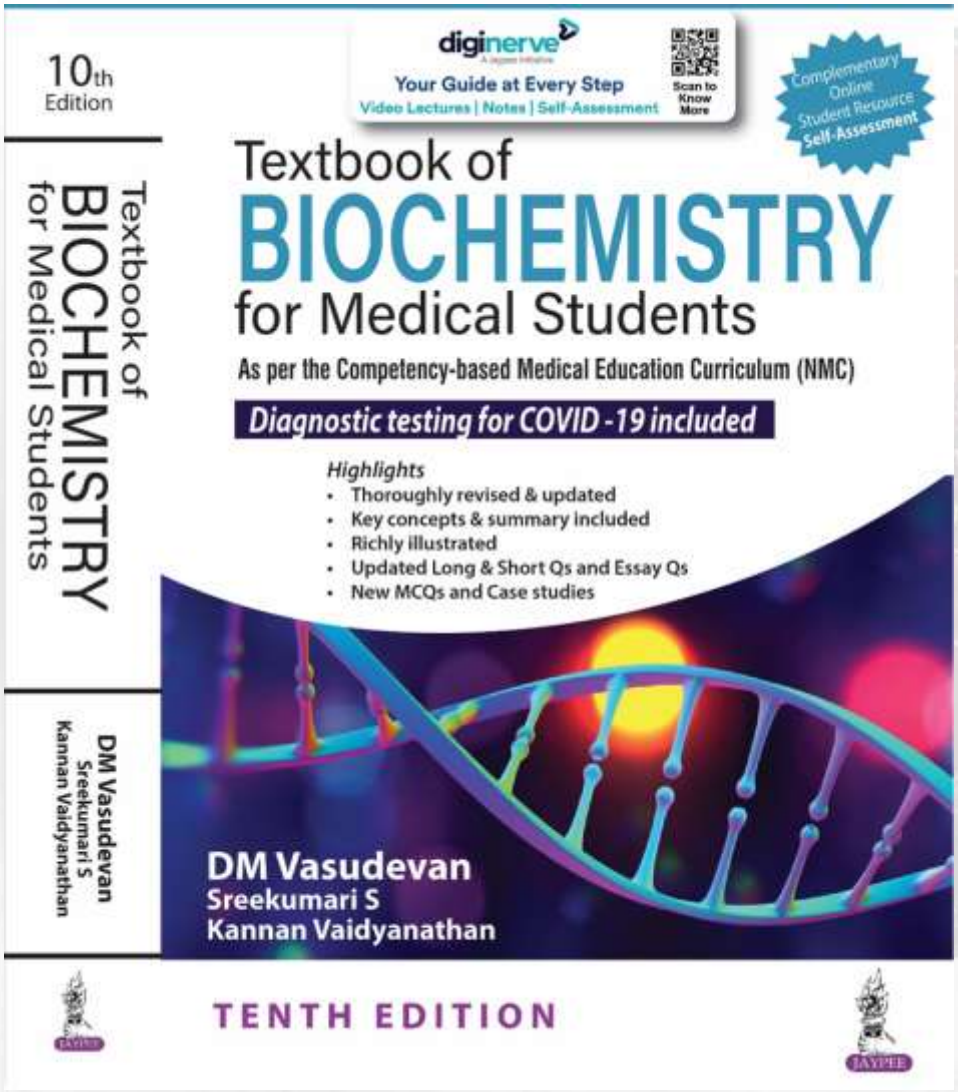
Chapter 43:

Extracellular matrix and Tissue proteins



Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

Extracellular Matrix

The **extracellular matrix** (ECM) constitutes the noncellular component of all tissues and organs. ECM actually plays an important part in the development, differentiation, and maintenance of the tissue and organ.

Components of the ECM

The major components are water, proteins, and polysaccharides. The cellular and protein microenvironment plays an important role in the interaction between the matrix and cellular components.

Functional Properties of the ECM

The two main components are proteoglycans and fibrous proteins. The biochemical and mechanical properties of each organ is dependent on its ECM composition. The posttranslational changes undergone by the ECM proteins are also significant in their functions.

Changes in ECM during Aging

A major change in aging is the stiffening of ECM. The junction proteins like cadherin, occludin, and catenin decrease with age and gaps occur at the junction between cells. The matrix metalloproteinase (MMP)-mediated degradation is enhanced, resulting in thinning of the basement membrane. As fibroblasts become senescent, they express more cytokines, MMPs, and plasminogen activator inhibitor (PAI). Along with the increased production of mitochondrial reactive oxygen species (ROS), a state of chronic inflammation occurs. The net effect is the destruction of the integrity of the elastin network and basement membrane. Inappropriate collagen cross-linking induced by the ROS, and enhanced glycation results in the stiffening of tissues. Aged tissue is less elastic and rigid, but mechanically weaker.

Changes in the ECM in Healing after Injury

The first response to injury is vascular damage and formation of fibrin clot causing monocytic infiltration to the damaged ECM. They differentiate into macrophages. The growth factors, MMPs, and cytokines secreted by the macrophages favour angiogenesis, fibroblast migration, and proliferation. These fibroblasts will lay down ECM proteins leading to transdifferentiation of fibroblasts to other types of cells like myofibroblasts, resulting in the deposition of ECM and synthesis of collagen bundles. The remodeled ECM will promote migration of cells to the wound site.

Collagen

The major structural protein found in connective tissue is the collagen. It is the most abundant protein in the body. About 25–30% of the total weight of protein in the body is collagen. It serves to hold together the cells in the tissues. It is the major fibrous element of tissues like bone, teeth, tendons, cartilage, and blood vessels.

Structure of Collagen

The **tropocollagen** is made up of three polypeptide chains. Depending on the amino acid variations, there are 19 types of collagens described. Type I is the most abundant form seen in the connective tissues in almost all regions of the body. Type II is mainly seen in cartilage and vitreous humor, Type III is in skin, lung, and vascular tissues and Type IV is seen in the basement membranes.

Each polypeptide chain of collagen has about 1,000 amino acid residues. Every third amino acid is the glycine. This repetitive amino acid sequence may be represented as Gly- X - Y- Gly - X - Y - ; where X and Y are other amino acids, most commonly proline and hydroxy proline. Moreover, 4-hydroxy proline, and 5-hydroxy lysine are found in fairly large proportions in collagen.

Synthesis of Collagen

The collagen is synthesized by fibroblasts intracellularly, as a large precursor, called procollagen. After the synthesis, it is then secreted outside the cell. The extracellular procollagen is cleaved by specific peptidases to form tropocollagen.

Post-translational Modifications

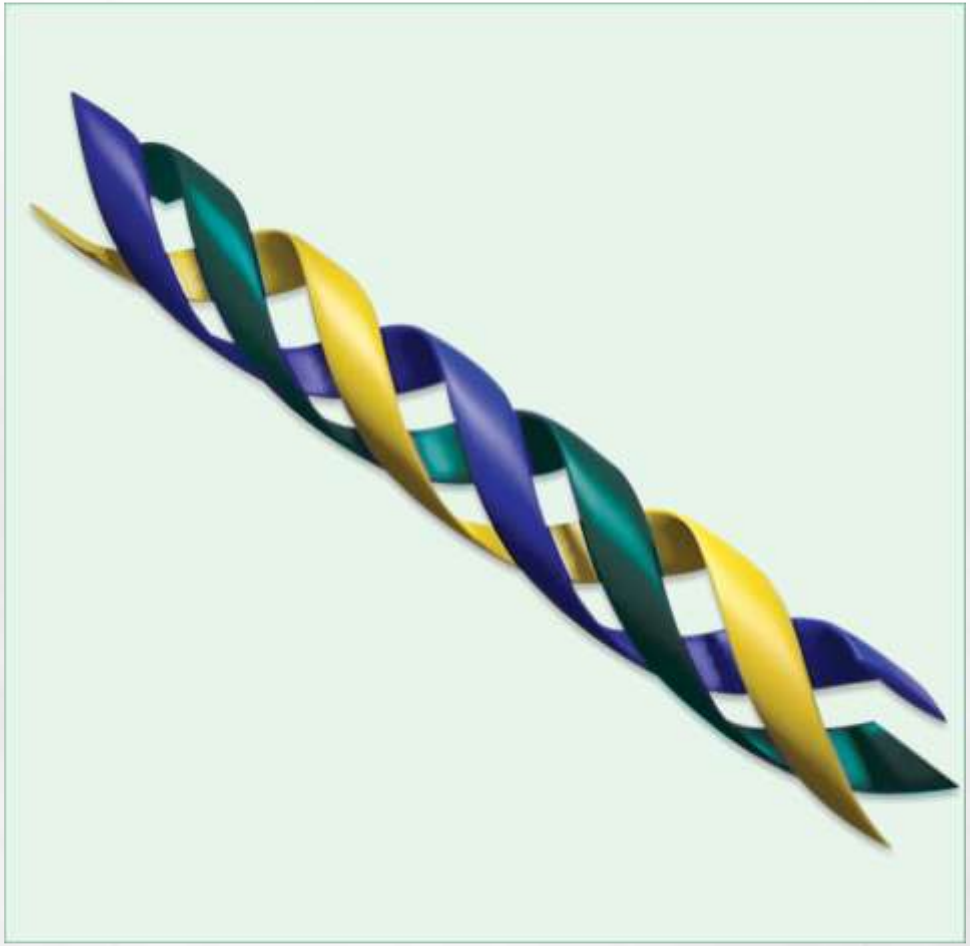
The **hydroxylation of proline and lysine** residues of collagen is a post translational modification taking place intracellularly. Prolyl hydroxylase and lysyl hydroxylase enzymes also contains ferrous iron at its active site and requires a reducing agent like **ascorbic acid** to preserve the iron in the reduced ferrous state. So, vitamin C deficiency leads to poor hydroxylation. It is the major biochemical defect in scurvy.

Extracellular Maturation of Collagen

Then the procollagen molecules are secreted. Outside the cell, procollagen is cleaved by specific peptidases. Finally, covalent cross-links are formed. Deficiency of the peptidase leads to **dermatopraxis**, where the skin is prone to be torn easily.

Triple-stranded Helix

The collagen is a rod-like structure. Each of the 3 polypeptide chains is held in a helical conformation by winding around each other. Thus a superhelical cable is made. The three strands are hydrogen bonded to each other. Glycine, because of its small size can fit into the crowded interior of the collagen triple helix.



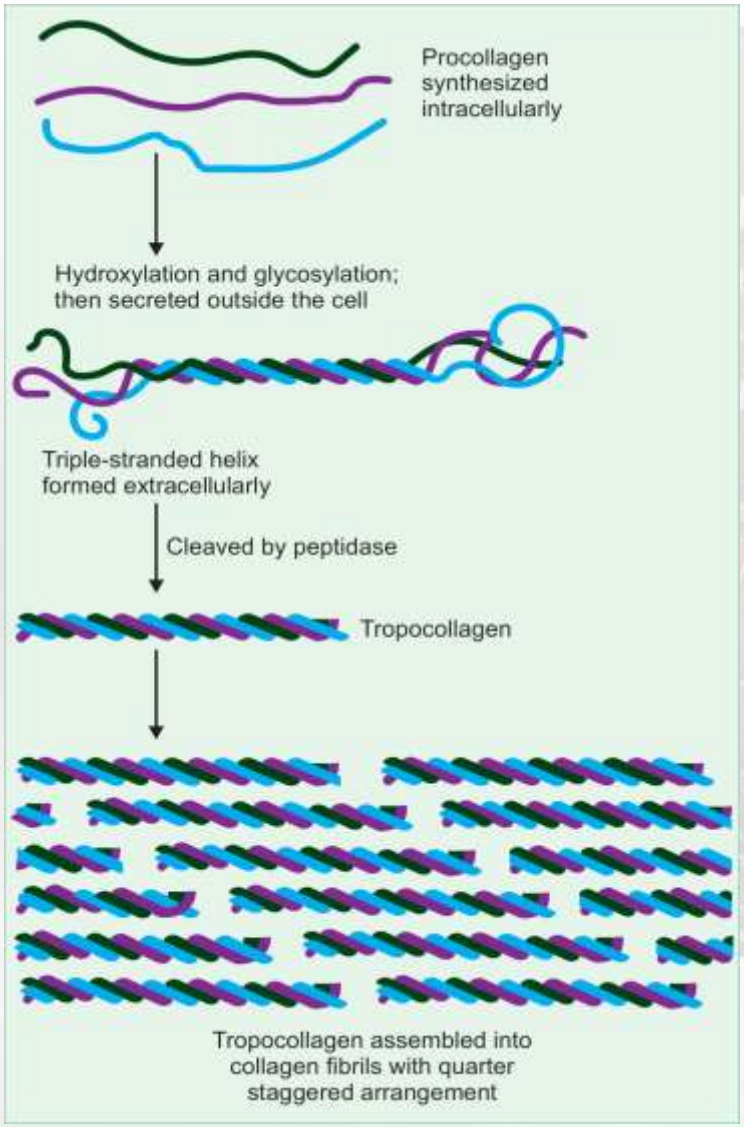
Triple-stranded collagen fiber.

Quarter Staggered Arrangement

The tropocollagen molecules are arranged in a "*quarter staggered array*" to form the collagen fibers. The structure repeats after each row. Thus, the collagen fiber has **triple-stranded, quarter staggered** arrangement. This arrangement helps in mineralization.

Cross-links in Collagen Fibers

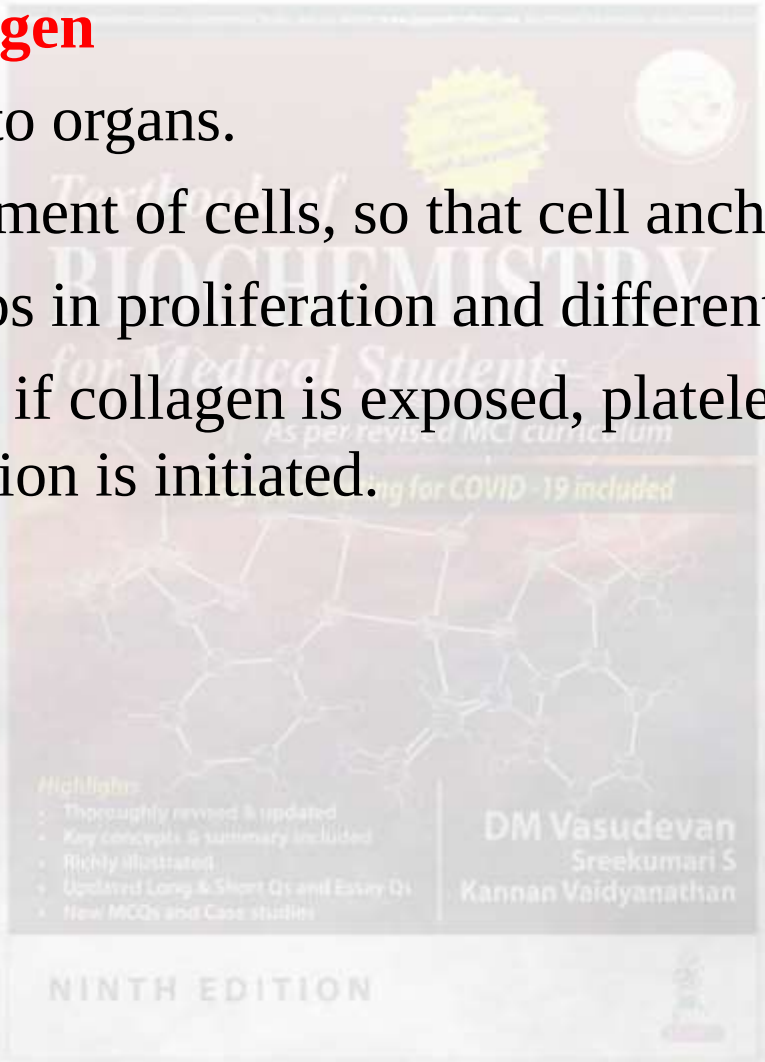
The collagen fibers are strengthened by covalent cross-links between lysine and hydroxy lysine residues. The cross-links are formed by **lysyl oxidase** which converts these amino acids into aldehydes. The aldehyde derivatives of lysine residues can form an aldol condensation. Such aldol crosslinks are formed near the amino terminal of the chains. These are called **aldol cross-links**. Lysyl oxidase is a copper containing enzyme, the copper ion being located at its active site. In **copper** deficiency, the cross-links of the collagen are reduced.



Maturation of procollagen into tropocollagen and assembly of collagen fibrils. Note the quarter staggered arrangement of collagen fibril. Each row moves one-fourth length over the last row; the 5th row repeats the position of the first row.

Functions of Collagen

- To give support to organs.
- To provide alignment of cells, so that cell anchoring is possible.
- This in turn, helps in proliferation and differentiation of cells.
- In blood vessels, if collagen is exposed, platelets adhere and thrombus formation is initiated.



Abnormalities in Collagen

Osteogenesis Imperfecta

It is inherited as a dominant trait. It is the result of a mutation which results in the replacement of a single glycine residue by cysteine in type I collagen. This change disrupts the triple helix near the carboxy terminus, hence the polypeptide becomes excessively glycosylated and hydroxylated. This results in **brittle bones** leading to multiple fractures and skeletal deformities.

Ehlers–Danlos Syndrome

The condition results from defective type III collagen formation due to defective lysyl oxidase or lysyl hydroxylase. It is characterized by weakening of collagen, loose skin, hypermobile, and lax joints.

Abnormalities in Collagen

Marfan Syndrome

It is inherited as autosomal dominant manner. Arachnodactyly (long digits), ectopia lentis (dislocation of lens), hyperextension of joints, and aortic aneurysm are the manifestations. This disease is produced by a defect in the gene, coding for a connective tissue protein, **fibrillin-1**. It is a component of microfibrils, which normally gives the substratum for deposition of elastin. So, fibrillin and elastin are deposited at lower concentrations.

Menkes Disease

Deficiency of copper results in defective function of lysyl oxidase, and reduced cross-linking of collagen results.

Deficiency of Ascorbic Acid

It is characterized by the defective hydroxylation of collagen. The collagen formed is weak, leading to fragility of blood vessels, poor wound healing, etc.

ELASTIN

Elastin is another ECF protein which provides the recoil to tissues that undergo repeated stretching (elasticity). They are found in the ligaments as well as in the walls of the blood vessels, especially large vessels like aorta. One-third of the residues are glycine. Proline is present in large amounts, so also alanine. Triple helix structure is absent. When elastin matures, **desmosine** cross-links are formed from 4 lysine residues. (Collagen has aldol crosslinks, while elastin has desmosine cross-links).

Pseudoxanthoma Elasticum

It is an inherited defect in the formation of elastin. Clinical manifestations are similar to Ehlers–Danlos syndrome.

Copper Deficiency

Copper deficiency blocks the formation of aldehydes, which are essential for cross-linking. Some lysine residues are oxidized by copper containing **lysyl oxidase.**

Muscle Proteins

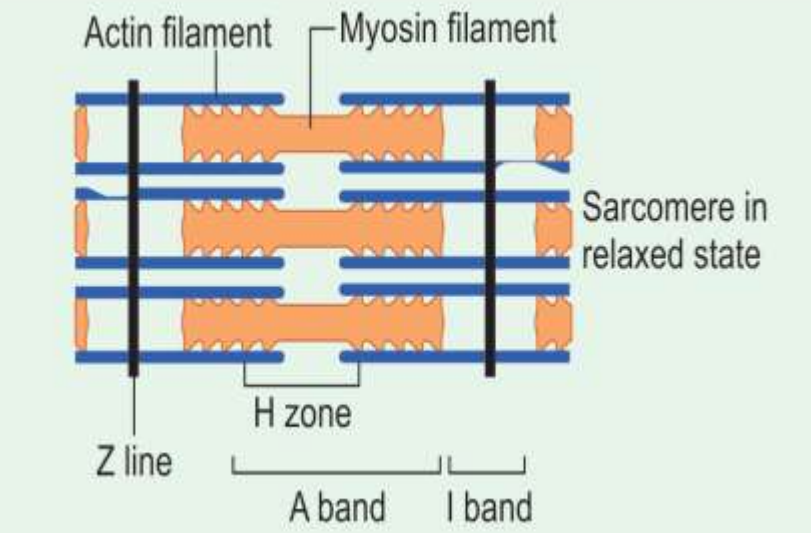
There are three types of muscle. The s**keletal muscle** or voluntary muscle is used for skeletal movement such as locomotion. **Smooth muscle** or involuntary muscle is found within the walls of organs and structures such as the esophagus, stomach, intestines, bronchi, blood vessels, etc. **Cardiac muscle** is found only within the heart.

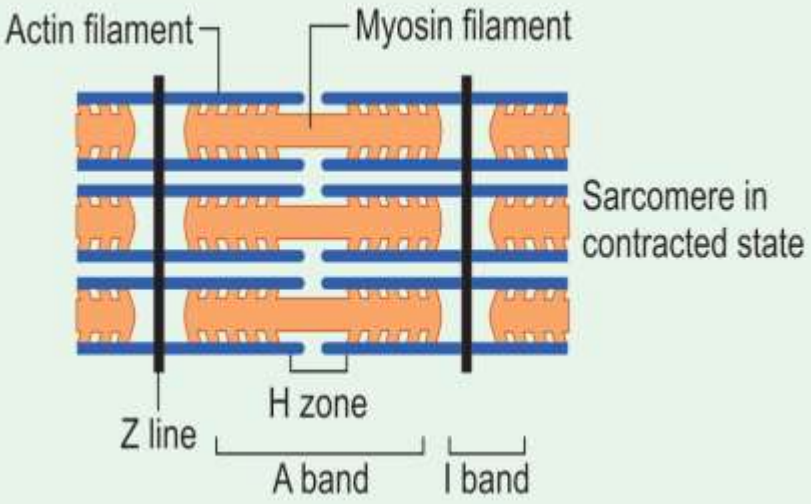
Striated muscle is made up of multinucleated cells bound by plasma membrane called **sarcolemma**. Each muscle cell contains myofibrils about 1 mm in diameter. The sarcoplasm of a muscle fiber houses unusually large amounts of glycosomes (granules of stored glycogen) and significant amounts of myoglobin, an oxygen-binding protein. The calcium concentration in sarcoplasma regulates the muscle contraction.

Muscle Proteins

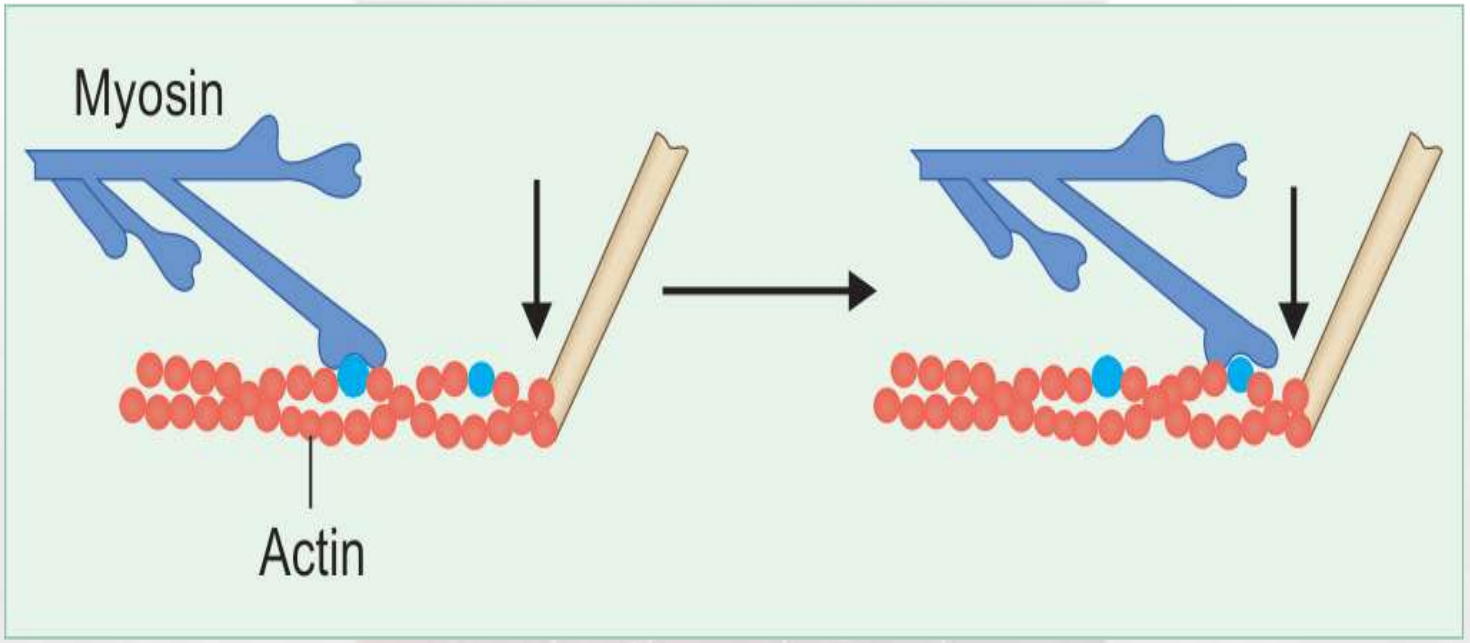
The functional unit of a myofibril is a **sarcomere**. The dark A bands and light I bands alternate regularly. The central H zone of A band is lighter, while the dark

M line is found in the middle of the H zone. The I band is bisected by a very dense narrow Z line. These bands are formed by variable combination of thick and thin filaments. The thick filaments have a diameter about 150 A whereas thin filaments have a diameter about 70 A. The thick filament is primarily **myosin** and thin filament contains **actin, tropomyosin,** and **troponin**. The Z line contains 2 actin molecules and M protein is located in the M line. The proteins of the thin and thick filaments can be separated into actin and myosin.





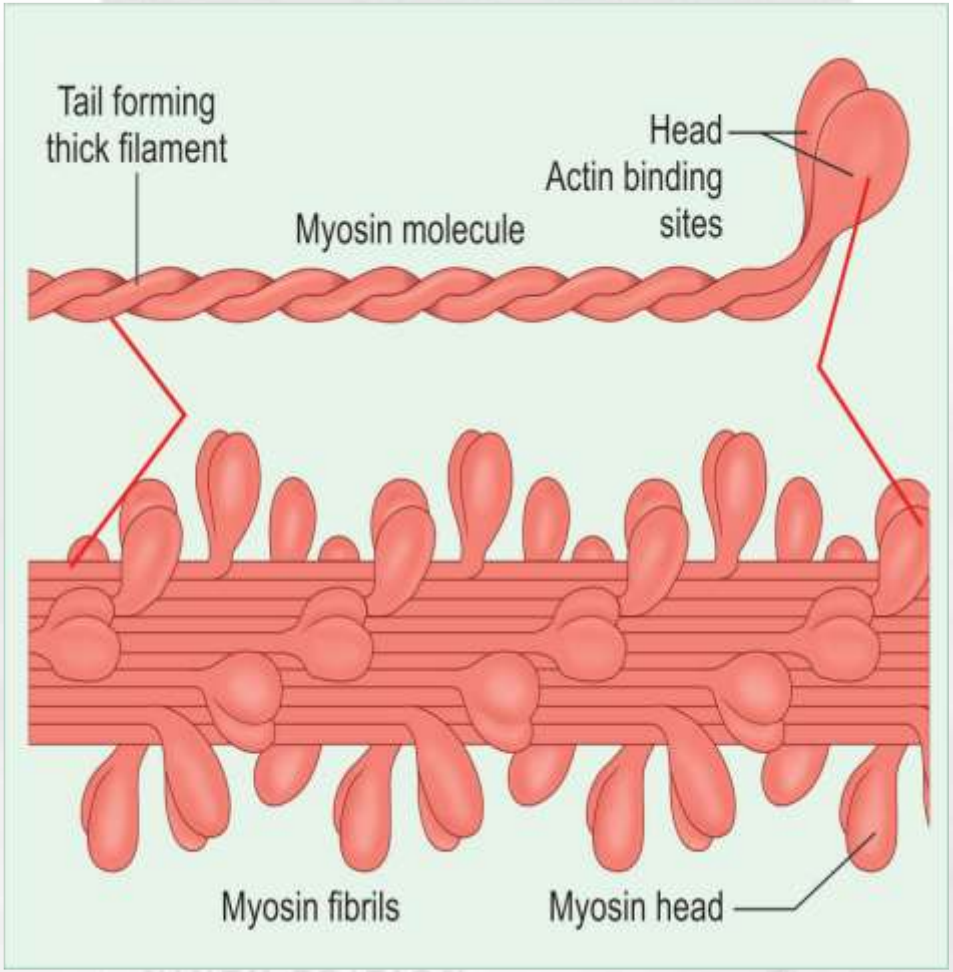
Sliding and shortening of actin and myosin is the basis of muscle contraction. Compare the distance of H zone in the upper picture (2,300 nm) and in the lower picture (1,500 nm).



During muscle contraction, myosin moves over actin filament.

Myosin

The thick filament consists mainly of myosin. (1) Myosin molecules assemble into filaments; (2) Myosin acts as the enzyme ATPase; (3) Myosin binds to actin polymer which is the major component of the thin filaments. The myosin molecules are large, each with 6 polypeptide chains; 2 identical heavy chains and 4 light chains. The myosin molecule has a double-headed globular end. At the head portion of each heavy chain, 2 light chains are bound. A thick filament is composed of approximately 400 myosin molecules, 200 arrayed on either side of the M line. The myosin molecules are most tightly packed in the regions represented by the light meromyosin (LMM) portion of the molecules.

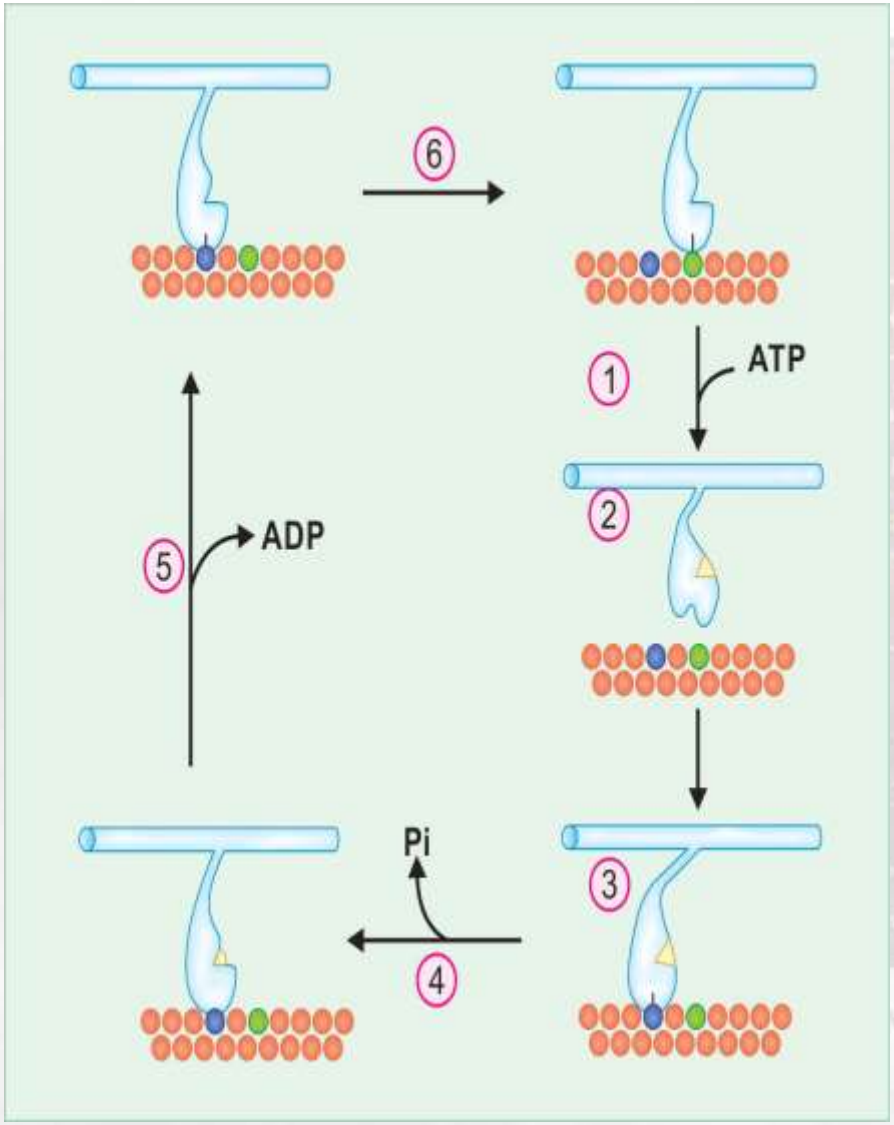


Heavy chain of myosin.

Actin

It is the major protein of the thin filaments. It can polymerize into a fibrous form, called F-actin, which is a helix of actin monomer. The muscle contraction results from the interaction of the actin and myosin, to form the actomyosin, with energy provided by ATP. When the two thin filaments that bind the cross bridges of a thick filament are drawn towards each other, the distance between Z lines becomes shorter. This results in contraction of muscle fibers. This needs energy from hydrolysis of ATP, effected by the ATPase activity of myosin.

The thin filament is made of actin, tropomyosin, and troponin. The contraction of skeletal muscle is triggered by nerve impulses which in turn release Ca2+. In the resting muscle, the Ca++ is within the sarcoplasmic reticulum. The nerve impulse releases Ca++ from the sarcoplasmic stores.



Cycle of events in muscle contraction.

1 = ATP binds.

2 = Myosin head dissociated from the actin filament.

3 = ATP is hydrolyzed by the myosin. ADP and Pi remain bound. Head pivots and binds with a new actin subunit.

4 = Pi is released, head pivots and moves the filament.

5 = ADP is released.

6 = Cycle repeats.

Muscle contraction-relaxation events

1. Nerve impulse releases acetylcholine (ACh) at motor end-plate. This ACh binds with the receptors.
2. Sodium-potassium conductance in neuromuscular end-plate, so that a potential is generated at the end-plate.
3. This is transmitted as the action potential in muscle fibers.
4. Depolarization; release of calcium ions from SR
5. Binding of calcium ions to TnC.
6. Cross-link formation between actin and myosin.
7. Sliding of thin filaments over thick filaments; muscle is contracted.
8. Calcium is pumped back into SR.
9. Release of calcium from troponin.
10. Actin and myosin are separated; muscle relaxed.

Troponins

The muscle contraction is modulated by troponin and tropomyosin through Ca++ which is the physiological regulator of muscle contraction. In the resting muscle, the Ca++ is within the sarcoplasmic reticulum. The nerve impulse releases Ca++ from the sarcoplasmic stores and increases its cytosolic concentration about 10 times (1–10 mM).

The troponin complex has 3 different polypeptide chains. Out of this, the **troponin-C** (TnC) binds calcium. The **troponin-I** (TnI), otherwise called "actomyosin-ATPase inhibitory element", binds to the actin and inhibits the binding of actin to myosin.

Troponin-T (TnT, 37kD) binds to the tropomyosin. Two isoforms of cardiac TnT, called TnT1 and TnT2 are present in adult human cardiac tissue. When Ca++ is released from the sarcoplasmic reticulum, a conformational change occurs.

Ca++ → Troponin → Tropomyosin → Actin → Myosin

Transduction of Chemical Energy to Mechanical Energy

The reservoir of high energy phosphate in skeletal muscleis **creatine phosphate**. The reaction (*Lohmann reaction*) is catalyzed by the **creatine kinase** (CK).

Creatine phosphate + ADP → ATP + Creatine

The resting muscle has a high concentration of the creatine phosphate (25 mM) when compared to ATP (4 mM). The creatine phosphate is therefore able to provide a high ATP concentration during the muscle contraction (In athletes, it is the major source of energy during the first 4 seconds of a short sprint). After that, the energy needs are met by the glycogen breakdown, glycolysis, TCA cycle, and oxidative phosphorylation.

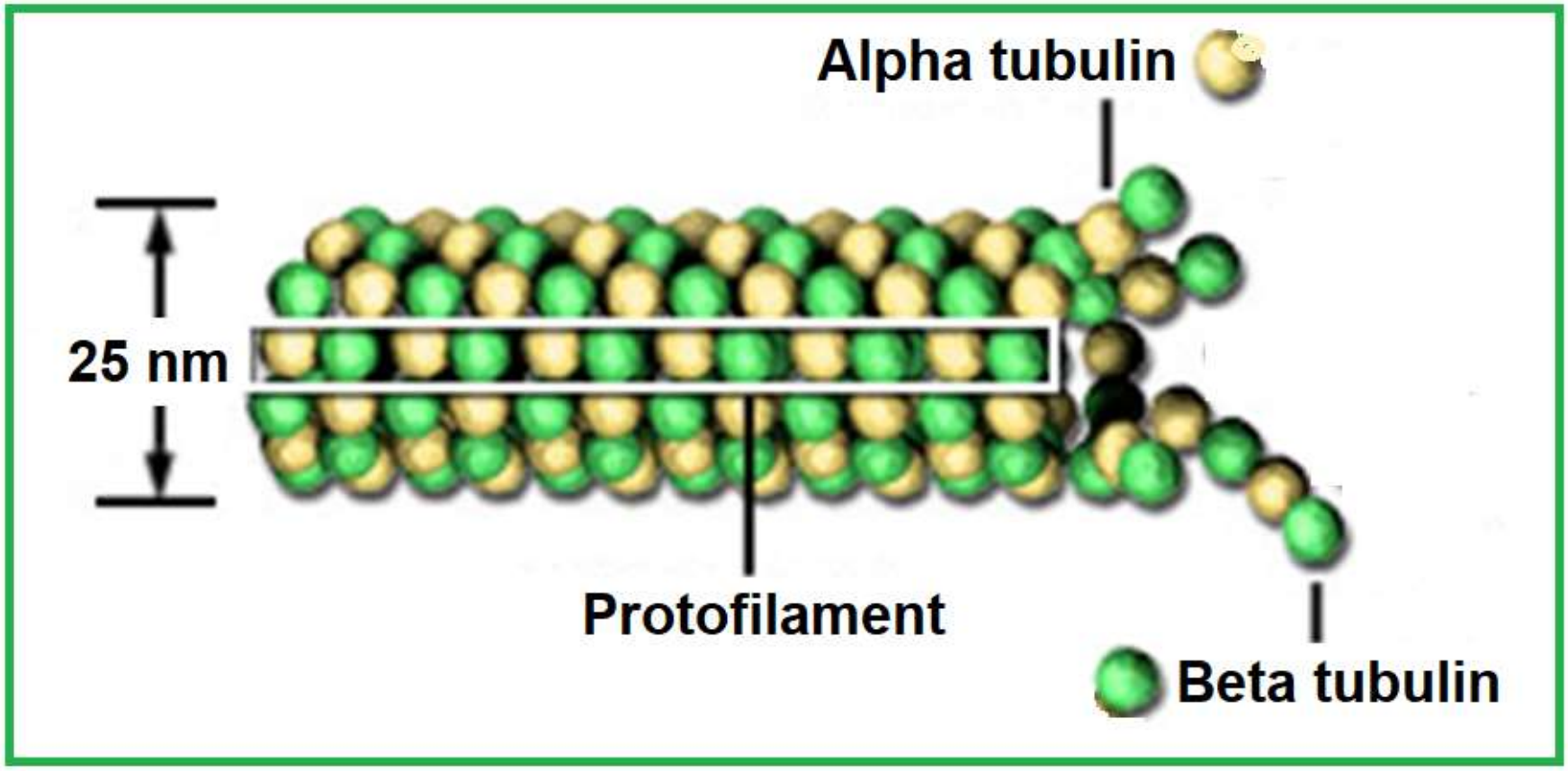
Biochemical Basis of Red and White Muscle Fibers

Muscles that depend predominantly on oxidative phosphorylation for ATP require abundant oxygen. To ensure its availability, these muscles store appreciable oxygen as oxymyoglobin. Oxidative, myoglobin-containing muscles are red in color because of their high myoglobin and mitochondrial content. Glycolytic muscles lack appreciable myoglobin and appear white. These muscles generally contain abundant stores of glycogen and generate most of their ATP from glycolytic reactions. A major functional difference between red and white muscle cells is that white fibers generate ATP from glucose by glycolytic pathway. On the other hand, in the red muscle, the pathway from substrate (glucose) to ATP is comprised by glycolysis plus TCA cycle plus electron transport chain. Consequently, fast-acting skeletal muscles are composed of predominantly glycolytic (white fibers) while slow-acting muscles maintain tone (red and oxidative).

Duchenne Muscular Dystrophy

It is a degenerative disease of muscle affecting only male children. The Duchenne muscular dystrophy (DMD) affects about one in 5,000 males at birth. It is the most common type of muscular dystrophy. It is transmitted as X-linked recessive manner. This gene produces a protein called **dystrophin** with 3,700 amino acids. It is one of the largest human genes known. Dystrophin is important to maintain the muscle cell membrane. Dystrophin connects the cytoskeleton of each muscle fiber to the underlying basal lamina (extracellular matrix). In DMD patients, the gene for dystrophin is mutated, and an altered protein is synthesized. The absence of dystrophin permits excess calcium to penetrate the cell membrane. Alteration in the calcium causes water to enter into the mitochondria, which then burst. Muscle weakness usually begins around the age of four in boys and worsens quickly. Females are spared from the disease, but are carriers. The presence or absence of the protein can be detected by using immunohistochemistry for dystrophin on muscle biopsy sample. Prenatal tests can tell whether the unborn child has the common mutations.

Microtubules



Microtubules

These are hollow cylinders about 25 nanometers in diameter, and form part of the **cytoskeleton** that gives structure and shape to a cell. They are composed of alpha and beta **tubulins**. The cytoplasmic microtubules are composed of **tubulin** (55 kD) and several accessory proteins called MAPs (**microtubular associated proteins**). In a microtubule, the subunits are organized in such a way that they all point in the same direction to form 13 parallel **protofilaments**.

The functions include formation of the mitotic spindle, and movement of the secretory granules (exocytosis or endocytosis). Microtubules help in the movements of cilia and flagella. The microtubule network interconnects the Golgi apparatus with the plasma membrane to guide secretory vesicles for export.

Lens Proteins

- Cataract is the cause for 70% of blindness. The eyes of older people and diabetics are prone to cataract formation.
- Being avascular, lens relies on the aqueous humor for the provision of oxygen and essential metabolites. The normal lens cells (but not the old cells) possess the usual protein synthesizing machinery.
- The proteolytic activity of the lens is quite low and is due to the presence of endogenous protease inhibitors.
- Lens tissue has a very active hexose monophosphate (HMP) shunt pathway and has the maximum concentration of NADPH.
- Lens also contains high quantity of ascorbic acid. They scavenge the free radicals and maintain the transparency of lens.

Crystallins

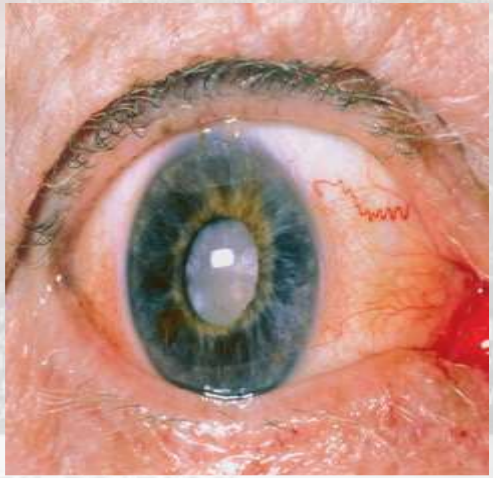
Major lens proteins are alpha, beta, and gamma crystallins. They undergo no replacement throughout the life of the individual. There is no turnover of these proteins. The orderly arrangements of the molecules make the lens proteins transparent.

Cataract

When the lens proteins change in their three dimensional structure, lens becomes opaque. In diabetes mellitus, when the blood glucose level is increased, lysine residues of these proteins are glycated. This leads to increased susceptibility for sulfhydryl oxidation and consequent aggregation of the proteins, resulting in opalescence and cataract. Protein aggregates with molecular weight more than 50 million will produce scattering of light, so that the light is not passed correctly to the retina.

Cataract

In the lens, the enzyme **aldose reductase** reduces monosaccharides to corresponding sugar alcohols; glucose to sorbitol and galactose to galactitol. These polyols do not readily cross cell membranes and hence accumulate; causing osmotic swelling, and consequent disruption of cell architecture. Thus, **diabetes mellitus** (increased glucose in blood) and galactosemia (high galactose level) cause cataract.



In cataract, the lens becomes opaque.

Demyelinating Diseases

Demyelinating disorders of the Central Nervous System	Demyelinating disorders of the Peripheral Nervous System
• Multiple sclerosis • Neuropathies (Vitamin B12 deficiency) • Central pontine myelin lysis • Tabes dorsalis (syphilitic myelopathy) • Progressive multifocal leukoencephalopathies • Leukodystrophies	• Guillain-Barre syndrome • Chronic inflammatory demyelinating polyneuropathy • Charcot-Marie disease • Copper deficiency • Progressive inflammatory neuropathy

Multiple Sclerosis (MS)

MS is a disease that results from demyelination and inflammation along axons. The lesions are scattered in the white matter, but often cluster near the ventricles. They are demyelinating plaques variable in size. Lesions of the optic nerves are common, because the optic nerve is an extension of the CNS.

In relapsing-remitting multiple sclerosis (RRMS), symptoms typically develop over days or weeks, with subsequent stabilization and gradual improvement over weeks to months. Then the patient may go for a relapse; and this cycle repeats. Each cycle will cause additional damage to nerve conduction. The course of the disease is unpredictable; the average survival after diagnosis is 15 to 20 years.

Multiple Sclerosis (MS)

There may be lesions of the optic nerve (blindness, optic atrophy), lesions of corticospinal tracts (motor paralysis, spastic hemiparesis), lesions of dorsal column sensory pathways (paresthesias) or lesions in cerebellar connections (ataxia and dysarthria) or lesions in the vestibular system (vertigo and nystagmus). Patients usually have intact intellect at the beginning, but may result in dementia late in the course.

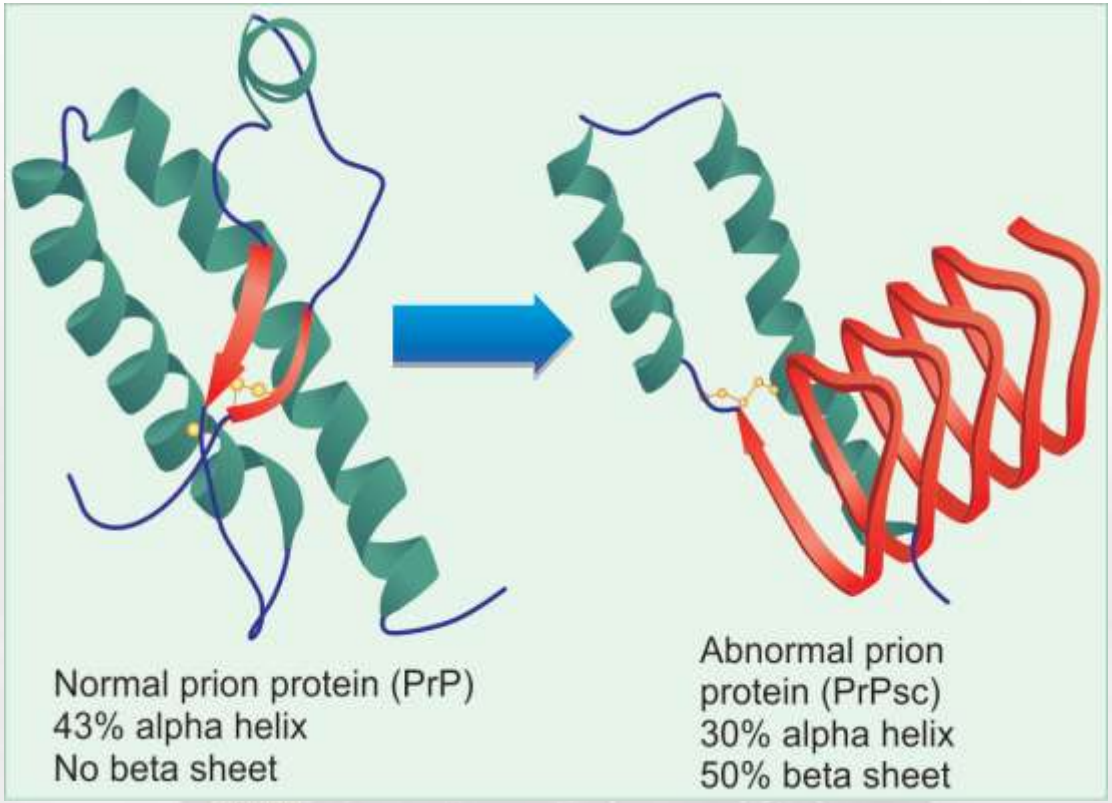
Laboratory tests include antinuclear antibodies (ANA) and tests for syphilis (RPR, VDRL, etc.) to rule out tertiary syphilis. Magnetic resonance imaging (MRI) will identify the multifocal white matter disease. A spinal fluid examination may show elevation of mononuclear white blood cells, and CSF oligoclonal IgG bands and increased globulin to albumin ratio.

PRIONS

There are a few diseases characterized by very long incubation period of many years. These “slow disease agents” are proved as prion proteins (PrP).

Prion Proteins

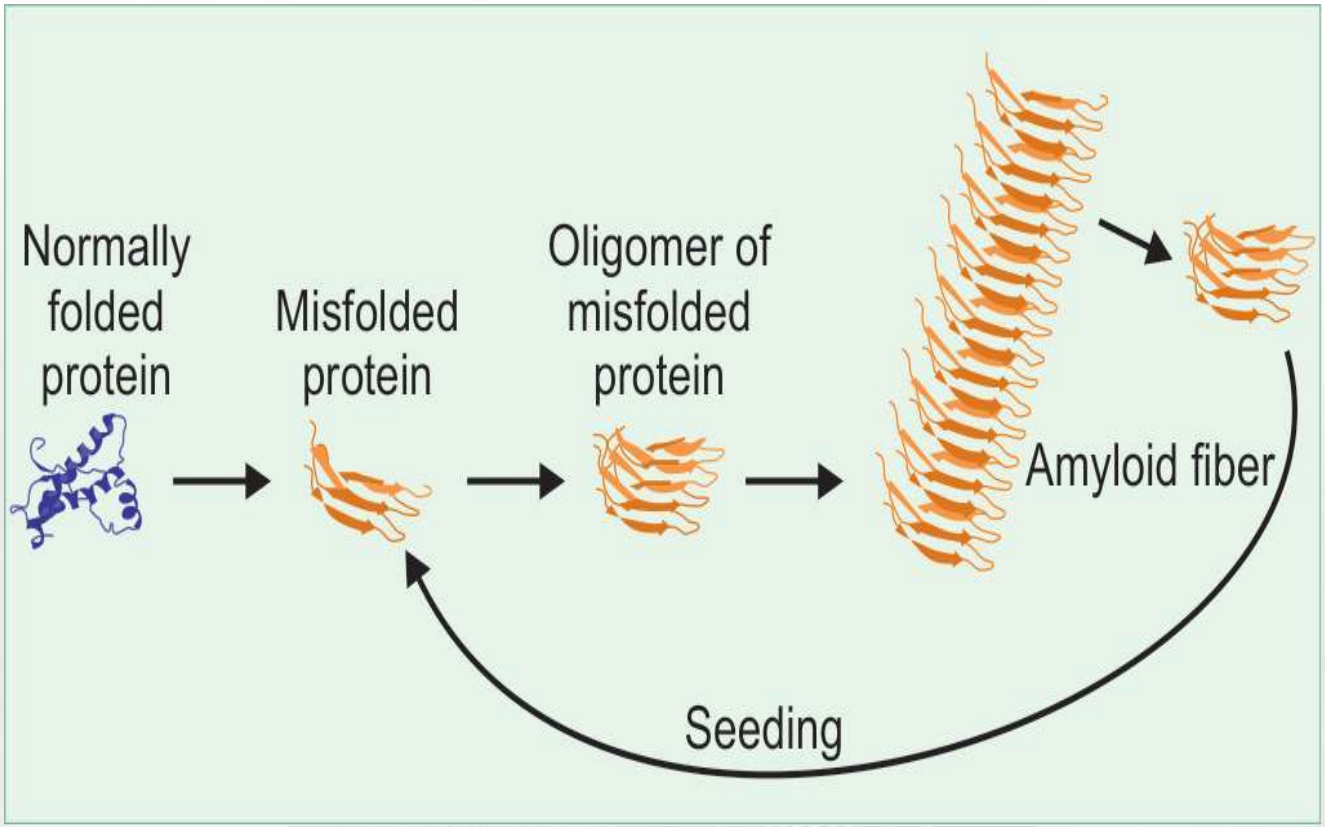
“Prions” is the acronym for “proteinaceous infective particles”. The PrP is a normal protein of 253 amino acids, found in leukocytes and nerve cells. The PrP molecules can undergo a change in structural conformation. The altered molecule is resistant to heat and proteolytic enzymes. The abnormal protein is called **PrPsc;** “sc” stands for scrapie, the disease in which it was first isolated. Thus, **prions are proteins with correct primary structure, but with abnormal tertiary structure**. The PrP is in alpha helical form; but PrPsc is in beta-pleated sheets.



Prions have correct primary structure, but have altered tertiary structure.

Abnormal Proteins can be Infectious

The "**seeding model**" explains that the infectious prion induces the nearby normal protein molecules to misfold to the abnormal form. These abnormal proteins now convert further normal proteins into the abnormal varieties, producing a "chain reaction" that generates new infectious materials.



Seeding model. Infectious protein misfolds further proteins.

Pathogenesis of Prion Diseases

The lysosomal enzymes could break down the normal PrP; but the abnormal PrPsc cannot be digested. Hence, the prions are accumulated inside the cells, and eventually the cell dies. One part of PrP can cause apoptosis (programmed cell death), which also leads to the loss of cells. As a group, they are also called **transmissible spongiform encephalopathies (TSF)**, because the brain becomes riddled with small holes like a sponge. Neurons degenerate, protein deposits may accumulate as plaques and glial cells grow larger. Clinically, rapidly progressive dementia sets in with neurological defects and ataxia. All the prion diseases are slowly progressive, but eventually become fatal.

Human Prion Diseases

Prion diseases in human beings are **Kuru**, Creutzfeldt-Jakob disease (**CJD**), Gerstmann-Straussler-Scheinker disease (**GSSD**) (familial CJD), and fatal familial insomnia (**FFI**). Cerebral cortex becomes sponge-like in CJD. Thalamus is affected in FFI. In Kuru, cerebellum is affected.

Prion Diseases in Animals

Classical example of the "slow disease" is **scrapie** disease in sheep. It is characterized by constant scratching; hence the name. The disease is manifested only about 10 years after the entry of the agent. In **Bovine spongiform encephalopathy (BSE)** (mad cow disease) the brainstem is affected. The cattle feed containing the infected meat from sheep suffering from Scrapie caused the entry of prions into millions of cows. Thus, it is obvious that prions had crossed the species barrier from sheep to cattle.

Alzheimer's Disease

About 5–10% of people above 60 years are affected by the Alzheimer disease (AD). It is characterized by slow progression of memory loss, confusion, dementia, hallucinations, personality changes, and finally patient enters into a vegetative state with no comprehension of the outside world. The patient may require round the clock care and protection. Death occurs about 10 years after the first onset of the symptoms. Omega-3 fatty acids and antioxidants may be helpful in the prevention of the disease.

Molecular Defects in AD

The pathological hallmarks of AD are neurofibril tangles in CNS, senile neuritic plaques, and cerebral amyloid deposition. The **neurofibrillary tangles** are paired helical filaments made up of **Tau protein**. Normal Tau is soluble and catabolized easily; but abnormal Tau is insoluble, cannot be degraded by the tissue cathepsins and are deposited around the neurons.

Amyloid Precursor Protein

Neuritic plaques are composed of **beta-amyloid precursor protein (APP)**; a 40 amino acid fragment derived from the APP (amyloid precursor protein). The APP is a normal constituent of human serum. A mutation in APP gene leads to production of an abnormal protein. Then APP then cannot be cleaved at appropriate position. A wrong cleavage produces beta-APP, which is precipitated around neurons as beta-amyloid. This is similar to prion action.

Familial AD and Apo-E4 gene

About 30% cases of AD have genetic background. Genes identified with AD are that coding for APP, presenilin-1, presenilin-2, AD3 or AD4. Another major susceptibility gene for AD is the **Apo-E4** (apolipoprotein E4) gene. The presence of Apo-E4 gene is the major risk factor for the AD. The **Apo-E2** gene reduces the risk of the AD.

Parkinsonism

Parkinsonism is a degenerative disease, affecting the muscular coordination. Two genes associated with Parkinson's disease have been reported. The first, called alpha-**synuclein**, is mutated in Parkinson's disease. The second, codes for a protein called **parkin**, which is associated with a juvenile form of Parkinson's disease.

Protein Misfolding Diseases

These are associated with the deposition of normally soluble proteins which become insoluble as the amyloid deposits. These illnesses include different forms of familial amyloidosis. A common feature in the altered conformation of the protein is the change from alpha helical to beta sheet structure, which makes the proteins resistant to normal proteolysis. The cause may be mutations, defects in chaperones or presence of inappropriate proteins. The misfolded proteins recruit more proteins into abnormal shape, to form plaques.

Biochemistry of Aging

Theories of Ageing

Mutation theory:

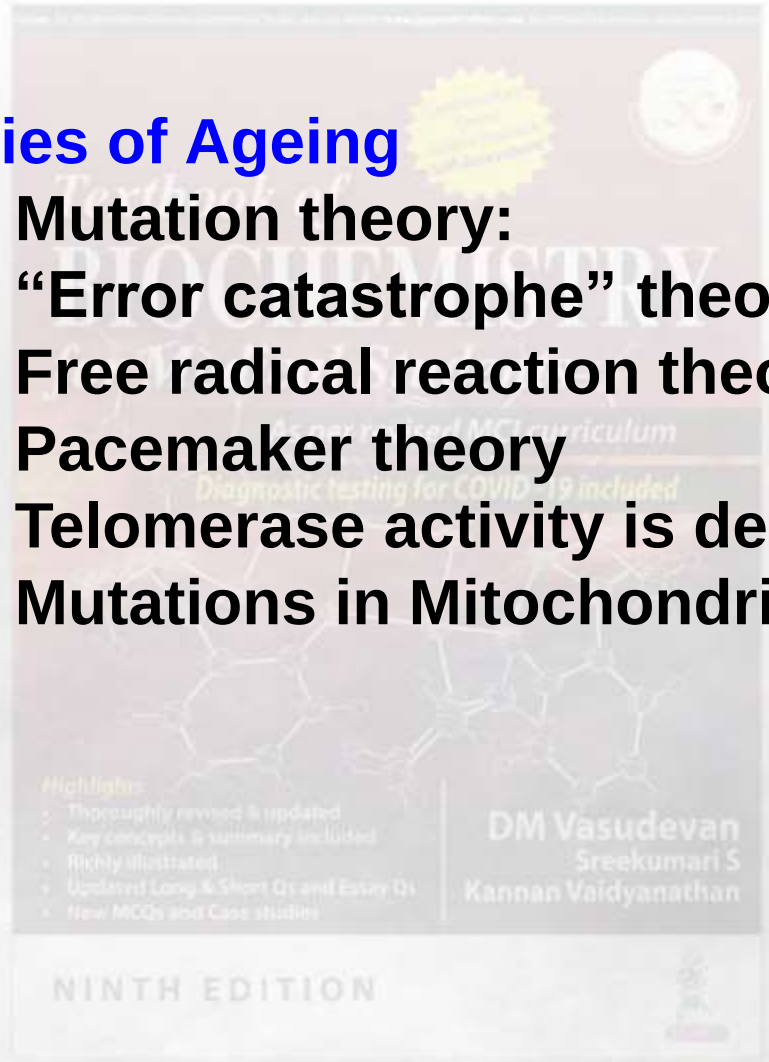
"Error catastrophe" theory.

Free radical reaction theory.

Pacemaker theory

Telomerase activity is decreased

Mutations in Mitochondrial DNA



Biochemistry of Aging

Change during ageing	Clinical manifestation
Sarcopenia	Loss of muscle mass
Osteopenia	osteoporosis
oxidative stress	Accumulation of age pigment, lipid peroxidation
Decreased antioxidant status	Type 2 diabetes mellitus, Coronary artery disease
Increased insulin resistance	Diabetes mellitus
Glycation of proteins	Accumulation of AGE in tissues
Loss of subcutaneous fat	wrinkles
Loss of elasticity of collagen	stiffening of joints and tissues