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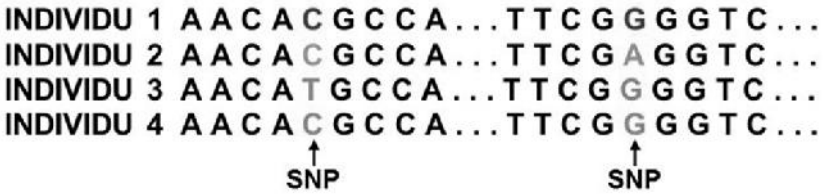
LECTURE 7: NUCLEOTIDE METABOLISM 51

Single Nucleotide Polymorphisms (SNPs)



All humans are ~99,7% identical at DNA sequence level, and yet all of us carry a significant number of 'glitches' in our genomes. The functional significance of minor SNPs are the basis for the diversity among humans

If you can't explain it simply,
you don't understand it well enough—Albert Einstein

EXAM

1. What is the basis for the diversity found among humans
   [a] Polypeptide [b] DNA [c] Genome [d] SNP
2. The following compound is a nucleotide
   [a] Cytidine [b] dTDP [c] Thymidine [d] Uridine
3. The following compound is a nucleotide of DNA
   [a] GMP [b] TMP [c] UMP [d] CMP
4. One of the pathways of nucleotide biosynthesis is
   [a] salvage [b] TCA cycle [c] plastids [d] catabolism
5. The first reaction of purine nucleotide biosynthesis is catalyzed by
   [a] GARS [b] GAT [c] PRAT [d] GAR

LECTURE OUTCOME

Students, after mastering materials of the present lecture, should be able

1. To explain the importance of nucleotides as the central cellular metabolisms
2. To explain *de novo* pathway of purine and pyrimidine nucleotide biosynthesis
3. To explain salvage pathway of purine and pyrimidine nucleotide biosynthesis
4. To explain the catabolism of purine and pyrimidine nucleotides

LECTURE OUTLINE

1. INTRODUCTION
2. NULEOTIDE BIOSYNTHESIS
   - A. ATP Structure
   - B. Purine Synthesis
   - C. The De Novo Purine Pathway

IV. ATP REGENERATION
   - -Glycolysis
   - -Krebs Cycle
   - -Electron Transport Chain

1. INTRODUCTION

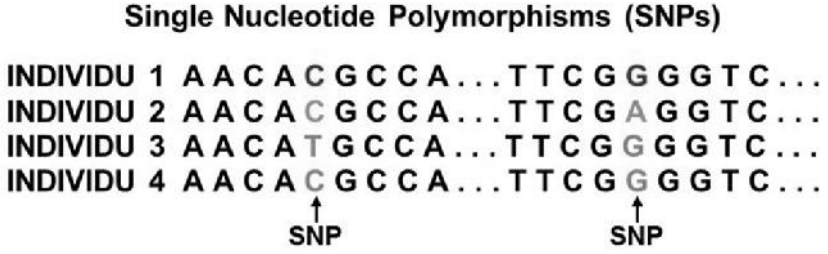
What is the importance of nucleotides ?

- Synthesis of nucleotides to provide an ample supply of nucleotides is essential for many life processes due to the central metabolic function of nucleotides in all cells as follows. (1)

1. Nucleotides are the activated precursors of nucleic acids. As such, they are necessary for the replication of the genome and the transcription of the genetic information into RNA. (2)
2. Adenine nucleotide, ATP, is the universal currency of energy. A guanine nucleotide, GTP, also serves as an energy source for a more select group of biological processes. ATP acts as the donor of phosphoryl groups transferred by protein kinases. (3)
3. Nucleotide derivatives such as UDP-glucose participate in biosynthetic processes such as the formation of glycogen (energy storage in animals). (4)
4. Nucleotides are essential components of signal-transduction pathways such as cyclic AMP and cyclic GMP (cAMP & cGMP) as second messengers that transmit signals both within and between cells. (5)
5. Nucleotides also provide bases for a number of essential coenzymes (NAD, NADP, FAD, and coenzyme A) (6)
6. Nucleotides are the precursors for purine alkaloids, and for the adenine moiety of cytokinin plant growth regulators. (7) (8)

7. A better understanding of nucleotides is further encouraged partly by the discovery and development of
   (i) anti-HIV drugs, and (9)
   (ii) the identification, cataloguing, and mapping of human SNPs (single nucleotide polymorphisms) (10)
8. Anti-HIV drugs is based on nucleoside such as NRTIs (nucleoside reverse-transcriptase inhibitors) which is the first drug used for the treatment of HIV that may develop into AIDS (acquired immunodeficiency syndrome). (11)
9. SNPs are variations in a DNA sequence that occur when a single nucleotide in the sequence is different from the norm in at least one percent of the population. (12)

10. Human genomes are 99.9% identical (Cooper *et al*., 1985), but a 0,1% difference leads to 3.2 million differences of 3.2 billion base pair genome in each person. (13) (14)
11. Most of the differences are due to SNPs which have no biological consequence in majority, but the rest SNPs have functional significance and are the basis for the diversity found among humans (15)



Eric Green's View of SNPs (Director of NHGRI)

2. BASIC STRUCTURE OF NUCLEOTIDES

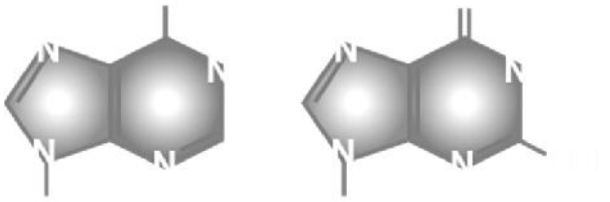
Components

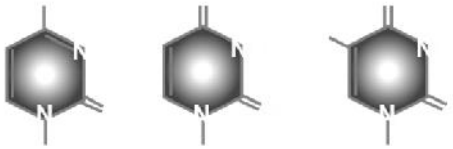
- A nucleoside consists of a purine or pyrimidine base linked to a sugar (ribose or deoxyribose), and a nucleotide is a phosphate ester of a nucleoside

  Nucleoside = Sugar + Base (16)
  Nucleotide = Sugar + Base + Phosphate

- A nitrogen base is a molecule that contains nitrogen and has the chemical properties of a base. (17) Nitrogenous bases are typically classified as the derivatives of two parent compounds, pyrimidine and purine

- The nitrogenous bases in DNA are adenine (A), guanine (G), thymine (T), and cytosine (C).
- The nitrogenous bases in RNA are the same, with one exception: adenine (A), guanine (G), uracil (U), and cytosine (C).





- Adenine, for example, forms adenosine, a nucleoside, when attached to ribose, and deoxy-adenosine when attached to deoxyribose. Adenosine triphosphate (ATP) is formed when three phosphate groups are added to adenosine.

Naming Conventions

- Nucleosides:
  - Purine nucleosides end in “-sine”
    - Adenosine, Guanosine
  - Pyrimidine nucleosides end in “-dine”
    - Thymidine, Cytidine, Uridine

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- Nucleotides:
  - Start with the nucleoside name from above and add “mono-”, “di-”, or “triphosphate”
    - Adenosine Monophosphate (AMP),
    - Cytidine Triphosphate (CTP),
    - Deoxythymidine Diphosphate (dTDP)

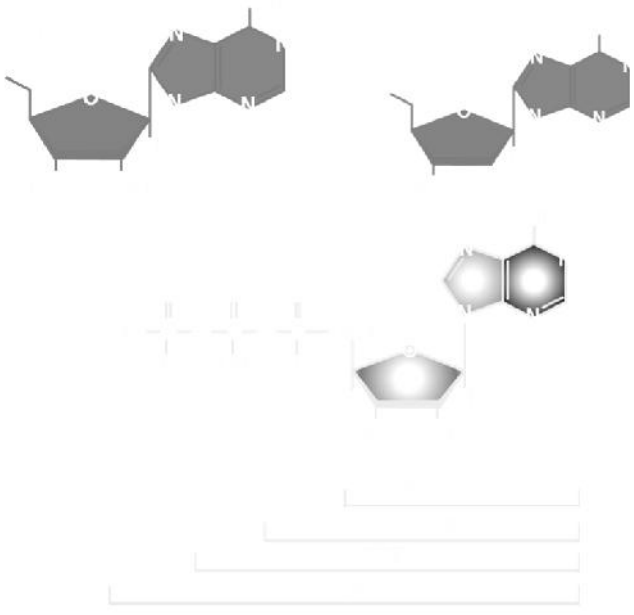
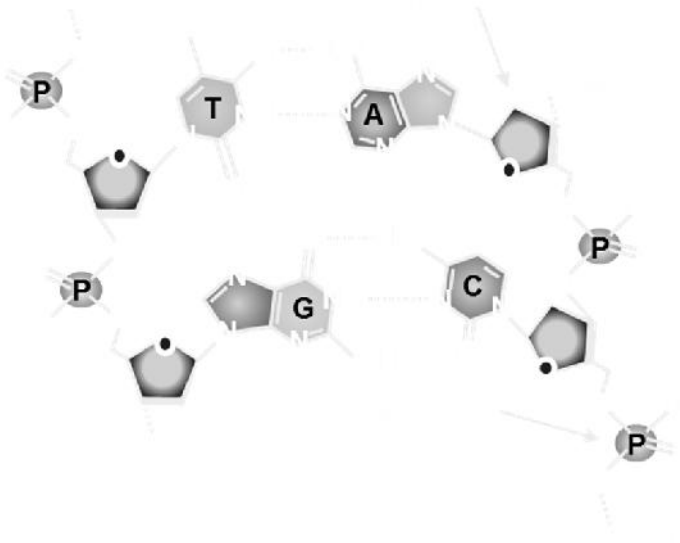
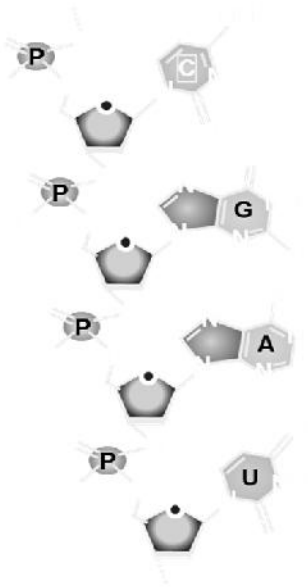


Table . Nomenclature of bases, nucleosides, and nucleotides

RNA		
Base	Ribonucleoside	Ribonucleotide (5 - monophosphate)
Adenine (A)	Adenosine	Adenylate (AMP)
Guanine (G)	Guanosine	Guanylate (GMP)
Uracil (U)	Uridine	Uridylate (UMP)
Cytosine (C)	Cytidine	Cytidylate (CMP)
DNA		
Base	Deoxyribonucleoside	Deoxyribonucleotide (5 - monophosphate
Adenine (A)	Deoxyadenosine	Deoxyadenylate (dAMP)
Guanine (G)	Deoxyguanosine	Deoxyguanylate (dGMP)
Thymine (T)	Thymidine	Thymidylate (TMP)
Cytosine (C)	Deoxycytidine	Deoxycytidylate (dCMP)



DNA



RNA

3. NUCLEOTIDE BIOSYNTHESIS

1. The biosynthesis of nucleotides proceeds through *de novo* pathways and salvage pathways (19)
2. In de novo (from scratch) pathways, the nucleotide bases are assembled from simpler compounds. (20)
   - The framework for a pyrimidine base is assembled first and then attached to ribose. (21)
   - In contrast, the framework for a purine base is synthesized piece by piece directly onto a ribose-based structure. (22)
   - These pathways comprise a small number of elementary reactions that are repeated with variation to generate different nucleotides, as might be expected for pathways that appeared very early in evolution. (23)

3. The de novo route of nucleotide synthesis has a high requirement for energy as compared that of the salvage pathway (23)
4. In salvage pathways, preformed bases are recovered and reconnected to a ribose unit. (24)
5. The enzymes of both of these biosynthetic routes are classified as “housekeeping” enzymes because they perform basic, cellular activities and are assumed to be present in low, constitutive levels in all cells. (25)
6. The de novo pathway is thought to reside in plastids, salvage cycle enzymes may be localized in more than one compartment. (26)

1. *De novo* Pathways

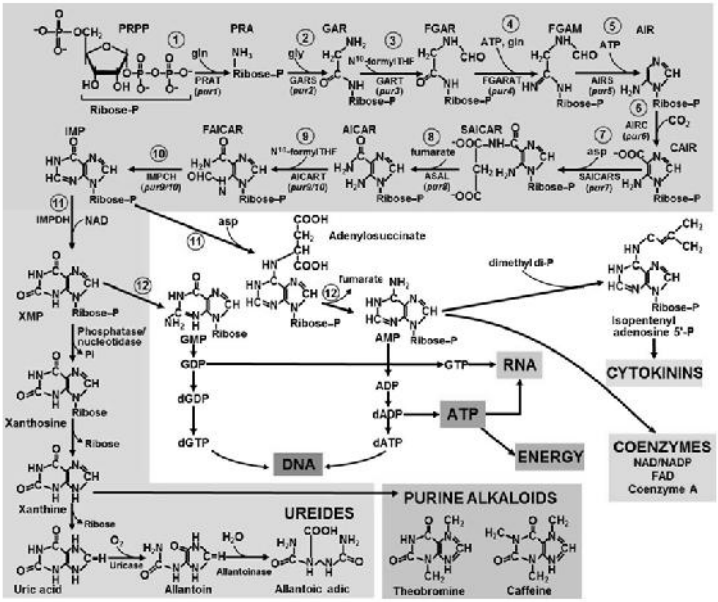
1. The *de novo* pathways of nucleotide biosynthesis are initially regulated by the formation of PRPP (*5-phospho-~-D-ribosyl 1-pyrophosphate*) from ribose 5-P (*ribose 5-phosphate*) and ATP catalyzed by PRPP *synthetase* with Mg^{2+} as a cofactor (27)

   Ribose 5-P + ATP ì PRPP + AMP
2. This reaction is activated by inorganic phosphate and inhibited by purine nucleotides. (28)
3. Ribose 5-P can be obtained from OPP (*oxidative pentose phosphate*) pathway or HMS (*hexose monophosphate shunt*). This is parallel with glycolysis in the breakdown of sugar (glucose) to form NADPH and pentose. (29) (30)

1.1 Purine Nucleotide Biosynthesis

1. The *de novo* pathway leading to the synthesis of AMP and GMP begins with the transfer of an amido (-NH_2) group from glutamine to PRPP catalyzed by PRAT (PRPP amidotransferase or ATase). (31)
2. There are ten reactions in the conversion of PRPP to IMP (inosine monophosphate) which is the precursor of purine nucleotide AMP. (32)
3. On the other reaction, IMP is converted to XMP (xanthine monophosphate) which is the precursor of purine nucleotide GMP. (33)

De novo pathway of purine nucleotide synthesis



ENZYMES

(1) PRAT (*phosphoribosylpyrophosphate amidotransferase*) atau ATase (*amido phosphoribosyltransferase*), (2) GARS (*glycinamide ribonucleotide synthetase*) atau GAR *synthetase*, (3) GART (*glycinamide ribonucleotide transformylase)* atau GAR *formyl transferase*, (4) FGARAT (*formylglycina-mide ribonucleotide amidotransferase*) atau FGAM *synthetase*, (5) AIRS (*aminoimidazole ribonucleotide synthetase*) atau AIR *synthetase*, (6) AIRC (*aminoimidazole ribonucleotide carboxylase*) atau AIR *carboxylase*, (7) SAICARS (*succinoaminoimidazolecarboxi-mide ribonucleotide synthetase*) atau SAICAR *synthetase*, (8) ASAL (*adenylosuccinate-AMP lyase*) atau *adenylosuccinate lyase*, (9) AICART (*aminoimidazolecarboximide ribonu-cleotide transformylase*) atau AICAR *formyl transferase*, (10) IMPCH (*inosine monophosphate cyclohydrolase*) atau IMP *cyclohydrolase*, (11) XDH/XO (*xanthine dehydrogenase/xanthine oxidase*), (12/11) SAMP *synthetase*, (13/12) *adenylosuccinase*, (14/13) IMP *dehydrogenase*, dan (15/14) GMP *synthetase.*

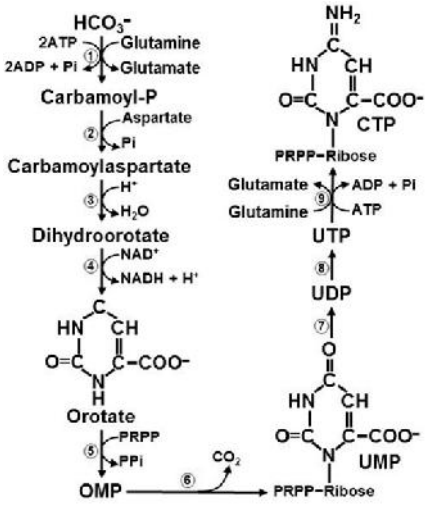
1.2 Pyrimidine Nucleotide Biosynthesis

1. The *De novo* pathway of pyrimidine nucleotide biosynthesis which is also referred to as the “orotate pathway” is usually defined as the formation of UMP from carbamoyl phosphate (CP). (34)
2. The organization, the control mechanism, and subcellular localization of the enzymes of the orotate pathway in plants are different from those in other organisms. (35) However, the sequence of biosynthetic events of pyrimidine nucleotides in plants is essentially the same as that in animals and microorganisms.
3. The orotate pathway consists of the six reactions with (36) the initial reaction catalyzed by CP synthetase (CPS) is the formation of CP by combination of carbonate, ATP and an amino group from glutamine.

De novo pathway of pyrimidine nucleotide synthesis

Figure 2. *De novo* biosynthetic pathway of pyrimidine nucleotides in plants. Enzymes shown are:

(1) Carbamoyl phosphate synthetase, (2) aspartate transcarbamoylase, (3) dihydroorotase, (4). dihydroorotate dehydrogenase, (5)-(6) UMP synthase (orotate phosphoribosyltransferase plus orotidine-5′-phosphate decarboxylase), (7) UMP kinase, (8) nucleoside diphosphate kinase, (9) CTP synthetase.

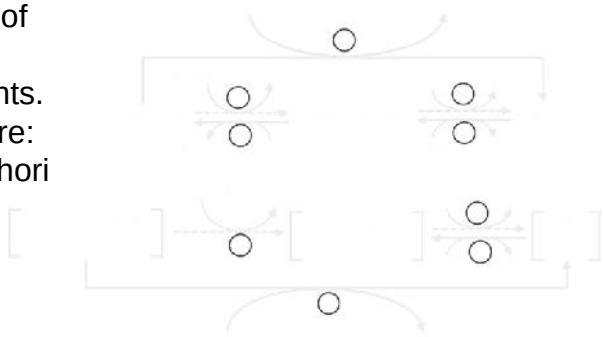


2. Salvage Pathways

2.1 Purine Nucleotide Biosynthesis

- The salvage pathway interconverts purine bases, nucleosides and nucleotides released as by-products of cellular metabolism (catabolism of nucleic acids or nucleotide cofactors). (37)
- This pathway is energetically favorable for a cell since only one salvage reaction requires ATP (phosphorylation of nucleosides to nucleotides). (38)
- For example, bases and nucleosides released from storage organs during germination or by senescencing leaves are recycled by this pathway. (39)
- Operation of the salvage pathway also reduces the levels of purine bases and nucleosides that may otherwise be inhibitory to other metabolic reactions. (40)

Salvage pathway of purine nucleotide synthesis

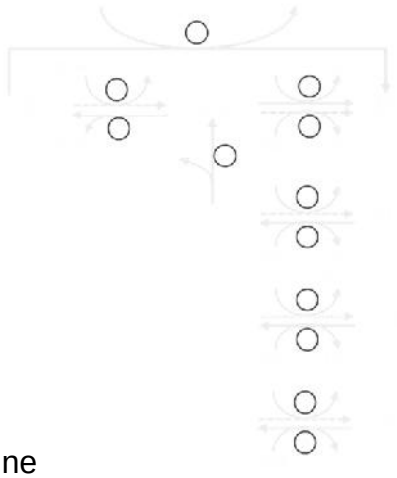


Salvage reactions of purine bases and nucleosides in plants. Enzymes shown are: (1) adenine phosphoribosytransferase, (2) adenosine phosphorylase, (3) adenosine kinase, (4) adenosine phosphorylase, (5) nucleoside nucleosidase, (6) inosine-guanosine phosphorylase, (7) inosine-guanosine kinase, (8) hypoxanthine-guanine phoshoribosyltransferase. Solid arrows: major reactions; dashed arrows: minor reactions.

2.2 Pyrimidine Nucleotide Biosynthesis

- In the salvage pathway of pyrimidine nucleotide biosynthesis, plant cells reutilize pyrimidine bases and nucleosides derived from the preformed nucleotides. (41)
- Of the bases, only uracil is directly reused via a specific phosphoribosyltransferase whereas the pyrimidine nucleosides, uridine, cytidine and deoxycytidine are exclusively salvaged to their respective nucleotides, UMP, CMP and dCMP. (42) (43)
- High activity of uridine/cytidine kinase and nucleoside phosphotransferase in plants may contribute the salvage of these nucleosides. (44)

Salvage pathway of pyrimidine nucleotide synthesis



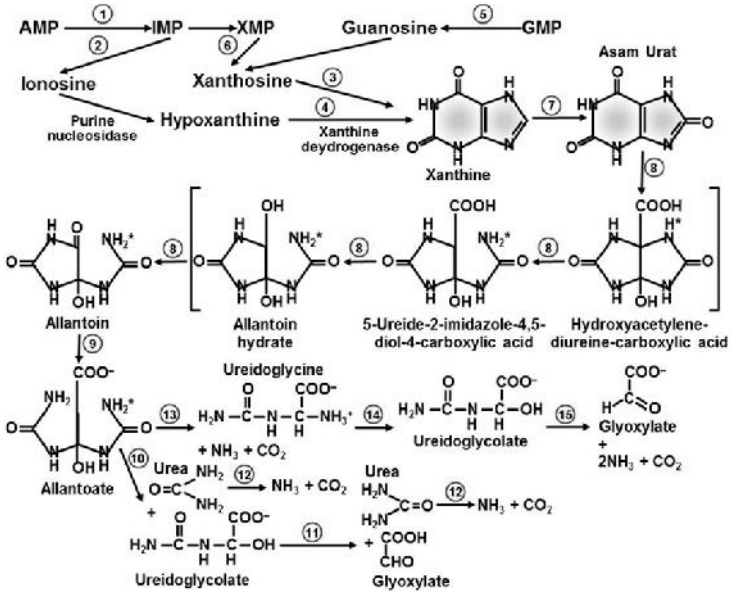
Pyrimidine salvage and related pathways in plants. Enzymes shown are: (1) Uracil phosphoribo-syltransferase, (2) uridine phosphorylase, (3) uridine kinase, (4) nucleoside phosphotransferase, (5) deoxycytidine kinase, (6) thymidine kinase, (7) cytidine deaminase, (8) uridine nucleosidase. Solid arrows: major reactions; dashed arrows: minor reactions

4. NUCLEOTIDE CATABOLISM

1. Catabolism of purine nucleotides

1. The end products of purine catabolism are different in different species. For example, uric acid is the end product of higher primates including man, however, allantoin is formed in other mammals. (45)
2. In most plants, purine nucleotides are degraded via ureides, allantoin and allantoate to NH_3 and CO_2 by the conventional purine catabolic pathway. (46)
3. In specific organs (e.g., roots) of ureide-accumula-ting plants, allantoin and/or allantoate are the end products of this pathway and they are translocated to other parts of the plant, such as shoots and leaves, where they are degraded completely. (47)

Catabolism of purine nucleotide

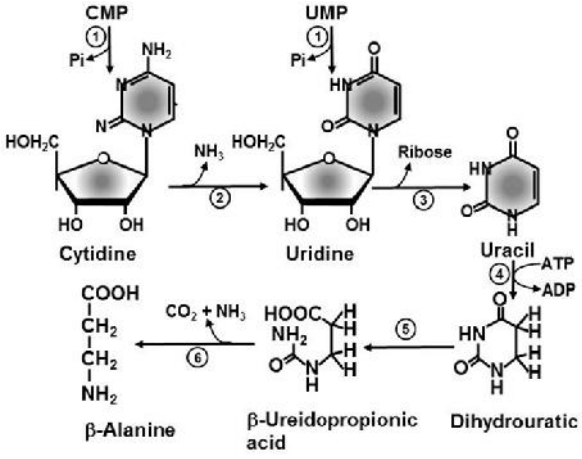


- Figure 5. Catabolism of purine nucleotides in plants. Enzymes shown are: (1) AMP deaminase, (2) IMP dehydrogenase, (3) 5′-nucleotidase, (4) inosine-guanosine nucleosidase, (5) guanosine deaminase, (6) guanine deaminase, (7) xanthine dehydrogenase, (8) uricase, (9) allantoinase, (10) allantoicase, (11) ureidoglycolate lyase, (12) urease, (13) allantoin deaminase, (14) ureidoglycine amidohydrolase, (15) ureidoglycolate hydrolase.

Catabolism of pyrimidine nucleotides

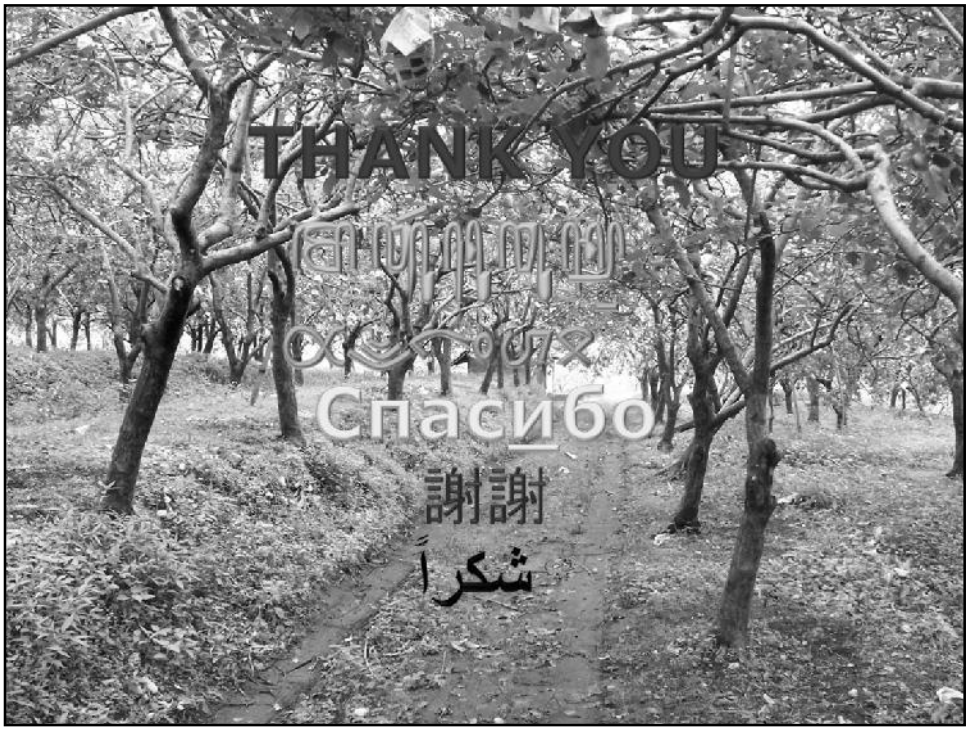
1. Pyrimidine nucleotides seem to be catabolised to pyrimidine bases, uracil and thymine, via their nucleosides. (47)
2. Uracil and thymine are catabolized by the three sequential reactions catalyzed by dihydrouracil dehydrogenase, dihydropyrimidinase and β-ureidopropionase. (48)
3. The end products of this pathway are either β -alanine or β -aminoisobutyrate depending upon whether uracil or thymine are catabolised by this pathway. In both cases, NH_3 and CO_2 are released as by products. (49)

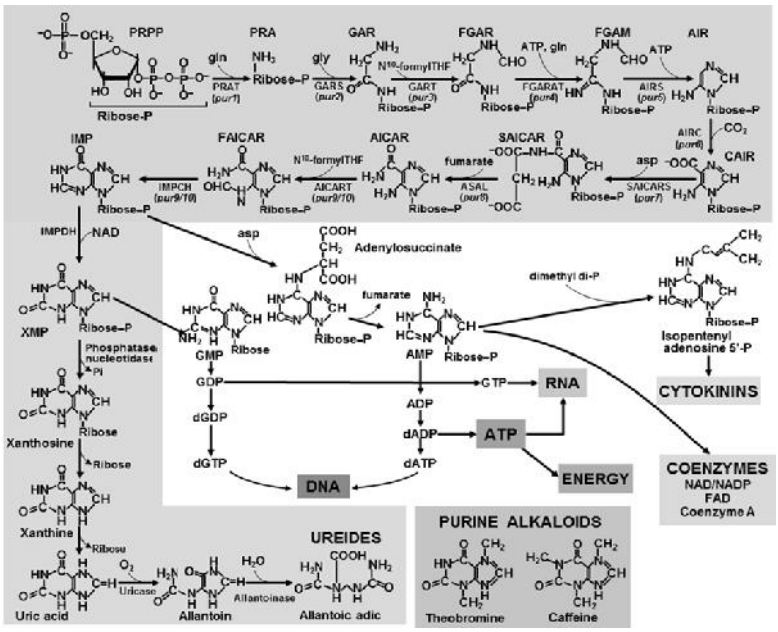
Catabolism of pyrimidine nucleotide



Catabolism of pyrimidine nucleotides in plants. Enzymes shown are: (1) 5′-nucleotidase, (2) cytidine deaminase, (3) uridine nucleosidase, (4) dihydrouracil dehydrogenase, (5) dihydropyriminase, (6) b-ureidopropionase.

3. Catabolism of cytosine nucleotides may proceed via uridine since cytosine is not the substrate of this pyrimidine reductive pathway and plants lack cytosine deaminase. (50)
4. Cytidine formed from CMP can be converted to uridine via cytidine deaminase. An Arabidopsis cDNA encoding cytidine deaminase (AT-CDA1) was recently cloned (51)





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4. The first committed step of the purine pathway, catalyzed by phosphoribosylpyrophosphate amidotransferase (PRAT), is one of a number of possible metabolic fates for both substrates (phosphoribosylpyrophosphate [PRPP] and Gln [gln]).
5. The final product of the pathway, inosine monophosphate (IMP), is the first purine nucleotide and provides the basic purine ring structure for the other purine nucleotides, xanthosine monophosphate (XMP) AMP, and GMP.
6. The last two of these are the building blocks of DNA and RNA, whereas AMP is the precursor for the cytokinin group of plant growth regulators and a number of important coenzymes. GTP and ATP participate in the energy metabolism of the cell.

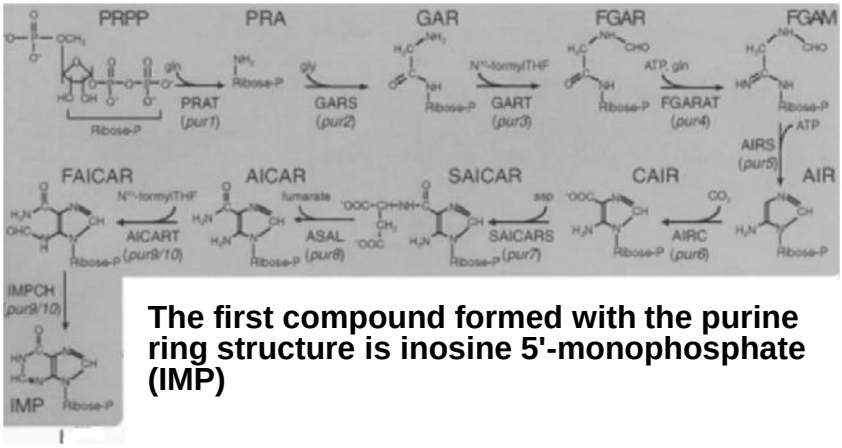
7. Although there are a number of routes that can generate the purine bases from IMP, in legume nodules the preferred route is through IMP dehydrogenase (IMPDH).
8. Both xanthosine and xanthine serve as precursors for the purine alkaloids (theobromine and caffeine) and their further oxidation yields the ureides, allantoin and allantoic acid.
9. Enzyme names are abbreviated as follows:
   - glycinamide ribonucleotide synthetase (GARS),
   - glycinamide ribonucleotide transformylase (GART),
   - formylglycinamide ribonucle-otide amidotransferase (FGARAT),
   - aminoimidazole ribonucleotide synthetase (AIRS),

- aminoimidazole ribonucleotide carboxylase (AIRC),
- succinoaminoimidazolecarboximide ribonucleotide synthetase (SAICARS),
- adenylosuccinate-AMP lyase (ASAL),
- aminoimidazolecarboximide ribonucleotide transformylase (AICART),
- inosine monophosphate cyclohydrolase (IMPCH), and
- xanthine dehydrogenase/xanthine oxidase (XDH/XO).

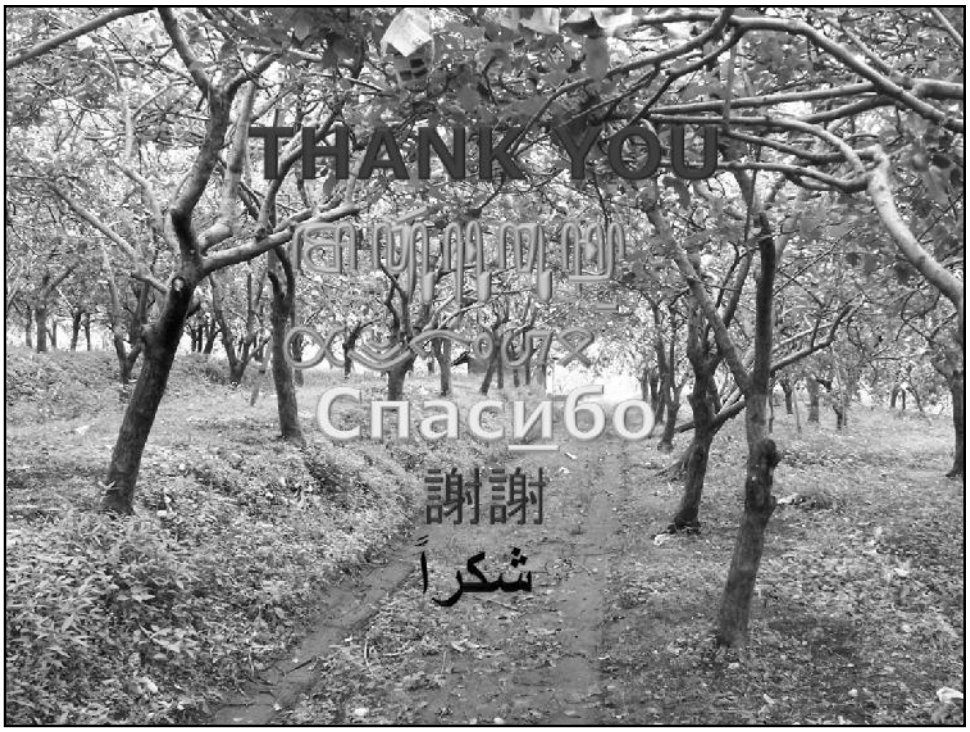
10. Substrate names are abbreviated as follows:
    - phosphoribosylamine (PRA),
    - glycinamide ribonucleotide (GAR),
    - formylglycinamide ribonucleotide (FGAR),
    - formylglycinamidine ribonucleotide (FGAM),
    - aminoimidazole ribonucleotide (AIR),

- carboximideaminoimidazole ribonucleotide (CAIR),
- succinoaminoimidazolecarboximide ribonucleotide (SAICAR),
- aminoimidazolecarboximide ribonucleotide (AICAR),
- formylaminoimidazolecarboximide ribonucleotide (FAICAR),
- XMP, GMP, Gln (gln), Gly (gly), N10-formyl tetrahydrofolate (N10-formyl THF), Asp (asp), and inorganic phosphate (Pi).

11. Genes encoding the enzymes of the purine pathway are shown in parentheses beneath the enzyme abbreviation.



The first compound formed with the purine ring structure is inosine 5'-monophosphate (IMP)


THANK YOU
Спасибо
謝謝
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