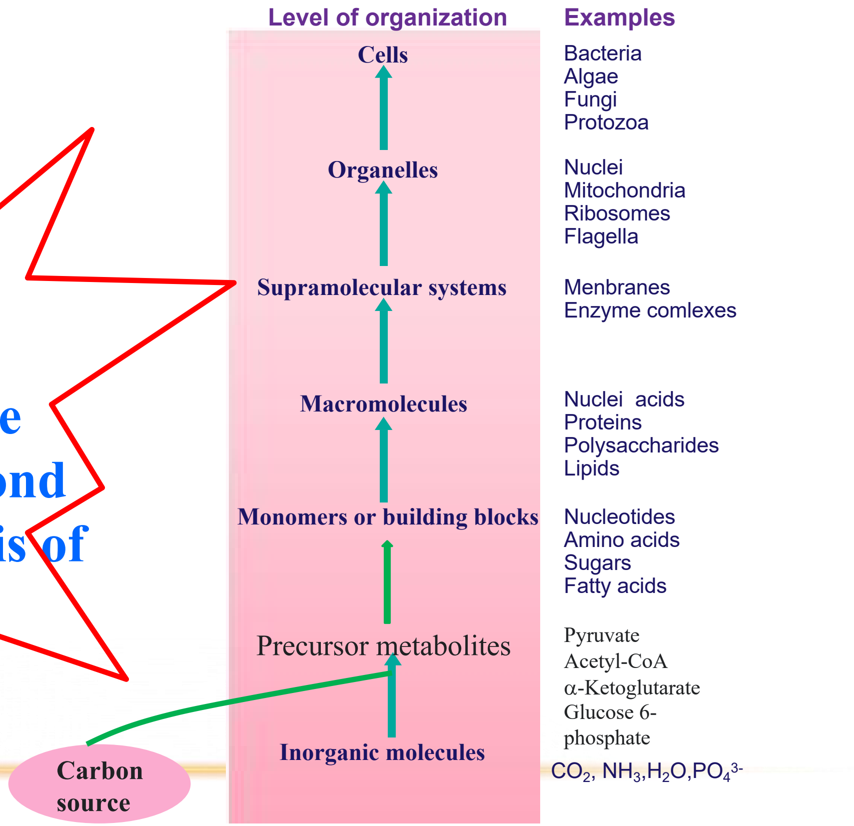
[a] Estimates for a cell with a volume of 2.25 μm³, a total weight of 1×10^{-12} g, a dry weight of 2.5×10^{-13} g, and a 20-minute cell division cycle.
[b] It should be noted that bacteria can contain multiple copies of their genomic DNA.

细胞的构建——生物合成导致复杂性不断提高

Principals governing biosynthesis

- Large molecules are made from small molecules.

The use of a few monomers linked together by a single type of covalent bond makes the synthesis of macromolecules highly efficient.



细胞的构建——生物合成导致复杂性不断提高

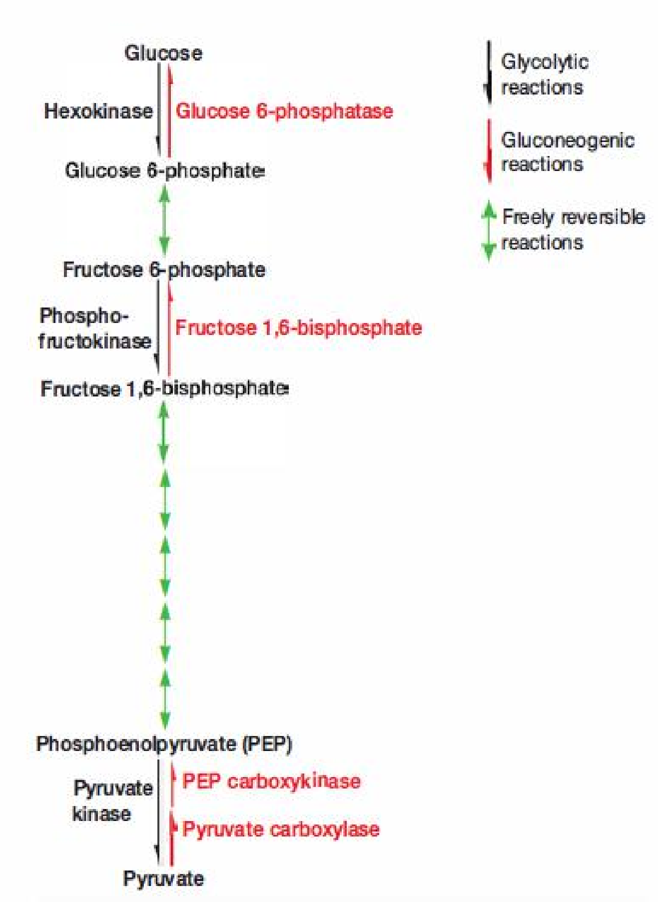
Many enzymes do double duty, but some enzymes function in only one direction(control)

*Embden Meyerh Pathway - an amphibolic pathway.

*Many reactions are catalyzed by enzymes that function in glycolysis and gluconeogenesis.

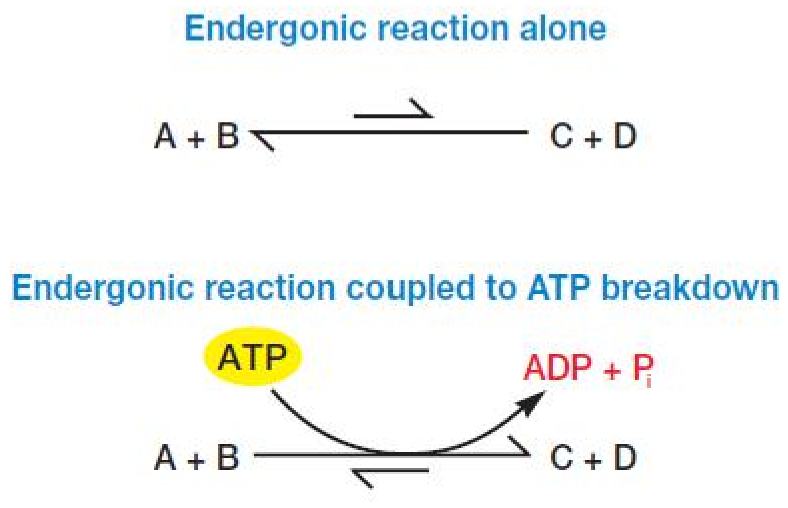
*Some glycolytic reactions are catalyzed by enzymes unique to glycolysis.

*Therefore, although they share several enzymes, the two pathways are distinct.



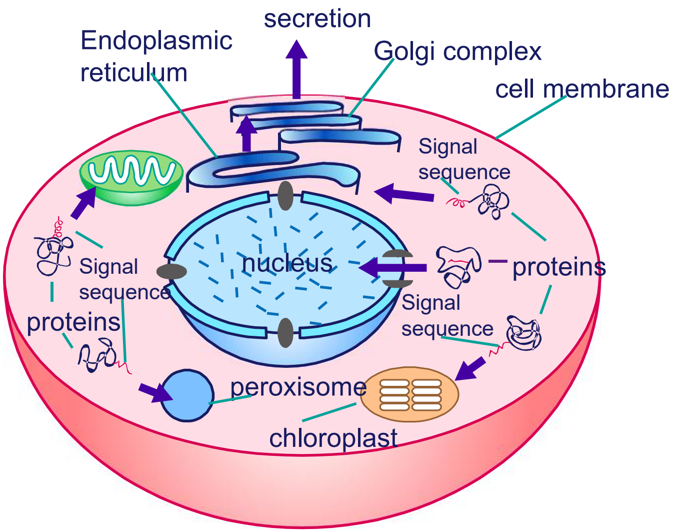
Operate irreversibly in the direction

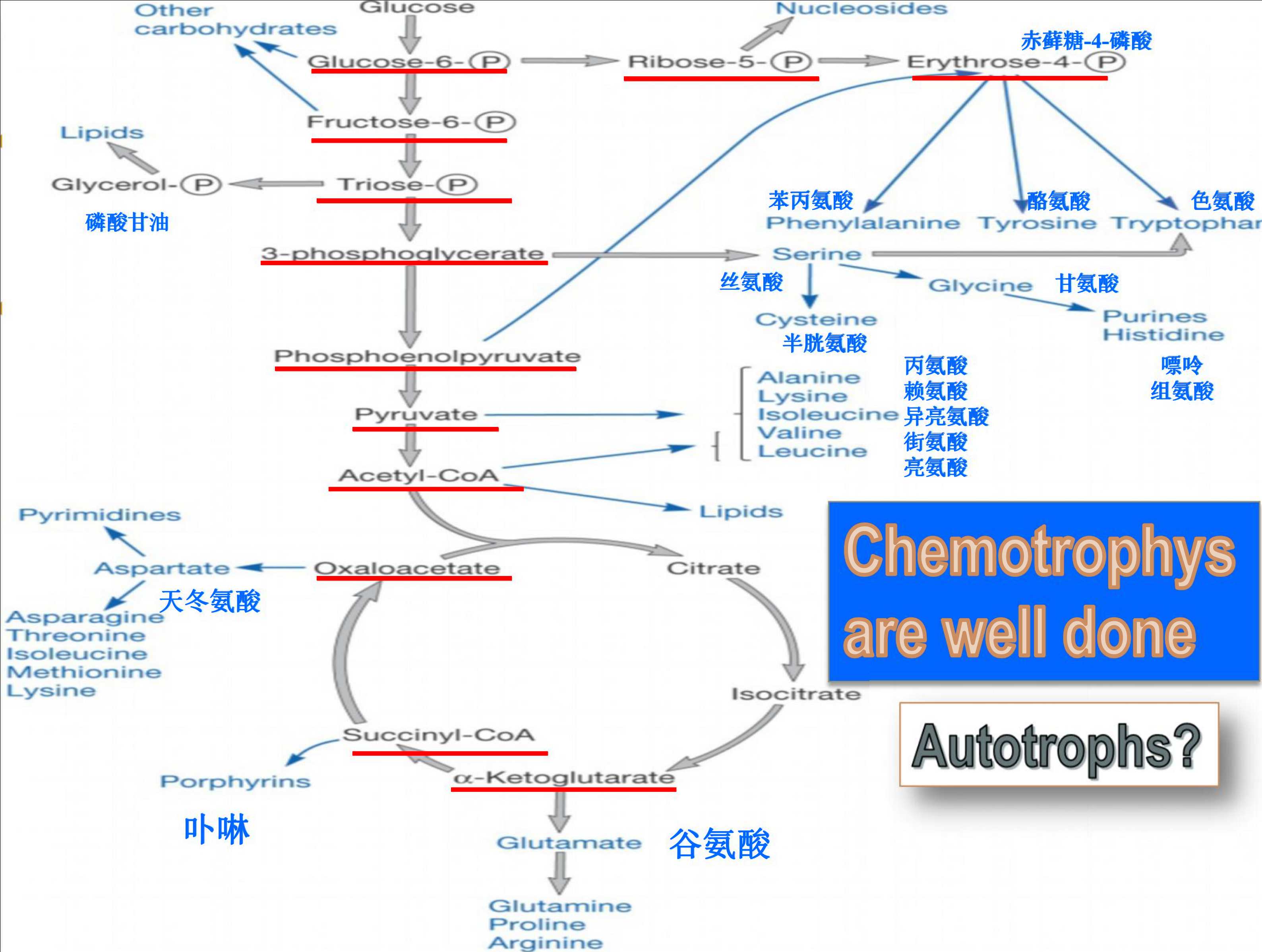
by connecting some biosynthetic reactions to the breakdown of ATP



Catabolism and anabolism can be physically separated and often use different cofactors

- Compartmentation makes it easier for catabolic and anabolic pathways to operate simultaneously yet independently.
- Usually catabolic oxidations produce NADH, NADPH often serves as the donor during biosynthesis.





Chemotrophys are well done

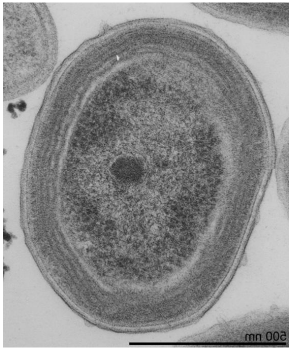
Autotrophs?

The fixation of CO_2 by Autotrophs-pathways

- The calvin cycle 卡尔文循环
- The reductive TCA cycle 还原的TCA循环
- The hydroxypropionate cycle 羟丙酸循环
- The acetyl-CoA pathway乙酰辅酶途径
- The 3-hydroxypropionate/4-hydroxybutyrate pathway 3-羟丙酸/4-羟丁酸途径

Calvin cycle

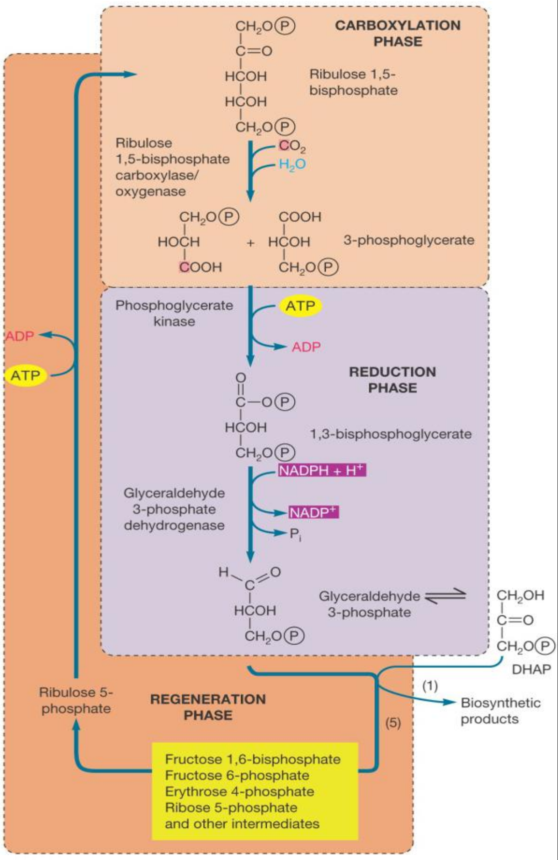
- used by most autotrophs to fix CO_2
- also called the reductive pentose phosphate cycle还原的戊糖磷酸循环
- in eukaryotes, occurs in stroma of chloroplasts



- in cyanobacteria, some nitrifying bacteria, and thiobacilli, may occur in carboxysomes（羧酶体）
  - inclusion bodies that may be the site of CO_2 fixation

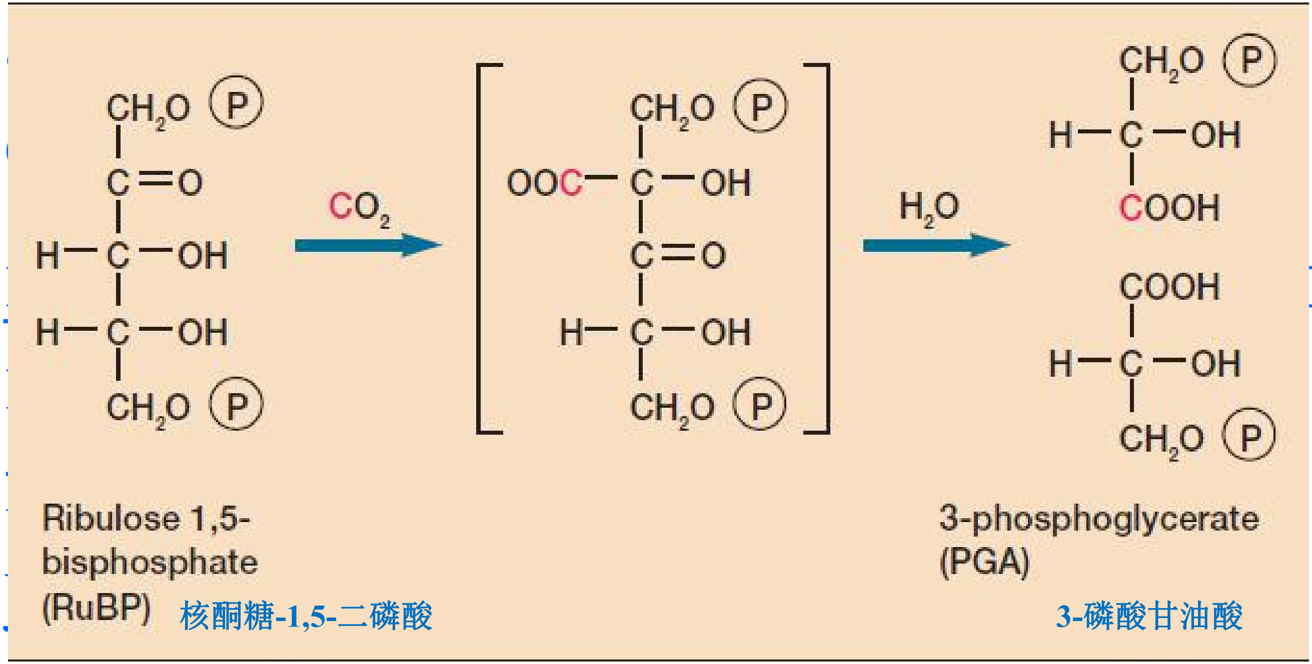
Calvin cycle

- consists of 3 phases
  - the carboxylation phase羧化期
  - the reduction phase还原期
  - the regeneration phase再生期
- 3 ATPs and 2 NADPHs are used during the incorporation of one CO_2

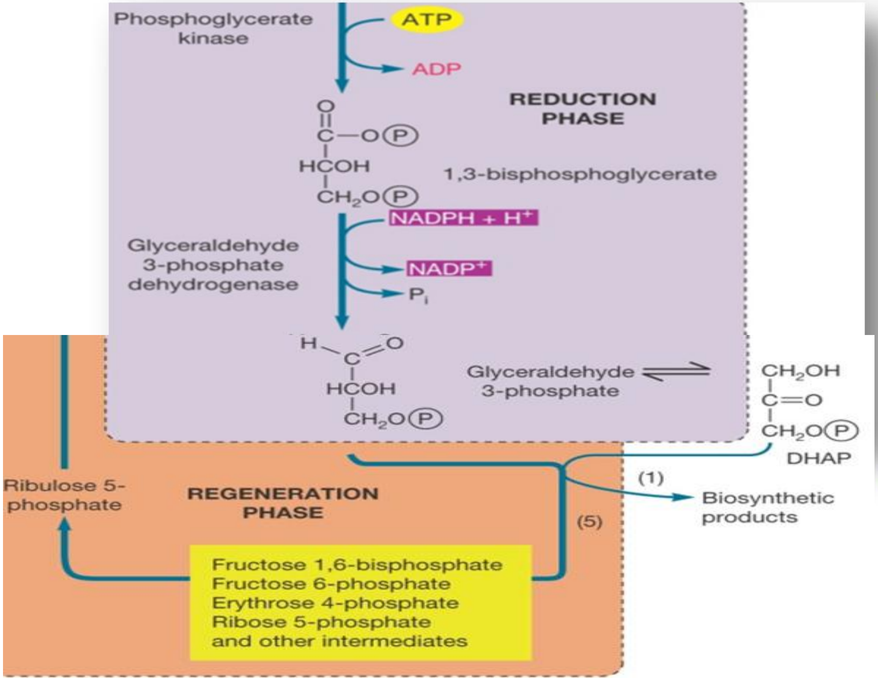


The carboxylation phase

- catalyzed by the enzyme ribulose 1.5-bisphosphate carboxylase 1,5-二磷酸核酮糖羧化酶



The reduction and regeneration phases



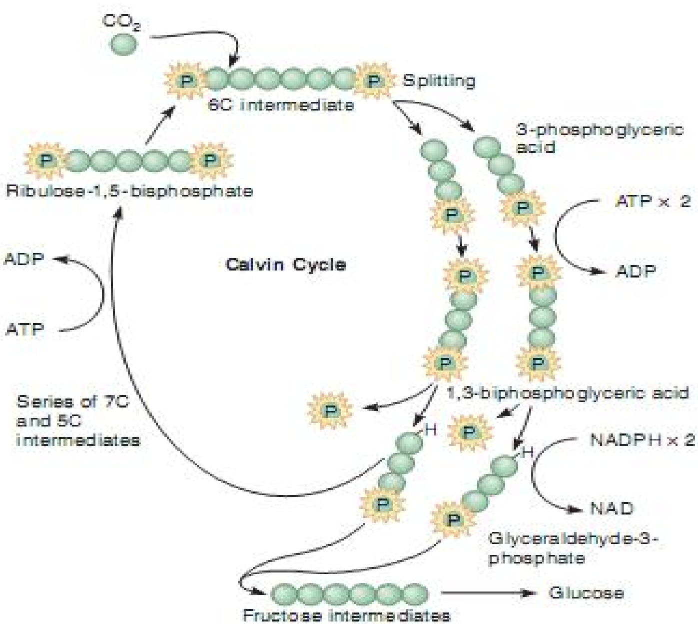
- 3-phospho-glycerate reduced to glyceraldehyde 3-phosphate
- RuBP regenerated
- carbohydrates (e.g., fructose and glucose) are produced

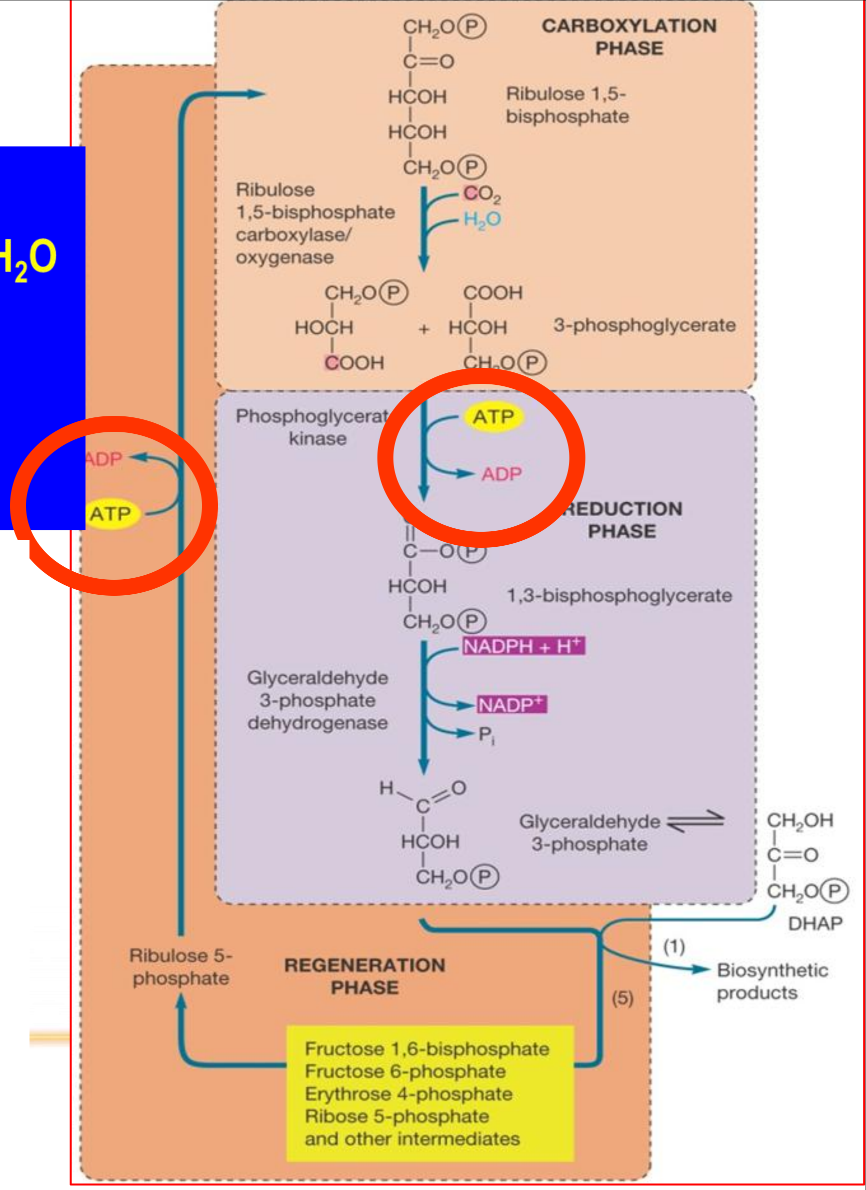
Summary

$$6CO_2 + 18ATP + 12NADPH + 12H^+ + 12H_2O$$

$$\downarrow$$

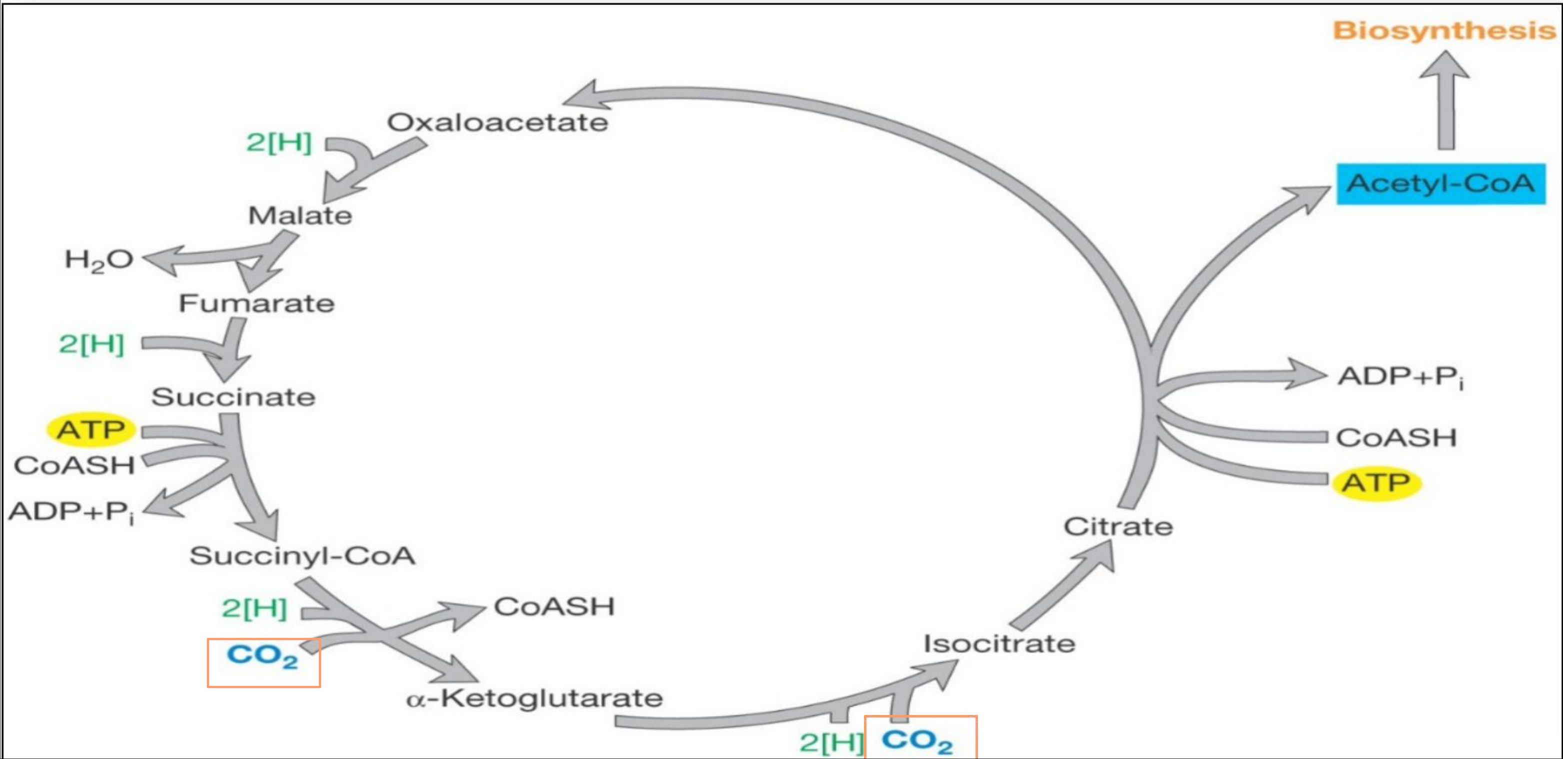
$$glucose + 18ADP + 18P_i + 12NADP^+$$





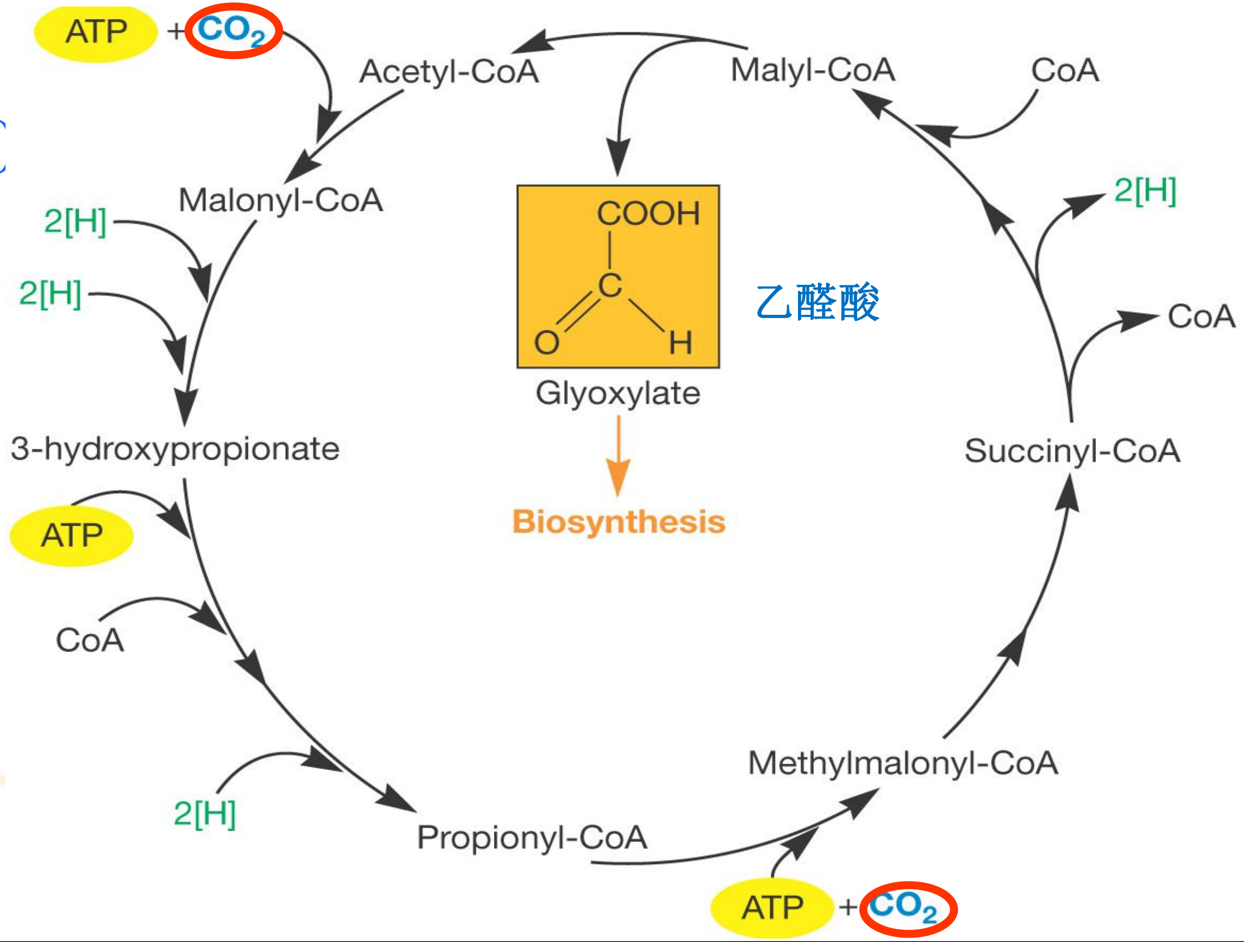
Other CO_2-fixation pathways

- the reductive TCA cycle还原的TCA循环
  - used by some chemolithoautotrophs
  - runs in reverse direction of the oxidative TCA



Other CO_2-fixation pathways

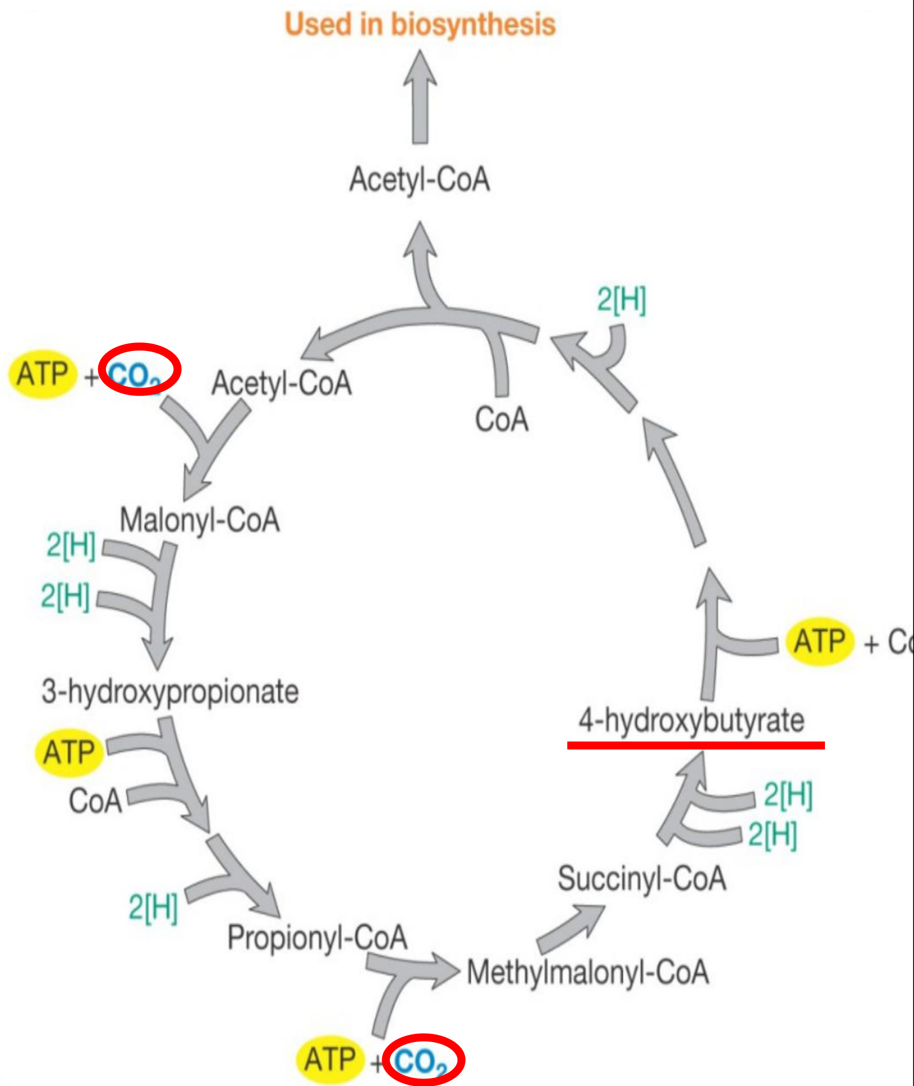
- the hydroxypropionate cycle羟丙酸循环: used by some archaeal genera and the green nonsulfur bacteria (also anoxygenic phototrophs)



The 3-hydroxypropionate/4-hydroxybutyrate pathway

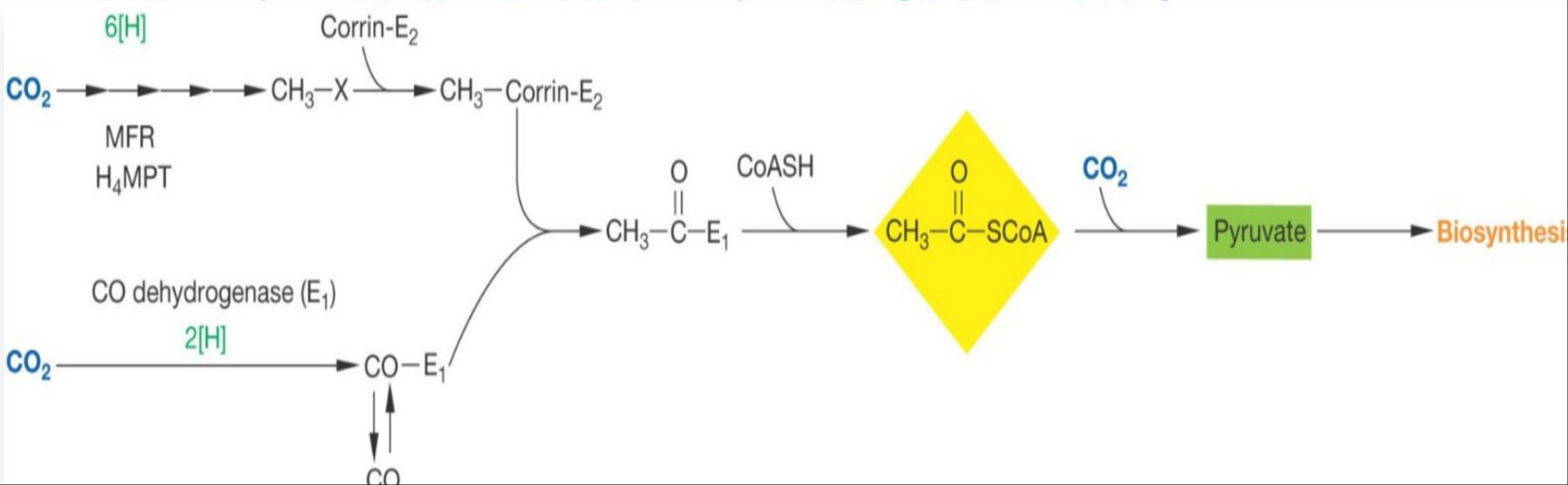
3-羟丙酸/4-羟丁酸途径

- first described in 2007 in an archae
- uses 3-hydroxypropionate cycle
- uses unique reaction to produce 4-hydroxybutryate



Other CO_2-fixation pathways***

- the acetyl-CoA pathway (*Methanobacterium thermoautotrophicum*) 乙酰辅酶途径
  - methanogens use portions of the acetyl-CoA pathway for carbon fixation
  - involves the activity of a number of unusual enzymes and coenzymes (see 20.3)



Discussion

- Can Heterotrophs carry out the fixation of CO_2? If yes, why and how do they fix CO_2?

The fixation of CO_2 by Heterotrophs

Anaplerotic Reactions添补反应

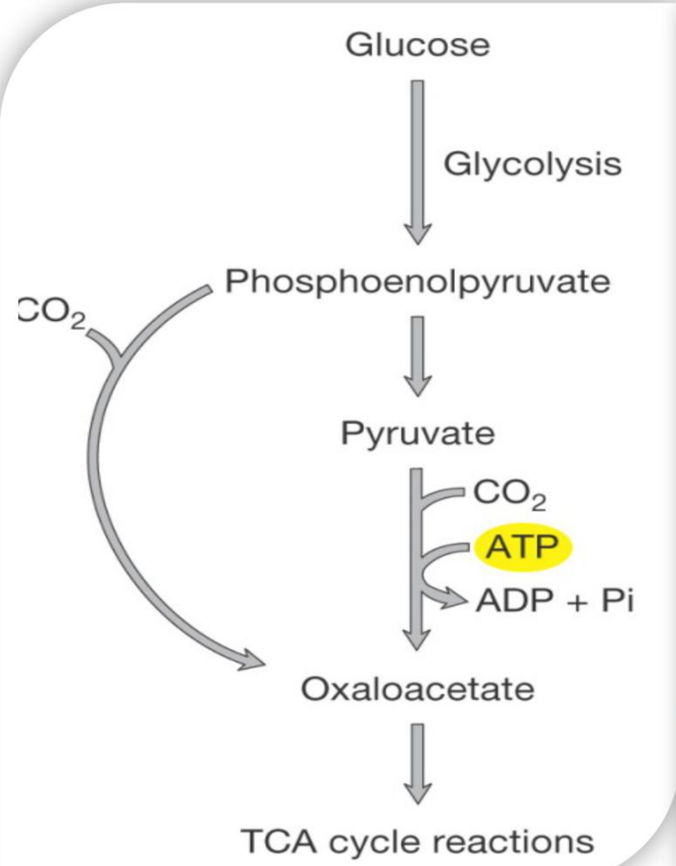
- phosphoenolpyruvate (PEP) carboxylase
  - phosphoenolpyruvate + CO_2 →

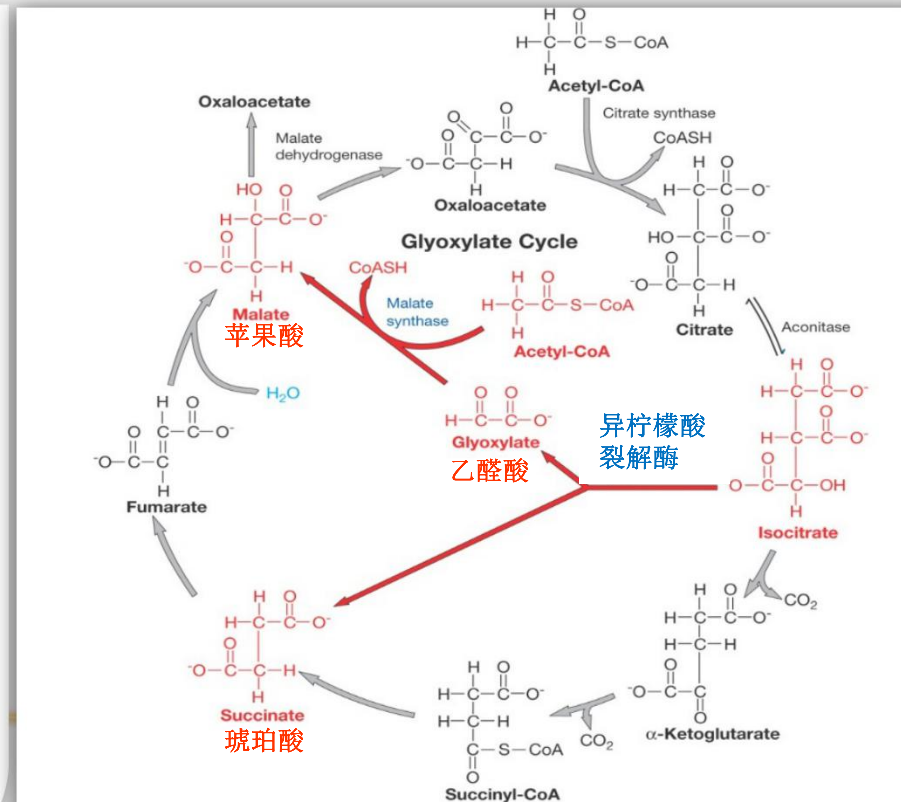
    （草酰乙酸）oxaloacetate + P_i
- pyruvate carboxylase羧化酶
  - pyruvate + CO_2 + ATP + H_2O →

    oxaloacetate + ADP + P_i
    - reaction requires the cofactor biotin生物素

Glyoxalate cycle乙醛酸循环

- other anaplerotic reactions are part of the glyoxalate cycle, a modified TCA cycle





Overall equation:

2 Acetyl-CoA + FAD + 2NAD$^+$+ 3H_2O ⟶ Oxaloacetate + 2CoA + $FADH_2$ + 2NADH + 2H

Synthesis of sugars and polysaccharides

Gluconeogenesis

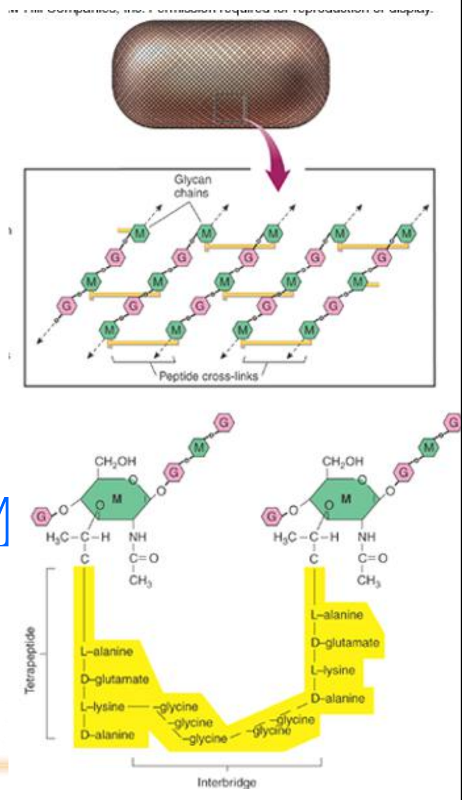
Monosaccharides

Polysaccharides

Peptidoglycan 肽聚糖

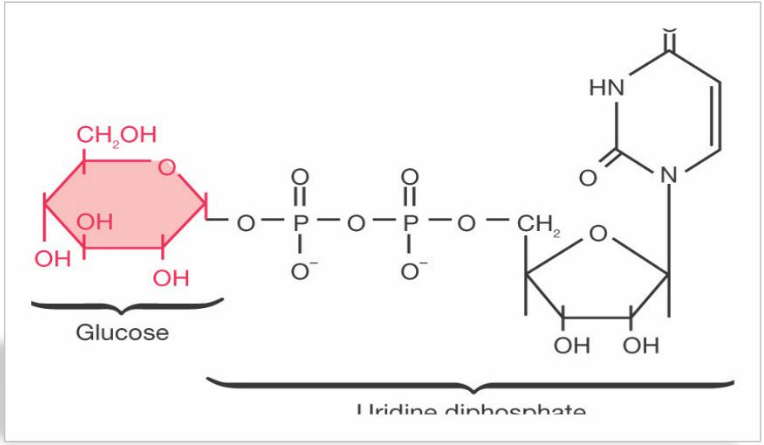
Peptidoglycan structure

- Meshlike(网 状) polymer of identical subunits forming long strands
  - two alternating sugars
    - *N*-acetylglucosamine (NAG)
      N-乙 酰 葡 萄 糖 胺
    - *N*- acetylmuramic acid(NAM
      N-乙 酰 胞 壁 酸
  - alternating D- and L- amino acids

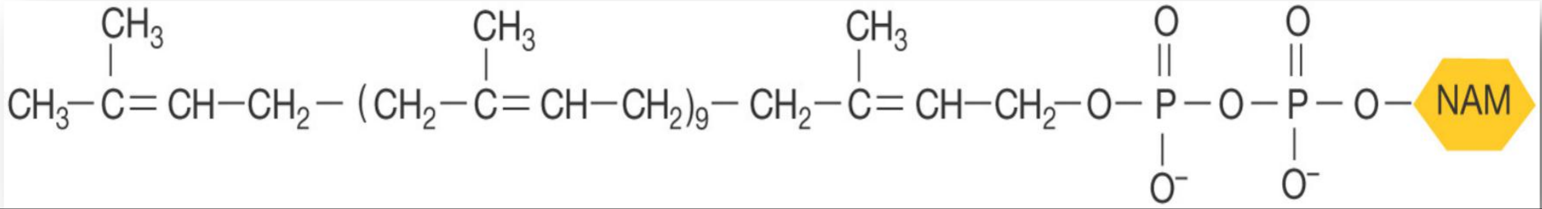


Peptidoglycan synthesis

- Carrier:
  - UDP尿苷二磷酸
  - Bactroprenol细菌萜醇
- Location:
  - cytoplasm;
  - membrane;
  - and periplasmic space



- Reaction:



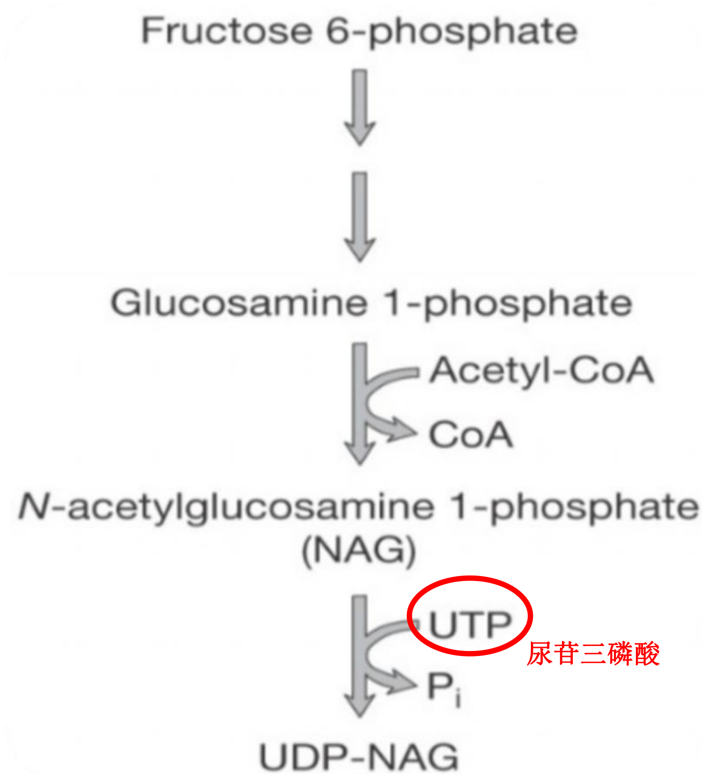
Discussion

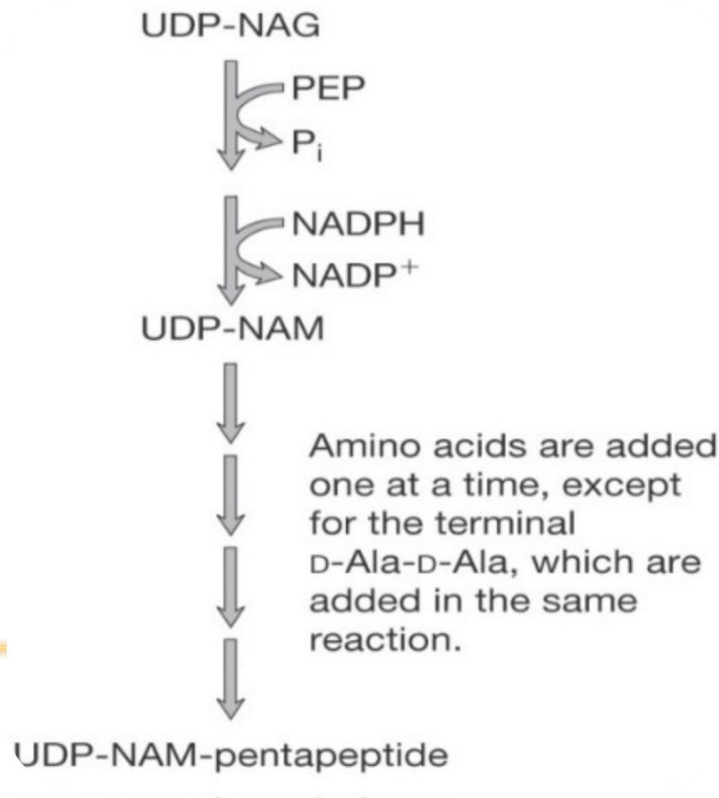
- **Describe the patterns of peptidoglycan synthesis seen in gram-positive cocci and in rod-shaped bacteria such as *E. coli*.**

  What is unusual about the synthesis of peptides that takes place during peptidoglycan construction?

（1）UDP derivatives of N-acetylmuramic acid and Nacetylglucosamine are synthesized in the cytoplasm

（2） Amino acids are sequentially added to UDP-NAM to form the pentapeptide chain

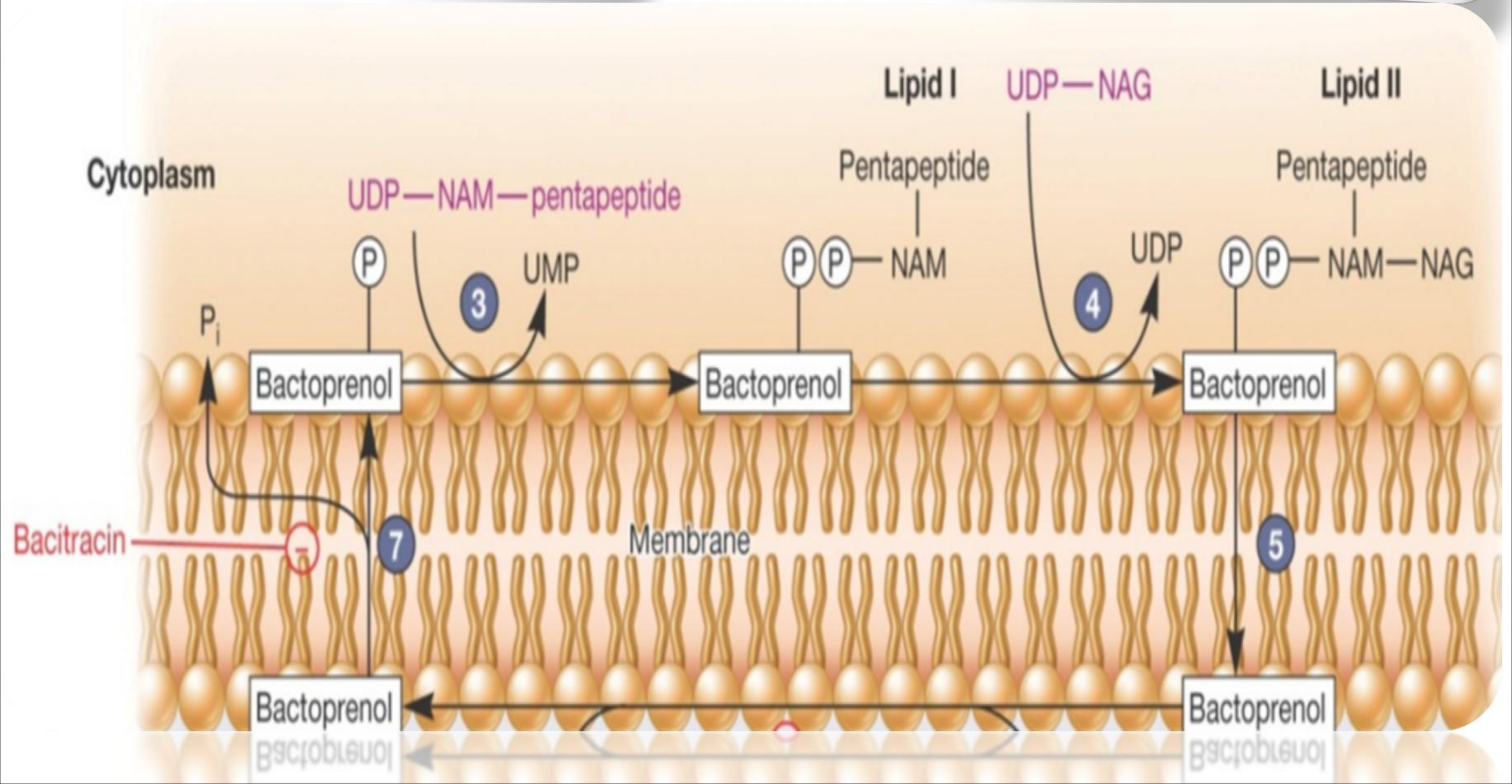




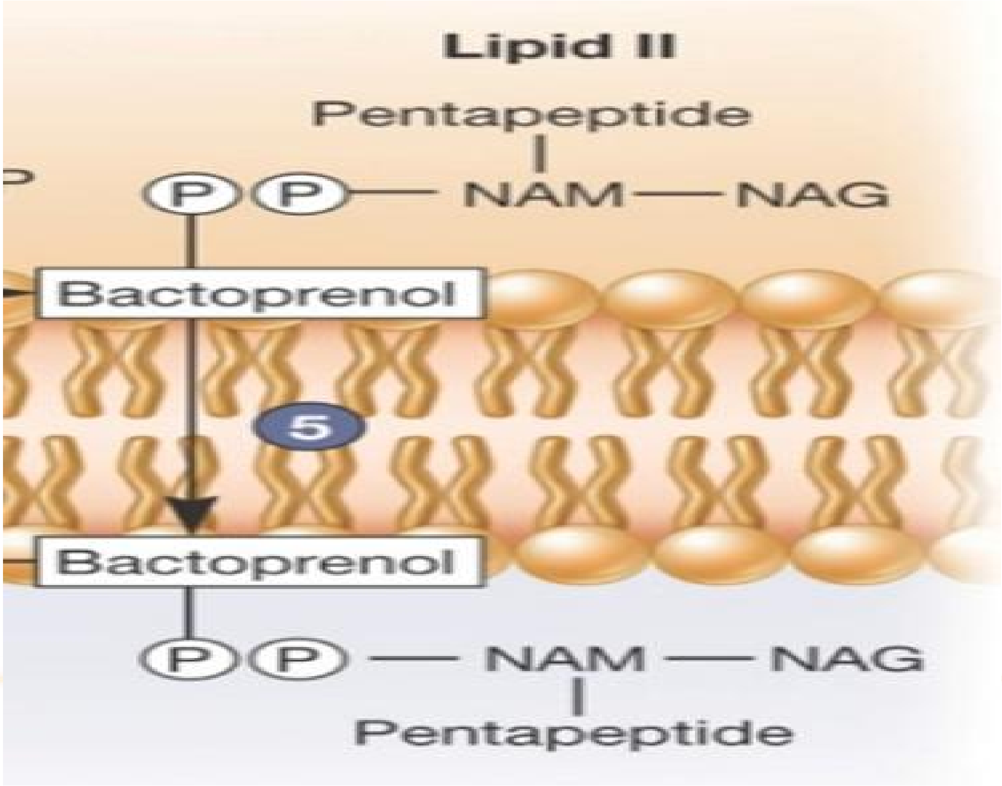
Bactoprenol is attached to N-acetylmuramic acid (NAM)

3 NAM-pentapeptide is transferred to bactoprenol phosphate. They are joined by a pyrophosphate bond.

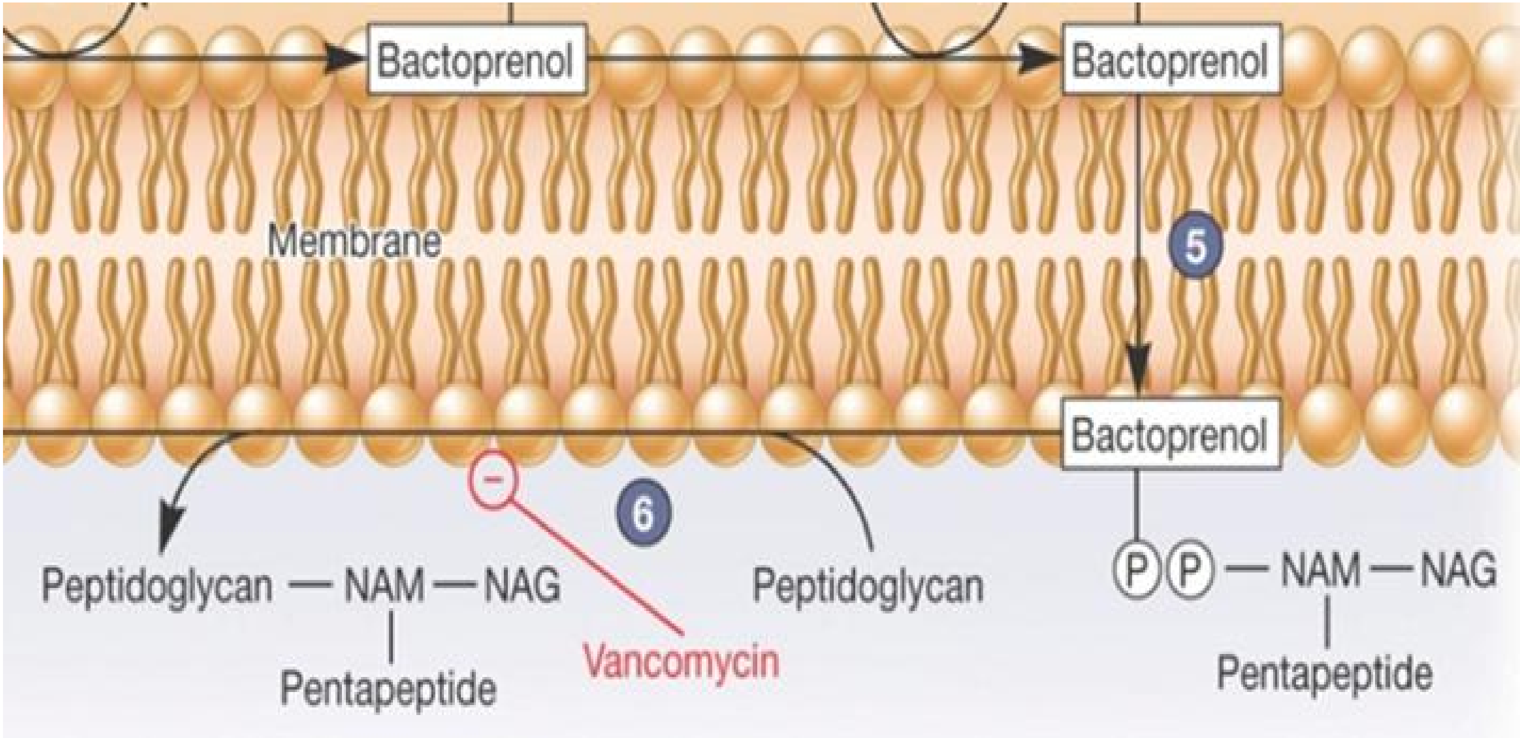
4 UDP transfers NAG to the bactoprenol-NAM-pentapeptide. If a pentaglycine interbridge is required, it is created using special glycyl-tRNA molecules but not ribosomes. Interbridge formation occurs in the membrane.



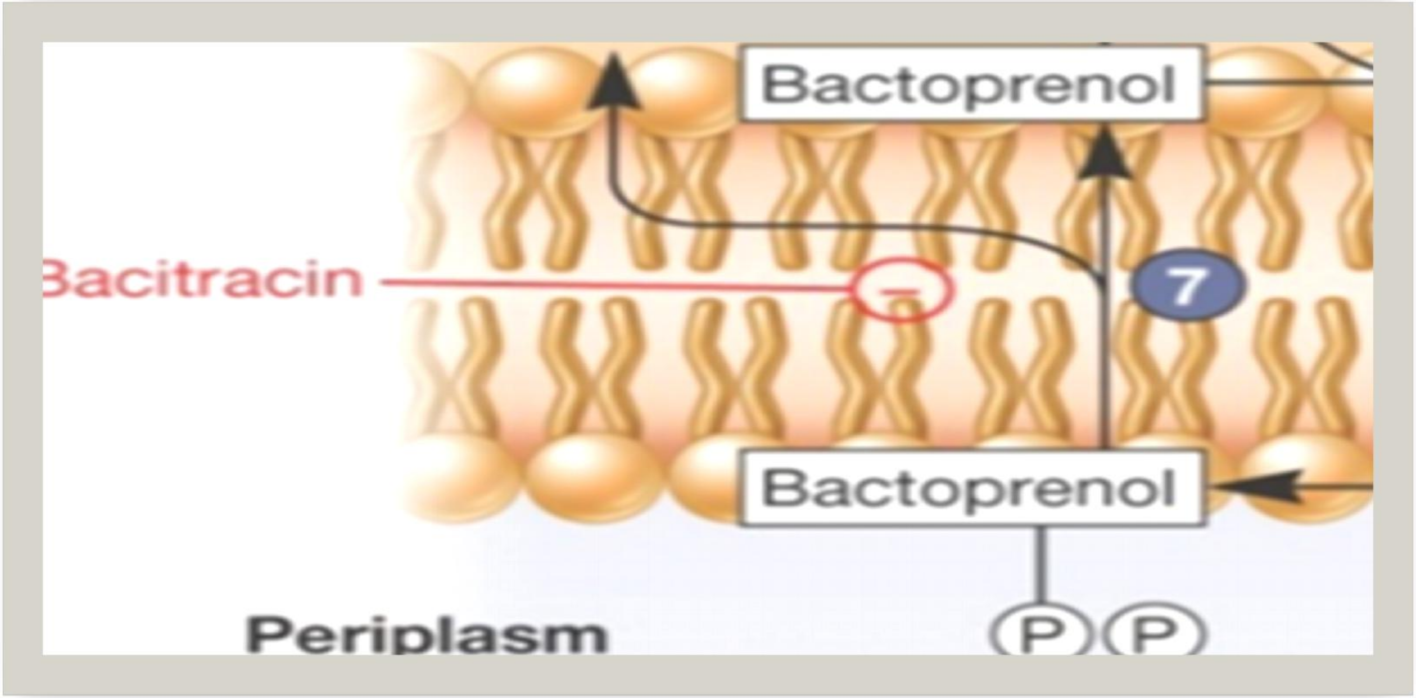
（5）The completed NAM-NAG peptidoglycan repeat unit is transported across the membrane



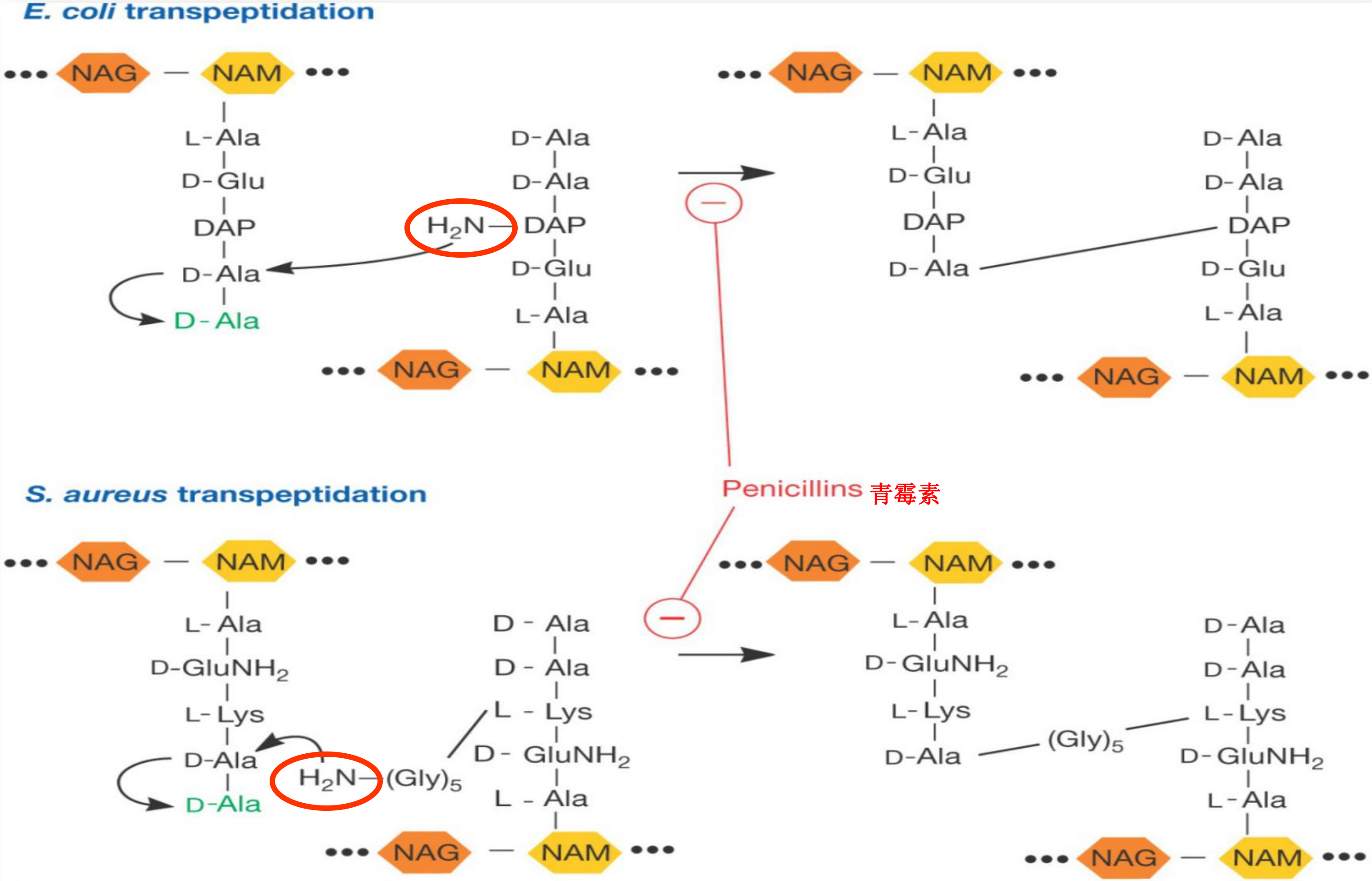
（6）The peptidoglycan unit is attached to the growing end of a peptidoglycan chain to lengthen it by one repeat unit.

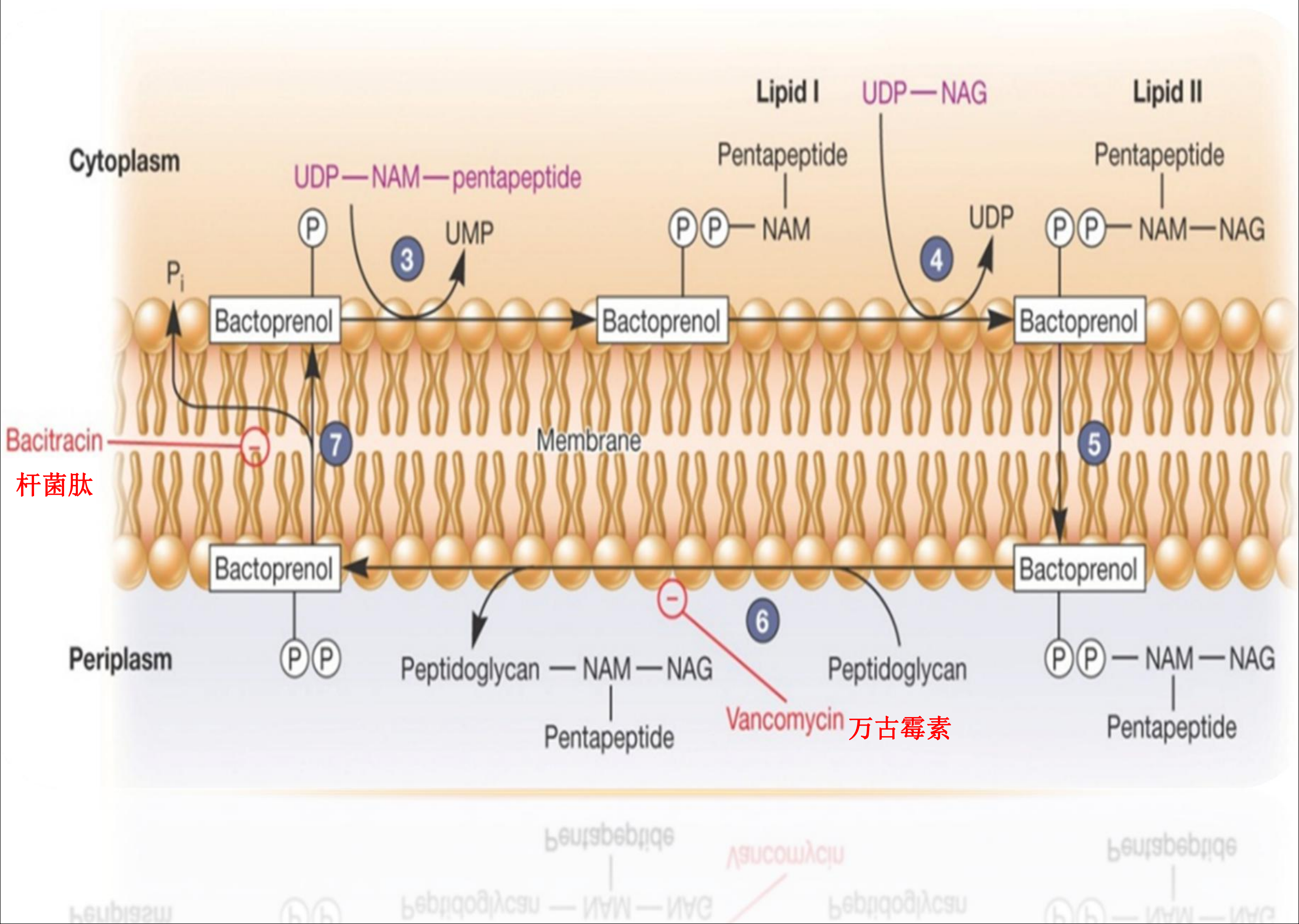


（7）The bactoprenol carrier returns to the inside of the membrane. A phosphate is released during this process to give bactoprenol phosphate, which can now accept another NAM-pentapeptide.



Finally, peptide cross-links between the peptidoglycan chains are formed by transpeptidation（转肽作用）




Cytoplasm
UDP—NAM—pentapeptide
P
UMP
3
Pi
Lipid I
Pentapeptide
P P — NAM
UDP—NAG
UDP
4
Lipid II
Pentapeptide
P P — NAM—NAG
Bactoprenol
Bactoprenol
Bactoprenol
Membrane
Bacitracin
杆菌肽
7
5
Bactoprenol
Bactoprenol
6
Periplasm
P P
Peptidoglycan — NAM — NAG
Pentapeptide
Vancomycin
万古霉素
Peptidoglycan
P P — NAM — NAG
Pentapeptide

Discussion

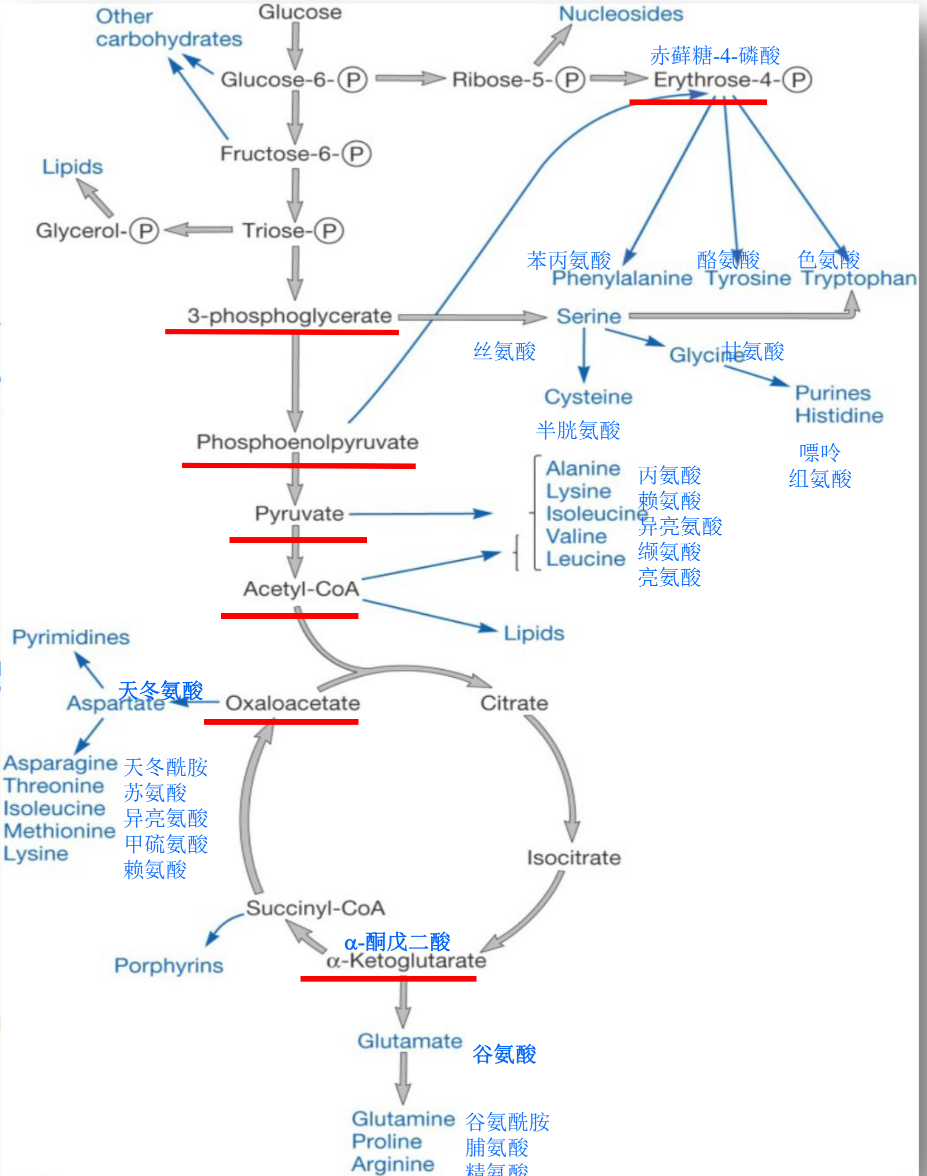
Intermediary carriers are in a limited supply—when they cannot be recycled because of a metabolic block, serious consequences ensue. Think of some examples of these consequences.

Control of cell wall formation

- Autolysins自溶素
  - carry out limited digestion of peptidoglycan
  - activity allows new material to be added to wall and division to occur
- inhibition of peptidoglycan synthesis can weaken cell wall and lead to lysis
- many commonly used antibiotics inhibit cell wall formation.

The synthesis of

- many precursor metabolites are u as starting substr for synthesis of amino acids
  - carbon skeleton is remodeled
  - amino group and sometimes sulfur added



Nitrogen Assimilation 氮的同化

- Nitrogen氮 is a major component of proteins, nucleic acids, coenzymes, and many other cell constituents.
- Few microorganisms can reduce nitrogen gas氮气 and use it as a nitrogen source.
- Most must incorporate同化 either ammonia氨 or nitrate硝酸盐.

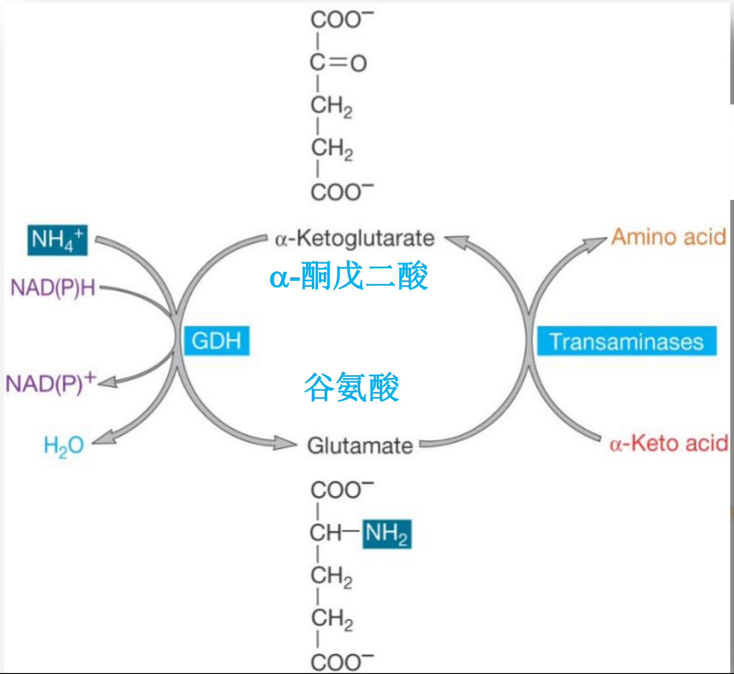
Ammonia incorporation into carbon skeletons

氨的掺入

- ammonia N can be directly assimilated by
  - glutamate dehydrogenase(GDH)谷氨酸脱氢酶

NADPH- or NADH-dependent

–high NH_3

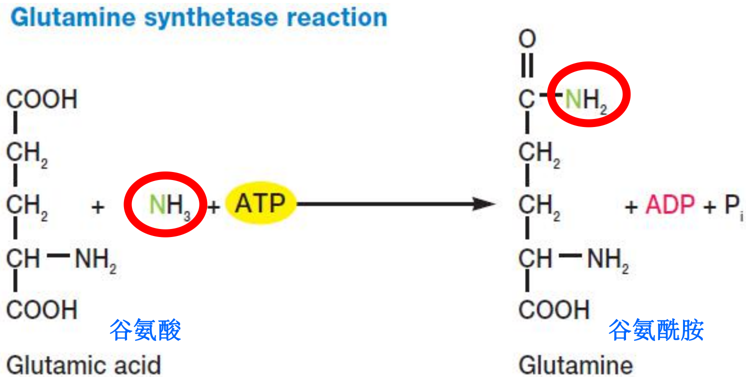


$$\alpha\text{-ketoglutarate} + NH_4^+ + NADPH\ (NADH) + H^+ \rightleftharpoons \text{glutamate} + NADP^+\ (NAD^+) + H_2O$$

α-amino group can be transferred to other carbon skeletons by enzymes called **transaminases**.

In many bacteria and fungi.

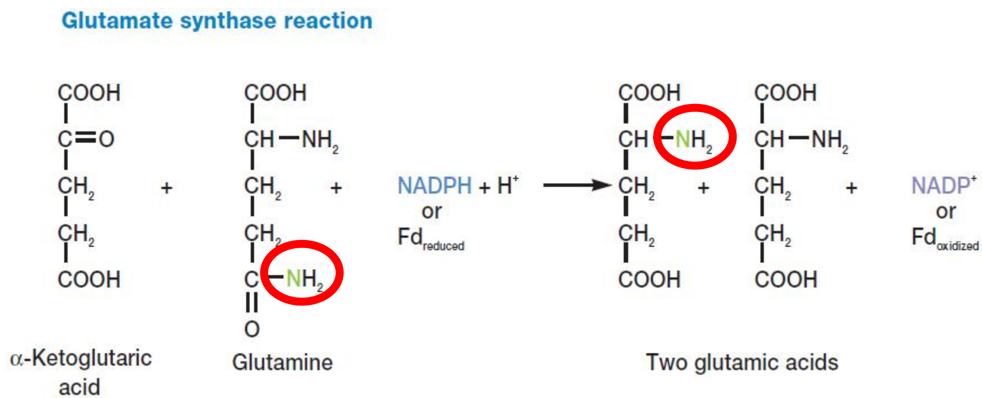
- **glutamine synthetase谷氨酰胺合成酶-glutamate -synthase谷氨酸合成酶systems**
  - **– low NH_3**



An amino donor

Both ATP and a source of electrons, NADPH or reduced ferredoxin, are required.

in *Escherichia coli, Bacillus megaterium*, and other bacteria.

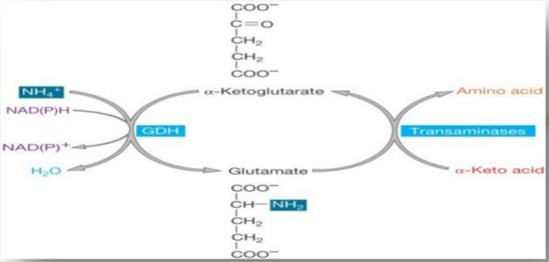


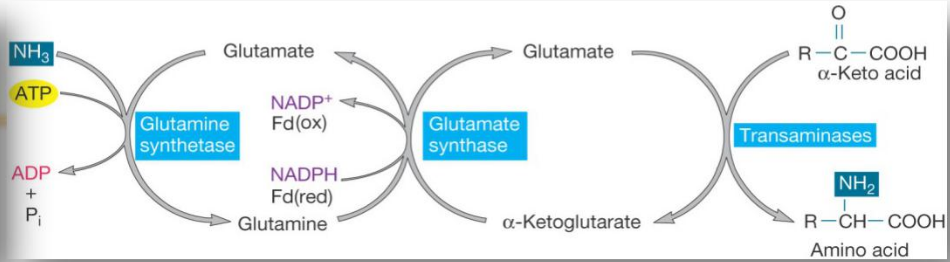
transaminase 转氨酶 activity

- once incorporated, nitrogen can be transferred to other carbon skeletons by transaminases
- Microorganisms have a number of transaminases

Where is NH_3 come from?

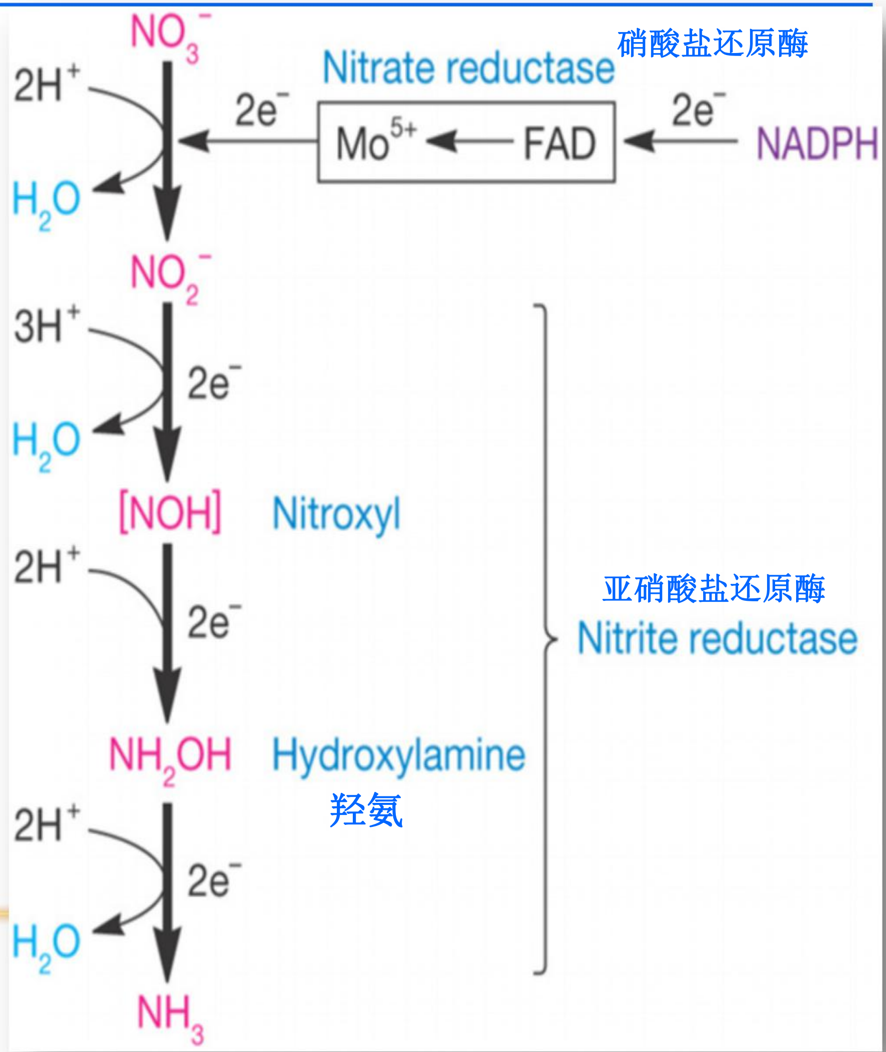
- ammonia may be used to synthesize all common amino acids when suitable transaminases are present.



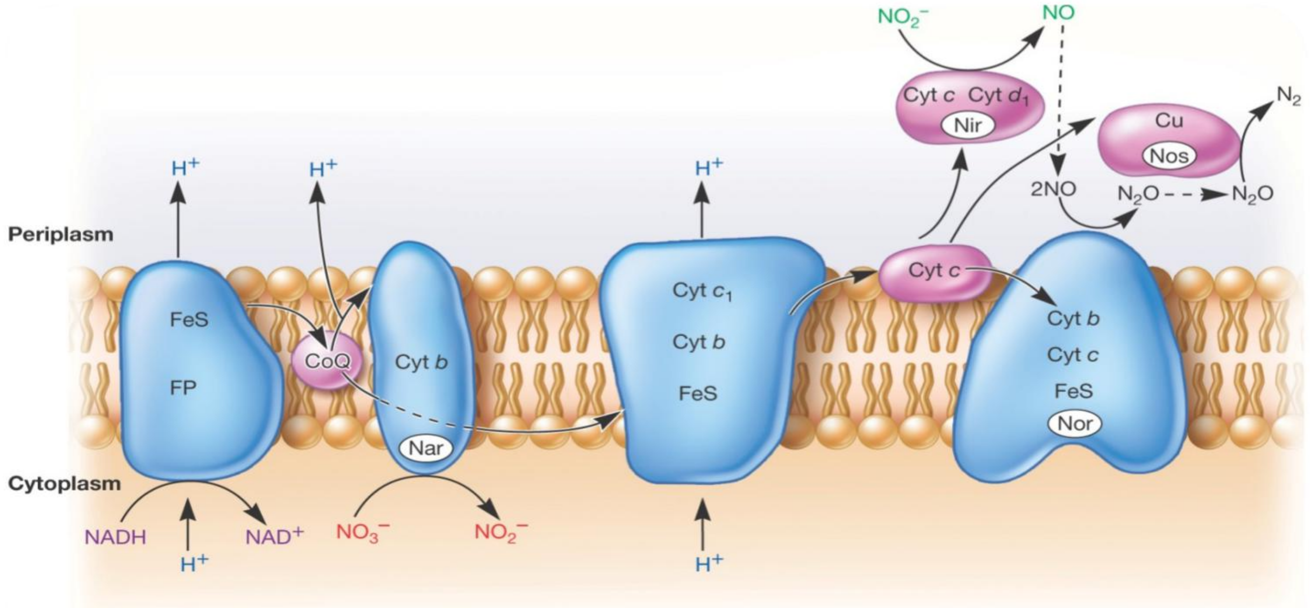


Assimilatory Nitrate Reduction硝酸盐同化还原

- used by bacteria to reduce nitrate to ammonia and then incorporate it into an organic form
- nitrate reduction to nitrite catalyzed by nitrate reductase
- reduction of nitrite to ammonia catalyzed by nitrite reductase



Is it similar to dissimilatory nitate reduction in anaerobic respiration?



dissimilatory nitrate reduction

Nitrogen fixation- from N_2 to NH_3

- The reduction of atmospheric gaseous nitrogen to ammonia
- only a few prokaryotes can carry out nitrogen fixation
- catalyzed by the enzyme nitrogenase固氮酶

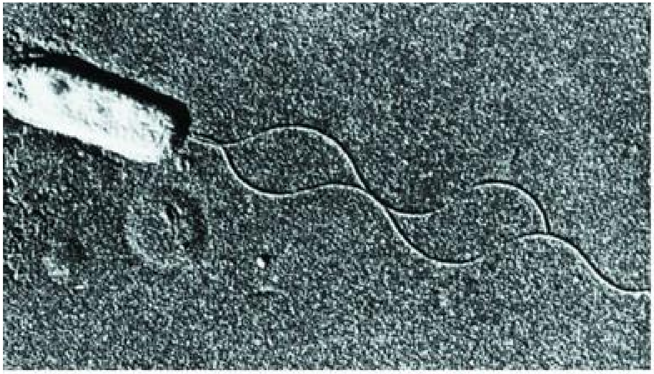
Nitrogen fixation- from N_2 to NH_3

A few prokaryotic microbe engaged in N_2 fixation

- **Free living chemotrophic bacteria and archaea(*Azotobacter, clostridium*, and *Methanococcus*)**自生固氮菌

共生固氮菌

- **Symbiotic associated with plant (*Rhizobium*)**
- **Cyanobacteria(*Nostoc*念珠蓝细菌, *Anabaena*鱼腥蓝细菌, and *Trichodesmium*束毛蓝细菌)**



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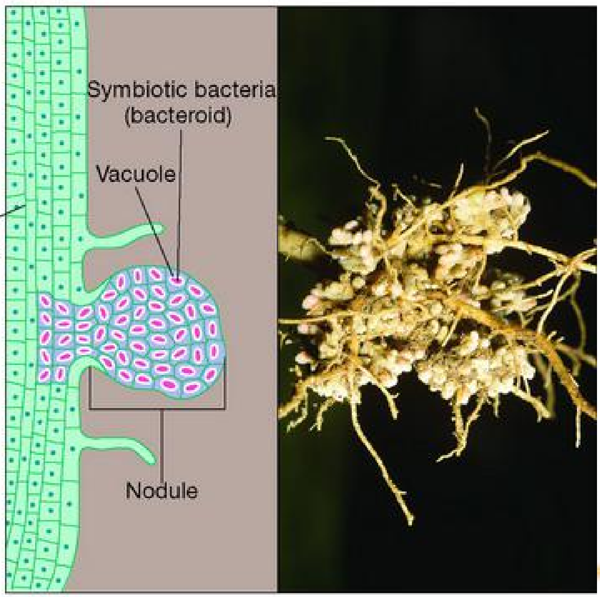
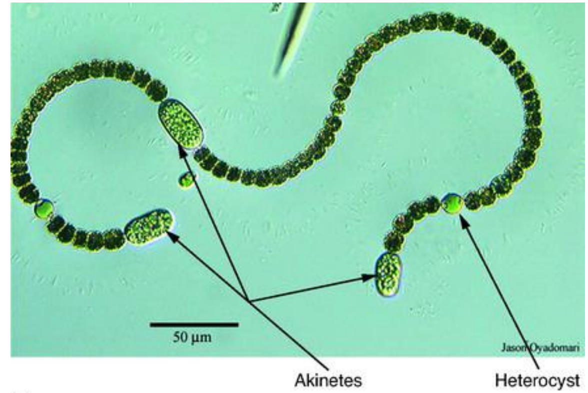
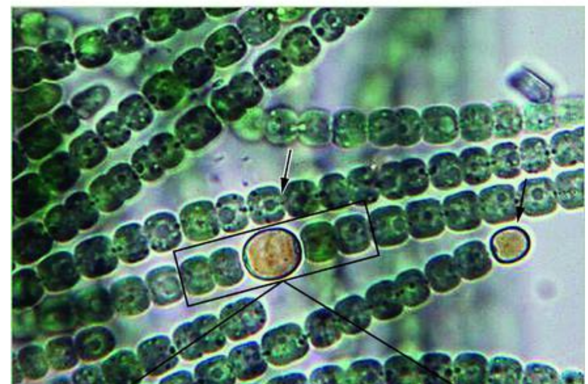


(b) (c)

Rhizobium



(a)



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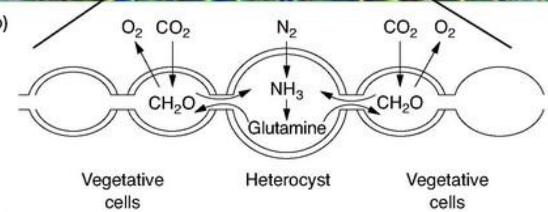


(c)

异形胞

Anabaena, with heterocysts

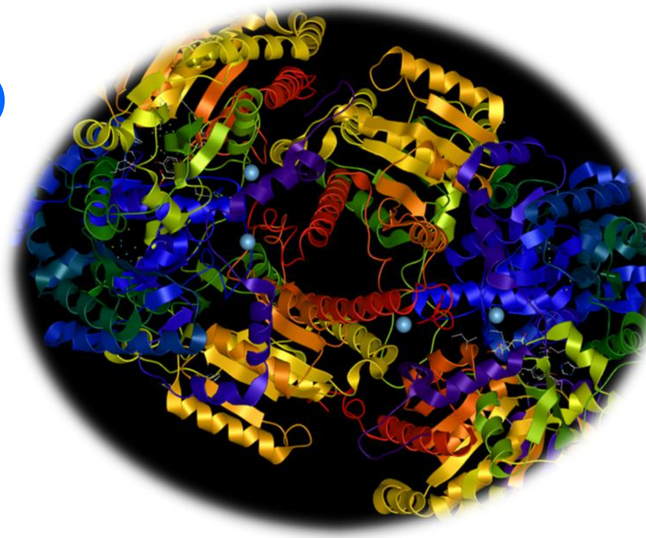
Catalyzed by nitrogenase (Mo-Fe-P+Fe-P)

*Nitrogenase consisting of two major protein components

钼铁蛋白

MoFe protein (MW 220,000)

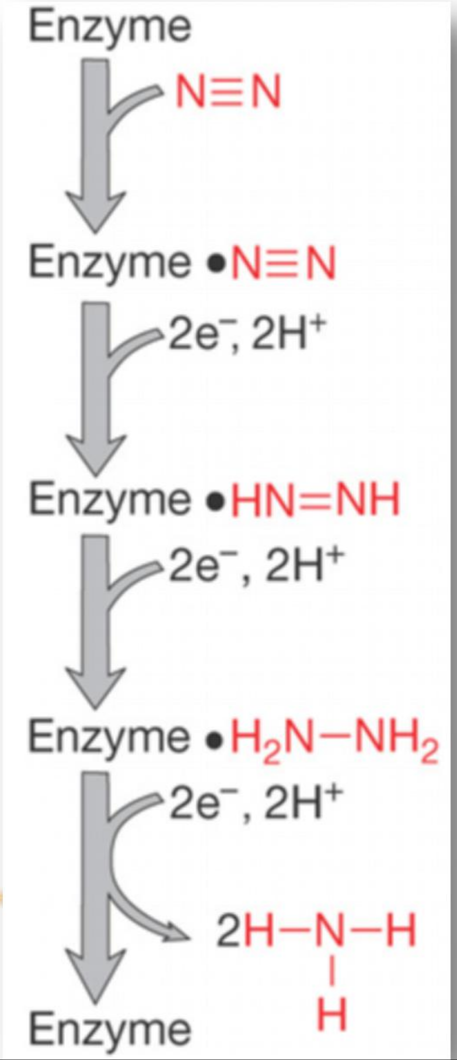
Fe proteins (MW 64,000)



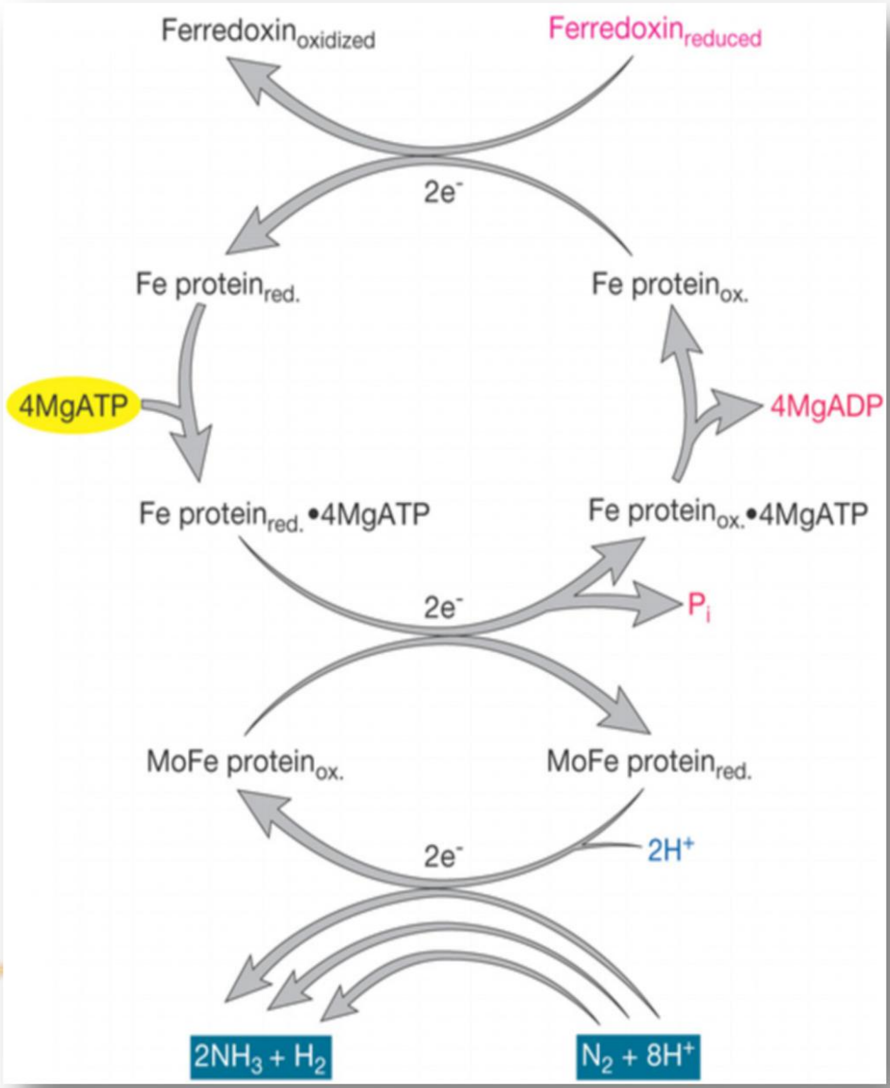
*Nitrogenase is quite sensitive to O_2

Mechanism of Nitrogenase Activity

- The reduction of molecular nitrogen to ammonia
- requires large ATP expenditure
- once reduced, NH_3 can be incorporated into organic compounds



* The reduction of N_2 to NH_3 occurs in three steps, each of which requires an electron pair.
* Ferridoxin is used as the electron donor.
* This process is repeated three times in order to reduce N_2 to two molecules of ammonia.
* reduction of protons to H_2



Summary of Nitrogen fixation

$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

Nitrogen reduction is expensive and requires a large ATP expenditure. Consume almost 20% of the ATP produced by the host plant.

Discussion

Nitrogenase is quite sensitive to O_2 and must be protected from O_2 inactivation within the cell. But Why the most of N fixation bacteria are aerobic?

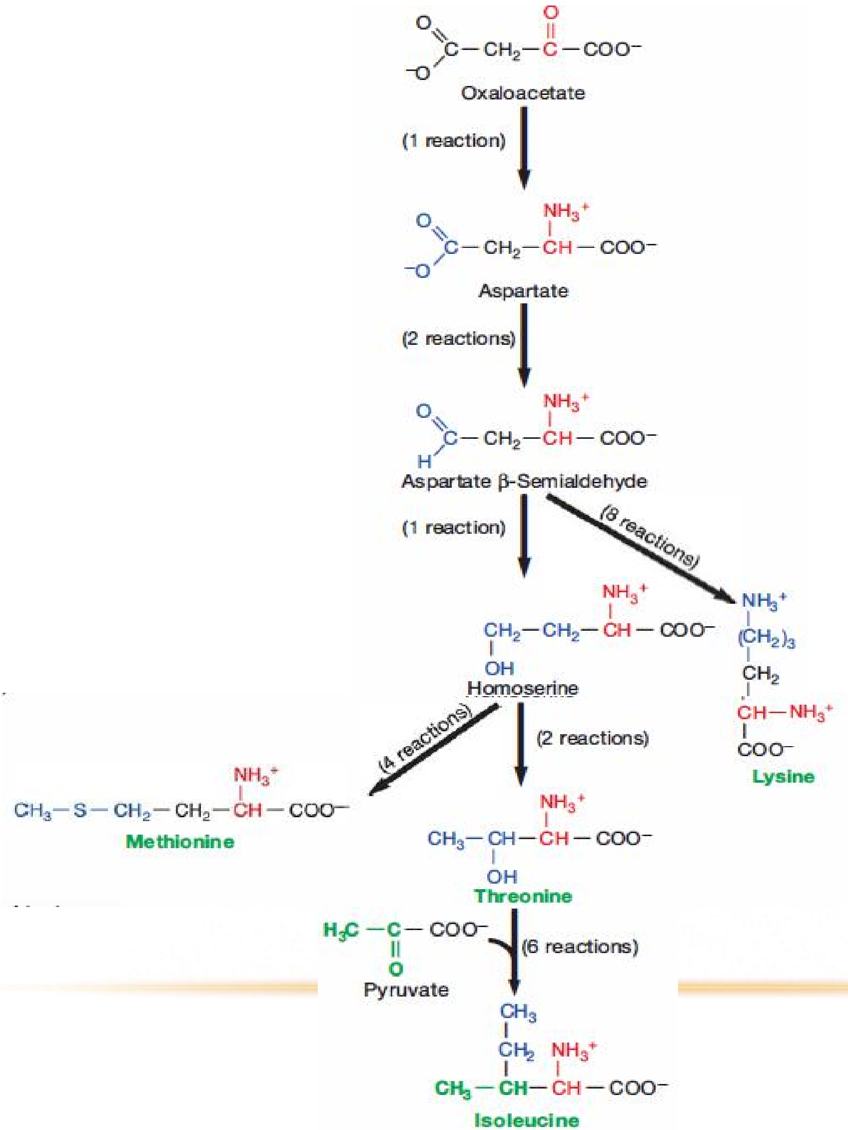
Discussion

Amino acid skeletons are derived from acetyl-CoA and from intermediates of the TCA cycle, glycolysis, and the pentose phosphate pathway.
How To maximize efficiency and economy in amino acid biosynthesis?

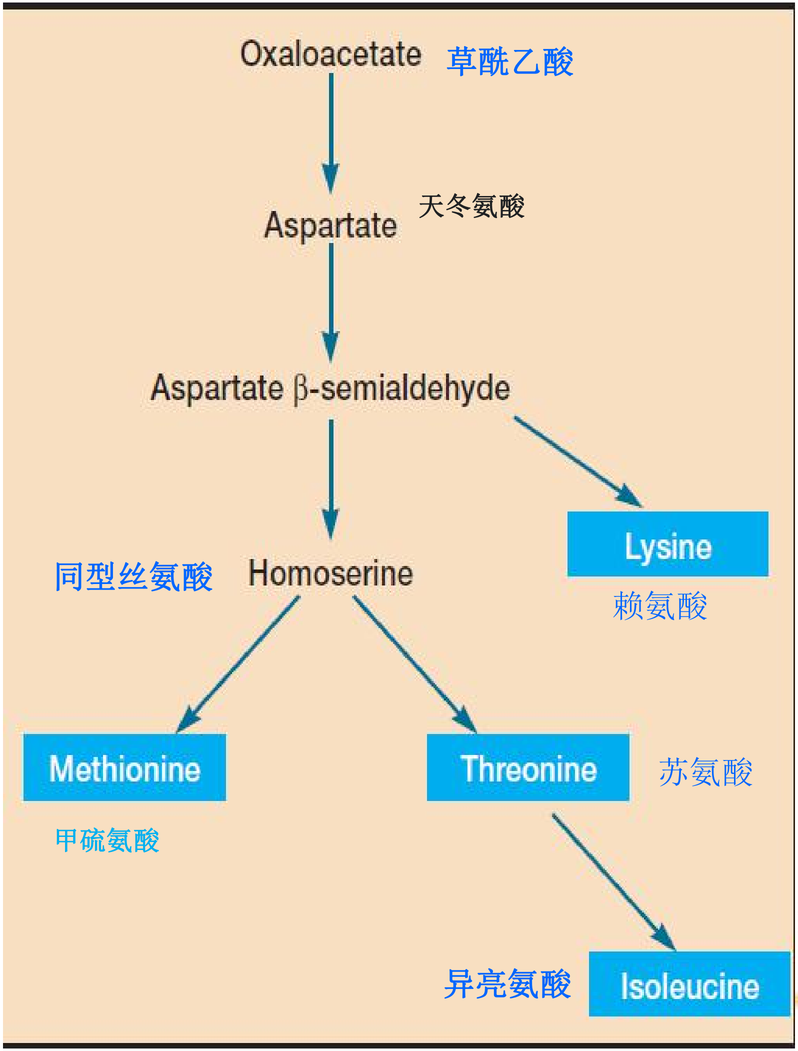
Amino acid biosynthesis –
branching pathways

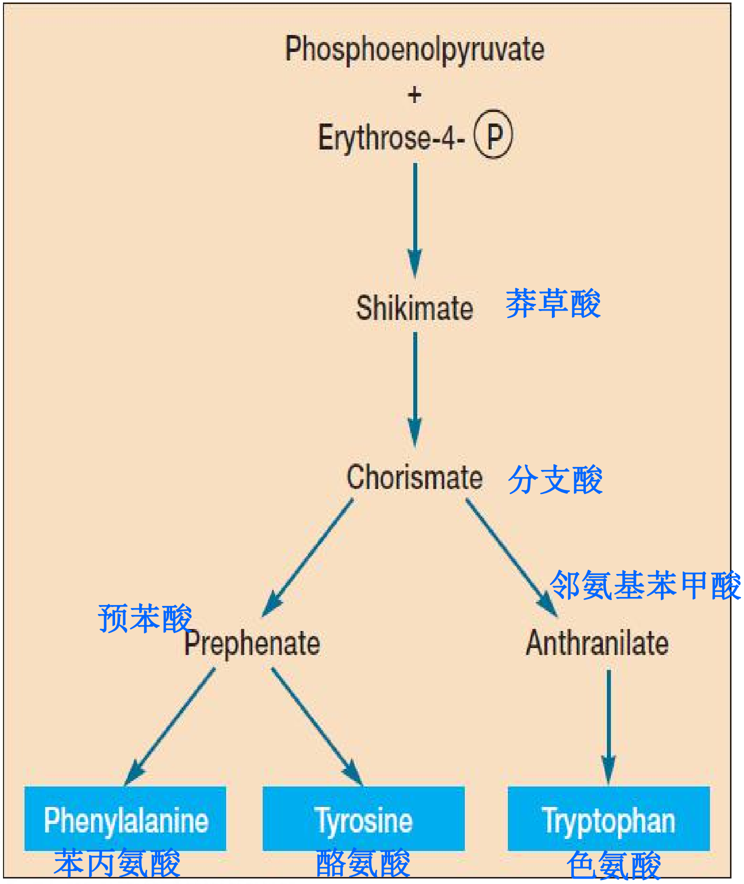
- used in the synthesis of multiple amino acids
- a single precursor metabolite can give rise to several amino acids
- biosynthetic pathways for aromatic amino acids also share intermediates

共同使用许多中间体



Oxaloacetate
(1 reaction)
Aspartate
(2 reactions)
Aspartate β-Semialdehyde
(1 reaction)
(8 reactions)
Homoserine
Lysine
(4 reactions)
(2 reactions)
Methionine
Threonine
Pyruvate
(6 reactions)
Isoleucine



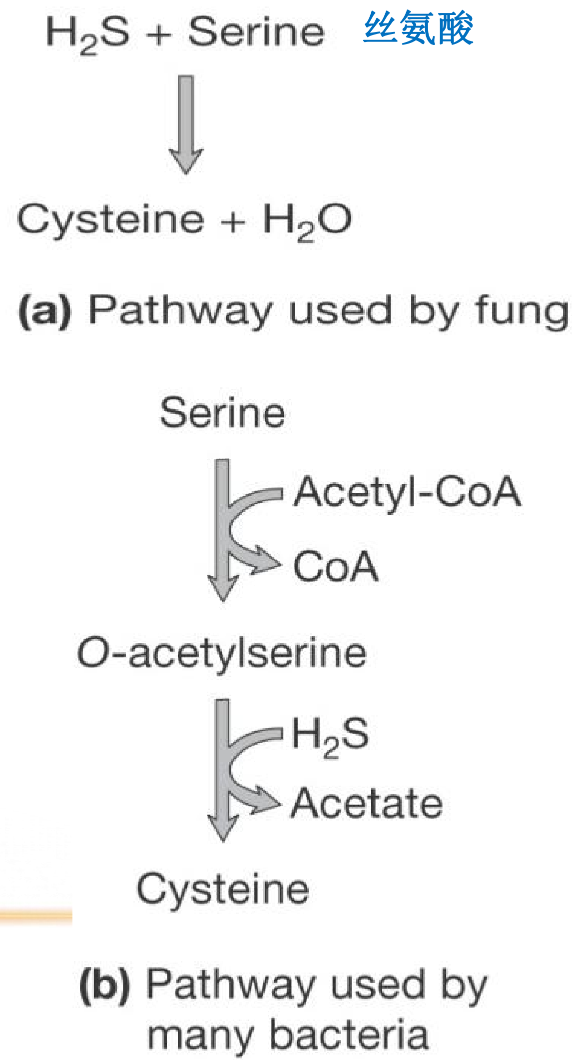


芳香族氨基酸合成

氨基酸合成的分支途径

Use of sulfate as a sulfur source

- sulfate = inorganic sulfur source
  - assimilatory sulfate reduction硫酸盐同化还原
    - sulfate reduced to H_2S and then used to synthesize cysteine半胱氨酸
    - cysteine can then be used to form sulfur containing organic



SUMMARY

- Building blocks synthesis
- Sugar-carbon fixation
- amino acids –nitrogen fixation

- Macromolecular synthesis
- Peptidoglycan

Thanks!

Discussion

1. Can Heterotrophs carry out the fixation of CO_2? If yes, why and how do they fix CO_2?

2. Describe the patterns of peptidoglycan synthesis seen in gram-positive cocci and in rod-shaped bacteria such as *E. coli*. What is unusual about the synthesis of peptides that takes place during peptidoglycan construction?

3. Intermediary carriers are in a limited supply—when they cannot be recycled because of a metabolic block, serious consequences ensue. Think of some examples of these consequences.

4. Nitrogenase is quite sensitive to O_2 and must be protected from O_2 inactivation within the cell. But Why the most of N fixation bacteria are aerobic?

5. Amino acid skeletons are derived from acetyl-CoA and from intermediates of the TCA cycle, glycolysis, and the pentose phosphate pathway.

How To maximize efficiency and economy in amino acid biosynthesis?