Supplemental Material

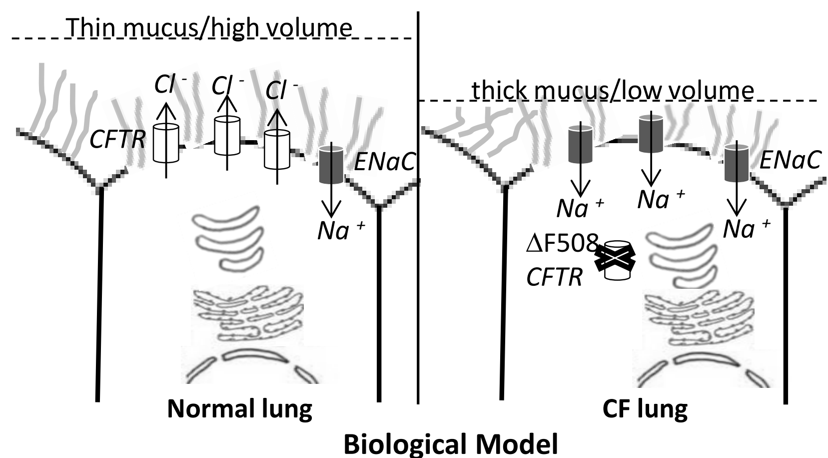
CBE—Life Sciences Education

Zagallo *et al.*

SUPPLEMENT 1. Problem-Sets that were given to students as worksheet handouts. These 4 problem-sets (Cystic Fibrosis, Cell Cycle, RTK Signaling, BRCA Tumors) were the focus of analysis in the paper.

CYSTIC FIBROSIS

Cystic Fibrosis (CF) is a genetic disorder that results from a mutation in an ion channel termed CFTR. The lung is one of the organs most affected by the disease. Thick mucus builds up on the surface of alveolar cells in the lung and patients are plagued with frequent lung infections. In individuals who do not have CF, CFTR allows chloride ions to flow from the inside of lung cells to the extracellular space. Extracellular chloride concentrations are high, relative to intracellular, as are extracellular sodium concentrations; intracellular potassium concentrations are high. At the steady state, water diffusion maintains an appropriately fluid mucus lining of the alveolar space. The most common CF-causing mutation (ΔF508) results in a mis-folded protein that is not properly modified in the Golgi, is degraded by the cell, and is almost absent from the cell surface. The biological model depicts the alveolar space in normal and diseased lungs. The current CF model includes increased levels of a sodium channel (ENaC) at the surface of lung cells when CFTR is missing from the cell surface. This mis-regulation of ENaC further exacerbates the salt and water balance in patient's lungs. The following experiments, from research over a number of years, have contributed to this model of CF disease.



Q1: Given what you know about typical membrane potentials and the chemistry of ions behavior, why does sodium flow in the direction shown in the model?

Q2: Based on the biological model, how would you predict that the intracellular concentration of Cl^- and Na^+ would be different in normal and CF patient lung cells? How do you predict this difference would affect the net diffusion of water between the mucus and the intracellular environment?

In Experiment 1 (Boucher, Cotton, Gatzy, Knowles, & Yankaskas, 1988)**, airway cells were collected from normal and CF patients and cultured. A microelectrode was used to measure potential differences (V_a) across the membranes of individual cells (similar to Patch Clamp analysis). Panel 1 is a representative example of the trace from a single sample for which the membrane potential was measured in a control solution (Ringer) and then after the introduction of a chemical that specifically inhibits ion flow through sodium channels (Amiloride).**

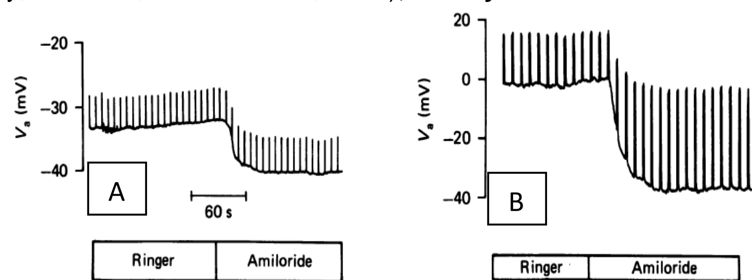


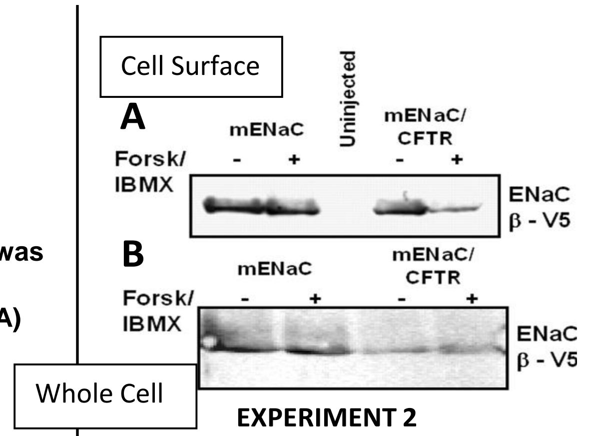
Fig. 8. Tracings of response of normal (*A*) and CF (*B*) cultures to exposure to amiloride (10^{-4} M, luminal). Note in these tracings V_a was monitored directly.

Experiment 1

Q3: What is the potential difference across the membrane of normal and CF cells before the Amiloride treatment? What might account for this difference between samples?

Q4: What is your interpretation of the sample data shown when cells are treated with Amiloride? What factors in the biological model could account for how each tissue responds to this drug?

In <u>Experiment 2</u> (Yan, Samaha, Ramkumar, Kleyman, & Rubenstein, 2004)**, Xenopus oocytes (which are large and easy to manipulate) were injected with mRNA message allowing cells to express either ENaC or ENaC and CFTR. Both sets of cells were stimulated with forskolin-IBMX (which activates CFTR to allow Cl^- flow) or with a control solution. Western blots were performed with an antibody specific to a portion of the ENaC protein; an artificial tag was included that allowed researchers to only detect ENaC on the cell surface through a biotinylation procedure (panel A) or to detect all ENaC in whole cell lysates (panel B).**



Q5: What is your interpretation of the data in Experiment 2?

Q6: How do these findings confirm the biological model? What do they add to the model?

The data in Experiment 2, and other findings, suggested a regulatory relationship between CFTR and ENaC. Recently, a group of researchers (<u>Experiment 3</u><u>(Grubb et al., 2012)</u>**) decided to explore this relationship further using a transgenic mouse that overexpresses the ENaC protein (βENaC mice); these mice have a CF-like phenotype with reduced volume of liquid on the airway surface (ASL) and mucus plugging of the airways. They crossed βENaC mice with mice that overexpressed human CFTR specifically in lung cells (hCFTR mice). They then added colored fluid on top of cultured airway cultures from each group of mice (including a normal control) and measured the height of the volume of liquid (ASL) as it eventually reached a steady state level, using confocal microscopy.**

For data figure used, please refer to Grubb et al. 2012.

Figure 8. Dysregulation of airway surface liquid (ASL) volume in β-ENaC and hCFTR/β-ENaC mouse cultures

EXPERIMENT 3

Q7: If CFTR has a direct effect on the cell surface levels of the ENaC protein, what results would you predict for Experiment 3? Draw your own model (adapting the biological model given) for what you might hypothesize would happen in this case.

Q8: What is your interpretation of the data in Experiment 3? Why does the volume of the water on the surface of the cells seem to change depending on the presence of ion channels?

Q9: Do the results of Experiment 3 confirm your model? Why or why not?

CELL CYCLE

This worksheet is based on work by Evans and Rosenthal et al. (1983) and Murray, Solomon and Kirschner (1989) (Evans, Rosenthal, Youngblom, Distel, & Hunt, 1983; Murray, Solomon, & Kirschner, 1989). The first set of data were obtained from dividing sea urchin eggs, when scientists had relatively little idea about what causes cells to progress through the cell cycle. The second set of data were obtained from a xenopus egg extract system. At the time of the second paper, scientists were hypothesizing the role of cyclin as a regulatory component of maturation promoting factor. At the point this paper was published, scientists were trying to functionally explain the exit of cells from mitosis using what they knew about MPF.

For image of biological model used, please refer to Gautier et al. 1990.

Figure 7. Diagram of the cdc2/Cyclin Cycle

Biological Model (Gautier et al., 1990)**.** Diagram of the cdc2/Cyclin Cycle. This simplified diagram illustrates the essentials of the relationship between cyclin, cdc2, kinase activity and the cellular consequences of that activity. Newly synthesized cyclin joins preexisting cdc2 to form pre-MPF, which lack kinase activity until the cdc2 subunit is dephosphorylated. Cyclin is phosphorylated as the cdc2 becomes active and active MPF is then able to initiate entry into mitosis. Cyclin is subsequently destroyed. This leads to loss of MPF activity and return to interphase.

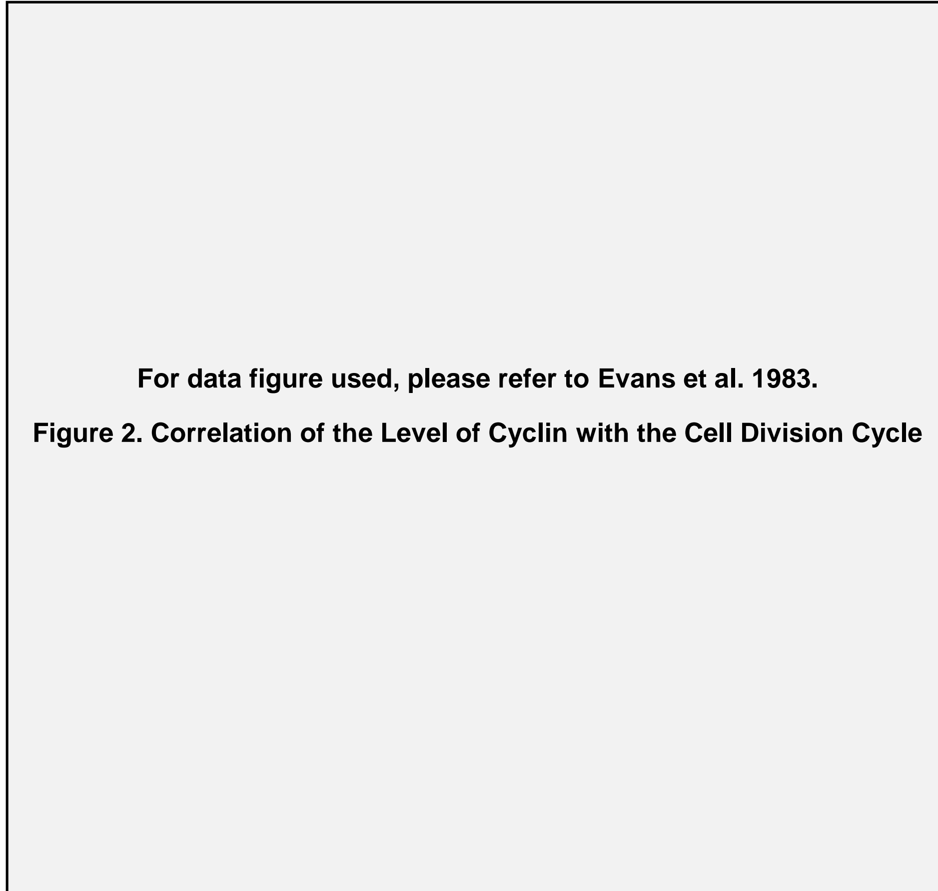


Figure 1. A suspension of urchin eggs was fertilized, and after 6 minutes radio-labeled methionine was added. Samples were taken for analysis on gel electrophoresis at 10 minute interviews, starting 16 minutes after fertilization. The autoradiograph of the electrophoretic gel was scanned for label density, and the data were plotted as shown. The percentage of cells undergoing division at any given time are plotted as the "cleavage index". The authors called Band A "cyclin".

Q1. Describe and interpret the data in Figure 1.

Q2. Relate these findings to the biological model.

For data figure used, please refer to Murray, Solomon, & Kirschner 1989.

Figure 3. Induction of mitosis by cyclin made in reticulocyte lysate

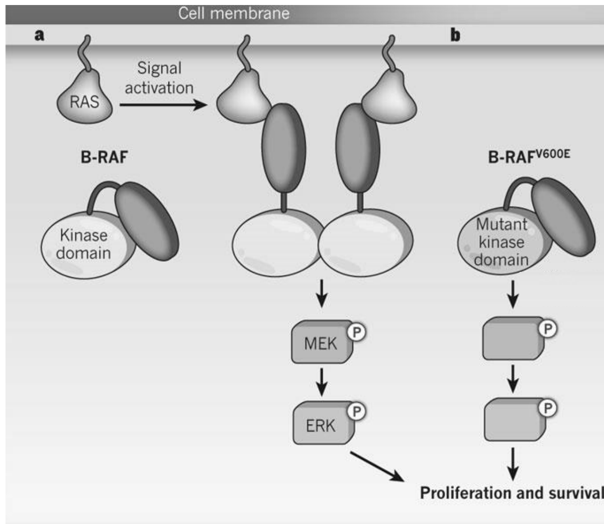
Figure 2. These experiments were performed by adding sperm nuclei to oocyte extracts as an artificial system to measure movement of the nuclei between mitosis and interphase. mRNA for the cyclin protein was added to the system to allow synthesis of cyclin proteins. The cylin mRNA was either for a version similar to wild-type (CYC wild type) or for a mutant version of the protein that was not able to be cleaved by proteolysis (CYCdelta90). Radiolabled- methionine was also added, so that when cyclin proteins were made they could be detected when run on gel. The times shown were minutes after the extracts were combined with the nuclei. The indicator of artificial entry into mitosis was measured by watching nuclear break down (NBD). Return of nuclei to an interphase-like state was also observed (Inter).

Q3. Describe and interpret the data in Figure 2.

Q4. Relate these findings to the biological model.

Q4. What does the experiment in Figure 2 add to the experiment in Figure 1?

RTK SIGNALLING



This model (Lavoie & Therrien, 2011) depicts how a mutant form of B-RAF can activate MEK and thus ERK, leading to cellular proliferation, even in the absence of signaling through RAS. The model zooms into a portion of the RTK pathway (some parts are not shown). Prior to the research shown here, scientists had shown that mutations in RAS were common in malignant tumors and that this was because constitutively active RAS could lead to signaling events that promoted unregulated cellular proliferation. The authors of the data figures below (Davies et al., 2002) are trying to demonstrate that mutations in B-RAF can also contribute to the development of cancer, independent of mutations in RAS.

The results in Table 1 show a screen of samples taken from various human tumors. Each line shows a different mutation found in the B-RAF gene. “Mel.” and “Mel STC” are two forms of melanoma. “Colo. ca.” is colorectal cancer.

Table 1 *BRAF* mutations in human cancer

BRAF mutations		Primary tumours					
Nucleotide	Amino acid	(1) Mel. STC	(2) Mel.	(3) Colo. ca.	(4) Ovarian*	(5) Sarcoma	(6) Other†
G1388A	G463E						
G1388T	G463V						
G1394C	G465A	1					
G1394A	G465E		1				
G1394T	G465V						
G1403C	G468A						
G1403A	G468E			1			
G1753A	E585K				1		
T1782G	F594L			1			
G1783C	G595R						
C1786G	L596V						
T1787G	L596R				1		
T1796A	V599E	11	5	2	3	1	0
TG1796-97AT	V599D						
	Total	12	6	4	5	1	0
No. samples screened		15	9	33	35	182	104
Per cent		80%	67%	12%	14%	0.5%	0%

Q1. What can you conclude from the data in Table 1? How do they support the idea that B-RAF is a clinically important oncogene? [some columns, from other types of samples, are deleted for simplicity]

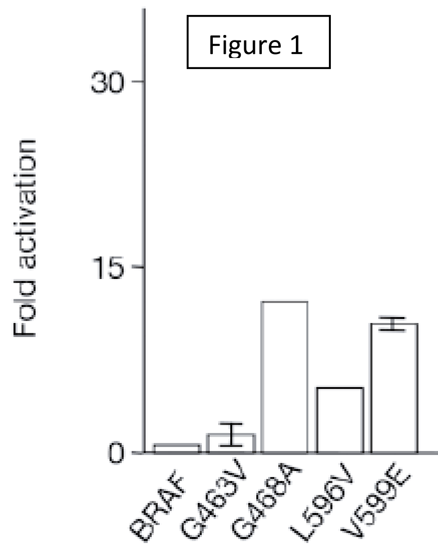


Figure 1. In this experiment, B-RAF protein was isolated and combined with additional proteins in vitro. The level of activation of MEK in vitro was then measured. B-RAF is the wild type version of the protein. G463V, G468A, L596V and V599E are four different B-RAF mutants. Fold activation is determined by dividing the level of activation measured by the level measured for the control (BRAF). Thus it represents the "fold" increase over baseline. <u>In a prior experiment, 89% of the mutations found in the cancer cells were within or very close to the activation segment of B-RAF, which is responsible for its protein kinase activity. Each of the mutants represented in this figure were from mutations found in the kinase domain of the protein.</u>

Q2. Describe and interpret the results of the experiment performed in Figure 1.

Q3. Explain how these results relate to the biological model.

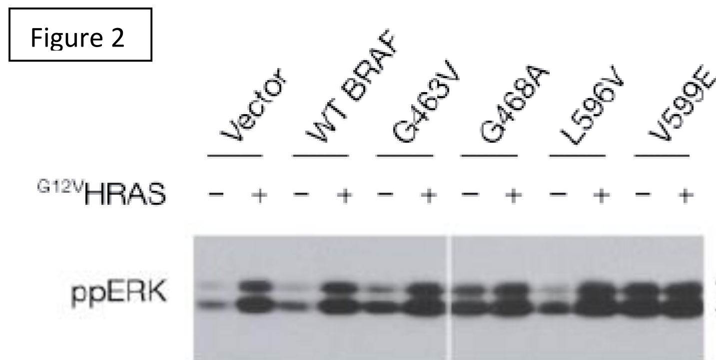
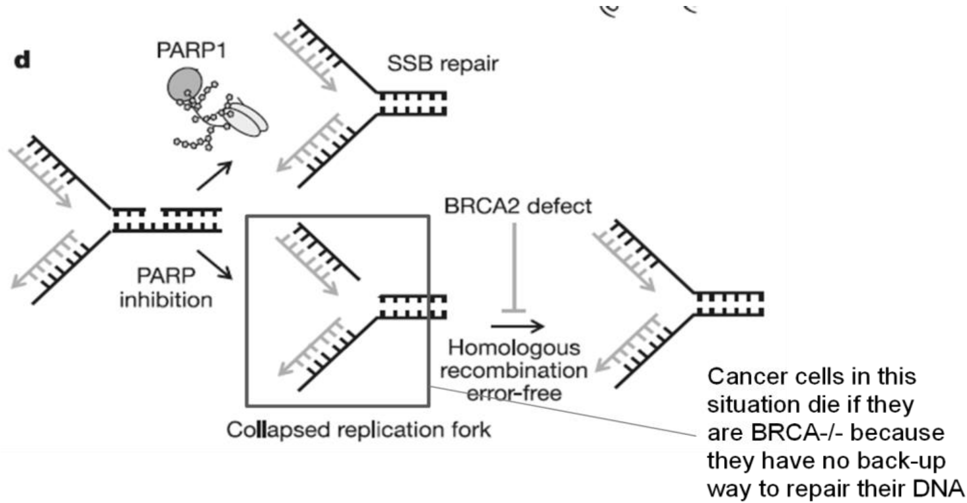


Figure 2. In this experiment, half of the cells were transfected with constitutively active Ras (G12V HRAS). Some cells were also transfected with wild type B-RAF or one of the B-RAF mutant proteins. Western blots were performed with antibodies that specifically bind to phosphorylated ERK proteins (ERK1/2) (the antibody does not bind to the ERK protein if it is not phosphorylated).

Q4. Describe and interpret the results of the experiment performed in Figure 2

Q5. Explain how these results relate to the biological model.

BRCA TUMORS



This model (Bryant et al., 2005) above explains why BRCA2 -/- tumors are thought to respond to PARP inhibitors. PARP is a normal cellular protein that is required for repair of single stranded break in DNA (something that happens all the time in cells). There are actually two PARP proteins referred to in this paper PARP1 and PARP 2. The authors' model suggest that PARP1 is the protein important here. In this model PARP inhibition is accomplished through treatment with specific drugs. The collapsed replication fork is thought to form when single stranded breaks cannot be repaired. The collapsed replication fork includes a double stranded break in DNA. Normally, these double stranded breaks can be repaired through another mechanism (homologous recombination error-free). This mechanism does not function properly in BCRA-/- cells. Thus BRCA-/- cells do not have an efficient way to repair these double stranded breaks. Data figures are from a published article (Bryant et al., 2005).

Q1: Looking at the model, explain what happens when PARP proteins are inhibited?

Q2: What is the function of wild type BRCA2 in this model?

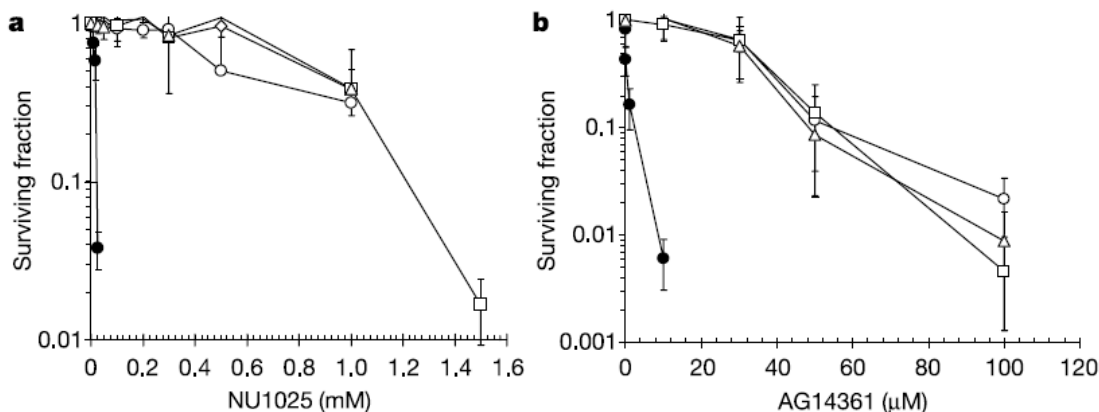


Figure 1 This assay looks at survival of cells in vitro. Cells were treated with increasing concentrations of two different PARP inhibitors (NU1025 and AG14361). Wild type cancer cells are shown by open circles; BBCA2 deficient cells are shown by filled circles. BRCA2 deficient cells with BRCA2 complemented (added back) in two different ways are shown by squares and triangles.

Q3. Describe and interpret the data shown in Figure 1 and explain how it relates to the model.

Q4. Describe and interpret the data shown in Figure 2 and explain how it supports the model.

Figure 2 Cultured cancer cells were treated with various forms of siRNA. RT-PCR for BRCA2, PARP1, PARP2, and β-actin was performed on mRNA collected from these cells (panel A). In a separate set of experiments, cancer cells treated with siRNA (as in panel a) were followed for 48 hours after siRNA treatment and survival rate was measured.

Figure 2

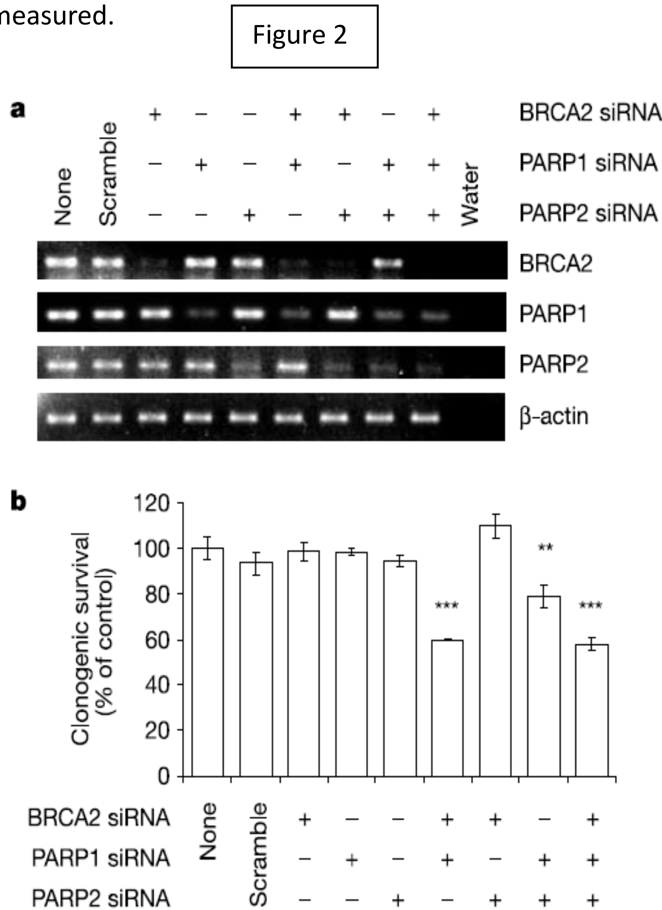


Figure 3

For data figure used, please refer to Bryant et al. 2005.

Figure 3. BRCA2-deficient cells fail to repair a recombination lesion formed by inhibitors of PARP

Figure 3. Cancer cells were cultured without (control) or with different concentrations of a PARP inhibitor (NU1025). Cells were examined for an indicator of double stranded breaks (panel b) or for an indicator of homologous recombination for repair of double stranded breaks (panel C). In both panels, black bars represent BRCA2 deficient cells and white bars represent BRCA2 deficient cells with BRCA2 added back (in other words, cells that contain BRCA2)

Q5. Describe and interpret the data shown in Figure 3 and explain how it supports the model.

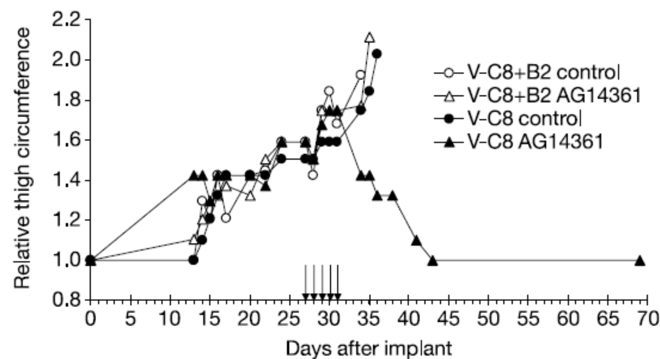


Figure 4. Cancer cells were injected into the thigh of mice to induce formation of a tumor. Then the growth of the tumor was measured over time. During the five days indicated by the arrows, mice were injected with either a PARP inhibitor (AG14361) or saline as a control. V-C8 is a BRCA2 deficient cancer cell line. V-C8+B2 is that same cell line with BRCA2 added back.

Q6. Describe and interpret the data shown in Figure 3 and explain how it supports the model.

SUPPLEMENT 2. Two examples of using formative and summative assessments for learning objectives that integrate content and scientific practice.

Sample Learning Objective 1 (one of the objectives for a protein trafficking unit which included receptor-mediated endocytosis): *Relate experimental data to the model of receptor-mediated endocytosis that explains familial hypercholesterolemia in the typical and JD cases.*

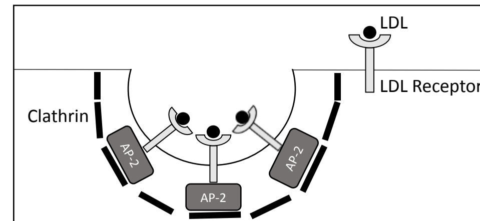
[Data for LDL binding and internalization are taken from Brown and Goldstein, 1974 (left) and Davis et al., 1986 (right).(Brown & Goldstein, 1974; Davis et al., 1986)]

Sample Clicker Question:

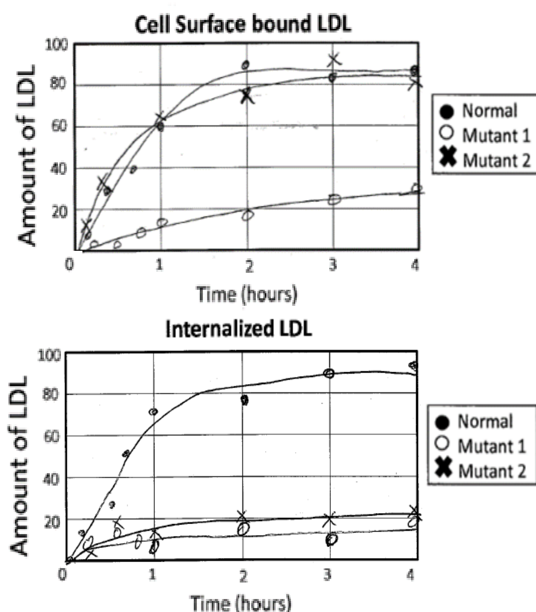
Which of the following did the researchers NOT know before the start of their study in the research article?

A) *familial hypercholesterolemia* could be caused by a mutation in the LDL receptor
B) LDL binds to receptors at the cell surface
C) The LDL receptor tail interacts directly with coated-pit components
D) LDL receptors enter the cell via endocytosis at coated pits

Sample Multiple-Choice Exam Question:



You are studying the inherited condition *familial hypercholesterolemia*, which causes afflicted patients to have profoundly elevated levels of cholesterol in their blood. You have identified two mutations in the LDL receptor in these patients, Mutation 1 (very common) and Mutation 2 (quite rare). You have cell lines that have been created from cells from these patients as well as from people without the condition. You are conducting an experiment to measure binding and internalization of LDL by cells that have each mutation, compared to "normal" cells. Here are your results:

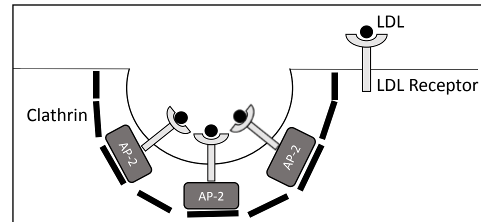


Which of the following is the MOST REASONABLE statement about the mutations in the LDL receptor?

A) Mutations 1 and 2 both affect the cytoplasmic region of the LDL receptor.
B) Mutations 1 and 2 both affect the extracellular region of the LDL receptor.
C) Mutation 1 affects the extracellular region of the LDL receptor and Mutation 2 affects the cytoplasmic region of the LDL receptor.
D) Mutation 1 affects the cytoplasmic region of the LDL receptor and Mutation 2 affects the extracellular region of the LDL receptor.
E) Mutations 1 and 2 both affect the transmembrane region of the LDL receptor.

Sample Short Answer Exam Question:

You are working with the Goldstein lab to provide evidence for their model of the interaction between membrane receptors and clathrin coated pits - shown to the right. You goal is to relate this model to the process of receptor mediated endocytosis, specifically you are investigating endocytosis of LDL. For your experiments you are using cultured cells and knocking down the AP2 adaptor protein using siRNA (or using scrambled siRNA). You are then providing cells with LDL and then collecting endocytosed vesicles via fractionation (a process that uses centrifugation to separate different parts of cells by their density). You perform Western blots for several proteins on the vesicles you collected. Your results are shown below.



A) **Describe** and **interpret** the results of your Western blot. **Relate** these results to the biological model.

Sample student answer: "In untreated cells, endocytosed vesicles contain AP2, LDL receptor, and clathrin, which the model predicts. When AP2 is knocked down in Lane 2, AP2 isn't present, indicating the siRNA was successful. LDL receptor also isn't present. Clathrin is present, indicating vesicles coated with clathrin were still endocytosed. The results for the untreated cells indicate that AP2, LDL receptor, and clatrhrin all ended up in the vesicle. When AP2 is absent, the vesicle still forms, but LDL receptor isn't present, indicating that AP2 is necessary for LDL receptors to bind to coated pits. This supports the model, which shows that APO2 connects LDL receptor to clathrin, allowing them to be endocytosed together. The scrambled siRNA results match the untreated indicating that technique did not cause abnormalities."

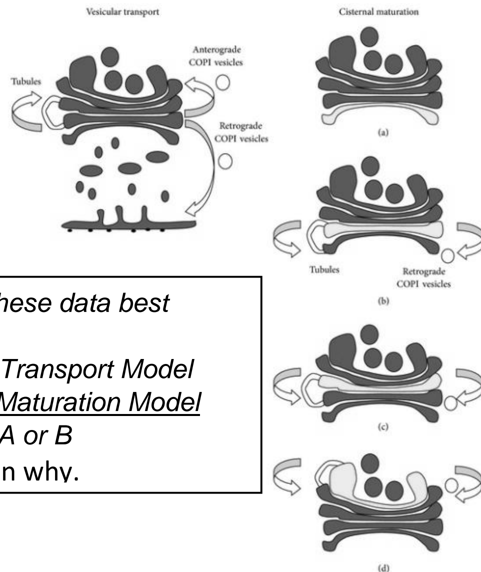
B) Next you perform an immuno-gold electron microscopy experiment in which you stain cells for the LDL receptor (just like we did in journal club worksheet). Describe the experimental results you would expect for each of your experimental conditions (untreated cells, AP2 siRNA, and scrambled siRNA) in your immuno-gold electron microscopy experiment. In other words, what would you likely see in each condition?

Sample student answer: "For untreated, you would expect LD receptor to be localized to the coated pits. For scrambled siRNA, you would expect the same. For AP2 siRNA, you would expect to see LDL receptor spread out all over the plasma membrane. Scrambled siRNA is you negative control."

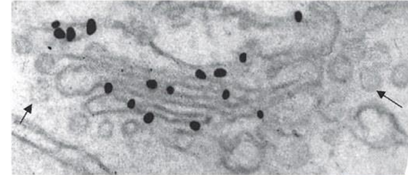
Sample Learning Objective 2 (one of the objectives for a protein trafficking unit which included Golgi transport): *Use experimental evidence to argue for the vesicular transport model or cisternal maturation model of transport of proteins through the Golgi complex.*

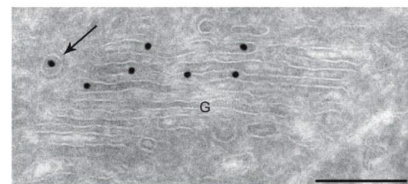
Model taken from Martinez-Menarguez 2013 (©Creative Commons); Electron micrographs from ©Mironov et. al., 2001 originally published in JCB. doi *10.1083/jcb.200108073* and ©Martinez-Menarguez et al., 2001 originally published in JCB; Fluorescent micrograph from Losev et. al 2006

Sample Clicker Question:



VSVG, secreted protein, in cisternae but not vesicles





Mannosidase II, Golgi enzyme,

Which model do these data best support?

A) Vesicular Transport Model

B) Cisternal Maturation Model

C) Could be A or B

Be ready to explain why.

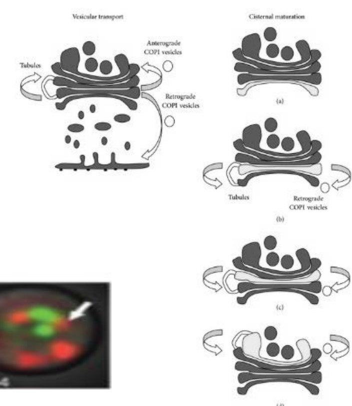
Sample Multiple-Choice Exam Question:

Which of the following is necessary for the Cisternal Maturation model of protein transport through the Golgi, but not true of the Vesicle Transport Model?

A) Golgi enzyme proteins move in vesicles in a retrograde direction.
B) Secreted proteins move in the retrograde direction.
C) Secreted proteins move in vesicles between cisternae.
D) Golgi cisternae each contain different enzymes that function to modify carbohydrates.

Sample Short Answer Exam Question:

In the fluorescent micrographs, each spot represents an individual Golgi cisternae. The arrow is pointing to the same cisternae at different points in time (time is passing from left to right). These cells were stained for a-manosidase I (a cis-Golgi enzyme), shown in green, and galactosyl transferase (a trans-Golgi enzyme), shown in red. **Describe and explain the results of your experiment AND explain which model the data support and how.**



Sample Student Answer: "The color of the individual Golgi cisternae is clearly changing colors as the time passes. The cisternae initially fluoresces green, then fluoresces red. This would indicate that the contents of the cisternae are not changing from manosidase I to galactosyl transferase. Since manosidase I is a cis-Golgi enzyme and galactosyl transferase is a trans-golgi enzyme, we can assume the cisternae is moving in an anterograde direction. The results of this experiment fit the cisternal maturation model in which the same cisternae move from cis to trans face, with a change in enzyme components along the way. In this model, it is the vesicles, that travel in the retrograde direction."

References for Supplements 1 and 2

Boucher, R., Cotton, C., Gatzy, J., Knowles, M., & Yankaskas, J. (1988). Evidence for reduced cl-and increased na permeability in cystic fibrosis human primary cell cultures. The Journal of Physiology, 405(1), 77-103.

Brown, M. S., & Goldstein, J. L. (1974). Familial hypercholesterolemia: Defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Proceedings of the National Academy of Sciences of the United States of America, 71(3), 788-792.

Bryant, H. E., Schultz, N., Thomas, H. D., Parker, K. M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J., Helleday, T. (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase. Nature, 434(7035), 913-917.

Creative Commons. For license link see http://creativecommons.org/licenses/by/3.0/legalcode

Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Bottomley, W. (2002). Mutations of the BRAF gene in human cancer. Nature, 417(6892), 949-954.

Davis, C. G., Lehrman, M. A., Russell, D. W., Anderson, R. G., Brown, M. S., & Goldstein, J. L. (1986). The JD mutation in familial hypercholesterolemia: Amino acid substitution in cytoplasmic domain impedes internalization of LDL receptors. Cell, 45(1), 15-24.

Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D., & Hunt, T. (1983). Cyclin: A protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell, 33(2), 389-396.

Gautier, J., Minshull, J., Lohka, M., Glotzer, M., Hunt, T., & Maller, J. L. (1990). Cyclin is a component of maturation-promoting factor from xenopus. Cell, 60(3), 487-494.

Grubb, B. R., O'Neal, W. K., Ostrowski, L. E., Kreda, S. M., Button, B., & Boucher, R. C. (2012). Transgenic hCFTR expression fails to correct β-ENaC mouse lung disease. American Journal of Physiology-Lung Cellular and Molecular Physiology, 302(2), L238-L247.

Lavoie, H., & Therrien, M. (2011). Cancer: A drug-resistant duo. Nature, 480(7377), 329-330.

Losev, E., Reinke, C. A., Jellen, J., Strongin, D. E., Bevis, B. J., & Glick, B. S. (2006). Golgi maturation visualized in living yeast. Nature, 441(7096), 1002-1006.

Martínez-Menárguez, J. A. (2013). Intra-golgi transport: Roles for vesicles, tubules, and cisternae. ISRN Cell Biology, 2013

Martinez-Menarguez, J. A., Prekeris, R., Oorschot, V. M., Scheller, R., Slot, J. W., Geuze, H. J., & Klumperman, J. (2001). Peri-golgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-golgi transport. The Journal of Cell Biology, 155(7), 1213-1224.

Mironov, A. A., Beznoussenko, G. V., Nicoziani, P., Martella, O., Trucco, A., Kweon, H. S., . . . Luini, A. (2001). Small cargo proteins and large aggregates can traverse the golgi by a common mechanism without leaving the lumen of cisternae. The Journal of Cell Biology, 155(7), 1225-1238. doi:10.1083/jcb.200108073 [doi]

Murray, A. W., Solomon, M. J., & Kirschner, M. W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. Nature, 339(6222), 280-286.

Yan, W., Samaha, F. F., Ramkumar, M., Kleyman, T. R., & Rubenstein, R. C. (2004). Cystic fibrosis transmembrane conductance regulator differentially regulates human and mouse epithelial sodium channels in xenopus oocytes. Journal of Biological Chemistry, 279(22), 23183-23192.

SUPPLEMENT 3. Coding Schemes for Qualitative Data Analysis

Model Use Coding Scheme (for transcipts)		
Code [a]	Description	Example
1. Productive model use [b]	Anytime students use model productively, including, but not limited to: - use model to set-up experiment - integrate model within claim building - instructor or TA uses model to explain - relate model at the end of claim building - check claim against model AND helps expand the claim	S1: in here, it says MEK activation is what it is, so I would say that um, basically it's showing that the mutant versions of BRAF have higher, are um, able to increase MEK activation, or have like a higher MEK activation than um, just wild-tupe RBAF. S2: What is the MEK activation leading to? Oh, it increases proliferation. S1: What? S2: So then the MEK increases proliferation and can cause it to be cancerous. Right here, that's where I'm getting it from, the MEK proliferation and survival [Gr.1 RTK Signaling]
2. Vague, unproductive model use [c]	When a student relates to the model by reiterating claims previously mentioned during claim building. Or when a claim is checked against the model but the claim does not change or progress in any way.	S2: so the data shows, G4 blah blah V5 [BRAF mutant], have a higher activation of MEK. [claim] […] S2: how do these results. B-RAF mutations S1: wait what are you writing? S2: for like how do these support the model? B-RAF mutations um allow for activation of MEK. S1: okay so second one. [move to next question] [Gr.23 RTK Signaling]
3. Missing model link: Attempt [d]	When students explicitly mention they need to use the model but do not progress further or wait for answers to be announced. Or when students incorrectly relate to the model.	S2: the folding is what's like, how much it's folding is like activating the kinase domain or, that's what's like making the cancer right? The mutations in BRAF… S1: how does this - I don't even get this model. S2: [inaudible] and BRAF increases the folding S1: I'm just gonna wait until she goes over the answers [Off-topic chatter]. S2: I don't know. Are we still talking about number 3? This model. S1: I don't know. [Inaudible] wait and see what she says. S2: I still don't understand this model S1: yeah S2: dude I'm still having a hard time understanding this. [Gr.17 RTK Signaling]
4. Too difficult to tell [d]	When students use/mention the model but there is not enough information in the dialogue to see how students are using the model. Or when coder cannot tell if model use is productive or vague/unproductive. Or when group eludes to relating their claim to the model but do not explicitly discuss the model link.	S3: Don't they both have BRCA deficient cells? S1: No this is the only one that is BRCA deficient. The squares and triangles were BRCA deficient, but they added back. You need a much smaller concentration of the inhibitor to cause cell death, or like to cause significant cell death [off-topic chatter]. S3: Then I'll just relate it to the model right? [May have related on their own]. S1: Yeah [Gr.22 BRCA Tumors]

[a] one code per figure discussion, [b] if no, go to code 2, [c] if no, go to code 3 or 4, [d] if no, code as "Missing model link: No attempt"

Presence of Argumentation and Other Common Strategies for Interpreting Data Coding Scheme (for transcripts)		
Code	Description	Example
Argumentation	At least 2 students working together to build claims (interpretations) through evidence (validly linked to claim to support or counter claim).	S2: mutations in BRAF are associated with the incidence of skin cancer, or melanoma **[INITIAL CLAIM]** S1: you can also get colorectal or ovarian, but you are right, skin cancer dominates S3: yeah you have 80 and 67 then you have 12 and 14 [percent] **[EVIDENCE]** S2: melanoma dominates as the primary tumor for these BRAF mutations, however **[CLAIM]** […] S1: these are both melanoma, the first 2. So you see really large percentages there **[EVIDENCE]** S2: so in other words, mutations in BRAF pretty much are gonna [inaudible] melanoma cancers S4: melanoma? S2: yeah S3: you have higher incidence of mutated BRAF than, in skin cancer, than in other cancers **[FINAL CLAIM]** [Gr.16 RTK Signaling]
De-coding the Data Representation	When student asks or describes what the symbols, numbers, lines, shapes, etc. presented in data mean/represent. This does not include referring to which panel represents what (i.e. 'a' is normal, 'b' is mutant).	S1: Like, what are these... S2: These are different types of mutants of the RAF. So they either added wild type RAF or various mutant forms of RAF. S1: Oh okay. S2: And I guess this plus means that it is constitutively active thing and the minus means it doesn't? S3: It does mean that or minus doesn't mean mutation, does it? S2: Cause it's like each RAS here... S3: Okay so plus means it does have it. S2: Yeah. S3: Okay. [Gr.6 RTK Signaling]
Rabbit Hole	When students spend 10 or more speaker turns (this can include instructor/TA) on a distracting feature of the data representation. The distracting feature must be a variable you can point out in figure. This does NOT include time spent on wrong experimental idea or theoretical concept.	S1: Yeah they do appear to be darker... S2: Yeah. They appear to be darker. S1: But then in ERK2 you have protein in every single case S3: I'm sure it's everywhere S2: How is ERK1 and ERK2 different though? Like in this pathway like? S3: What are the differences? S2: Are they both like the orange things? [reference to target model] S3: Isn't ERK1 like in one pathway and ERK2 goes in another pathway S2: That's what I was thinking, but it really doesn't seem like ERK on the left is phosphorylated and it says both are... S3: yeah I don't know where we see ERK1 and ERK2, where they are at like separately S2: yeah I don't know, that's what I am saying S1: yeah I don't get it. I don't know the difference between ERK1 and ERK2 S2: I don't know how that makes a difference here. [Gr. 16 RTK Signaling]

Rabbit Hole Aversion (RHA)	When a student and/or TA/instructor explicitly redirects focus from a distracting variable or unfruitful path. This includes if the distracting variable was 1 speaker turn AND someone explicitly says, "Don't worry about it" or similar. This does NOT include when the focus shifts without some explicit redirection (i.e. 1 speaker turn on a distracting variable and then another speaker turn on appropriate variable without some redirect comment in between).	S1: Um… So I don't get though like what is the difference between ERK1 and ERK2? S2: I think it's just different forms of this Kinase, like they're different kinds of this Kinase. S1: Right. But I mean like… is ERK2 and this pathway or, I mean, what is the… S2: I think there's just multiple ERKs like just, don't worry about it. Like MEK phosphorylates ERK when there's more than one like…types 1 and 2… I think. [Gr.5 RTK Signaling]
Noticing Patterns	When students point out what is the same or different in the data, or when try to notice a trend or pattern in the data that is not yet a claim or interpretation. This must happen BEFORE claim building.	S1: That one very similar to the constitutively active, so that one like was like most prevalent mutation. […] um… the G463V kind of, like there's a little bit, I mean it's obvious it's more than the H-RAS. And then same with G468A, it's a little bit higher. [Gr.5 RTK Signaling]
Noticing Patterns Within Claim Building	When students notice a trend or pattern or point out same/difference in data DURING the claim building.	S1: So like it shows that with the BRCA2 in there, it doesn't really have an effect on death [claim]. And then S2: So when you have no BRCA2 function do you... S1: Since it can still do it the other way it doesn't really have an effect. And then on the next one when it adds PARP one, it can still do PARP2 or BRCA2 so it doesn't really have much of an effect. Same thing with the third column. S2: And when you knock them both out S1: Right. But once you get rid of that BRCA2 and the PARP, it has a significant drop [claim]. So yeah. [Gr.1 BRCA Tumors]

Written Interpretation Quality: Validity and Generativeness Coding Scheme (for students' written responses)		
Score	Validity (V)	Generativeness (G)
4	NA	Hypothesis requires new experiment
3	Contains all listed points (below) and no mistakes	Proposes an interpretation that ties to the model/biology beyond the immediate variables in the data figure
2	Contains at least one listed point (below)	Makes an inference about some aspect of the figure
1	Contains none of the listed points	Does not move past a literal description of the figure/data

<u>Listed points for "Cystic Fibrosis" Problem Set</u>

Fig.1
- There is a greater drop in CF cells treated with Amiloride because CF cells have more Na channels that can be affected by the Amiloride treatment (more channels = bigger affect).
- Amiloride treatment makes both normal and CF cells more negative than before Amiloride treatment because less positive ions are flowing in, making the inside of the cell more negative.

Fig.2
- Cl- flow through Cl channels decreases cell surface levels of ENaC without affecting total levels of ENaC.
- Cl- flow either directly or indirectly inhibits ENaC from reaching the cell surface or removes it from the cell surface, but does NOT inhibit synthesis/expression of ENaC.

<u>Listed points for "Cell Cycle" Problem Set</u> (This problem set presented in the Supplementary Figure has been revised by the instructor since the data were collected for this study. The version presented here is the one currently used by the instructor. Figure 1 in the original version is the same as Figure 2 in the current version. Figure 2 here is no longer present in the current version, but can be found in Murray, Solomon, & Kirschner, 1989).

Fig.1
- WT cyclin is degraded before interphase while cycΔ90 (nondegradable cyclin) inhibits entry into interphase.
- Cyclin must be degraded to enter interphase or to exit mitosis (or to deactivate the MPF).
- As long as some amount of cyclin is present, regardless if some gets degraded, the cell cannot enter interphase. (Students may equate this to: nondegradable cyclin is the "dominant" phenotype or overcomes the degradable cyclin phenotype).

Fig.2
- Cells with cycΔ90 are stalled in mitosis (specifically prophase).
- Cells with cycΔ90 cannot enter interphase to continue the cell cycle and divide/cleave, hence why there is still only one cell (one nuclei). Alternatively, cells with WT cyclin can degrade the cyclin and enter interphase to continue the cell cycle and divide, hence why there are 14 nuclei. Or some mechanism for why cell cannot enter interphase mentioned.

<u>Listed points for "RTK Signaling" Problem Set</u>

Fig.1
- BRAF mutants have higher MEK activation compared to WT.
- BRAF mutants can activate the downstream signaling cascade more than WT to increase cell proliferation and survival (supporting that BRAF is an oncogene).

Fig.2
- In WT, ERK is phosphorylated only in the presence of HRAS because active RAS phosphorylates BRAF which phosphorylates MEK which phosphorylates ERK.
- Some BRAF mutants activate ERK in the absence of HRAS.

<u>Listed points for "BRCA Tumors" Problem Set</u>

Fig.1
- BRCA deficient cells are more sensitive to PARP inhibition (die quicker at lower doses of drug).
- The phenotype can be rescued to wild-type condition by complementing BRCA back in.
- Cells without both DNA repair mechanisms (BRCA and PARP) will die because there is no back-up mechanism to repair DNA.

Fig.3
- In B, treatment with the PARP inhibitors causes more double stranded breaks for both BRCA + or – cell types (because you need PARP to fix double stranded breaks).
- In C, BRCA deficient cells are not able to repair double stranded breaks. Cells with BRCA are able to repair double stranded breaks.
- Cells without both BRCA and PARP will have DNA damage that they cannot repair because there is no back-up mechanism to repair.

Fig.4
- Thigh circumference decreases after PARP inhibition drug treatment ONLY in the BRCA deficient mice.
- When the thigh circumference decreases in BRCA deficient mice, the tumor is shrinking because the tumor cell are dying because they have no back-up mechanism to repair their DNA when PARP is inhibited.