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CONJUGATED PROTEINS: STRUCTURE, FUNCTIONS AND METABOLISM

Textbook for students of international faculty
Speciality: 7.120 10001 «General Medicine»

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This textbook is recommended to use for students of international faculty (the second year of study) for independent work at home and in class. It is created as additional manual for study of Biochemistry for students of international faculty.

Александрова К.В.

Складні білки: структура, функції та метаболізм. Начально-методичний посібник для студентів міжнародного факультету спеціальності 7.120 10001 «Лікувальна справа»/ К.В. Александрова, Н.В. Крісанова, Д.Г. Іванченко, Н.П. Рудько,.- Запоріжжя : ЗДМУ, 2016. – 100 с.

INTRODUCTION

The educational process for students of medical department requires the use not only the basic literature but also that one which is discussed as additional literature sources. This is because each day we have new scientific researches in biochemistry, later which can improve our understanding of theoretical questions this subject. Sometimes it is difficult for students to find out the main important notions for study of biochemistry in basic literature that is recommended. This manual is proposed by authors as additional one for study of conjugated proteins: their structure, properties, functions and metabolism in human organism.

Authors

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Chapter 1

LIPOPROTEINS: STRUCTURE, PROPERTIES, FUNCTIONS AND METABOLISM

Created by ass.pr.Ivanchenko D.G., ass.pr. Krisanova N.V.

Lipoproteins: classification, composition and function

Lipoproteins (LP) are conjugated proteins containing lipid component as the non-protein part. They are represented in humans by LP found in blood plasma and by LP found in nervous tissue. First LP are the transport form for lipids in the blood stream. In this chapter these LP will be described mainly.

Any class contains all the lipids (except high fatty acids, because they are transported mainly by plasma albumins) and special simple proteins named apolipoproteins. The main class of LP that is formed in the small intestine wall is Chylomicrons (ChM). ChM are used for transport of lipids from the small intestine across the lymph and blood to the liver. But there are other classes of LP in the blood plasma, too:

VLDL – Very Low Density Lipoproteins;

LDL - Low Density Lipoproteins;

HDL – High Density Lipoproteins;

IDL - Intermediate Density Lipoproteins.

A class name according density is associated with the separation method – ultracentrifugation. It is made so: sucrose solutions with various concentrations are added step by step to the centrifugal test tube, and the blood plasma of patient is plotted on the surface of this mixture. Then there is the centrifugation using special centrifuge that gives 50000g. Centrifugation is complex and lasts about 24 hours. All LP of plasma are separated after this time, and may be allocated, using special pipette from this test tube. Lipoproteins of blood plasma are represented in figure 2 and in table 1. The information placed in figure 1 gives the understanding of three important things about LP:

1. All LP are micelle particles in blood plasma, surface of which is created by monolayer of phospholipids and some proteins; most hydrophobic lipids are placed inside micelle (triacylglycerols, cholesterol esters).

2. The diameter of micelle particle for each class is not the same: the highest diameter is found for Chylomicrons (ChM), and the least diameter is for HDL.

3. The biggest density is found for HDL, and the least density is for ChM.

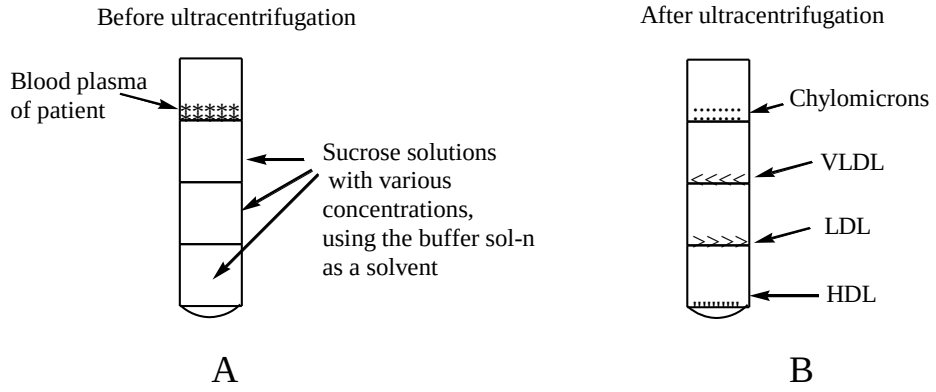


Figure 1. **Ultracentrifugation:** *(A) - preparation of the mixture content before centrifugation; (B) - separated layers of LP in the test tube after centrifugation.*

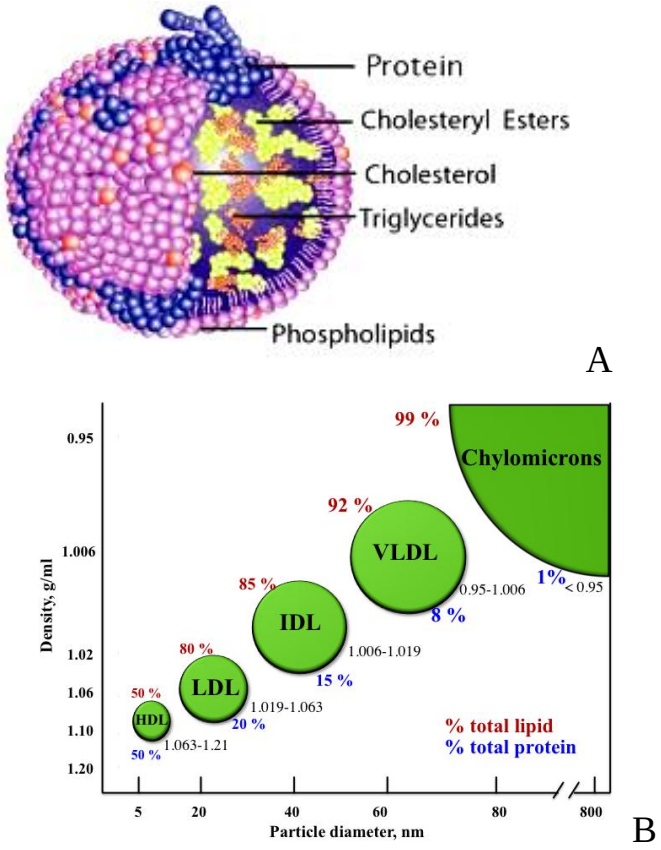


Figure 2. **Micelle particle for lipoprotein molecule in blood plasma (A); a diagram for density of each class of LP (B).**

Table 1. **Main characteristics of blood plasma lipoproteins**

LP class	Main lipids represented	Apoprotein class represented in LP	Density, g/ml	Diameter of micelle , Å
ChM	TG>>CHL	A-I, A-II, A-IV, B-48 C-I, C-II, C-III, E	<0,95	800-5000
VLDL	TG >> CHL	B-100, C-I, C-II, C-III, E	<1,006	300-800
IDL	TG = CHL	B-100, E	1,006-1,019	250-350
LDL	CHL > TG	B-100	1,019-1,063	180-280
HDL	CHL >> TG	A-I, A-II, C-I, C-II, C-III	1,125-1,210	50-90
TG - triacylglycerols; CHL - cholesterol; ChM -chylomicrons				

The charge of micelle particles for each class of LP in the blood plasma is also not the same, that is because, it is possible to use electrophoresis method for separation of LP. It is made so:

Poly Acryl Amide Gel (PAAG) may be used as a carrier for plotting of blood serum. pH of buffer solution must be about 7,4-7,6 and after electrophoresis (about 1.5 hours), and after the painting of fractions may be found result shown in figure 3:

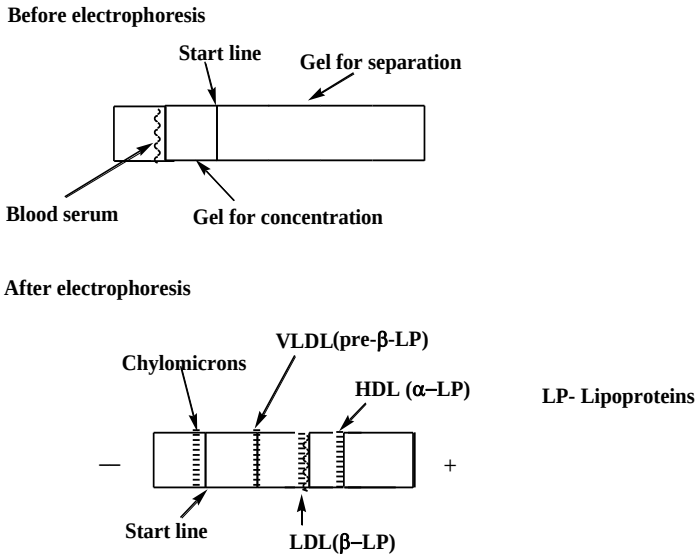


Figure 3. **Electrophoresis for LP separation with the use of PAAG**.

All LP contain the same lipids but in various concentrations:

[Triacylglycerols] : VLDL > ChM > LDL > HDL

[Total Cholesterol]: LDL> VLDL ≥ HDL >ChM

[Cholesterol esters]: LDL> VLDL ≥ HDL >ChM

[Cholesterol free]: LDL ≥ VLDL > HDL ≥ ChM

[Glycerophospholipds]: HDL > LDL > VLDL > ChM

[Proteins]: HDL > LDL > VLDL > ChM

Table 2. **Classes of Lipoprotein Particles** *(PL –Phospholipids, ChL- Cholesterol, ChLE - Cholesteryl esters, TAG – Triacylglycerols)*

Class	Diametr (nm)	Density (g/ml)	Composition (weight %)*				
			Surface components			Core lipids	
			Protein	PL	ChL	ChLE	TAG
Chylo-microns	75-1200	0.930	2	7	2	3	86
VLDL	30-80	0.930-1.006	8	18	7	12	55
IDL	25-35	1.006-1.019	19	19	9	29	23
LDL	18-25	1.019-1.063	22	22	8	42	6
HDL2	9-12	1.063-1.125	40	33	5	17	5
HDL3	5-9	1.125-1.210	45	35	4	13	3
Lp(a)	25-30	1.040-1.090					

*Data from Havel, R. J., and Kane, J. P. (1995) in *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II (Scriver C. R., Beaudet A.L., Sly W. S., and Valle D., eds), pp. 1841 – 1852, McGraw-Hill, New York.

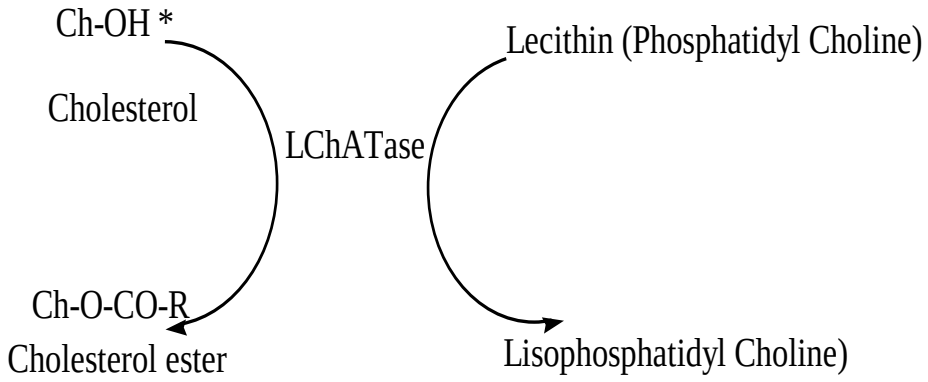
It should be noted that high fatty acids (HFA) are not transported by LP, as a rule, their content in LP is less than 1% from all HFA present in blood plasma. Albumins produced by the liver are in transport of HFA in the blood.

There are about 9 classes of apoproteins that are in the composition of LP, some of them represented in tables 1, 2. These types of proteins are used for the

contact with receptors in target cells or with other lipoproteins during their interactions in the blood stream, other function may be discussed for them also (table 2). Besides them, there are in the structure of LP:

1) Triacylglycerol lipase – linked with VLDL. It catalyzes lipolysis of TG in VLDL during the transformation of VLDL to IDL, and then to LDL.

2) Lecithin Cholesterol Acyl Transferase (LChATase). Its action is represented in the figure N3. *LChATase* is linked to HDL-precursors (nascent form; apoprotein ratio A_1:E=1:10) that are synthesized in the liver, and then move to the blood stream and are converted into HDL with apoprotein ratio A_1:E=7:2 (remnant form). HDL-precursor has Cholesterol in a free form and high concentration of Lecithin. Remnant form of HDL has high concentration of Cholesterol esters. The task of *LChATase* to form cholesterol esters from excess cholesterol free, that is taken up from peripheral tissues (figure 4) to be transported the liver in the composition of remnant HDL.



* -- Cholesterol (Ch-OH) from a cell of peripheral tissue or Cholesterol in HDL-precursor

Figure 4. **The action of Lecitin Cholesterol Acyl Transferase.**

Table 3. **Properties of Major Plasma Apolipoproteins**

Designation	**№ residues**	**Mass (kDa)**	**Source**	**Function**
A-I	243	29		Major HDL protein, cofactor of LCAT
A-II	–	17.4	Liver and intestine	Unknown
A-IV	376	44.5	Intestine	Unknown
B-100	4536	513	Liver	VLDL formation; ligand for LDL receptor
B-48	2152	241	Intestine	Chylomicron formation, ligand for liver Chm receptor
C-I	57	6.6	Liver	Inhibition of Cholesteryl ester transfer protein
C-II	79	8.9	Liver	Cofactor for lipoprotein lipase
C-III	79	8.8	Liver	Inhibits lipoprotein lipase and hepatic lipase
D	–	31	Many tissues	Structural component of lipocalins (are a family of proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids)
E	299	34	Liver, VLDL	Ligand for Chm receptor
(a)	Variable			Ligand for liver chylomicron receptor

VLDL are synthesized in the liver to transfer lipids from the liver to the blood, then there is their conversion to LDL: VLDL→ IDL → LDL (in the blood).

The concentration of TG becomes lower in LP during this conversion (due to the action of two enzymes: Triacylglycerol lipase linked with VLDL and Endothelial Triacylglycerol lipase of blood vessels. They cleave the ester bonds
In structures which are analogs to which are shown in fig.5 these enzymes form free glycerol and fatty acids.

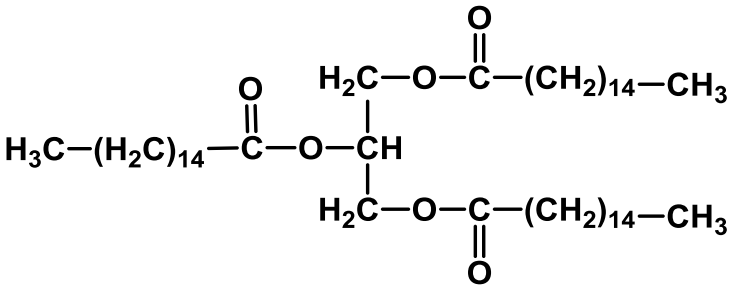


Figure 5. **Tripalmitin structure**.

A composition of apoproteins is changed, too: VLDL contain [apoprotein B_{48} and B_{100}] < [apoprotein E]; LDL contain [apoprotein B_{100}] >> [apoprotein E].

Apoprotein B_{100} is a site of LDL binding with apoB100 receptor on the outer surface of cytoplasm membrane of peripheral cell (endothelial cell of blood vessel or another type of cell where Cholesterol is in need).

LDL is discussed as main transport form for Cholesterol from the blood to peripheral tissues: adrenal cortex, gonads, vessel wall, skin (figure 5). Scientists supposed that they are the most atherogenic LP, because they stimulate the development of atherosclerosis of arteries when they are in high levels in the blood.

Metabolism of chylomicrons

Chylomicrons are produced in the small intestine wall after resynthesis of lipids there, using molecules of apoprotein E and B48 (the latter is produced only there). It should be noted, that this class of LP is produced after lipids intake from food sources, and it is absent in healthy person blood on empty stomach (to be

without food about 12-14 hours). Nascent form of ChM is reach in TG and apoprotein E, during circulation in the blood stream they are derived into remnant form. It means that content of TG is decreased due to lipolysis by endothelial TG-lipase, and the content of apoprotein E is increased due to the contact of Chylomicrons with other classes of LP in the blood. Chylomicrons promote also the transport of dietary cholesterol from the small intestine wall to the liver, and utilization of them in the liver is discussed as receptor-depended endocytosis due to the linkage of apoprotein E with its receptor on the cellular membrane of hepatocytes. Catabolism of chylomicrons is found also in the brain marrow, spleen and adipose tissue. ChM are in need to transfer fat-soluble vitamins also which are used in hematopoiesis.

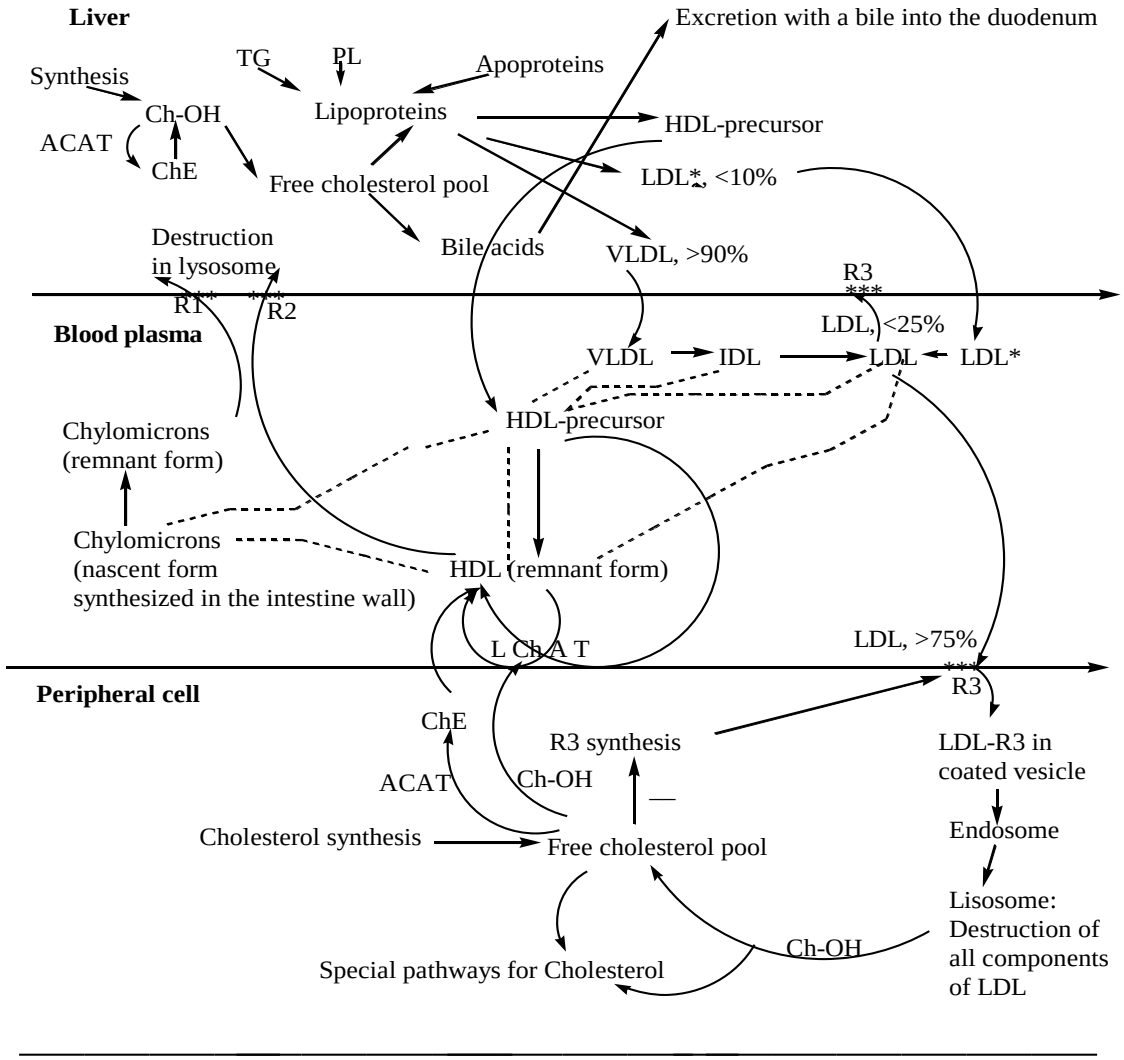
Metabolism of VLDL and LDL

About 10% of nascent VLDL is produced in the small intestine wall, other 90% of VLDL is produced in the liver. Synthesis of VLDL in the liver is stimulated by the increase of free high fatty acids content, which are transported to the liver in complexes with albumins, and by the increase of cholesterol synthesis rate too. All the excess lipids, which are produced in the liver may be involved in the formation of nascent VLDL. That VLDL, which are produced in the intestine wall are differ in the composition of apoproteins: apoprotein B48 is found in their content but not in VLDL produced in the liver. All these molecules move to the blood stream and undergo special conversions:

VLDL → IDL → LDL , it means:

- Degradation of TG due to Lipoprotein lipases
- The decrease of Lecithin content
- The increase of total cholesterol content
- The decrease of apoprotein E content
- The increase of apoprotein B100 content

Those conversions give remnant VLDL and prepare LDL for the degradation in peripheral tissues mainly (fig.6, 7).



Ch-OH, Cholesterol; ChE, Cholesterol ester; PL, Phospholipids; TG, Triacylglycerols; ACAT - Acyl-CoA Cholesterol Acyltransferase; LChAT - Lecithin Cholesterol Acyltransferase; R1- ApoE receptor; R2- Apo A1 receptor; R3- ApoB100 receptor; LDL* - LDL synthesized in the liver; IDL -Intermediate Density Lipoproteins.

---------- Interaction between different classes of lipoproteins

Figure 6. **The metabolism of plasma lipoproteins in humans**

About 25% of LDL is produced in the liver as nascent form, and 75% LDL is produced from VLDL in the blood. Utilization of LDL is also receptor-depended endocytosis mechanism (M. Brown, I. Coldstein, 1985), and content of apoB100-receptor in peripheral cell is the main factor to control the rate of LDL destruction in this cell (fig.6). LDL-apoB100-receptor complexes move in cytoplasm as endosomes, which later coupled with lysosomes for the destruction inside them. This mechanism helps to promote normal levels of Cholesterol and LDL in the blood stream to prevent the development of atherosclerosis.

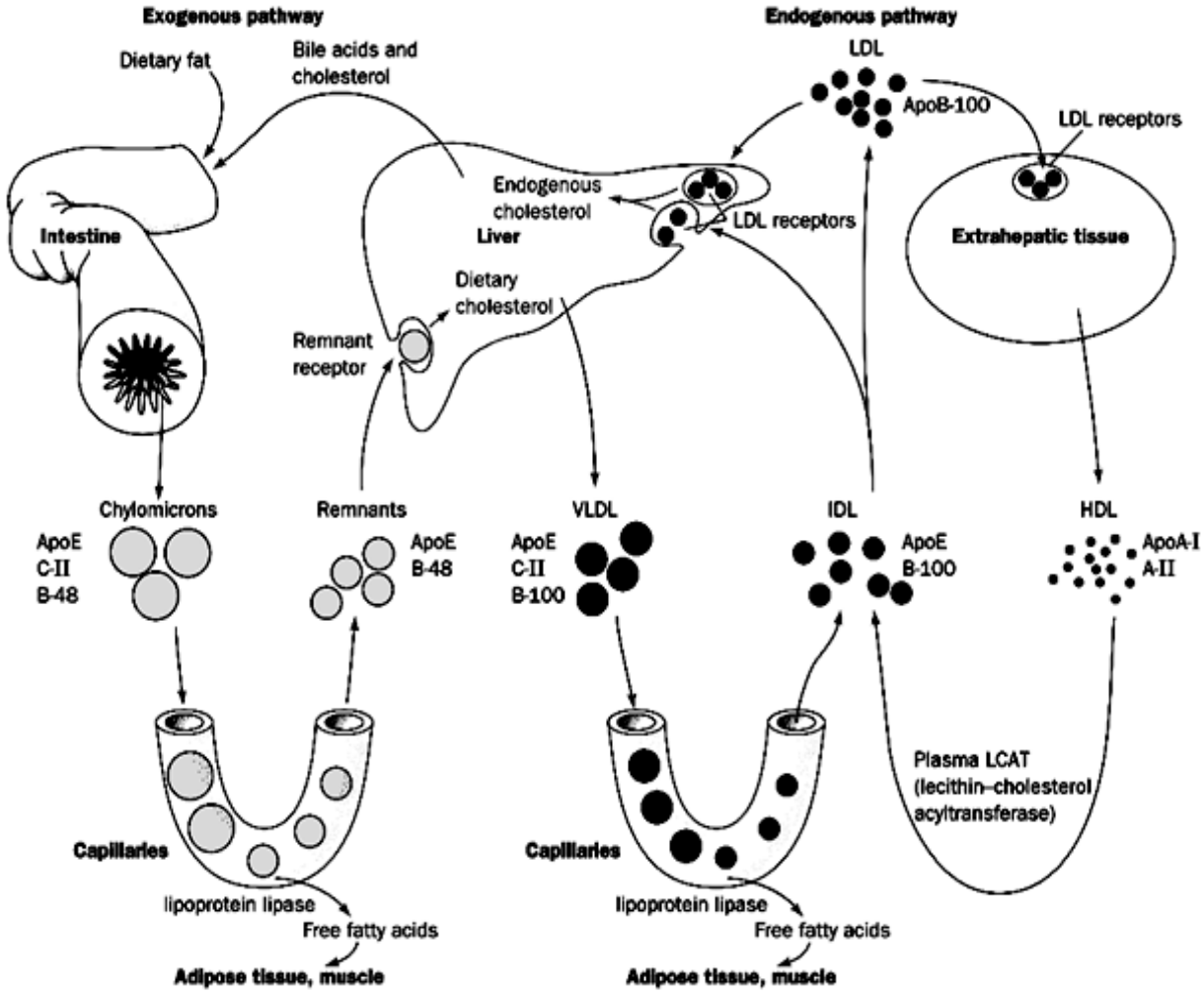


Figure 7. **Model for plasma triacylglycerol and cholesterol transport in humans**.

Receptor can move back (time of life 1-2 days/ 150 cycles) to cellular membrane. Synthesis of this receptor is inhibited when there is the accumulation of free cholesterol in this peripheral cell (fig. 8).

This situation can change the life span of LDL from 2.5days up to 5-6 days in the blood circulation. As the result LDL may be modified and will be recognized as foreign molecules by antibodies in the blood plasma.

The excess cholesterol is removed from peripheral tissues by HDL, but before its esterification must be made by *LChATase* linked to HDL.

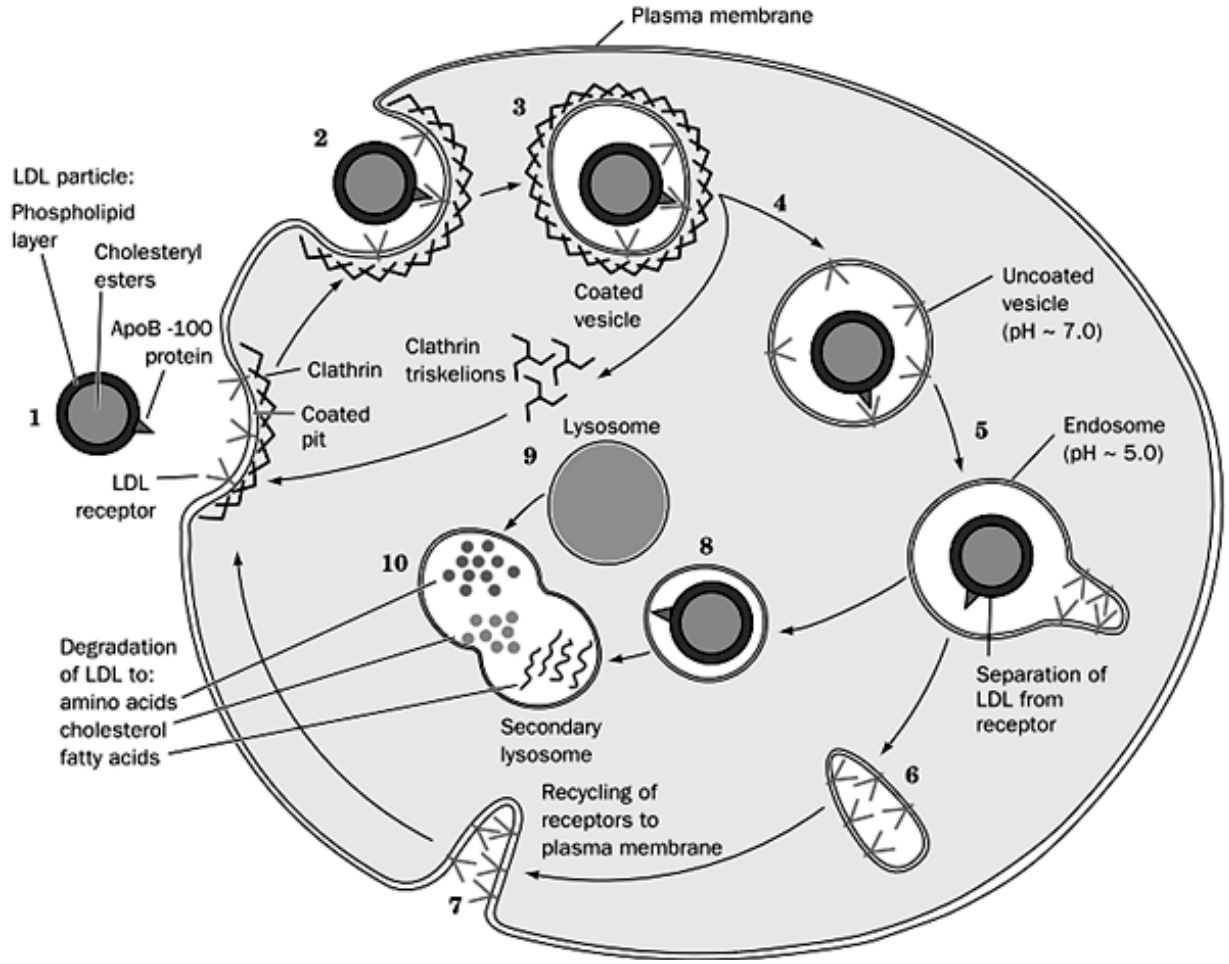


Figure 8. **Receptor-depended endocytosis mechanism for utilization of LDL.**

Metabolism of HDL

HDL nascent form (HDL*, apoprotein ratio A_1:E=1:10) is produced mainly by the liver (Stein Y, Stein O, 1987) as three fractions which later are derived into remnant form of HDL (apoprotein ratio A_1:E=7:2). LChATase is placed inside these complexes to make conversion with the formation of cholesterol esters. Main conversions when HDL* →… → HDL are:

- The decrease of TG
- The increase of Cholesterol esters (LCAT)
- The decrease of Lecithin (LCAT)
- The increase of Lisophosphatidyl choline (LCAT)
- The decrease of apoprotein E
- The increase of apoprotein A1

These conversions prepare HDL for destruction in the liver mainly, because the content of apoA1-receptor in the liver is the highest among all types of tissue. The function of LChATase promotes the removal of excess cholesterol from peripheral tissues, and its location gives ability for HDL to be as transporter of cholesterol from peripheral tissues to the liver, that is because they are named as anti-atherogenic lipoproteins. This property also is promoted by the ability of HDL to move to extracellular matrix, because diameter of their particles is less then for other classes of LP (figure 2), to take excess cholesterol from endothelial tissue of blood vessels for the destruction of it in the liver up to bile acids. Excretion of the bile containing potion of free cholesterol and bile acids as salts to duodenum is the way to decrease cholesterol content in human organism.

Atherosclerosis of blood vessels as the main disease associated with disorders in lipoprotein metabolism

All the conditions for the development of this disease in human person:

Hypercholesterolemia state more then 5.72 mmole/L associated with:

- High concentrations of VLDL with normal levels of LDL;
- Elevated LDL with normal VLDL;
- Elevation of both lipoprotein fractions (LDL and VLDL);
- Decrease of HDL levels lesser then 0.9 mmole /L

The most predictive relationship is the CHL_{LDL}:CHL_{HDL} ratio, and it must be higher then 3.5 to cause the development of this disorder.

Scientists said that CHL_{LDL} is abnormal when it is higher then 5.0 mmole/L and for CHL_{HDL} lesser then 0.9 mmole/L.

Main reasons for the accumulation of cholesterol and its transport forms in blood plasma

Primary reasons - genetic defects associated with:

- deficiency of apoB-100 receptors to LDL in the liver
- deficiency or decrease of the activity of lipoprotein lipase needed for triacylglycerols degradation in VLDL

• deficiency of Lecithin Cholesterol Acyl Transferase linked with HDL

The appearance of special proteins:

• Lipoprotein (a) levels in the blood are higher then 0.3 g/L

• β-VLDL (similar to IDL but not converted to LDL, rich in a content of CHL

• Apoprotein E4 is represented in high content instead of other isoforms of apoE.

Secondary reasons of atherosclerosis may be associated with development of:

• Diabetes mellitus: VLDL↑, HDL↓, CHL ↑

• Hypothyroidism: LDL ↑, HDL↓ or normal, CHL ↑

• Renal insufficiency:VLDL↑, HDL↓, LDL ↑ or normal

• Nephritic syndrome: VLDL↑, HDL↓, LDL ↑, CHL ↑

• Cholestasis: CHL ↑, HDL↓, LDL ↑

In any case the accumulation of LDL is dangerous, because the increase of their content and circulation time in the blood can cause the increase of:

• The rate of LDL penetration to the extra-cellular matrix of arterial wall to be utilized there

• The rate of LDL modification to produce from them foreign for humans lipoproteins (FLP) thus to stimulate the production of special antibodies for FLP by immune system

• The FLP-antibody complex is utilized by macrophages across the pene-tration of them to the extra-cellular matrix of arterial wall

• Uptake of LDL and FLP-antibody complex by macrophages in the arterial wall is an important event in the pathogenesis of atherosclerosis. When macrophages become overloaded with cholesterol esters, they are converted to "foam cells", the classic components of atheromatous plaques.

Additional Reasons of Hyperlipoproteinemies development in humans

Hyperlipoproteinemia is a metabolic disorder characterized by abnormally elevated concentrations of specific lipoprotein particles in the plasma. According the classification (Fridrickson E., et all) hyperlipoproteinemies are divided in five types:

- ***Type I (Hyperchylomicronemia).*** Defect in human organism: decreased lipoprotein lipase, altered ApoC-II. Diagnostic results: very high level of chylomicrons in the blood serum on empty stomach. Slight higher levels for VLDL and triacylglycerols. Xanthomatosis in patients is associated with this type of hyperlipoproteinemia, and obesity may be in patient, too.
- ***Type II (Hyper-β-lipoproteinemia). Subtype IIa***. Defect in human organism: LDL receptor deficiency. Diagnostic results: high levels of LDL and total cholesterol in patients.
- ***Type II (Hyper-β-lipoproteinemia). Subtype IIb***. Defect in human organism: decreased LDL receptor and increased ApoB. Diagnostic results: high levels of LDL, VLDL, cholesterol and triacylglycerols. Ischemic heart disease and hypertension is observed at patients.
- ***Type III (Dis-β-lipoproteinemia).*** Defect in human organism: ApoE-II synthesis. Diagnostic results: high levels of IDL in the blood plasm that are absent at healthy adults, high levels of cholesterol. These changes associated with problems in heart system: atherosclerosis of blood vessels, thrombosis may be at patient too.
- ***Type IV (Hyper-pre-β-lipoproteinemia).*** Diagnostic results: high levels of VLDL, but LDL are slight higher or normal. Chylomicrons are absent. Diabetes mellitus with obesity and ischemic heart disease are associated with this type of hyperlipoproteinemia.
- ***Type V (Hyper-pre-β-lipoproteinemia accompanied with Hyperchylomicronemia).*** Diagnostic results: high levels of chylomicrons and VLDL. Xanthomatosis is represented, also. This state may be in patients with latent form of insulin-independent diabetes mellitus, but ischemic heart disease is not observed at patient, in this case.

The treatment of hypercholesterolemia state depends upon its reason. It may include:

1) ***Cholesterol free diet*** (for all reasons of this state);

2) ***Unsaturated High fatty acids*** must be in high concentration in food products (for all reasons of this state)

Fish oils. These contain high levels of omega-3 fatty acids, which have a number of properties that could explain why fish oils or a diet high in oily fi sh have a protective effect:

• They lower the blood level of VLDL and therefore they lower plasma level of LDL.

• They decrease the formation of blood clots.

• They decrease the formation of thromboxane A_2 and prostacyclin I_2 in favour of thromboxane A_3 and prostacyclin I_3, changes which protect against thrombosis.

• They have a hypotensive effect.

The major omega-3 fatty acid in fish oil is eicosapentaenoic acid, which contains five double bonds compared with only four present in the omega-6 fatty acid, arachidonic acid. When eicosapentaenoic acid is substrate for eicosanoid production, it gives rise to prostacyclins and thromboxanes of the three series whereas when arachidonic acid is substrate, it gives rise to the two series, thromboxane A_2 and prostacyclin I_2. Thromboxane A_3 has much less of a thrombolytic effect than thromboxane A_2 whereas prostacyclin I_3 has more of an antithrombotic activity than prostacyclin I_2. Hence, the risk of formation of a thrombus is decreased when omega-3 fatty acids are the substrate for the cyclooxygenase. There is considerable epidemiological evidence that fish oils are protective against atherosclerosis;

3) ***Antioxidants.*** These are naturally occurring compounds that have the ability to lower the levels of free radicals: they include vitamins C and E, the carotenoids and the flavonoids. Vitamin E and the carotenoids are particularly important in preventing oxidation of the unsaturated fatty acids within the LDL particle and within membranes of cells;

4) ***Physical activity.*** Evidence for the beneficial effects of physical activity on the development of atherosclerosis first arose from a series of epidemiological

studies. This activity is now known to cause several changes, all of which are benefi cial in decreasing the risk of development of atherosclerosis. These are:

- a fall in the total serum level of cholesterol;
- an increase in the serum HDL-cholesterol level;
- a fall in the serum LDL-cholesterol level;
- a fall in the plasma triacylglycerol level;
- loss of weight;
- reduction of blood pressure.

It also increases the sensitivity of tissues to insulin, which may provide better control of the blood glucose level to minimise the risk of damage to LDL by glycosylation;

5) ***The use of drugs – inhibitors for β-hydroxy-β-methylglutaryl-CoA reductase***: Lovastatin, Mevastatin;

6) ***The use of drug – Cholestyramine resin*** to block the reabsorption of bile acids in the small intestine. In this case the cholesterol is utilized in higher quantity up to bile acids.

7) ***The use of drugs Clofibrate an gemfibrozil*** that divert the hepatic inflow of free fatty acids into oxidation, thus decreasing the secretion of VLDL by the liver. This drugs stimulate the hydrolysis of VLDL triacylglycerols by lipoprotein lipase;

8) ***The use of drug Probucol.*** It increases the rate of LDL catabolism via receptor-independent pathways, but its antioxidant properties may be more important in preventing accumulation of oxidized LDL in arterial walls;

9) ***The use of Nicotinic acid*** to reduce the flux of fatty acids by inhibiting of adipose tissue lipolysis thereby inhibiting VLDL production by the liver.

Features of LP metabolism at obesity

Obesity is an abnormal increase in the body weight due to excessive fat deposition. Men and women are considered as obese if their weight due to fat (in adipose tissue), respectively, exceeds more than 20 % and 25 % of body weight. Obesity is essentially an excessive accumulation of triacylglycerols in fatty tissue that is the net result of excessive energy intake compared to energy usage. Severe

forms of the disease are most likely to have a predominantly genetic basis and this is probably polygenic. The ‘thrifty gene’ hypothesis also describes the disturbance that a modern environment, including higher energy intake and decreased physical activity, has on otherwise advantageous genetic variations. While the physical consequences of obesity, such as arthritis, are debilitating and costly, the metabolic consequences are the drivers behind the modern epidemics of insulin resistance, diabetes, fatty liver disease, coronary artery disease, hypertension and polycystic ovary syndrome. The pathophysiological mechanisms behind these diseases are probably a combination of the toxic metabolic effects of free fatty acids and adipokines - the numerous messengers that adipose tissue has been discovered to produce.

Under the Obesity development there is the increase of VLDL (containing mostly triacylglycerols) levels through increased production and decreased clearance of triacylglycerols rich lipoproteins due to lack of stimulation of lipoprotein lipase. At Obesity HDL content is also lower in men and women of all ages and ethnicities. While LDL levels are not consistently elevated in obesity, LDL is smaller and denser and more atherogenic in patients with this pathology.

Cholesterol ester transport protein (CETP) exchanges triacylglycerols from VLDL to LDL in exchange for cholesterol esters. This results in triglyceride rich LDL particles that are rapidly lipolysed by hepatic lipase leaving smaller denser LDL particles. Small dense LDL can more easily be oxidised or glycated possibly leading to less identification by the LDL receptor and decreased clearance. Possibly small dense LDL is also more likely to get through endothelial fenestrations.

CETP also exchanges triglycerides from VLDL to HDL in exchange for cholesterol esters. This similarly results in triglyceride rich HDL particles that are rapidly lipolysed by hepatic lipase allowing HDL to be cleared from the circulation.

High fasting triglyceride levels (i.e. VLDL) predict the presence of small dense LDL in diabetes, non-diabetics and hypopituitarism (decreased secretion of

pituitary gland hormones). Remarkably the value of triglyceride that predicts the small dense LDL phenotype is about 1.5 mmol/L, the same level used in the definitions of metabolic syndrome. More specifically the logarithm of the triglyceride concentration is inversely related to particle size. The total cholesterol to CHL_{HDL} is more predictive than the CHL_{LDL}: CHL_{HDL} ratio because it mathematically includes also the component TG/HDL which is crucially abnormal in insulin resistance and predicts the presence of small dense LDL.

There is strong evidence to suggest that obesity has genetic basis. Thus, a child born to two obese people has about 25% chances of being obese. One gene namely *ob gene*, expressed in adipocytes (of white adipose tissue) producing a protein named *leptin,* is closely associated with obesity.

Leptin is regarded as a body weight regulatory hormone. It binds to a specific receptor in the brain and functions as a lipostat. When the fat stores in the adipose tissue are adequate, leptin levels are high. This signals to restrict the feeding behaviour and limit fat deposition. Further, leptin stimulates lipolysis and inhibits lipogenesis. Any genetic defect in leptin or its receptor will lead to extreme overeating and massive obesity. Treatment of such obese individuals with leptin has been shown to reverse obesity.

During starvation, leptin levels fall which promote feeding, and fat production and its deposition.

Obesity is a major problem in developed and increasingly in underdeveloped countries. There is particular concern about the marked increase in obesity in children, which can lead to major health problems in later life and will result in a massive increase in financial expenditure on health provision in the future: some obese children are now developing type 2 diabetes as young as 12.

There are at least three concerns about the diet of children in developed countries since they may lead to disease in adulthood or even earlier. The concerns are related to:

• the fat content;

• the trans-fatty acid content;

• the sugar (sucrose) content.

Fat content. The large amount of fat (as a high percentage of energy intake) is one factor that can lead to obesity, which increases the risk of developing type 2 diabetes. The increased availability of "fast food" and snacks that contain a high percentage of fat are temptations to children, in whom appetite is large, to accommodate sufficient intake of food to support growth. If the energy intake is higher than expenditure, obesity can result.

Trans-fatty acids. The phospholipids in the plasma and in membranes of all cells contain long-chain polyunsaturated fatty acids (PUFA). During periods of growth and development of organs, PUFAs are required for phospholipid synthesis. The PUFAs are, of course, obtained from dietary triacylglycerol and phospholipids. The double bonds in most natural fatty acids are cis not trans. Nonetheless trans-fatty acids do occur in dietary fats. If the diet contains trans-fatty acids, they might be incorporated into the phospholipids along with the cis-fatty acids and hence into membranes. The presence of these abnormal fatty acids will modify the structure of the phospholipids which could impair the function of the membrane. There are two main sources of trans-fatty acids in the diet: foods produced from ruminants contain trans-fatty acids due to the activity of bacteria in the rumen; commercial hydrogenation of oils results in conversion of some cis into trans bonds. These artificially hydrogenated fats are used in the preparation of children's favourite foods such as potato crisps (chips), biscuits and pastries, and fast food such as beefburgers. Cells particularly exposed to such fatty acids are the endothelial cells. Damage to membranes of endothelial cells can lead to local inflammation and predispose to atherosclerosis.

Sugar. The hydrolysis of sucrose in the intestine produces both glucose and fructose, which are transported across the epithelial cells by specific carrier proteins. The fructose is taken up solely by the liver. Fructose is metabolised in the liver to the triose phosphates, dihydroxyacetone and glyceraldehyde phosphates. These can be converted either to glucose or to acetyl-CoA for lipid synthesis. In addition, they can be converted to glycerol 3-phosphate which is required for, and

stimulates, esterification of fatty acids. The resulting triacylglycerol is incorporated into the VLDL which is then secreted. In this way, fructose increases the blood level of VLDL.

The metabolism of VLDL by lipoprotein lipase in the capillaries in many tissues results in the formation of low density lipoprotein (LDL), which is atherogenic, so that diets high in sucrose are a risk factor for development of atherosclerosis. Many children in developed countries now consume large quantities of soft drinks containing sucrose or fructose. According to the above discussion, this could lead to atherosclerosis in later life.

Laboratory work. The determination of β-lipoproteins (LDL) content in the blood serum

The principle of the method: β-Lipoproteins precipitate in the presence of calcium chloride and heparin: the turbidity is appeared. It is explained that heparin can form with β-lipoproteins a complex, which is precipitated in the presence of calcium chloride. The concentration of β-lipoproteins in the blood serum correlates with the rate of turbidity.

The course of laboratory work:

Pour 2 mL of 0.27 % calcium chloride solution and 0.2 mL of blood serum in a test tube, mix. Determine the optical density of this solution (E_1) apposite 0.27 % of calcium chloride solution at red-colour filter in cuvettes (5 mm thick layer). A solution from experimental cuvette transfer again into the test tube, add 0.04 mL of 1 % heparin solution, mix and exactly (in 4 minutes later) determine the optical density of this one again (E_2) with the same condition. Calculate LDL content according to the formula:

$X= (E_2 - E_1) \cdot 1000$, where

X - concentration of LDL in the blood serum, mg %;

E_1 - optical density of experimental sample before heparin adding;

E_2 - optical density of experimental sample after heparin adding;

1000 – empiric recalculation coefficient.

Clinical significance of β-lipoproteins (LDL) content determination in the blood serum

The content of β-lipoproteins in the blood serum is normal when it equals 300-450 mg% or 3.0-4.5 g/L.

The increased β-lipoproteins content is observed at states: hyperlipoproteinemies such types as IIa, IIb, III (Fredrikson classification), which correlates with the increase of the total cholesterol content in plasma. The specified conditions promote the development of atherosclerotic damages of the vessels at patients with a hypertension, myocardial ischemia (MI) or at diseases, which are accompanied with development of secondary hyperlipoproteinemes at diabetes mellitus (obvious and the latent form), hypothyroid edema, nephritis syndrome, chronic kidney insufficiency.

TASKS FOR SELF-CONTROL on CHAPTER 1

1. Point out the type of lipoproteins transporting great bulk of triacylglycerols by blood from intestine to tissues:

A. VLDL

B. LDL

C. HDL

D. Chylomicrones

E. Apoproteins

2. Which statement is correct about high density lipoproteins (HDL)?

A. Lecithin cholesterol acyltransferase is activated by apoC-II in HDL

B. Nascent HDL pick up triacylglycerols from peripheral cells

C. Lecithin cholesterol acyltransferase produces bile acids from cholesterol in HDL

D. HDL are synthesized in the adrenal gland

E. HDL transfer cholesteryl esters to liver

3. Which one of the following properties is not characteristic of LDL?

A. LDL is smaller than both VLDL and chylomicrones

B. LDL contains more cholesteryl esters than triacylglycerols

C. The major protein component of LDL is apoB-48

D. LDL are more dense than chylomicrons

E. LDL have specific high affinity receptors in most cells

4. Which statement is not associated with situation of fat deposition?

A. Insulin secretion is increased

B. Blood free fatty acid levels are elevated

C. Hormone sensitive triacylglycerol lipase activity is increased

D. Blood VLDL and chylomicron levels are elevated

E. Lipoprotein lipase activity is increased

5. Point out the atherogenic lipoproteins:

A. Nascent chylomicrones

B. Fatty acid-albumins complex

C. Low density lipoproteins

D. High density lipoproteins

E. Remnant chylomicrones

6. Which of the following statements explains correctly metabolic alterations that are specific for persons disposed to obesity beside people having standart weight?

A. Coupling of respiration with oxidative phosphorylation is much more

B. There is any genetic defect in leptin

C. Rate of Krebs cycle reactions is higher

D. Calorie intake is much less

E. Rate of fatty acid β-oxidation is much less

7. All of the following statements regarding hypercholesterolemia (type IIa hyperlipidemia) are correct except:

A. There is an increased risk of coronary artery disease

B. The blood serum cholesterol levels are increased

C. The blood serum triacylglycerol levels are elevated

D. The blood serum low density lipoprotein (LDL) levels are high

E. It is due to a deficiency of LDL receptors

8. Name biochemical indexes of blood plasma to make diagnosis for the development of atheroclerosis of blood vessels in patient:

A. Cholesterol total

B. LDL content

C. Cholesterol content in fractions of LDL and HDL

D. HDL content

E. All named indexes are in need

9. Choose the name of special regulatory protein whose content in adipose tissue correlates with event in patient named obesity:

A. Leptin

B. Ankirin

C. Spectrin

D. Insulin

E. Angiotensin II

10. This lipoprotein class is not produced in humans on empty stomach , name it:

A. HDL

B. IDL

C. LDL

D. Chylomicrons

E. VLDL

11. Point out the type of lipoproteins transporting great bulk of triacylglycerols by blood from liver to tissues:

A. VLDL

B. LDL

C. HDL

D. Chylomicrones

E. Apoproteins

12. What enzyme is attached to the luminal surface of endothelial cells in capillaries that hydrolyzes triacylglycerols in lipoproteins:

A. Triacylglycerol lipase

B. Diacylglycerol lipase

C. Monoacylglycerol lipase

D. Lecithin cholesterol acyl transferase

E. Lipoprotein lipase

13. Point out the lipoproteins of the blood plasma containing the highest mass of triacylglycerols:

A. HDL

B. LDL

C. IDL

D. Chylomicrons

E. VLDL

14. Point out the lipoproteins of the blood plasma containing the highest mass of proteins:

A. HDL

B. LDL

C. IDL

D. Chylomicrons

E. VLDL

15. The lipids are transported by lipoproteins in the blood. Specify the lipoproteins that are formed in the small intestine wall after high lipids intake:

A. HDL

B. Chylomicrons

C. LDL

D. VLDL

E. IDL

16. What substance **is not** component of a lipoprotein:

A. Cholesterol

B. Triacylglycerol

C. Phospholipid

D. Apoprotein

E. Transferrin

17. Choose the separation method for lipoproteins of the blood plasm:

A. Radioimmunal Assay

B. Extraction

C. Electrophoresis

D. Salting-out

E. Photocolorimetry method

18. Point out the lipoproteins of blood plasma that keep the biggest amount of cholesterol ester:

A. HDL

B. LDL

C. IDL

D. Chylomicrons

E. VLDL

19. Continue properly the proposition: “Lipoprotein lipase located in the vascular endothelium of adipose tissue…:

A is increased in activity during fasting, when lipid storage is diminished”

B is inhibited by heparin”

C removes fatty acids from triglycerides of very low density lipoproteins (VLDL)”

D. removes fatty acids from triglycerides of high density lipoproteins (HDL)”

E. hydrolyzes triglycerides to fatty acids and glycerol during lipolysis in the adipocyte”

20. Point out the most atherogenic lipoproteins of the blood plasm:

A. HDL

B. LDL

C. IDL

D. Chylomicrons

E. Alpha-lipoproteins

Chapter 2

NUCLEOPROTEINS METABOLISM

Created ass.pr. Krisanova N.V.

*S*YNTHETIC PATHWAYS FOR NUCLEIC ACIDS REPLICATION

Replication is ***the synthesis of two complementary DNA strands from deoxyribomononucleoside triphosphates on parental DNA template due to the function of special multienzyme system named Replisome.***

The double-helical model of DNA suggested that the strands can separate and act as templates for the formation of a new, complementary strands.

Conservative replication would occur if, after replication and cell division, the parental DNA strands remained together in one of the daughter cells and the newly synthesized DNA strands went to the other daughter cell.

Semi conservative replication would occur if, after replication and cell division, each daughter cell received one parental DNA strand and one newly synthesized complementary strand for which the parental strand was the template.

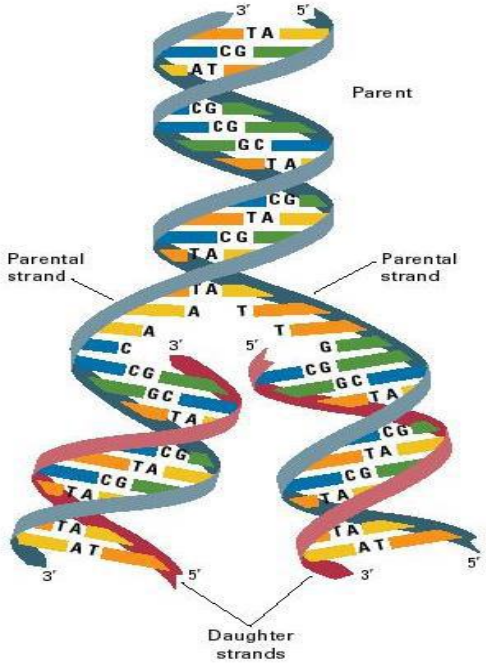


Figure 1. **A picture for formation of two double helical structures of DNA in semi conservative replication.**

All the ways to control replication in E.Coli may be across:

- Dna A gene regulation
- Dna A activity regulation
- Ori C blocking
- DNA methylation

1. Prokaryotic Replication

Replication of prokaryotes is much better understood than is replication in eukaryotes. The basic requirements and components of replication are the same for prokaryotes as for eukaryotes. Therefore, an understanding of how prokaryotes replicate provides much insight into the understanding of how eukaryotes replicate.

Consideration of the influence of chromosome structure on DNA replication in bacteria and eukaryotes must also take into account the different organization of DNA in the cell. The bacterial chromosome is associated with the cell membrane but otherwise is exposed to the entire intracellular environment. In contrast, eukaryotic DNA replication occurs in a distinct compartment in the separation of proteins that may influence the initiation of DNA replication. Within the nucleus, initiation of eukaryotic DNA replication occurs at pre-replicative complexes that are established prior to the beginning of S-phase, and these presumably contain the initiator and other replication proteins (Adachi and Laemmli 1994; Diffley et al. 1994). A similar initiation complex may exist at the membrane-bound bacterial replicator, since Dna A protein from E. coli is a lipid-binding protein and is associated with the membrane (Sekimizu and Kornberg 1988; Sekimizu et al. 1988a,b). Thus, in both cell types, initiation may actually occur on a solid-state support, albeit that the supports and environment may be quite different.

Basic requirements for DNA synthesis

1. ***Substrates*** - deoxynucleoside triphosphates: d-ATP, d-GTP, d-CTP, d-TTP. Cleavage of the high energy phosphate bonds (two) provides the energy for the phospho diester bond formation in a new strand.

2. ***Template*** **-** DNA replication can't occur without a template. A template is required to direct the addition of the appropriate complementary nucleotide to the newly synthesized DNA strand. In semi conservative replication, each strand of parental DNA serves as a template. Then, each template strand and its complementary strand serve as the DNA in daughter cells.

3.

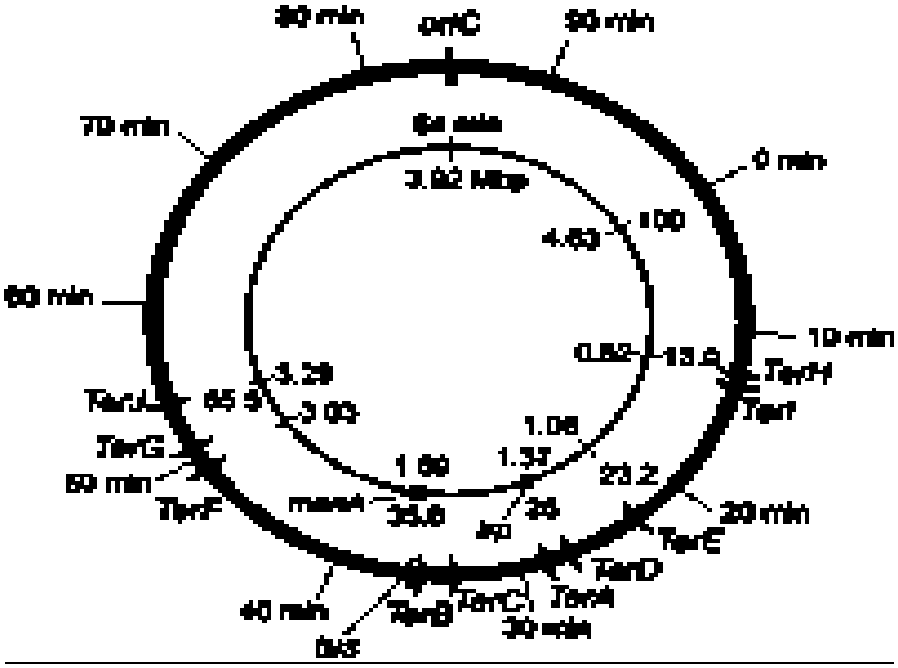
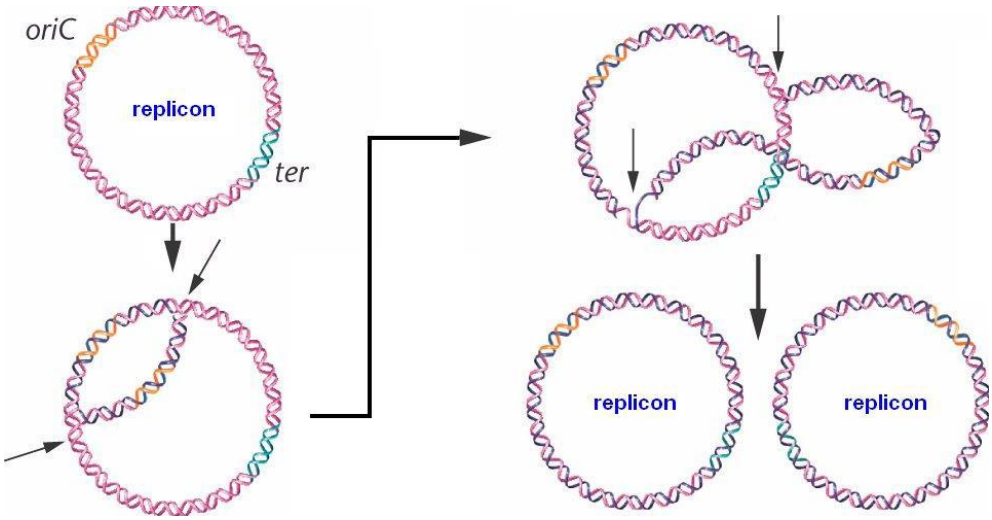


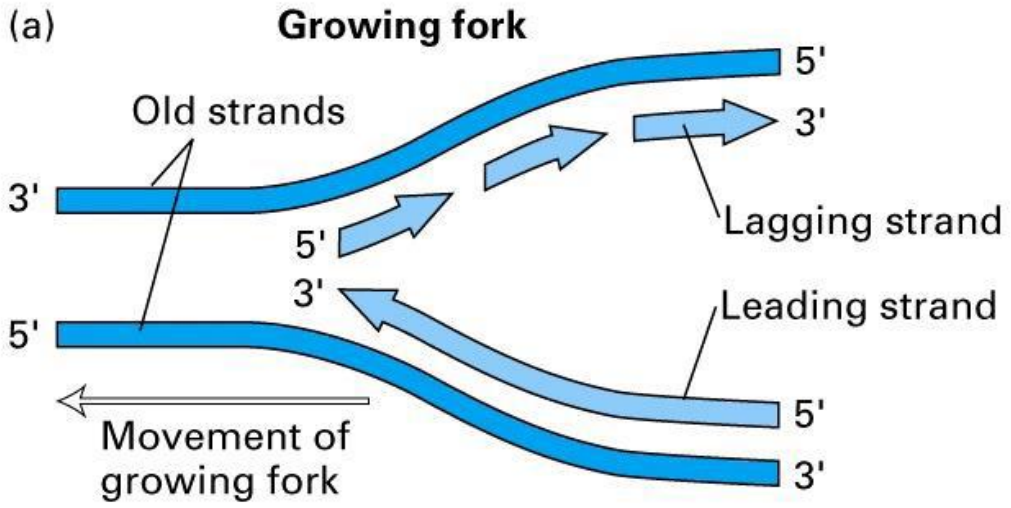
Figure 2. **Circular DNA molecule found in E.coli.**

Its replication is from ori C, bidirectional, it is finished in 40 minutes due to ten termination sequences (Ter) placed opposite ori C.

Initiation phase of replication in E.coli.

DNA replication in *Escherichia coli* initiates at *ori C*, the unique origin of replication, and proceeds bidirectionally. This creates two replication forks (fig. 2), that invade the duplex DNA on either side of the origin. The forks move around the circular chromosome at a rate of about 1,000 nucleotides per second and so meet about 40 min after initiation in a region opposite *ori C* (fig.2). In this region are located a series of sites, called termination or *Ter* sites, that block replication forks moving in one direction but not the other (fig. 2). This event creates a "replication fork trap" that allows forks to enter but not to leave the terminus region (fig.3, a,b).





(b)

Figure.3 . **(a) - Steps of replication in E.coli; (b) - Direction of replication synthesis of leading and lagging strands**

3. Enzymes for replication

The Replisome. It is believed that all replication enzymes and factors are part of a large macromolecular complex named a Replisome. It has been suggested that the replisome may be attached to the membrane, and that instead of the replisome moving along DNA during replication, DNA is passed through the stationary Replisome. The Replisome is a multiprotein complex made up of the dna A protein, DnaB helicase, the DnaG primase, and the Pol III holoenzyme. Each replicated strand commences with a short RNA primer synthesized by DnaG

primase recruited from solution by interaction with DnaB. Single-stranded DNA is protected by SSB proteins.

Dna A protein (E.coli) is required for proper initiation of replication at the origin C. When Dna A-ATP binds to ori C it twists the DNA and promotes the separation of DNA-strands in the AT-rich region to produce a single-stranded bubble or "open complex" (fig.3). The next step is the recruitment of the (DnaBC) complex to DnaA to obtain the pre-replicative Complex, which is stimulator of primosome complex. Four or five Dna A-ATP molecules interact with the (DnaBC) complex via the N-terminal of the replicative DnaB helicase and their common binding to oriC (Seitz et al., 2000).

Dna B is a monohexameric helicase. Its function is the unwinding of double-stranded DNA employing the hydrolysis of ATP, this activity is maintained as the elongation phase proceeds. Helicase activity provides single-strand templates for replication. dna B protein is the principal helicase of E.coli replication. It is a component of a primosome. In the normal process of replication, DnaB is at the front of the replisome. It is a ring-shaped homohexameric enzyme that translocates in the 5'-to-3' direction on the lagging-strand template to unwind double-stranded DNA in front of the DNA polymerase III holoenzyme, the multisubunit replicase that simultaneously synthesizes both strands.

Primosome . DNA synthesis can't start without a primer which prepares the template strand for the addition of nucleotides. Because, new nucleotides are added to the 3′ end of a primer, new synthesis is said occur in a 5′ to 3′ direction .

Primosome is a complex of proteins, a hexamer of dna B protein, dna C protein and several other proteins n, n′, n″, i. Primosome complex may be named as Primase. The primosome complex primes DNA synthesis at the origin. Using ATP hydrolysis, the primosome moves with the replication fork, making RNA primers for Okazaki fragment synthesis. It also makes the primer that initiates leading strand synthesis at the origin. Primers are not shorter than 12 and up to 29 ribonucleotides.

One strand (the leading strand) is replicated continuously, while the other (lagging) strand is synthesized discontinuously in a series of Okazaki fragments (fig.3,b). The replicative RNA-priming enzyme, DnaG primase is recruited by DnaB for the priming of each new fragment on the discontinuous strand. DnaB is physically associated with the replicase through the special subunit of the holoenzyme .

SSB. The single-stranded sections that result from helicase action are coated with single-stranded DNA-binding proteins (SSBP). Their functions are:

- to enhance the activity of helicase and to bind to single-stranded template DNA until it can save as a template.
- to protect single-stranded DNA from degradation by nucleases and may block formation of intra-strand duplexes of hairpins that can slow replication.

SSBP is displaced from single-stranded DNA when DNA undergoes replication.

Topoisomerase. Forks for replication represent unwound parental template DNA strands to which newly synthesized complementary DNA are paired. Positive super coils would build up in advance of a moving replication fork if it was not for the action of topoisomerase. It introduces "nicks" in one strand of the unwinding double helix allowing the unwinding process to proceed; alters the supercoiling of DNA. Topoisomerase is named as *gyrase* in some microbial organisms.

Initiation phase of replication requires the presence of all enzymes described before to produce first primer. The elongation phase starts from the moment of first round of DNA-polymerase action to make complementary linkage of first deoxyribonucleoside monophosphate to the chain of primer.

Elongation phase

Leading strand synthesis

It is the continuous synthesis of the daughter strand in a 5′ to 3′ direction. DNA-polymerase III catalyzes leading strand synthesis, continuously (in prokaryotes). (Fig. 4)

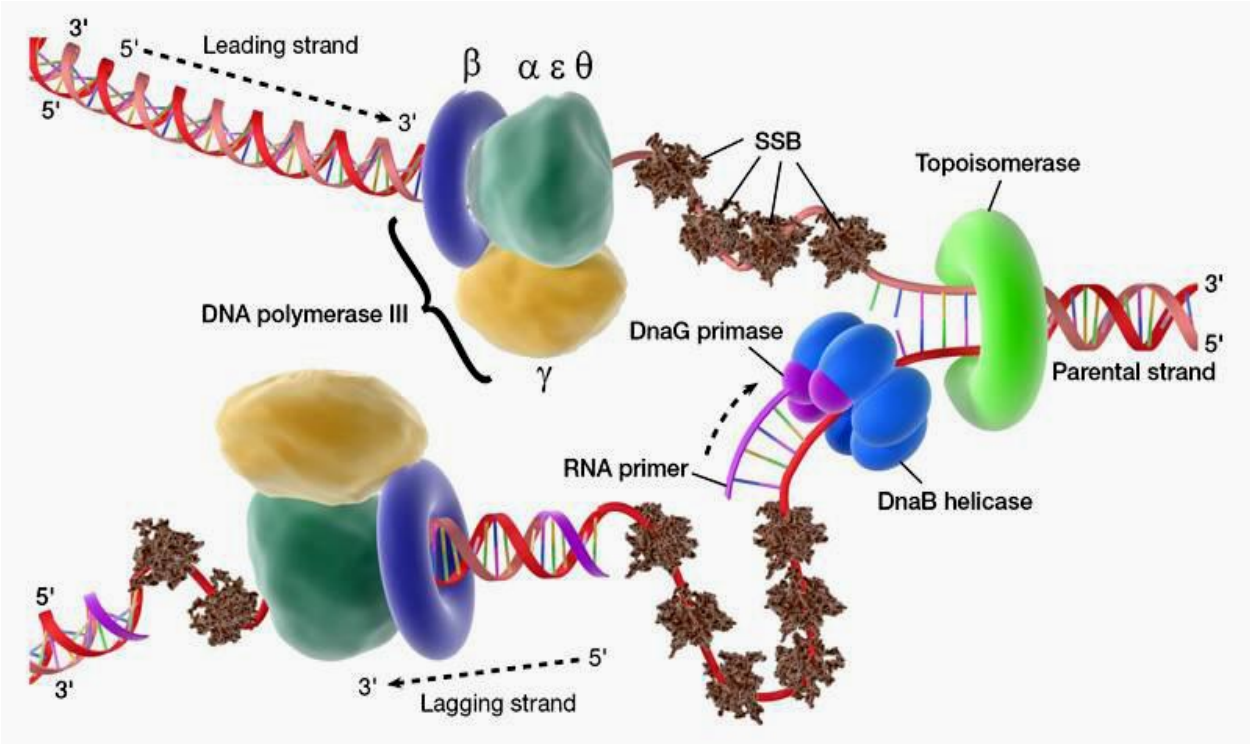


Figure 4. **Replisome complex function in elongation phase of replication (E.coli).**

Elongation phase

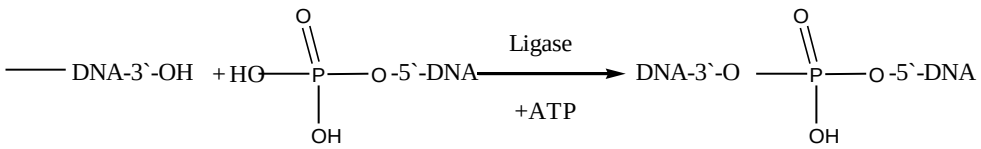
Lagging strand synthesis

This strand is made discontinuously (fig.4). The resulting short fragments are named Okazaki fragments, and they are synthesized by ***DNA-Polymerase III***, too. Synthesis of each new Okazaki fragment takes place until it reaches the RNA primer of the preceding Okazaki fragment. This effectively leaves a nick between the newly synthesized Okazaki fragment and the RNA primer.

Okazaki fragments are later joined by ***DNA ligase*** to make a continuous piece of DNA. ***DNA polymerase I*** uses its nick-translation properties to hydrolyse the RNA primer (5′ to 3′ exonuclease activity) and replace it with DNA fragment.

This lagging strand synthesis also occurs in a 5′ to 3′direction.

DNA ligase catalyses the formation of phospho diester bond between the adjoining fragments by the following reaction:



DNA polymerase II is a minor polymerase in E.coli. It may be involved in some DNA repair processes, but E.coli mutants lacking this enzyme show no replication or growth deficiencies. Polymerase II has proofreading activity (3` to 5` exonuclease activity) but lacks excision-repair activity.

The Termination of replication

Termination sequences in the parental DNA strands direct the termination of replication are placed in circular parental DNA molecule quite oppositely the ori C region (fig.2). A specific protein, Ter-binding protein (TUS), binds to these sequences and prevents the Helicase (or dna B protein) from further unwinding of DNA. This facilitates the termination of replication. In the *E. coli* chromosome, the *Ter* sequences were placed so as to form a "replication fork trap" that would allow a replisome to enter the region between the two Ter sites but not to leave. *Ter* sequences were also found in a variety of other plasmids as well as in other bacteria (30), and the number of *Ter* sites identified in the *E. coli* chromosome also increased, first to 4, then to 5, and finally to 10, after the publication of the entire genome sequence and an in-depth study of nucleotide substitutions by Coskun-Ari and Hill (1998).

2. Eukaryotic replication

The mechanism is similar to that of prokaryotic replication. It is semi conservative and proceeds bidirectionally from many origins. Replicons are basic units of replication. A replicon encompasses of all the DNA replicated from the growing replication forks originating from a single origin. There are estimated to be about 100000 replicons per cell in mammal. The large number of replicons is needed to replicate the large mammal genomes in a reasonable period of time. It takes about 8 hours to replicate the human genome. The duration of replication in eukaryotic cell may only once and during the S-phase of cell life. Factors for stimulation: Cyclins E, A (special proteins for regulation) and cyclin-dependent protein kinases involved in the initiation phase of replication.

The eukaryotic replication rate is about ten times slower than prokaryotic replication rate. Eukaryotes contain at least three different nuclear DNA polymerases: α, β and δ; and one mitochondrial DNA polymerase - γ .

DNA polymerase α is probably analogous to polymerase I but it plays no role in DNA repair.

DNA polymerase β acts in DNA repair synthesis.

DNA polymerase δ is probably analogous to polymerase III and responsible for leading strand synthesis.

DNA polymerase γ replicates mitochondrial DNA.

Telomerase

Whereas the genomes of essentially all prokaryotes are circular, the chromosomes of human beings and other eukaryotes are linear. The free ends of linear DNA molecules introduce several complications that must be resolved by special enzymes. In particular, it is difficult to fully replicate DNA ends, because polymerases act only in the 5′ → 3′ direction. The lagging strand would have an incomplete 5′ end after the removal of the RNA primer. Each round of replication would further shorten the chromosome. The first clue to how this problem is resolved came from sequence analyses of the ends of chromosomes, which are called *telomeres* (from the Greek *telos,* "an end"). Telomeric DNA contains hundreds of tandem repeats of a hexanucleotide sequence. One of the strands is G rich at the 3′ end, and it is slightly longer than the other strand. In human beings, the repeating G-rich sequence is AGGGTT.

How are the repeated sequences generated? An enzyme, termed ***telomerase,*** that executes this function has been purified and characterized. When a primer ending in GGTT is added to the human enzyme in the presence of deoxynucleoside triphosphates, the sequences GGTTAGGGTT and GGTTAGGGTTAGGGTT, as well as longer products, are generated. Elizabeth Blackburn and Carol Greider discovered that the enzyme contains an RNA molecule that serves as the template enzyme carries the information necessary to generate the telomere sequences. The exact number of repeated sequences is not crucial From its amino acid sequence,

this component is clearly related to reverse transcriptases, enzymes first discovered in retroviruses that copy RNA into DNA. Thus, ***telomerase is a specialized reverse transcriptase that carries its own template.*** Telomeres may play important roles in cancer-cell biology and in cell aging.

Cells which have an unlimited capacity such as male germ cells and the majority of human cancers have high levels of telomerase activity, the level and frequency of telomerase activity in more than 85% of all cancers highlights the critical role telomerase plays in tumor progression. Telomerase activation is the most common general marker for cancer cell to date making it in attractive target for new cancer diagnostics and therapeutics.

Something about regulation of replication in eukaryotes

The opening of the DNA helix by locked CMG-complex (a protein complex that is involved in unwinding DNA during replication) (fig.7), and stabilization of separated DNA strands after the binding of replication protein A (RPA, it is similar to SSB-proteins in action) facilitates the recruitment of DNA replication enzymes to begin DNA synthesis.

Cdt1 is a protein encoded by the gene Cdt1, and it is a key licensing factor which, along with the proteinCdc6, functions to license DNA by forming the pre-replication complex.

The interplay of S phase-kinases with Cdt1 and other components on the pre-replicative complex prevents the reformation of this complex, thus 'licensing' occurs only once per cell cycle at any given origin.

1) Cdt1 is subject to proteolysis as the cell cycle progresses through S and G2.

2) Cdt1 is inhibited by Geminin which specifically binds to Cdt1 during S, G2, and early mitosis. Geminin both inhibits Cdt1 activity during S phase in order to prevent re-replication of DNA and prevents it from ubiquitination and subsequent proteolysis.

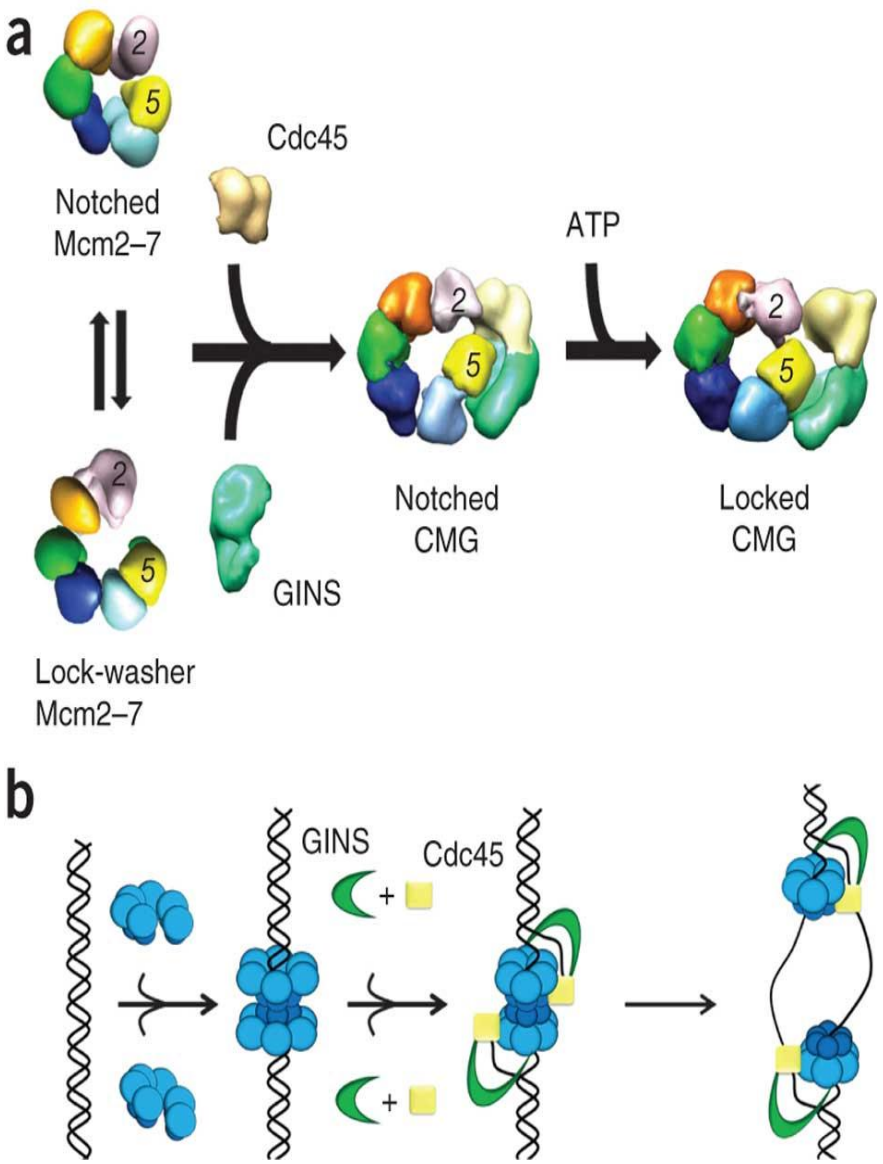


Figure 5. **CMG-complex composition and function at initiation of replication in eukaryotic cell**

CMG-complex is composed from:

- MCM2-7 – multichain protein complex - twisted dimmer; it is in conformation as two rings. The central channel, formed by these two staggered rings, has four constriction points that would restrict the movement of duplex DNA with tight grips and a kink at the interface of the two rings that would deform the bound DNA
- GINS – multichain protein complex linked with other key proteins at the fork to maintain an active replisome progression complex (RPC)

- Cdc45 - is a protein that in humans is encoded by the CDC45Lgene; required to the initiation of DNA replication.

Table 1.

Differences in DNA replication in Prokaryotes and Eukaryotes

Prokaryotes	Eukaryotes
Five polymerases (I, II, III, IV, V)	Five polymerases (α, β, γ, δ, ε)
Functions of polymerase:	Functions of polymerase:
I is involved in synthesis, proofreading, repair, and removal of RNA primers	α: a polymerizing enzyme
II is also a repair enzyme	β: a repair enzyme
III is main polymerizing enzyme	γ: mitochondrial DNA synthesis
IV, V are repair enzymes under unusual conditions	δ: main polymerizing enzyme
	ε: function unknown
Polymerase are also exonucleases	Not all polymerases are exonucleases
One origin of replication	Several origins of replication
Okazaki fragments 1000-2000 residues long	Okazaki fragments 150-200 residues long
No proteins complexed to DNA	Histones complexed to DNA

Drugs that effect replication

1. Antimetabolites which reduce or inhibit the production of the substrate for replication:

- ***5-Fluorouracil*** (analog of uracil)
- ***Methotraxate*** (analog of folic acid) that inhibits dihydrofolate reductase, regeneration of tetrahydrofolate is blocked, d-TMP synthesis is damaged)
- ***6-Mercaptopurine, 8-azoguanine and thioguanine.***

2.Substrate analogs: Azidothymidine

3.Antiviral drugs used to treat human immunodeficiency virus (HIV) infections

Cytosine arabinoside (cytoribine): it is a potent myelonic antileukemia drug. Upon incorporation into DNA, it is believed to alter the structure of DNA and make it more prone to breakage.

3. Intercalators are drugs, usually with aromatic ring, that insert between adjacent, stacked base pairs. Intercalation causes a physical block as well as disruption or change in the DNA conformation that inhibits the action of replication enzymes.

Anthracycline glycosides – antibiotics produced by a strain of Streptomycetes.

Actinomycin D (anticancer activity), it is beneficial in treating Wilm's tumor in children when used in combination with surgery, radiotherapy and other chemiotherapeutic drugs.

Table 2.

Genetic diseases associated with defects in DNA repair systems

Disease	Symptoms	Genetic defect
Xeroderma pigmentosum	Frecklelike spots on skin, sensitivity to sunlight, predisposition to skin cancer	Defects in nucleotide-excision repair
Cockayne syndrome	Dwarfism, sensitivity to sunlight, premature aging, deafness, mental retardation	Defects in nucleotide-excision repair
Trichothiodystrophy	Brittle hair, skin abnormalities, short stature, immature sexual development, characteristic facial features	Defects in nucleotide-excision repair
Hereditary nonpolyposis colon cancer	Predisposition to colon cancer	Defects in mismatch repair
Fanconi anemia	Increased skin pigmentation, abnormalities of skeleton, heart, and kidneys, predisposition to leukemia	Possibly defects in the repair of interstrand cross-links
Ataxia telangiectasia	Defective muscle coordination, dilation of blood vessels in skin and eyes, immune deficiencies, sensitivity to ionizing radiation, predisposition to cancer	Defects in DNA damage detection and response
Li-Fraumeni syndrome	Predisposition to cancer in many different tissues	Defects in DNA damage response

Drugs that damage DNA

a) ***Alkylating agents*** (strong electrophils) become linked to many cellular nucleophils, in particular to the seventh nitrogen in the guanine residue of DNA. After replication mutation can be, or cross-linking of double helix.

b) ***Platinum-coordination complexes (cis-platin).*** They lead to the formation of cross-links between adjacent guanines in DNA. They are drugs for testicular and ovarian cancers.

c) ***Bleomycins*** bind to DNA and interact with oxygen and Fe^{2+} to cause DNA breakage.

d) ***Inhibitors of replicative enzymes***: DNA polymerase inhibitors or topoisomerase inhibitors (***Nalidixic acid and Fluoroquinolones***). Use: treatment of urinary tract infections.

TRANSCRIPTION

Introduction

Multiple steps are required to produce functional cellular RNAs. Although some of these steps are common to the production of all RNAs others depend on the class of RNA being produced.

Three functionally distinct classes of RNA are produced in prokaryotes, and four are produced in eukaryotes.

m-RNA of prokaryotes

It is in need to know that:

1) most prokaryotic m-RNAs are polycistronic. That is they carry the information for the production of multiple polypeptides;

2) not all portions of prokaryotic m-RNA code for polypeptides:

a) the 5`-ends of m-RNA contain sequences that are never translated into protein (leader sequences or 5`-untranslated regions);

b) the 3`-ends contain sequences that are never translated into protein (trailer seq. or 3`-untranslated regions);

c) if the m-RNA is polycistronic, the sequences between that code for proteins (cistrons) are called the intercistronic regions or spacers.

m-RNA accounts for only 5% of the total cellular RNA in prokaryotes. Their life-time is short (just a few minutes).

m-RNA of eukaryotes

mRNAs of eukaryotic cell are monocistronic. They are formed from large precursors that are named heterogenous nuclear RNA (hnRNA). Like prokaryotic m-RNA, eukaryotic m-RNA contains leader and trailer sequences.

1) Leader seq. has 7-methylguanylate attached 5`to 5` triphosphate linkage –a cap.

2) Trailer sequence is a polyadenylate tail (200-300 adenylate residues at the 3`end) (fig.6)

3) m-RNA accounts for only 3% of the total cellular RNA in eukaryotes;

4) they exhibit half-lives on the order of hours to days.

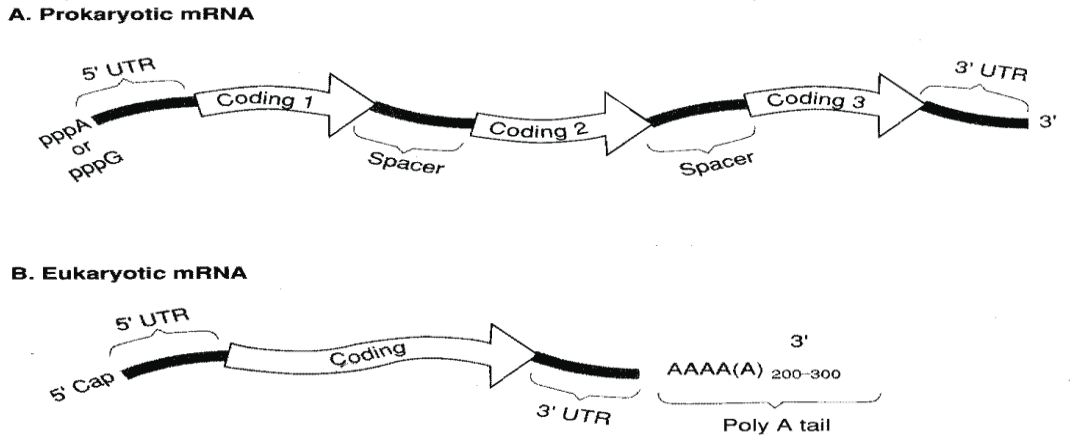


Figure 6. **Schematic representation of typical prokaryotic and eukaryotic messenger RNAs (mRNAs).** *The coding regions are indicated with open arrows.*

A) A polycistronic prokaryotic mRNA with three coding regions is shown. The coding regions are separated by noncoding spacer sequences. Flanking the proximal and distal coding sequences are noncoding 5' and 3' untranslated regions *(UTRs).* The 5' end of the mRNA is a purine nucleoside triphosphate.

B) A monocistronic eukaryotic mRNA is shown. The single coding region is flanked by a 5' and 3' UTR. The 5' end has a 7-methylguanylate cap and the 3' end has a polyadenylate *(poly A)* tail.

r-RNA of prokaryotes

Three kinds: 23S r-RNA; 16s r-RNA; 5S r-RNA. They arise from the processing of a large 30S precursor r-RNA. r-RNAs account for 80% of the total cellular RNA in prokaryotes.

r-RNA of eukaryotes

They are typically bigger than prokaryotic.

Four kinds: 28S r-RNA, 18S r-RNA, 5,8S r-RNA, 5S r-RNA. They arise from 45 precursor r-RNA; the 5S r-RNA is a transcription product of separate gene.

t-RNA of prokaryotes

1. Average size of t-RNAs is about 80 nucleotides.
2. All t-RNAs arise from processing of large precursor.
3. They are heavily modified post-transcriptionally.
4. They account for 15% of the total cellular RNA in prokaryotes.

t-RNA of eukaryotes

They are very similar in positions 1-4 to prokaryotic t-RNA.

Besides t-RNAs of eukaryotes have numerous other small RNAs that serve a variety of functions. These RNAs are divided in two groups according to their location: cytoplasmic and nuclear. The latter are associated with proteins in small nuclear ribonucleoprotein or snurps. Snurps function in splicing reactions needed to process hnRNA to m-RNA.

1. Transcription in E.coli (prokaryotic cell)

The process of RNA synthesis from nucleoside triphosphates (ATP, GTP, UTP, CTP) directed by a DNA template due to function of RNA-polymerase is termed Transcription and it proceeds in three phases:

1. Initiation: ***Transcriptional initiation does not require a primer.***

Promoter sequences are responsible for directing RNA polymerase to initiate transcription at a particular point of one DNA strand, only. In the figure 7 (below) you can see the sequence features of typical prokaryotic promoter. Conserved promoter sequence elements *(enclosed boxes),* are shown relative to the start point of transcription. The start point precedes the coding region so that the transcripts have a 5' untranslated region *(UTR).*

For most prokaryotic genes, they are conserved sequences that are necessary to promote accurate initiation of transcription.

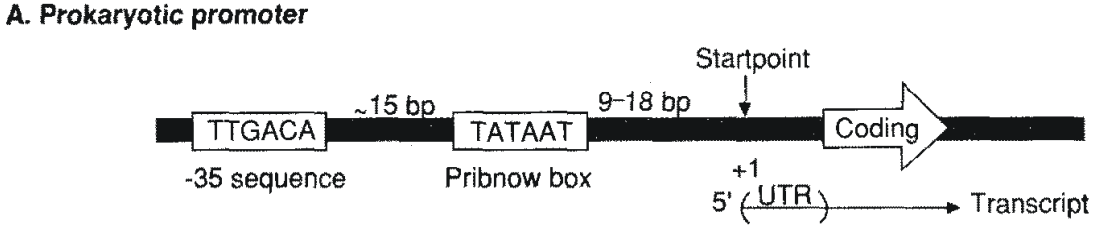


Figure 7. **Prokaryotic promoter sequences**

The promoters have three sequence elements:

1) ***Initiation site (start point).*** The start point is usually purine nucleotide residue.

2) ***Pribnow box***. There is a sequence called so which exists 9 to 18 base pairs upstream of the start point. A typical Pribnow box is either identical to or very similar to the ***sequence TATAAT.*** It has also been called –10 sequence because it is usually found 10 base pairs upstream of the start point.

3) ***The –35 sequence*** is a component of typical prokaryotic promoters. This sequence is very similar to the sequence TTGACA. It is named –35 sequence because it is typically found 35 base pairs upstream of the start point.

Initiation factors are needed to initiate transcription:

The prokaryotic σ-factor placed in holo-enzyme of RNA-polymerase is required for accurate initiation of transcription. σ-factor enables the RNA-polymerase holoenzyme (fig. 8) to recognize and bind tightly to the promoter sequences.

Process of initiation:

a) upon binding, the σ-factor facilitates the opening or melting of the DNA double helix; Sigma *(σ)* factor mediates initiation of prokaryotic transcription. Sigma factor enables the RNA polymerase holoenzyme to recognize and bind tightly to the promoter where it facilitates the initiation of transcription. After initiation, the sigma factor dissociates within the time it takes for new chain growth to proceed 10 nucleotides.

b) then holoenzyme catalyzes the formation of a phospho diester bond between the first two monomers. The first nucleotide is usually purine nucleoside triphosphate (ATP or GTP); A holoenzyme is a core enzyme with an additional subunit σ, it is required for proper initiation of transcription. A core enzyme consists of two α_2-subunit and two β`-subunit, it is required for the elongation steps of RNA synthesis.

c) The released σ-factor can combine with free core-enzyme to form another holoenzyme that can initiate transcription.

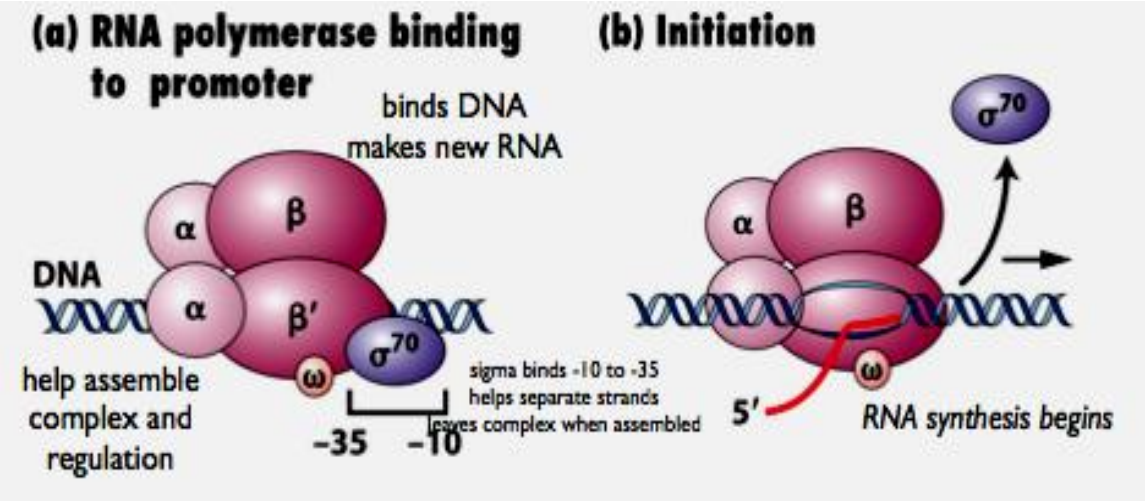


Figure 8. **RNA polymerase of E.coli in initiation phase of transcription.**

2. Elongation

This phase proceeds after the formation of the first phospho diester bond. By the time 10 nucleotides have been added, the σ-factor dissociates. The core enzyme then continues the elongation of the transcription A single strand of DNA acts as a template to direct the formation of complementary RNA during transcription. Substrates are four ribonucleoside triphosphates : ATP, GTP, UTP, CTP.

Except for the first nucleoside triphosphate, subsequent nucleotides are added to the 3`-hydroxyl of the preceding nucleotide. Therefore, RNA chain growth proceeds in the 5` to 3` direction.

3. Termination

There are two basic classes of termination events in prokaryotes:

1) Factor-independent termination.

Particular sequences of DNA strand can:

a) cause the core enzyme to terminate transcription;

b) share several common features.

c) All these sequences can code an inverted repeat , which - when transcribed – can form a stable stem-loop structure (fig. 9):

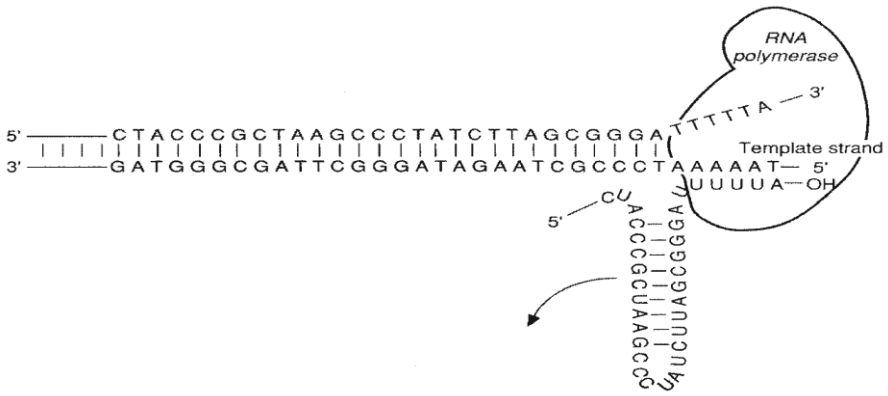


Figure 9. **A formation of a stable stem-loop in termination phase of transcription.**

While transcription pauses at the guanine-cytosine (GC)-rich sequences, a stable stem—loop structure forms in the RNA. This causes displacement—and subsequent termination—while the uracil (U)-rich sequence, which is only weakly base-paired to the template, is being synthesized. The formation of a stable stem-loop induces the displacement of the transcript when RNA polymerase synthesizes the U-rich segment. Displacement occurs easily because only weak adenine-uracil bonds hold the transcript to the template.

2) Factor-dependent termination

Particular sequences act as termination sequences in the presence of factor rho (ρ). Rho-dependent termination sequences do not appear to share common structural features as do the factor-independent termination sequences. Rho binds as a hexamer to the forming transcript at these unique sequences. Rho is an ATPase. The exact mechanism that Rho uses to terminate transcription is unknown but it requires the cleavage of ATP by Rho.

2. Transcription and Processing of mRNA in eukaryotes

The process of transcription in eukaryotes is similar to that in prokaryotes, although there are some differences:

- ***Eukaryote genes are not grouped in operons as are prokaryote genes.***
- ***Each eukaryote gene is transcribed separately, with separate transcriptional controls on each gene.***

- Whereas prokaryotes have one type of RNA polymerase for all types of RNA, ***eukaryotes have a separate RNA polymerase for each type of RNA***. One enzyme for mRNA-coding genes such as structural proteins. One enzyme for large rRNAs. A third enzyme for smaller rRNAs and tRNAs.

- ***Prokaryote translation begins even before transcription has finished, while eukaryotes have the two processes separated in time and location*** (remember the nuclear envelope). In prokaryotes, m-RNA is not post-transcriptionally processed. It may be only for precursor of r-RNA and t-RNA. Enzymes ribonucleases P, D, III are used.

After eukaryotes transcribe an RNA, the RNA-transcript is extensively modified before export to the cytoplasm Eukaryotic m-RNA is formed from extensive processing of a large precursor named hn-RNA:

- ***A cap of 7-methylguanine (a series of an unusual base) is added*** to the 5' end of the mRNA; this cap is essential for binding the mRNA to the ribosome. Cap formation is a multistep process that begins during transcription or immediately after. Caps serve two functions:

1. m-RNAs with caps are translated more efficiently;
2. caps help stabilize m-RNAs by protecting them from digestion by ribonucleases that degradate RNA from 5`-end

- ***A string of adenines (as many as 200 nucleotides known as poly-A) is added to the 3' end of the mRNA after transcription***. ***Polyadenylation is made*** (fig.10) The function of a poly-A tail is not known, but it can be used to capture mRNAs for study. The signal that identifies the site of polyadenylation lies within the hn-RNA. The sequence AAUAAA (cleavage polyadenylation signal) directs a cleavage of the RNA being transcribed to a point 11 to 30 nucleotides downstream. Polyadenylation occurs after capping before splicing; it helps to stabilize m-RNA. (Poly A polymerase adds several hundred adenylate residues to the free 3' end of the RNA formed from the cleavage reaction.

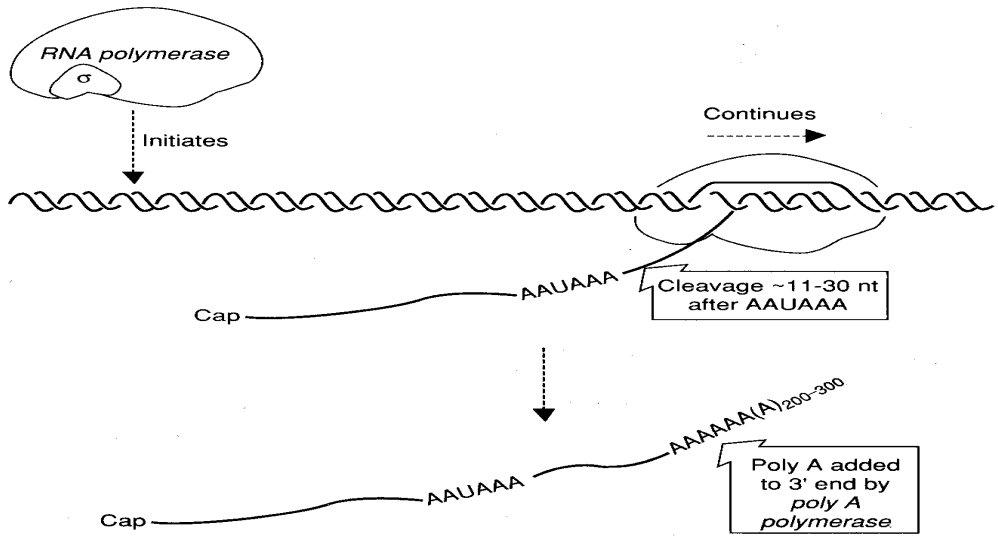


Figure 10. **Polyadenylation of hn-RNA during processing.**

- *Introns are cut out of the message and the exons are spliced together before the mRNA leaves the nucleus.* There are several examples of identical messages being processed by different methods, often turning introns into exons and vice-versa. Protein molecules are attached to mRNAs that are exported, forming ribonucleoprotein particles (mRNPs) which may help in transport through the nuclear pores and also in attaching to ribosomes. The process by which non-coding sequences are removed to form a functional m-RNA is named ***splicing.*** Splicing occurs through a multistep process that is catalyzed by a large ribonucleoprotein complex called ***splicesome***. Splicesomes are made of five snurps that contain five snRNA (u_1,u_2,u_4,u_5,u_6). The ***snRNAs*** are responsible for recognition of conserved sequences in introns and the bringing together of RNA sequences into perfect alignment for splicing. The first step is a cleavage at the 5' intron/exon junction. The 5'-phosphate of the conserved guanylate of the 5' intron/exon junction is then covalently linked to the 2'-hydroxide of the adenylate located in the branch site. After formation of this intermediate, lariat-like structure, a second cleavage at the 3' intron/exon junction occurs. The two exons are then ligated together and the lariat-like structure is lost, to eventually be degraded.

Transport of m-RNA from nucleus to the cytoplasm is coupled to splicing and does not occur until the splicing is complete. Regulation of gene expression is often at the level of splicing.

3. The regulation of transcription in E.coli . LAC-operon theory

Experimental investigation of LAC-operon in E.coli proved some notions in the regulation of transcription.

Some terms for understanding of this chapter:

Cistron (structural gene) – the sequence of DNA strand that codes the structure of one polypeptide chain of protein.

Inducible gene – transcription of this gene can be in the presence of inducer, only.

Inducer – special regulator-substance. It has affinity to special protein-repressor to block its linkage to gene-operator sequence.

Constitutive expression of genes – there is independent transcription of genes

Operon – a site of DNA strand that contains ***promoter sequence, gene-operator and one or more cystrons.***

Gene-regulator – gene coding the structure of protein-repressor.

Protein-repressor – protein that can bind to gene-operator to stop RNA-polymerase action.

Gene-operator - sequence of DNA strand placed between promoter sequence and structural genes, and it has affinity to protein repressor.

LAC-operon of E.coli contains three structural genes that keep information about enzymes: Gene X – β-Galactosidase; Gene Y – Galactoside permiase; Gene Z – Galactoside acetylase (fig.11).

All three genes are transcribed in a single m-RNA. ***Lactose is inducer*** of this transcription.

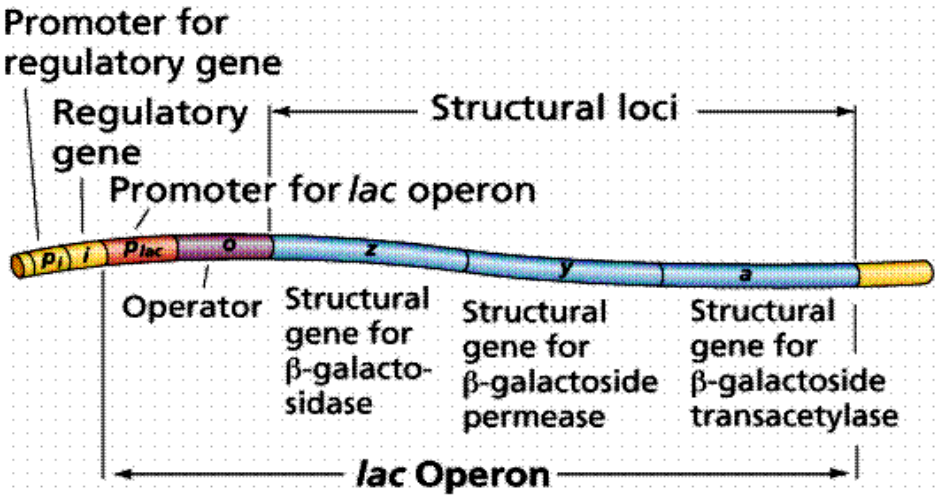


Figure 11. **The composition of LAC-operon in E.coli.**

In the presence of inducer (lactose) protein-repressor cannot attach the gene-operator, and holoenzyme of RNA-polymerase can move along the DNA single strand to form primary transcript. Inducer blocks the conformation of protein-repressor (active form) to allow the transcription of LAC-operon (fig.12)

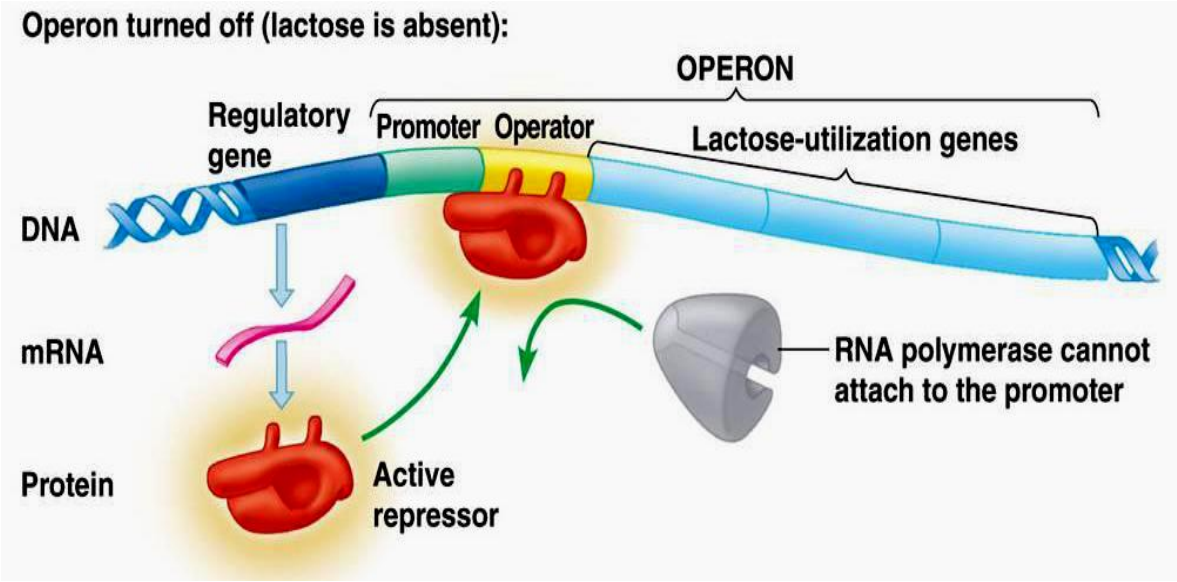


Figure 12. **The function of protein-repressor in control of transcription of LAC-operon.**

Positive regulators of transcription are: CRP-protein –catabolite gene reactive protein; cAMP (fig.13)

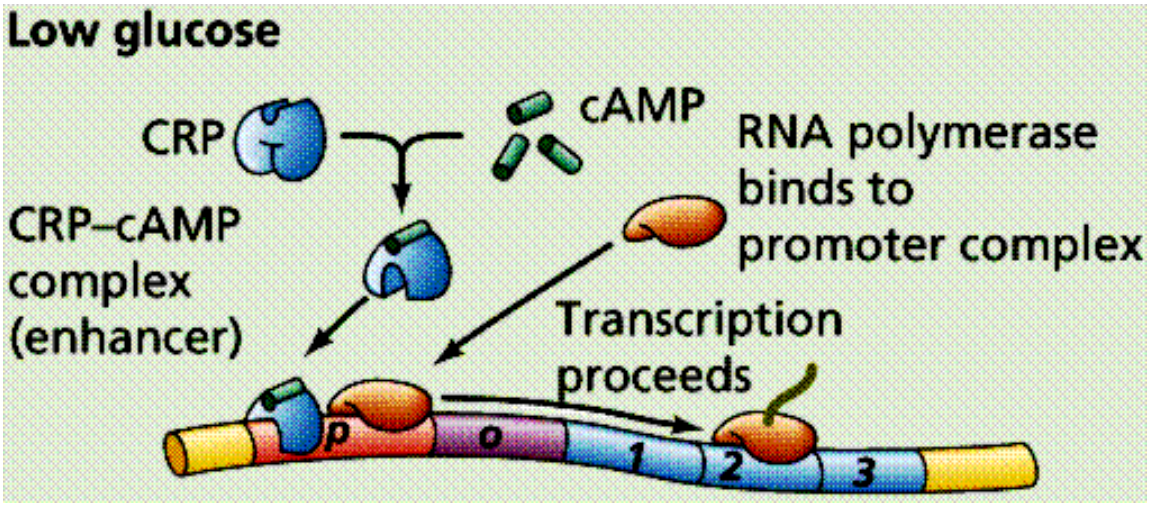


Figure 13. **CRP-cAMP complex is enhancer of RNA-polymerase linkage to promoter sequence.**

These two factors in a complex are required for activation of Sigma *(σ)* factor in the structure of holo-enzyme of RNA-polymerase to attach to promoter sequence when the inducer is present. Their content depends on the content of sources for carbon atom in the cell such as glucose or glycerol. The higher the content of glucose the lower the content of cAMP. Glucose and glycerol are considered as suppressor for transcription on LAC-operon.

4. Transcription Regulation in Eukaryotes

An eukaryotic cell contains in DNA molecules about 20,000–25,000 genes.

- Some of these are expressed in all cells all the time. These so-called housekeeping genes are responsible for the routine metabolic functions (e.g. respiration) common to all cells.
- Some are expressed as a cell enters a particular pathway of differentiation.
- Some are expressed all the time in only those cells that have differentiated in a particular way. For example, a plasma cell expresses continuously the genes for the antibody it synthesizes.
- Some are expressed only as conditions around and in the cell change. For example, the arrival of a hormone may turn on (or off) certain genes in that cell.

Protein-coding genes have :

- ***exons*** whose sequence encodes the polypeptide;

- ***introns*** that will be removed from the mRNA before it is translated;
- ***a transcription start site***
- a promoter :
    - ***the basal or core promoter*** located within about 40 bp of the start site
    - ***an "upstream" promoter***, which may extend over as many as 200 bp farther upstream
- ***enhancers***
- ***silencers***

Adjacent genes (RNA-coding as well as protein-coding) are often separated by an ***insulator*** which helps them avoid cross-talk between each other's promoters and enhancers (and/or silencers).

Transcription start site

This is where a molecule of RNA polymerase II (pol II, also known as RNAP II) binds. Pol II is a complex of 12 different proteins (shown in the figure 16 in yellow with small colored circles superimposed on it).

The start site is where transcription of the gene into RNA begins is the basal promoter (fig.14).

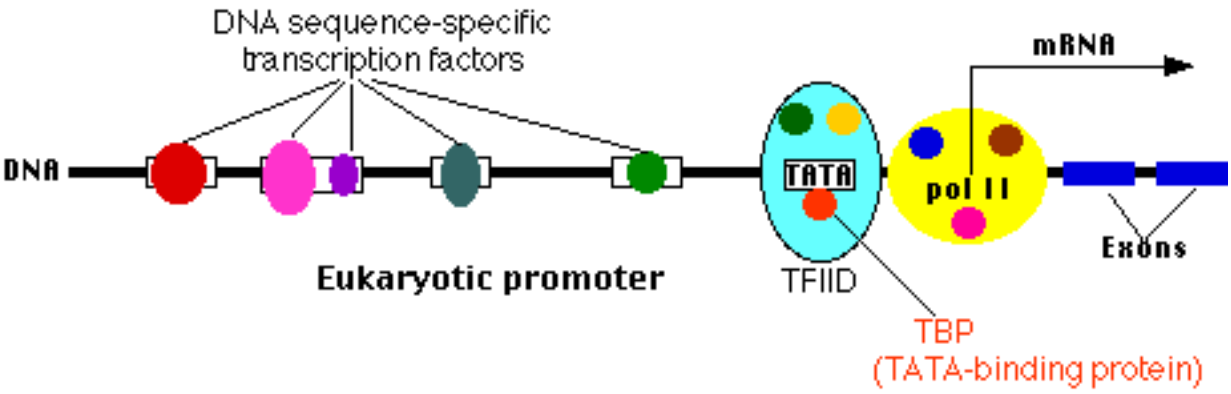


Figure 14. **The composition of basal promoter in DNA of eukaryotic cell.**

The basal promoter contains a sequence of 7 bases (TATAAAA) called the TATA box. It is bound by a large complex of some 50 different proteins, including:

1) Transcription Factor IID (TFIID) which is a complex of TATA-binding protein (TBP), which recognizes and binds to the TATA box (fig.16);

2) 14 other protein factors which bind to TBP — and each other — but not to the DNA.

3) Transcription Factor IIB (TFIIB) which binds both the DNA and pol II.

The basal or core promoter is found in all protein-coding genes. This is in sharp contrast to the upstream promoter whose structure and associated binding factors differ from gene to gene.

Although the figure 16 is drawn as a straight line, the binding of transcription factors to each other probably draws the DNA of the promoter into a loop.

Many different genes and many different types of cells share the same transcription factors — not only those that bind at the basal promoter but even some of those that bind upstream. What turns on a particular gene in a particular cell is probably the unique combination of promoter sites and the transcription factors that are chosen.

Transcription factors represent only a small fraction of the proteins in a cell. Hormones exert many of their effects by forming transcription factors. The complexes of hormones with their receptor represent one class of transcription factor. Hormone "response elements", to which the complex binds, are promoter sites.

Enhancers

Some transcription factors ("Enhancer-binding protein") bind to regions of DNA that are thousands of base pairs away from the gene they control. Binding increases the rate of transcription of the gene. Enhancers can be located upstream, downstream, or even within the gene they control. How does the binding of a protein to an enhancer regulate the transcription of a gene thousands of base pairs away?

- One possibility is that enhancer-binding proteins — in addition to their DNA-binding site, have sites that bind to transcription factors ("TF") assembled at the promoter of the gene.

This would draw the DNA into a loop (as shown in the figure 15).

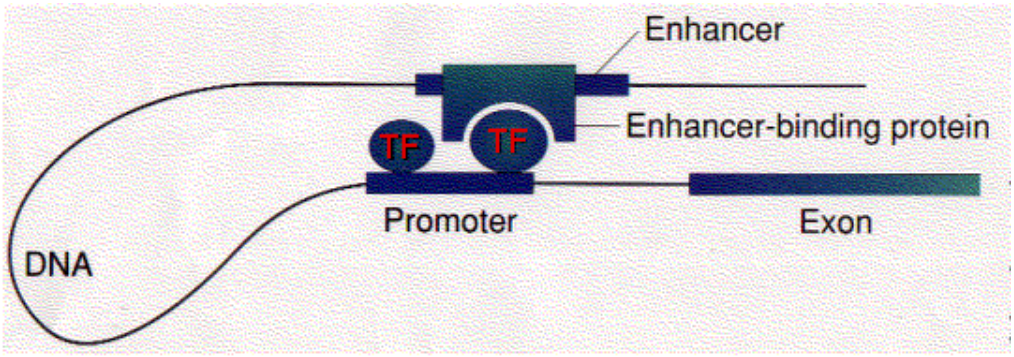


Figure 15. **The change of DNA strand conformation when transcription factor (TF) binds to enhancer sequence.**

Enhancers can turn on promoters of genes located thousands of base pairs away. Like promoter-proximal elements, many enhancers are cell-type-specific. For example, the genes encoding antibodies (immunoglobulins) contain an enhancer within the second intron that can stimulate transcription from all promoters tested, but only in B lymphocytes, the type of cells that normally express antibodies. Analyses of the effects of deletions and linker scanning mutations in cellular enhancers have shown that they generally are composed of multiple elements that contribute to the overall activity

What is to prevent an enhancer from inappropriately binding to and activating the promoter of some other gene in the same region of the chromosome?

One answer: ***an insulator*** (fig.16).

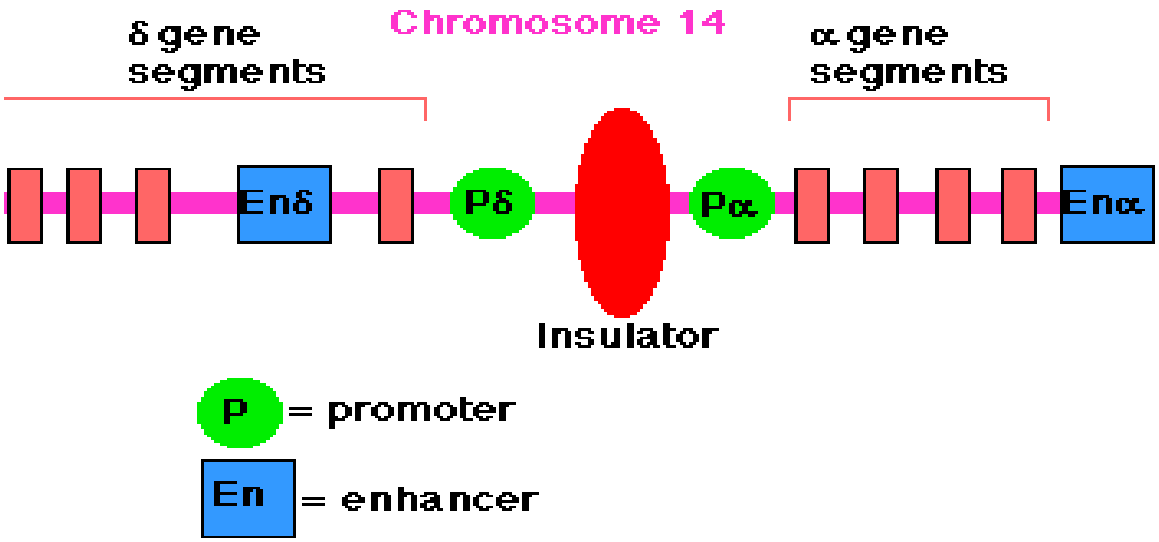


Figure 16. **A location of insulator in DNA strand of chromosome 14.**

Insulators are stretches of DNA (as few as 42 base pairs may do the trick) which located between the enhancer(s) and promoter or silencer(s) and promoter of adjacent genes or clusters of adjacent genes. Their function is to prevent a gene from being influenced by the activation (or repression) of its neighbors.

Silencers

Silencers are control regions of DNA that, like enhancers, may be located thousands of base pairs away from the gene they control. However, when transcription factors bind to them, expression of the gene they control is repressed.

Features of transcription control by different factors

Transcription activators and repressors are generally modular proteins containing a single DNA-binding domain and one or a few activation domains (for activators) or repression domains (for repressors). The different domains frequently are linked through flexible polypeptide regions

Among the most common structural motifs found in the DNA-binding domains of eukaryotic transcription factors are the C2H2 zinc finger, homeodomain, basic helix-loop-helix (bHLH), and basic zipper (leucine zipper). All these and many other DNA-binding proteins contain one or more helices that interact with major grooves in their cognate site in DNA.

The transcription-control regions of most genes contain binding sites for multiple transcription factors. Transcription of such genes varies depending on the particular repertoire of transcription factors that are expressed and activated in a particular cell at a particular time.

Combinatorial complexity in transcription control results from alternative combinations of monomers that form heterodimeric transcription factors and from cooperative binding of transcription factors to composite control sites.

Activation and repression domains in transcription factors exhibit a variety of amino acid sequences and three-dimensional structures. In general, these functional domains interact with co-activators or co-repressors, which are critical to the ability of transcription factors to modulate gene expression.

Cooperative binding of multiple activators to nearby sites in an enhancer forms a multiprotein complex called an *enhancesome*. Assembly of enhancesomes often requires small proteins that bind to the DNA minor groove and bend the DNA sharply, allowing bound proteins on either side of the bend to interact more readily. The function of some hormones is associated with enhancer-genes function.

Steroid Hormone Receptors and their Response Elements

Steroid hormone receptors are proteins that have a binding site for a particular steroid molecule. Their response elements are DNA sequences that are bound by the complex of the steroid bound to its receptor. The response element is part of the promoter of a gene. Binding by the receptor activates or represses, as the case may be, the gene controlled by that promoter.

It is through this mechanism that steroid hormones turn genes on (or off). It is shown (fig.17) that glucocorticoid receptor, like all steroid hormone receptors, is a zinc-finger transcription factor; the zinc atoms are the four yellow spheres. Each is attached to four cysteines (shown in dark green).

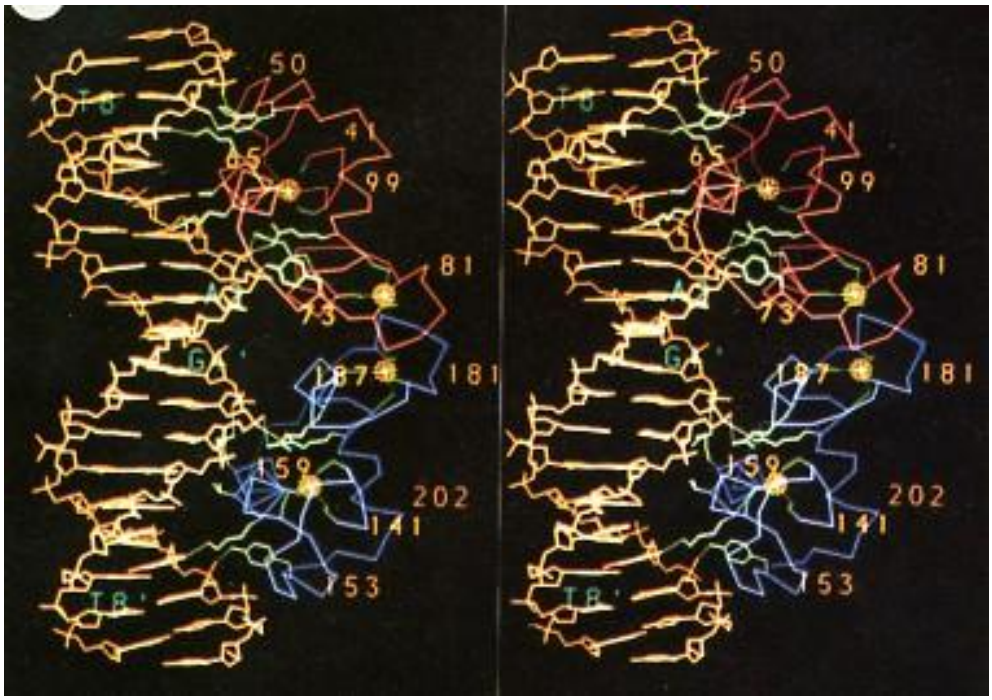


Figure 17. **A stereoscopic view of the glucocorticoid response element** *(DNA, the double helix shown in yellow at the left of each panel)* **with the glucocorticoid receptor** *(a protein homodimer, right portion of each panel) bound to it (by F.B. Sigler, 1999)*

The DNA sequence of the glucocorticoid response element is

5' AGAACAnnnTGTTCT 3'

3' TCTTGTnnnACAAGA 5'

where n represents any nucleotide. (Note the inverted repeats.)

For a steroid hormone to turn gene transcription on, its receptor must:

- bind to the hormone
- bind to a second copy of itself to form a homodimer
- be in the nucleus, moving from the cytosol if necessary
- bind to its response element
- activate other transcription factors to start transcription.

Each of these functions depend upon a particular region of the protein (e.g., the zinc fingers for binding DNA). Mutations in any one region may upset the function of that region without necessarily interfering with other functions of the receptor.

NUCLEOPROTEINS DEGRADATION & NUCLEOTIDE METABOLISM

Nucleoproteins and their non-protein part (nucleic acids) are the most important molecules in a cell because they are involved in the main processes promoted the keeping of genetic information and its transformation into the structure of proteins needed for the cell. The metabolism of nucleic acids is composed from anabolic pathways (DNA synthesis - replication; RNA synthesis – transcription) and their catabolic pathways (degradation) up to terminal products for humans (uric acid, urea, carbon dioxide and water). All these pathways are associated with the metabolism of nucleotides: their synthetic ways and degradation, too.

The breakdown of nucleoprotein containing DNA may be in the beginning both in gastrointestinal tract (GIT) and in tissues. Enzymes which are in cleavage of phosphor diester bonds of polynucleotide chains are named respectively the type of nucleic acid***: DNA-nuclease or RNA-nuclease***. The removal of phosphate from nucleotide molecules is catalyzed by ***special phosphatases*** (may be ***5'-nucleotidase*** in name), and nucleosides are formed. Destruction of nucleosides is found mainly in human tissues? But about 3% of their total content is derived into

terminal products of destruction in large intestine. Complete way destruction of nucleoprotein in GIT is represented in fig. 18.

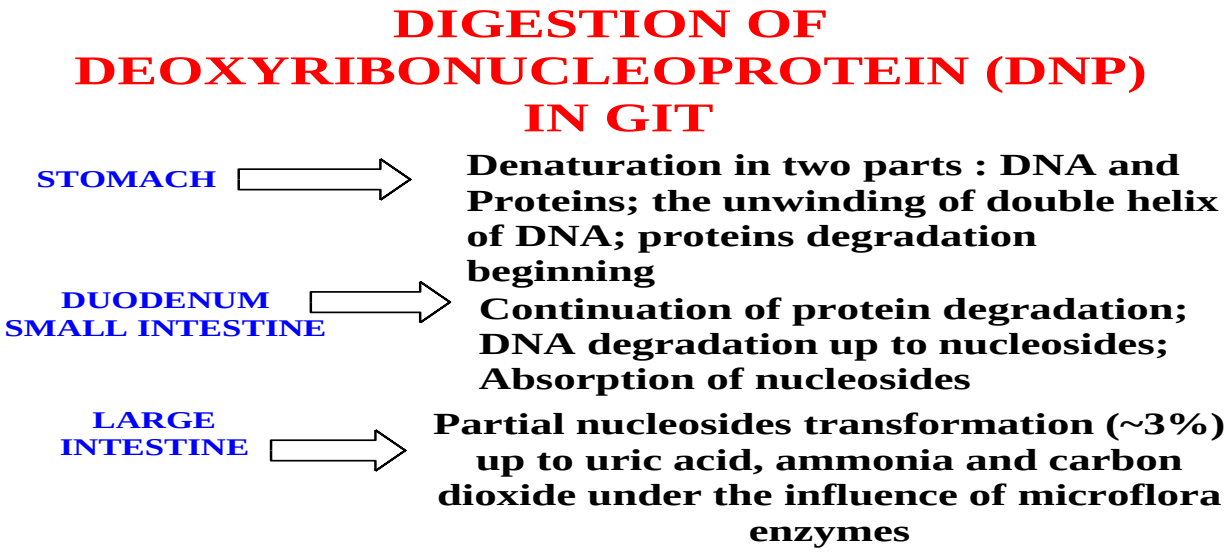


Figure 18. **Digestion steps of deoxyribonucleoproteins in gastro-intestinal tract.**

Therefore more then 95% of dietary nucleosides are destructed in human tissues. Their structure is shown in fig.19.

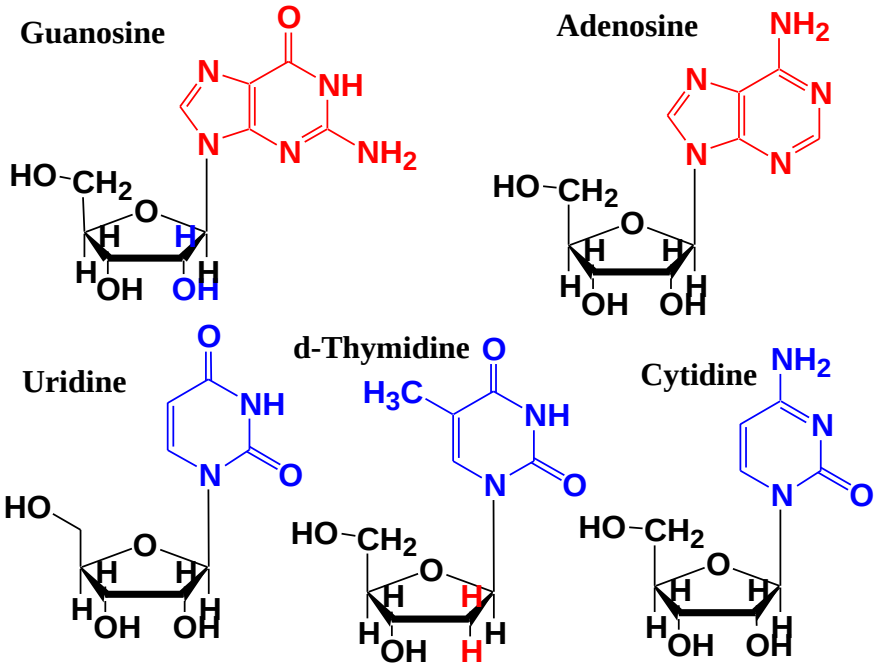


Figure 19. **Main nucleosides discussed for their metabolism in humans.** (*coloured red - purine derivatives; coloured blue - pyrimidine derivatives)*

Degradation of purine nucleosides in cells

Let us consider the figure 20 that explains this process. Adenosine is involved in hydrolytic deamination to form inosine (1), then we have to consider its dephosphorylation due to special phosphorylase. Hypoxanthine is formed as a product in this step (2). Xanthine oxidase catalyzed two reactions: conversion of hypoxanthine to xanthine (3) and then there is formation of uric acid (4). The latter enzyme is flavoprotein, keeps Mo^{2+} and four Fe^{2+}-centres. Allopurinol is inhibitor of Xanthine oxidase.

Guanosine is converted to guanine due to special phosphorylase (5), and then we can consider the deamination of guanine (6) to form xanthine (fig. 20).

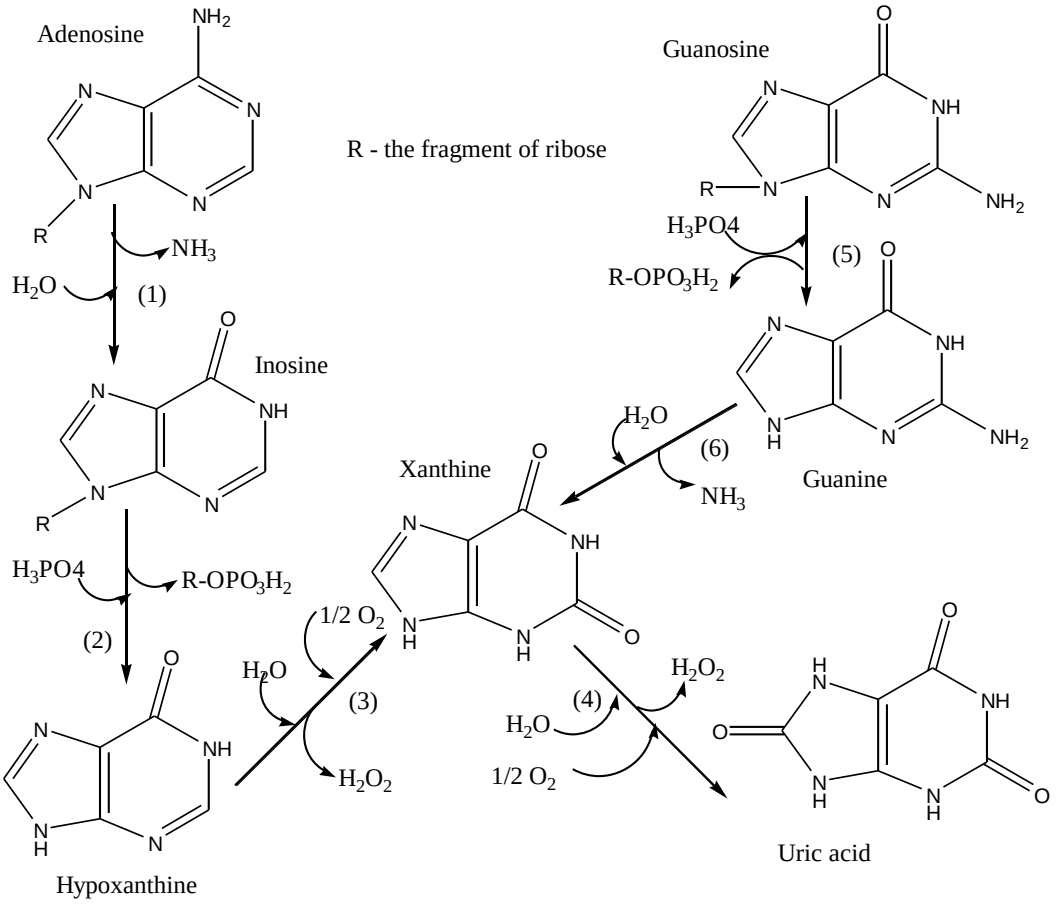


Figure 20. **Degradation of purine nucleosides up to uric acid**.

Uric acid may be in two forms: enol- and keto-form. Sodium ions form with enol-form of uric acid salt that is known as sodium urate (fig. 21).

Conversion for uric acid and formation of sodium urate

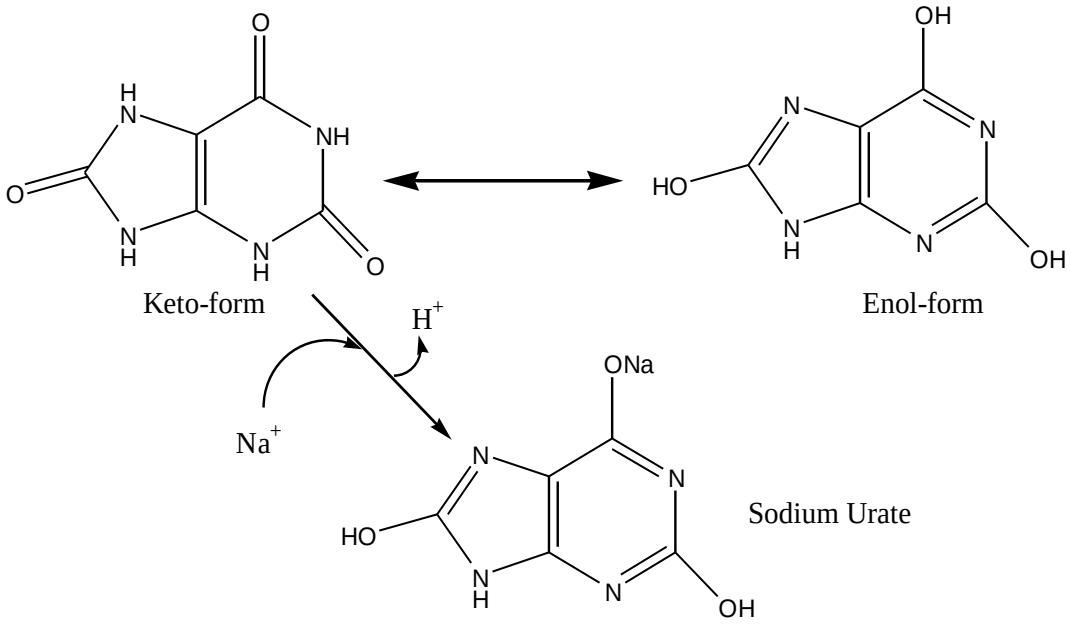


Figure 21. **Enol- and keto- forms of uric acid; the formation of monosodium urate.**

Degradation of pyrimidine nucleosides

Uridine is formed due to deamination of cytidine, and then there are two catabolic pathways for two nucleosides in human tissues (fig.22): uridine and thymidine destructions. Step by step they are destructed to give the end-products: urea (using the way for ammonia utilization), carbon dioxide, beta-alanine and β-aminoisobutyrate. β-alanine is involved in transamination with pyruvate to give formyl acetate that is cleaved into acetyl-CoA. Other way for its utilization may be: it is involved in the synthesis of Anserine and Carnosine. The latter substances are used in muscles for to increase the myosin-ATPase activity.

β-aminoisobutyrate is involved in direct deamination to form hydroxybutyrate, acetyl-CoA is formed after its oxidation. Due to the formation of acetyl-CoA in both cases we can consider β-alanine and β-aminoisobutyrate as energy sources, that is because acetyl-CoA is involved in Krebs cycle in any type of cell.

Excretion of β-aminoisobutyrate increases in leukemia and severe X-ray radiation exposure due to increased destruction of nucleic acids.

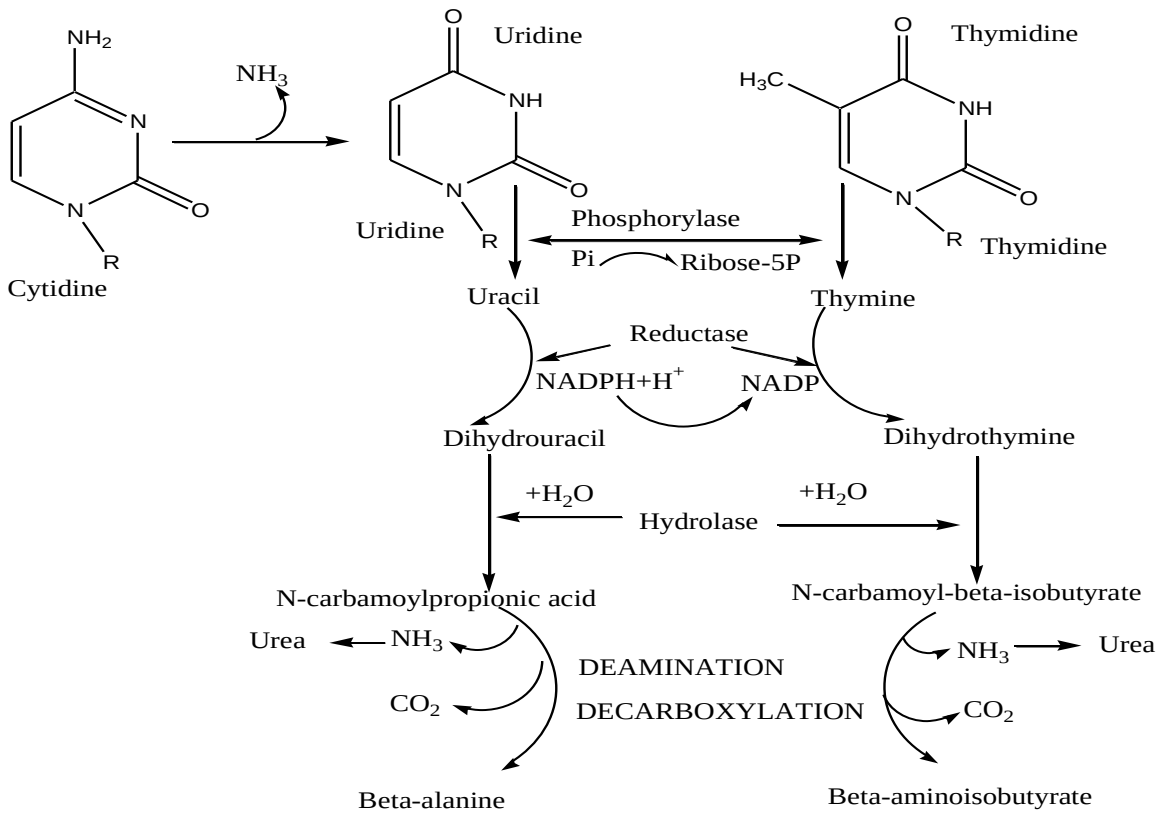


Figure 22. **Degradation of pyrimidine nucleosides**

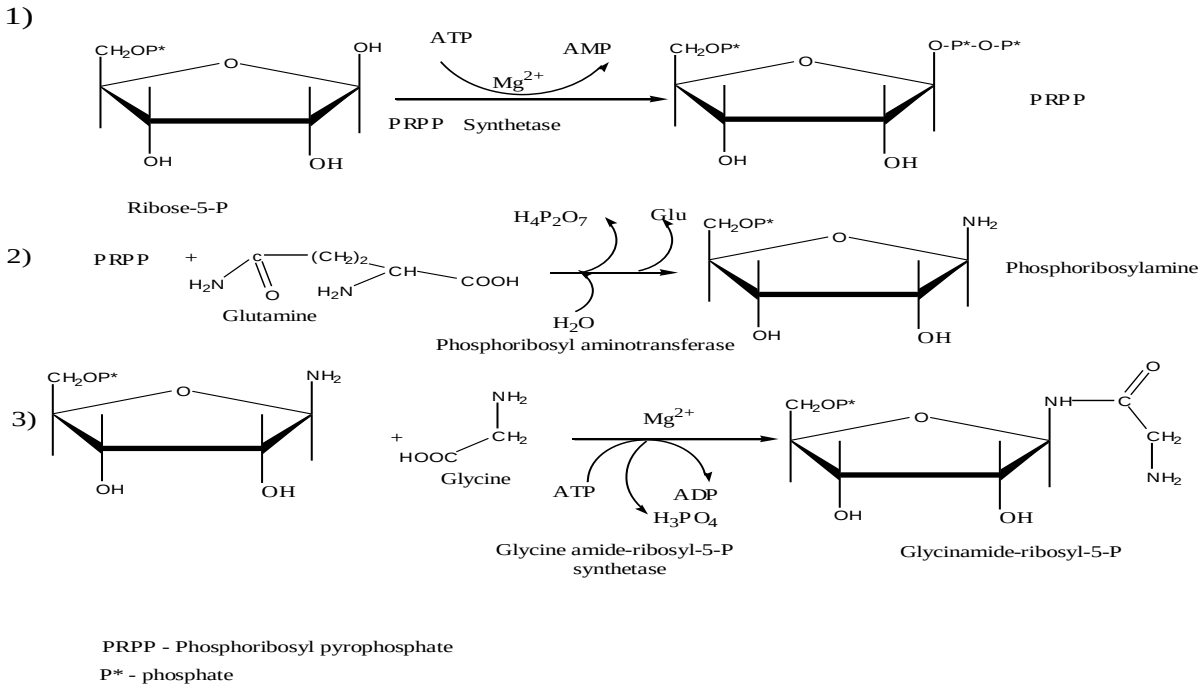
Synthesis de novo of purine nucleotides

This type of synthesis is very important, first of all, for strong vegetarians (in all types of tissue) and for tissues, where we have to consider the high rate of proliferation processes (epithelial tissues, skin, bone marrow, liver).

The first three reactions are very important for regulation of the process (Fig.23).

Then there are eight reactions that give the metabolite – Inosine monophosphate (IMP) (Fig. 24). Nitrogen atom $N^{(9)}$ is from Glutamine (reaction 2). Carbon atoms $C^{(4)}$ and $C^{(5)}$ and nitrogen $N^{(7)}$ are from Glycine (reaction 3). Carbon $C^{(8)}$ is from methenyl- tetrahydrofolic acid (THFA) (formyl fragment is formed, reaction 4). $N^{(3)}$ is incorporated from Glutamine (reaction N5). Then there is formation of imidazole fragment, the bond $C^{(8)}$-$N^{(9)}$ is formed due to dehydration (reaction N6). After that aminoimidazol-ribosyl-5-Phosphate is carboxylated in

position $C^{(5)}$ (reaction N7), $C^{(6)}$ is incorporated in the structure from carbon dioxide.



PRPP - Phosphoribosyl pyrophosphate
P* - phosphate

Figure 23. **The initial reactions of purine nucleotide synthesis**

Reaction N8 is due to synthetase that catalyzed the attachment of the fragment from aspartate. The product Amino imidazole succinyl carboxamide ribosyl-5-Phosphate is formed and $N^{(1)}$ is incorporated. Reaction N9 is the liberation of succinyl group as fumarate. Carbon atom $C^{(2)}$ is added (reaction N10) from formyl-THFA. Reaction N11 is a ring closure by enzyme – IMP cyclohydrolase and IMP is formed.

Four ATP are used for IMP synthesis (reactions 1, 3, 5, 6). The formation of IMP is discussed as first phase of synthetic pathway**:**

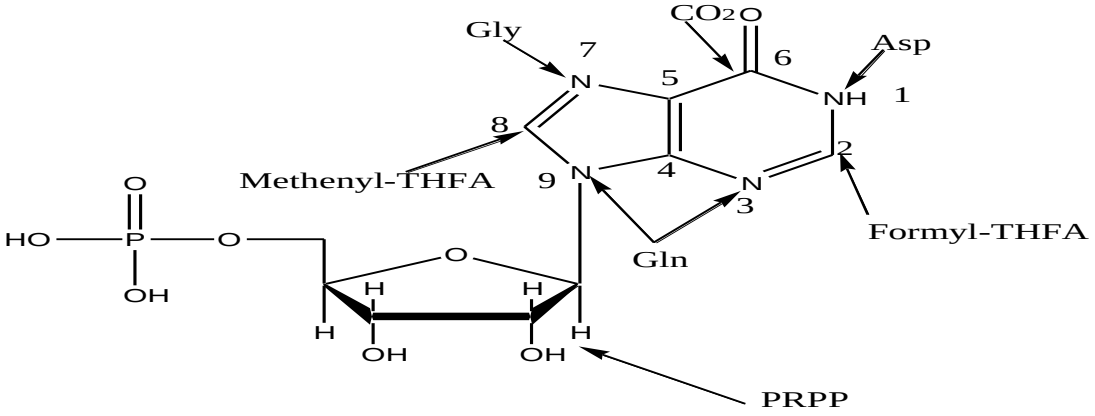


Figure 24. **A key intermediate metabolite for synthesis of purine nucleotides - Inosine Mono Phosphate (IMP).** (*THFA- Tetra Hydro Folic Acid*)

The next phase of purine nucleotide synthesis is the formation of AMP or GMP from IMP (fig. 25). Two reactions (1, 3, fig.25) are used for AMP synthesis from IMP, and GTP is used as energy source for reaction (1). Aspartate is used in the step (1, fig.25) to add nitrogen in a form of amino group instead of keto- group in position 6 of inosine fragment in IMP.

Two reactions are used for GMP synthesis from IMP, and ATP is used as energy source in the step (4, fig.25). Glutamine is a donor of amine group for position 2 of guanine fragment in GMP.

Two reactions (1, 3) are used for AMP synthesis from IMP, and GTP is used as energy source for reaction (1). Aspartate is used in the step (1) to add nitrogen in a form of amino group instead of keto group in position 6 of inosine fragment in IMP.

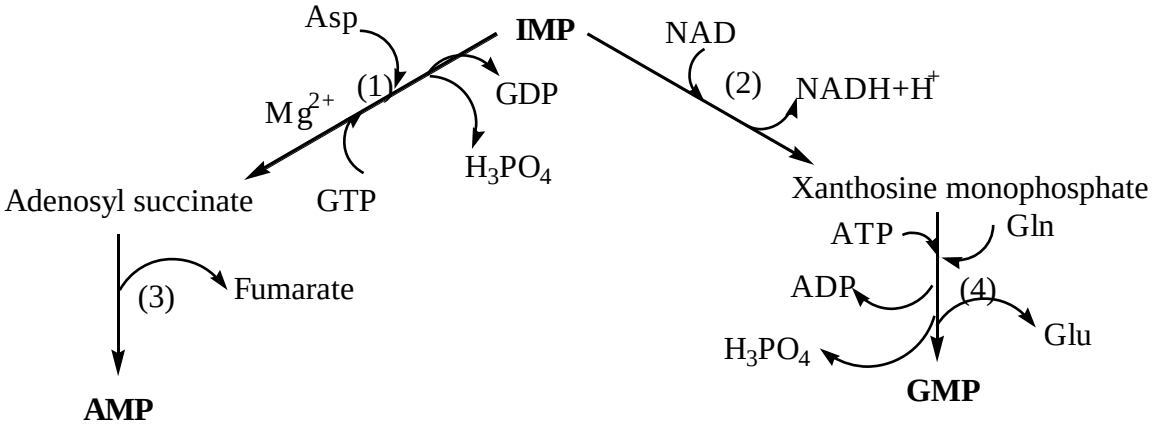


Figure 25. **The second phase of purine nucleotide synthesis.**

Energy requirement for complete pathway per 1 mol of AMP or GMP is 5 ATP.

Special vitamins intake for complete pathway: B_9, B_{12}, B_5 (PP) (B_{12} is required for formation of folic acid derivatives).

GMP and AMP are considered as precursors for ATP and GTP.

For example:

The most important way for ATP formation is oxidative phosphorylation!

Some metabolites from purine nucleotide degradation are involved in the synthesis of IMP, AMP, GMP. Those reactions are named as salvage reactions (fig.26). They are in the liver, brain, polymorphonuclear leukocytes, lymphocytes.

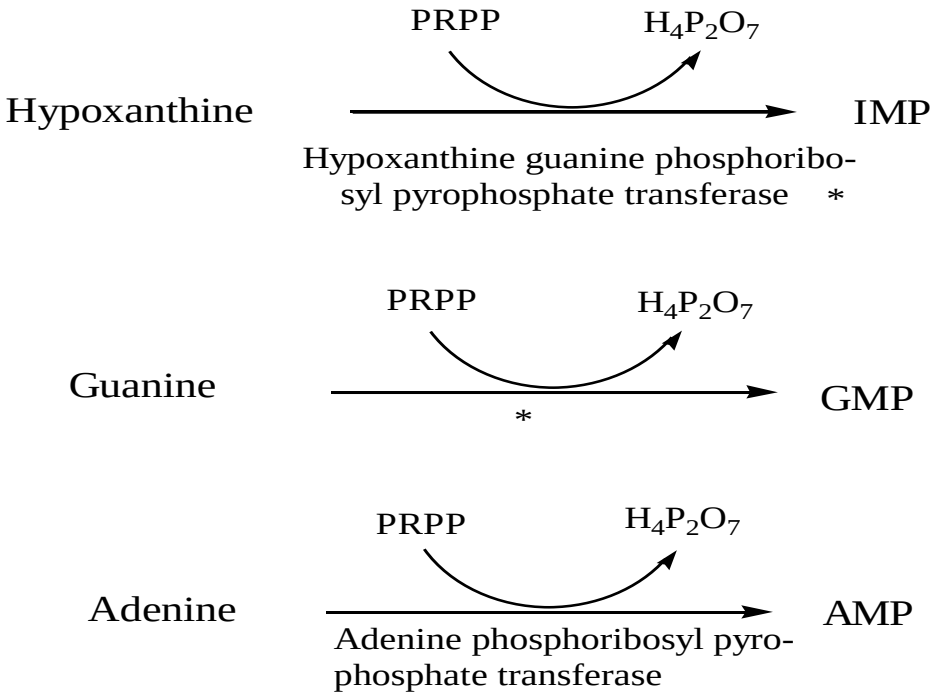


Figure 26. **Salvage reactions duration in humans.**

Pyrimidine nucleotide de novo synthesis

Uridine monophosphate is synthesized in five reactions from carbamoyl phosphate and aspartic acid (fig. 27). Carbamoyl phosphate may be synthesized in our tissues in two ways:

1) from ammonia due to carbamoyl phosphate synthetase I (placed in the liver, only);

2) from glutamine as donor of amine group for carbamoyl phosphate due to carbamoyl phosphate synthetase II, found in all the tissues, except nervous tissue. So, this synthesis we can consider as the way for ammonia utilization, too, but in the liver, only.

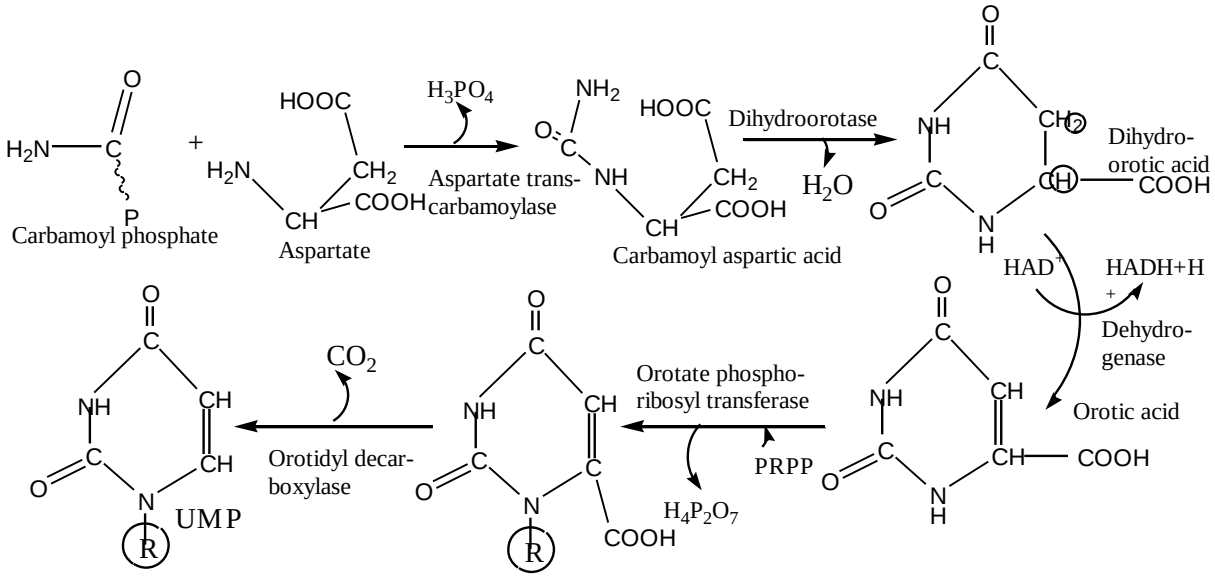


Figure 27. **The synthesis of UMP; R – ribose-5-P fragment.**

The UMP is considered as the precursor for UTP (reaction 1) and CTP (reaction 2):

$$UMP \xrightarrow[ATP\ \ ADP]{kinase} UDP \xrightarrow[ATP\ \ ADP]{kinase} UTP \quad (1)$$

$$UTP + Gln + ATP \xrightarrow[Synthetase]{} CTP + ADP + H_3PO_4 + Glu \quad (2)$$

The synthesis of dTMP requires three steps (fig. 28):

Step 1. The function of special multienzyme system that is used for transformation of riboderivative (UDP) to deoxyriboderivative (dUDP). This multienzyme system includes two enzymes:

- Ribonucleoside diphosphate reductase containing Thioredoxin as a non-protein part. During the first reaction the reduced form of Thioredoxin becomes the oxidized one;
- Thioredoxin reductase (NADPH – the non-protein part of enzyme) is used for transformation of oxidized form of Thioredoxin again to the reduced one.

Step 2. The function of dUDP phosphatase to form dUMP as a product.

Step 3. The function of Deoxythymidilate synthetase to form dTMP using special derivative of THFA.

Then again we can consider the transformation of dTMP to dTTP due to the action of special kinases.

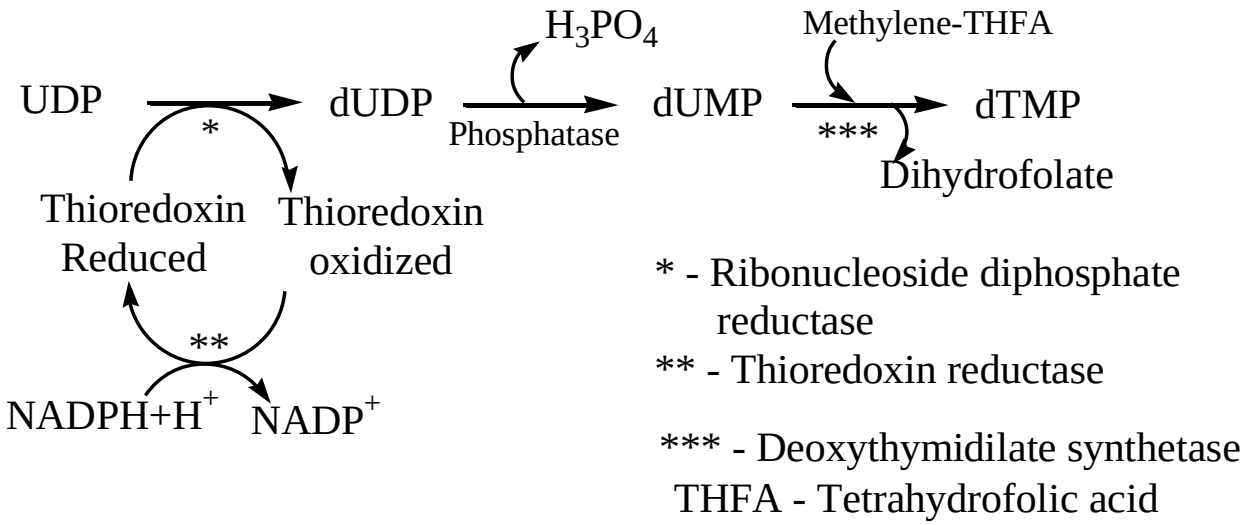


Figure 28. **The synthesis of dTMP from UDP.**

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The Regulation of nucleotide synthesis

- AMP, ADP GMP, GDP, TDP are considered as allosteric inhibitors for PRPP-synthetase when they are accumulated in cytoplasm (fig. 29).
- High concentration of GMP is allosteric inhibitor for Phosphoribosyl aminotransferase (reaction 2) in the purine nucleotide synthesis.
- The regulation of second stage of synthesis, respectively: GTP accumulation causes the stimulation of AMP synthesis (adenylosuccinate lyase), ATP accumulation causes the stimulation of GMP synthesis (GMP-synthetase)
- The accumulation of PRPP is a positive factor in stimulation of Carbamoyl phosphate synthesis and then the synthesis of UMP.
- But the accumulation of UDP in a cell is considered as a factor for inhibition of discussed reaction.
- The accumulation of CTP is the factor for inhibition of Carbamoyl aspartate formation during the synthesis of UMP

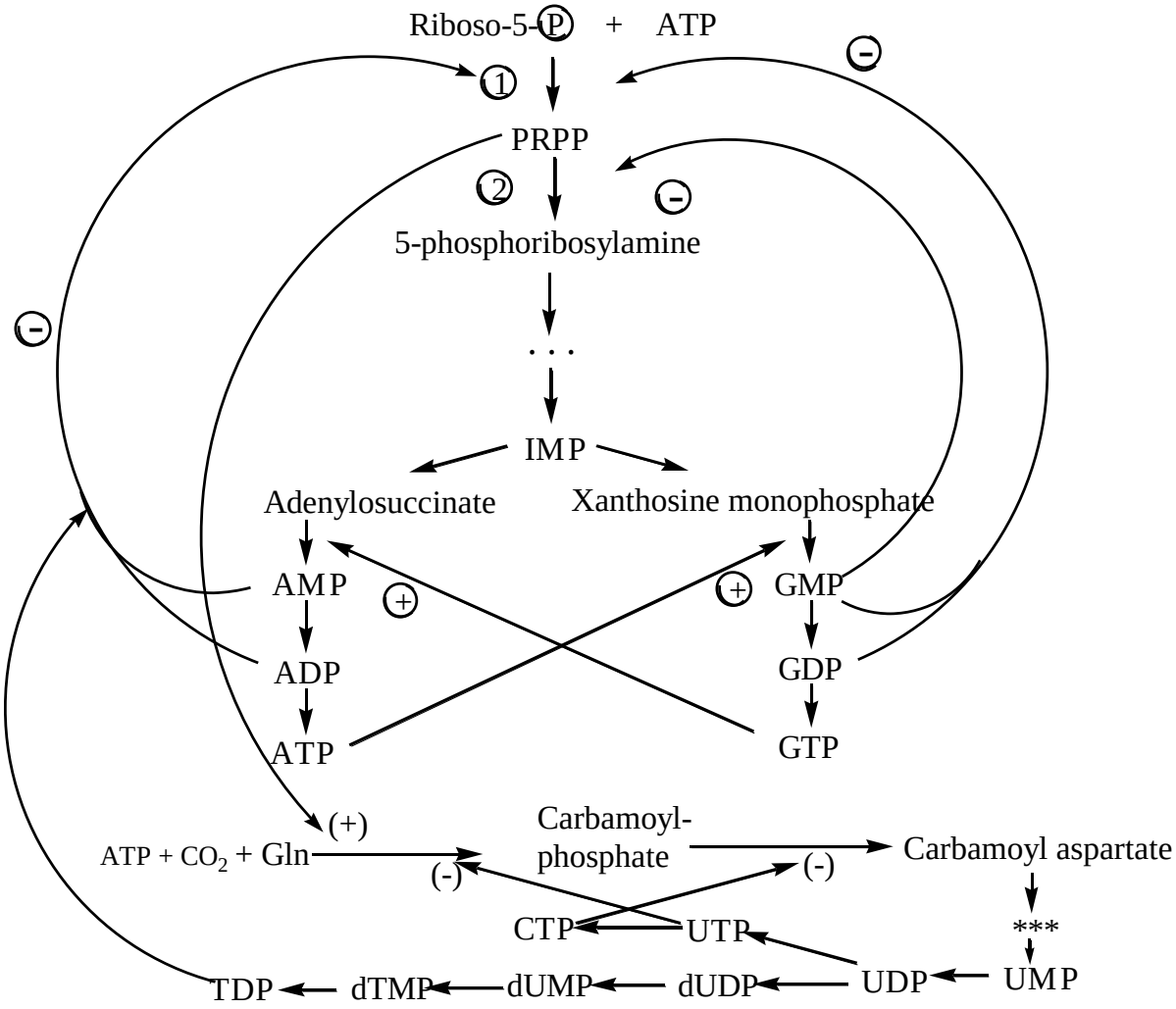


Figure 29. **The regulation of purine nucleotide synthesis**

Clinical disorders of nucleotide metabolism

Net excretion of total uric acid in healthy humans averages 400-600 mg/day (24 hours). Many pharmacologic and naturally occurring compounds influence renal absorption and secretion of sodium urate. High doses of aspirin competitively inhibit both urate excretion and reabsorption.

Urates (monosodium urate salts) are present in human fluids. Urates are far more soluble in water then uric acid. ***The lower the temperature of the medium the lower the solubility of urates in water.***

Normal value of urates in the blood plasma is not more than 0,42 mmol/l (for men) and 0,3 mmol/l (for women). The values, which are higher, provide the state named ***hyperuricemia.***

In hyperuricemia, serum urate levels exceed the solubility limit; this causes the crystallization of sodium urate in soft tissues and joints to form deposits named ***tophi.*** This event causes an inflammatory reaction, later acute gouty arthritis, which can progress to chronic gouty arthritis. Inflammation and erosion of the joints occur when leukocytes engage the deposited crystals and consequently rupture, releasing lysosomal enzymes. Sodium urate crystals in the urinary tract impair renal function, too.

Hyperuricemia is the obligatory component of gout appearance, but only in 15% of patients from all having this state.

Factors, which can cause the gout in patients with hyperuricemia:

1. Overcooling of human organism. The solubility of sodium urate is lower under low temperature. The rate of urate accumulation in joints is higher in this case.
2. The sharp change of diet in patient with hyperuricemia. If you are patient with hyperuricemia and have the diet with animal food products you cannot become strong vegetarian before consultation with doctor.

Hyperuricemia may be secondary to other disease such as cancer, psoriasis, chronic renal deficiency.

Treatment of gout

Allopurinol is the drug that blocks the action of Xanthine oxidase for production of uric acid. This drug is oxidized by xanthine oxidase to oxypurinol. Oxypurinol binds tightly to xanthine oxidase, inhibiting its ability to oxidase xanthine or hypoxanthine. It is an example of suicide inhibition.

The reaction of allopurinol action with PRPP used in HGPRT reaction results in decrease in PRPP levels and thus a decrease in de novo purine synthesis.

Colchicine is an anti-inflammatory drug that is used only to treat gout. It inhibits leukocyte movement by affecting microtubules.

Inherited disorders of purine metabolism

PRPP synthetase can have abnormal features:

1. Superactive (increased V max) → purine overproduction → gout
2. Resistance to feedback inhibition → purine overproduction → gout
3. Low Km for ribose-5-P→ purine overproduction → gout

Hypoxanthine guanine phosphoribosylpyrophosphate transferase (HGPRT)

1. Partial deficiency → purine overproduction → gout
2. Complete deficiency (Lesch-Nyhan syndrome) → purine overproduction, the main clinical symptoms: self-mutilation, mental retardation, and death in yearly childhood.

Lesch-Nyhan syndrome:

Several forms of HGPRT deficiency have been identified:

1) in one form patients have normal levels of this enzyme, but the enzyme is inactive;

2) the patients have en enzyme that is apparently unstable; its activity is higher in young red cells than in old.

The symptoms: hyperuricemia, gout, urinary tract stones, and neurological symptoms of mental retardation, self-mutilation, and then death in young age.

The basis of neurological symptoms is unknown. However, brain cells normally have much higher levels of purine salvage enzymes than other cells and may normally use salvage pathways to a greater extent.

Treatment by allopurinol reduces the uric acid formation but does not alleviate the neurological symptoms.

Xanthine oxidase:

Complete deficiency → xanthine renal lithiasis, hypouricemia associated with ***xanthinuria.***

von Gierke's disease (glucose-6-phosphatase deficiency)

Purine overproduction and hyperuricemia in von Gierke's disease (glucose-6-phosphatase deficiency) occurs secondarily to enhanced generation of the PRPP

precursor ribose-5-phosphate. In addition, associated lactic acidosis elevates the renal threshold for urate, elevating total body urates.

TASKS FOR SELF-CONTROL on CHAPTER 2

1. Which of the following compounds **may be not** used in the the conversion of ribonucleotide to deoxyribonucleotide?

A. Ribonucleotide reductase
B. Thioredoxin
C. NADPH
D. ATP
E. ADP

2. Which of the following compounds contributes to the structure of **both** purine and pyrimidine nitrogenous bases during nucleotide biosynthesis?

A. Aspartic acid
B. Methylene-THF
C. Carbamoyl phosphate
D. Glycine
E. Phosphoribosyl pyrophosphate (PRPP)

3. Two enzymatic activities are supplied by two domains of a single protein. A defect in this bifunctional enzyme causes orotic aciduria. Choose the correct name for these domains:

A. Carbamoyl phosphate synthase II & aspartate transcarbamoylase
B. Dihydroorotase & dihydroorotate dehydrogenase
C. Orotate phosphoribosyltransferase & orotidine monophospfate decarboxylase
D. Phosphoribosyl pyrophosphate (PRPP) synthase & PRPP glutamyl amidotransferase
E. Adenosine deaminase & xanthine oxidase

4. Lesch-Nyhan syndrome is due to the complete deficiency in hypoxanthine guanine phosphoribosyl transferase (HGPRTase) and as result impossibility to produce certain product. Point out it:

A. Urea

B. Uric acid

C. GMP

D. AMP

E. Phosphoribosyl pyrophosphate (PRPP)

5. Which of the following nucleotides **does not** undergo a ring deamination during catabolism?

A. GMP

B. AMP

C. CMP

D. ATP

E. UMP

6. The first purine nucleotide that is formed de novo in metabolism is:

A. IMP

B. AMP

C. GMP

D. XMP

E. ADP

7. The regulatory enzyme in the pyrimidine synthesis in animals is:

A. Aspartate transcarbamoylase

B. Carbamoyl phosphate synthase II

C. Dihydroorotase

D. Dihydroorotate dehydrogenase

E. Orotate phosphoribosyltransferase

8. Point out the main substrates for the replication:

A. ATP, GTP, TTP, CTP

B. ADP, GDP, TDP, CDP

C. dATP, dGTP, dTTP, dCTP

D. dATP, dGTP, dUTP, dCTP

E. dADP, dGDP, dTDP, dCDP

9. Thymine-thymine dimers may be formed under ultraviolet light exposure and may result in the block the DNA replication. Choose the enzyme particitating in the reparation of this damage:

A. UV specific endonuclease
B. UV specific exonuclease
C. UV sensitive DNA polymerase
D. UV sensitive RNA polymerase
E. UV dependent reparase

10. Choose the chemical nature of the primer required for the synthesis of DNA:

A. Neutral fat
B. Olygosaccharide
C. Polypeptide
D. Olygoribonucleotide
E. Olygodeoxyribonucleotide

11. Retroviruses possess RNA as genetic material. They use a special enzyme for formation of DNA from RNA. Name the enzyme:

A. RNA polymerase III
B. Primase
C. Reverse transcriptase
D. Topoisomerases
E. DNA ligase

12. Which of the following enzymes catalyzes the formation of phosphodiester bond between the adjoining Okazaki fragments?

A. RNA polymerase III
B. Primase
C. DNA polymerase III
D. DNA ligase
E. DNA polymerase I

13. Point out the specific protein which can bind to the growing RNA, terminate transcription and release RNA:

A. RNA polymerase III

B. RNA polymerase II

C. RNA polymerase II

D. DNA polymerase III

E. ρ (rho) factor

14. Point out an inhibitor of transcription:

A. Rifampin

B. Methotraxate

C. ATP

D. GTP

E. Allopurinol

15. Which of following descriptions is not correct for promoter :

A. A promoter is a region of DNA that facilitates the transcription of a particular gene

B. A promoter is typically located near the gene that will be transcribed

C. A promoter is recognized by RNA polymerase

D. A promoter represents critical elements that can work in concert with other regulatory regions (enhancers, silencers etc) to direct the level of transcription of a given gene

E. A promoter stops the transcription by formation of hairpins of newly synthesized RNA

16. During replication of DNA, which one of the following enzymes produces the Okazaki fragments?

A. DNA Polymerase I

B. DNA Polymerase II

C. DNA Polymerase III

D. RNA Polymerase I

E. RNA Polymerase II

17. Replication and transcription are similar processes mechanistic terms because both:

A. Use RNA primers for initiation

B. Use deoxyribonucleotides as precursors

C. Are semi conserved events

D. Involve phosphodiester bond formation with elongation occurring in the 5’-3’ direction

E. Use DNA polymerase III

18. Excessive ultraviolet radiation is harmful to life. The damage caused to the biological systems by ultraviolet radiation is by:

A. Inhibition of DNA synthesis

B. Formation of thymidine dimmers

C. Ionization

D. DNA fragmentation

E. Deamination of DNA

19. Restriction enzymes which have revolutionized the field of genetic engineering have been found in:

A. Bacteria

B. Humans

C. Viruses

D. Plants

E. Animals

20. Formation of Okazaki fragments occurs in:

A. Transcription

B. Reverse Transcription

C. Translation

D. Replication

E. Reparation

Chapter 3

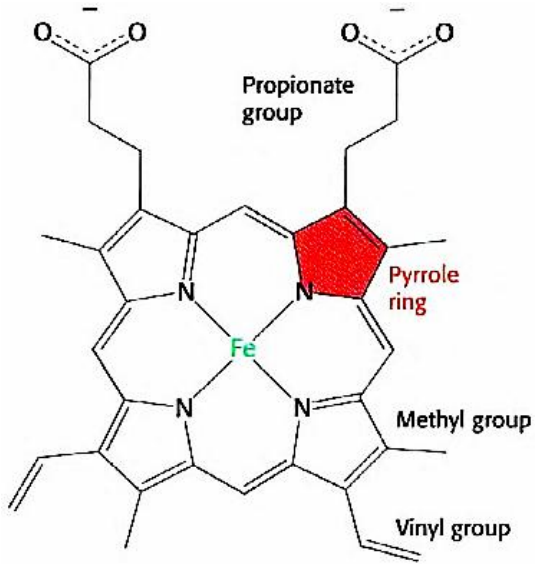
HEAMOPROTEINS: STRUCTURE, PROPERTIES, FUNCTIONS AND METABOLISM

Created by ass.pr. Ivanchenko D.G., as.pr. Rudko N.P.

Common notions about haemoproteins

Haemoprotein contains polypeptide chain and haem as non-protein component. The species character of a haemoglobin is primaly due to its polypeptide moiety, while the haem is the same in haemoproteins of any type. The structure basis of prosthetic group in most haem-containing proteins is the porphyrin ring derived from a tetrapyrrolic compound, porphyrin. The unsubstituted porphyrin is called porphyn. In a haem molecule, porphyn is represented by protoporphyrin IX. The chelated comlex of protoporphyrin IX with Fe (II) is called haem.

Heme consists of protoporphyrin IX and a central iron Fe2+ ion. The organic component, named protoporphyrin IX , is made up of 4 pyrrole rings linked by methine bridges to form a tetrapyrrole ring. 4 Methyl groups, 2 vinyl groups, and 2 propionate as side chains are attached (fig. 1).



The iron ion lies in the center of the protoporphyrin, bonded to the 4 pyrrole nitrogen atoms. The iron ion can form 2 additional bonds one on each side of the heme plane. These binding sites are named as the fifth and sixth *coordination sites*.

Figure 1. **The heme structure**

The fifth coordination site is occupied by the imidazole ring of a histidine residue from the protein chain. This histidine is referred to as the proximal histidine.

Examples of Haemoproteins

Haemoglobin is the quantitatively most important haemoprotein**.**

- It is the carrier of oxygen and carbon dioxide in the blood.
- The body of adults contains about 750 grams of haemoglobin; it is replaced every 120 days.
- Normal hemoglobin turnover requires synthesis of new 6-8 grams hemoglobin daily, a process which uses about 14% of the dietary amino acids. 300 milligrams of haem are synthesized daily for conjugation with the newly synthesized globin protein.

In subunit of hemoglobin, the sixth coordination site of the heme remains unoccupied; this position is available for binding O2**.** The iron ion lies approximately 0.4 Ä outside the porphyrin plane because an iron ion, in this form, is *slightly too large* to fit into the well-defined hole within the porphyrin ring. The linkage of hemoglobin with oxygen gives derivative oxyhemoglobin (fig.2), and carbon dioxide transport is available due to this protein if carbhemoglobin is produced.

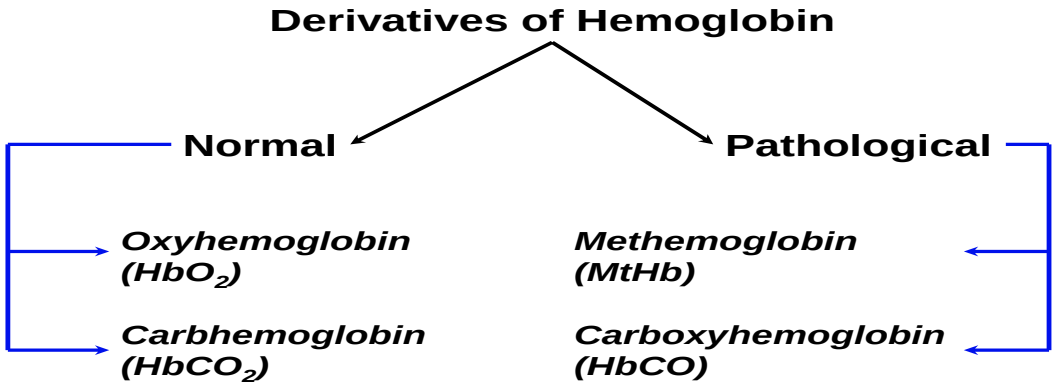
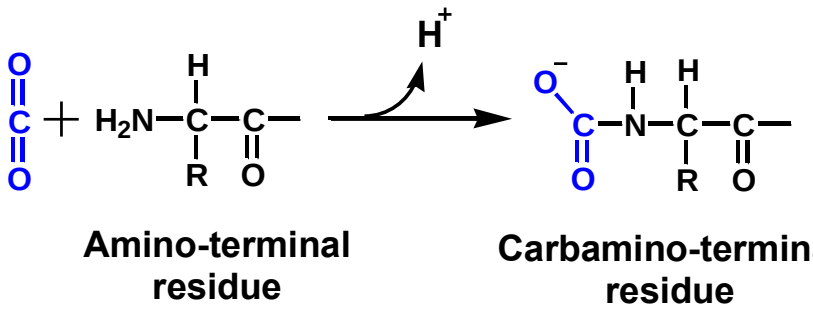
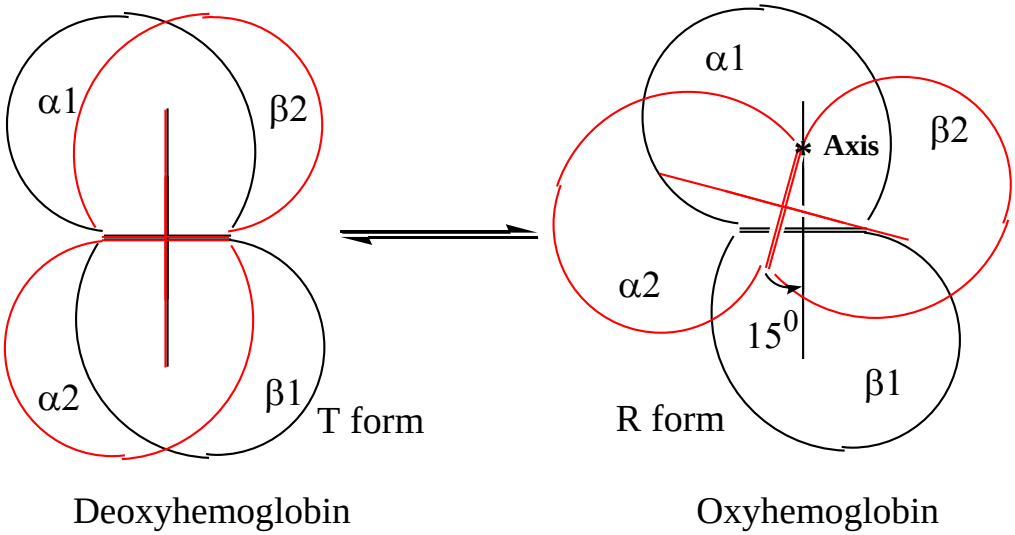


Figure 2. **Normal and Pathological derivatives of hemoglobin in humans**

Linkage of CO2 is not using the Fe2+ , because CO2 binds as a carbamate group to the α-amino group at the amino-terminal end of each globin chain, forming carbaminohemoglobin:



The bound carbamates also form additional salt bridges that help to stabilize the ***T- state*** and promote the release of oxygen. Transition from T-form to the R-form is more probable if each heme group is oxygenated:



Factors influenced the hemoglobin affinity to oxygen

In the lungs, at pH 7.4 and high PO2, hemoglobin shifts to the R form, with a lower affinity for protons

An increase in PCO2 and a decrease in pH are both characteristic of actively metabolizing cells, these cells promote the release of oxygen from hemoglobin : R-form shifts to the T-form

Pathological derivatives of hemoglobin

In haemoglobin Fe^{2+} does not change its valency during binding or release of oxygen. But it can be oxidized by oxidation agents to Fe^{3+}, giving rise to

haemming. The resulting compound is called ***methaemoglobin*** and it can't function as oxygen carrier. So, Heme contains Fe3+ instead Fe2+. It is formed:

- Under the influence of strong oxidizing agents (superoxide ion, etc.)
- At the deficiency of methemoglobin reductase, glucose-6-phosphate dehydrogenase, glutathione reductase

Carboxyhaemoglobin (HbCO) is forming during carbon monooxide (CO) poisoning. CO is very toxic gas because it binds with a higher affinity (much tighter) to haeme does oxygen. Since CO has a higher affinity than oxygen, oxygen cannot displace it. In this way, CO acts as much like a potent competitive inhibitor. There can be plenty of oxygen available, but the hemoglobin bond to CO will not carry it; therefore the oxygen is not available for tissues. CO effectively binds irreversibly to the haem in hemoglobin molecule.

HbA_{1c}. The hyperglycemia causes this glucosylation of lysine residues mainly in beta-subunits of hemoglobin to form HbA_{1c}. This is the formation of Shift (S) base between glucose and the amino group of terminal amino acid of the β-chains. Then the S base becomes the more stable amino ketone. In diabetes mellitus the concentration of HbA_{1c} may reach 12% or more of the total hemoglobin. The amount of HbA_{1c} becomes a good indicator of blood glucose levels over 2-4 month period of disease duration.

Cytochromes are present in the mitochondria and in the endoplasmic reticulum.

- They participate in important electron transfer reactions.
- The most important are *b*, c_1, *c*, aa_3, b_5, P450.
- Their half life is about 132 hours.

Catalase and peroxidase utilize toxic hydrogen peroxide.

- These enzymes protect the body against uncontrolled oxidation by hydrogen peroxide.
- Their half life is about 20 hours.

Tryptophan oxygenase is a haemoprotein of intermediary metabolism**.**

- It catalyzes the conversion of tryptophan to N-formylkynurenine, using oxygen as the oxidant.
- Its half life is only about two hours.

These are only a few of the many haemoproteins.

Haem Biosynthesis

Mitochondria are not only the powerhouse of the cell, supplying abundant ATP through the coupling of active proton pumping across their inner membrane with the transfer of electrons along the respiratory chain, they are also the alpha and omega of haem biosynthesis.

The overall pathway of haem biosynthesis begins in the mitochondria, with the condensation of succinyl CoA coming from the citric-acid cycle with glycine to form δ-aminolevulinate, which is expedited forthwith into the cytoplasm. There, the synthesis of the tetrapyrroleporphyrin nucleus continues apace, until the oxidative decarboxylation of coproporphyrinogen III to protoporphyrinogen IX is accompanied by its transport back into the mitochondria whence it came, to undergo oxidation of its methylene groups to protoporphyrin IX and insertion of iron to yield the end product, haem. The two major sites of haem biosynthesis are erythroid cells, which synthesize around 85 % of the body's haem groups, and the liver, which synthesizes most of the remainder. ***A major function of haem in liver is as the prosthetic group of cytochrome P450, the importance of which is detoxification.*** The liver cell must synthesize cytochrome P450 throughout its lifetime in quantities that vary with conditions. In contrast, the developing erythroid cell only engages in haem synthesis when it differentiates, and then it is a one-time synthesis in vast quantities to accompany globin production and ensure the haemoglobin content that will last for the erythrocyte life-time. Haem and globin synthesis cease upon red cell maturation. This means that haem synthesis in liver and erythroid cells is regulated in a quite different way.

In the liver, the main control site is ***δ-aminolevulinate synthase***, which is regulated by haemin, the Fe (III) oxidation product of haem, by three mechanisms:

1) feedback inhibition, *2)* inhibition of transport of the enzyme from its site of synthesis in the cytosol to the mitochondria, and *3)* repression of the enzyme synthesis (figure 3).

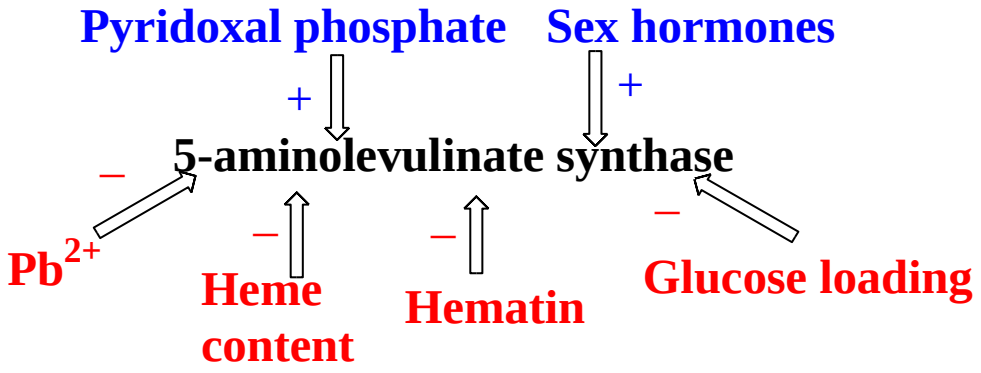


Figure 3. **Factors to control activity of δ-aminolevulinate synthase**: *coloured blue -stimulators of activity; coloured red -suppressors of activity.*

In differentiated erythroid cells (reticulocytes), haem stimulates protein synthesis, inducing synthesis of globin to ensure that haem and globin are synthesized in the correct ratio for assembly into haemoglobin, but also induces the synthesis of the haem biosynthetic pathway enzymes. ***The control of haem synthesis in erythroid cells seems to be at the level of ferrochelatase***, and ***porphobilinogen deaminase***, rather than δ-aminolaevulinate synthase. However the translation of δ-aminolaevulinate synthase mRNA is regulated by iron availability, increasing when iron is abundant.

Ferrochelatase (protohaem ferrolyase), is the terminal enzyme in haem biosynthesis, catalysing the incorporation of ferrous iron into protoporphyrin IX. They are monomeric proteins of molecular weight between 36–40 kD. Mutations in the ferrochelatase gene in humans can cause ***erythropoietic protoporphyria***.

Haemoglobinopathies as disorders of haemoglobin synthesis

They are genetic diseases in which haemoglobin subunits are mutated. More then 150 of haemoglobinopathies have been described. In some there are no symptoms or impairment, whereas in others there may be severe impairment.

Sickle cell anemia (HbS is instead of HbA_1, homozygous defect) was one of the first hemoglobinopathies to be described (fig.4). HbS is formed when valine

replaces glutamic acid in the sixth position of the β-chains. The α-chains are normal. HbS polymerization and deoxygenation is indicated within red blood cells. The polymerization leads to the characteristic sickle-shaped cell. The symptoms of Sickle cell anemia in homozygous can be severe. The patients are anemic and their red blood cells have an average life span of 10 to 15 days instead of normal 120 days. A sickle cell crisis can be extremely painful and can lead to cumulative organ damage. Death often occurs in early adulthood. Heterozygotes are said to have sickle cell trait. They are usually symptoms free except under condition of low P_{O2}. Persons with sickle cell trait show an increased resistance to malaria.

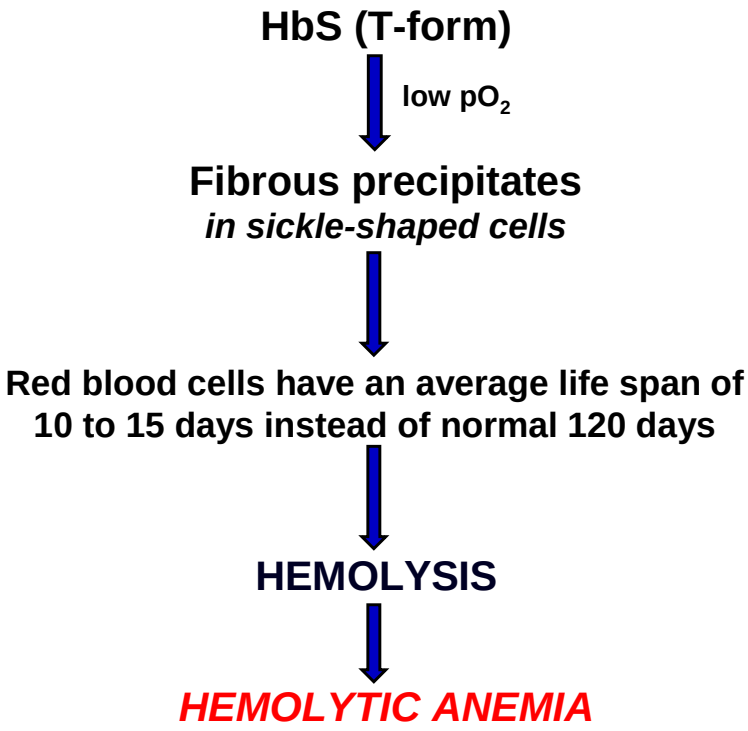


Figure 4. **Events in sickle-cell anemia development.**

Thalassemias are hemolytic anemias that arise from insufficient production either the α- or β-chains of haemoglobin.

Thalassemias are classified according to the number of copies of mutated genes that the patient carries. Individuals with β-thalassemias (insufficient production of β-chain) may have either one (homozygous) or two (heterozygous) mutated genes. Because α-chain genes are duplicated, from one to four α-chain genes may be mutated.

Haem Degradation

Haem is mainly found in the human organism as a prosthetic group in erythrocyte haemoglobin. Most of the haem which is degraded comes from haemoglobin.

Since in the steady state 6-8 grams of haemoglobin are synthesized daily, 6-8 grams must also be degraded, around 100–200 million aged erythrocytes per hour are broken down in the human organism. This gives rise to about 300 milligrams of haem. Haem is not reutilized, so it must be degraded and excreted. The degradation process starts in reticuloendothelial cells in the spleen, liver, and bone marrow.

Although haem is not recycled, **its iron** is conserved.

- Normally, senescent and damaged erythrocytes are sequestered by the spleen, which processes them in a manner that preserves their iron content.
- If haemolysis occurs, haemoglobin (with its iron) is released into the plasma.

Possible causes of **haemolytic anemia** include:

- Genetic defect of some enzymes of haem synthesis.
- Megaloblastic anemia (pernicious anemia) appears when absorption of vitamin B_{12} is prevented by lack of intrinsic factor.
- Folic acid deficiency causes megaloblastic anemia.
- It may by causes by exogenous factors:
  - Prolonged treatment by antibiotics;
  - Poisoning by some products of chemical industry;
  - X-ray radiation.
- Some special disorders of brain marrow: ostheomyelosclerosis, ostheopetrosis.
- It may be caused by deficiency of two enzymes in erythrocytes:
  - Glucose-6-phosphate dehydrogenase,
  - Pyruvate kinase.
- Methaemoglobinemia: intake of excess oxidants (various chemicals and drugs).

In the plasma, oxyhaemoglobin dissociates into alpha-beta dimers, which can escape through the glomerular filtration system of the kidney to appear in the urine. To prevent this, there is a plasma protein, haptoglobin, which binds the dimer and delivers it to the reticuloendothelial system for processing, then activates the haem to prepare it for degradation.

Any free haem is bound to another plasma protein, haemopexin, which then transports it to the liver for degradation. Turnover of haem proteins, particularly haemoglobin, potentially leads to release of free haem into extracellular fluids, where it can be a source of free radical formation and a major source of iron for invading bacterial pathogens. Protection is afforded by haemopexin, a 60-kD serum protein whose structure has been recently determined, which binds haem with high affinity, and delivers the haem to target cells such as liver via specific receptors. Internalization of the haem–haemopexin complex releases haem for intracellular degradation by haem oxygenase, stimulates intracellular protective mechanisms including induction of haemoxygenase 1 and the anti-apoptotic transcription factor NFkB. In this way, haem binding and transport by haemopexin provides protection against both extracellular and intracellular damage by free haem, limits access by pathogenic organisms to haem, and conserves iron by recycling the haem iron. The importance of haem–haemopexin as a cellular iron source is probably rather limited under normal physiological conditions.

Haem is degraded in two steps to bilirubin, which is conjugated with UDP-glucuronic acid and excreted.

The first reaction is cleavage of the haem ring by a microsomal haem oxygenase (HO, fig.5). The substrates for the reaction are: haem, three molecules of oxygen, NADPH. The reaction is a cleavage of the ring between the I and II pyrrole rings:

- The alpha-methylene group is released.
- The product is symmetric with respect to the propionic acid groups.

Terminal products for this reaction are : 1) biliverdin (green-coloured); 2) carbon monoxide (this is the only endogenous source of carbon monoxide); 3) Fe^{3+} ; 4) $NADP^{+}$.

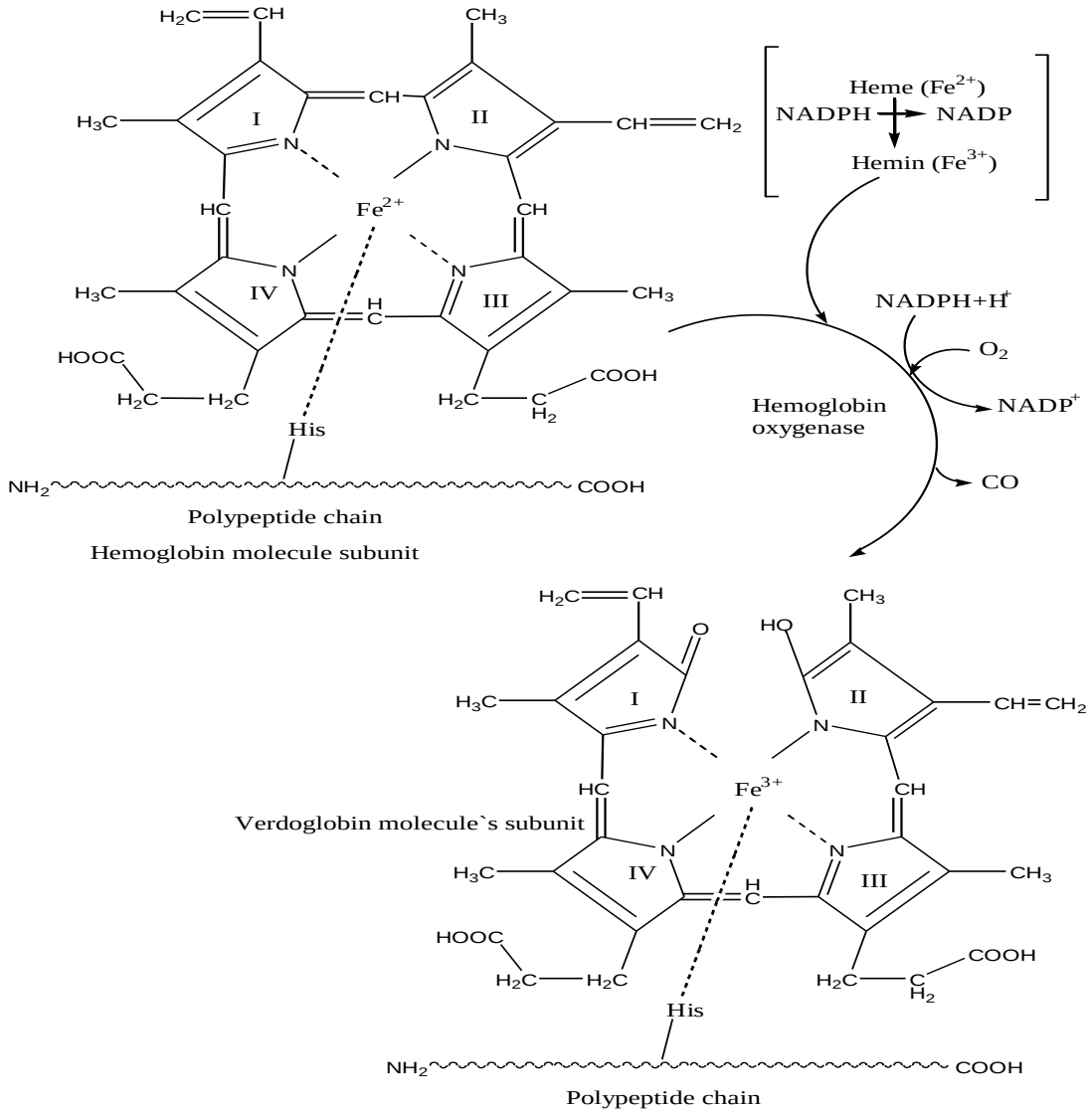


Figure 5. **Hemoglobin oxygenase action in the breakdown of hemoglobin**

The enzyme haem oxygenase catalyses the NADPH, O_2 and cytochrome *P*450 reductase-dependent oxygenation of haem to iron, CO and biliverdin. Humans and other mammals have two isoenzymes, HO-1 and HO-2, which are products of separate genes. Human HO-1 is a 288-residue protein which is found at highest levels in the spleen, where recycling of erythrocytes takes place, but is also found in liver, where haem derived from cytochrome *P*450 is degraded, and in other tissues. It is regulated at the transcriptional level by porphyrins, metals,

progesterone, and a variety of other molecules, and is involved in response to oxidative stress, ischemia, hypoxia and other disease states. Human HO-2, a 316-residue protein, is constitutively expressed at high levels in the testis and some regions of the brain. This has led to the proposal that the principal role of HO-2 is the production of CO as a neural messenger. Both HO-1 and HO-2 have *C*-terminal extensions with membrane anchors that are both localized in the microsomal membrane.

In the first step, a ferrous haem–O_2 complex is thought to undergo an internal electron shift to form a ferric peroxyhaem intermediate, which then reacts regiospecifically with the α-bridge carbon to form α-hydroxyhaem. A second O_2 then reacts with α-hydroxyhaem, forming verdoglobin and liberating CO. A third O_2 next reacts with verdoglobin to form an enzyme-bound Fe(III)–biliverdin complex. Fe^{3+} and biliverdin IXa are released from the enzyme. At various steps, reducing equivalents are required. The microsomal haem oxygenases of animals derive these reducing equivalents from NADPH via the microsomal NADPH-cytochrome *P*450 reductase.

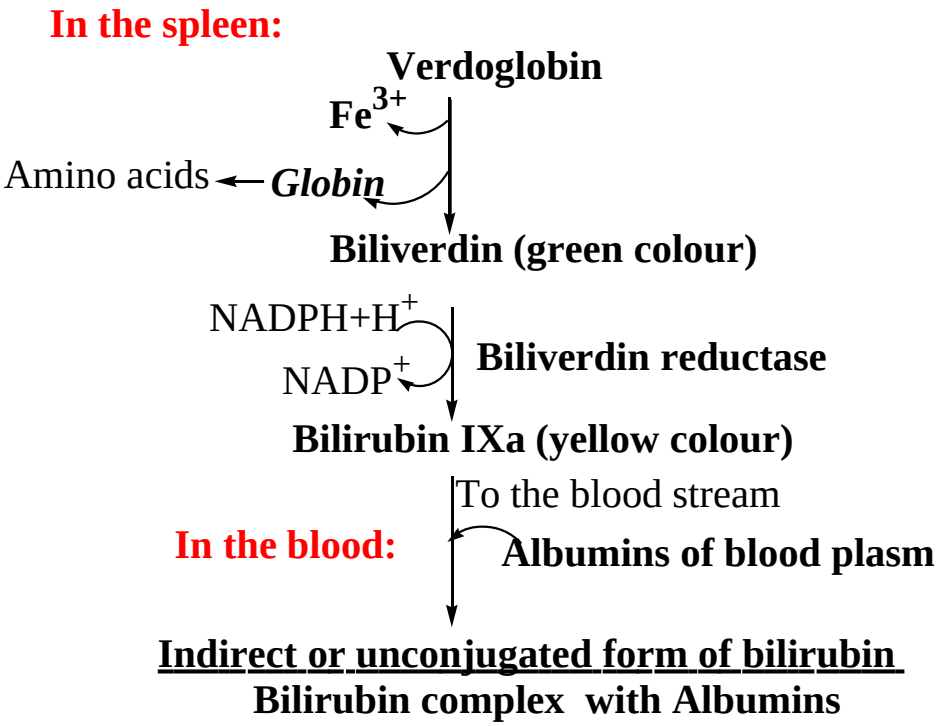


Figure 6. **The formation of indirect or unconjugated bilirubin in the blood plasma.**

In the second reaction biliverdin reductase reduces the central methene bridge of biliverdin, producing orangecolored bilirubin. The color change from purple to green to yellow can be easily observed *in vivo* in hematoma state (fig.6).

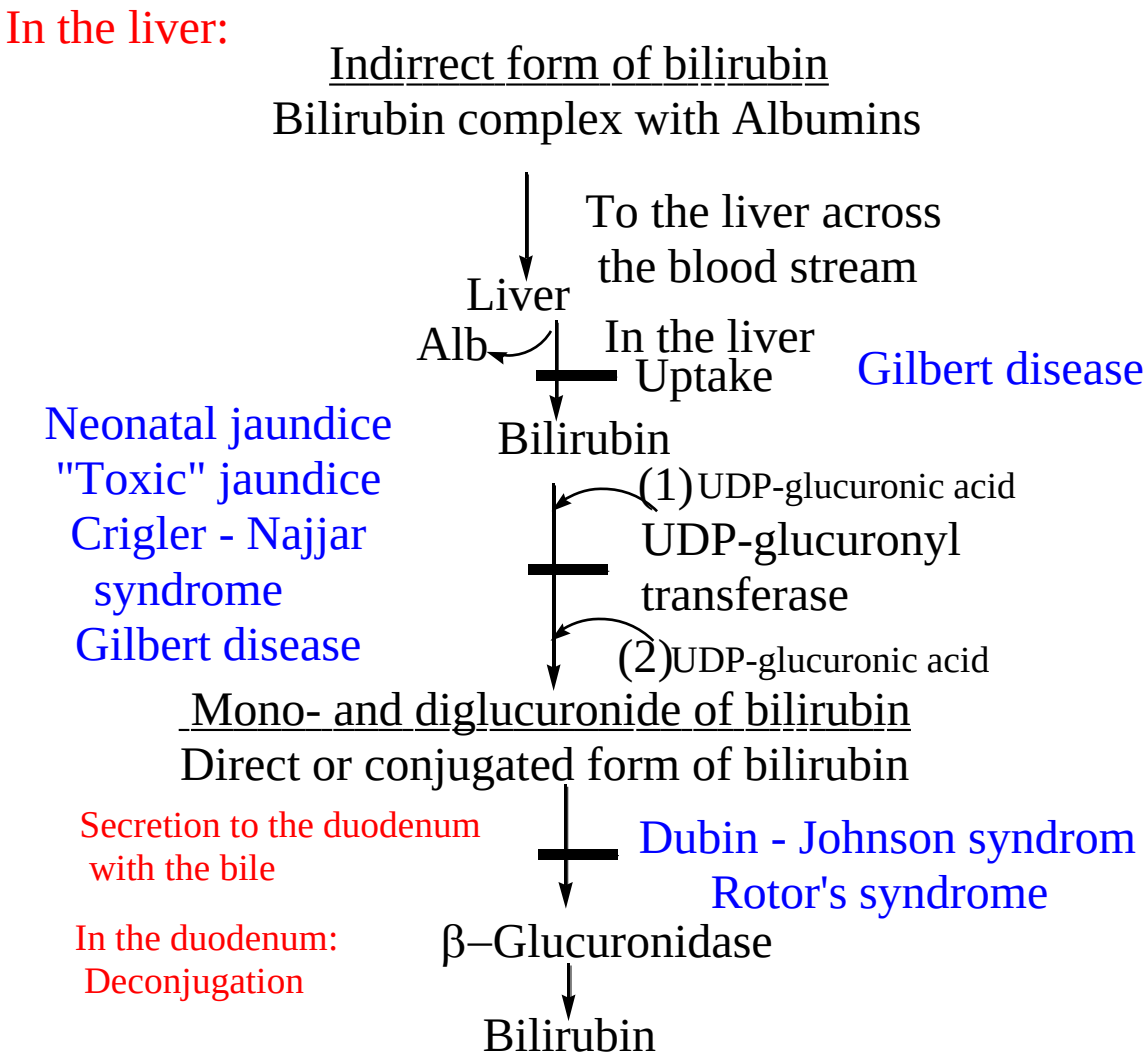


Figure 7. **Bilirubin transformations in the liver.**

Bilirubin. The high lipid solublity of bilirubin determines its behavior and its further metabolism. ***Its lipid solubility dictates:***

- that it is soluble in the lipid bilayers of cell membranes.
- that it must be transported in the blood by a carrier; the physiological carrier is serum albumin. Some drugs that also bind to albumin can lead to an increase in free bilirubin.

Bilirubin must be conjugated to a water-soluble substance. This increased its water solubility, decreases its lipid solubility and makes easier its excretion. Conjugation is accomplished by attaching two molecules of glucuronic acid to it in a two step process. The substrates are bilirubin (or bilirubin monoglucuronide) and UDP-glucuronic acid.

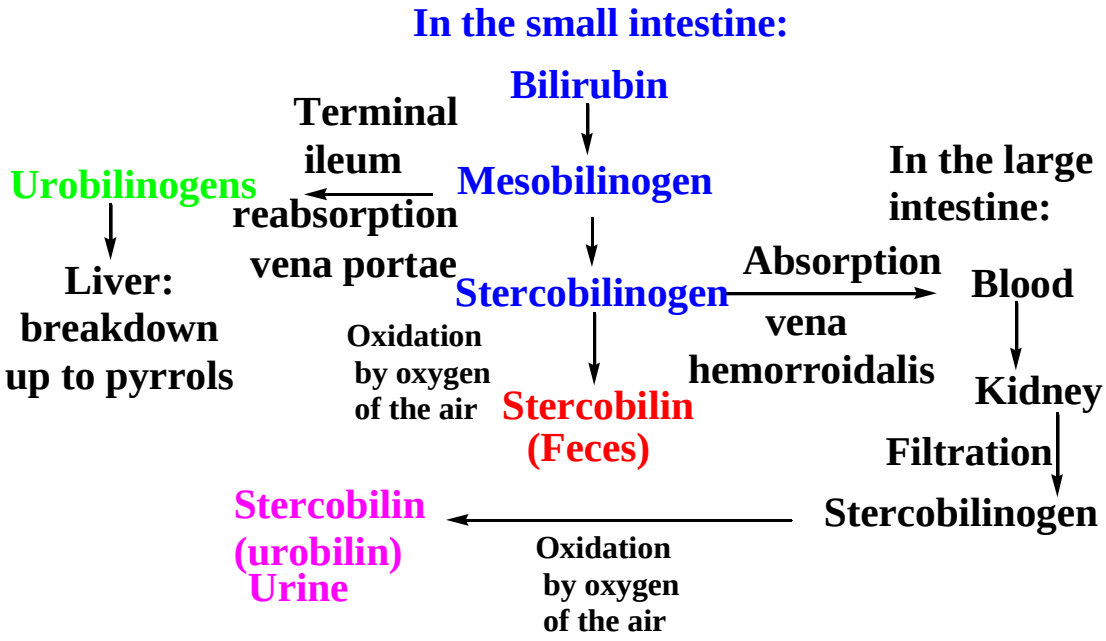


Figure 8. **All the processes for bilirubin in the gastrointestinal tract**.

The reaction is a transfer of two glucuronic acid groups sequentially to the propionic acid groups of the bilirubin. The major product is bilirubin diglucuronide (direct bilirubin).

Glucuronide synthesis is the rate-determining step in hepatic bilirubin metabolism. Drugs such as *phenobarbital*, for example, can induce both conjugate formation and the transport process.

The bilirubin glucuronides are then excreted by active transport into the ***bile***, where they form what are known as the ***bile pigments*** (fig.8). Some of the bilirubin conjugates are broken down further in the intestine by bacterial *glucuronidases.* The bilirubin released is then reduced further via intermediate steps into colored dark brown mesobilirubinogen. The portion of it is reabsorbed and across vena portae is transported to live for destruction up to pyrrols. When high levels of haem degradation are taking place, mesobilinogen appears as ***urobilinogen*** in

blood circulation, in the urine where oxidative processes darken it to form **urobilin**.

Colorless ***stercobilinogen***, some of which is oxidized again into orange to yellow-colored stercobilin under air oxygen. The end products of bile pigment metabolism in the intestine are mostly excreted in feces, but a small proportion in urine.

The increased allocation of urobilinogen bodies (urobilin bodies) with urine is named ***urobilinuria***.

Urobilinuria (with determination of urobilinogen) more often occur at hepatocellular diseases of a liver (a hepatitis, a cirrhosis, poisonings, etc.), the cardiovascular pathology accompanying with stagnant damage of the liver. Urobilinogen reabsorbed from intestines and via portal vein does not undergo usual transformations for it because of functional failure of liver and it is takes out with urine.

Determination of bilirubins in the blood serum.

The determination of total bilirubin and its fractions has a big clinical significance. It is noticed, that jaundice appears when the total bilirubin level in blood exceeds 27-34 mmole/L (>10 mg/L). An elevated bilirubin level is known as *hyperbilirubinemia.* When this is present, bilirubin diffuses from the blood into peripheral tissue and gives a yellow color (jaundice). The easiest way of observing this is in the white conjunctiva of the eyes. Jaundice can have various causes.

Major causes of jaundice (fig. 6, 7, 8)

I. Pre-hepatic

Hemolytic jaundice

- is accompanied with increased hemolysis of erythrocytes (incompatible blood transfusion, malaria, sickle-cell anemia and other causes see above);
- results in increased production of bilirubin;
- here more bilirubin is conjugated and excreted than normally, but the conjugation mechanism is overwhelmed, and an abnormally large amount of unconjugated bilirubin is found in the blood.

II. Hepatic

Neonatal jaundice

- results in a temporary condition due to production of insufficient levels of UDP-glucuronyl transferase by the infant;
- currently, the irradiation of jaundiced infants during neonatal life by fluorescent lights is the most common treatment of neonatal hyperbilirubinemia. The products from the irradiation of bilirubin are more soluble than bilirubin and can be excreted by the liver into the bile without conjugation with glucuronic acid.

Gilbert's disease

- may be caused by an inability of the hepatocytes to take up bilirubin from the blood or reduced activity of UDP-glucuronyltransferase;
- as a result, unconjugated bilirubin accumulates.

Neonatal "Physiological jaundice"

- are conditions in which conjugation is impaired due to a deficiency in UDP-glucuronyl transferase or there probably is reduced synthesis of the substrate for that enzyme, UDP-glucuronic acid. UDP-glucoronate is formed under NAD-dependent UDP-glucosyl dehydrogenase;
- ***Neonatal jaundice*** (physiologic jaundice) usually resolves after a few days by itself;
- in severe cases, however, unconjugated bilirubin can cross the blood–brain barrier and lead to brain damage (*kernicterus*).

Crigler-Najjar syndrome

- Crigler-Najjar syndrome, type I: deficiency in UDP-glucuronyl transferase; the desease is fatal within the first 2 years of life; children have been treated with phototherapy; phenobarbital has no effect;
- Crigler-Najjar syndrome, type II: some activity of the UDP-glucuronyl transferase is retained; addition of second glucuronyl group is defective; treatment with Phenobarbital;
- unconjugated bilirubin is retained by the body.

Dubin-Johnson syndrome

- is associated with inability of the hepatocytes to secrete conjugated bilirubin after it has been formed;
- conjugated bilirubin returns to the blood.

III. Post hepatic

Biliary obstruction

- obstruction in the bile duct (gall stones, tumors);
- blood levels of conjugated bilirubin increase.

LABORATORY WORKS. ***DETERMINATION OF TOTAL, DIRECT AND INDIRECT BILIRUBINS IN THE BLOOD SERUM (BASED ON THE VAN DEN BERGH REACTION)***

THE PRINCIPLE OF THE METHOD

The reaction is a coupling of bilirubin with a diazonium salt (Erlich's reagent) to form a colored complex. Only conjugated bilirubin is water soluble and reacts directly. This is called the **DIRECT bilirubin.** To measure the unconjugated bilirubin bound to albumin, alcohol (caffeine reagent) is added to release it into solution, where it can now react. This is called the **INDIRECT bilirubin**.

The intensity of the colouring is proportional to the concentration of bilirubin and can be determined due to photocolorimetry.

THE COURSE OF THE WORK:

Pour 0.5 ml of blood serum (dilute with 0, 9% sodium chloride solution 1:1) into each test-tube (two). Add 1.75 ml of 0.9% NaCl solution and 0.25 ml of Erlich's reagent into one test-tube (determination of conjugated bilirubin). Add 1.75 ml of caffeine reagent and 0.25 ml of Erlich's reagent to the 2-nd one (determination of total bilirubin). Shake up both test tubes and let them stay on the table for 5 min (conjugated bilirubin) and 20 min (total bilirubin).

Measure the optical density of every test tube opposite water at a green colour filter in cuvettes (5 mm). If colouring in the test tube is slight, add 3 drops

of 30% NaOH solution into each test-tube. The content of total and conjugated bilirubin is determined using the slope of the plot. The difference between total and conjugated bilirubin is non-conjugated bilirubin of the blood serum.

Blood serum of healthy people contains: total bilirubin 3.5-20.5 μmole/L; non-conjugated bilirubin - < 12 μmole/L; conjugated bilirubin - < 7μmole/L.

Qualitative determination of bile pigments in the urine. The test for bile pigments in the urine (Gmelin`s test)

THE PRINCIPLE OF THE METHOD:

The reaction is based on ability of the bile pigments to be oxidized by strong nitric acid. As the result the various colouring products are obtained: for biliverdine (green colour), bilicyanin (blue colour), cholelethine (yellow colour).

Gmelin's test can be carried out in a test tube or on filter paper. This test is very sensitive and can estimate the bilirubin at dilution 1:80 000.

THE COURSE OF THE WORK:

Pour 1-2 ml of concentrated nitric acid into a test-tube. Stratify cautiously an equal volume of urine over the wall of the test tube. Don’t mix! The colour rings will appear in the presence of the bile pigments. The green, blue, violet, red and yellow rings indicate the various pathology states. The brown ring is typical for healthy people.

Urobilinogen determination in the urine (Bogomolov`s reaction)

THE PRINCIPLE OF THE METHOD:

Urobilinogen, stercobilinogen (urobilinogen bodies) and their oxidized forms: urobilin, stercobilin (urobilin bodies) are pigments that are formed from bilirubin in intestines. Urobilin with copper sulfate gives pink-red compound.

THE COURSE OF THE WORK:

Before carrying out of the test add 1-2 drops of iodine solution to urine for oxidation of the urobilinogen bodies in urobilin ones.

Add 2 ml of the saturated copper sulfate solution to 10 ml of the urine. If there is the turbidity of solution, add some drops of strong hydrochloric acid. This gives the transparent solution. Add 2-3 ml of chloroform by drops and shake up.

The layer of chloroform is coloured in rose-red color at the urobilin bodies presence.

TASKS FOR SELF-CONTROL on CHAPTER 3

1. Which fragment is not a structural component of haemoglobin molecule:

A. Pyrrole rings

B. Globin

C. Ferric ion

D. Propionyl

E. Histidine residue

2. Which one of the following compounds is not a chromoprotein:

A. Haemoglobin

B. Myoglobin

C. Catalase

D. Amylase

E. Cytochrome b

3. Which of the following statements is incorrect concerning bilirubin or its metabolism?

A. Its formation involves two NADPH-requiring reactions

B. It is carried by albumin in the blood

C. It originates from haem

D. It and its derivatives are excreted in the urine

E. It is made more soluble in the liver by the conjugation with UDP-glucuronate

4. Congenital erythropoietic prophyria is due to a defect in the enzyme used for haem synthesis. Name it:

A. Uroporphyrinogen III cosynthetase

B. Coproporphyrinogen oxidase

C. Uroporphyrinogen I synthetase

D. Uroporphyrinogen decarboxylase

E. Photoporphyrinogen oxidase

5. A patient is suffering from lead poisoning. This inhibits the following Zn-containing enzyme:

A. Uroporphyrinogen decarboxylase

B. Uroporphyrinogen III co-synthase

C. Amino-levulinic acid synthase

D. Amino-levulinic acid dehydratase

E. Porphobilinogen deaminase

6. What level of organization for haemoglobin A is changed mostly during the transformation of its oxygenated form to deoxygenated one:

A. Primary structure

B. Quaternary structure

C. Sextic structure

D. Tertiary structure

E. Secondary structure

7. A critical enzyme in the conversion of haem to biliverdin is:

A. Biliverdin oxygenase

B. Biliverdine reductase

C. Biliverdine hydroxylase

D. Haem reductase

E. Haem oxygenase

8. In a patient, already diagnosed as suffering from a carcinoma of pancreas head, the tumor provokes a gradual compression of the common bile duct and the patient begins to develop jaundice. The findings related with bilirubin metabolism that you expect to see in this patient include:

A. High concentration of unconjugated bilirubin in serum, dark urine, and light-colored stools

B. High concentration of conjugated bilirubin in serum , dark urine (bilirubinuria) and dark stools

C. High concentration of conjugated bilirubin in serum , urine with a normal color and dark stools

D. Normal concentration of conjugated bilirubin in serum, dark urine and dark colored stools

E. High concentration of conjugated bilirubin in serum, dark urine (bilirubinuria) and light- colored stools

9. Which of the following statements is true about the Bohr effect?

A. The affinity of haemoglobin for O_2 is improved by high concentrations of CO_2

B. The acidic environment of an exercising muscle allows haemoglobin to bind O_2 more strongly

C. The lowering of pH shifts the oxygen dissociation curve of haemoglobin to the right (lower affinity)

D. In the lungs, the presence of higher concentrations of O_2 promotes the binding of CO_2 and H^+

E. In the lungs, the presence of higher concentrations of H^+ and CO_2 allows haemoglobin to become more oxygenated.

10. Which of the following compounds are the substrates for haem synthesis:

A. Glycine and succinyl-CoA

B. Glutamine and acetyl-CoA

C. Glutamate and malonyl-CoA

D. Serine and palmityl-CoA

E. Glycine and acetyl-CoA

11. The examination of primary structure of haemoglobin revealed substitution of the glutamic acid by valine in the sixth position from N-terminal of beta-chains. What inherited pathology is it typical for?

A. Crigler-Najjar syndrome

B. Hemoglobinosis

C. Thalassemia

D. Gilbert's disease

E. Sickle-cell anemia

12. Point out the normal form of hemoglobin that is represented mostly in the blood of adults:

A. HbA1

B. HbA2

C. HbF

D. HbS

E. HbC

13. One of the hemoglobin forms is dominated after the child's birth. This form retains in adults, but in smaller concentration. Point out it:

A. HbA1

B. HbA2

C. HbF

D. HbS

E. HbC

14. Point out the first pigment that is produced in spleen after HbA cleavage:

A. Biliverdin

B. Verdoglobin

C. Bilirubin diglucuronide

D. Stercobilin

E. Mesobilirubin

15. Point out a bile pigment appears in the urine as a result of its elevated excretion in obstructive jaundice:

A. Mesobilirubin

B. Conjugated biliribin

C. Unconjugated bilirubin

D. All the bilirubin forms

E. Biliverdin

16. Point out the tissue enzyme that takes part in the heme cleavage:

A. Cycle oxygenase

B. Lipooxygenase

C. Heme oxygenase

D. Biliverdin reductase

E. Hydroxylase

17. Choose the form of the bile pigment that is normal urine component:

A. Haemoglobin

B. Stercobilinogen (urobilinogen)

C. Mesobilirubin

D. Unconjugated bilirubin

E. Conjugated bilirubin

18. A patient who suffers from congenital erythropoietic porphyria has skin photosensitivity. The accumulation of what compound in the skin cells can cause it?

A. Uroporphyrinogen I

B. Uroporphyrinogen II

C. Protoporphyrin

D. Coproporphyrinogen III

E. Haem

19. Specify the substance that is used for the incomplete denaturation of haemoglobin:

A. Sulfuric acid

B. Nitric acid

C. Toluene

D. Sodium hydroxide

E. Urea

20. Examination of initial molecular structure revealed substitution of the glutamic acid by valine. What inherited pathology is it typical for?

A. Minkowsky-Shauffard disease

B. Hemoglobinosis

C. Thalassemia

D. Favism

E. Sickle-cell anemia

LITERATURE

1. Davidson V. L. Biochemistry: textbook / V. L. Davidson, D. B. Sittman. - USA : Harwal Publishing, 1994. – 584 p.
2. Denke M. A. Excess body weight. An underrecognized contributor to high blood cholesterol levels in white American men / M. A. Denke, C.T. Sempos, S. M. Grundy // Arch. Intern. Med. – 1993. – №. 153. - P. 1093–1103.
3. Echols H. Nucleoprotein structures initiating DNA replication, transcription, and site-specific recombination / H. Echols // J. Biol. Chem. -.1990. – Vol. 25, № 265. - P. 697-700.
4. Influence of mild to moderately elevated triglycerides on low density lipoprotein subfraction concentration and composition in healthy men with low high density lipoprotein cholesterol levels / M. Halle [and others] // Atherosclerosis. – 1999. – № 143. – P. 185–192.
5. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles / G. M. Reaven [and others] // J. Clin. Invest. – 1993. – № 92. – P. 141–146.
6. Koolman J. Color Atlas of Biochemistry: textbook / J. Koolman, K., H. Roehm. – 2nd edition. – Stuttgart-New York : Thieme, 2005. – 467 p.
7. Lieberman M. Medical Biochemistry: textbook / M. Lieberman; A. Marks, C. Smith. - 2nd ed. - New York : Lippincott Williams & Wilkins, 2007. – 540 p.
8. Low-density lipoprotein particle size in type 2 diabetic patients and age matched controls / J. M. Stewart [and others] // Ann. Clin. Biochem. – 1994. - № 31. – P. 153–159.
9. Marshall J. W. Clinical Chemistry : textbook / J. W. Marshall, S. K. Bangert.- Fifth edition. – China: ”Mosby”, 2004. – 422 p.
10. Regulation of low-density lipoprotein particle size distribution in NIDDM and coronary disease: importance of serum triglycerides / S. Lahdenpera [and others] // Diabetologia.- 1996. –№ 39. – P. 453–461.

11. Seven-year changes in physical fitness, physical activity, and lipid profile in the cardia study. Coronary Artery Risk Development in Young Adults / B. Sternfeld [and others] // Ann Epidemiol. – 1999. –№ 9. – P. 25–33.
12. Sikaris Ken A. . The Clinical Biochemistry of Obesity / Ken A. Sikaris // Clin. Biochem. Rev. – 2004, № 25(3). - P. 165–181.
13. Smith C. Basic Medical Biochemistry: A Clinical Approach: textbook / C. Smith, A. Marks, M. Lieberman. - 2nd ed. - New York : Lippincott Williams & Wilkins, 2009. - 920 p.
14. Specificity and enzymatic mechanism of the editing exonuclease of Escherichia coli DNA polymerase III / S. Brenowitz [and others] // Biol. Chem. – 1991. - Vol. 12, № 266. - P. 888-892.
15. Taskinen M. R . Lipoprotein lipase in diabetes / M. R. Taskinen // Diabetes Metab. Rev. -1987. –№ 3. – P. 551–570.
16. Williams P. T. Associations of age, adiposity, menopause, and alcohol intake with low-density lipoprotein subclasses / P. T. Williams, R. M. Krauss // Arterioscler. Thromb. Vasc. Biol. – 1997. - №17. – P. 1082–1090.

ANSWERS TO TASKS FOR SELF-CONTROL:

on chapter 1

1	2	3	4	5	6	7	8	9	10
D	E	C	C	C	B	C	C	A	D
11	12	13	14	15	16	17	18	19	20
A	E	D	A	B	E	C	B	C	B

on chapter 2

1	2	3	4	5	6	7	8	9	10
D	A	C	C	E	A	B	C	A	D
11	12	13	14	15	16	17	18	19	20
C	D	E	A	E	C	D	B	A	D

on chapter 3

1	2	3	4	5	6	7	8	9	10
C	D	D	A	D	B	E	E	C	A
11	12	13	14	15	16	17	18	19	20
E	A	C	B	B	C	B	C	E	E