# Biochemistry of nervous system

Lecture # 32

Lecturer: Alexander Koval

# Treats of Muscular and Nervous Tissue Metabolism

- These tissues has many common in their structure and functions. Both…
  - are of high energy needs;
  - prefers aerobic metabolism;
  - has a good deal of mitochondria;
  - are excitable.

# Introduction

- **Nervous system** is an internal communications network that enables an animal to adjust to changes in its environment.
- The **human nervous system** – especially the brain – makes people different from other animals. The human brain enables people to speak, solve difficult problems, and produce creative ideas.
- The brain collect information from outside, sends instructions to various muscles via other pathways so that the body can respond to the information. The nervous system also regulates internal functions, such as breathing, digestion, and heartbeat. All of a person's movements, sensations, thoughts, and emotions are products of his or her nervous system.

# Nerves

- The nervous system is made up of billions of special cells called neurons or nerve cells.
- Cordlike bundles of neuron fibers are called **nerves**.
- The nerves form a network of pathways that conduct information rapidly throughout the body.

# Brain Metabolism: Glucose

- The brain must generate ATP in large quantities to maintain the membrane potentials essential for transmission of nerve impulses.
- Under normal conditions the brain utilizes only glucose: about 60% of the total glucose of a human at rest.
  - About 120 g of glucose/day: ≈1760 kJ – about 15% of the total energy consumed each day.
- The brain's requirement for glucose remains constant.

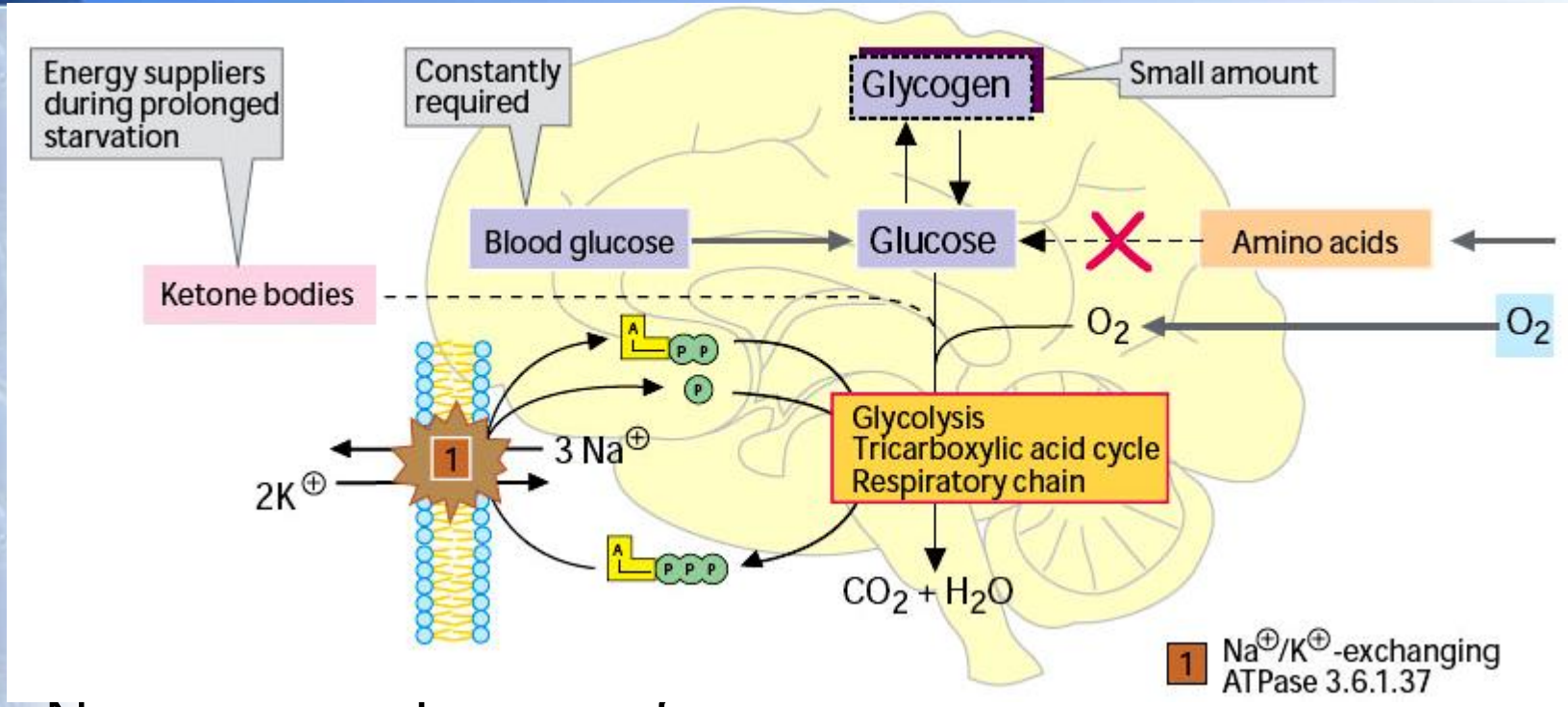
# Brain Metabolism: Oxygen

- The brain is a highly aerobic organ: its metabolism utilizes some 20% of the total oxygen consumed in human.
- The brain has little glycogen reserves, the supply of both oxygen and glucose cannot be interrupted, even for a short time. Otherwise, anoxic brain damage results.
  - However, the brain can adapt during fasting to use ketone bodies instead of glucose as a major fuel.

# Nervous Cells

- The nervous system is made up of billions of special cells called neurons or nerve cells. Cordlike bundles of neuron fibers are called nerves. The nerves form a network of pathways that conduct information rapidly throughout the body.
  - Neurons are the big cells – 1000 times bigger rather hepatocyte.
  - Neurons are surrounded by neuroglia cells (1:10).
  - Neuroglia cells are the main formation of hematoencephalic barrier.

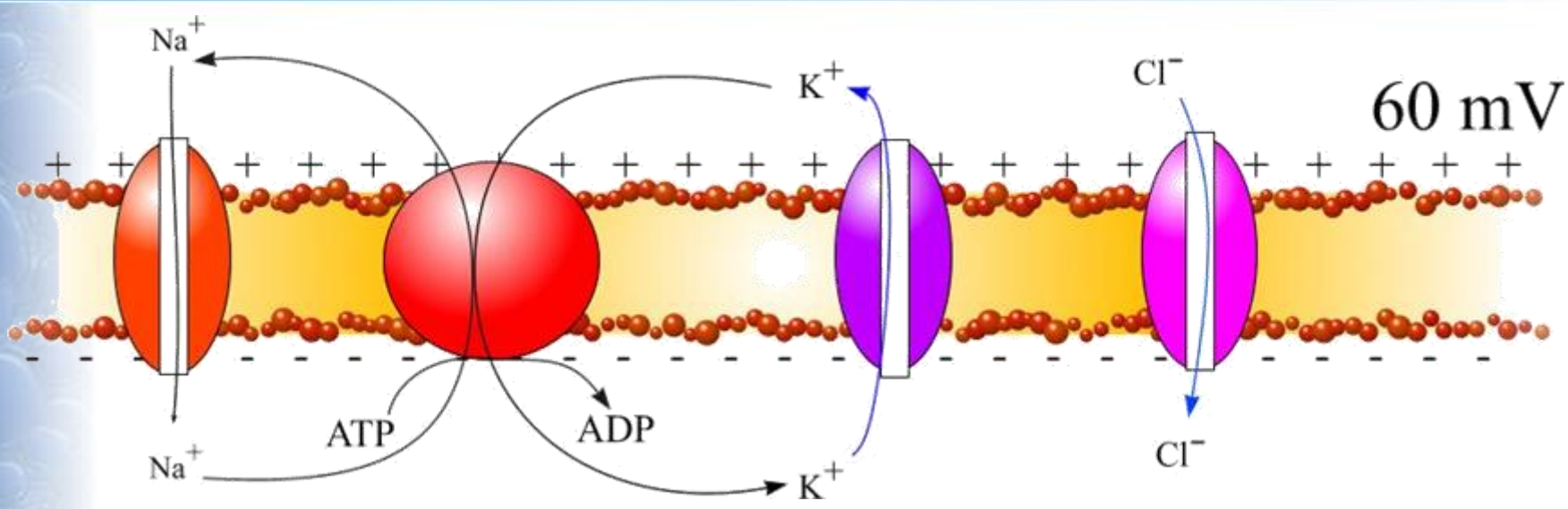
# Energy Metabolism of the Brain




- Neurons need energy for:
  - Electrogenesis;
  - Protein synthesis;
  - Neuromediator synthesis.

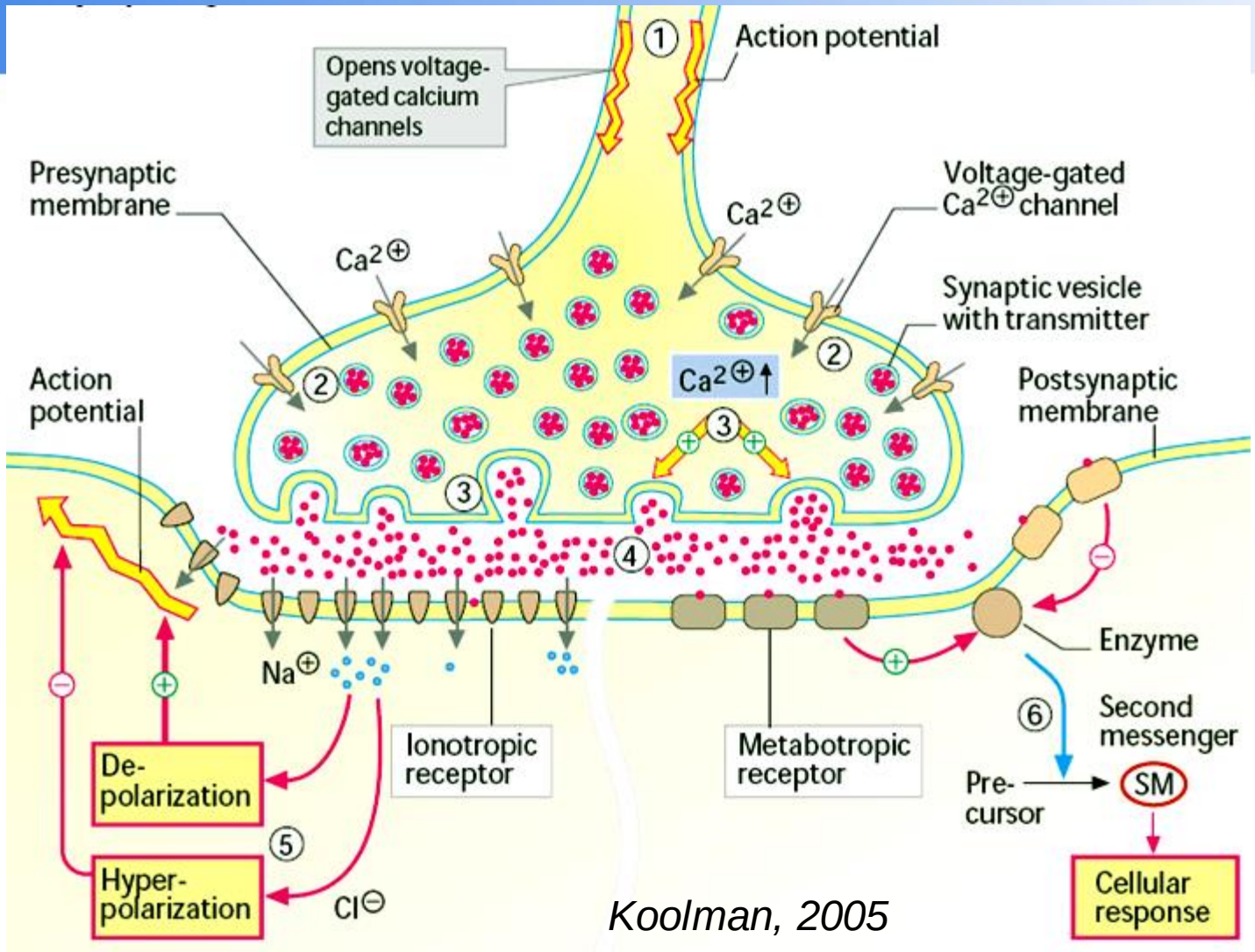
*Koolman, 2005*

# Electrogenesis Mechanism




- The main ATP consumption is by $Na^+/K^+$-ATPase.
- The resting potential 60 mV is formed.
- After impuls have passed (action potential, through ion channels), the resting potential should be restored again.

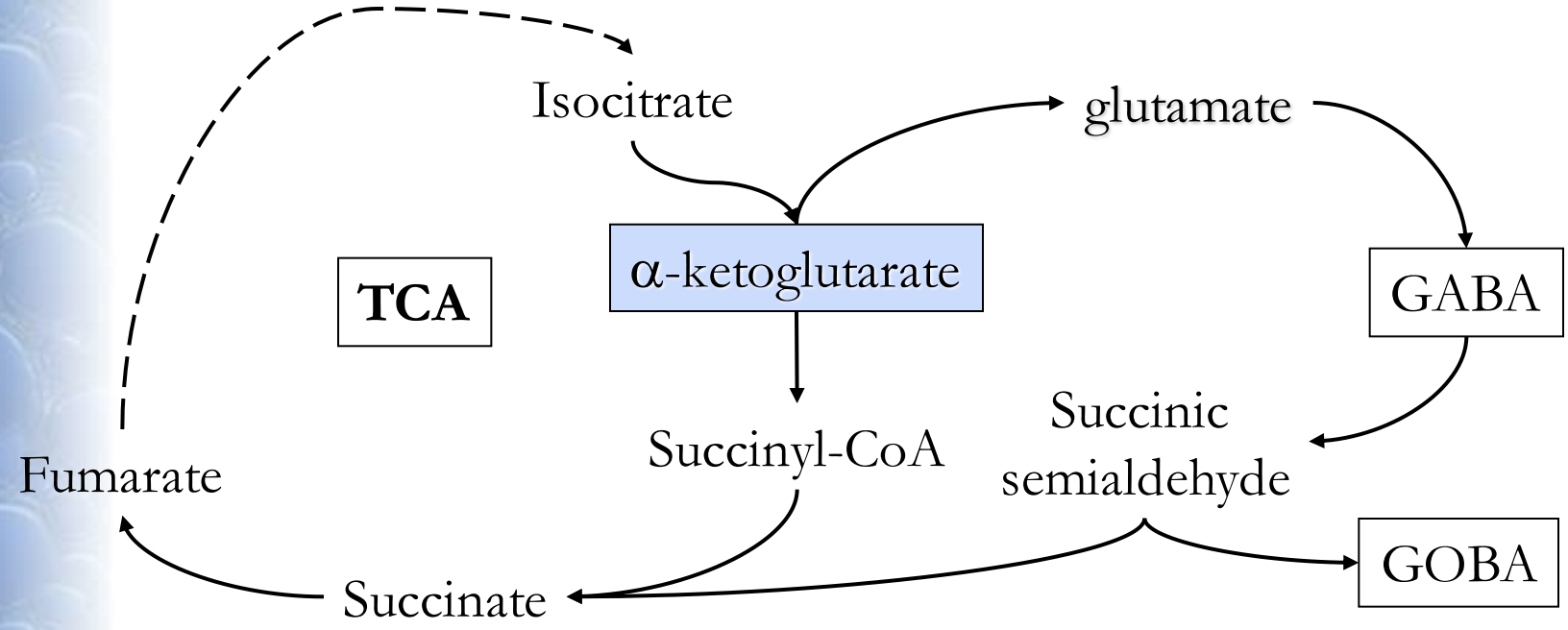
# Synaptic Signal Transmission




*Koolman, 2005*

# Roberts Cycle

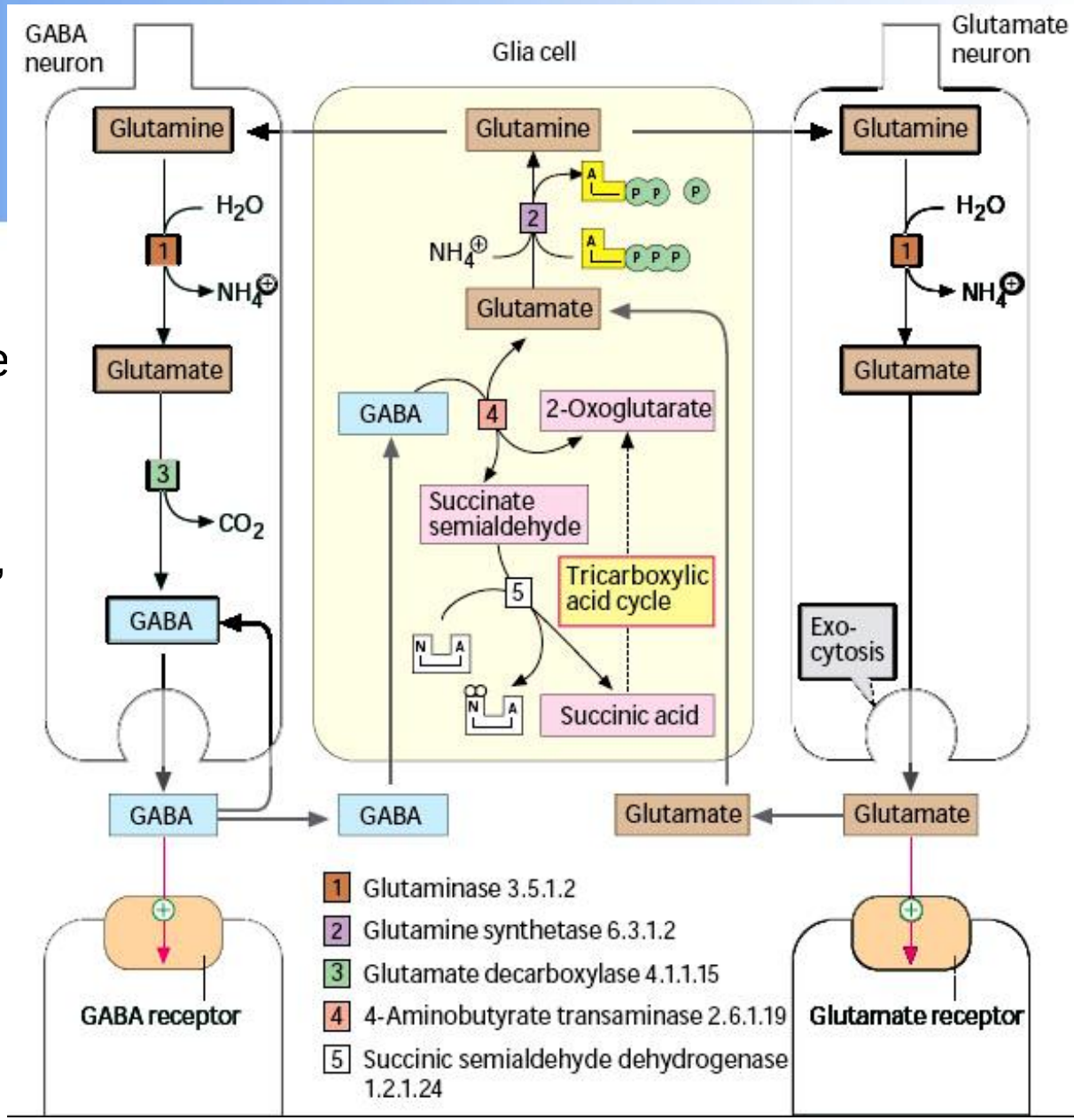
- In hypoxia nervous cells produce GABA – inhibiting neuromediator.
  - It is formed from α-ketoglutarate or glutamate.



# Glutamate, Glutamine, and GABA

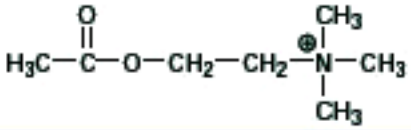
- Since glutamate and GABA as transmitters must not appear in the extracellular space in an unregulated way, the cells of the neuroglia (center) supply “glutaminergic” and “GABAergic” neurons with the precursor **glutamine** (Gln), which they produce from glutamate with the help of *glutamine synthetase.*

*Koolman, 2005*

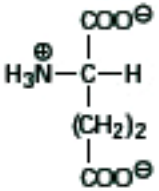


# The Main Neuromediators in Nervous Tissue

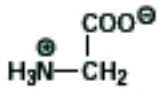
**Acetyl-choline**



**Amino acids:** Glutamate, Glycine, Dopa

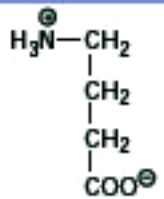


Glutamate

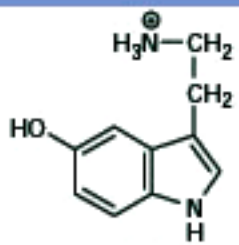


Glycine

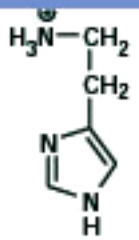
**Biogenic amines:** γ-Aminobutyrate (GABA), Dopa, Dopamine, Norepinephrine, Epinephrine, Serotonine, Histamine



GABA



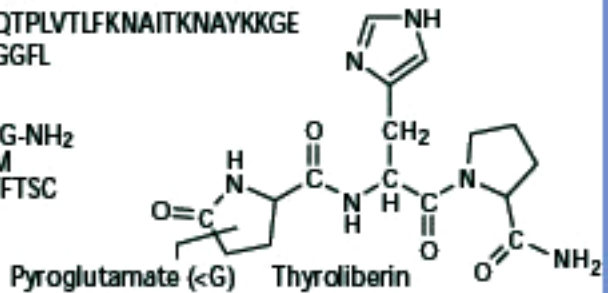
Serotonin



Histamine

**Peptides:**

| | |
|---|---|
| β-Endorphin | YGGFMTSEKSQTPLVTLFKNAITKNAYKKGE |
| Met- and Leu-enkephalin | YGGFM und YGGFL |
| Thyroliberin (TRH) | <GHP-$NH_2$ |
| Gonadoliberin (GnRH) | <GHWSYGLRPG-$NH_2$ |
| Substance P | RPKPQQFFGLM |
| Somatostatin | AGCKNFFWKTFTSC |
| Angiotensin II | DRVYIHPF |
| Cholecystokinin (CCK-4) | WMDF-$NH_2$ |
| and many others | |



Pyroglutamate (<G) Thyroliberin

**Purine derivatives:** ATP, ADP, AMP, Adenosine

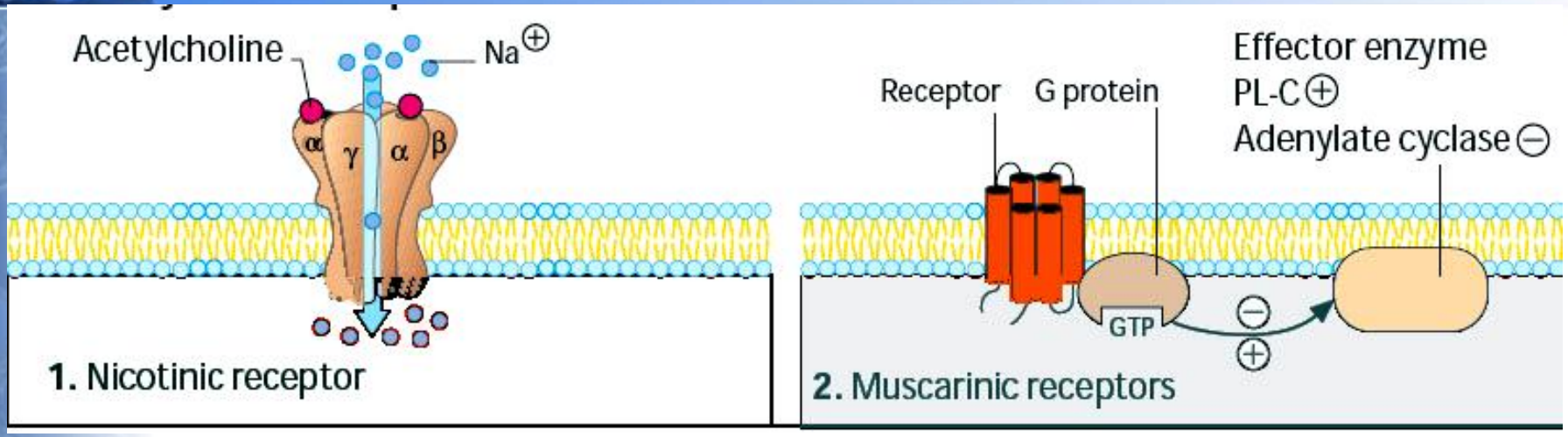
*Koolman, 2005*

# Receptors for Neurotransmitters

| Ionotropic | | | | Metabotropic | | |
|---|---|---|---|---|---|---|
| **Receptor** | **Transmitter** | **Ion(s)** | **Effect** | **Receptor** | **Transmitter** | **Effect** |
| Acetyl-choline (nicotinic) | Acetyl-choline | $Na^{\oplus}$ | $\oplus$ | Acetylcholine (muscarinic) M1, M3, M5, M2, M4 | Acetylcholine | $[Ca^{2\oplus}]\uparrow$ $[cAMP]\downarrow$ |
| 5HT3 | Serotonin | $Na^{\oplus}$ | $\oplus$ | $5HT_1$ $5HT_2$ $5HT_4$ | Serotonin " " | $[Ca^{2\oplus}]\uparrow$ $[cAMP]\uparrow$ $[cAMP]\downarrow$ |
| GABAa | GABA | $Cl^{\ominus}$ | $\ominus$ | $\alpha_1$ $\alpha_2$ $\beta_1, \beta_2, \beta_3$ | Norepinephrine " " | $[Ca^{2\oplus}]\uparrow$ $[cAMP]\uparrow$ $[cAMP]\downarrow$ |
| Glycine | Glycine | $Cl^{\ominus}$ | $\ominus$ | | | |
| AMPA<br>NMDA<br>Kainate | Glutamate<br>Glutamate<br>Glutamate | $Na^{\oplus}$ $K^{\oplus}$<br>$Na^{\oplus}$ $K^{\oplus}$ $Ca^{2\oplus}$<br>$Na^{\oplus}$ $K^{\oplus}$ | $\oplus$<br>$\oplus$<br>$\oplus$ | $D_1, D_5$ $D_2, D_3, D_4$ | Dopamine " | $[cAMP]\uparrow$ $[cAMP]\downarrow$ |
| | | | | $\delta, \kappa, \mu$ | Opioids | $[cAMP]\downarrow$ |

*Koolman, 2005*

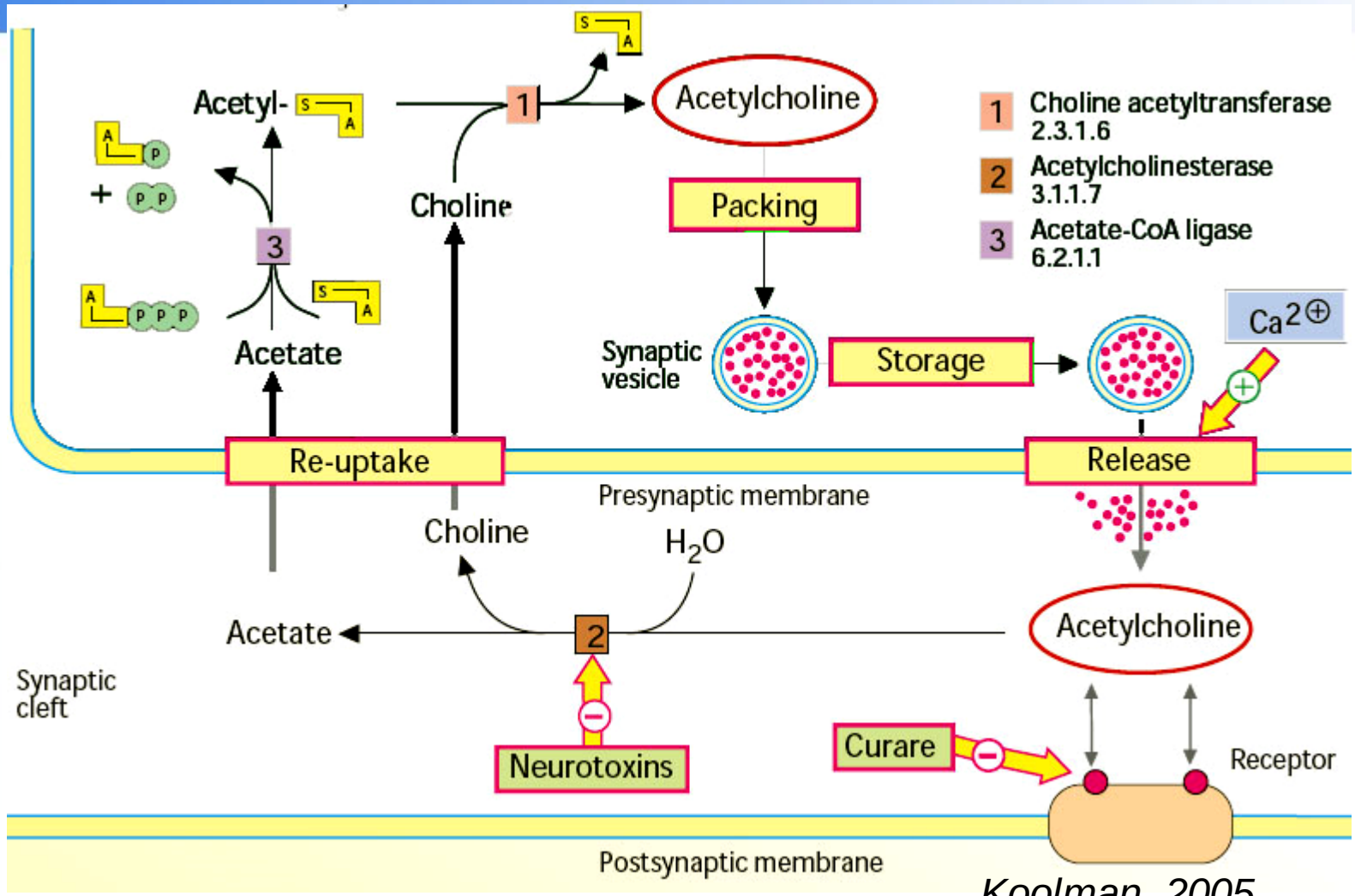
# Acetylcholine receptors




- There are 2 types of Acetylcholine receptors:
  - The **nicotinic ACh receptor** responds to the alkaloid *nicotine* contained in tobacco (many of the physiological effects of nicotine are based on this). The nicotinic receptor is ionotropic.
  - The **muscarinic ACh receptors** (of which there are at least five subtypes) are metabotropic.
    - Their name is derived from the alkaloid *muscarine,* which is found in the fly agaric mushroom (*Amanita muscaria*), for example. Like ACh, muscarine is bound at the receptor, but in contrast to ACh, it is not broken down and therefore causes permanent stimulation of muscle.

*Koolman, 2005*

# Metabolism of Acetylcholine

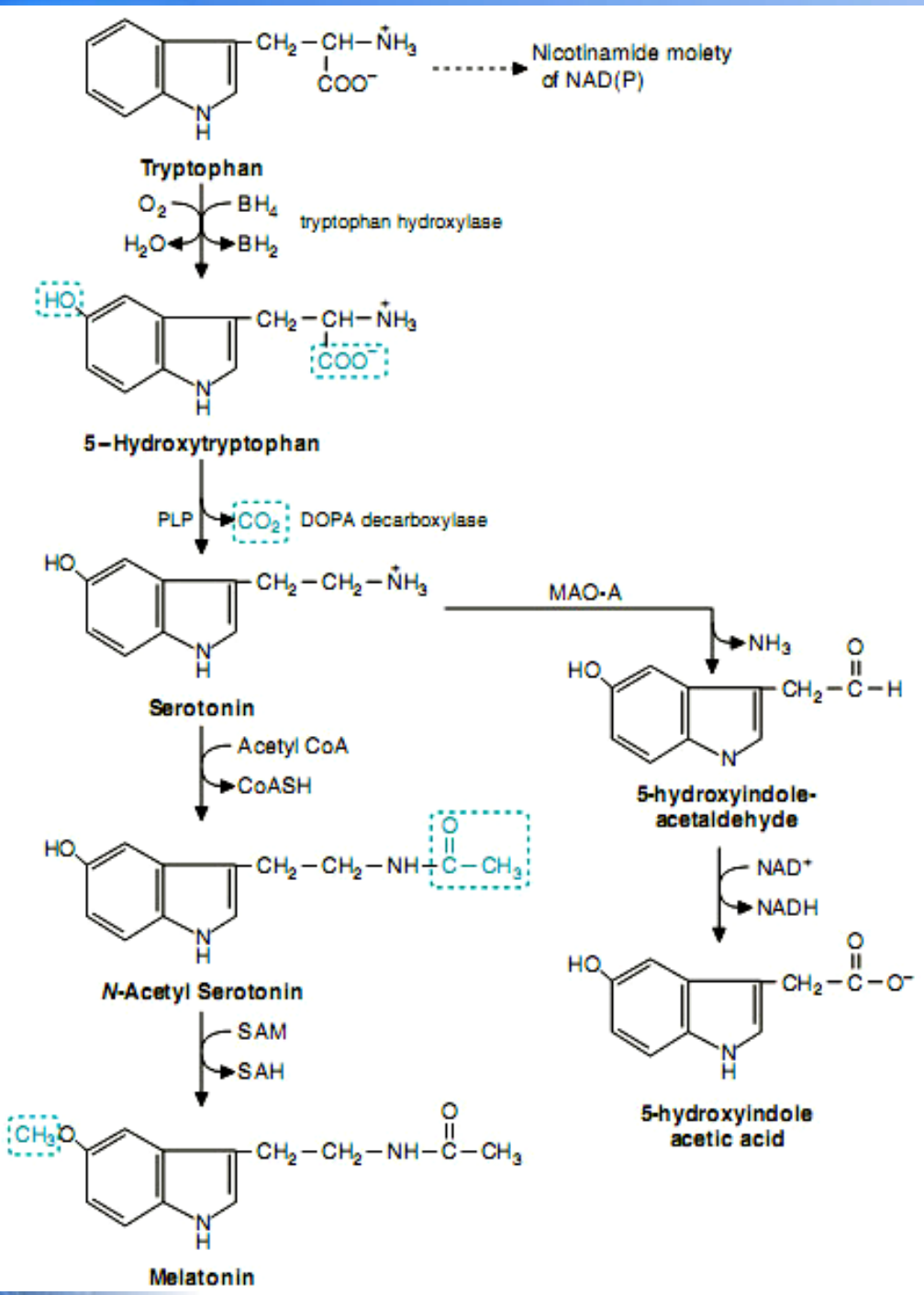



*Koolman, 2005*

# Biosynthesis of Catecholamines

*Koolman, 2005*

# Synthesis and Inactivation of Serotonin




Thank you
for your attention

# Biochemistry of Muscular Tissue

## Myocardial Metabolism

Lecture # 33

Lecturer: Alexander Koval

# Content

- Introduction
- General Structure of Skeletal Muscle
- General Structure of Smooth Muscle
- Muscle Metabolism: Fuels
  - Energy Fuels: Glycogen
    - Lactate, Alanine, Cori Cycle
  - Energy Fuels: Proteins
- Energy Reserve: Creatine Phosphate
- Proteins of Muscle

# Introduction

- The main function – movement (all levels).
- Muscular contraction involves specific proteins and ions ($Ca^{2+}$).
- Clinical importance:
  - Some muscular diseases (Duchenne-type muscular dystrophy, malignant hyperthermia, heart value cardiomyopathies).

# Muscle Metabolism: Fuels

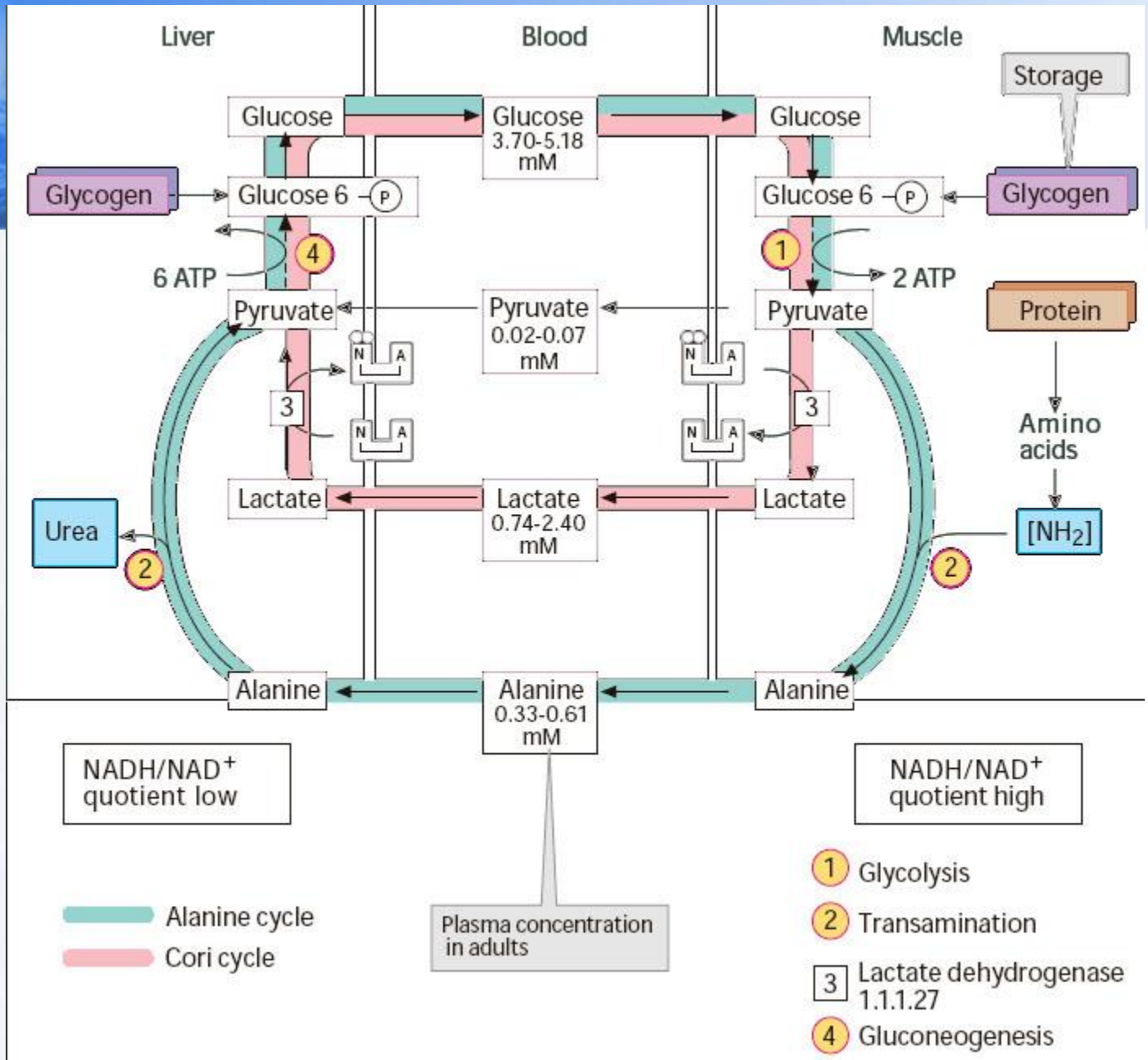
- Muscle can utilize a variety of fuels - glucose, fatty acids, and ketone bodies.
- Skeletal muscle varies widely in its energy demands and the fuels it consumes, in line with its wide variations in activity.
- In resting muscle, fatty acids represent the major energy source;
  - during exertion, glucose is the primary source.
  - Early in a period of exertion, glucose comes from mobilization of the muscle's glycogen reserves.

# Energy Fuels: Glycogen

- Skeletal muscle stores about ¾ of the total glycogen in humans,
- The liver stores the most of the rest.
  - Glucose from muscle glycogen cannot be released from the cell.
  - Muscle lacks the enzyme glucose-6-phosphatase, so glucose phosphates from glycogen cannot be converted to glucose.

# Lactate, Alanine, Cori Cycle

- During exertion, the rate of glycolysis in muscle exceeds that of the citric acid cycle $\Rightarrow$ **lactate** accumulates and is released.
- Another metabolic product is **alanine** (transamination from **pyruvate** in the glucose-alanine cycle).
- Both lactate and alanine are transported through the bloodstream to the liver, where they are reconverted (in gluconeogenesis) to glucose – the **Cori cycle**.


Liver
Blood
Muscle
Storage
Glucose
Glucose
3.70-5.18
mM
Glucose
Glycogen
Glucose 6 P
Glucose 6 P
Glycogen
4
1
6 ATP
2 ATP
Pyruvate
Pyruvate
0.02-0.07
mM
Pyruvate
Protein
N A
N A
N A
N A
3
3
Amino
acids
Lactate
Lactate
0.74-2.40
mM
Lactate
Urea
[NH2]
2
2
Alanine
Alanine
0.33-0.61
mM
Alanine
NADH/NAD+
quotient low
NADH/NAD+
quotient high
Alanine cycle
Cori cycle
Plasma concentration
in adults
1 Glycolysis
2 Transamination
3 Lactate dehydrogenase
1.1.1.27
4 Gluconeogenesis

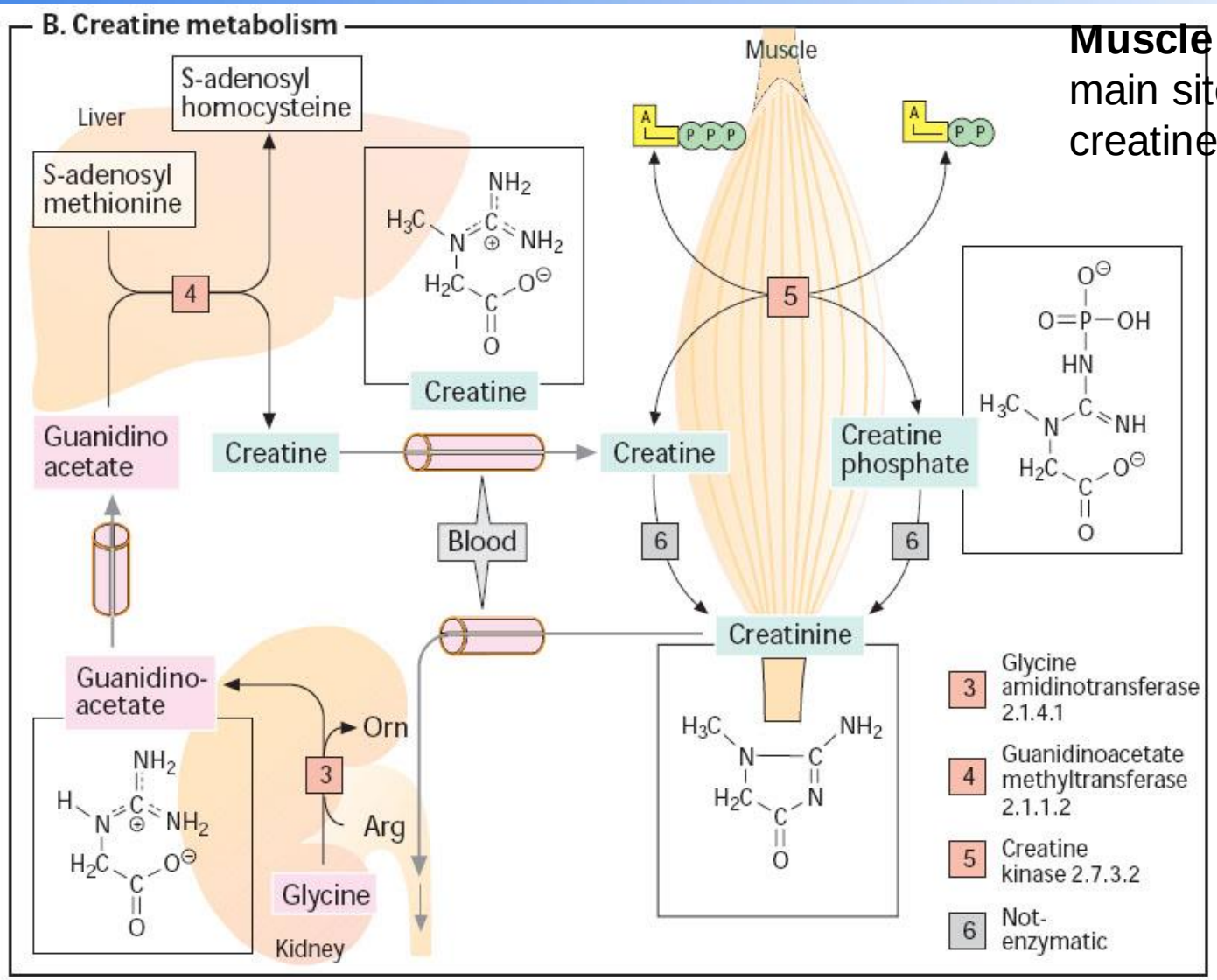
# Energy Fuels: Proteins

- Muscle contains another readily mobilizable source of energy – its own protein.
- The breakdown of muscle protein to meet energy needs is both
  - energetically wasteful and harmful to an animal, which must move about in order to survive.
- Protein breakdown is regulated so as to minimize amino acid catabolism except in starvation.

# Energy Reserve: Creatine Phosphate

- Muscle has an additional energy reserve in creatine phosphate, which generates ATP without the need for metabolizing fuels (see here).
- This reserve is exhausted early in a period of exertion and must be replenished, along with glycogen stores, as muscle rests after prolonged exertion.

# Creatine Metabolism




**Muscle** is the main site of creatine function.

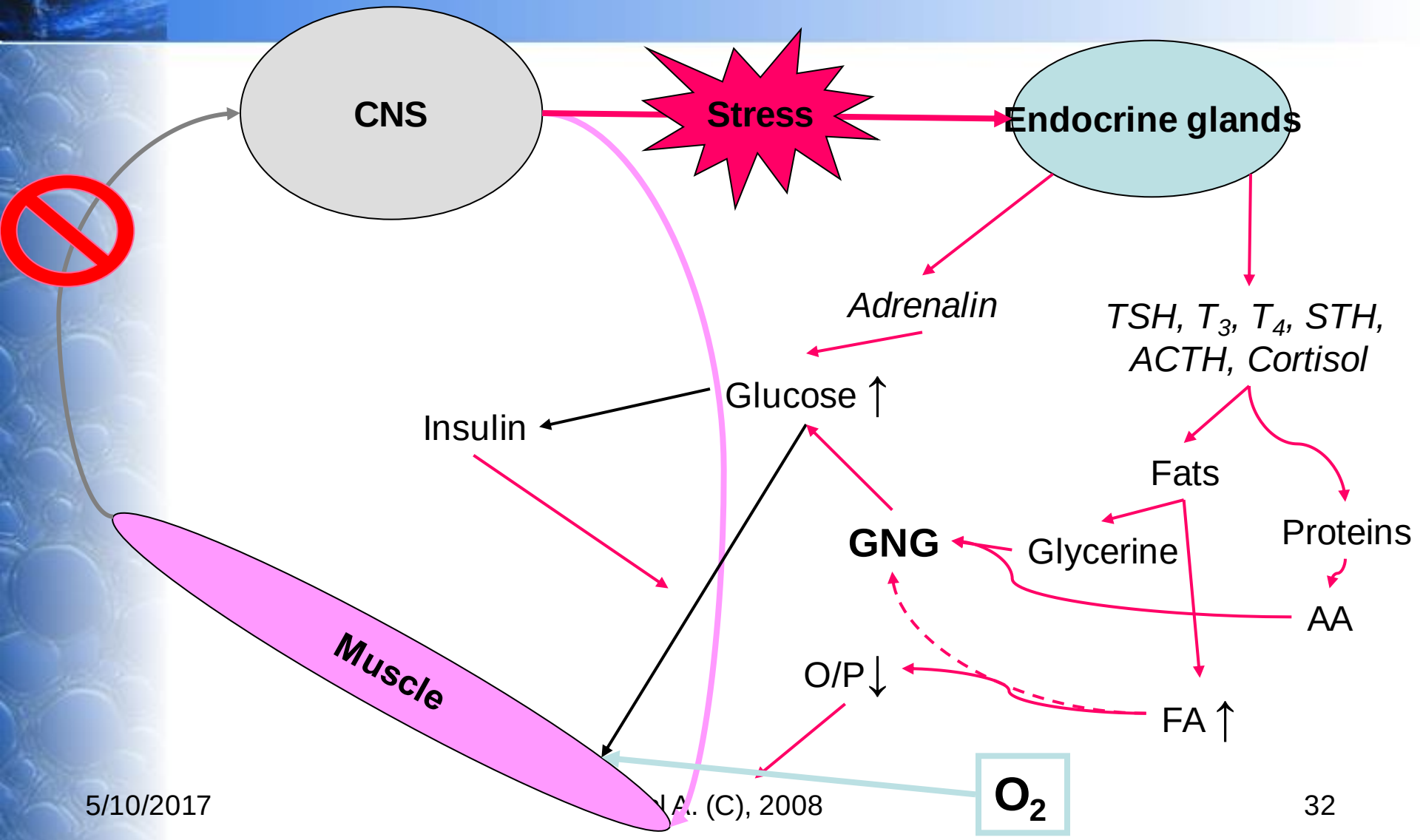
# Hypokinetic Syndrome

- **Hypokynetic syndrome** (HKS) is the essential restriction of the mobility.
- The mobility decreased 20 fold last 100 years.

# HKS Patogenesis (1st step)

- Proprioceptive information deficiency.
- Stress (reaction of the organism on the proprioceptive information deficiency)
  - Counter insular hormones effect: catecholamines, $T_3$, $T_4$, glucocorticoides, etc:
    - Activated proteolysis, lipolysis, GNG.
    - Increased blood FA.
    - Uncoupling of oxidation and phosphorylation.
    - Activated catabolic processes in the organism.
    - Increased heat production.

# Hypokinetic Syndrome Formation



# HKS Patogenesis (2nd step)

- Increased oxygen consumption (**hypokinetic paradox**)
- Muscular mass decreased
  - proteolysis
- Bone tissue resorption, osteoporosis, decreased mineral metabolism.
  - Decreased physical activity, piezoeffect.
- Loss of electrolytes $Na^+$, $K^+$, $Ca^{2+}$ with urine.
  - Due to decreased cell number.
- Increased rate of spontaneous mutations.
  - High intracellular NADH $\Rightarrow$ ROS generation.

# HKS Patogenesis (conclusion)

- **Hypokinetic syndrome is a** dissipative process, which includes the structure's catabolism, and its convertation into the heat, irradiated in the environment.

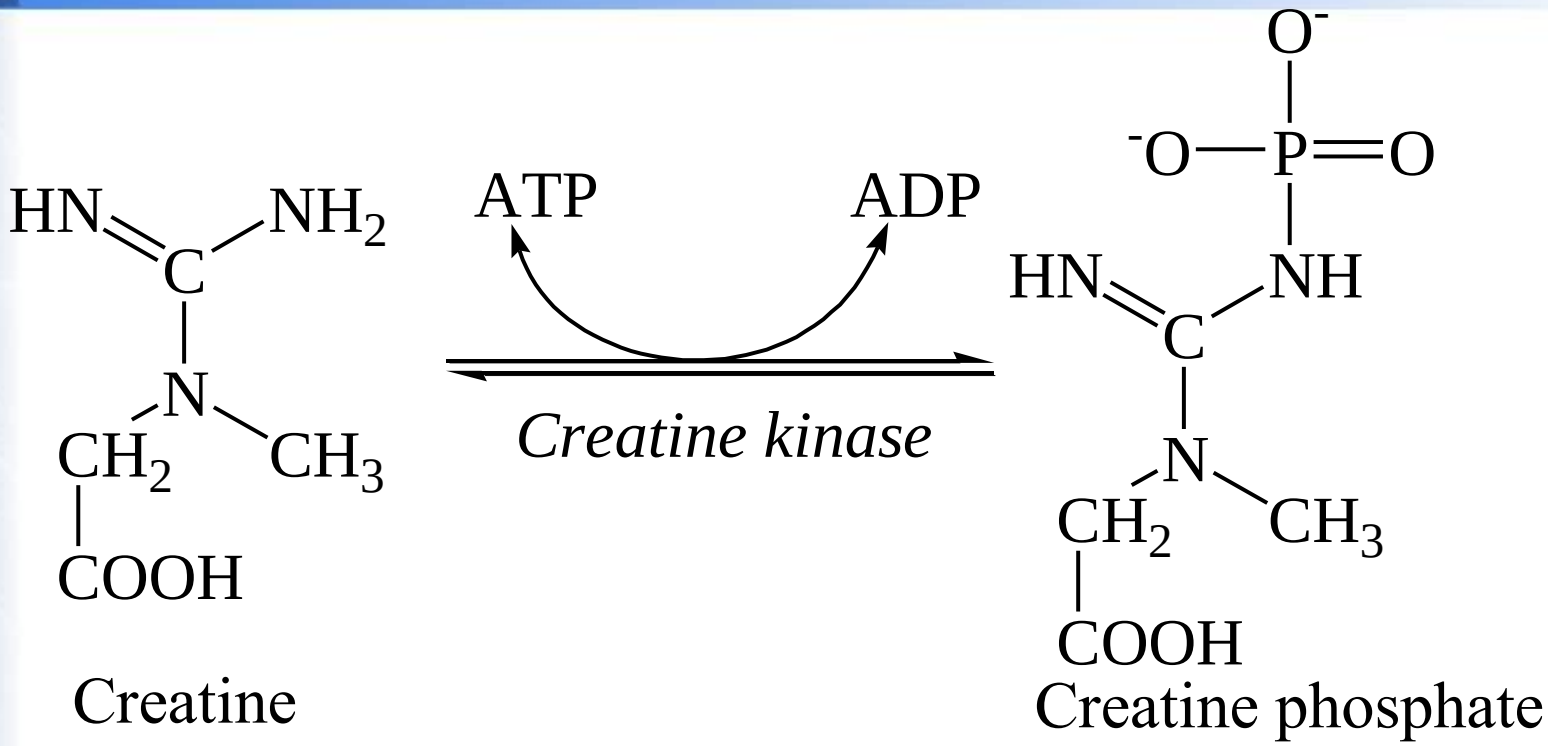
# Heart Mucsle

- Many mitochondria: 25-30%.
- Cardiomyocytes stops division early.
- Very fast proteins turnover, especially of contractile ones.
  - 1 month full turnover.

# Myocardial Biochemistry

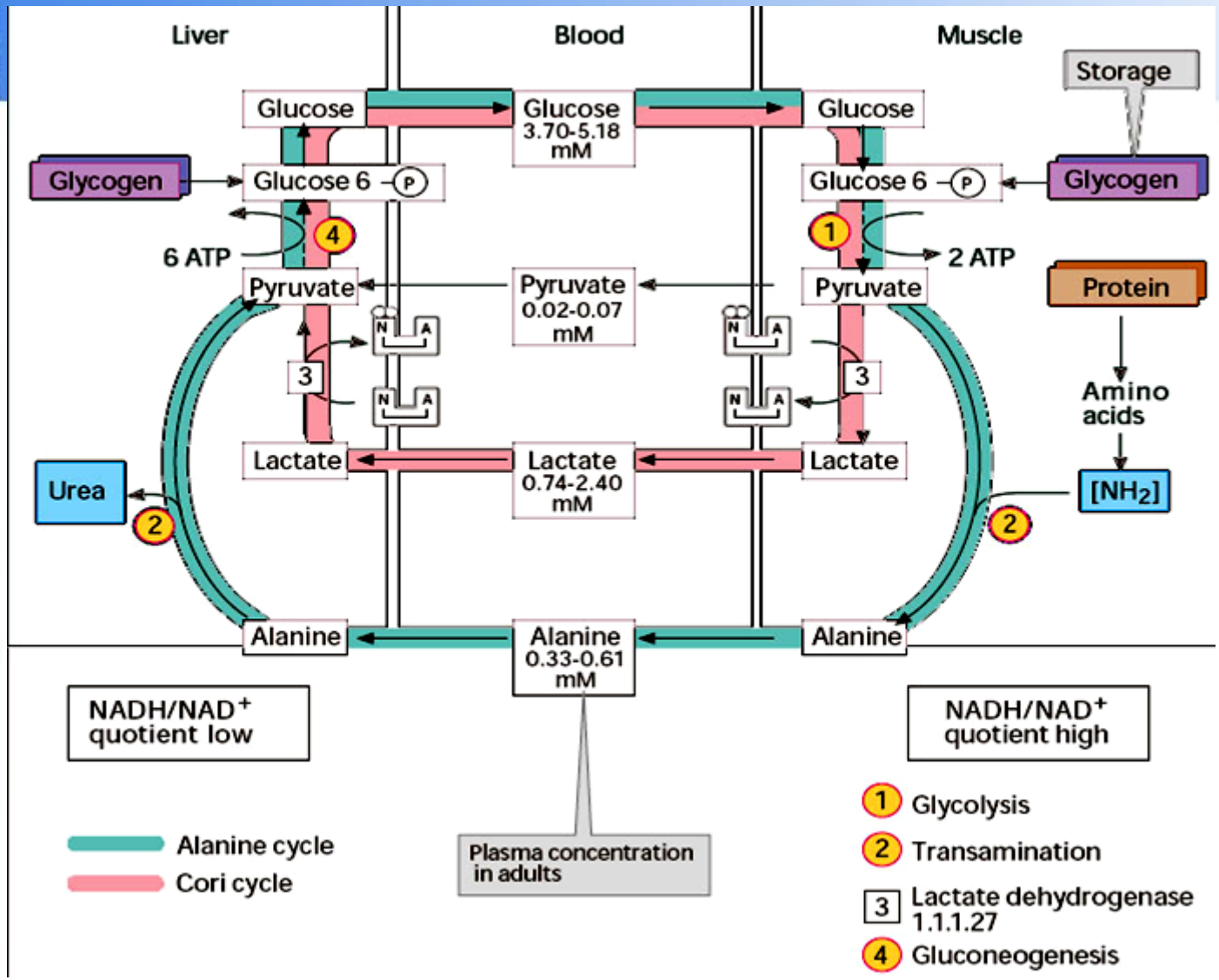
- Aerobic tissue (7-20% of the total $O_2$) $\Rightarrow$ aerobic isoenzymes.
  - LDH1 and LDH2
  - CPK2 (MB-isoenzyme).
- High rate of TCA reactions, FA $\beta$-oxidation, very low anaerobic glycolysis.
- Energy substrates – FA, glucose, lactate. Ketone bodies.
  - Very active is absorption of unsaturated FA – oleinic acid – from the blood.
- Intensive AA metabolism $\Rightarrow$ ALT, AST.
- SR is well formed, $Ca^{2+}$ however enters from out the cell.
- High sarcolemmal ATPase activity.

# Energy Reserve: Creatine Phosphate

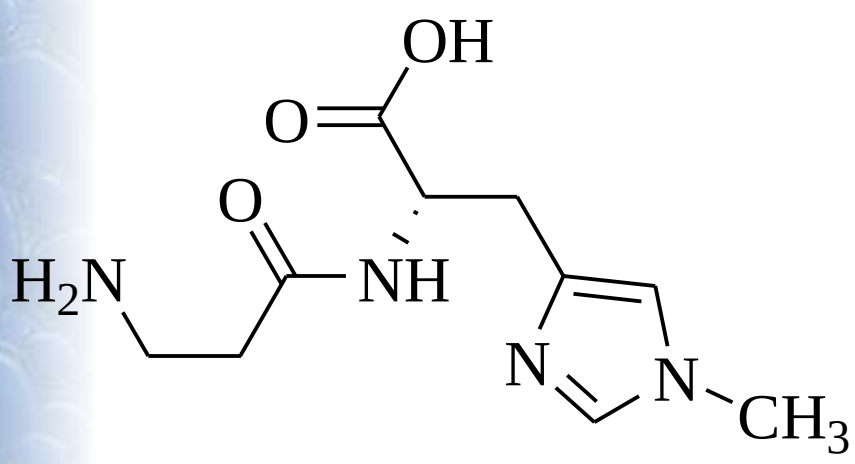



- Extra energy reserve in the muscle – creatine phosphate.

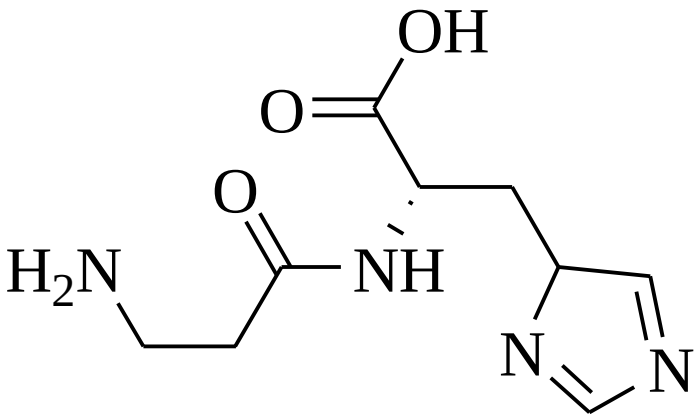
# Creatine Metabolism



# Dipeptides: Anserine & Carnosine



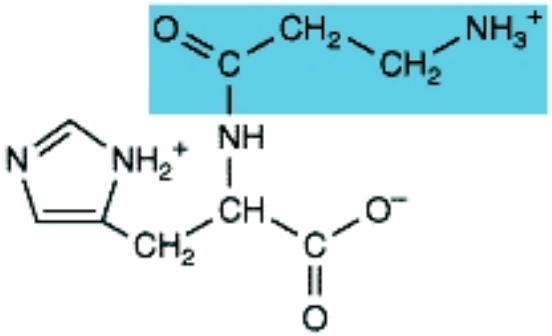

Anserine



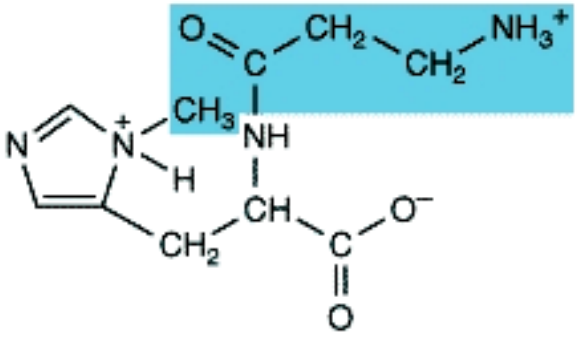

Carnosine

- These buffer dipeptides prevents acidification.

# Dipeptides: Anserine & Carnosine



**Carnosine**



**Anserine**

- The β-alanyl dipeptides carnosine and anserine (*N*-methylcarnosine) activate myosin ATPase, chelate copper, and enhance copper uptake.
- β-Alanyl-imidazole buffers the pH of anaerobically contracting skeletal muscle.
  - Biosynthesis of carnosine is catalyzed by *carnosine synthetase* in a two-stage reaction that involves initial formation of an enzyme-bound acyl-adenylate of β-alanine and subsequent transfer of the β-alanyl moiety to L-histidine.
  - Hydrolysis of carnosine to β-alanine and L-histidine is catalyzed by *carnosinase*.
  - The heritable disorder **carnosinase deficiency** is characterized by **carnosinuria**.

# Energy Metabolism in the Muscles (cont'd)

- Hydrolylis ATP rate exceeds its synthesis.

$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{2-} + H^+$$

- ADP is accumulated, but <u>is used in no reaction</u> but (myo)**adenylatekinase:**

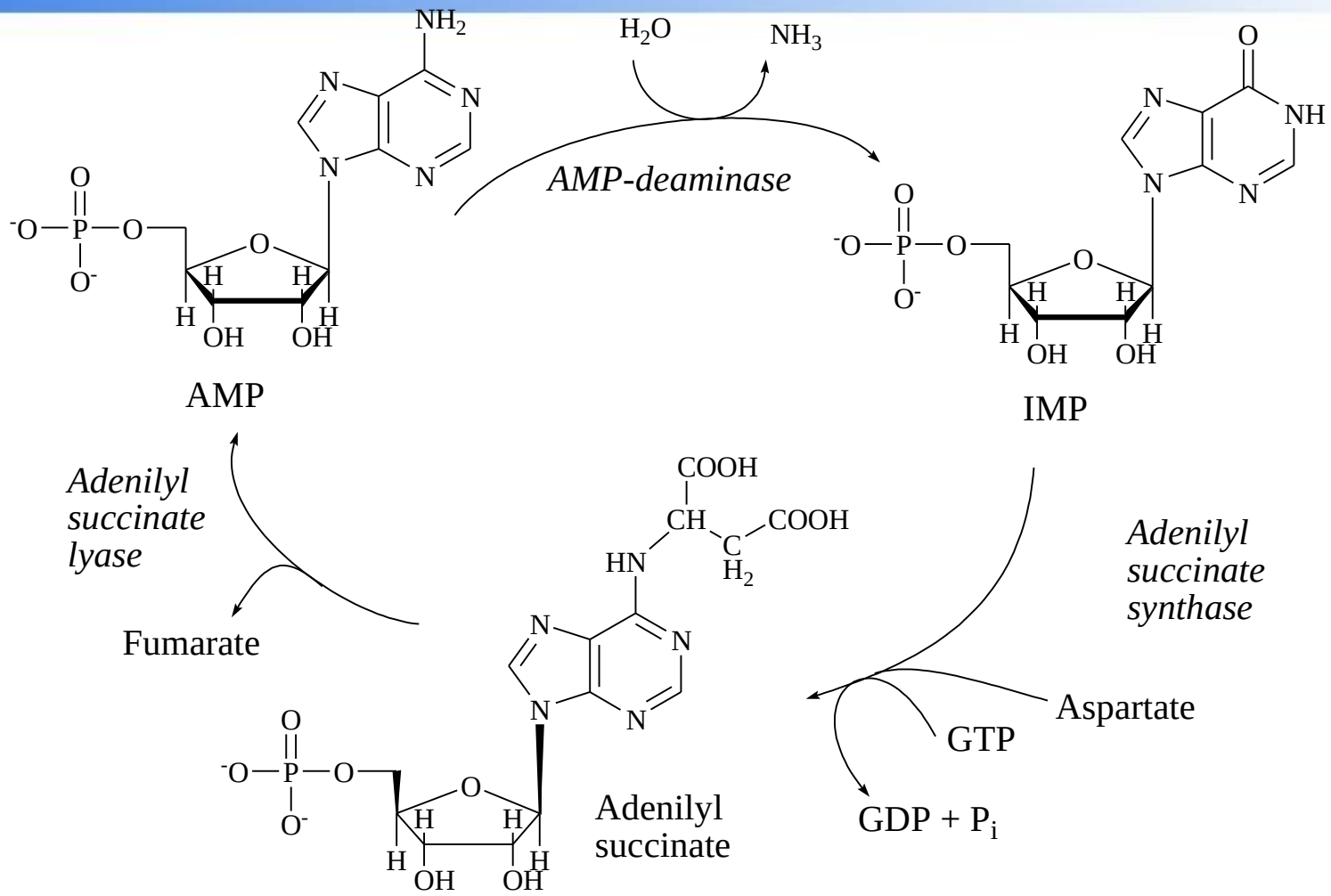
$$2\ ADP3- \leftrightarrow\ ATP4- + AMP$$

(*myoadenylatekinase*)

- AMP ↑. Decreased by **AMP deaminase**.

$$AMP \rightarrow IMP + NH_3$$

# Purine Nucleotide Cycle



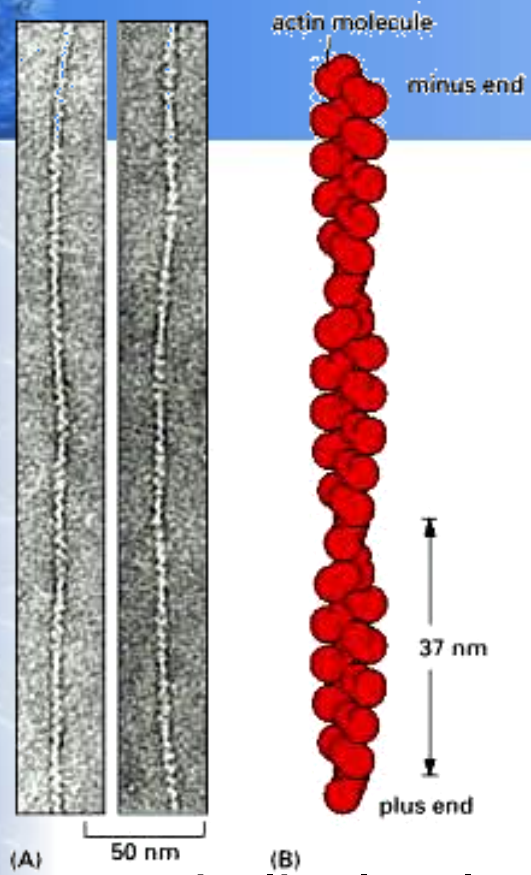
# Ways or ATP utilization in the muscle

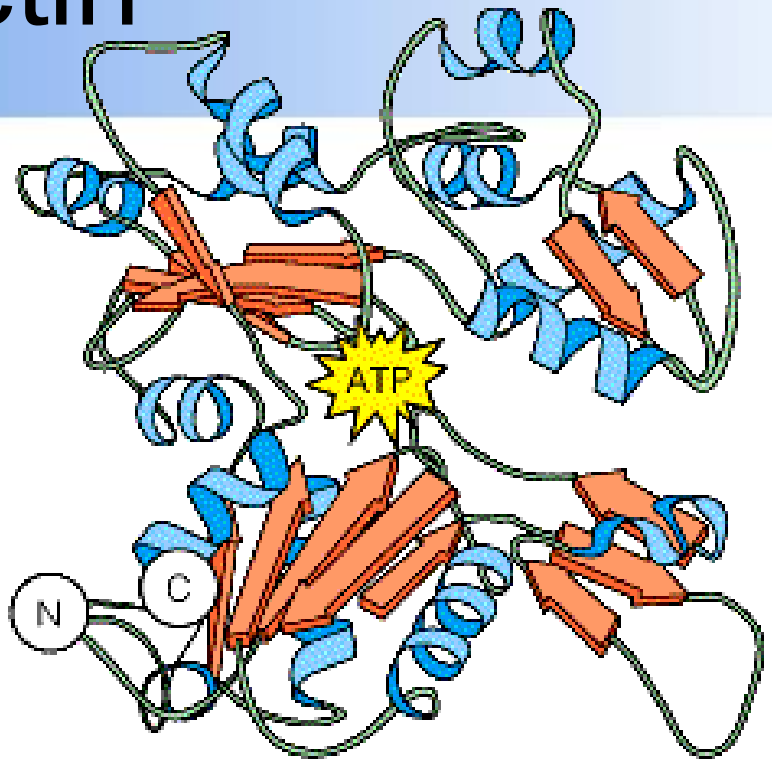
1. **Mechanic work** – muscular contractility.
2. **ATPase work** – provide the electrical component. :
   1. $Na^+/K^+$-ATP-ase – maintains the resting potential; substrate transportation.
   2. $Ca^{2+}$- ATP-ase – muscular relaxation,
   3. $Mg^{2+}$- ATP-ase – ATP stabilisation, enc.
3. **Muscular relaxation** – ATP-dependent process ($Ca^{2+}$ pumping out the sarcoplasm).

# Proteins of Muscle

- The major proteins in muscle are **actin** and **myosin**.
- They associate into a complex, called *actomyosin*, which is organized into a highly ordered structure having the ability to do work very efficiently.
- Actin and myosin are also found in many other kinds of cells besides muscles and are involved in several kinds of cellular and intracellular motions (e.g., cell motility and changes of cell shape).

# Actin



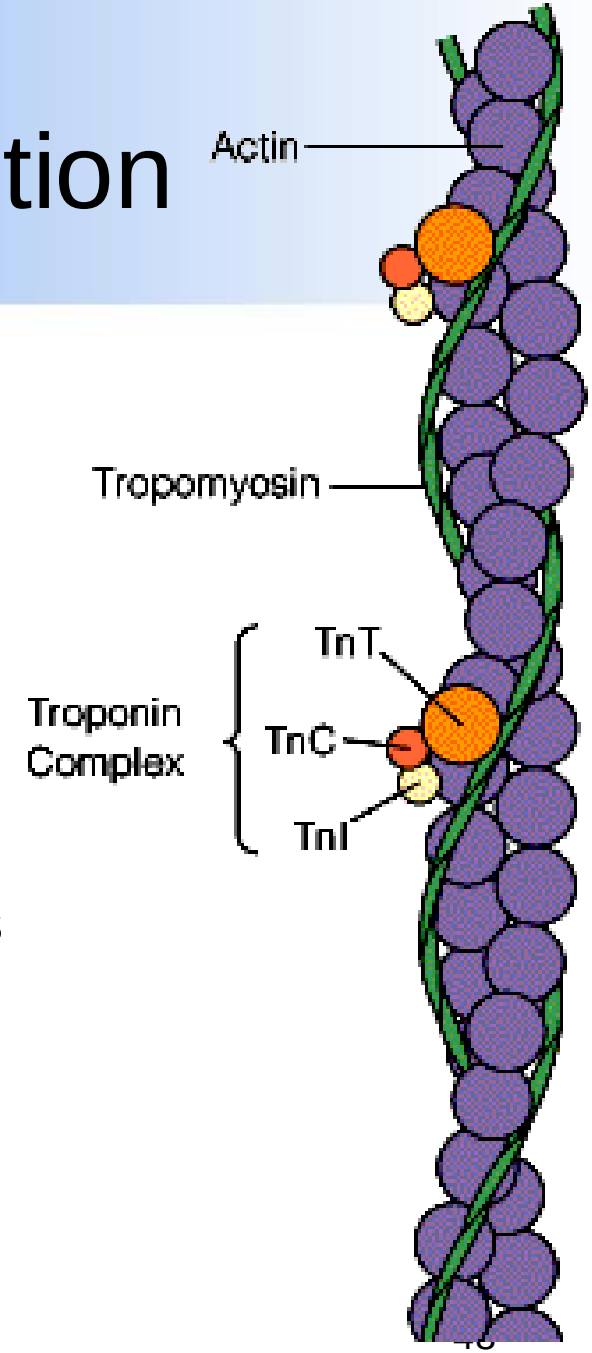

- Long, helical polymer (fibrous actin, or F-actin) of a globular protein monomer (G-actin).
- The structure of the G-actin monomer is a two-domain molecule with a mass of 42,000 Daltons.

# Actin (cont'd)

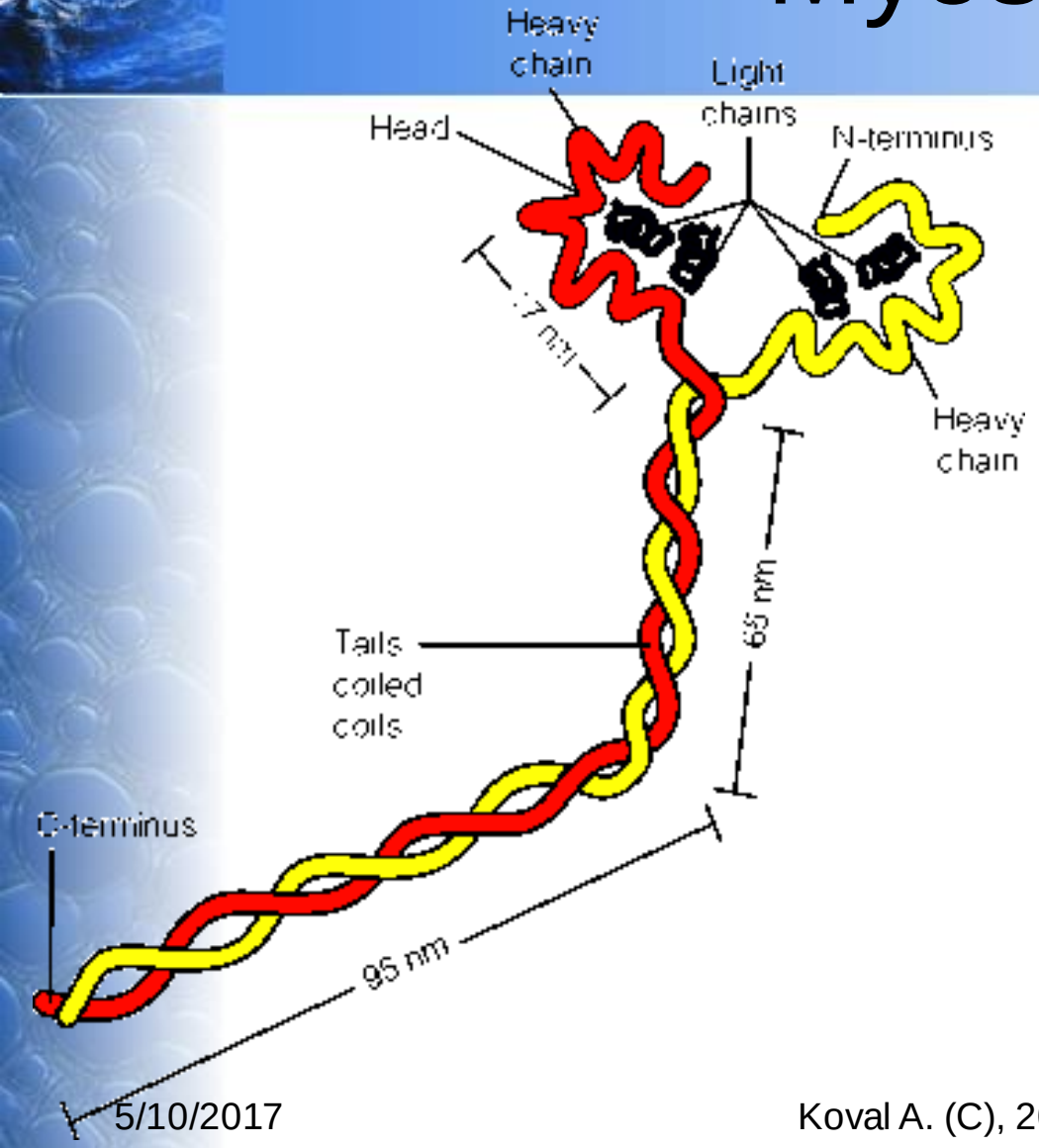
- The binding of ATP by a G-actin monomer leads to polymerization (i.e., the formation of F-actin). The ATP is hydrolyzed, but the ADP is retained in the actin.
- Within F-actin filaments, the G-actin monomers are arranged in a two-strand helix.
- Actin filaments carry sites on each subunit that can bind to **myosin**.

# Thin Filaments Composition

- Thin filaments are composed of
  - **F-actin** arranged in a helix,
  - **tropomyosin** (a fibrous protein that exists as elongated dimers lying along, or close to, the groove in the F-actin helix),
  - and three small proteins called **troponins I, C, and T**.
- The presence of tropomyosin and the troponins inhibits the binding of myosin heads to actin unless calcium is present at a concentration of about $10^{-5}$M.
  - In resting muscle, $Ca^{2+}$ concentrations are ~$10^{-7}$M, so cross bridges cannot occur.



# Myosin

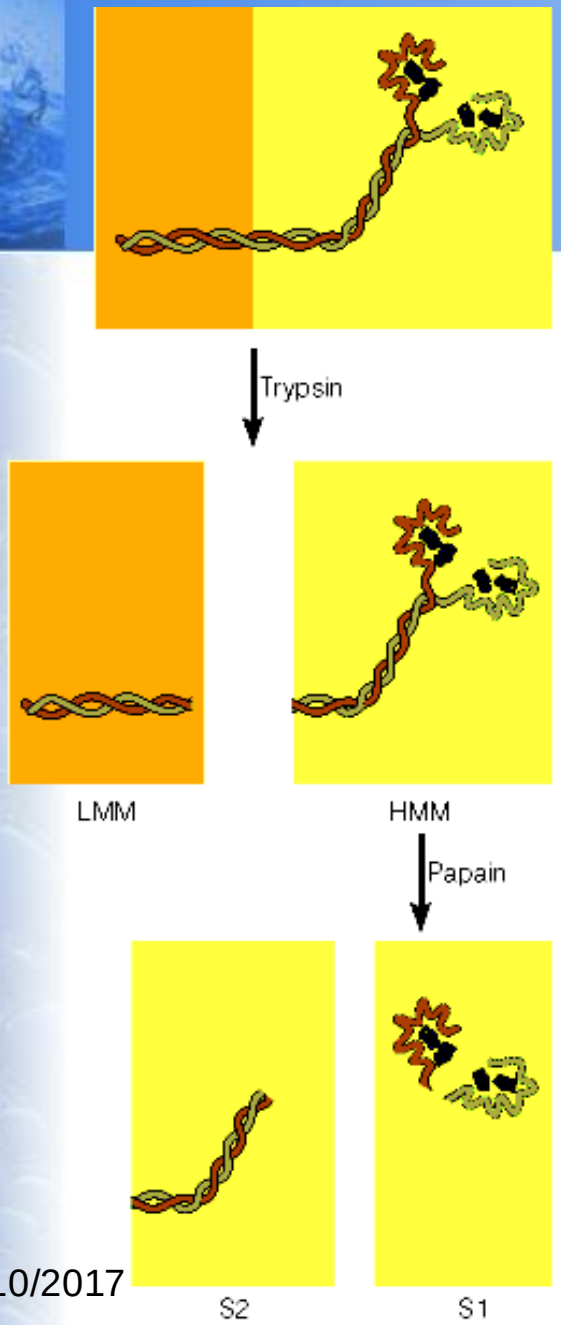



- The functional **myosin** molecule is composed of six polypeptide chains:
  - two identical heavy chains (M = 230,000)
  - and two each of two kinds of light chains (M = 20,000).
- Together they form a complex of molecular weight 540.000.

# Myosin (cont'd)

- The heavy chains have long $\alpha$-helical tails and globular head domains.
- The $\alpha$-helical tails are interwound into a two-strand coiled coil and the light chains are bound to the globular head domains.
- Between each head domain and tail domain is a flexible

# Light and Heavy Meromyosines

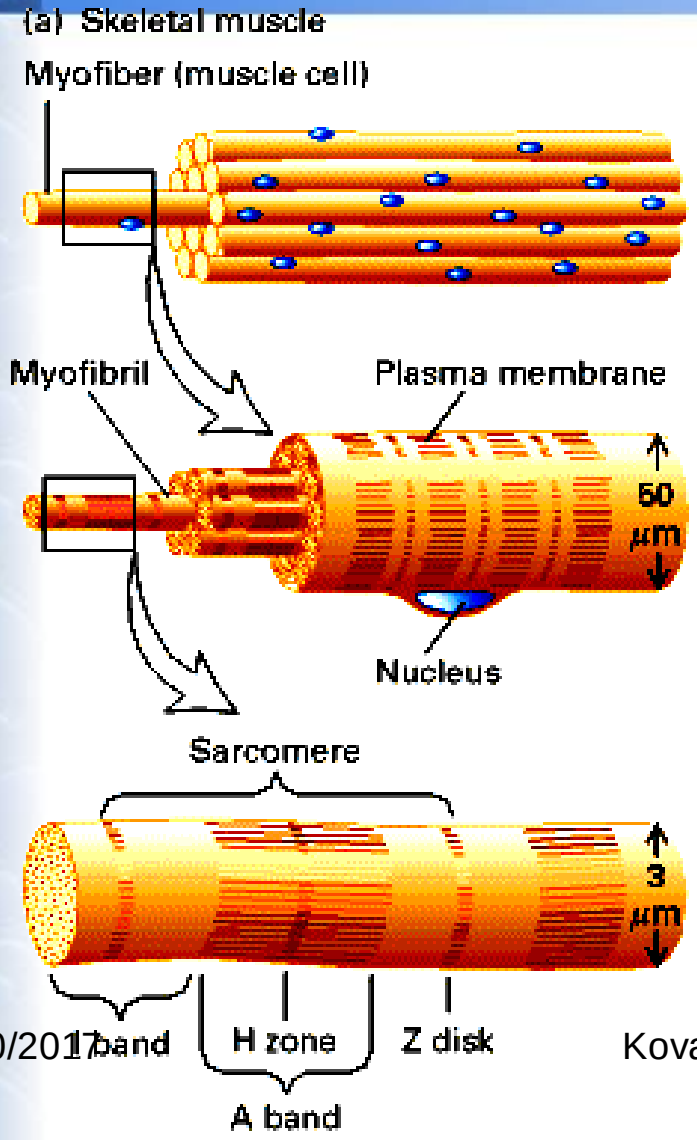



- The myosin molecule can be cleaved by proteases.
- The tail domain can be cleaved at a specific point by *trypsin* to yield fragments called **light meromyosin** and **heavy meromyosin**.

# Domains of the Myosin

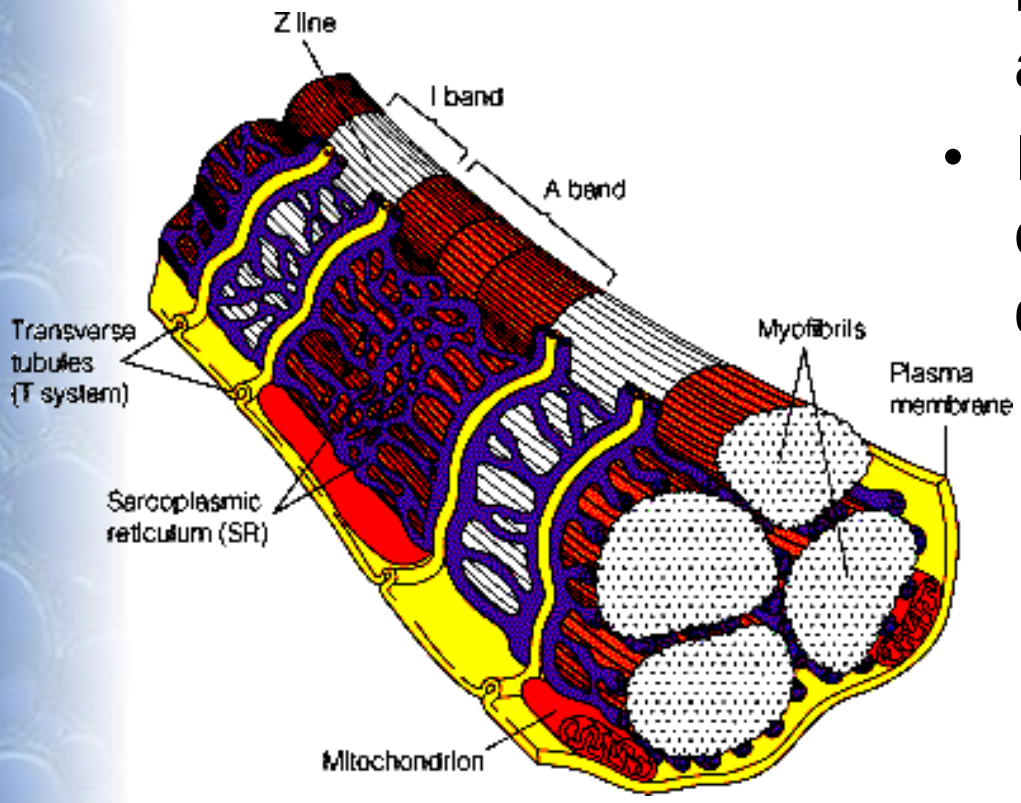
- Myosin exhibits aspects of both fibrous and globular proteins, and its functional domains play quite different roles.
  - The tail domains have a pronounced tendency to aggregate, causing myosin molecules to form thick bipolar filaments.
  - The head domains, with their attached light chains, are often called headpieces; they have a strong tendency to bind to actin.

# General Structure of Skeletal Muscle




- Skeletal muscle tissue is composed of bundles of multinucleated muscle cells, or **myofibers**.
- Each muscle cell is packed with bundles of actin and myosin filaments, organized into **myofibrils** that extend the length of the cell.
- Packed end to end in a myofibril is a chain of **sarcomeres**, the functional units of contraction.
- The internal organization of the filaments gives skeletal muscle cells a **striated appearance**.

# Muscle Fiber Structure




- Within a muscle fiber are myofibrils, which are arranged in bundles.
- Individual myofibrils contain the structurally distinct regions:
  - Myofibrils have thin filaments composed of actin and thick filaments composed of myosin.
  - Arrangement of the thick and thin filaments in a myofibril produces the distinctive pattern.

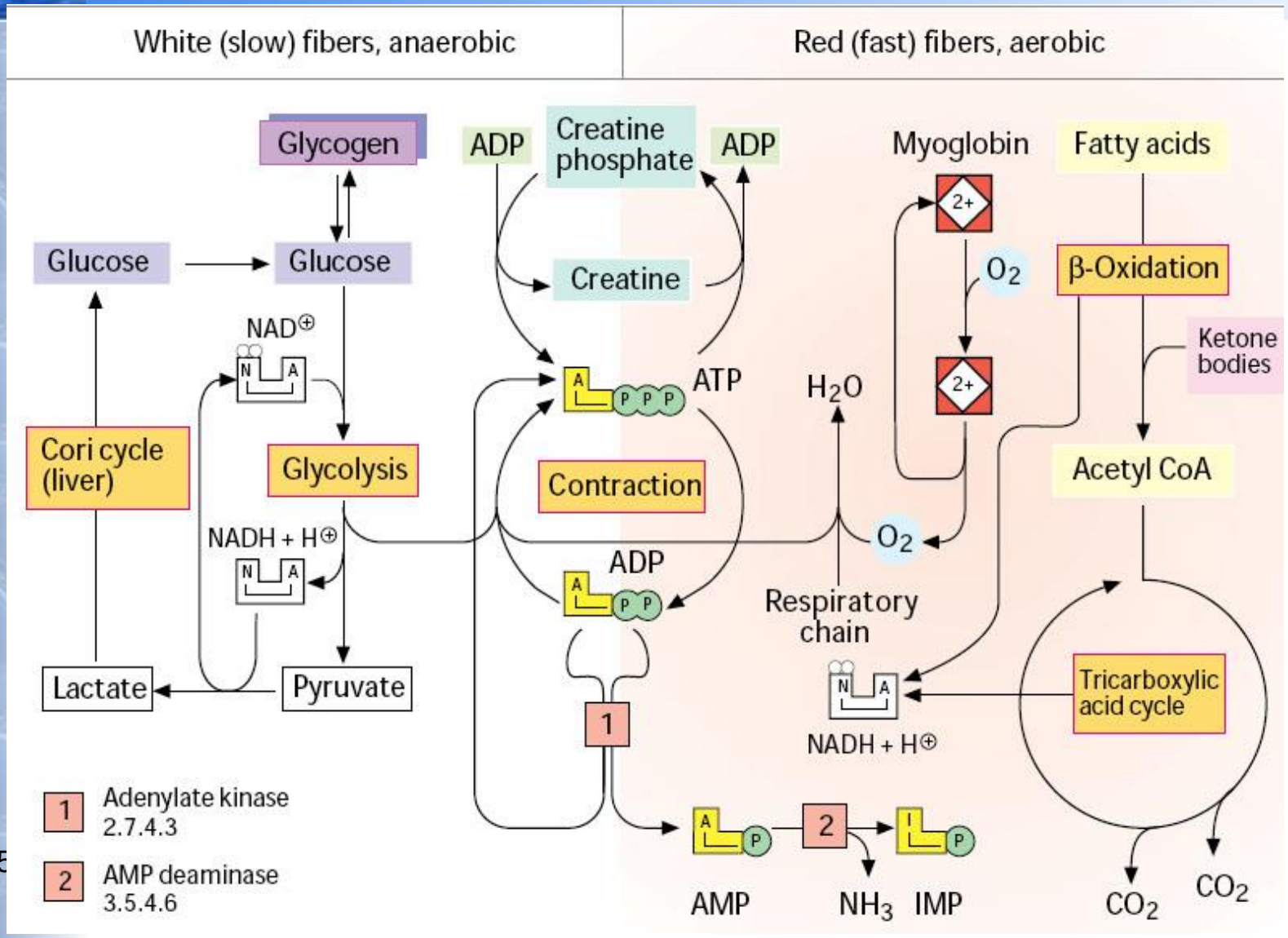
# Red and White Muscles

- Striated muscle can be divided into two categories:
- red muscle, designed for relatively continuous use, and
- white muscle, employed for occasional, often rapid motions.
  - Red muscle owes its dark color to abundant heme proteins. It is well supplied with blood vessels and, therefore, with hemoglobin. It has many mitochondria with cytochromes and it has large stores of myoglobin. Red muscle depends heavily on aerobic metabolism in mitochondria, so the primary energy source in red muscle is the oxidation of fat.
  - White muscle, on the other hand, relies on glycogen as a primary energy source.

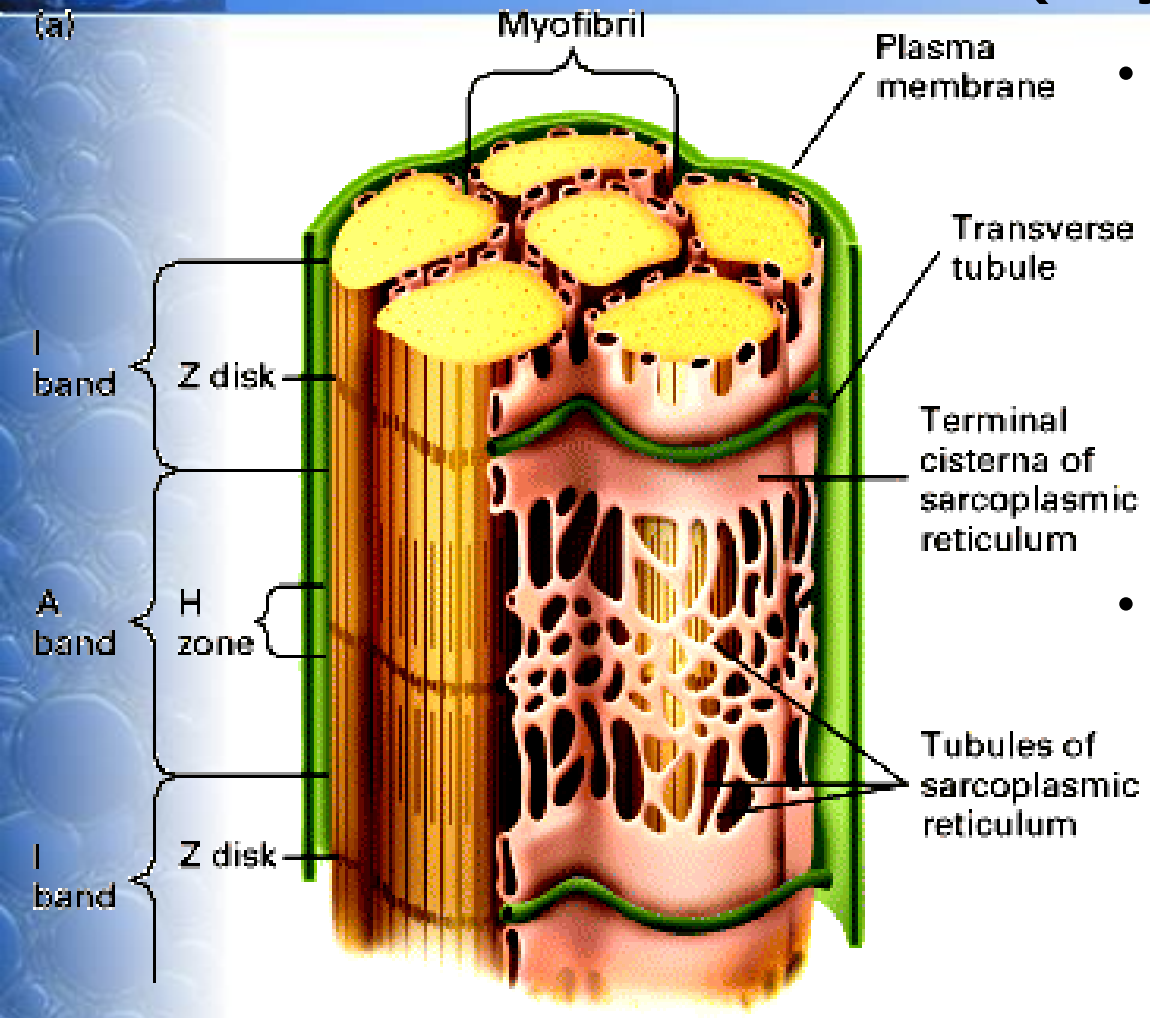
# Energy Fuels in White and Red Muscle

| | Red | White |
|---|---|---|
| Relative fiber size | Small | Large |
| Mode of contraction | Slow twitch | Fast twitch (about 5 times faster) |
| Vascularization | Heavy | Lighter |
| Mitochondria | Many | Few |
| Myoglobin | Much | Little |
| Major stored fuel | Fat cells | Glycogen in muscle |
| Main source of ATP | Fatty acid oxidation | Glycolysis |

# Energy metabolism in the white and red muscle fibers

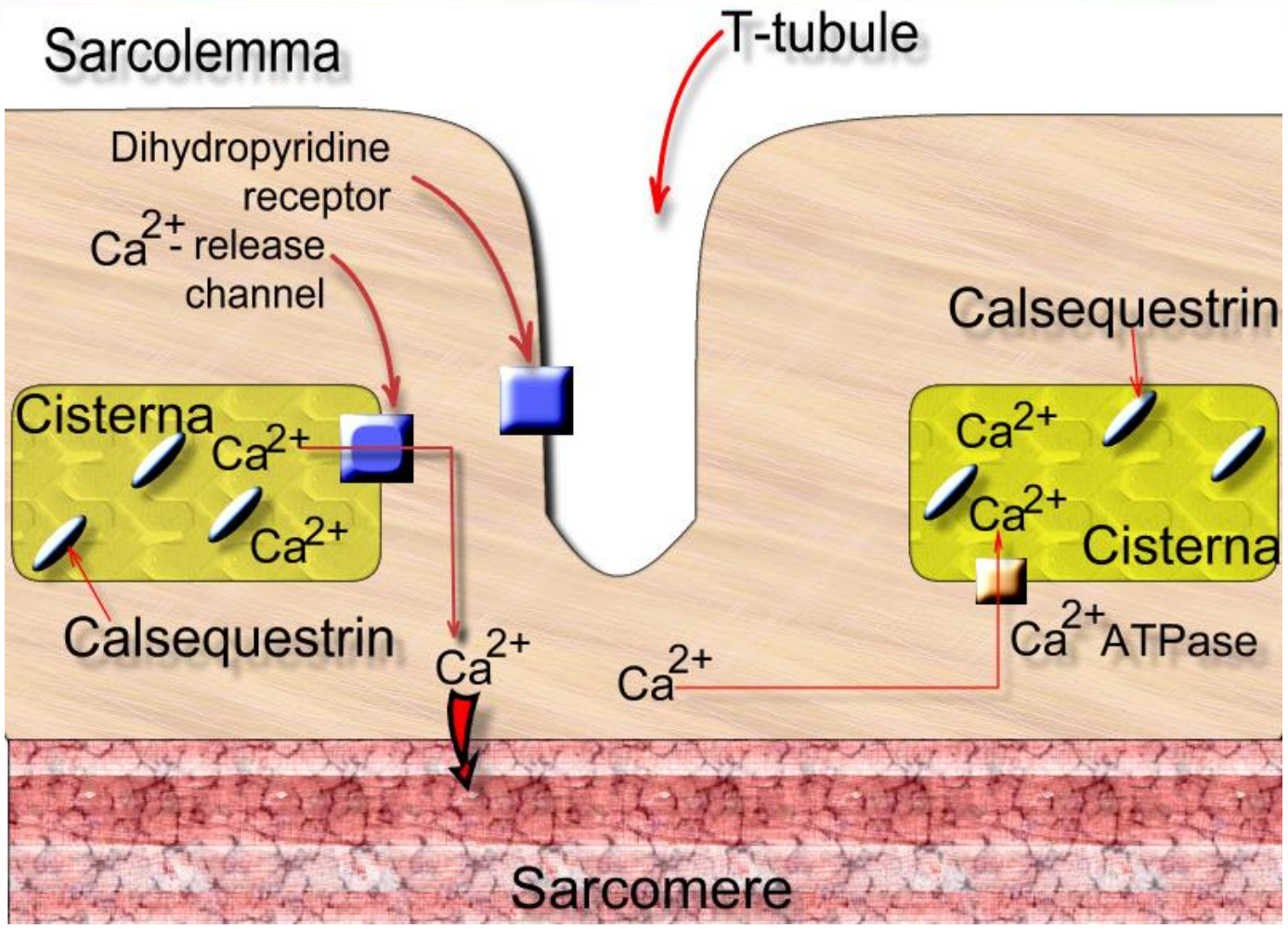


# Three-dimensional Drawing of a Muscle Cell (Myofiber)

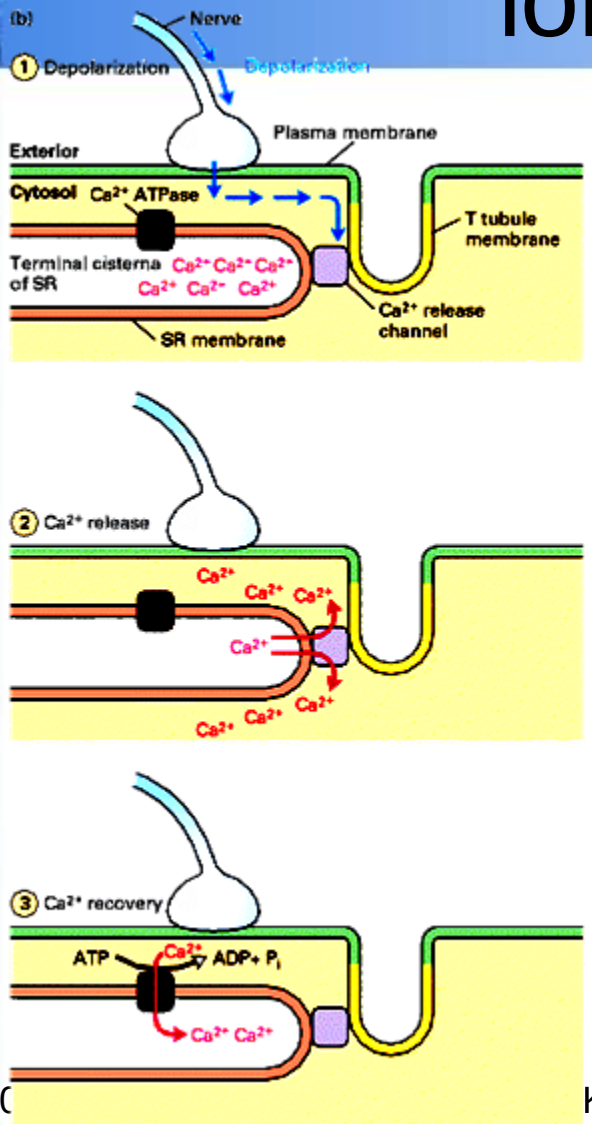



- The transverse (T) tubules, which are invaginations of the plasma membrane, enter myofibers at the Z disks, where they come in close contact with the terminal cisternae of the SR, forming triads.
- The terminal cisternae store $Ca^{2+}$ ions and connect with the lacelike network of SR tubules that overlie the A band.

# $Ca^{2+}$ Release and Recovery by SR



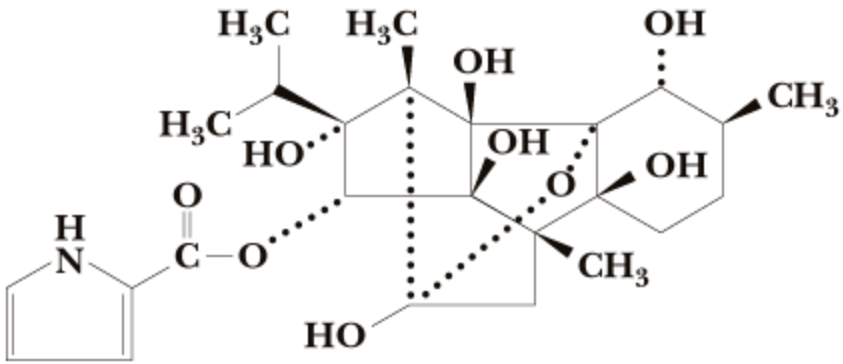
# Release and recovery of $Ca^{2+}$ ions by the SR




- Depolarization of a muscle cell (step **1**) induces the release of $Ca^{2+}$ ions stored in the SR via $Ca^{2+}$ release proteins in the SR membrane (step **2**).
- Subsequently, $Ca^{2+}$ ATPases in the SR membrane pump $Ca^{2+}$ ions from the cytosol back into the SR, restoring the cytosolic $Ca^{2+}$ concentration to its resting level within about 30 milliseconds (step

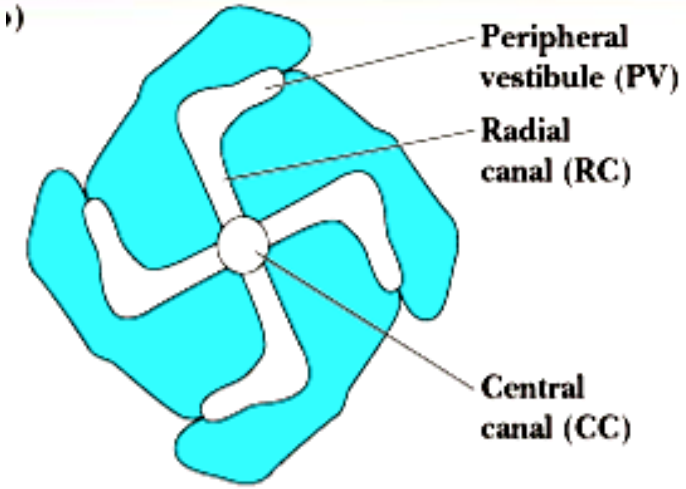
# $Ca^{2+}$ Release Channel – Ryanodine Receptor

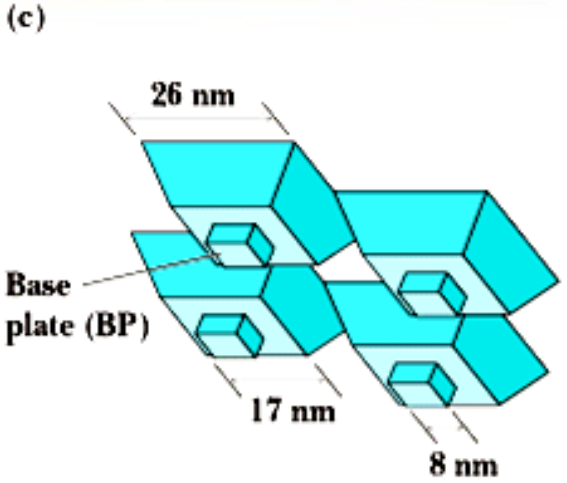
- The $Ca^{2+}$ release channel on the sarcoplasmic reticulum is known as the **ryanodine receptor** (**RYR**). It is the major source of calcium required for muscle excitation-contraction coupling.
- **Ryanodine** is a plant alkaloid. It binds to RYR specifically and modulates its activities.
- There are 3 isoforms of this receptor, **RYR1, RYR2** and **RYR3**.
  - RYR1 – in skeletal muscle
  - RYR2 – in heart muscle a
  - RYR3 – in brain.

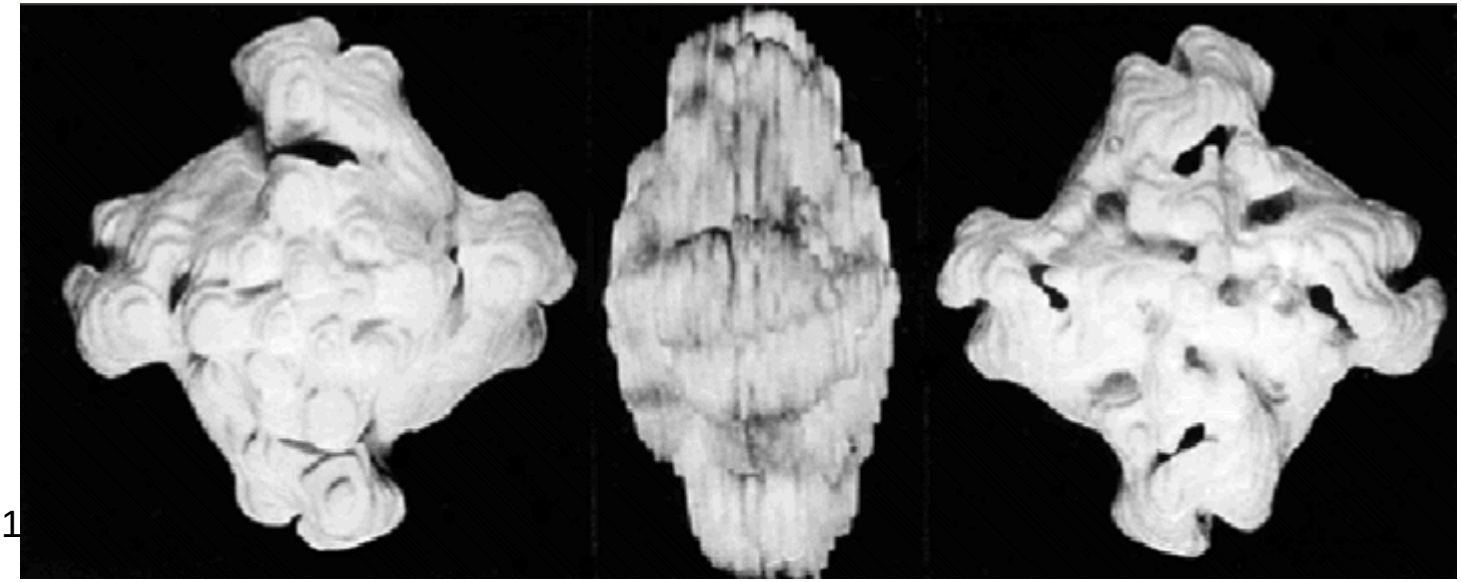


Ryanodine

# Structure of RYR



# Ryanodine Receptor 1 (RYR1)

- 5,032-amino acid protein; molecular mass of 563.5 kD, which is made without an N-terminal sequence.
  - 10 + 2 potential transmembrane sequences.
- Hydrophilic part of the protein constitutes the cytoplasmic domain.
- Potential calmodulin-binding sites.
- **Abnormalities**:
  - Central core disease;
  - Susceptibility to malignant hyperthermia;
  - Minicore myopathy with external ophthalmoplegia.

# Central Core Disease

- **Central core disease** is characterized pathologically by the presence of central core lesions extending the length of type I muscle fibers.
  - The **cores** are regions of sarcomeric disorganization, absent mitochondria, and lack of oxidative activity.
- Ultrastructural studies show changes in the sarcoplasmic reticulum and t-tubules.
- Central core disease is one of the conditions that produces the “floppy infant”.
  - Central core disease was the first described (Shy and Magee, 1956) example of a stationary muscle disorder.
- Central core disease can be caused by mutation in the ryanodine receptor-1 gene (RYR1).

# Ryanodine Receptor 2 (RYR2)

- The channel is a tetramer comprised of 4 RYR2 polypeptides and 4 FK506-binding proteins.
  - Protein kinase A phosphorylation of RYR2 dissociates FKBP12.6 and regulates the channel open probability (Marx et al., 2000).
- **Abnormalities**:
  - Ventricular tachycardia, stress-induced polymorphic (autosomal dominant);
  - Familial arrhythmogenic right ventricular dysplasia;
  - In failing human hearts RYR2 is PKA hyperphosphorylated, resulting in defective channel function due to increased sensitivity to calcium-induced activation.

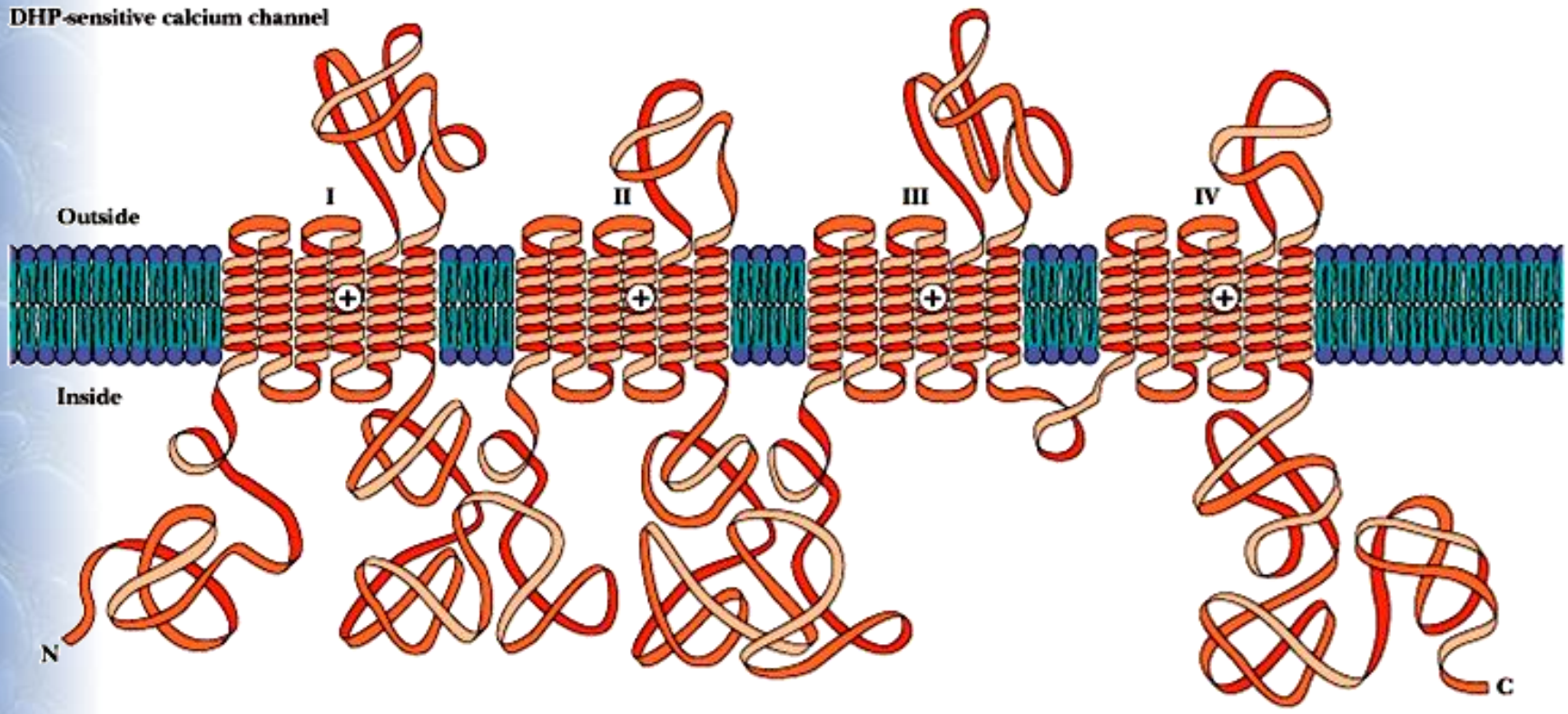
# Ryanodine Receptor 3 (RYR3)

- Last time it was found new ryanodine receptor 3.
- The novel ryanodine receptor is expressed mainly in brain.
  - 4,872 amino acids;
  - shared characteristic structural features with the skeletal muscle (RYR1) and cardiac (RYR2) ryanodine receptors.
- The brain ryanodine receptor gene is transcribed in smooth muscle, also in skeletal muscle during the postnatal phase of muscle development.
- At the moment being RYR3 is studied actively.

# Voltage-sensitive $Ca^{2+}$ Channels (Dihydropyridine Receptors, DHPR)

- The major type of voltage-sensitive $Ca^{2+}$ channels in skeletal muscle is the slowly inactivating L-type.
  - Sensitive to calcium channel blockers such as 1,4-dihydropyridines (DHP), phenylalkylamines, and benzothiazepines.
  - Play a key role in excitation-contraction coupling.
- The DHP-sensitive L-type $Ca^{2+}$ channel from skeletal muscle is an oligomeric protein composed of 2 high molecular weight polypeptide subunits ($\alpha$-1 and $\alpha$-2) and 3 smaller units ($\beta$, $\gamma$, and $\delta$).
  - The $\alpha$-1 subunit confers the structural features needed for $Ca^{2+}$ channel function and also contains the binding sites for the $Ca^{2+}$ channel blockers.

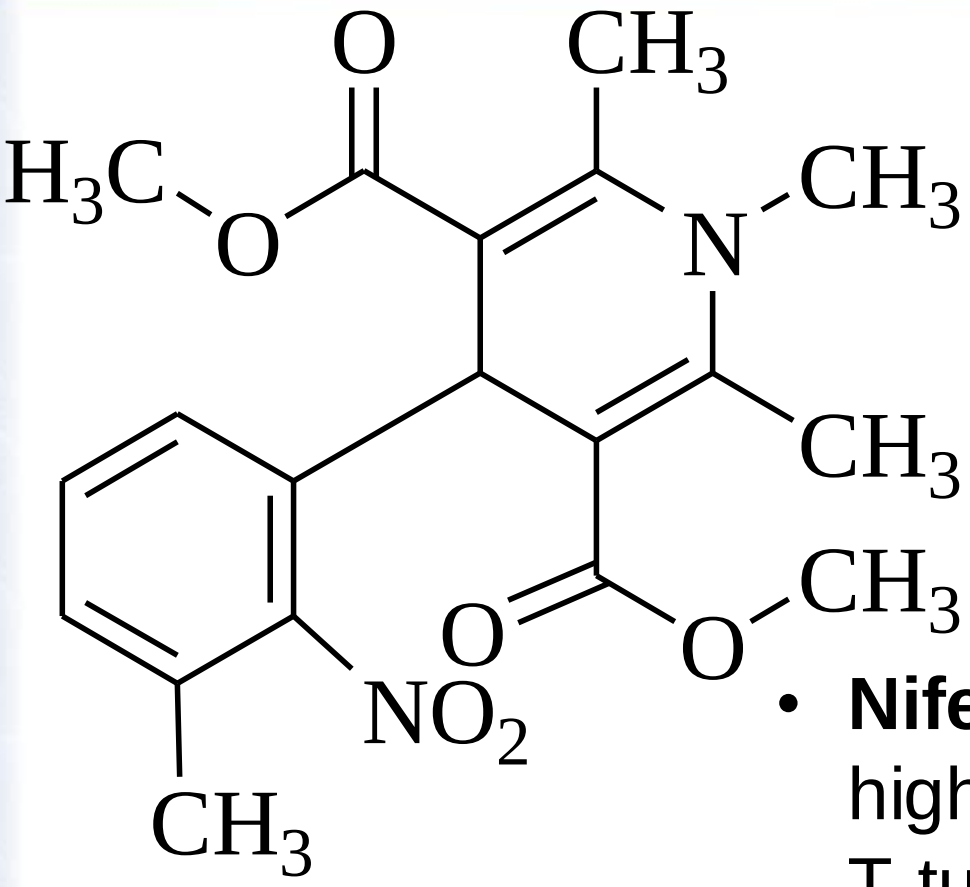
# The Structure of Dihydropyridine Receptor (α-1)



# Abnormalities of DHPR

- Hypokalemic periodic paralysis;
- Susceptibility to thyrotoxic periodic paralysis;
- Susceptibility to malignant hyperthermia.

# Nifedipine




- **Nifedipine** binds with high affinity to DHPR of T-tubules.

# Calsequestrin

- **Calsequestrin**, an acid glycoprotein located in the luminal space of the terminal cisternae of the sarcoplasmic reticulum, binds calcium ion and is believed to function as a storage protein for calcium.
  - Skeletal muscle sarcoplasmic reticulum (SR) contains **fast-twitch skeletal muscle** isoform of calsequestrin (CASQ1).
  - Cardiac muscle SR contains a **cardiac** isoform of calsequestrin (CASQ2).

# Calsequestrin (cont'd)

- The CASQ2 protein serves as the major calcium ion reservoir within the sarcoplasmic reticulum of cardiac myocytes and is part of a protein complex that contains the ryanodine receptor.
  - Human gene has 11 exons, 5 amino acids near the COOH terminus of the sequence are lacking in the human protein.
- Abnormalities:
  - missense mutation in the CASQ2 gene as the cause of autosomal recessive **catecholamine-induced polymorphic ventricular tachycardia** in Bedouin families from Israel.

# Calsequestrin: calmitine

- **Calmitine** is a mitochondrial calcium-binding protein specific for fast-twitch muscle fibers.
  - It is absent in patients with **Duchenne** and **Becker** types of **muscular dystrophy**.
- The sequence of calmitine is identical to calsequestrin.
- Calmitine represents the $Ca^{2+}$ reservoir of mitochondria.

# Other Muscular Proteins

- Major proteins of striated muscle (myosin, actin, tropomyosin, and the troponins).
  - Myosin and actin – 65% of the total muscle protein,
  - Tropomyosin and the troponins – 5%.
- Numerous other proteins play important roles in the maintenance of muscle structure and the regulation of muscle contraction.
- 25% of the total myofibrillar protein.
- The regulatory proteins can be classified as either **myosin-associated proteins** or **actin-associated proteins.**

# Myosin-associated Proteins

- The myosin-associated proteins include three proteins found in the M disks.
- The M disks consist primarily of
  - **M protein** (165 kD),
  - **myomesin** (185 kD), and
  - **creatine kinase** (a dimer of 42-kD subunits).
- **Creatine kinase** facilitates rapid regeneration of the ATP consumed during muscle contraction.
- The association of M protein, **myomesin**, and **creatine kinase** in the M disk maintains the structural integrity of the myosin filaments.

# Other Myosin-associated Proteins

- Several other myosin-associated proteins have also been identified, including
  - **C protein** (135 kD),
  - **F protein** (121 kD),
  - **H protein** (74 kD), and
  - **I protein** (50 kD).
- The C protein is localized to several regularly spaced stripes in the A band.
- C protein inhibits myosin ATPase activity at low ionic strength but activates it at physiological ionic strength.
- The roles of F, H, and I proteins are not yet understood.

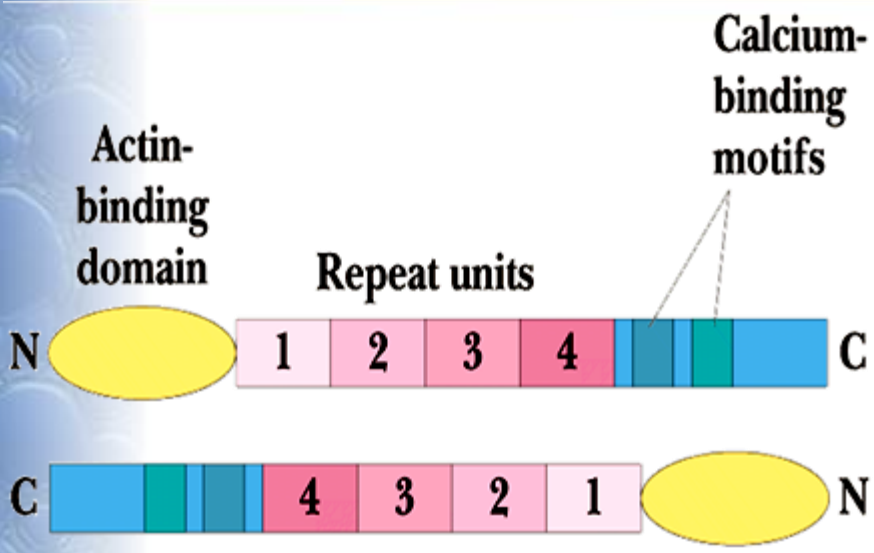
# Actin-associated Proteins

- Actin-associated proteins (other than tropomyosin and the troponins) include **α-actinin** (a homodimer of 95-kD subunits), **β-actinin** (a heterodimer of 37-kD and 34-kD subunits), **γ-actinin** (a 35-kD monomer), and **paratropomyosin** (a homodimer of 34-kD subunits).

# Actinins

- $\alpha$-Actinin is found in the Z lines and activates contraction of actomyosin. It is thought to play a role in attachment of actin to the Z lines.
- $\beta$-Actinin consists of three domains:
  - an N-terminal, actin-binding domain;
  - a central domain consisting of four repeats of a 122-residue sequence; and
  - C-terminal domain that contains two EF-hand, calcium-binding domains.

# Actinins (cont'd)

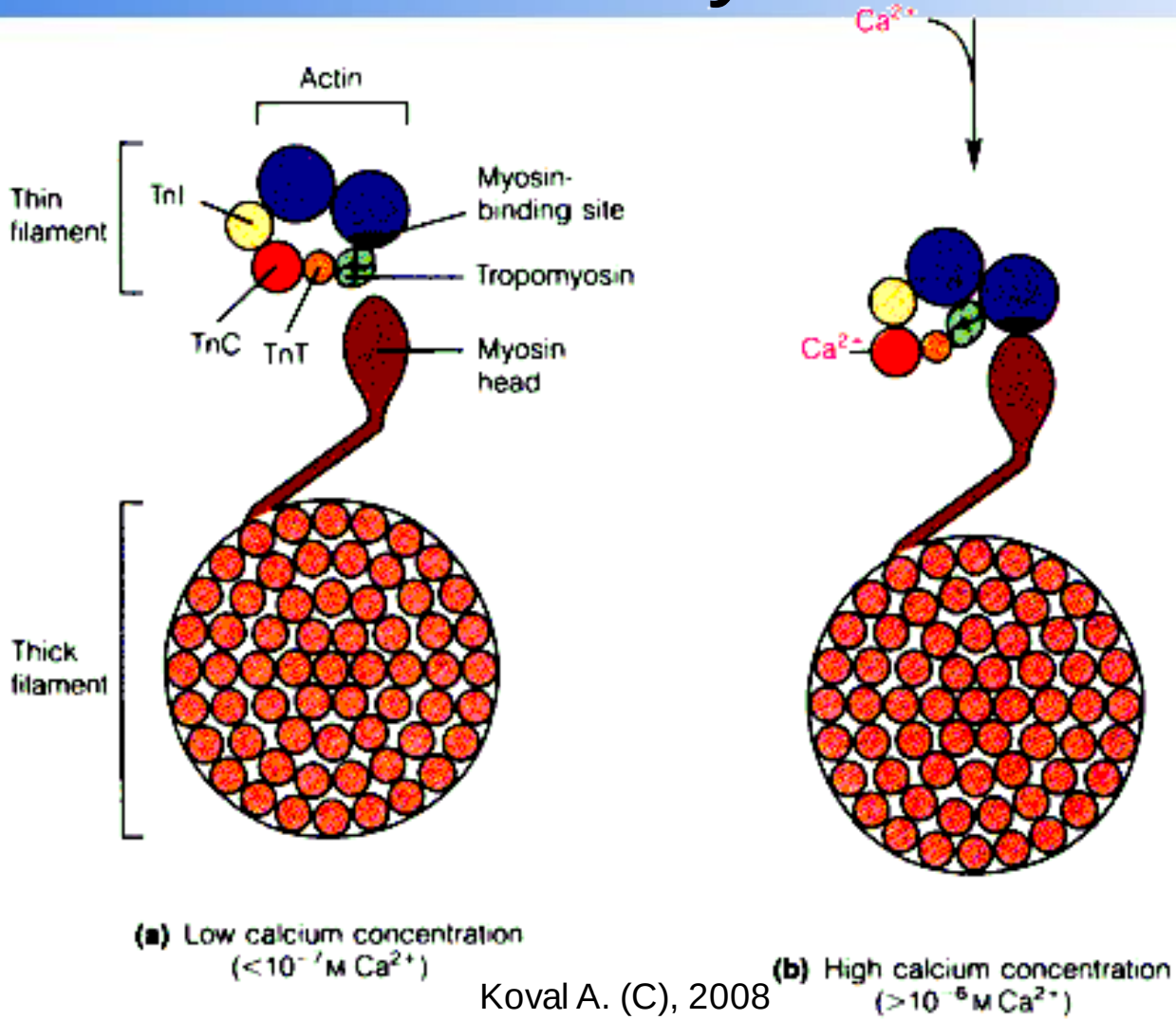



- α-Actinin exists as a homodimer of antiparallel subunits, illustrated here in terms of their primary structure.
  - The N-terminal, actin-binding domain and the C-terminal, EF-hand domains are separated by a central domain consisting of four repeats of 122-residue sequence.

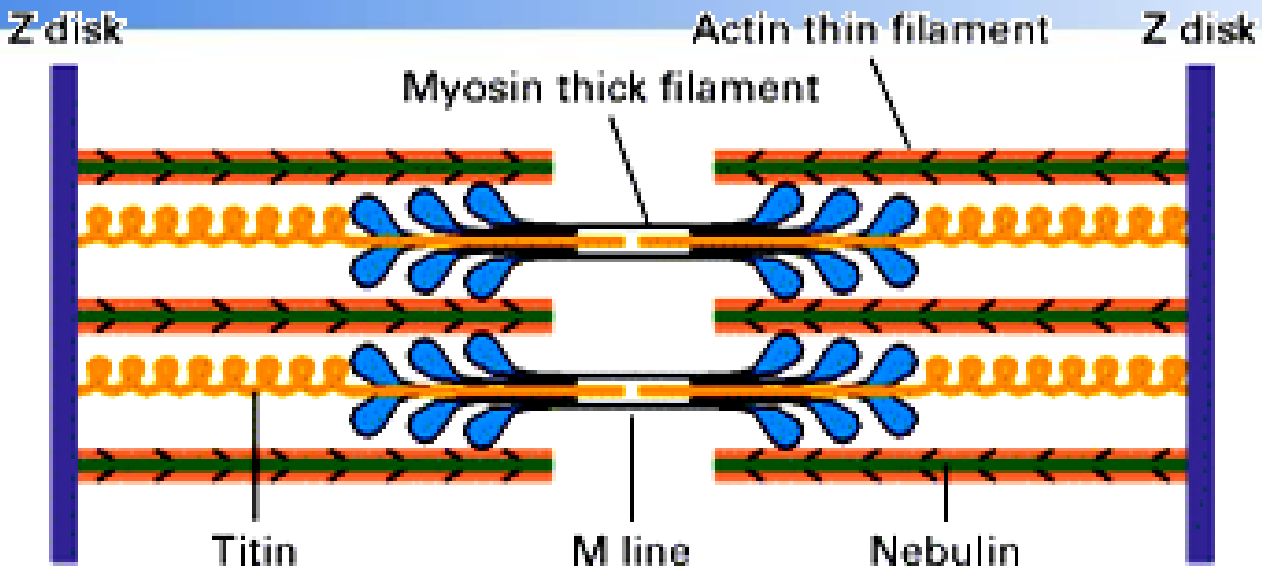
# Actinins (cont'd)

- The four central repeats in $\alpha$–actinin are highly homologous with the 106-residue repeat sequences of **spectrin,** the major structural protein of the red blood cell cytoskeleton.
  - The repeating segments of both $\alpha$-actinin and spectrin are thought to consist of bundles of four -helices.
- $\beta$-Actinin acts as an actin-capping protein, specifically binding to the end of an actin filament.
- $\gamma$-Actinin also inhibits actin polymerization, but its location in thin filaments is not known with certainty. Paratropomyosin is similar to tropomyosin, but appears to be located only at the A band–I band junction.

# The Regulation of Muscle Contraction by Calcium



# The titin-nebulin filament system

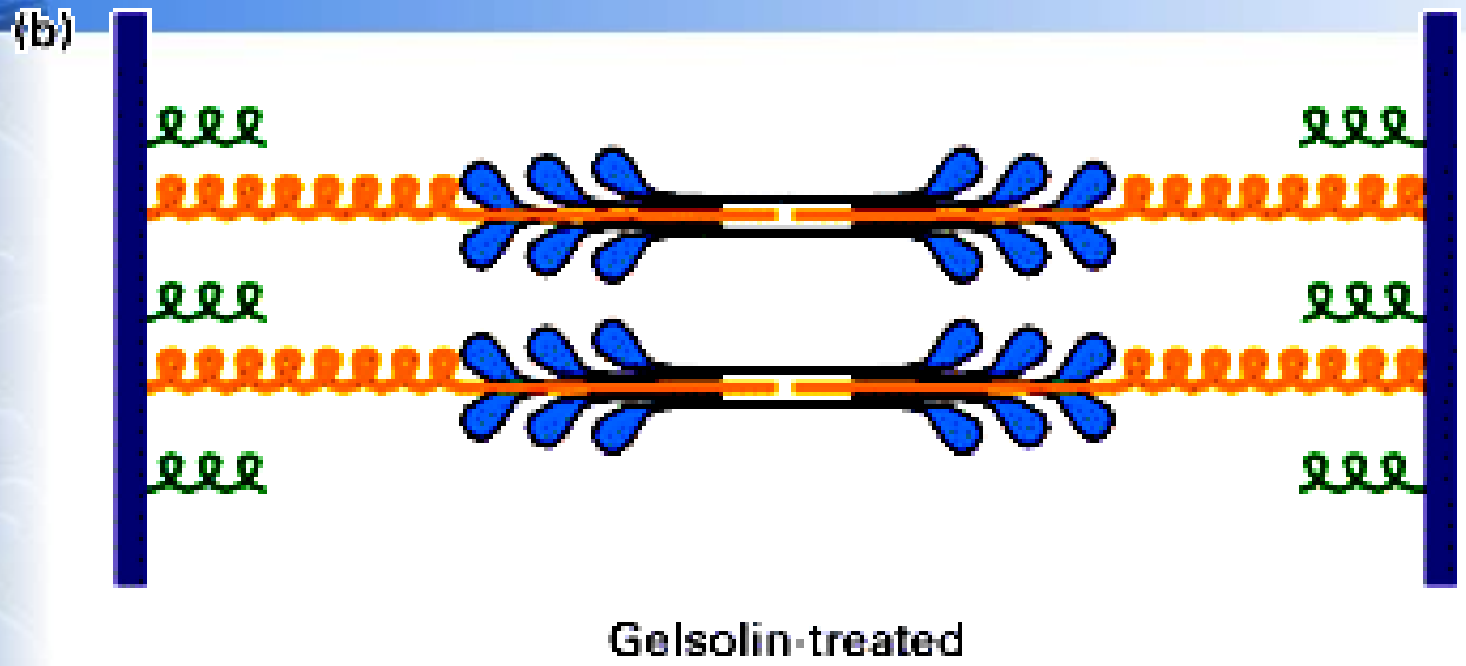



- A titin filament attaches at one end to the Z disk and spans the distance to the middle of the thick filament.
  - Thick filaments are thus connected at both ends to Z disks through titin.
- Nebulin is associated with a thin filament from its (+) end at the Z disk to its (-) end.

# The titin-nebulin filament system (cont'd)

- **Titin dynamics**
  - The large titin and nebulin filaments remain connected to thick and thin filaments during muscle contraction and generate a passive tension when muscle is stretched.

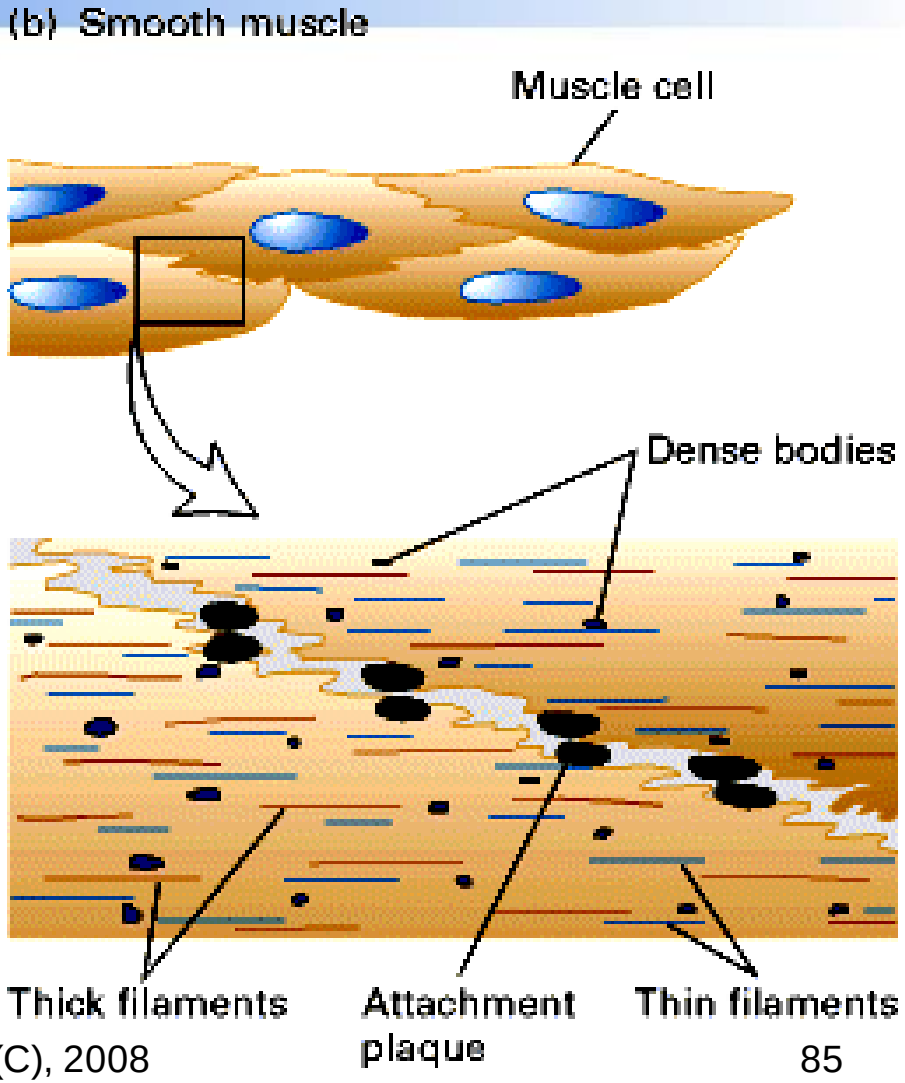
# Gelsolin-treated Sarcomere



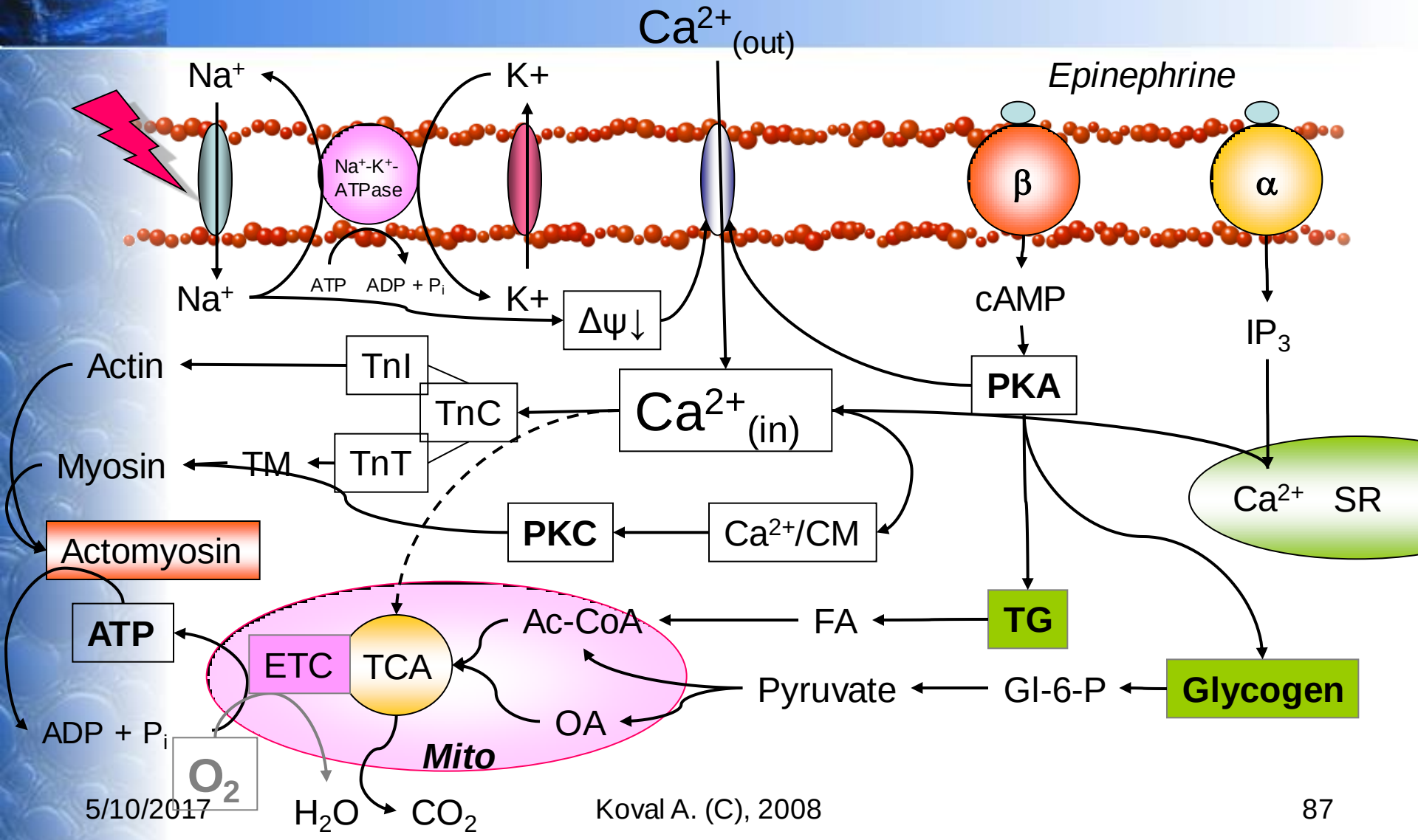

- To visualize the titin filaments in a sarcomere, muscle is treated with the actin-severing protein **gelsolin**, which removes the thin filaments.
- Without a supporting thin filament, nebulin condenses at the Z disk, leaving titin still attached to the Z disk and thick filament.

# General Structure of Smooth Muscle

- Smooth muscle is composed of loosely organized spindle-shaped cells that contain a single nucleus.
- Loose bundles of actin and myosin filaments pack the cytoplasm of smooth muscle cells.
- These bundles are connected to dense bodies in the cytosol and to the membrane at attachment plaques.



# Electromechanic Coupling



# $Ca^{2+}$ - Key Element of EMC

- $Ca^{2+}_{in}$ – $10^{-7}$ - $10^{-8}$ M.
- $Ca^{2+}_{out}$ – $10^{-3}$ M
- Calcium activates the number of enzymes:
  - TCA Dehydrogenases: Pyruvate DH, isocytrate DH, $\alpha$-ketoglutarate DH, (malate DH – in certain tissues);
  - TG lipases.
  - Calcium-dependent calmululine kinases (in smooth muscles)
- Calmoduline (CM) – is conservative protein. Binds 4 $Ca^{2+}$.

# Rigor complex formation

- **Rigor complex** is produced as a result of progressive oxygen deficiency $\Rightarrow$ ATP$\downarrow$ $\Rightarrow$ $Ca^{2+}\uparrow$ $\Rightarrow$ actin-myosine complex is not dissociated.
- After ≈2-3 h Rigor complex is destroyed (lysosomal proteases action) $\Rightarrow$ muscles again become soft.


Thank you
for your attention