To Stick or Not to Stick: PKA and its Role in Platelet Coagulation

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Have you ever gotten a cut and wondered how and what makes it heal? When you get a cut, platelets stick together, or aggregate, to form a seal in a process called coagulation. A platelet is a cell fragment that is found in the blood and is involved in blood clotting. Too little blood clotting can end in wounds not healing properly. Too much clotting can cause heart attacks and strokes. Protein Kinase A (PKA) regulates the ability of platelets to aggregate. The Milwaukee Academy of Science SMART Team (Student Modeling A Research Topic) modeled the PKA protein using 3D printing technology. Kinase proteins are responsible for adding a phosphate group to other proteins to activate them through a process called phosphorylation. PKA is made up of four subunits: two Regulatory Subunits (R-Subunits) and two Catalytic Subunits (C-Subunits). When the R-subunits are bound to the C-subunits, PKA is inactive. When a small molecule, cyclic adenosine monophosphate (cAMP), binds to the R-subunits, the C-subunits are released, thus activating the kinase. When active, the C-subunit attaches a phosphate to a target protein, such as Rap1b. Rap1b is an important player in the regulation of platelet aggregation and is located on the inner cell membrane. When Rap1b is phosphorylated, it detaches from the cell membrane and enters the cytosol. As a result, the platelets are not able to aggregate. In essence, it is the behavior of PKA that determines whether platelets aggregate or remain free-floating, which in turn makes up PKA an incredibly important factor in understanding heart attacks and strokes.

**Abstract**

**Diseases & Importance**

- Heart attacks, also known as myocardial infarctions, are caused by a blood clot that blocks coronary arteries. The coronary arteries bring blood and oxygen to the heart. A heart attack can occur as a result of plaque buildup on the inside of arteries. If the plaque is ruptured, platelets will coagulate around the rupture and will potentially stop blood flow. If the blood is blocked, the heart tissue can’t receive oxygen and heart cells die and cannot be replaced. Heart attacks are the leading cause of death in the United States, affecting 1.2 million people annually and killing 600,000 of those.

- Blood clots

  - Blood clotting may seem all bad when dealing with heart attacks and strokes, but clotting is not always a bad thing. As you may know when you cut yourself platelets stick together or coagulate to form a seal, preventing additional blood loss. If platelets didn’t coagulate, any kind of injury to a blood vessel would result in uncontrolled bleeding. Severe blood loss can also result in hypovolemic shock, which is also characterized by coma and possibly death.

- Strokes

  - When blood flow to a portion of the brain is blocked, neural tissues are deprived of life sustaining oxygen and glucose. Brain cells will begin to die within six minutes of oxygen deprivation and cause permanent brain damage in a condition known as a stroke. They are two major types of stroke, ischemic stroke and hemorrhagic stroke. Ischemic stroke happens when a blood vessel that brings blood to the brain is blocked. It can happen in two ways: a clot may break off from a part of the body and travel up to the brain or a clot may form in an artery that is already very narrow. A hemorrhagic stroke occurs when a blood vessel in part of the brain becomes weak and bursts open, causing blood to leak into the brain. Strokes are the third highest cause of death in the United States.

**Phosphorylation of Rap1b reduces its membrane association**

A) Representation of a single experiment. B) A summary of four experiments. The left side of each panels shows the localization and phosphorylation state of Rap1b from isolated mouse platelets. The right side of each panels shows that when platelets are detached into the cytosol and travel to phosphorylate Rap1b.

**Platelets**

A platelet is an oddly shaped and colorless cell fragment that is found in the blood. Platelets are heavily involved in blood clotting. The main purpose of platelets is to clot (coagulate or stick) when a blood vessel is broken. This may sound like a good thing because platelets heal wounds but platelets can clot abnormally and create issues for our health. In heart attacks, platelets cling to ruptured plaque and coagulate as if it were healing a wound. As a result, the platelets block blood flow causing a heart attack. If platelets clot inside a blood vessel in the brain, blood flow to that part of the brain is blocked, killing brain tissue and creating a stroke.

**Anatomy of Protein Kinase A (PKA)**

Protein Kinase A (PKA) is an important protein involved in the clotting process. PKA is a platelet peripheral protein, which means it is located on the inner membrane of platelets. A kinase is an enzyme and an ATP carrier. Kinases carry around ATP (yellow) to transfer a phosphate to other proteins in order to activate or deactivate them in a process known as phosphorylation. If you look closely you will be able to see 4 parts and 2 units to each part. These units are called subunits. There are 4 subunits in all, 2 red being catalytic (red) and the 2 being regulatory (blue). At first the units are all bound together and attached to AKAP, which anchors PKA to the platelet inner cell membrane. When 4 cyclic AMPs, a messenger molecule, attach to regulatory subunits, the catalytic units detach into the cytosol and travel to phosphorylate Rap1b.

**Summary**

- Heart attacks, strokes, and blood clots are all events in the human body that occur due to platelet activity.
- Platelets are oddly shaped cell fragments in the body which aggregate to form a clot and heal wounds.
- Platelet aggregation is controlled by a platelet peripheral protein called Protein Kinase A (PKA).
- PKA is made of two catalytic subunits and two regulatory subunits.
- When PKA is activated by cyclic AMP (cAMP), the catalytic subunits break off and add a phosphate group to another protein called Rap1b.
- Whether or not Rap1b is phosphorylated determines whether or not a platelet will aggregate.
- If PKA’s function can be controlled, researchers can hopefully prevent unnecessary platelet aggregation, thereby preventing heart attacks and strokes.

**Legend**

- Regulatory Subunit
- Catalytic Subunit
- ATP used for phosphorylation
- 4 cAMPs bind to the regulatory subunits causing the catalytic subunit to break off.
- 3. The catalytic subunit binds with Rap1b. When connected, the catalytic subunit transfers one phosphate from ATP to Rap1b resulting in: PHOSPHORYLATION!
- 5. When Rap1b is the platelet’s cytosol, the platelet avoids aggregating with other platelets. When it is attached to the inside of the cell membrane, the platelet will aggregate with other platelets.
- 1. These are the regulatory and catalytic subunits before phosphorylation.
- 2. Here 4 cAMPs bind to the regulatory subunits causing the catalytic subunit to break off.
- 4. Then Rap1b breaks off from the inside of the membrane into the cytosol of the cell.

**Platelet Cytosol**

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- Regulatory Subunit
- Catalytic Subunit
- ATP
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