

Modeling the Cell Adhesion Molecule E-Cadherin

SMART Team
Students A-Z

Teacher: Ms. Teacher

Mentor: Dr. Mentor

E-Cadherin is a transmembrane protein essential for adherens junctions involved in organ and tissue development and integrity. Adherens junctions provide direct connections between adjacent cells and are crucial to maintaining epithelial membranes and blood vessel integrity. Adhesion of neighboring cells via E-cadherin occurs through a salt bridge interaction between the N-terminal Aspartate-1 of E-cadherin protein on one cell and Glutamate-89 of the opposing E-cadherin protein from the adjacent cell. Additional contacts are provided by hydrophobic and hydrogen-bonding between residues Trp2, Asp90, and Met92 located at the binding interface. E-cadherin is a classical Ca^{2+} -dependent cadherin and loss of function may contribute to developmental abnormalities. Balance between maintenance and remodeling of cell adhesion junctions is required for normal embryonic development. For example, in the development of the embryonic heart, cadherin proteins facilitate connections between adjacent epithelial cells of proepicardial tissue. Cells of the proepicardium must migrate over the surface of the developing heart and then differentiate into the endothelial and smooth muscle cells of the mature coronary vasculature. Understanding the mechanisms of formation and dissolution of adherens junctions provides important insight into cardiac development and may provide direction for new cancer therapies.

Heart development requires reversible cell contacts.

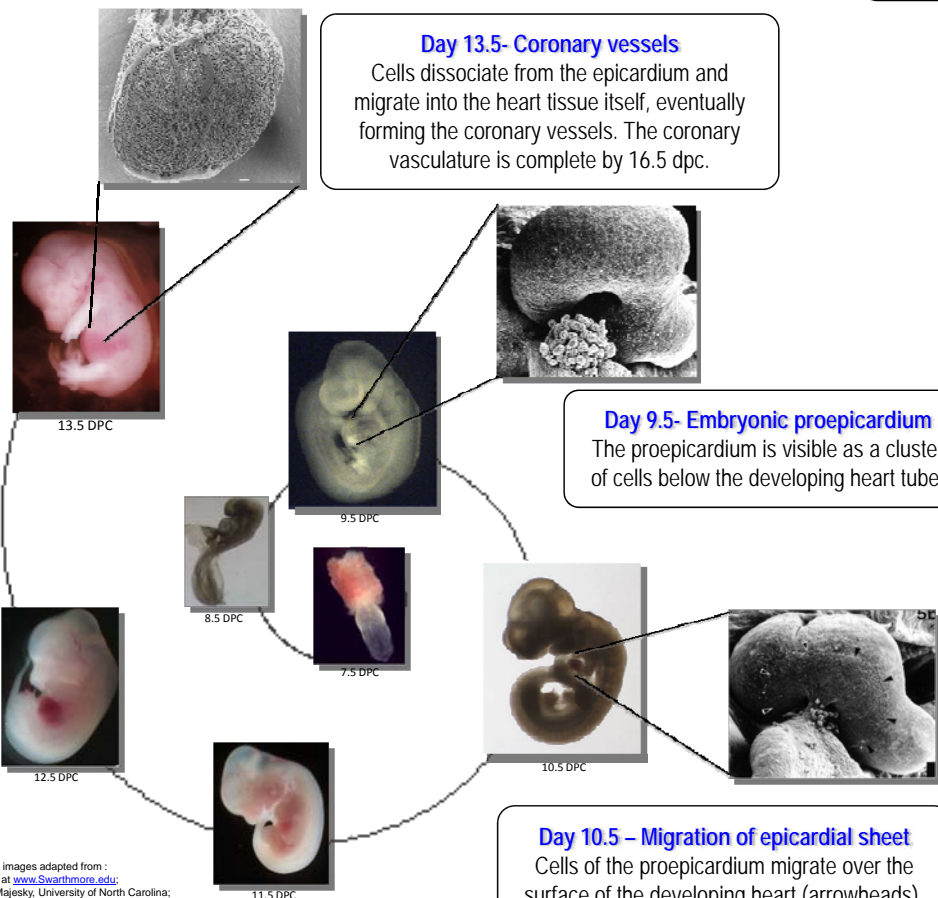
Heart development begins with formation of the heart tube, a structure that eventually develops into the chambers and muscular tissue of the heart. The heart also requires a cardiovascular system, which develops from a structure of cells called the proepicardium. During vascular formation, cells of the proepicardium undergo a series of associations and dissociations as they migrate over the surface of the heart. Mouse embryos lacking functional VE- or N-cadherin die by 9.5 to 10.5 dpc due to severe vascular defects, suggesting that adherens junctions play a role in embryonic cardiovascular formation.

Cadherins mediate Ca^{2+} -dependent, reversible adhesion in epithelial tissues and blood vessels.

Cell-cell adhesion is necessary for the formation of tissues within the body. Cadherins are transmembrane proteins that support cell-cell adhesion at adherens junctions. These junctions, along with others known as gap and tight junctions, promote direct contact between adjacent cells. The three primary types of classical cadherins display tissue-specific expression: epithelial cadherin (E-cad), vascular endothelial cadherin (VE-cad), and neuronal cadherin (N-cad). Interactions between cadherins in adjacent cells are reversible depending on the presence or absence of calcium ions. This reversibility is crucial for tissue development and function.

Day 13.5- Coronary vessels

Cells dissociate from the epicardium and migrate into the heart tissue itself, eventually forming the coronary vessels. The coronary vasculature is complete by 16.5 dpc.

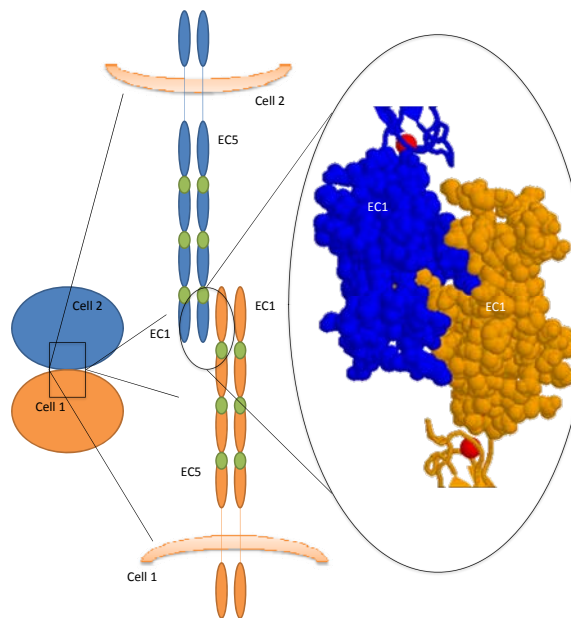


Day 9.5- Embryonic proepicardium

The proepicardium is visible as a cluster of cells below the developing heart tube.

Day 10.5 - Migration of epicardial sheet

Cells of the proepicardium migrate over the surface of the developing heart (arrowheads), forming the epicardium.

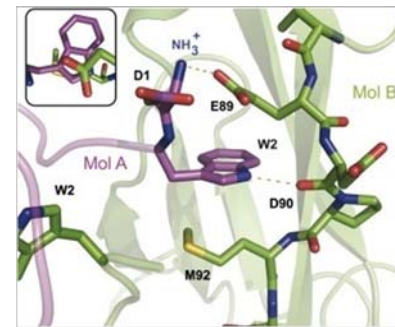


Calcium is essential for the stabilization of homophilic cadherin interactions.

Calcium ions act to maintain the integrity of cadherin molecules by stabilizing the structure and preventing unfolding. The calcium binds covalently to a negatively charged amino acid in each individual domain. This allows the domain to take on a stiff yet elastic ordered shape that supports interaction with cadherin on a neighboring cell. When calcium is removed, the cadherin molecule becomes disordered and easily denatured, causing cadherin molecules to dissociate.

Detailed interaction between opposing EC1 domains of E-cadherin.

A salt bridge between the N-terminal $^{\text{A}}\text{NH}_3^+$ and the carboxyl group of $^{\text{B}}\text{Glu89}$; main chain hydrogen bonds between $^{\text{A}}\text{Va3-NH}$ and $^{\text{B}}\text{Lys25-CO}$ as well as between $^{\text{A}}\text{Asp1-CO}$ and $^{\text{B}}\text{Asn27-NH}$; a hydrogen bond between NE of $^{\text{A}}\text{Trp2}$ and carbonyl group of $^{\text{B}}\text{Asp90-CO}$.



Get "Hooked" on *Brugia malayi* Asparaginyl tRNA Synthetase



SMART Team: Students A-Z

Advisor: Mrs. Teacher

Mentor: Dr. Mentor

I. Abstract

A partnership between high school students and a scientist enabled the Brown Deer SMART Team (Students Modeling A Research Topic) to explore the structure and function of asparaginyl-tRNA synthetase (AsnRS), a potential drug target to treat lymphatic filariasis, and to build a 3D physical model of the protein. Lymphatic filariasis results from mosquitoes transferring the nematode, *Brugia malayi*, to host lymph nodes, leading to swelling of affected limbs. AsnRS hooks asparagine to tRNA, used during protein synthesis. AsnRS is a member of the aminoacyl tRNA synthetase (AARS) family, a set of structurally heterogenous enzymes, specific for each amino acid. AARS are potential drug targets as they are essential for survival and are structurally different between species. AARS also functions as an immunosuppressant, blocking interleukin 8 receptors in humans. Current research for treatment targets parasitic AARS. If multiple functions could be mapped to the same region of the protein, a single drug could target these functions. Inhibition of the tRNA-aminoacylation function of AsnRS would prevent protein synthesis, thus causing death of the parasite. Preventing AsnRS from blocking interleukin 8 receptors, would act as an immunostimulant in humans. Further research on this family of enzymes could provide alternative therapies to treating parasitic diseases.



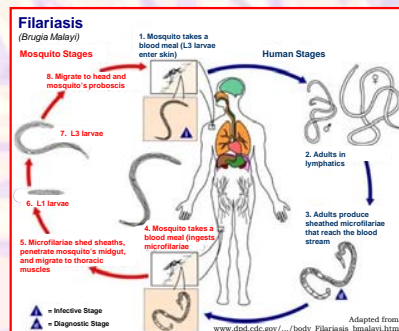
Over 200 million people worldwide are affected by lymphatic filariasis. The distribution of the disease, as of 2004, is represented by the green colored countries.

II. Disease Aspect

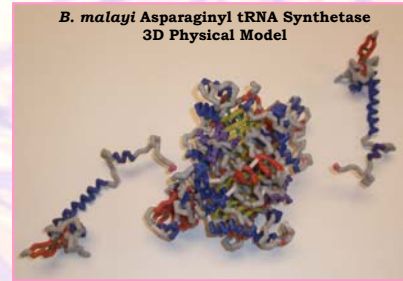
The nematode, *Brugia malayi*, buries itself in the lymph nodes causing scar tissue to form, inflaming the glands. This restricts the circulation of lymph, making the leg swell to an abnormal size, as seen in the figure below.



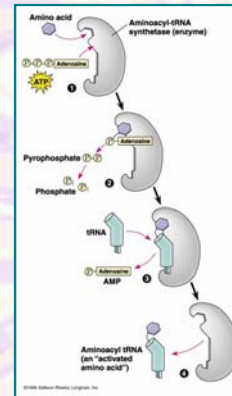
B. malayi is dormant during normal daylight hours. The worm can be found in the bloodstream from 10 pm to 2 am causing difficulty in diagnosing the disease. The complex life cycle of the nematode is pictured below:



III. AsnRS Structure and Function



B. malayi asparaginyl tRNA synthetase (AsnRS) functions as an immunosuppressant in humans and is essential for protein synthesis. Asparagine binds to the amino acids (shown in purple in the model above), and ATP binds to the beta sheets (highlighted yellow in the model above).



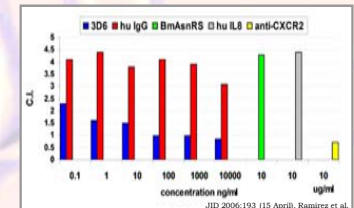
The main function of AsnRS is to attach asparagine to tRNA by the two step process shown in the diagram above. Disruption of this process interrupts protein synthesis, causing the death of the worm.

IV. Blocking AsnRS Receptors

Research Focus: Identify drugs which will inhibit protein synthesis in *B. malayi* and be an immunostimulant in humans.

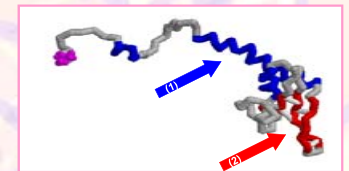
Experimental Objective: Find a chemical that affects chemotaxis by blocking the part of AsnRS important to receptor interaction.

Results:

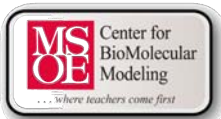


Conclusion: The experiment demonstrated that 3D6, a monoclonal antibody, subdues the chemotaxis, or movement, towards AsnRS; however, IgG allows AsnRS to continue attracting molecules. Because 3D6 blocks chemotaxis, it was concluded that it does so by blocking the part of AsnRS crucial to receptor interaction.

V. Drug Target



Since the receptor binding site and the tRNA binding site are in close proximity, it might be possible to design a drug to block both. The drug would attach to the hook shown at site (1) in the diagram above, which inhibits protein synthesis in the parasite. Site (2) shown above depicts the region that blocks immunosuppression. Finding a single drug could lead to treatment of lymphatic filariasis.



I'm a PC (Pyruvate Carboxylase)... ...and diabetes was not my idea!



Students' Names
Advisor: Teacher Name
School Name and Address
Mentor Name and Affiliation

ABSTRACT

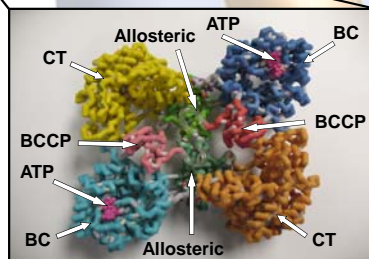
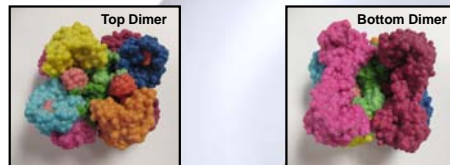
NIH estimates that 23 million Americans have diabetes, and 6.2 million are undiagnosed. If untreated, diabetes can cause complications, including heart disease and neuropathy. Type II diabetes patients cannot regulate glucose due to insulin resistance or deficiency. Pyruvate carboxylase (PC) plays an important role in insulin release from pancreatic β cells. Abnormal PC activity has been correlated with type II diabetes. PC is a dimer of dimers, each monomer a single chain with four domains: N-terminal biotin carboxylase (BC), central carboxyltransferase (CT), C-terminal biotin carboxyl carrier protein (BCCP), and allosteric domains. PC catalyzes the conversion of pyruvate to oxaloacetate (OAA). The process begins when biotin is carboxylated at the BC active site. The BCCP domain transfers the carboxybiotin to an active site in the CT domain. OAA is formed at the CT domain by adding a carboxyl group to pyruvate. Researchers concluded that the BCCP domain swings between active sites on opposite chains, instead of sites on the same chain. The Brown Deer SMART Team (Students Modeling A Research Topic), in collaboration with MSOE, built a model of PC using 3D printing technology illustrating this movement of the BCCP domain. Current research is focused on increasing PC activity through controlling a binding site in the allosteric domain, which may increase insulin production. *Supported by a grant from NIH-NCRR-SEPA.*

I. What's PC?

A possible contributing factor of type II diabetes is a decrease in activity in the enzyme, pyruvate carboxylase (PC), found in pancreatic β cells. PC catalyzes the ATP-dependent carboxylation of pyruvate to oxaloacetate. Normally, PC is involved in a cycle that leads to the release of insulin resulting in glucose absorption from the bloodstream, lowering blood sugar. Reduced PC activity correlates with reduced insulin release. Without proper release of insulin, cells do not absorb glucose and blood sugar increases. When the blood sugar level is too high, it can profoundly harm body tissues leading to complications including heart and kidney disease, stroke, hypertension, and blindness.

PC is a Tetramer

PC is a tetramer composed of two identical dimers, perpendicular to each other, with two chains each, as shown below. Before activation by acetyl coenzyme A (acetyl CoA), the chains on both dimers are equidistant from their partner on the same face. After activation by acetyl CoA, the top chains move together and become active, forcing the bottom chains to move apart.



Models created from adapted PDB file 2QF7.

- CT – Reaction site for Pyruvate & Carboxyl group
- BCCP – Carries Carboxyl group from BC to CT
- BC – Releases Carboxyl group via ATP
- Allosteric – Holds CT to BC and BCCP and allows BCCP movement

II. Why Is PC Important In Diabetes?

- Glucose is transported into pancreatic β cells by a specific glucose transporter.
- Glucose is metabolized through glycolysis, resulting in pyruvate.
- Pyruvate enters the mitochondria. About half of it combines with CoA and enters the TCA cycle (illustrated with green arrows). The other half is carboxylated by PC to generate oxaloacetate (illustrated with black arrows).
- Oxaloacetate is converted to malate and malate exits the mitochondria where it is converted to pyruvate by malic enzyme. Pyruvate can then re-enter the mitochondria. Steps 3 and 4 are part of the pyruvate-malate shuttle. High throughput through this shuttle raises cytosolic NADPH levels.
- Elevated cytosolic NADPH results in the release of insulin from the cell by influencing the activity of a number of other cytosolic enzymes.

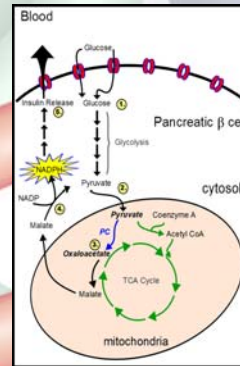
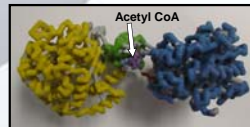


Diagram created by Dr. St. Maurice, Marquette University for Brown Deer SMART Team 2010

IV. Where Does PC Go From Here?

When acetyl CoA (indicated in purple to the lower left) binds to PC, it makes PC more efficient by pushing the two chains on the top face of the tetramer closer together, allowing for faster carboxylation transfer. As of now, scientists are looking for ways to create a molecule more specific to PC that makes PC even more efficient, thereby overcoming deficiencies in the pancreatic β cell insulin release, lowering blood sugar and possibly allowing for a treatment for type II diabetes.

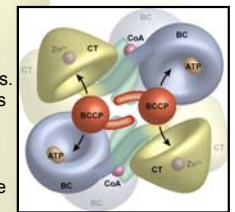


III. How Does PC Work?

IMAGE (Black is Deactivated)	CONSTRUCT	S.A. (MMOL MIN ⁻¹ MG ⁻¹)	ACTIVITY RELATIVE TO CONTROL
	Control	2.5 ± 0.2	100%
	CT Deactivated	0.12 ± 0.02	5%
	BCCP Deactivated	0.0025 ± 0.0008	0.1%
	Chain A CT, Chain B BCCP Deactivated	0.49 ± 0.06	20%

The data above is the result of experiments done by deactivation of specific domains on PC through the process of site specific mutagenesis. Enzymatic activity measured by spectrophotometry, demonstrates that the BCCP domain swings from the BC on the same chain to the CT on the adjacent chain. Model areas colored black in the table represent the deactivated domains.

The simplified model on the right illustrates the swinging action of the BCCP domain from the BC domains to the CT domains on adjacent chains. In the BC domain, a carboxyl group is attached to a tethered biotin on the BCCP domain of the same chain via energy from the hydrolysis of ATP. The BCCP domain then swings to the CT domain on the adjacent chain, where its tethered biotin is decarboxylated. The released carboxyl group then reacts with pyruvate to form oxaloacetate.



V. Conclusion

Pyruvate carboxylase is distributed throughout various body tissues. In pancreatic β cells, reduced PC levels correlate with decreased insulin release. Since PC takes part in a cycle that releases insulin into the bloodstream, it may play an important role in the onset of type II diabetes. PC is allosterically activated by acetyl CoA. As of now, scientists are looking for a molecule that will activate PC more efficiently than acetyl CoA, thereby raising PC activity levels in the pancreas and decreasing the chance of contracting type II diabetes.

OmpR: Outer Membrane Protein Gene Regulator

Regulating *Xenorhabdus nematophila*'s "Appetite for Destruction"

Abstract

OmpR, a regulator of outer membrane protein genes, is a transcription factor necessary for the nutrition gathering strategy of the bacterium *Xenorhabdus nematophila*. Bacteria absorb food through membrane pores, which change in size to optimize food intake and to protect themselves from toxins.

X. nematophila has sensor proteins in the inner membrane. When food touches an inner membrane receptor (EnvZ), the receptor transfers phosphate to OmpR forming OmpR-P, which can bind to DNA. When nutrients are abundant, OmpR-P binds to OmpC, a gene promoting formation of a small pore, allowing food uptake yet limiting influx of toxins. When food is scarce, OmpR-P binds to OmpF, a gene promoting formation of a large pore, allowing more food intake and growth of a flagellum enabling movement to a nutrient rich location.

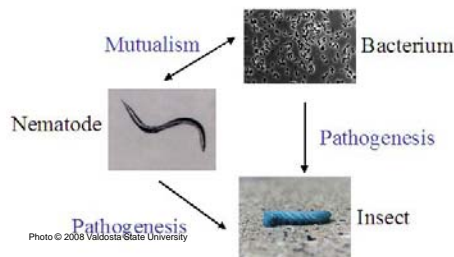
The OmpR gene is also responsible for production of antibiotic compounds that combat a broad range of microorganisms. *X. nematophila* often forms a mutualistic relationship with nematodes. The bacterium-nematode pair seek to inhabit and eventually kill certain insects, benefiting from the nutrients provided by the insect's corpse.

Symbiosis in Nature

The bacterium *Xenorhabdus nematophila* engages in both parasitic and mutualistic relationships. The bacterium is released inside the insect host and produces antibiotics that suppress microbial competition. However, the nematode that also resides in the insect host is immune to the antibiotics.

X. nematophila utilizes the OmpR/EnvZ regulatory system to produce toxins that are involved in killing the insect host (pathogenesis). The toxins produced by the bacteria can be employed as natural pesticides to keep crops healthy. Since the toxins are specifically designed to target insects, they can kill pests without harming the plants.

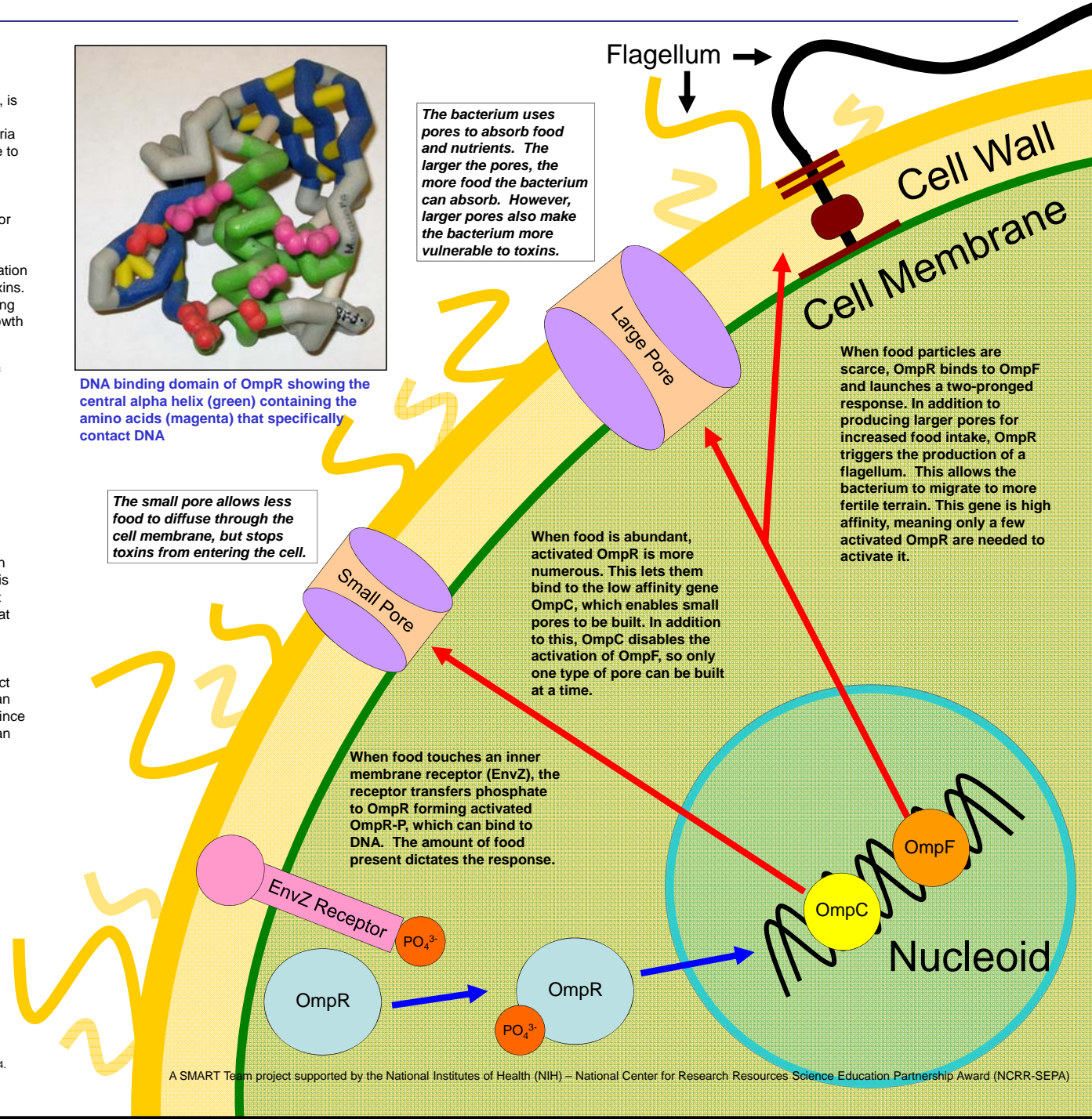
Xenorhabdus host interactions



DNA binding domain of OmpR showing the central alpha helix (green) containing the amino acids (magenta) that specifically contact DNA

The small pore allows less food to diffuse through the cell membrane, but stops toxins from entering the cell.

The bacterium uses pores to absorb food and nutrients. The larger the pores, the more food the bacterium can absorb. However, larger pores also make the bacterium more vulnerable to toxins.



The Way the Armadillo Repeats and the Helix You Can't C

THE ROLE OF B-CATENIN IN CELL DIVISION AND COLON CANCER

SMART Team Student Names

Advisor: Teacher Name

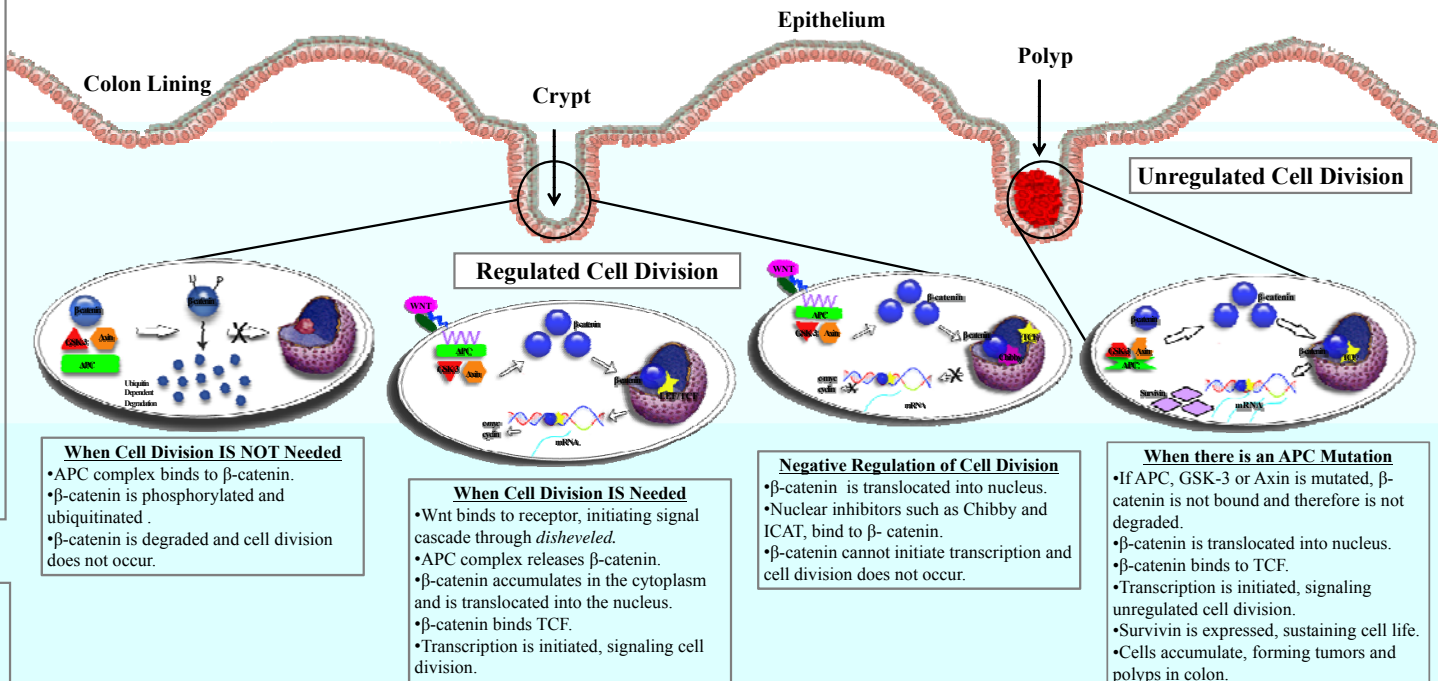
Mentors: Mentor Name and Affiliation



Abstract

Colon cancer is the fourth most lethal cancer in the U.S. As food passes through the colon, water and vitamins are absorbed and epithelial cells are sloughed off and replaced by a tightly regulated cell division process. Unregulated division can lead to the formation of polyps and tumors. β -catenin plays a role in regulating cell division and was modeled by the Messmer SMART Team (Students Modeling A Research Topic) using 3D printing technology. In non-dividing cells, a multi-protein complex of APC, GSK-3 and Axin phosphorylates β -catenin, signaling its degradation, thereby preventing cell division. When cell division is needed, a Wnt signal cascade causes the complex to release β -catenin, stabilizing the protein for nuclear translocation and binding to TCF, a transcriptional activator, thus triggering cell division. Competitive binding of the inhibitor proteins, ICAT and Chibby, to β -catenin negatively regulates this process. In colon cancer, mutations in APC or Axin impede binding of the complex to β -catenin, preventing degradation, leading to increased nuclear localization, binding to TCF and deregulated cell division. Additionally, survivin, an anti-apoptotic protein that enables tumor cell survival, is upregulated. Understanding β -catenin's structure could help design drugs to promote binding of inhibitors to prevent the unregulated cell division of cancer. *Supported by a grant from NIH-NCRR-SEPA*

β -Catenin and Cell Division



Colon Cancer

Cancer is a disease caused by unregulated cell division affecting various parts of the human body, one of them being the colon.

- Colon cancer is the third most diagnosed cancer, and the second leading cause of cancer-related deaths in the United States.
- In 2009 in the US, over 106,000 people were diagnosed with colon cancer and approximately half are expected to die from it.
- The risk of developing colon cancer is 1 in 19, with a slightly higher risk in men than in women.



Normal Colon Colon with a polyp Colon with a tumor

Cell Division in the Colon

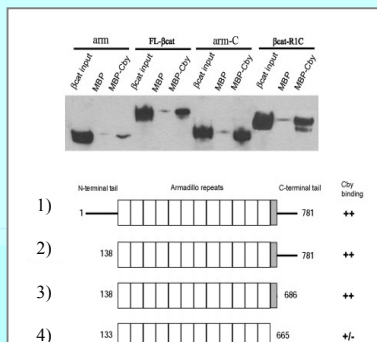
The colon is responsible for the final stage of the digestive system. It absorbs nutrients and water from digested food and eliminates waste from the body. As food passes through the colon, dead cells slough off and must be replaced through cell division. β -catenin, a multifunctional glycoprotein, plays a pivotal role in this process through its involvement with the Wnt signaling pathway and the APC protein. About 85 % of colon cancers are caused by mutations in APC.

β -catenin



β -catenin consists of an N-terminal tail followed by 12 armadillo repeats (ARM) and a final helix called helix C, which is located on the C-terminal end of the protein. The armadillo repeats and helix C bind transcription factors which are crucial positive and negative regulators of cell division. These regions of the protein are potential drug targets for cancer prevention and treatment.

Role of Helix C in Binding to Chibby



Xing, Yi. (2008). Crystal structure of a full length β -catenin. *Structure*, 16, 478-487.

Purpose: To determine what part of β -catenin's structure is important in binding to the nuclear transcription inhibitor, Chibby.

Method: Truncated mutant fragments of β -catenin were produced and assayed for strength of binding compared to intact protein. Fragments tested were 1) full length β -catenin; 2) β -catenin minus the N-terminus; 3) β -catenin minus N-terminus and all but 21 amino acids of the Helix C domain and 4) β -catenin minus the N-terminus and all of Helix C.

Results: Binding to Chibby is weakest when the protein is missing Helix C but is as strong as the intact protein when 21 amino acids of Helix C are present.

Conclusion

β -catenin plays an important role in Wnt signaling of cell division in processes from embryogenesis to cell replacement to tissue repair and wound healing to cancer through its interaction with proteins such as the transcriptional activator, TCF. Nuclear inhibitor proteins such as ICAT and Chibby interfere with the binding of β -catenin to TCF and thus negatively regulate gene activation and cell division. As β -catenin/TCF activation of gene expression has been implicated in multiple diseases from developmental disorders to colon cancer to Alzheimer's, understanding the structural basis of Chibby binding to β -catenin and how this inhibits TCF binding and cell division, is critical in the development of medications and treatments of these diseases

APC: The Key to Colon Cancer

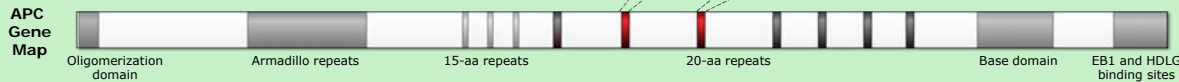
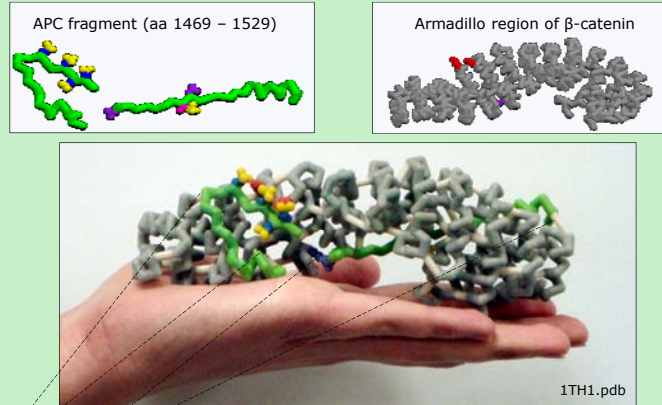
The APC protein and its role in controlling the cell cycle

SMART Team: Student Names
 Teachers: Teacher Names and School Affiliation
 Mentor: Mentor Name and Affiliation

Abstract

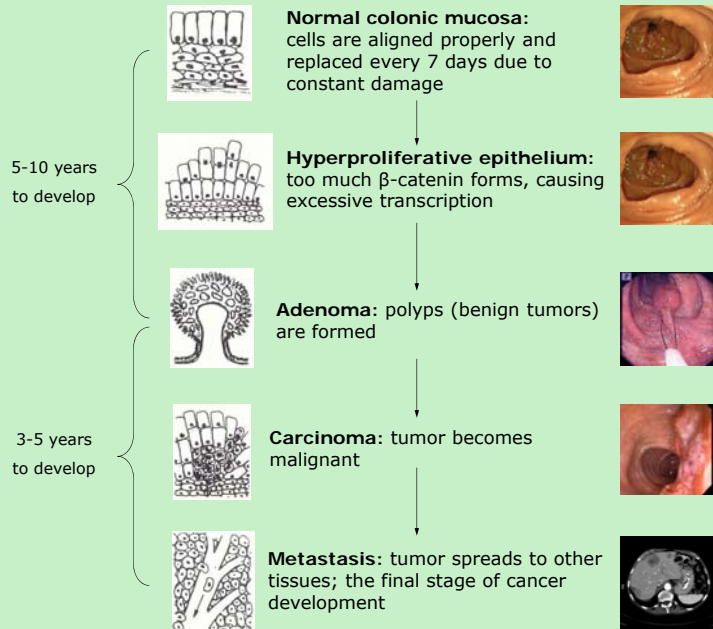
Colorectal cancer affects 1 in 18 Americans, and is linked to mutations in the Adenomatous Polyposis Coli (APC) gene. The rapid division of colonocytes is regulated by the Wntless Type (Wnt) signaling pathway, mediated by β -catenin. In the nucleus, β -catenin binds to Transcription Cell Factor (TCF) and initiates transcription of cell cycle proteins. Alternatively, β -catenin binds to the 20-amino acid repeat region of the APC protein with the help of the scaffold protein axin. The enzyme GSK-3 then phosphorylates threonine and serine residues of the APC protein and subsequently β -catenin. Phosphorylated β -catenin is degraded, slowing mitosis. Mutations in APC allow β -catenin to accumulate, resulting in hyperproliferation of colonocytes, an early step in colon cancer development. As such, better understanding of APC and its function could potentially lead to better diagnosis and treatment in colorectal cancer.

An APC fragment (shown in green) bound to β -catenin's armadillo region (gray). The APC fragment includes two phosphorylated 20-amino acid repeats.

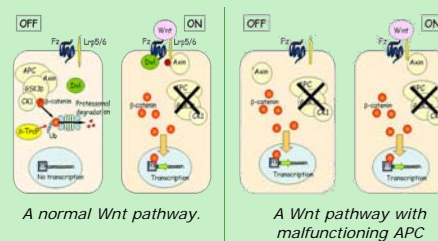


Multi-Hit Hypothesis

Development of colon cancer requires multiple sequential changes in colonocytes.



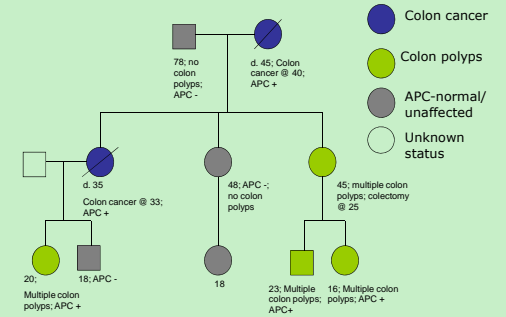
The Wnt pathway is a cell signal cascade regulating cell division. Without the Wnt signal, the cell does not divide; with Wnt, division is promoted. When APC is mutated, mitosis occurs whether or not Wnt is present.



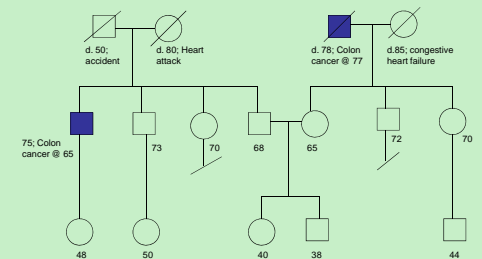
Diagrams from Cell Microscopy Center, University Medical Center Utrecht: http://www.cmc-utrecht.nl/people/Madelon_Maurice/projects/madelon.html

A SMART Team project supported by the National Institutes of Health (NIH)-National Center for Research Resources Science Education Partnership Award (NCCR-SEPA).

Familial vs. Sporadic Colon Cancer



This pedigree portrays a family that has passed down a single APC mutation from generation to generation. Notice how two of the three offspring of the original parents have either early-onset (before the age of 50) colon polyps or colon cancer.



This pedigree portrays a family in which sporadic colon cancer has occurred. Unlike the pedigree above, colon cancer is not common in this family. There are only two occurrences in unrelated males, and cancer appears late in life.

Think About It...

Considering the multi-hit hypothesis, it may take a decade for a polyp to become cancerous. When discovered early, the risk of dying from colon cancer drops considerably. Therefore, it is imperative to get a colonoscopy at least by your 50th birthday. Also, because colon cancer can be a result of hereditary factors, people with a familial history of colon cancer should consult with their physician about when they should start screenings. They may also wish to consult with a genetic counselor.

Special thanks to Sara K. Svendsen, MS, Certified Genetics Counselor, for providing the pedigrees for our poster.

$\alpha_{IIb}\beta_3$: The Key to Platelet Aggregation (and Clotting)

High School SMART Team:

Students A-Z

Advisors: Ms. Teacher Research Mentor: Dr. Mentor

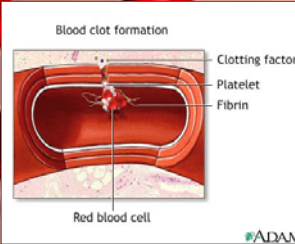
The Story of $\alpha_{IIb}\beta_3$

Blood coagulation, or the clotting of blood, is a vital process in the body wherein a damaged area of a blood vessel is blocked by platelets and fibrin to stop bleeding until it can be repaired. This process involves proteins known as integrins, a kind of integral membrane protein, which mediate cell-cell and cell-surface interactions. Integrin $\alpha_{IIb}\beta_3$, comprised of two glycoprotein subunits and acting as a transmembrane protein on the surface of platelets, and plays a crucial role in the clotting process by acting as a receptor for proteins that mediate the interaction of one platelet to another.

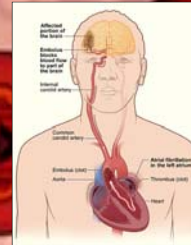
When a blood vessel is damaged, proteins under the endothelial layer of the blood vessel are exposed at the site of injury. Other receptors cause platelets to bind to the site of damage. This initial binding causes the platelets to become activated, which causes the release of many substances supportive of the clotting process, and also results in the activation of $\alpha_{IIb}\beta_3$. It is vital that the activation of $\alpha_{IIb}\beta_3$ is controlled, as an overly large amount of activated $\alpha_{IIb}\beta_3$ would cause excessive clotting. During activation, the structure of $\alpha_{IIb}\beta_3$ changes dramatically, converting from a bent, inactive conformation, into a comparatively 'straight' protein. These changes occur in the integrin because a salt bridge between the intracellular domains of α_{IIb} and β_3 is broken. As a result of this conformational change, amino acids are exposed which form the binding site for several plasma proteins which adhere to the damaged blood vessel, including fibrinogen, von Willebrand Factor, fibronectin, and vitronectin. The binding of fibrinogen cross-links the platelets and results in platelet aggregation at the site of damage. Despite intensive study, the changes leading to the activation of $\alpha_{IIb}\beta_3$ functions are still being clarified. However, because blood clotting plays a role in cardiovascular diseases, scientists are working hard to fully understand the structure and function of $\alpha_{IIb}\beta_3$ and its role in platelet aggregation.

Why Does Clotting Matter?

Blood clotting is a delicate balance that requires the rapid ability of platelets to activate and stem blood flow. If there is too much activation of platelets, there will be **too much** clotting, forming a clot in the blood vessel. This could lead to a variety of dangerous health conditions, including heart attack and stroke. **Too little** clotting can cause severe bleeding (exsanguination). An improper amount of clotting may be due to an insufficient amount of activated $\alpha_{IIb}\beta_3$.



Clotting in a blood vessel



Source: National Heart, Lung, and Blood Institute

Excessive clotting may cause a stroke, leading to brain damage.

Related Medical Conditions

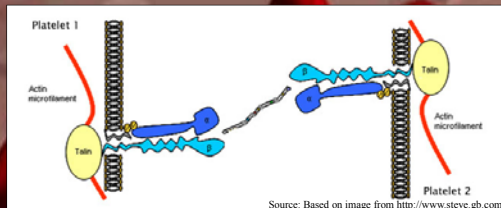
In **Glanzmann's thrombasthenia**, $\alpha_{IIb}\beta_3$ is missing or abnormal due to genetically defective proteins. This abnormality leads to improper clotting and a bleeding disorder.

$\alpha_{IIb}\beta_3$ also plays a role in neonatal alloimmune thrombocytopenia (NATP), where the mother's body attacks a fetus' platelets. This is caused by genetic polymorphism at amino acid 33 in $\alpha_{IIb}\beta_3$. The amino acid there varies - roughly 85% of people have leucine and 15% proline. If a mother has one and the fetus another, the mother forms antibodies against the fetus' platelets, causing NATP. This puts the child at risk of hemorrhage.

Heart attacks and strokes occur when there is a shortage or complete lack of blood flow through a specific portion of the heart. Such occurrences may derive from excess platelet build up at a damaged location on the wall of the blood vessel.

What are integrins?

Integrins are integral membrane proteins on the surface of cells and act as receptors, receiving and sending signals through the cells through inside-out or outside-in communication. In the case of $\alpha_{IIb}\beta_3$, this signal, activated when a platelet comes into contact with damaged blood vessel, triggers the binding of fibrinogen (or the clotted form called fibrin), helping to aggregate platelets into clots.



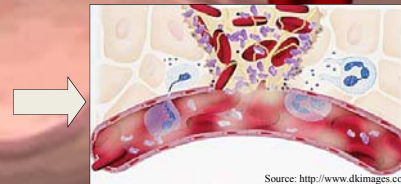
Source: Based on image from <http://www.steve.gb.com>

Here, one $\alpha_{IIb}\beta_3$ integrin on the surface of a platelet interacts with an $\alpha_{IIb}\beta_3$ integrin on another through binding of bivalent fibrinogen. This process mediates the binding of one platelet to another, known as platelet aggregation.

The $\alpha_{IIb}\beta_3$ Activation Process

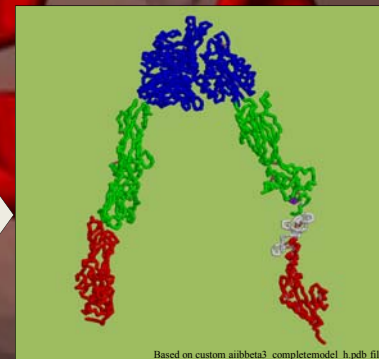


$\alpha_{IIb}\beta_3$ is 'closed' in its inactive state, where it is held together by a salt bridge and is unable to bind fibrinogen to the extracellular domain due to the closed position of the legs in the intracellular domain.



Source: <http://www.dkimages.com>

Blood vessel damage **signals** $\alpha_{IIb}\beta_3$ when the platelet is exposed to the sub-endothelial tissues of the damaged blood vessel.

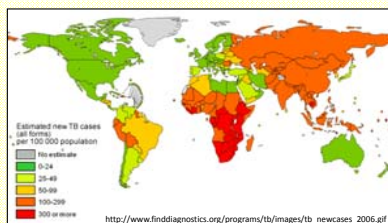


Based on custom aIIb beta3 completemodel h.pdb file

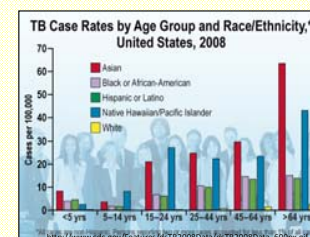
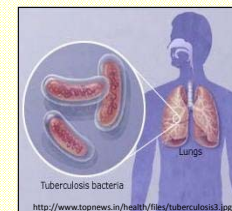
This process leads $\alpha_{IIb}\beta_3$ to activate, and it in turn begins to facilitate the binding of fibrin. In this active form, which we modeled, the legs are separated and the extracellular domain, formerly bent, is upright. Fibrinogen binds at the blue top, where the α and β subunits meet.

Abstract: One-third of the world's population is infected by *Mycobacterium tuberculosis* (*M.tb*). Two million people die each year from tuberculosis (TB), the disease caused by this bacterium. TB primarily affects the lungs and is easily transmitted. One way to kill *M. tb* might be to inhibit the enzyme dihydrofolate reductase (DHFR). DHFR catalyzes the production of tetrahydrofolate by transferring a hydrogen ion from NADPH (nicotinamide adenine dinucleotide phosphate) to dihydrofolate, thereby releasing tetrahydrofolate and NADP⁺. Tetrahydrofolate is essential to the bacteria's survival, and is a cofactor that is needed for the synthesis of the DNA base thymine. Isoniazid is one antibiotic already used to treat TB by targeting several TB proteins that are necessary for building *Mycobacterium tuberculosis* cell walls and inhibiting DHFR. Unfortunately, strains of *M. tb* are evolving resistance to isoniazid, so next generation antibiotics are needed. A variation of isoniazid could be designed to avoid resistance, and to inhibit DHFR, thereby targeting bacterial DNA synthesis. The Valders SMART team, using rapid three dimensional printing technology, created a physical model of DHFR and a possible inhibitor of tetrahydrofolate production. *Supported by a grant from NIH-NCCR-SEPA.*

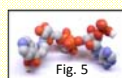
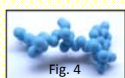
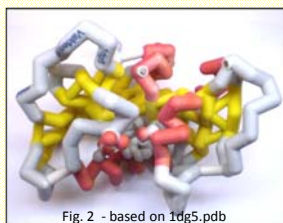
Introduction: Two million people die each year from tuberculosis (TB). The bacterium that causes this disease, *Mycobacterium tuberculosis*, infects over one third of the world's population. This study is devoted to modeling a new drug for Trimethoprim, an antibiotic used to treat TB that is losing effectiveness due to drug resistance. We are modeling a possible inhibitor of tetrahydrofolate production that could effectively prevent TB cell multiplication and could possibly be used as an antibiotic to treat the infection.



Disease Aspect: TB primarily affects the lungs and can be transmitted by coughing and sneezing. The active infection of tuberculosis has symptoms that include a mild fever, weight loss, night sweats and persistent coughing. The treatment takes place over a six month span and involves a cocktail of four anti-TB drugs. Not everyone that is infected with TB gets sick, which is called latent TB infection. This occurs when the bacteria live in the body but do not cause visible symptoms. At this stage, TB is not contagious, but if untreated it can become active causing the bacteria to multiply. If TB goes untreated, it can be fatal.

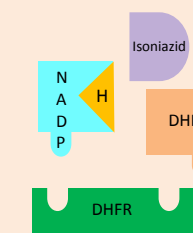


The Problem: Bacteria are becoming resistant to current drugs, like trimethoprim (Fig. 1). The goal of this research is to find a drug that binds to dihydrofolate reductase (DHFR) (Fig. 2) like trimethoprim does. Isoniazid has the potential to be this alternative drug. The natural process shown to the right, models the normal activity of DHFR in a bacterial cell. The production of tetrahydrofolate (THF) allows for the synthesis of DNA that is needed for bacterial cell (TB) growth.

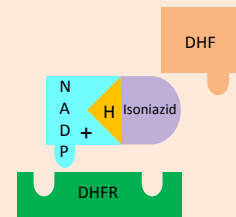


A Novel Approach: Isoniazid is a pro-drug (Fig. 3). It is consumed in its inactive form and then converted to an active form in the liver. The process of this activation allows the potential for drug resistance to develop. The lab of Dr. Daniel Sem hopes to engineer a form of isoniazid that does not require this step, therefore hopefully preventing future drug resistance. The inhibited process modeled to the right shows how isoniazid may prevent THF production. This process is believed to mimic that of trimethoprim which inhibits DNA synthesis in other bacteria. Fortunately, human DNA synthesis would not be inhibited because there is only a 26% sequence alignment between human DHFR and *M. tuberculosis* DHFR.

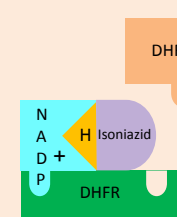
Method of Study: Nuclear magnetic resonance, or NMR, is used by scientists to understand the structure and function of proteins. Nuclei of atoms (in this case, nitrogen atoms) have spin when in a magnetic field, and when a second electromagnetic field is applied, the atoms "flip", and this phenomenon is visualized as an NMR spectrum (Fig. 6), with each backbone amide (N-H) of the protein represented as one "spot" - called a cross-peak. One cross-peak has been expanded to show how it shifts upon a protein binding to an inhibitor. Such data could be used to determine where (and how tightly) a molecule like NADP⁺ or trimethoprim binds to DHFR. Related experiments can be used to calculate a 3-D structure of the protein-drug complex.



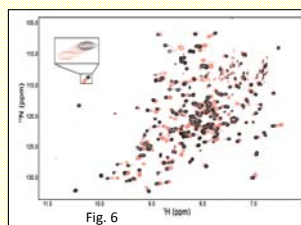
1. Isoniazid is consumed in an inactive form and then metabolized to an active form, the "radical".



2. The radical binds nonenzymatically to NADP⁺ to form the "adduct". (Fig. 5)



3. The adduct binds to DHFR and prevents DHF and NADPH from binding. THF does not form causing the bacteria to die.



NMR Binding Analysis:

To confirm that a molecule like NADP⁺, isoniazid, or trimethoprim binds to DHFR, an NMR titration experiment could be done. The spots in this spectrum correspond to amides in a protein (one of each amino acid) - and they change their location in the spectrum when increasing amounts of the binding molecule are added. The different colors represent spectra with more of the binding molecule added.

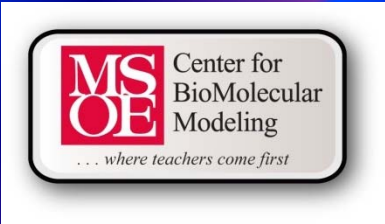
Conclusion: Tuberculosis causes two-million deaths every year worldwide. A common antibiotic used to treat other bacterial infections, by inhibiting DHFR, is trimethoprim. Isoniazid, used to treat TB, also inhibits DHFR - but it is losing effectiveness. It is hoped that a modified version of Isoniazid can be designed, based on structures of trimethoprim bound to DHFR. Isoniazid inhibits THF restricting production of thymine which inhibits tuberculosis cell replication, therefore killing the TB bacteria. Isoniazid is a pro-drug so it is consumed in an inactive form and then converted to an active form inside the liver. This creates potential for form resistance. Scientists hope to have the modified drug skip this step. The new version could then be used as a treatment for the third of the world population that is infected with tuberculosis.

References:

- Argyrou et al. Nature Structural & Molecular Biology. 2006 May;Vol (13)5:408-413
- Li et al. Journal of Molecular Biology. 2000;295:307-323

2009-2010 SMART Team

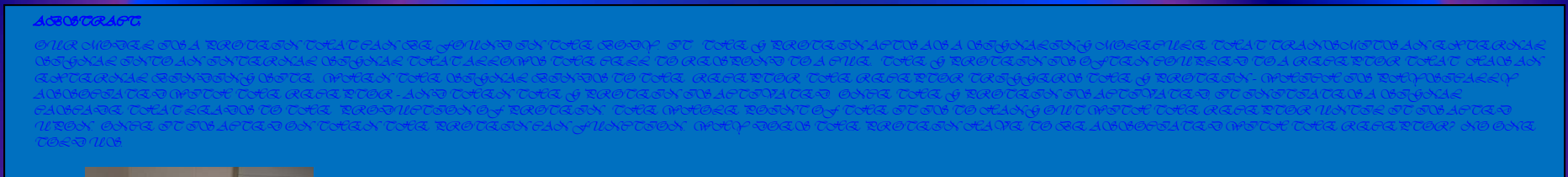




Our Model



Shannon Colton, Center for BioMolecular Modeling, Milwaukee School of Engineering, Milwaukee, WI

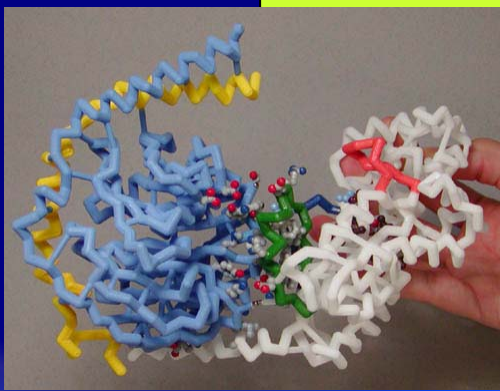


...where teachers come first

Conclusion:
In conclusion, our SMART Team has covered that science can be a lot of hard work, it can be very challenging, but it can also be lots of fun. Science Rocks!!!

•The toxin that is released from *E. coli* is called Shiga Toxin. The toxin has two main parts to its structure, a catalytic domain and a binding domain. The binding domain is responsible for targeting the toxin to specific cells. In this case, the toxin is specifically looking for cells that have the Gb3 receptor located on the cell surface. Once the Shiga Toxin locates this receptor, the toxin will dock onto the surface of the cell.

•From this point, the Shiga Toxin is brought into the cell via receptor-mediated endocytosis enclosing the toxin within an endosome. The endosome travels to the ER retrogradely through the Golgi apparatus. At this point, the catalytic domain of the Shiga Toxin is brought to the cytoplasm and it targets the ribosome.

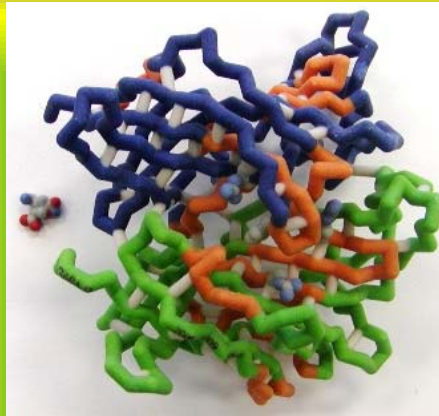


If this protein is missing, bad things happen to the body. Cells cannot talk to one another anymore and that means that the cells cannot tell each other what to do and that means that the body cannot function and that means that the cells will die and that means that the body will die, too.



Our Model is Cool!

Shannon Colton, Center for BioMolecular Modeling, Milwaukee School of Engineering, Mke, WI



*Function of the protein:
This protein is made of three parts. It can be found in a cell.*

*THE TOXIN THAT IS RELEASED FROM *E. COLI* IS CALLED SHIGA TOXIN. THE TOXIN HAS TWO MAIN PARTS TO ITS STRUCTURE, A CATALYTIC DOMAIN AND A BINDING DOMAIN. THE BINDING DOMAIN IS RESPONSIBLE FOR TARGETING THE TOXIN TO SPECIFIC CELLS. IN THIS CASE, THE TOXIN IS SPECIFICALLY LOOKING FOR CELLS THAT HAVE THE Gb3 RECEPTOR LOCATED ON THE CELL SURFACE. ONCE THE SHIGA TOXIN LOCATES THIS RECEPTOR, THE TOXIN WILL DOCK ONTO THE SURFACE OF THE CELL.

*FROM THIS POINT, THE SHIGA TOXIN IS BROUGHT INTO THE CELL VIA RECEPTOR-MEDIATED ENDOCYTOSIS ENCLOSING THE TOXIN WITHIN AN ENDOSOME. THE ENDOSOME TRAVELS TO THE ER RETROGRADE THROUGH THE GOLGI APPARATUS. THE CATALYTIC DOMAIN OF THE TOXIN IS BROUGHT TO THE CYTOSOL AND IT TARGETS THE RIBOSOMES.

cannot function and that means that the cell will die and that means that the body will die, too.



Conclusion:

In conclusion, our SMART Team has discovered that science can be a lot of hard work, can be very challenging, but it can also be lots of fun.

Science Rocks !!!



...where teachers come first