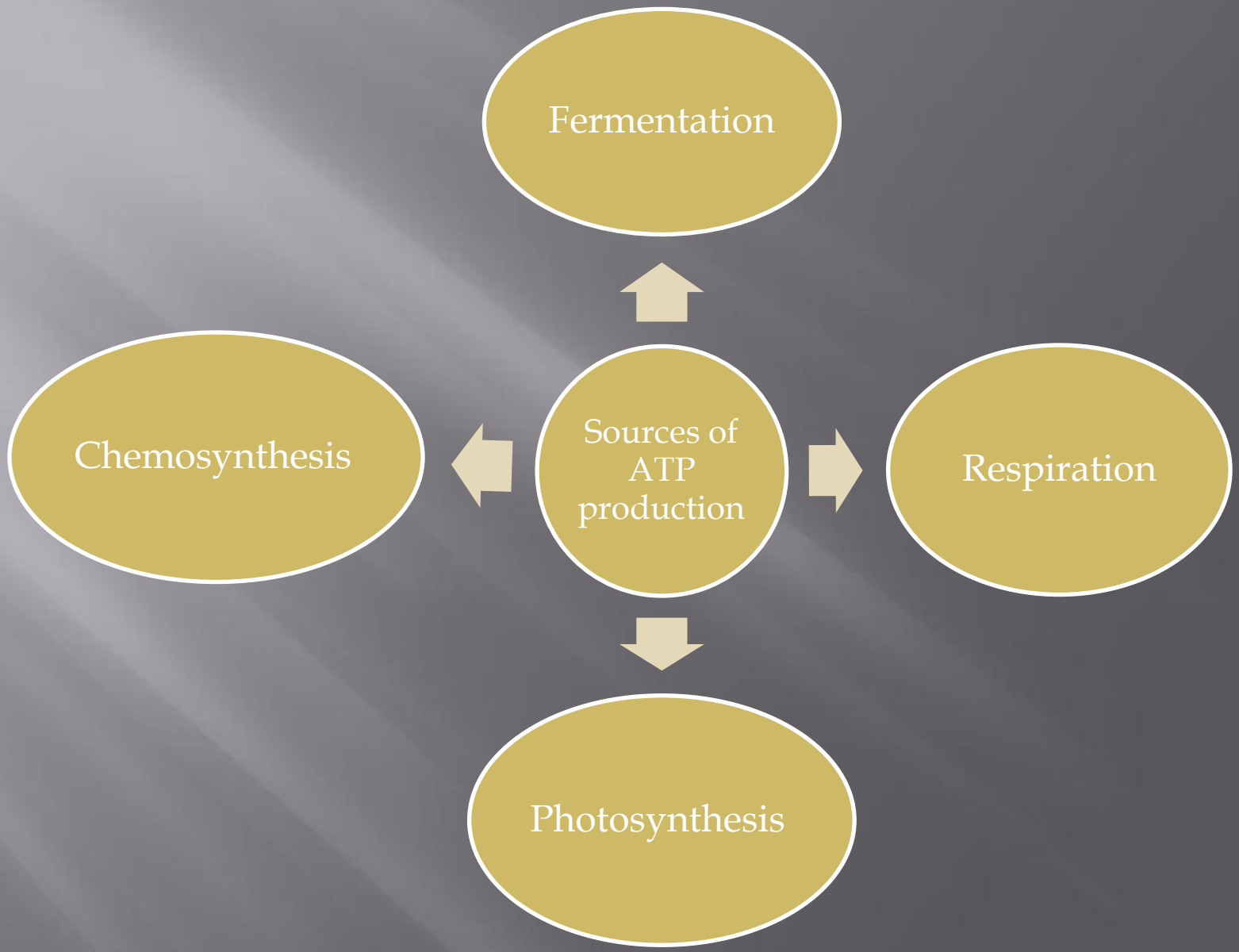
MICROBIAL BIOCHEMISTRY (COURSE 42)

Eukaryotes & prokaryotes differences

Differences	Eukaryotes	prokaryotes
Examples	Cells of higher organisms like plants & animals Protozoa, fungi & most algae	Cells of lower organisms like all bacteria & small group of blue green algae
Nucleus	Present with nuclear membrane & multiple chromosomes	Single naked chromosome
Membrane bound organelles	Present	Absent
steroids	Present	Absent
Muramate & diaminoplmelate in the cell wall	Absent	Present
Specialized properties • Carbon storage • Nitrogen fixation • Obligate anaerobiosis	Absent	Present

- Anaerobiosis is a mode of photosynthesis that doesn't release O2 and drives energy from oxidation of inorganic compounds

Energy Production (ATP synthesis)


Fermentation
Chemosynthesis
Sources of ATP production
Respiration
Photosynthesis

Fermentation

=

Energy yielding rearrangement of the atoms of substrates without next oxidation

+

It yields various organic compounds in large amounts

Respiration

It is the net oxidation of substrates at the expense of molecular oxygen

Complete respiration yields only CO_2 & H_2O and several times as much ATP per mole of substrate

Fermentation

- The term fermentation is extended to a process catalyzed by microbes by which metabolic energy is derived

- It is a mechanism in which O2 is replaced by another inorganic electron acceptor, thus fermentation require anaerobic condition

- The anaerobic phase of carbohydrate metabolism is referred to glycolysis, the initial pathway in carbohydrate metabolism

- Anaerobes: microorganisms can derive all of their metabolic energy from glycolysis which occur in cytosol, thus the action of bacteria on carbohydrates is the fermentation

Glycolysis

- Definition: it is the conversion of glucose or glycogen to lactate or ethanol

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Biological importance of glycolysis

1. It is the major source of energy in the form of ATP molecules
2. It is the major source of energy in certain organisms that are devoid of mitochondria as in prokaryotes
3. It is the major source of pyruvic acid & lactic acid
4. It is an important source of glucose via reversal of glycolysis
5. It links carbohydrate metabolism with lipid metabolism & amino acid metabolism

Phases of glycolysis

1- energy investment

(5 reactions)

2- energy generation

(5 reactions)

Glycolysis

Energy yield from glycolysis

In energy investment phase

- 2 moles of ATP are lost:

1. One in the first reaction(activation of glucose)
2. The second in reaction 3 (activation of fructose-6-phosphate)

- These 2 reaction are irreversible

In energy generation phase

- Total 4 moles of ATP are gained
- 2 ATP moles are gained from reaction 7 (from 1,3 bisphosphoglycerate by phosphoglycerate kinase)
- Other 2 ATP moles are gained from reaction 10 (from phosphoenol pyruvate via pyruvate kinase)

- Thus the net yield of ATP is 2 ATP moles per one molecule of glucose when oxidized anaerobically
- But under aerobic condition the 2 NADH+H are oxidized through electron transport give 6 ATP
- Thus the net energy yield in glycolytic pathway are 8 ATP moles per one glucose molecule

- Under anaerobic condition (absence of oxygen) the reduced NAD must be reoxidized to allow glycolysis to continue
- This occurs by

Reduction of pyruvate to lactate via lactate dehydrogenase

Alternative fates of pyruvate

Alternative fates of pyruvate

- Since the total NAD in the cell is very limited, fermentation would cease very rapidly if the NADH+H weren't reoxidized in the further metabolism of pyruvate

- Microbes have evolved a variety of pathways for this purpose, some of which also yield additional ATP

- A variety of fermentations, yielding quite different products, are based on the glycolytic pathway. These are as follows:

1. Lactic: *Streptococcus Lactobacillus*
2. Alcoholic: many yeasts, few bacteria
3. Mixed acid: most *Enterobacteriaceae*
4. Butanediol: *Aerobacter*
5. Butyric: Clostridium
6. Propionic: *Probionibacterium*

1- Lactic fermentation

- It is one step reaction catalyzed by NAD linked lactic dehydrognase, reduces pyruvate to lactate
- This fermentation is specific for *streptococcus lactobacillus* & is identical with the glycolysis in mammalian cells

- This type of fermentation is also called homolactic fermentation because it forms only lactate
- It is also characteristic of *Lactobacillus casei* and *Streptococcus cremoris*
- It also occurs in certain pathogens like *Pneumococci* which are included by taxonomists among the lactic bacteria

2- Alcoholic fermentation

- This fermentation is characteristic of yeast
- Its value in the leavening of bread as well as in the fermentation of beverages

3- Mixed acid fermentation

- It is the conversion of pyruvate by *Enterobacteria,* including *E.coli* into acetyl coA & ethanol and either formic acid or CO2 & H2

4- Butanediol fermentation (Acetoin fermentation)

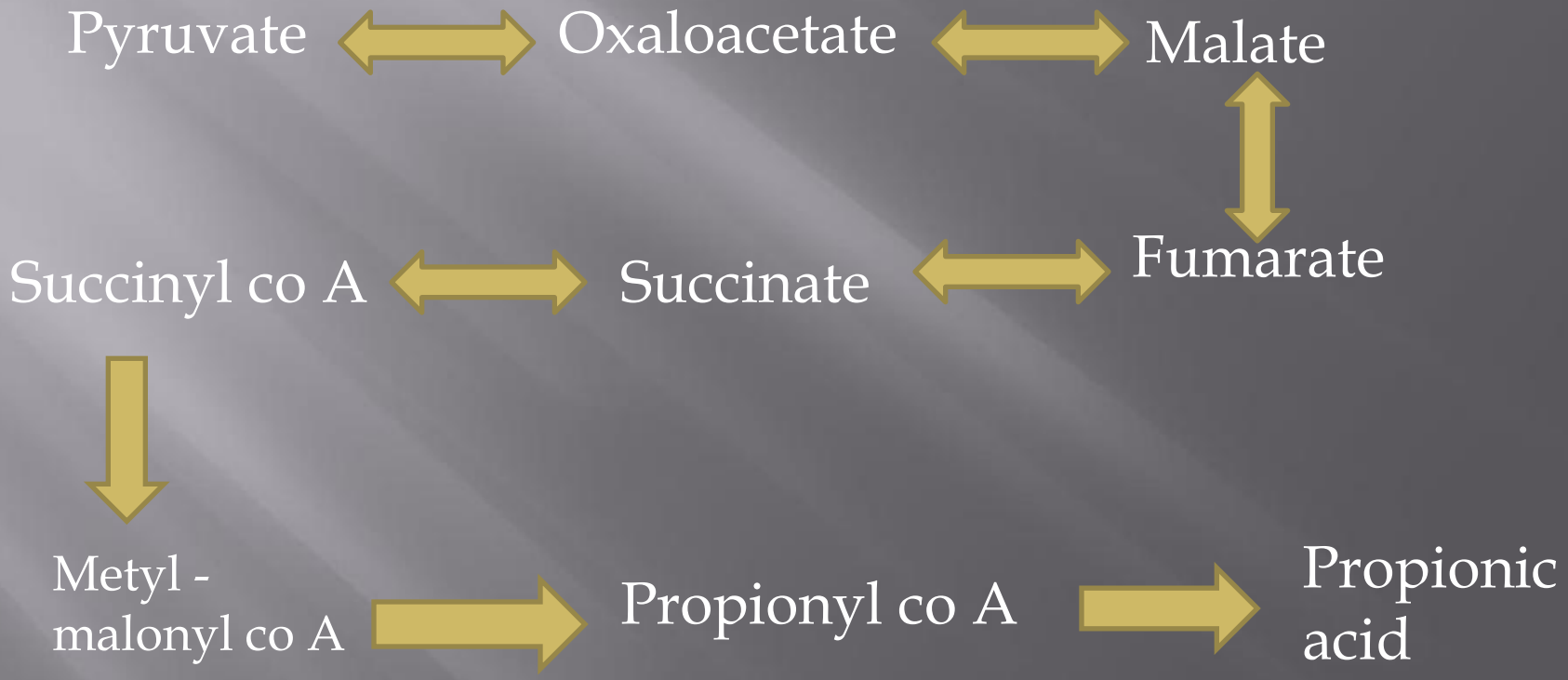
- This pattern of fermentation is observed in *Aerobacter,* certain other *Enterobacteriaceae* & some species of *Bacillus*

5- Butyric fermentation

- This fermentation is carried out by bacteria of the genus Clostridium and by the rumen organisms *Eubacterium* (*Butyribacterium*) & *Butyrivibrio*

- Bacteria of genus clostridium contain the ferredoxin depenedent hydrogenase that is needed for this interconversion

6- Propionic fermentation



- Propionic acid producing bacteria are especially numerous in the digestive tract of ruminant
- The carboxylation of pyruvate to oxaloacetate requires ATP
- The propionic acid bacteria save one equivalent of ATP by using carboxytransferase

- A second molecule of ATP is saved by linking directly the conversion of succinate to succinyl coA to the cleavage of propionyl coA to propionate
- Note that more carboxyl groups and CO2 molecules are formed in this fermentation than in the regular lactic acid fermentation
- The net gain is one molecule of ATP

Question

- What is the biological significance of fermentation

- Write on the metabolic pathways of pyruvate in the following microorganisms

1. *Streptococcus cremoris*
2. *Enterobacteria*
3. *Clostridium*

- Enumerate the various types of fermentation in different bacteria
- Compare between butyric & propionic fermentation

Respiration

- Bacteria fall into several groups with respect to the effect of oxygen on their growth & metabolism
- Obligate aerobes: e.g. *Tubercle bacillus* and some spore forming bacilli require oxygen but lack fermentation
- Obligate anaerobes: can grow only in the absence of oxygen e.g. *Clostridia* & *Propionibacteria*

- Facultative organisms: can grow with or without air and they shift in its presence to a respiratory metabolism e.g. many yeasts & *enterobacteria*

- Aerotolerant anaerobes: resemble facultative but their metabolism remains fermentative e.g. most lactic acid bacteria

- Obligate aerobes & facultative organisms adapted to a respiratory metabolism contain a complete electron transport chain in which electrons flow from NADH+H (or directly from a few substrates such as succinate or lactate) to a flavoprotein & then via several cytochromes to oxygen

- ATP is generated in the accompanying process of oxidative phosphorylation

- Most cytochromes are absent from strictly fermentative organisms
- Some of fermentative organisms can carry out a limited respiration e.g. lactobacillus delbrueckie can oxidize glucose through flavoprotein which in turn is directly reoxidized by air
- This short respiratory chain yields little or not ATP but it may serve to scavenge dissolved oxygen & thus to make anaerobic growth possible

Electron transport chain (respiratory chain)

- In bacteria, like higher organisms the oxidation of glucose depends on the conversion of pyruvate to acetyl coA & Co2
- Acetyl coA is then oxidized via citric acid cycle to Co2 &H2O to give energy
- Each oxidative reaction in this cycle is accomplished at the expense of the reduction of NADH+H (except that succinate is coupled directly to reduction of a flavoprotein)

Site of respiratory chain

- It occurs in mitochondria which is composed of 2 membranes , outer membrane & inner with a space filled with water fluid in between
- The surface of both membranes are sprinkled with thousands of small particles
- The outer particles are concerned with the oxidation reactions that supply hydrogen (electrons) from citric acid cycle or/and fatty acid oxidation

- These electrons are then taken by carriers (as coenzymes NAD or FAD) which shuttles them across the liquid filled space between the membranes to the inner particles

- Inner particles are concerned with all steps of respiratory chain where electrons are transferred in several steps ending by oxidation with molecular oxygen to form water

- There is a problem in the mitochondrial membrane itself which is impermeable to NADH

- This problem is solved by 2 shuttle system which are responsible for entry the electrons from external cytosol into internal mitochondria

- In the electron transport chain, the 2 hydrogen equivalents are transported inside mitochondria in several oxidation- reduction sequences until they are accepted by tne oxygen to form water

- The chain require oxidase & dehydrogenase enzymes (oxido-reductase)
- Synthesis of ATP occurs in the inner particles of mitochondria, hence it is the site of coupling the oxidation with ATP synthesis (oxidative phosphorylation)
- ATP is formed in 3 sites through the respiratory chain starting with NADH, 3 moles of ATP are formed while starting with FAD, only 2 moles of ATP are formed

Question

- Write on the electron transport chain in *tubercle bacillus*

AUTOTROPHIC METABOLISM (PHOTOSYNTHESIS & CHEMOSYNTHESIS)

- The metabolism in animals & plants is heterotrophic (means feeding on others) because these organisms depend on organic compounds as food stuffs

- On other hand, some microbes don't depend on organic compounds but use different sources of energy & reducing power to form organic compounds needed for their growth

- Thus its metabolism is autotrophic (self feeding) metabolism

Autotrophic process can be divided into 2 classes

Photosynthesis

Chemosynthesis

Photosynthesis

- Drives energy and reducing power from the absorption of visible light & is found in algae and a few bacteria as well as in higher plants
- In this process, a quantum of visible light is absorbed by a molecule of chlorophyl which eject an electron
- This electron is accepted by ferredoxin, while the oxidized chlorophyl oxidizes the terminal cytochrome of an electron transport system

- The system functions in 2 different ways:

1. Cyclic photophosphorylation
2. Non cyclic phosphorylation

- In the cyclic photophosphorylation, the electron is transported from the reduced ferrodoxin to the oxidizes cytochrome through a chain of quinones & cytochromes similar to the electron transport chain

CHEMOSYNTHESIS

- This drives energy from the respiration (oxidation) of inorganic electron donors
- It is found only in certain bacteria which are also known as lithotrophs (litho = stone)

- Some of these organisms use O2

- Examples:

1. Hydrogen bacteria
2. Sulphur bacteria
3. Iron bacteria
4. Nitrifying bacteria

- Others use other electron acceptors as following:

1. Denitrifiers
2. Methane bacteria
3. *Clostridium aceticum*

- Some of chemo-autotrophic bacteria play an essential role in the geochemical nitrogen cycle where N is converted to NH3 which is stabilized in the soil by oxidation through the action of nitrifying bacteria to non-volatile nitrate

- This can be reduced by plants to organic amino compounds

Catabolic, anabolic & amphibolic pathways

- Catabolism of glucose in *E.coli* occurs mainly via glycolysis
- Glucose can also pass through an alternative minor pathway which has primarily an anabolic role rather than catabolic
- The major energy-yielding pathways of glucose, pyruvate oxidation & the citric acid cycle were considered purely as catabolic process

- Various specific pathways of biosynthesis originate from various catabolic pathways

- Therefore both glycolysis & citric acid cycle are now designated as amphibolic (amphi= either)

Hexose Monophosphate Shunt (HMS)
Pentose Phosphate Pathway (PPP)

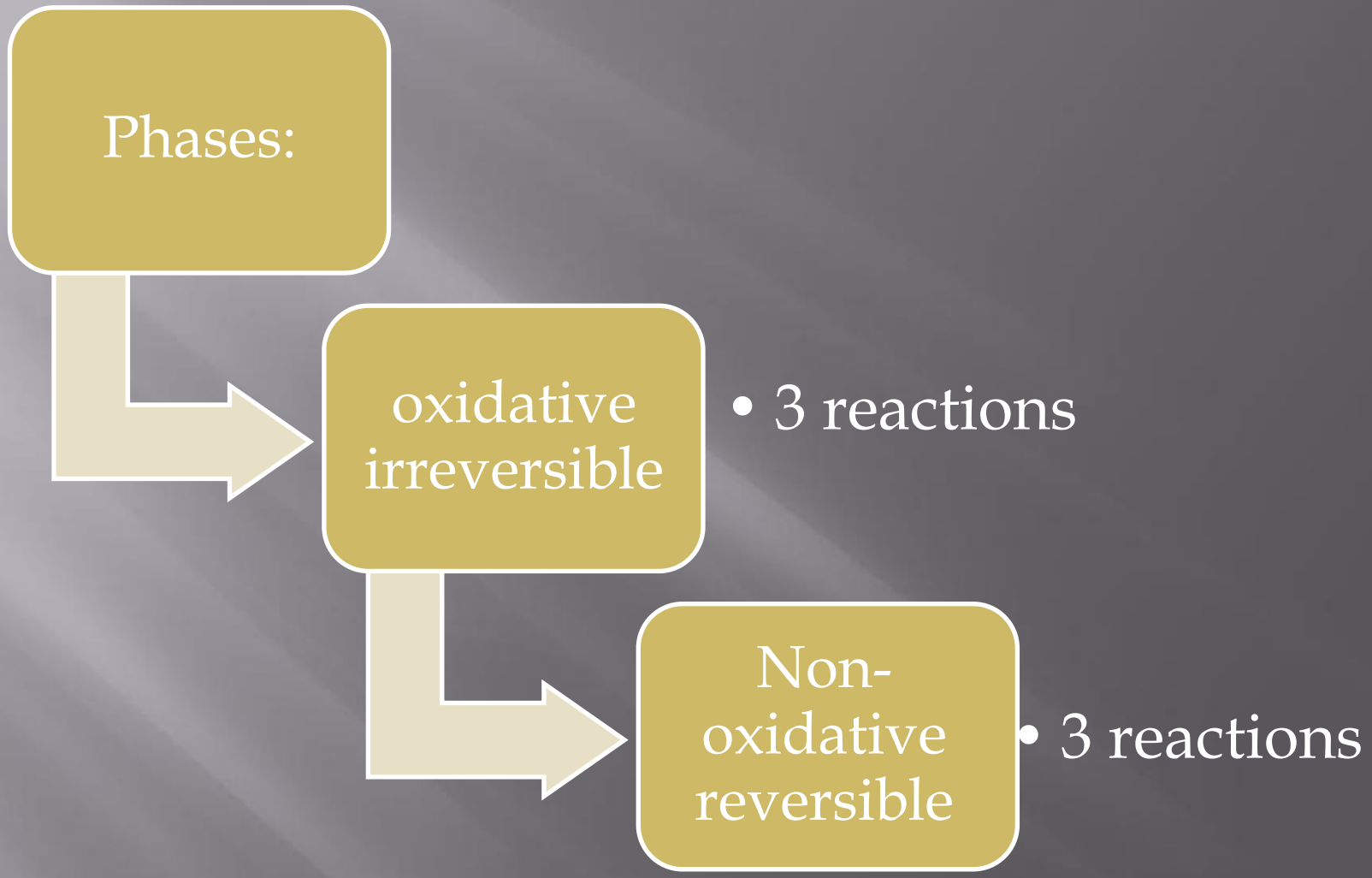
- Site: in cytosol of tissues which are characterized by F.A or steroid synthesis namely adipose tissue, liver, mammary gland, testis, RBCs, adrenal cortex & thyroid

- Function

I. Production of pentoses which are used for synthesis of ATP, NAD, Co A, FAD, DNA & RNA

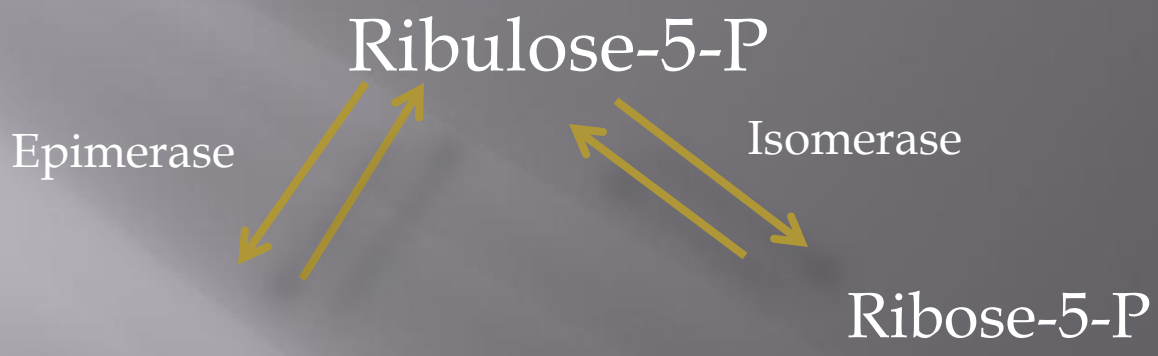
II. Generation of NADPH+H which is required for synthesis of fatty acids, adrenal cortical hormone, cholesterol, RBCs health, glucuronate, non essential a.a, coenzyme for cytochrome P450

III. Complete oxidative degradation of pentoses into hexoses than through glycolysis

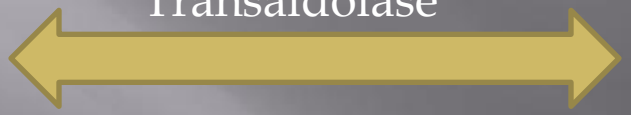

Phases:
oxidative irreversible
• 3 reactions
Non-oxidative reversible
• 3 reactions

Oxidative irreversible reactions (generation of NADPH+H)

Non-oxidative reversible reactions


Ribulose-5-P
Epimerase
Isomerase
Ribose-5-P

- C5+ C5  C3 + C7

- C7 + C3  C4 + C6

- C5 + C4  C3 + C6

- The net result of these reactions is the formation of 2 hexoses and 1 triose from 3 pentoses

Aerobic oxidative process kreb's cycle

- Biological importance of kreb's cycle

I. It provides energy in the form of ATP
II. Formation of water & CO2
III. It has an anabolic role providing intermediates for biosynthesis of glucose, F.A, amino acids & succinyl CoA

Over all yield of ATP from kreb's cycle

- There are 5 oxidative steps in kreb's cycle responsible for ATP production from one molecule of pyruvate as follow:

- Oxidation of pyruvate via NAD into acetyl CoA (3 ATP)

- Oxidation of isocitrate via NAD into α ketoglutarate (3 ATP)

- Oxidation of α ketoglutarate via NAD into succinyl CoA (3 ATP)
- Oxidation of succinate via FAD into fumarate (2 ATP)
- Oxidation of malate via NAD into oxaloacetate (3 ATP)
- Further GTP mole is also added in the conversion of succinyl CoA to free succinate

- So 15 ATP moles are formed from one pyruvate & 30 moles of ATP from 2 pyruvates

Glyoxylate cycle

Biosynthesis from 2-C compound

- This cycle enables plants & bacteria to grow on acetate
- Many bacteria & plants are able to grow on acetate (or other compounds that yield acetyl CoA as fat & pyruvate)
- They make use of a metabolic pathway that converts 2 carbon acetyl units into four carbon units (succinate) for energy production & biosynthesis

Glyoxylate cycle

Differences between citric acid cycle & glyoxylate cycle

i. 2 moles of acetyl CoA enter per turn of glyoxylate cycle compared with one in the citric acid cycle

ii. Citrate is isomerized to succinate in glyoxylate cycle without formation of succinate as in citrate acid cycle

iii. Instead of being decarboxylated into α ketogluterate, isocitrate is cleaved by isocitrate & glyoxylate

iv. Acetyl CoA condenses with glyoxylate by malate synthesis to form malate which is then oxidized to oxaloacetate

- The sum of these cycle is

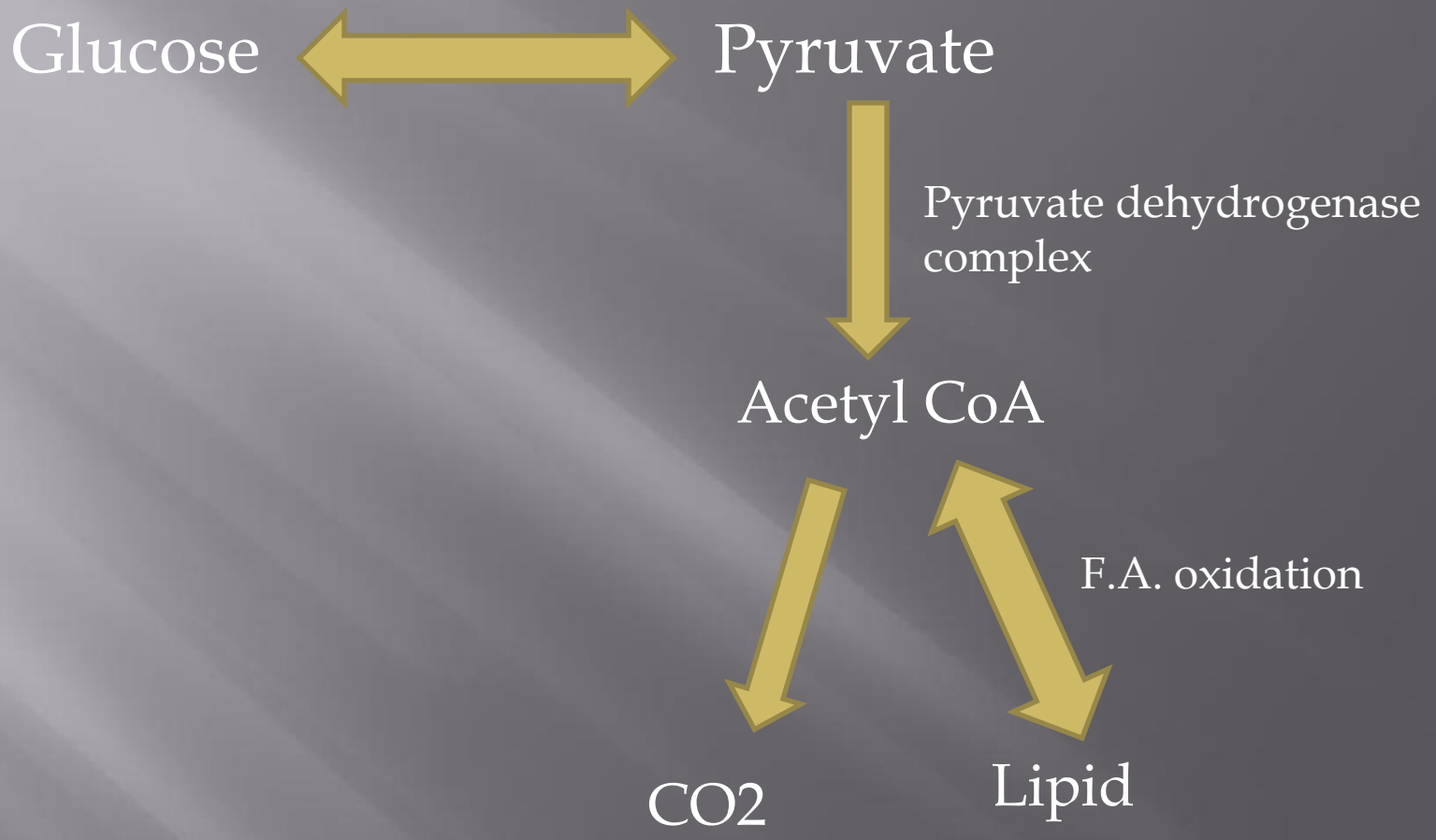
2 acetyl CoA + NAD + 2 H2O

Succinate + 2 CO2 + NADH+H

- The 2 enzymes of the glyoxylate bypass have been found in a variety of organisms grow on acetate as sole C source but their formation is repressed by the simultaneous supply of a more rapidly used substrate such as glucose or succinate

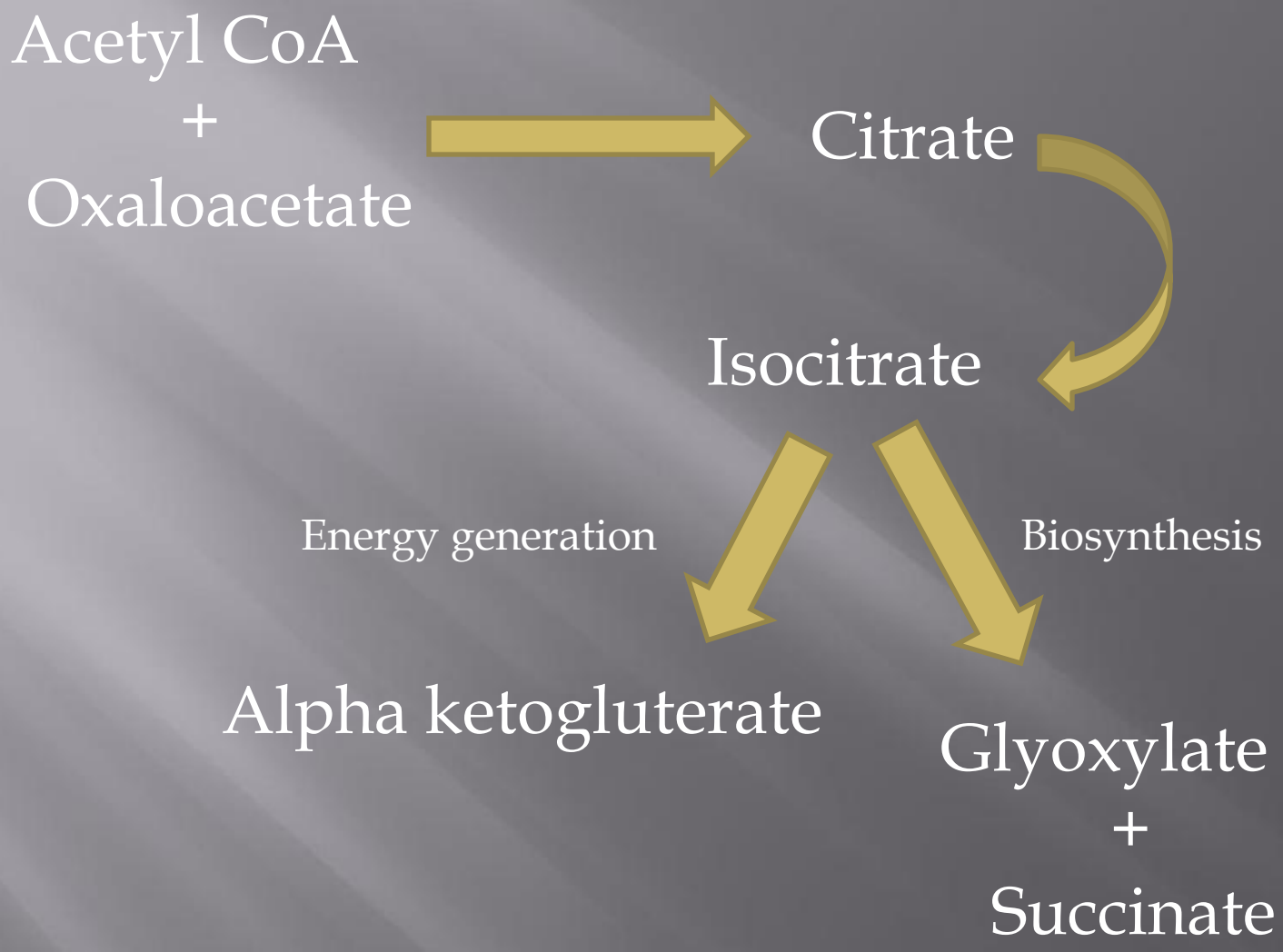
- The need for the glyoxylate cycle arises from the irreversibility, in most organisms, of the oxidative carboxylation of pyruvate to acetyl CoA & CO_2

- This permit photosynthetic bacteria & certain other obligate anaerobes to form pyruvate by reductive carboxylation



- The formation of acetyl CoA from pyruvate is a key irreversible step

Isocitrate has 2 major fates in some bacteria & plants


Acetyl CoA
+
Oxaloacetate
Citrate
Isocitrate
Energy generation
Biosynthesis
Alpha ketogluterate
Glyoxylate
+
Succinate

Amino acid synthesis
&
Metabolism

Sources of amino acid synthesis

- The carbohydrates metabolic pathways constitute the main sources for the amino acid biosynthesis

Pathways

Synthesis of serine

Synthesis of glycine

Serine give cysteine by condensation with homocysteine as follow:

Pyruvate family

- Synthesis of alanine occurs by transamination via ALT (alanine transferase) as follow:

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Biosynthesis of valine & isoleucine

- The pyruvate & aspartate families both make contributions to the synthesis of isoleucine

- The reactions leading to valine are catalyzed by the same enzymes that catalyze the corresponding reactions in isoleucine biosynthesis

Biosynthesis of Di-aminopimelate (DAP)

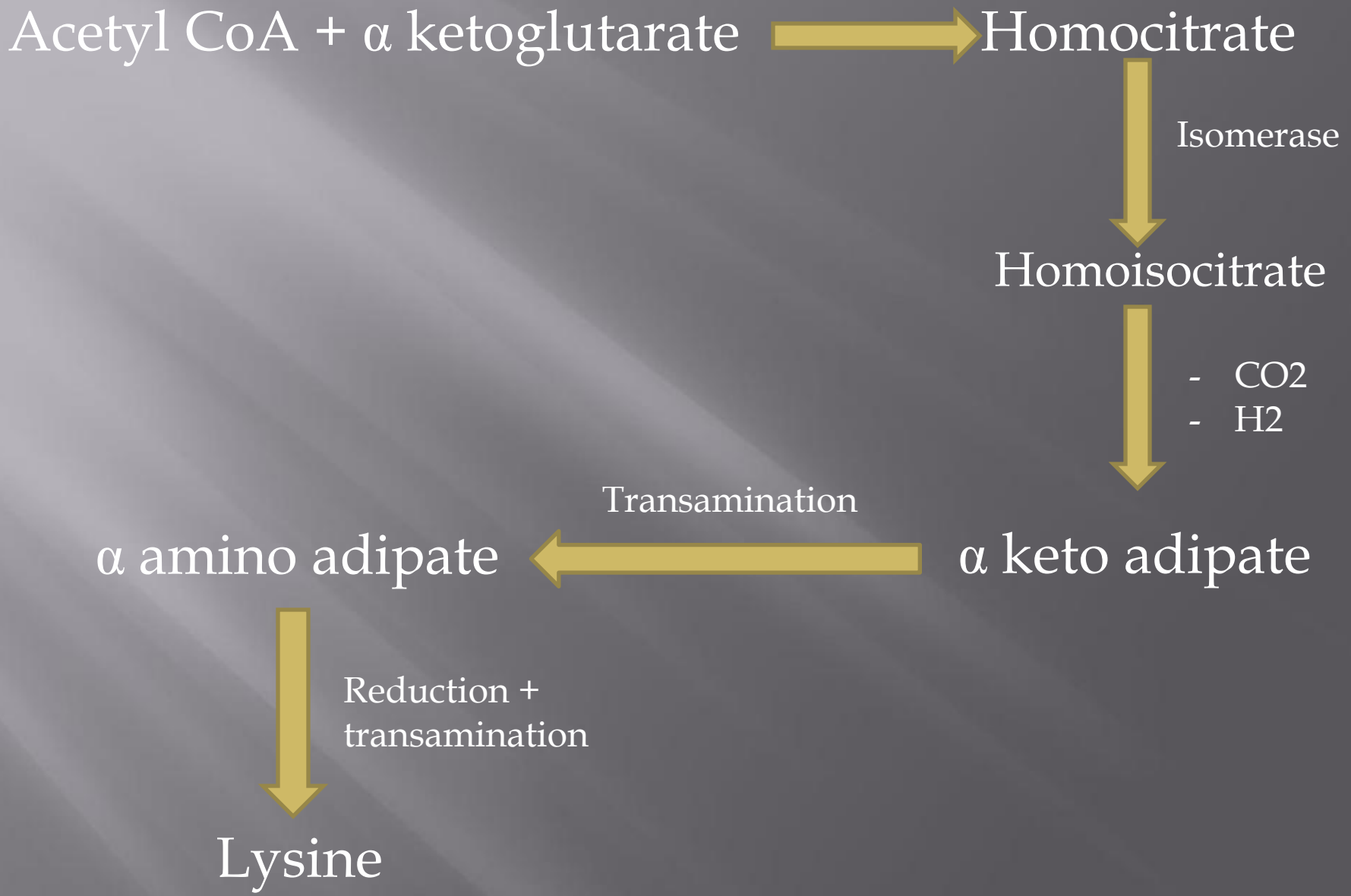
- Also the pyruvate & aspartate both contributes to the synthesis of DAP


Pyruvate + Aspartic semialdehyde
- H2
Di picolinic acid
(spores)
Dihydrodipicolinic acid
Reduction
Tetra hydropicolinic acid
+ succinyl CoA
Isomerase
Meso DAP
Diaminopimelate
- CO2
Lysine

- A few bacterial species incorporate this compound into wall but more use its derivative meso-DAP

Biosynthesis of lysine

- Quite different biosynthetic routes have been found in diferrent organisms
- In fungi whose walls don't contain DAP lysine is synthesized by a route involving the 6-C α amino adipate rather than the 7-C. DAP
- The DAP pathway is found in higher plants


Acetyl CoA + α ketoglutarate
Homocitrate
Isomerase
Homoisocitrate
- CO2
- H2
α keto adipate
Transamination
α amino adipate
Reduction + transamination
Lysine

Synthesis of leucine

- Leucine formation involves elongation of the chain of valine by 1C through addition of an acetyl group to α keto-isovalerate followed by oxidative decarboxylation

- This is analogous to a series in the citric acid cycle which converts oxaloacetate to α ketoglutarate

α keto-isovalerate + acetic acid

Aceto-isocaproic

Oxidative decarboxylation

Alpha keto-isocaproic

Transamination

Leucine

Synthesis of alanine

- Alanine is usually derived like aspartate by transamination of the corresponding α keto-acid pyruvate

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Synthesis of glutamate

- Glutamate serves as the starting material for the synthesis of glutamine, proline & argenine
- In the fungi, lysine is also included in the glutamate family
- The direct amination of α ketoglutarate leads to glutamate

- The most direct route to glutamate (and therefore amino group formation) is that exhibited by many bacteria when grown in a medium containing an ammonium salt as the sole nitrogen source

- The reaction involves a reductive amination catalyzed by glutamate dehydrogenase

- In *E.coli,* this enzyme is specified for NADPH as the hydrogen donor, as might be expected for a biosynthetic reaction involving a reductive step

- Studies of the glutamate dehydrogenase from green plants indicate that they require a very high concentration of ammonia to be effective

- In fact, an alternative pathway is responsible for glutamate biosynthesis in most plants

- Thus in green plants, as well as many bacteria & fungi, the formation of the amino group of glutamate usually originate from the amide group of glutamine

Chemical reactions

- The reaction is catalyzed by glutamate synthase
- Glutamine synthase is a highly regulated enzyme
- In *E.coli,* this enzyme is composed of 2 identical subunits arranged in 2 hexameric rings

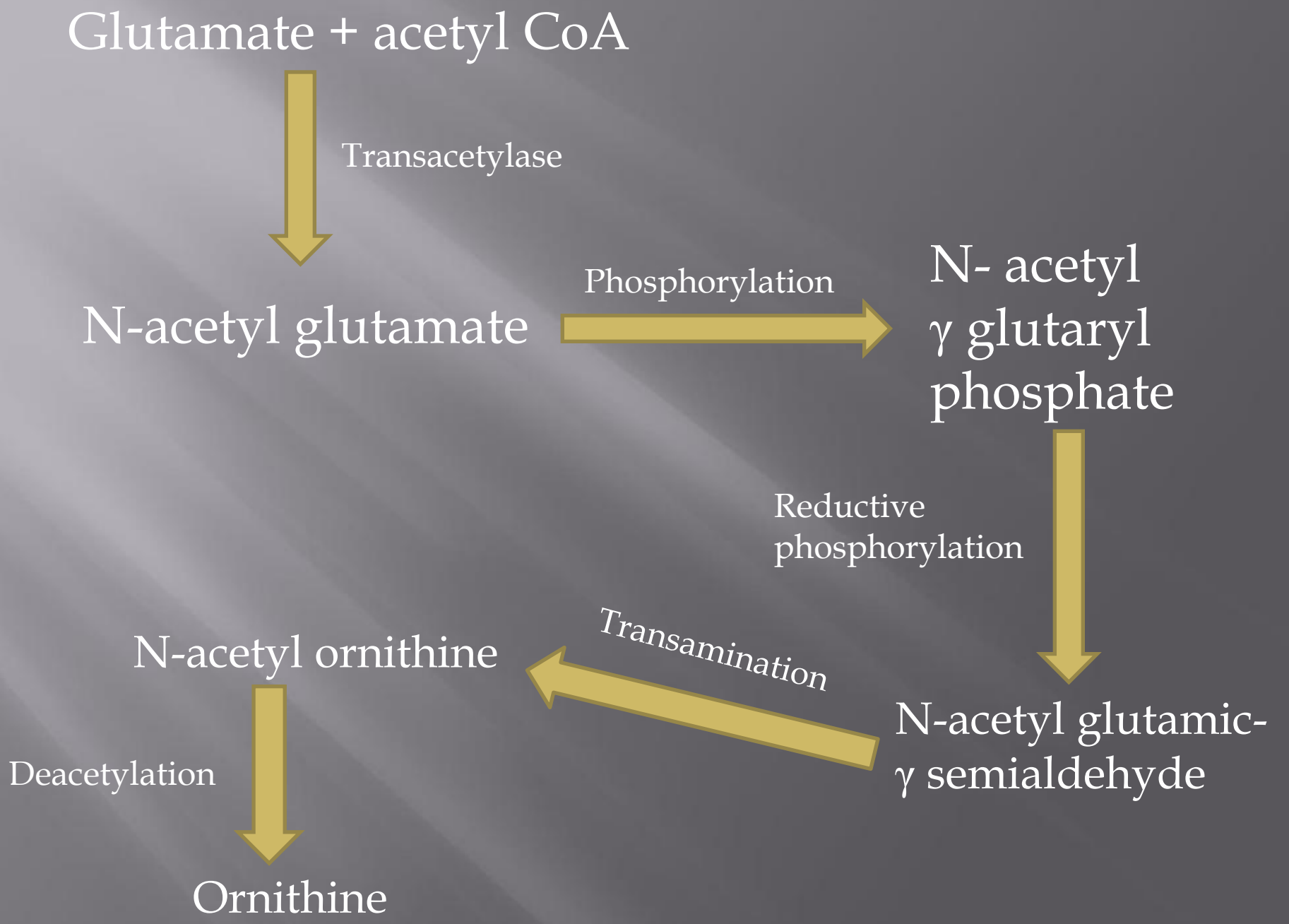
- Activity of glutamate synthase enzyme is negatively regulated by eight nitrogenous compounds: carbamyl phosphate, glucosamine-6-phosphate, tryptophane, alanine, glycine, histidine, cytidine triphosphate & AMP

- These compounds, with the exception of alanine & glycine receive the amide nitrogen from glutamine during their biosynthesis

- Glutamate is a precursor for other amino acids
- In most bacteria, as in higher organisms, glutamate serves, via pyridoxal phosphate, as the source of the α amino group of all other amino acids & of N atoms in certain other cell components
- The amide group of glutamine also provides N at a higher energy level in the synthesis of various compounds (purines, pyrimidines, carbonyl-P, histidine, tryptophan & amino suger)

- Finally, glutamate provides the C skeleton as well as an N atom in proline & ornithine (a precursor of arginine)

Biosynthesis of ornithine


Glutamate + acetyl CoA
Transacetylase
N-acetyl glutamate
Phosphorylation
N- acetyl
γ glutaryl
phosphate
Reductive
phosphorylation
N-acetyl glutamic-
γ semialdehyde
Transamination
N-acetyl ornithine
Deacetylation
Ornithine

Biosynthesis of proline

Biosynthesis of arginine & urea

Biosynthesis of aspartate

- Aspartate is synthesized by transamination of oxaloacetate
- Like glutamate, it is converted (via aspartyl adenylate) to an amide aspargine
- Its metabolic pathways include the following:

I. It is the precursor of the pyrimidine ring

II. It is the precursor of threonine synthesis

III. It is the precursor of N atoms in the purine ring

IV. It is the precursor of nicotinamide in bacteria

V. It is the precursor for methionine synthesis as follow:

Chemical reactions

- In enterobacteriaceae, the intermediate preceding of cystathionine is O-succinyl homoserine but in bacilli ,it is O- acetyl homoserine

- The methyl group is derived from 5-Met HF in *E.coli* but via cobamide (a derivative of vit. B12) in aerobacter

Biomedical importance of methionine

- It participates in the initiation step of protein synthesis as forming methionyl tRNA
- It provides S-adenosyl methionine, a derivative in which the energy of the S-CH3 bond is increased by conversion to the sulfonium ion
- It provides the methyl group for a variety of methylations including the synthesis of betaine, carnosine, melatonin, anserine, sarcosine, methyl nicotinamide, adrenaline, choline, creatine & thymine (for DNA & RNA synthesis)

- It provides the (CH2)3-NH2 portion of spermidine
- Putrescine (diamine) & spermidine (triamine) are polyamines
- They are not amino acids & are formed directly from ornithine & methionine
- In *E.coli,* the diamine putrescine & the triamine spermidine in about a 4:1 ratio constitute about half the total product of the arginine pwayath

- Spermine (a tetramine) which is present in eukaryotes hasn't been found in bacteria

- However, exogenous spermine (which is present in yeast extract & meat extract) can be taken up by *E.coli* and then replaces the endogenous spermidine

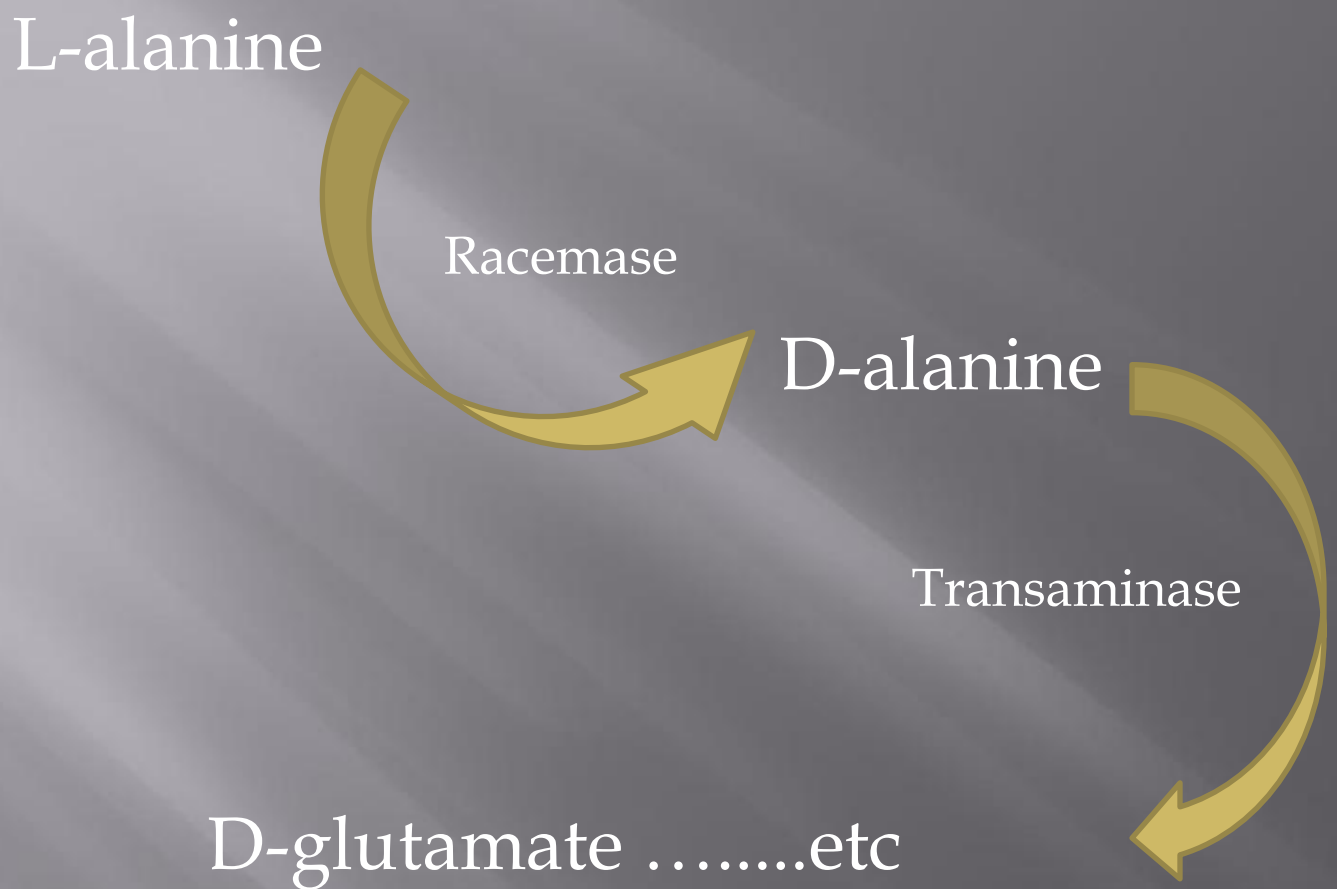
- These polyamines bind to nucleic acids in vitro and increase the resistance of double stranded nucleic acids to strand separating agents and to enzymatic attack

- Thus they influence DNA functionin the cell
- The bulk of the polyamine in cell is probably bound to ribosomes
- Ribosomes extracted from bacteria contain considerable spermidine
- The best defined function of polyamines isn't in cell but in viruses
- Nearly half the changes on DNA in the T4 phage particle are neutralized by polyamines

- Polyamines are required for the growth of some bacteria (pasteurella, mycoplasma)
- Polyamines play one or more essential roles in metabolism
- Putrescine can be synthesized in *E.coli* via decarboxylation either of arginine or of its precursor ornithine. The latter pathway is more economical

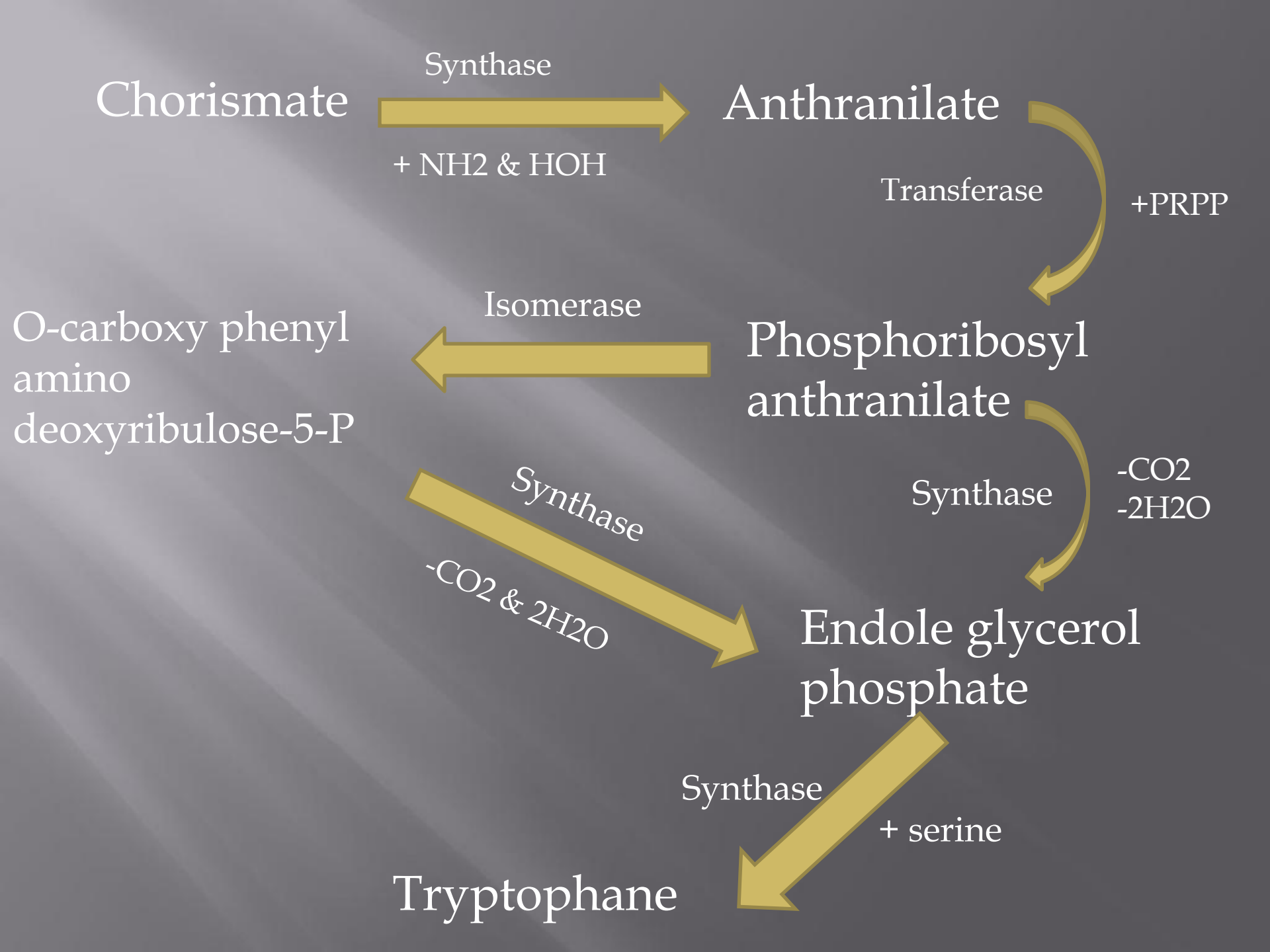
- D-amino acids are present in bacteria in cell wall polypeptides and some in capsules and antibiotic products

- Their synthesis involves a racemase that act on alanine plus a special transaminase that can transfer the D- amino group to various keto acids


L-alanine
Racemase
D-alanine
Transaminase
D-glutamate ……..etc
+
pyruvate

Biosynthesis of aromatic compounds

- Chorsimate is a common precursor of the aromatic amino acid family
- It represents the major branch point. In one branch yield the skeleton of tyrosine & phenylalanine. In other branch tryptophan is produced
- Chorismate is synthesized from erythrose-4-P & phosphoenol pyruvate
- The synthesis of tryptophan from the branched point compound chorismate in *E.cloi* is as follow

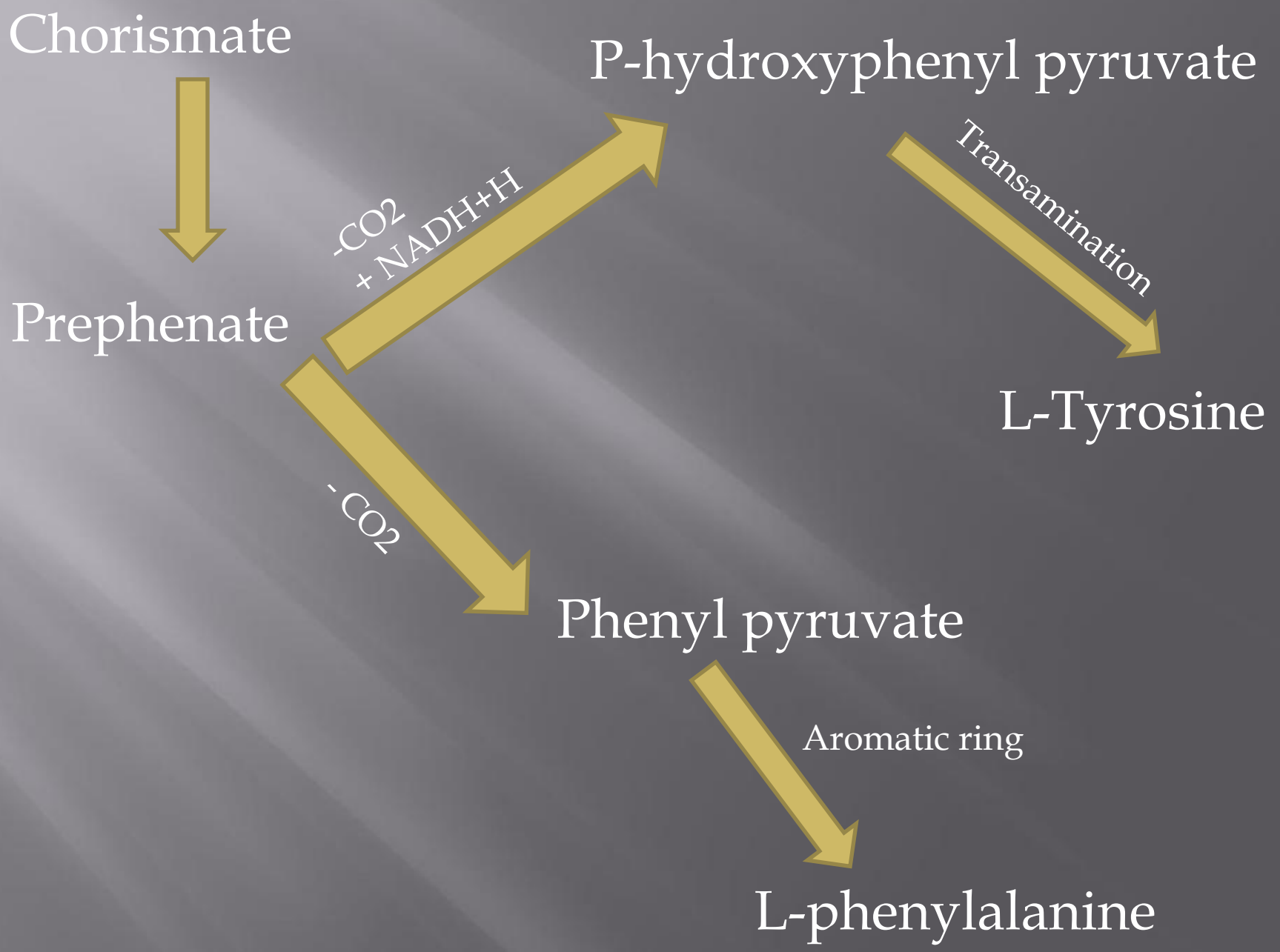

Chorismate
Synthase
+ NH2 & HOH
Anthranilate
Transferase
+PRPP
Phosphoribosyl anthranilate
Isomerase
O-carboxy phenyl amino deoxyribulose-5-P
Synthase
-CO2
-2H2O
Synthase
-CO2 & 2H2O
Endole glycerol phosphate
Synthase
+ serine
Tryptophane

- Among different organisms the fine enzyme activities required for tryptophan synthesis are distributed on different proteins
- All these enzymes exist in the cell as a single multienzyme (& multiprotein) complex
- Metabolic flow to tryptophan is controlled by inhibition of anthranilate synthase by tryptophan
- In many cases, the amino acid pathway branches so that 2 or more amino acids are formed

Synthesis of Histidine

- The starting point for histidine biosynthesis is PRPP (phospho-ribosyl-pyrophosphate) as in the purine pathway

Synthesis of phenylalanine & tyrosine


Chorismate
P-hydroxyphenyl pyruvate
-CO2
+ NADH+H
Transamination
Prephenate
L-Tyrosine
- CO2
Phenyl pyruvate
Aromatic ring
L-phenylalanine

- A wide variety of D-amino acids are found in bacteria. For example: the peptidoglycans of bacteria contain both D-alanine & D-glutamateD- lysine or D-ornithine is found in the glycopeptide of some gram +ve organisms

- The capsule of the anthrax bacillus is composed of a nearly pure homopolymer of D- glutamate in γ linkage

- A wide variety of D-amino acids have been found in antibiotics e.g. D-valine in actinomycine C1 & valinomycine

NUCLEIC ACIDS STRUCTURE & METABOLISM

Nucleotide metabolism

- Composition: each nucleotide is composed of 3 parts

1. A hetero cyclic nitrogenous bases (purines & pyrimidines)
2. A pentose
3. A phosphoryl group

- Functions

- Building blocks of nucleic acids (DNA & RNA)
- Components of many coenzymes (NAD& FAD)
- Activators for the transfer & transformations of sugars, wall peptides & complex lipids
- Constituents of some antibiotics

- Play important roles in all major aspects of metabolism. ATP, for example, is the major substance used by all organisms for the transfer of chemical energy from energy yielding reactions to energy requiring reactions such as biosynthesis

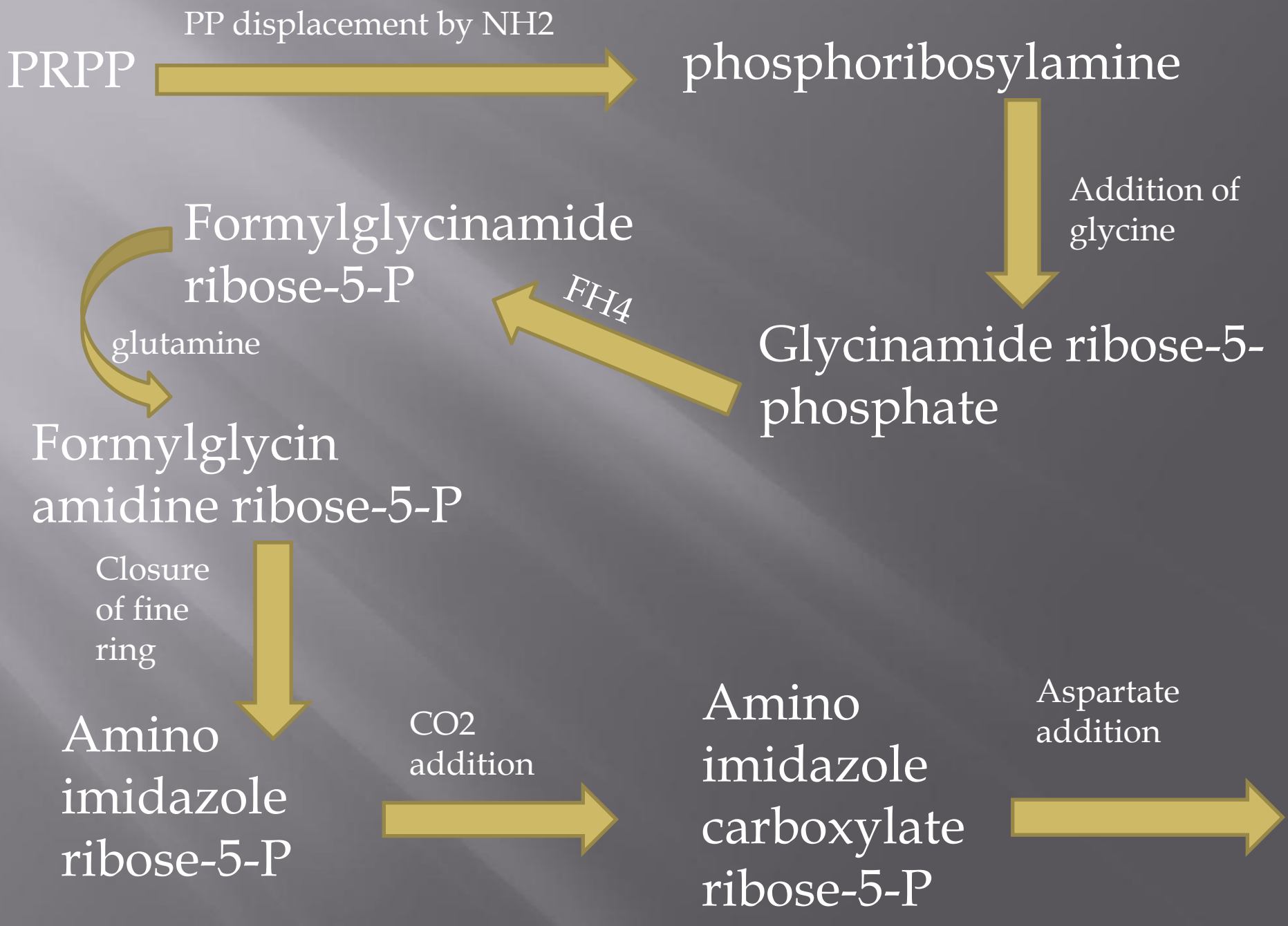
- Other nucleotides are activated intemediates in the synthesis of carbohydrates, lipid, proteins & nucleic acids

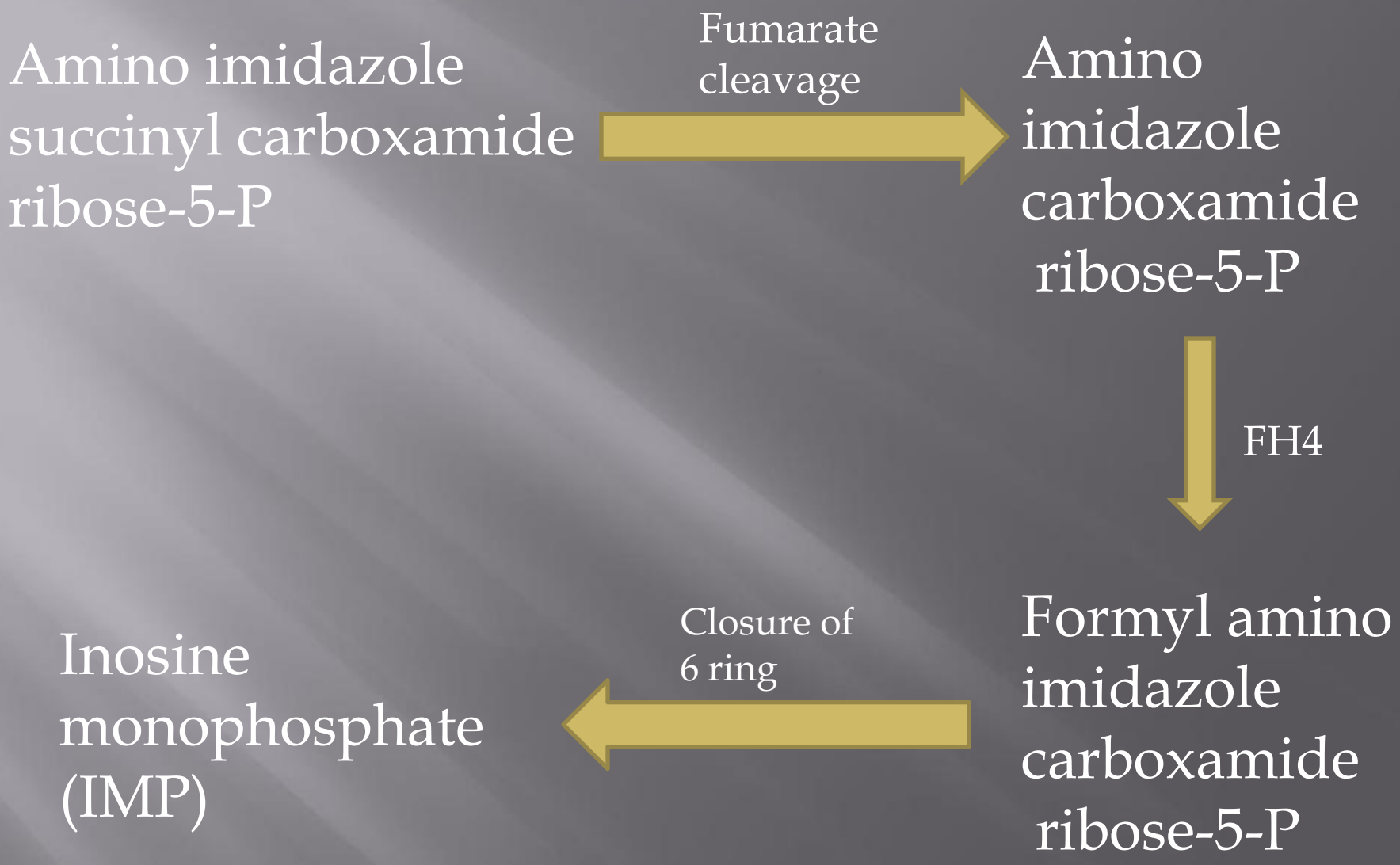
Nucleotide biosynthesis

- The ribose-5-P can be formed in E.coli both oxidatively, from glucose-6-P, & non oxidatively (PPP)
- Ribose-5-P enters nucleotide biosynthesis from its pyrophosphate derivative (PRPP)
- Deoxyribose residues (for DNA synthesis) are formed by NADPH2-linked reduction of the ribose of any ribonucleotide

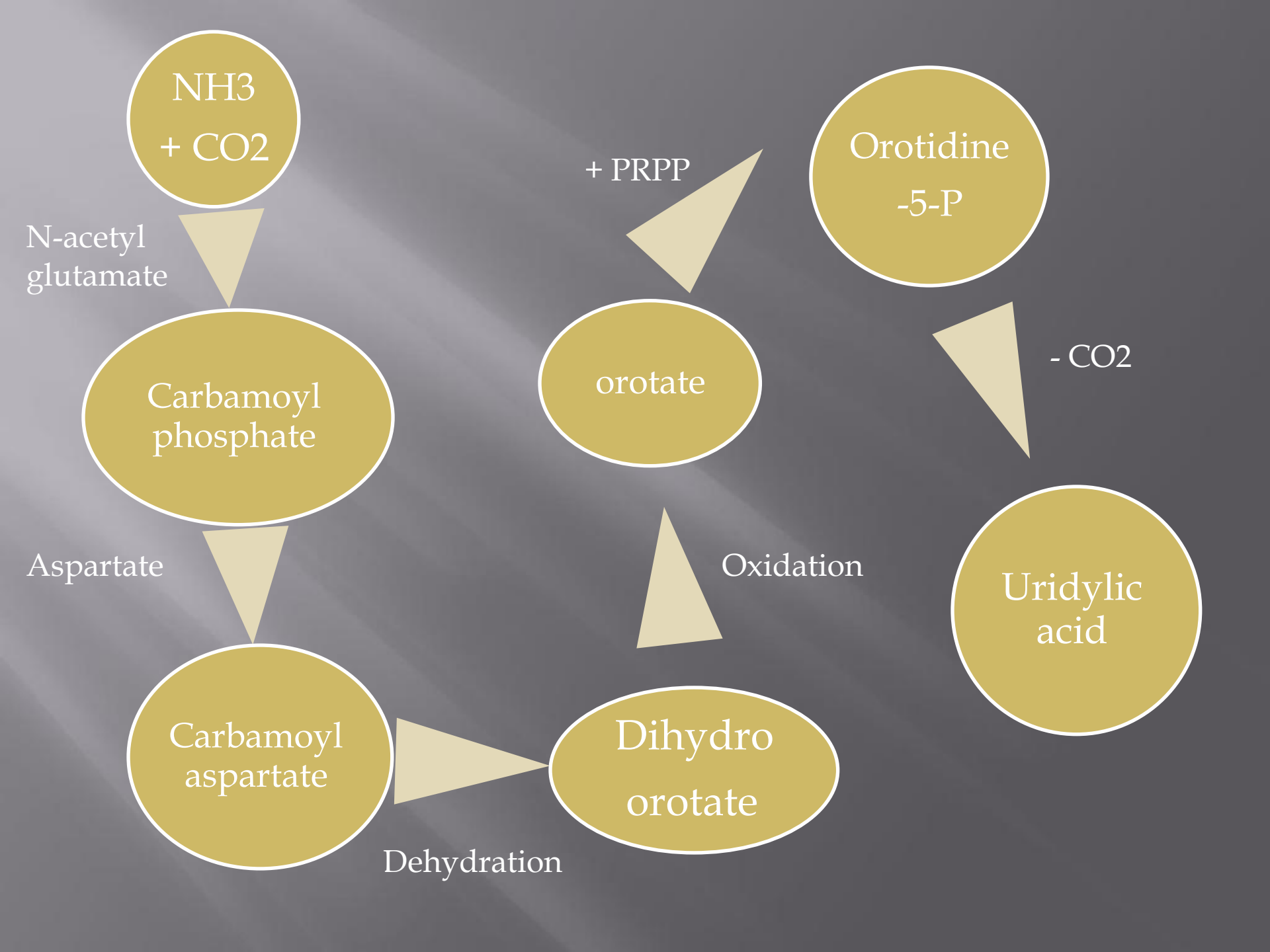
Purine synthesis

Preparatory phosphorylation step


PP displacement by NH2
PRPP
phosphoribosylamine
Addition of glycine
Formylglycinamide ribose-5-P
FH4
glutamine
Glycinamide ribose-5-phosphate
Formylglycin amidine ribose-5-P
Closure of fine ring
Amino imidazole ribose-5-P
CO2 addition
Amino imidazole carboxylate ribose-5-P
Aspartate addition


Amino imidazole succinyl carboxamide ribose-5-P
Fumarate cleavage
Amino imidazole carboxamide ribose-5-P
FH4
Formyl amino imidazole carboxamide ribose-5-P
Closure of 6 ring
Inosine monophosphate (IMP)

Biosynthesis of pyrimidine


NH3
+ CO2
N-acetyl
glutamate
Carbamoyl
phosphate
Aspartate
Carbamoyl
aspartate
Dehydration
Dihydro
orotate
Oxidation
orotate
+ PRPP
Orotidine
-5-P
- CO2
Uridylic
acid

Uridylic acid

Thymidylic acid

Cytidylic acid

- Nucleotide sugars are donors in transglycosylation reactions in the formation of wall polysaccharides in bacteria (e.g. in *pneumococci*)

- Over 60 nucleotide sugars have now been isolated as wall precursors among which the following UDP-D-glucose, UDP-acetyl glucosamine, UDP-galactose, UDP-galactouronate, GDP-mannose, GDP-fucose…….etc

Structure of nucleic acid

- They are formed of building units called nucleotide
- Each nucleotide is formed of nitrogenous base, pentose & phosphoric acid
- Nitrogenous bases are connected to C1 of the pentose (which may be ribose or deoxyribose)phosphoric acid is connected to C5 of the pentose

- The nucleotide are interconnected by phosphodiester bond so that the hydroxyl group of C3 of a nucleotide is connected to the hydroxyl group of the phosphoric acid is attached to C5 of the neighboring nucleotide

- The nucleic acid molecule has a polarity, presence of OH group attached to the phosphoric acid present at the 5 carbon end & the presence of free OH group attached to C3 end

- The 5 end of nucleic acid molecule is written in the left side & the 3 end on the right side where the sequence of nucleotide runs from 5 to 3 end

The detailed structure of DNA

- DNA is present in the form of double stranded molecule contains deoxyribose & thymine in stead of uracil

- The polymer of one strand possesses 2 ends, 5′ phosphate terminus & 3′ hydroxyl terminus so that it runs in the 5′to 3′ direction & called non coding strand

- The other strand runs antiparallel to the 1st one i.e. in the 3′ to 5′ direction & called coding strand that used for synthesis of mRNA during transcription process

- The 2 strands are complementary but not identical to each other
- The complementarity is explained by base pairing role which means that adenine (A) is linked to thymine (T) through 2 hydrogen bonds & guanine (G) is linked to cytosine (C) through 3 hydrogen bonds
- Thus the linkage is through hydrogen bonding

- If one strand has sequence of
  TAGCCTATC
- The other strand will be
  ATCGGATAG

- The 2 strands are coiled to give the form of twisted ladder

- The conformation of the DNA helix leads to the formation of 2 grooves (major & minor grooves)

- The concentration of (A) nucleotides equals that of (T) nucleotides while the concentration of (C) nucleotides equals that of (G) nucleotides
- The 2 strands are forming helical structure along a longitudinal axis
- In the structure of DNA the hydrophilic deoxyribose & phosphoric acid projects outward forming the backbone of the molecule protect DNA
- The nitrogenous bases (hydrophobic part) present in the core of the molecule

GENOME

- Genome is the whole DNA content of the nucleus
- The length of the nuclear DNA is about 1 meter while the cell is 20 μm in diameter
- So the DNA content must be condensed to occupy a small space called chromosome where the condensed structure of the genome is called chromatin

- Chromatin is formed of 3 components

1. Double strands nuclear DNA

2. Histones (basic proteins) which are positively charged linked to the negatively charged DNA molecules by ionic bonds. These histones are of five types H1, H2A, H2B, H3 & H4

3. Non histone protein (protamine which is also basic protein)

- Eight molecules of histones (2H2A, 2H2B, 2H3 & 2H4) are present in the form of beads around them 2 turns of a string of DNA are rapped
- These building units of chromatin are called nucleosomes (or nucleoids)
- A linker DNA is present in between the nucleosomes which appear to be serounded by H1 histone

- Genome contain a total number of about 10,000,000 genes (in human)
- Less than 10% of which are function genes, so gene is a part of genome that codes for a certain function e.g. gene of insulin
- Each gene occupies a well defined position or a chromosome (locus) and thus it is a segment of DNA molecule
- Each character (gene) is controlled by a pair or series of genes occupying the same locus on homologus chromosom

- In gametes (ova, sperm) the genome is haploid, it is half the number in somatic cells (the human gametes contain 23 chromosomes)
- In somatic cells the genome is diploid (double the amount of gametes)
- Each human somatic cell contains 23 pairs of homologus chromosomes
- Each one of the genes is an allele, these alleles may be similar (homozygous BB) or different (heterozygous BB)

- The 2 alleles determines the genotype , one allele may be dominant (phenotype) & the other allele will be recessive

BB	Bb	bb
black	black	yellow

In eukaryotes genome

- The amount of DNA per cell is the same for every diploid cell of any species
- In eukaryotes cells, some genes are found in many copies
- Each gene consists of coding sequences called exons that are interrupted by non coding sequences called introns number of which vary from one gene to another
- Exact function of introns aren't known but it is suggested to promote recombination

Genome of bacteria & viruses

- The chromosome of *E.coli* is a circular continuous double stranded DNA of 1mm long when fully extended
- It contains 4.7 million base pairs

- Bacterial chromosome is bidirectionally replicated started at specific nucleotide sequence known as the origin of replication& ended at termination site DNA polymerase adds nucleotides at 3′ end of both strands forming a structure resembling to Greek letter theta, hence it called theta replication

- There are additional DNA molecules found in bacteria which are much smaller than bacterial chromosomes containing from 2 to 30 genes
- These molecules are called plasmids which are circular and self replicating DNA. Thus it is a replicating piece of DNA
- Certain plasmids can move into & out of the bacterial chromosomes

- Some bacterial cells may contain one or more copy of plasmids while others not receive any copies of the replicated plasmid

- Two of the most important types of plasmids are sex factor (F plasmids) & drug resistance (R plasmids)

- Plasmids exists as a covalently closed circular piece of double stranded DNA that has the capability of replicating autonomously and it is this property that leads to its isolation & physical recognition

- 2 common properties of plasmids are mentioned

1. Many plasmids contain genes uninvolved in either replication or incompatibility. Such genes can encode properties like antibiotic resistance
2. The ability of plasmid to promote the transfer of the plasmid itself from one cell to another termed a property of conjugation which is defined as the unidirectional transfer of genetic information between cells by cell-to-cell contact

F-plasmids

- F-plasmids or fertility factor of *E.coli* contains 25 genes, many of which control the production of F Pilli which are long rod-shaped protein structure that extend from the surface of cells containing F-plasmids (which known as male or Ft cells)

- Cells that lack F-plasmids are known as female (recipient) or F-cells

- Ft cells can attach themselves to F-cells by the pilli & transfer the F-plasmid to them through cytoplasmic bridges thus recipient cells become Ft cells

- This process of transfer of F-plasmid is known as conjugation

R-plasmid

- R-plasmid are plasmids that carrying genes for resistance to antibiotics or antibacterial drugs
- Resistance genes can be also transferred from plasmids to the bacterial chromosome, to viruses & to bacterial of other species
- Therefore infectious drug resistance has been found among an increasing number of types of pathogens

- Typically, only a few copies of these large plasmids exist in a single cell
- They are passed from mother to daughter cells at cell division by conjugation
- Drug resistance in bacteria is due to the synthesis of enzymes that break down the drug or that set up a new enzymatic pathway

Viruses

- Viruses are obligate parasites and can't multiply outside the cell
- Viruses consist of a molecule of nucleic acid enclosed in a protein coat or capsid
- They contain no cytoplasm or ribosomes or other cellular components

- They can move from cell to cell in which utilizing enzymes and organelles to replicate their nucleic acid & synthesize new coat protein

- Within the host cell the viral nucleic acid directs the production of new viruses using the nucleotides, amino acids, ATP & cell metabolic machinery

- The nucleic acid of viruses (viral chromosome) may be either DNA or RNA, single or double stranded, circular or linear

- Viral chromosomes vary from 5,400 nucleotides to 18o,000 nucleotides

- In viruses, genes are closely packed with very little intergenic DNA & even some genes may overlap

- In prokaryotes. In general genes may be allocated in different strands of DNA

Basis & principal of DNA typing

- The length of the phosphodiester link is a 1nm while the bases are a bout 0.33 nm thick so that in the straight ladder like structure there would be a gap between them

- The faces of bases are hydrophilic and in the straight structure they would be exposed to H2O molecules (unstable solution)

- What can be done to bring the hydrophobic bases together to exclude H2O from their faces?

- It can be achieved by collapsing them together by sloping the phosphodiester link or more accurately hydrophobic forces cause the bases to collapse together

- The base pair still lie flat stacked on top of each other
- Thus the arrangement still permits base-pair stacking and the exclusion of water from between them but the stacking can ‘t be exactly vertical

- Instead successive base pair must rotate slightly relative to one another such that approximately 10 base pairs (in B-form of DNA) are required to rotate through one complete turn

- The bases are separated by 3.4A° along the helix axis and each base is rotated 36° in relation to the previous base (complete cyclic turn is 360°)

- This means that in (B type of DNA), the helical structure repeats at intervals of 34A° (every 10 base pairs)

- The helices are right handed as you more along a strand or a groove you continuously turn clockwise , alternatively, imagine you are driving in screw, holding the screw driver in your right hand

- The structure of the double helix is such that there is a major and a minor groove
- These 2 grooves are present in B-DNA and arise because the glycosidic bonds of a base pair aren't diametrically opposite each other
- The minor groove contains the pyrimidine O-2 & the purine N-3 of the base pair and the major groove is on the opposite side of the pair

- The major groove is slightly deeper than the minor one
- Each groove is lined by potential hydrogen bond donor & acceptor atoms
- In the minor groove, N3 of adenine & guanine and O-2 of thymine & cytosine, can serve as hydrogen acceptors and the amino group attached to C-2 of guanine can be a hydrogen donor

- This B-DNA form is the normal form that exists in cells
- However, DNA can adopt different configurations in special circumstances
- When dehydrated, the double helix is more squat in shape and the bases are tilted, this is known as the A form which may exist in spores

- Another form is known as Z (because the polynucleotide backbone zigzags) in this the double helix is left handed which occur in short synthetic DNA molecules with alternating purine & pyrimidine bases provided the solution is of high ionic strength

- Complete denaturation of a molecule of DNA leads to separation of the 2 complementary strands

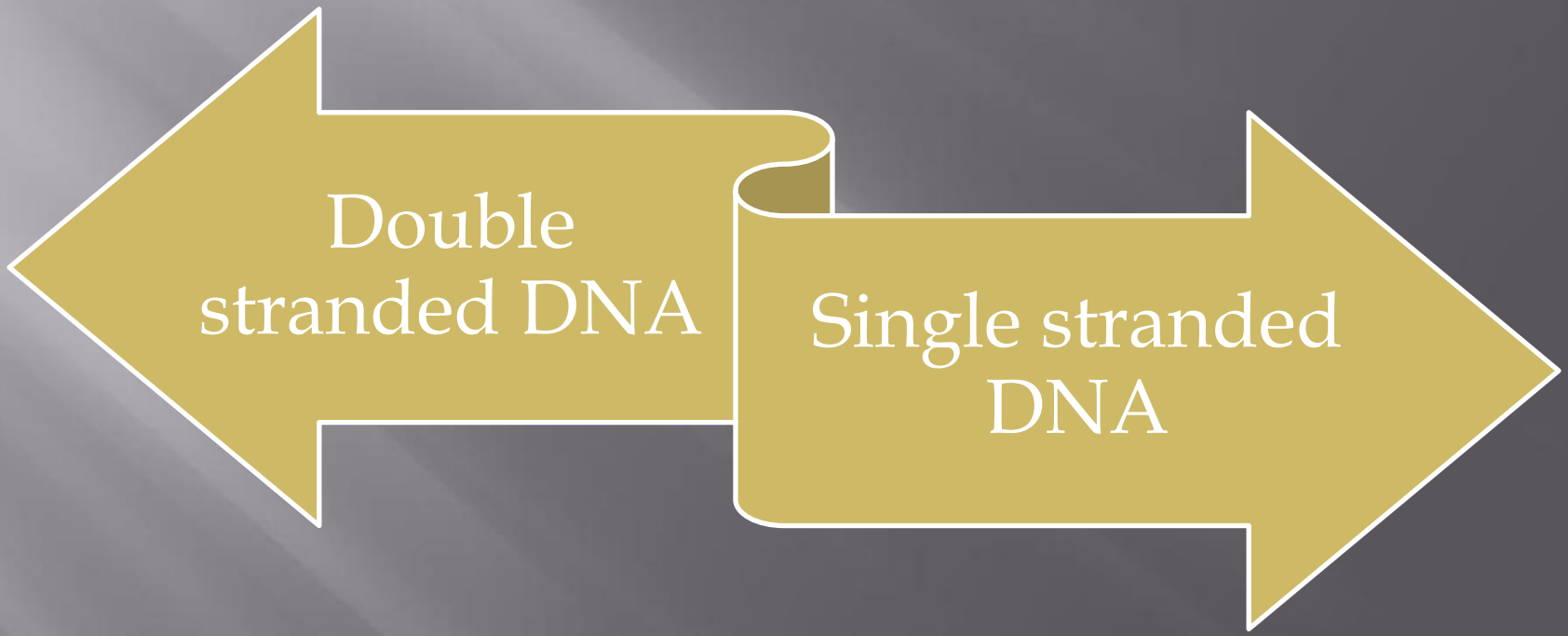
- If a solution of denatured DNA is cooled quickly the denatured strands remain separated
- If the temperature is just below Tm (the melting temperature) a process known as annaeling, the double stranded structure can be reformed
- This is termed hybridization when the nucleic acid is from different sources

- Hybridization can be used to compare the homology (degree of complementarity) of nucleic acids from different sources

- DNA is broken into pieces of moderate length and is denatured

- The denatured fragments from the 2 species are mixed and those that have similar nucleotide sequence tend to hybridize whereas those where the sequences are very different don't

Types of DNA



Single stranded DNA

Present in few viruses

Double stranded DNA

Linear

Helical

Circular

Helical

Has 6 forms

(A, B, C, D, E, Z)

Linear

-In most somatic cells

-Inside the nucleus

-In some DNA viruses

-Codes for cellular protein

Circular

-The polarity is los

-present in mitochondria

-Also in some DNA virus

-Codes for mitochondrial protein

-Most species of bacteria contain small circular extra-microsomal DNA called plasmids

- According to helical structure there are 6 types of DNA (A, B, C, D, E & Z) which are different according to the following factors:

I. The number of base pairs found per 360° turn of the helix
II. The pitch (angle) between each base pair
III. The helical diameter of the molecule
IV. The handedness (right or left) of the double helix direction

- Inter-conversion of one type to another might happen in vivo depending on salt concentration and hydration conditions
- The B-form is the dominant form of DNA and has a pitch of 3.4 nm per turn
- Minor and major grooves are formed to which specific proteins interact with exposed atoms of nucleotide
- Each single turn contains 10 base pair (pb)

- Z- DNA is the least twisted (12 pb /turn) and has only one type of groove that may not bind to proteins

- Z-DNA contains some 5-methylated deoxycystidine nucleotides loss of which leas to the reversion of Z- form to B-form, thus affecting gene activity

Functions of DNA

- It transfer the genetic information into the daughter cells by the replication
- It acts as a template for the transcription of mRNA which in turn required for protein biosynthesis

DNA replication in prokaryotes & eukaryotes

Definition

- It means the duplication of DNA of mother cell producing two copies of DNA in the daughter cell

- Replication is semi-conservative in nature which means that each daughter cell will receive a DNA strand from the mother cell and a newly synthesized strand from the bases in the nucleus complementary to the mother strand

Requirements

1. Substrate (for different deoxynucleotide triphosphate (dNTP))
2. RNA primer
3. DNA template
4. Enzymes (DNA polymerase, helicase, topoisomerase, ligases, reverse transcriptase)
5. Proteins
6. High energy phsphate compounds

Steps of replication in prokaryotes & eukaryotes are the same but there are some differences as follows:

	Prokaryotes	Eukaryotes
Enzymes responsible for replication are	DNA polymerase Ⅰ, Ⅱ, Ⅲ	DNA polymerase α, β, γ
Replication occur at	A single site called “oric”	Multiple sites

- Polymerase lll is responsible for DNA replication
- Polymerase l is responsible for DNA repair
- But in eukaryotes polymerase α is responsible for DNA replication and β for DNA repair
- γ polymerase is responsible for replication of mitochondrial DNA

Steps in prokaryotes

1. Separation of the two complementary DNA strands
2. Formation of the replication fork
3. Base pairing between DNA template and DNA primer
4. Chain elongation
5. Excision of RNA primers and their replacement by DNA
6. These steps can be summarized in only 4 steps namely separation, initiation, elongation and termination

Separation of the double stranded DNA molecule

- This occurs by enzymes and proteins as follows:

1) Helical unwinding proteins or helicases are attached to the single stranded DNA near the site of initiation of replication and more to the double stranded DNA separating the 2 strands

- These helicases break down the bonds between the bases at the expense of ATP (needs energy) where 2 ATP molecules are consumed for each base pair breaking

2) Helical destabilizing protein become attached non enzymatically to the single stranded DNA at the sited initiation

- They prevent rejoining of the bases and protect the single stranded DNA from hydrolysis by nucleases

3) During separation of double stranded DNA super twisting of the DNA can occur which makes a further separation of DNA very difficult

- Topoisomerase I (swinelase) makes a nick (space) in a single strand in the double helix allowing each strand to rotate in the opposite direction of the nick prevents over twisting

4) Topoisomerase Ⅱ (gyrase) introduces negative super twisting to facilitate separation and unwinding

- The nick is sealed and closed again
- As a result of these preparatory steps a structure called replication fork is formed
- Replication moves up and down the double stranded DNA i.e. replication occur in both directions

Base pairing

- DNA polymerase lll begins the process of replication by adding free bases to the 3 OH end of the RNA primer
- This is done according to the base pairing rule A-T, G-C using DNA strand as a template

- DNA polymerase III builds the new DNA strand in the 5 to 3 direction

- This means that one strand is built in the direction of replication fork (leading strand) while the other strand is built away from the replication fork (lagging strand)

Leading strand	Lagging strand
Grows in the direction of replication fork	Grows away from the replication fork
It is built in a continuous manner	It is built in the form of multiple fragments called okazaki fragments which are linked later
RNA primer is rare	RNA primer is common

Chain elongation

- DNA polymerase III catalyzes the chain elongation using the 3 hydroxyl group of the RNA primer as the acceptor of the first deoxyribonucleotide

- The sequence of nucleotides that are added is dictated by the base sequence of the template strand with which the incoming nucleotides are paired

Excision of RNA primers & their replacement by DNA (Termination)

- DNA polymerase III continues to synthesize DNA on the lagging strand until the enzyme miss-pairs a nucleotide with the DNA template

- When this occurs, the RNA is excised by DNA polymerase I

- The gaps left after excision of RNA primers is built according to the base complementarity by DNA polymerase I

- The 5 end of DNA fragment built by DNA polymerase III is linked to the 3 end of the DNA fragment built by the DNA polymerase I by a ligase enzyme at the expense of ATP

- Ligase links any cuts in the DNA

Eukaryotes DNA replication

- The process of eukaryotic DNA replication closely follows that of prokaryotic DNA synthesis

- Some differences such as the multiple origins of replication in eukaryotic cells versus single origin of replication in prokaryotes

- Eukaryotic single stranded DNA binding proteins and ATP-dependent DNA helicases have been identified, whose functions are analogous to those of the prokaryotic enzymes previously discussed

- In contrast, RNA primers are removed by RNase H (reverse transcriptase enzyme) also called RNA dependent DNA polymerase

- In cells, the flow of genetic information appears to be in one direction from DNA to RNA and from RNA to protein

- One species of viruses called retroviruses has a mechanism for reversing the first step in this flow i.e. from RNA to DNA

- The retroviruses contain single stranded RNA nucleic acid and a viral enzyme called reverse transcriptase

- Reverse transcriptase uses the RNA as a template for the synthesis of viral DNA which then becomes integrated into host chromosome

- Reverse transcriptase moves along the template RNA in the 3′-5′ direction, synthesizing the DNA product in the 5′-3′ direction
- Examples of retroviruses are human immuno-deficient virus and some tumor viruses
- Reverse transcriptase enzymes are important in recombinant DNA technology

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