SKELETAL MUSCLE FIBERS

A skeletal muscle fiber is surrounded by a plasma membrane called the sarcolemma, which contains sarcoplasm, the cytoplasm of muscle cells. A muscle fiber is composed of many fibrils, which give the cell its striated appearance.

EXCITATION-CONTRACTION COUPLING

Motor End-Plate and Innervation. At the NMJ, the axon terminal releases ACh. The motor end-plate is the location of the ACh-receptors in the muscle fiber sarcolemma. When ACh molecules are released, they diffuse across a minute space called the synaptic cleft and bind to the receptors.

Skeletal Muscle

The Three Connective Tissue Layers. Bundles of muscle fibers, called fascicles, are covered by the perimysium. Muscle fibers are covered by the endomysium.

THE SARCOMERE

The sarcomere, the region from one Z-line to the next Z-line, is the functional unit of a skeletal muscle fiber.

Muscle Fiber Contraction

A cross-bridge forms between actin and the myosin heads triggering contraction. As long as Ca²⁺ ions remain in the sarcoplasm to bind to troponin, and as long as ATP is available, the muscle fiber will continue to shorten.
Muscle Fiber Relaxation

Ca++ ions are pumped back into the SR, which causes the troponyin to reposition the binding sites on the actin strands. A muscle may also stop contracting when it runs out of ATP and becomes fatigued.

The conversion of muscle into meat

After slaughter, the muscle undergoes various biophysical changes and events that convert it into meat. This process can be divided into three phases:

1. Pre-rigor phase during which collagen content mainly contributes to the toughness
2. Rigor phase during which further toughening occurs due to muscle shortening
3. Tenderization phase or resolution of rigor during aging during which the muscles undergo a series of changes and observe a remarkable improvement in tenderness

ATP AND MUSCLE CONTRACTION

Sequelae of Muscle Contraction: (a) The active site on actin is exposed as calcium binds to troponin. (b) The myosin head is attracted to actin, and myosin binds actin at its actin-binding site. (c) During the power stroke, the phosphate generated in the previous contraction cycle is released. This results in the myosin head pivoting toward the center of the sarcomere, after which the phosphate is released. (d) A new molecule of ATP attaches to the myosin head, causing the cross-bridge to detach. (e) The myosin head hydrolyzes ATP to ADP and phosphate, which returns the myosin to its cocked position.

Muscle Extensibility and Rigor Mortis

Start

Onset

Completion

CP = creatine phosphate, an immediate reservoir for replenishing ATP
SR = sarcoplasmic reticulum, a storehouse in muscle that binds calcium (muscle relaxes) and releases calcium (muscle contracts)

Muscle Activation in Contraction

When a sarcomere contracts, the Z lines move closer together, and the I band becomes smaller. A band stays the same width. At full contraction, the thin and thick filaments overlap.

SOURCES OF ATP IN MUSCLE

Muscle Metabolism: (a) Some ATP is stored in a resting muscle. As contraction starts, it is used up in seconds. More ATP is generated from creatine phosphate, which is also decreased. ATP and ADP are then converted to lactic acid if oxygen supply is limited. (b) Aerobic respiration is the conversion of glucose and oxygen to carbon dioxide, water, and ATP. Approximately 95% of the ATP required for resting or moderately active muscles is provided by aerobic respiration, which takes place in mitochondria.

The sliding filament model of contraction

When a sarcomere contracts, the Z lines move closer together, and the I band becomes smaller. A band stays the same width. At full contraction, the thin and thick filaments overlap.
meat tenderness

• Tenderness of meat is the most important quality distinguishing feature of meat in consumer evaluation.
• Various pre-slaughter and post-slaughter factors and their mutual effects influence tenderness of meat.
• The most important pre-slaughter factors include:
  • Post-slaughter transformations, including:
    - rigor mortis
    - aging

Aging

• It was observed that in the ageing process one can distinguish changes in the:

  1. ultrastructure of muscle fibers:
    - weakening of myofibrils
    - fragmentation
  2. changes in the area of the Z-line and the I-band
  3. degradation of myofibrillar and cytoskeletal proteins:
    These changes lead to obtaining the final meat tenderness.

Factors affecting the tenderness during aging

Post-mortem aging results in optimum improvements in the tenderness of meat; however, it does not ensure uniformity in the tenderness as it is influenced by several genetic and environmental factors.

1. Temperature
2. Time of aging
3. pH
4. Fiber-type composition
5. Sarcomere length
6. Proteolysis

Post-mortem proteolysis and candidate proteolytic systems

proteolytic systems present in a muscle which can participate in the postmortem proteolysis and tenderization:

• calpains
• cathepsins
• Proteasomes
• The caspase system

Major intracellular proteolytic systems

Lysosome system

Cathepsins are a group of enzymes comprised of both exo- and endo-peptidases.

categorized into peptidase families:
- cysteine (cathepsins B, H, L and X),
- aspartic (cathepsins D and E)
- serine (cathepsins G)

The autophagy-lysosome system primarily degrades non-specific cell components, including proteins and microorganisms, contained by isolation membranes.

The four digestive processes mediated by the lysosome:
Major intracellular proteolytic systems

**Proteasomes**
- The proteasome is a multisubunit protease complex.
- Proteasomes are ubiquitously expressed in the body and are abundant in skeletal muscle.
- The proteasome (26S) consists of:
  - 25S regulatory subunits
  - 19S multicatalytic structure
- The 20S proteasome, also known as the multisubunit proteolytic enzyme complex (MPE).
- Proteolysis by the proteasome is an ubiquitin-dependent process.
- At least four ubiquitin proteins must attach to the lysine residue of the target substrate.
- The polyubiquitinated proteins are subsequently recognized by the proteasome, which removes the ubiquitin chain and degrades the substrate.

**The ubiquitin–proteasome proteolytic system**
Ubiquitin is attached by the ubiquitin activating enzyme, E1, followed by the transfer to a ubiquitin carrier, E2, and finally to the catalytic proteolysis enzyme, E3. The proteasome contains a regulatory and a catalytic subunit.

**The calcium-dependent proteolytic system (calpains)**
- Calpains are a large family of nonlysosomal cysteine proteases which are present in almost all eukaryotes and a few bacteria.
- The first calpain discovered and purified by Dantzig et al. in 1976 was calpain 1.
- Neutral proteases activated by cardiazal, called calpains, occur ubiquitously in animal cells.
- They are unusual proteases in that they require calcium for their activity.
- They are intracellular proteases with optimum activity at neutral pH.
- The system comprises several isoforms of the proteolytic enzyme calpain and their endogenous inhibitors, calpastatin.
- In mammals, there are two large subunit members, one small subunit member, and one endogenous inhibitor.

**Comparison of calpain and other intracellular proteolytic systems**
- Calpains differ from other major intracellular proteolytic components such as proteasomes and lysosomal proteases functioning in autophagy; these systems eliminate and recycle their substrates by degradation. Calpains act by proteolytic processing, as in the activation of conventional proteolytic enzymes (PKC).
- Calpains are unique in that they directly recognize substrates, whereas proteasomes and autophagy rely on other systems (ubiquitin and autophagy). Their function is the degradation of a protein (ubiquitin) substrate that is bound specifically to a unique ubiquitin carrier (ubiquitin A and B) followed by its transfer to a ubiquitin conjugating enzyme (E1 and E2). The transfer is to a ubiquitin ligase (E3) followed by its transfer to a ubiquitin carrier (ubiquitin A and B) followed by its transfer to a ubiquitin conjugating enzyme (E1 and E2). The transfer is to a ubiquitin ligase (E3). The conjugation of ubiquitin to a target protein is catalyzed by the ubiquitin ligase, and only then is targeted to the ubiquitin ligase. Ubiquitin conjugation of ubiquitin is necessary to ensure recognition of a polyubiquitin chain (UBQ) that leads to the 26S proteasome (B) followed by ubiquitination of the substrate by proteasome (C). Free and reusable ubiquitin is released by deubiquitinating enzymes (UUBs).

**The calpain system**
- They are a large family of nonlysosomal cysteine proteases which are present in almost all eukaryotes and a few bacteria.
- The first calpain discovered and purified by Dantzig et al. in 1976 was calpain 2.
- Neutral proteases activated by cardiazal, called calpains, occur ubiquitously in animal cells.
- They are unusual proteases in that they require calcium for their activity.
- They are intracellular proteases with optimum activity at neutral pH.
- The system comprises several isoforms of the proteolytic enzyme calpain and their endogenous inhibitors, calpastatin.
- In mammals, there are two large subunit members, one small subunit member, and one endogenous inhibitor.
Calpain homologues

Calpains, which are classified by two criteria: 
structure and distribution.

By structure:

1. Classical calpains:
   - Typical calpains contain a penta-EF hand in domain IV that can bind Ca\(^{2+}\), the calpain small subunit (only calpains 1, 2, and 9 have been shown to dimerize), or calpastatin.
   - Nine human classical calpains (CAPN1–3, 8, 9, 11–13) have been shown to bind Ca\(^{2+}\), the calpain small subunit, or calpastatin.

2. Non-classical calpains:
   - Atypical calpains (5, 6, 7, 10, 13, and 15) lack a penta-EF hand in domain IV and are unable to bind the calpain small subunit or calpastatin.

By distribution:

1. Tissue-specific calpains:
   - Six human calpain genes are tissue-specific:
     - calpain 3 (skeletal muscle)
     - calpain 6 (placenta)
     - calpain 8 (smooth muscle)
     - calpain 9 (stomach)
     - calpain 11 (testes)
     - calpain 12 (skin after birth)

2. Ubiquitous calpains

The calpain system

Ubiquitous and tissue-specific calpains:

1. Tissue-specific calpains:
   - Six human calpain genes are tissue-specific:
     - calpain 3 (skeletal muscle)
     - calpain 6 (placenta)
     - calpain 8 (smooth muscle)
     - calpain 9 (stomach)
     - calpain 11 (testes)
     - calpain 12 (skin after birth)

2. Ubiquitous calpains

Calpain homologues

ubiquitous calpains have basic roles in the cell,
tissue-specific calpains are involved in specific cell functions.

- Accordingly, defects in ubiquitous calpains can be lethal,
- whereas defects in tissue-specific calpains may cause tissue-specific phenotypes, such as the:
  - muscular dystrophy caused by CAPN3 mutations
  - cardiomyopathy
  - traumatic ischaemia

Phylogenetic tree and schematic structures of human calpains.

Functional roles of calpain

Although its physiological function is still not fully understood, it is implicated in a variety of calcium-regulated cellular processes such as:

- Adhesion, modulation – Spreading and Motility
- Cell Death, apoptosis and Destruction
- cell proliferation
- signal transduction pathways
- cell cycle progression
- Cell differentiation
- membrane fusion
- regulation of the cytoskeleton
- platelet activation

Regulation of its activity has been implicated in various pathological conditions such as:

- Alzheimer
- Parkinson’s
- Huntington’s
- Type 2 Diabetes Mellitus
- Neuronal degeneration
- Muscular Dystrophy
- Metastasis
- Cancer

Schematic structures of calpain superfamily members.

The calpain system
Calpains and mitochondria

In endothelial cells, Co-oxidative stress causes mitochondrial Ca2+-calpain-1 cleavage of the Na+/Ca2+-exchanger leading to mitochondrial Ca2+ accumulation. Also, activated calpain-1 cleavesBid, inducing cytochrome c release and apoptosis.

In renal cells, calpains 1 and 2 promote apoptosis and necrosis by cleaving cytoskeletal proteins, which increases plasma membrane permeability and cleavage of caspases.

Calpain-10 cleaves electron transport chain proteins, causing decreased mitochondrial respiration and excessive activation, or inhibition of calpain-10 activity induces mitochondrial dysfunction and apoptosis.

calpains and Motility

Calpain-3 can cleave effector molecules such as FAK, talin, and vinculin, possibly resulting in changes in actin cytoskeleton, adhesion complex turnover, or detachment of integrin sites.

Potentially, the accompanying protein complexes might lead to inhibition of membrane processes.

Cleavage of single-stranded DNA might be important for the formation of small calpain clusters during the early stages of self-spreading, whereas formation of the self-spreading ring leads to cell spreading.

Interaction of ATP with calpain-2 small subunit (2S) may also mediate cell spreading.

Proteins of the actin cytoskeleton, such as FHOD1, might also regulate cell migration in mouse embryo, possibly by promoting cell adhesion formation.

The balance required for degradation of rac, Rac and FHOD1 levels to be determined, as do the genome's effect by proteolysis of many 200 other calpain substrates.

The calpain system

Calpain substrates

- Among the >100 proteins identified as calpain substrates are:
- transcription factors
- transmembrane receptors
- Signaling enzymes
- cytoskeletal proteins

Gastrointestinal-tract specific calpains, CAPN8, CAPN9

Single nucleotide polymorphisms (SNPs) reported for human CAPN8 and CAPN9 include nonsense mutations and missense mutations that changes amino acid residues highly conserved among calpain family members. These most probable functions of CAPN8 and CAPN9. Actually, an in vitro assay showed that these two CAPN8 and CAPN9 did not have any activity.

CAPN3 and muscular dystrophy

Pathogenic mutations of human CAPN3 are responsible for limb-girdle muscular dystrophy type 2A (LGMD2A, also called “calpainopathy’). CAPN3 is essential for skeletal muscle functions, and, since then, studies on CAPN3 has been developing in relation with LGMD2A pathogenic mechanisms. We have shown that expression and genetic analysis on mouse genetically modified calpain-3 deficient mice, showed that loss of protease activity of CAPN3 is responsible for LGMD2A. Moreover, CAPN3 expression changes in cellular localization by activity-dependent, and this movement is important for stress response of skeletal muscle.
The calpain system

Structure of calpains

µ-calpain and m-calpain are heterodimers composed of a similar 80 kDa catalytic subunit and a 28 kDa small subunit.

The small subunit has two domains (V, VI):
- Domain V is rich in glycine and is the site for phospholipid binding.
- Domain VI contains five Ca²⁺-binding sites also known as EF-hand motif.

Domains within calpains

- Catalytic subdomain
- Subdomains
- EF-Hand motifs
- Calcium-binding sites
- EF-Hand-like sites

Structure of Calpain 3

Originally referred to as p94, calpain 3 is a 94 kDa calpain isoform having sequence homology of approximately 50% with the large domain of µ- and m-calpain. It may form homodimers in vivo as it lacks the 28 kDa small subunit.

This calpain protease is specific to skeletal muscle and is activated in the presence of Ca²⁺ and phospholipid.

Structure of Calpastatin

- Calpastatin, an endogenous inhibitor of µ- and m-calpain,
- is a 70–80 kDa protein.
- and has an N-terminal domain
- and four repeating domains (I, II, III, IV).
- Each of the four repeating domains is able to inhibit one calpain molecule.
- calpastatin must bind domain II and domain IV or VI to inhibit calpains.

The calpain system

Mechanisms of action in calpains

- Calpain exists in the cytosol as an inactive enzyme.
- Translocates to membranes in response to increases in the cellular Ca²⁺ level.
- At the membrane, calpain is activated in the presence of Ca²⁺ and phospholipids.
- Autocatalytic hydrolysis of domain I takes place during activation.
- Dissociation of 30K from 80K occurs as a result.
- Activated calpain or 80K hydrolyses substrate proteins at membranes or in cytosol after release from membranes.

- In the absence of Ca²⁺, two protease subdomains Ila and IIb are separated by structural constraints imposed by domain interaction. Ca²⁺-induced structural changes that release the constraints are prerequisite for activation to form a functional catalytic site.

- There are at least three different Ca²⁺-binding sites in m-calpain:
  - two calmodulin-like domains IV and VI
  - an acidic loop region in C3-like domain III
  - and a protease domain II
**Activation mechanism of calpain by Ca**

Binding of Ca and phosphorylated (PL) to m-calpain induces conformational changes, which brings Ilb and Ih closer together to form a functional calpain site and causes dissociation of 80k from 80k, resulting in 80k homodimer formation. There are at least two calcium binding sites in each subunit: one weak, low-affinity site (VI, red sphere) and two high-affinity sites (IV, purple spheres). These three calcium binding sites are in each ILb and Ih. C115 and 156 in ILb and C115 and 156 in Ih form a salt bridge in the absence of calcium ions. Nt, N terminus residue.
Calpain 3 may function in conjunction with ubiquitin ligases to mediate sarcomere remodeling.

The calpain system

Calpain 3 (red) is anchored to the sarcomere at the N line and M line, through its association with titin (not shown). The contractile proteins are highly organized and entwined and cannot be degraded in the proteasome without the initiating step of proteolytic dissociation of this complex (for simplicity, only actin and myosin are shown). Data suggest that calpain 3 is one enzyme that performs the initial proteolytic cleavage that allows E3 ubiquitin ligases to ubiquitinate the peptides and targets them for degradation in the proteasome.

Major intracellular proteolytic systems

The caspase system

- Caspases are a family of cysteine aspartate-specific proteases.
- To date, 14 members of the caspase family have been identified.
- These highly specialized enzymes create the intracellular network signaling proteolysis.

Proteolysis activation of caspases may occur with the participation of enzymes such as:

1. granzyme B (a strong activator of procaspases 3 and 7)
2. cathepsin G
3. calpains
4. cathepsin D

Apoptosis is the organized dismantling of the cell, characterized by:
- cell shrinkage,
- DNA fragmentation,
- chromatin condensation,
- membrane blebbing
- the formation of apoptotic bodies without inducing an inflammatory response.

The caspase system

- Caspases are activated via three main pathways:
  1. The cell death pathway or extrinsic pathway is triggered by cell surface receptors and initiator caspases 8 and 10 are activated via this pathway.
  2. The intrinsic pathway involves caspase 9 and is activated in response to environmental stress such as hypoxia and ischemia.
  3. The ER mediated pathway is activated via stress directly upon the ER, for example disruption in Ca^2+ homeostasis, which in turn activates initiator caspase 12.

Effector caspases are activated by initiator caspases upstream and once activated target and cleave specific substrates, resulting in cell disassembly.

To date more than 280 caspase targets have been identified including myofibrillar and cytoskeletal proteins.

Major intracellular proteolytic systems

The caspase system

- Caspases can be activated early in pathological events associated with hypoxia/ischemia
- which is not that dissimilar to the hypoxic conditions in muscle after slaughter.
- In meat animals the process of exsanguination occurs after slaughter, depriving all cells and tissues of nutrients and oxygen.
- After death muscle continues to metabolise and therefore muscle cells will presumably engaged in the process of cell death, with apoptosis rather necrosis considered to be the most likely process of cell death.
- Therefore it has been hypothesised that the process of slaughter and exsanguination could initiate the apoptotic pathways and caspase activity may contribute to early post-mortem proteolysis and meat tenderisation.

Schematic representation of conventional calpain substrates and major events of tumor cell pathophysiology.
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MEAT TENDERNESS

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Thanks for attention to my awesome presentation