

Exercise 6: Muscular Tissue and Function



Figure 6.1 Illustration of skeletal muscles based on anatomical preparations from 17th century scholar Bernardino Genga (https://commons.wikimedia.org/wiki/File:Genga_36.jpg).

Exercise 6 Learning Goals

After completing this lab, you should be able to:

- Describe the structural and functional differences between skeletal, cardiac and smooth muscle cells
- Describe the anatomical organization of a skeletal muscle including connective tissue, muscle tissue, muscle cells and contractile proteins
- Describe the specialized organelles associated with skeletal muscle cells
- Explain which organelles are important for muscle contraction

Pre-Lab Exercise 6

Pre-Lab Activity 6.1: Skeletal Muscle

Skeletal muscle is organized into bundles of muscle cells. Muscle cells are commonly known as fibers due to their long, cylindrical appearance. These bundles of muscle fibers are held together by different layers of connective tissues. This connective tissue is continuous with the tendon, which attaches the muscle to the bone. So how is it all organized together? There are three layers of continuous connective tissue. From superficial to deep the connective tissue layers are:

1. Epimysium- covering the **entire muscle belly** and continuous with tendons
2. Perimysium- covering bundles of muscle fibers in units called **fascicles** and continuous with the epimysium
3. Endomysium- covering **individual muscle fibers** and continuous with perimysium

To conclude, whole muscles are made up of bundles of fascicles and each fascicle is made up of bundles of muscle fibers.

In the diagram below, fill in the blanks indicating the three layers of connective tissue

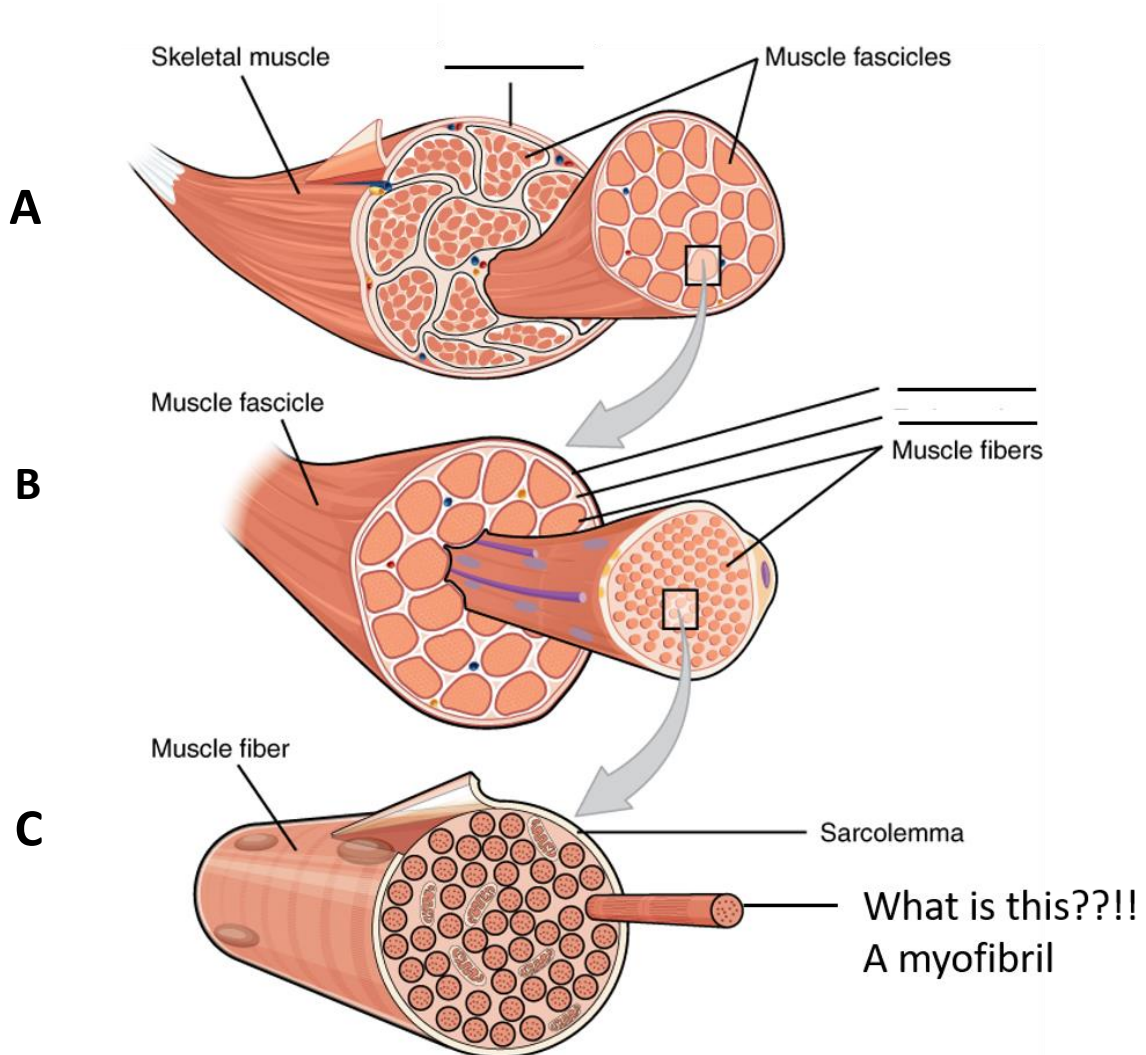


Figure 6.2 Organization of muscle (A) muscle belly (B) muscle fascicle (C) Muscle fiber (edited from <http://cnx.org/contents/14fb4ad7-39a1>)

Pre-Lab Exercise 6.2: Anatomy of a Muscle Fiber

In the diagram below, fill in the blanks indicating the different organelles of a muscle fiber

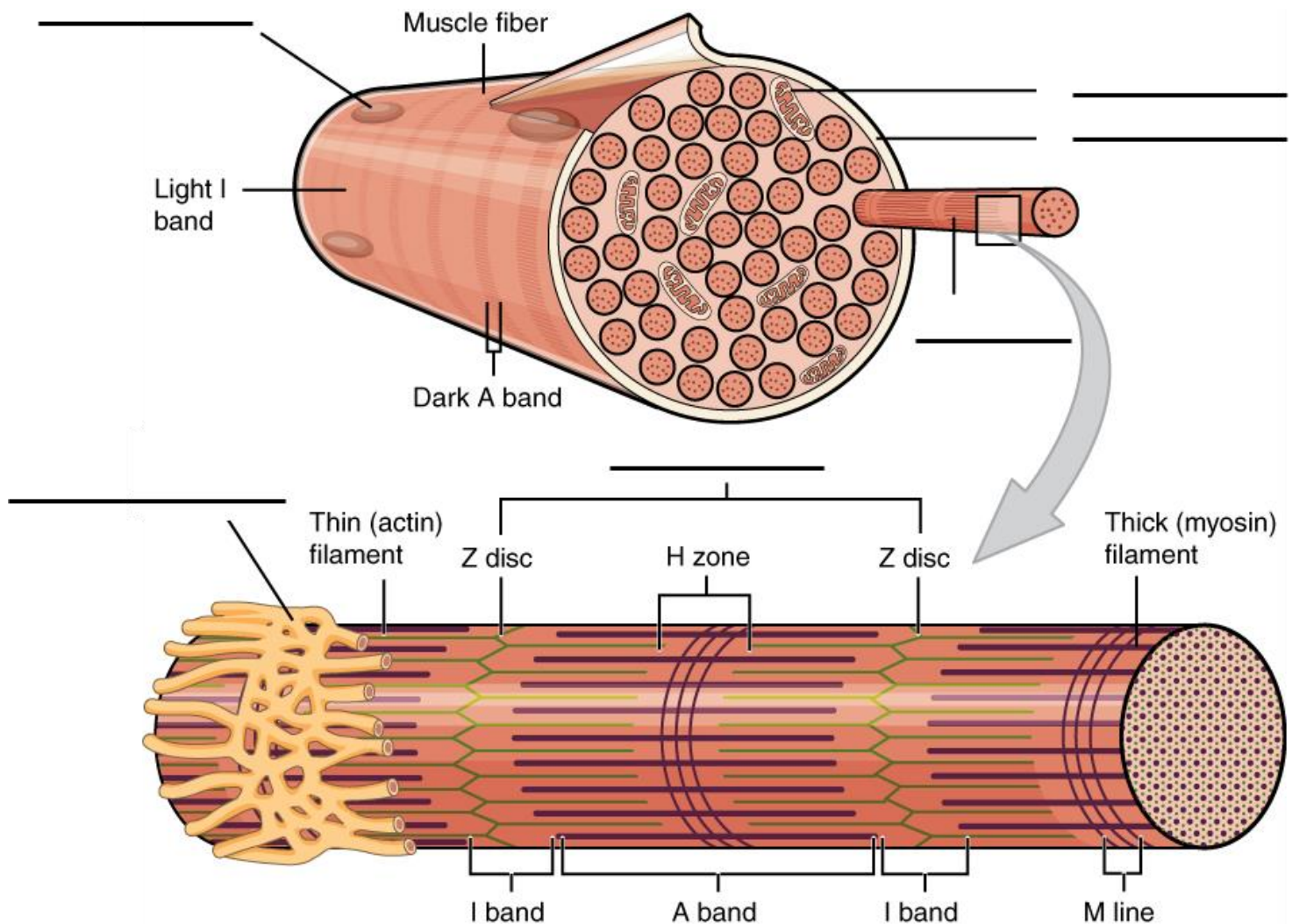


Figure 6.3 Anatomy of a muscle fiber (edited from <http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@15.1>)

Pre-Lab Activity 6.3: Myocyte Questions

Answer the following questions regarding the parts of the muscle fiber.

1. Describe the **arrangement** of muscle fibers in a skeletal muscle including levels of connective tissues?

2. What is a **sarcolemma** and what is its **main function**?

3. Why do muscle fibers require **more mitochondria** than a typical cell?

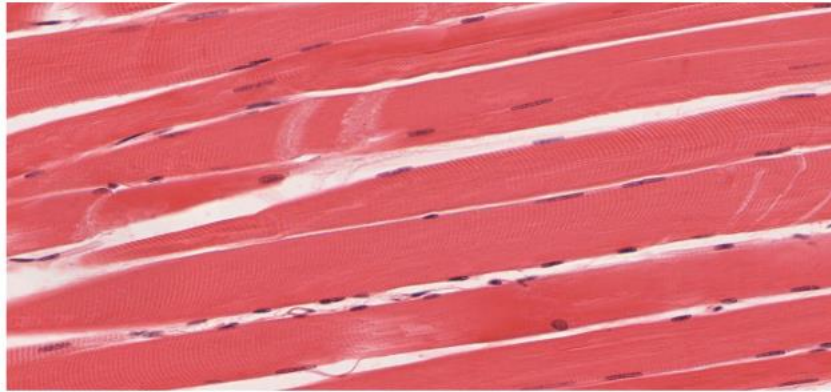
4. What is a **sarcoplasmic reticulum** and what is its **main function**?

5. Where in a muscle fiber is the **contractile unit** (i.e. sarcomeres) found?

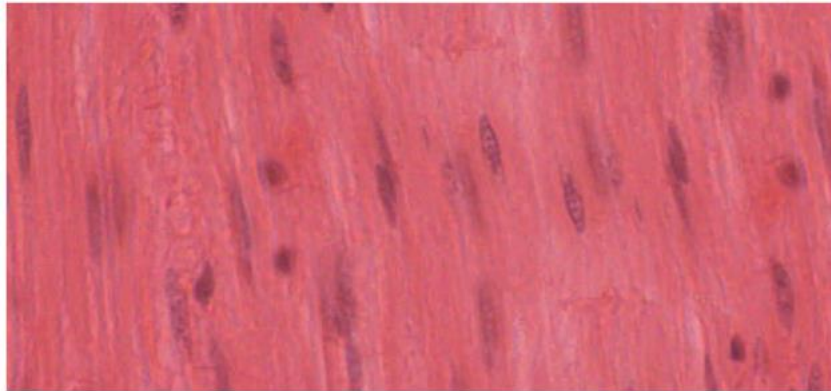
6. What are the **two major proteins** in a muscle fiber that are responsible for contraction? What other protein plays a major role in the contraction cycle?

Lab Exercise 6 Activities

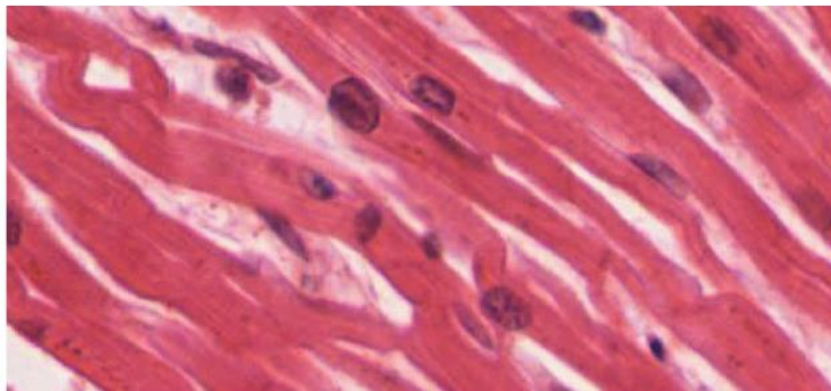
Reference Photos: Muscle Tissue Types



(a)



(b)



(c)

Figure 6.4 Longitudinal sections of the three types of muscle tissue (a) skeletal muscle (b) smooth muscle (c) cardiac muscle (<http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@15.1>)

Lab Activity 6.1: Identifying Differences between Skeletal, Cardiac, & Smooth Muscle

Set up your compound microscope and select one muscle composite slide (all three muscle types are present on one slide)

When looking at your slide make sure you are looking at a longitudinal section and not a cross-section of each muscle. It is much easier to visualize the features of individual muscle cells in this orientation

Complete the table below

Characteristics	Skeletal	Cardiac	Smooth
Location			
Number of nuclei			
Location of nuclei			
Striated or non-striated			
Branching fibers?			
Intercalated discs?			
Cell/fiber length			
Contraction time			
Endurance			

From the pre-lab exercise, we know that skeletal muscle is striated in appearance. But what causes these striations? Within skeletal muscle cells (known as fibers) there are proteins that cause the muscle cell to shorten. This shortening will end up moving a bone that the muscle is attached to and those striations are proteins that help the skeletal muscle to contract. You may have noticed that these proteins are small, but muscles are big!

Lab Activity 6.2: Anatomy of the Sarcomere

The sarcomere contains proteins arranged in a way that allows the muscle to contract and shorten (the whole point of a muscle!). As you can see in Figure 6.4, bundles of perfectly organized sarcomeres are found within myofibrils (and myofibrils make up each individual muscle fiber).

The contractile proteins within each sarcomere are known as either **thick or thin filaments**. Thin filaments are made up of the protein **actin**. Thick filaments are made up of the protein **myosin**. There are also regulatory proteins troponin and tropomyosin that determine whether thick and think filaments will interact to initiate a muscle contraction (see **steps of sarcomere shortening** below)

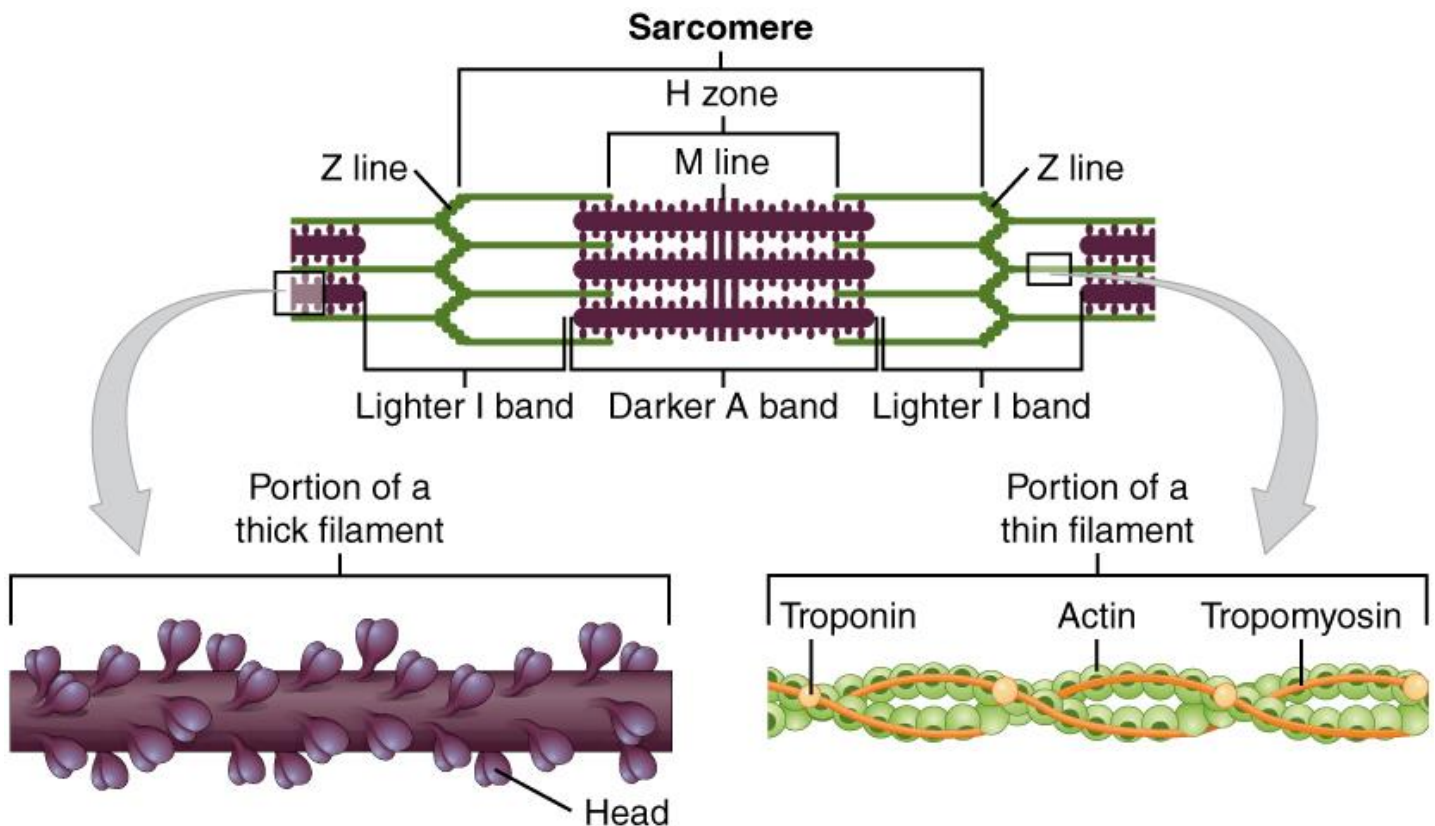


Figure 6.5 Anatomy of a sarcomere (<http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@15.1>)

Activity 6.2.1: Answer the following questions regarding the parts of the sarcomere.

1. Which proteins make up the **thin filament**?
2. Which protein makes up the **thick filament**?

3. Define the **H zone**.
4. What do the **Z lines** represent?
5. What does the **M line** represent?
6. Why is the **I band** lighter and the **A band** darker?
7. What is the role of **tropomyosin**?
8. What is the role of **troponin**?
9. Why do the **thick filaments** have heads?

Lab Activity 6.2.2: Sliding-Filament Theory

The whole point of a muscle (and sarcomere) is to shorten when stimulated. But how does a muscle shorten? And what materials are required to stimulate this shortening?

How does a sarcomere shorten?

Interlocking your fingers helps to visualize how a sarcomere can shorten. Place both hands in front of your face with your palms facing you. Your fingers represent the proteins myosin and actin. Your wrists represent Z lines (discs). Interlock your fingers and move them closer together as in Figure 6.6 below. As your fingers (filaments) slide past each other, your wrists (Z lines) get closer together and therefore 'shorten'. **Note that your fingers do not have to shorten in order to bring your wrists closer together. This is how a sarcomere shortens. The filaments 'slide' over each other bringing the Z lines (discs) closer together.**

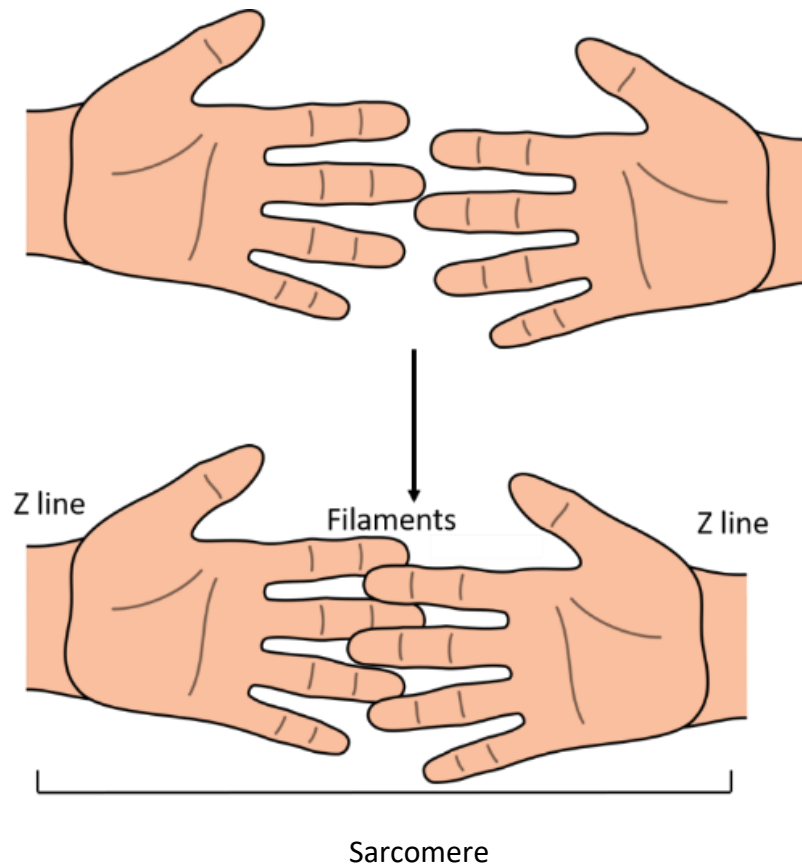


Figure 6.6 Visualizing the shortening of a sarcomere using your fingers. Source: OpenStax A&P

Steps of Sarcomere shortening

Sarcomeres shorten by the heads of myosin (thick filament) attaching to myosin binding sites on actin (thin filament). This attachment is called a **cross bridge**. Using ATP, the myosin head swivels (called the **power stroke**), bringing the two ends of the sarcomere closer together. The process is represented in Figure 6.7.

1. **Calcium** from the sarcoplasmic reticulum is released in response to a muscle action potential from a nerve impulse. Calcium binds to troponin, which causes tropomyosin to change shape and **expose myosin binding sites on the actin**.
2. The myosin head (energized by ATP breaking down to ADP + P) then binds to the actin, forming the **cross bridge**.
3. The myosin head swivels (**power stroke**), pulling the actin closer to the M line or center of the sarcomere, narrowing the H Zone. ADP + P molecules are released from the myosin head
4. The myosin head detaches from the actin when a new molecule of ATP binds to the myosin head
5. The new ATP molecule breaks down, releasing energy that re-orientates the myosin head to reposition itself for a new cycle of muscle contraction

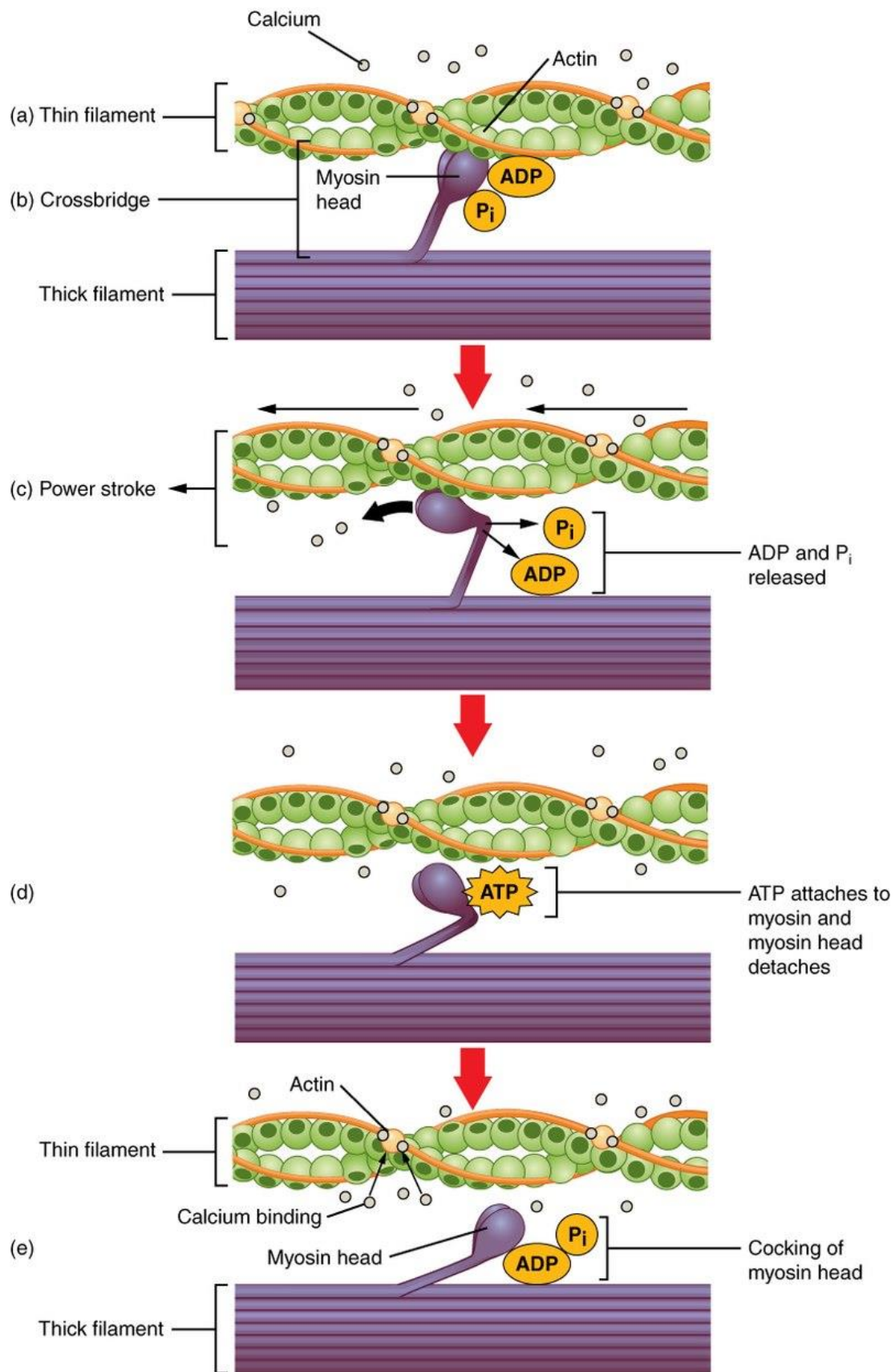


Figure 6.7 Steps of one muscle contraction cycle (<http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@15.1>)

Lab Activity 6.3: Skeletal Muscle Contraction – Effects of ions & ATP

Materials needed:

- Section of glycerinated skeletal muscle in 50% glycerol (rabbit)
- Vials containing the following solutions
 - 0.25% ATP plus 0.05 M KCl plus 0.001 M MgCl_2 in distilled water (ATP + ions)
 - 0.25% ATP in triple distilled water (ATP only)
 - 0.05 M KCl plus 0.001 M MgCl_2 in distilled water (ions only)
- Teasing needle
- Pipette
- Petri dish
- Dissecting microscope
- Four microscope slides
- Four coverslips
- Compound microscope
- Wax pencil
- Ruler (marked in millimeters)

Procedure

BEFORE STARTING THE PROCEDURE PLEASE MAKE SURE YOUR HANDS AND INSTRUMENTS/MATERIALS ARE CLEAN.

1. From your instructor, obtain a 2 cm length of glycerinated skeletal muscle and place it in a **clean** petri dish with a small amount of glycerol
2. Place the petri dish under a dissection microscope. With teasing needle/fine pointed forceps divide the skeletal muscle into 10 **very thin groups** of fibers. Single fibers will demonstrate the greatest contraction. **Strands of muscle exceeding 0.2 mm in cross-sectional diameter are too thick to use.**
3. Transfer one strand of muscle from the petri dish to a slide. Place a coverslip over the muscle and use a **compound microscope at low magnification** to examine the striations of the muscle fibers.
4. Label three other microscope slides A, B and C.
5. On slide A, add a tiny drop of glycerol and transfer 3 strands of muscle to the slide. Make sure the strands are arranged parallel to one another and with the slide. The strands of muscle fibers **MUST** be straight to make them easier to measure.
6. Using a **dissection microscope** and a ruler, measure the lengths of each strand (3 total). Record the averages of these lengths (***in millimeters***) in the table below under the heading 'Resting Length'.
7. Flood the strands on slide A with several drops of the solution containing ATP plus ions (KCl and MgCl_2). Observe the reaction of the fibers.
8. After 45 seconds, re-measure each strand using the dissection microscope and ruler. Record the average of these lengths in the table under the heading 'Contracting Length'.
9. Put a coverslip over the muscle strands in slide 1 and examine them at **low power using a compound microscope**. Compare the strands treated with ATP plus salt solution (Slide A) to the untreated strands (from step 3) and note any differences.
10. Repeat steps 5-9 using slide B (ATP only), three new muscle fiber strands and the solution containing **ATP only**
11. Repeat steps 5-9 using slide C (ions only), three new muscle fiber strands and the solution containing **ions only**
12. After recording muscle strand lengths in the table below dispose of biological material by following directions from the instructor.

Data Collection

Solution applied to tissue	Resting length (mm)	Contraction length (mm)	Change in length (contraction-resting)	% change in length [(change/resting) x 100]
A. ATP and ions				
B. ATP only				
C. Ions only				

Activity 6.3.2 Answer the following questions based on the results of your experiment:

1. Which solution caused the largest change in muscle length? Why do you think that particular solution worked?
2. What is ATP's role in muscle contraction?
3. What is the role of calcium ions in muscle contraction?

Lab Activity 6.3.3: Sarcomere at Rest vs. Contracted

1. Sketch a sarcomere 'at rest' and label all of its parts.
2. Sketch a sarcomere 'during contraction' and label all of its parts.
3. Describe the changes in length of the sarcomere as it goes from resting to contracted.