Appendix 1: Biology of WNT Pathway

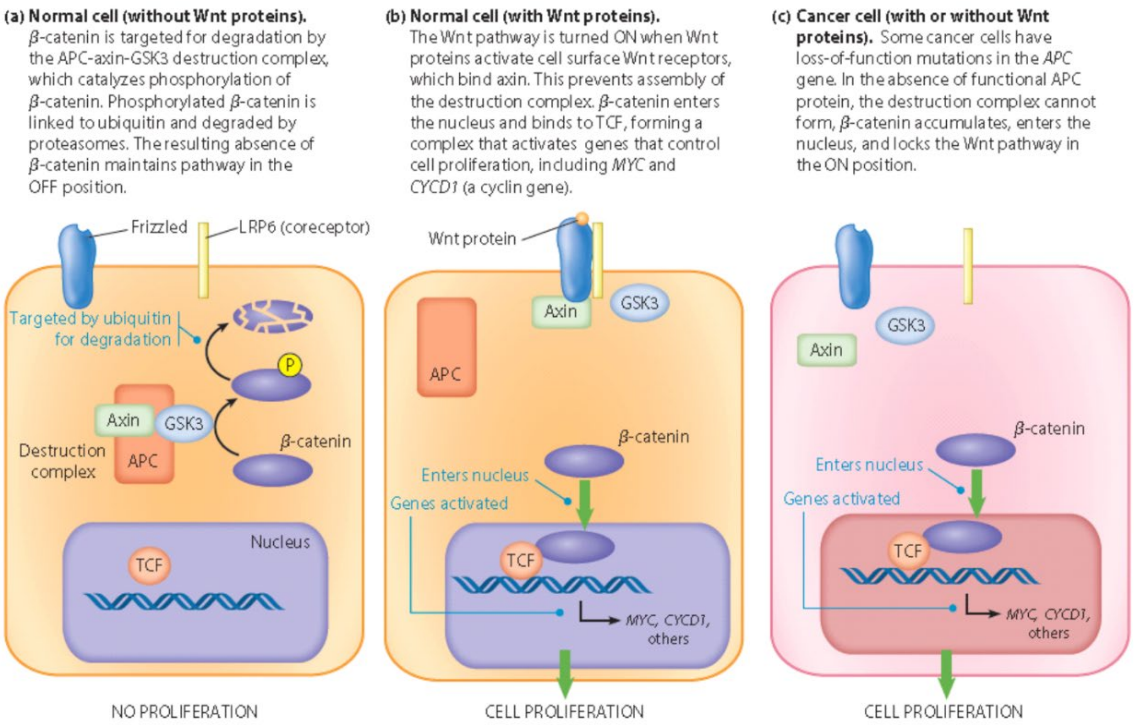


Figure 26-19 The Wnt Signaling Pathway. In normal cells, shown in **(a)** and **(b)**, the Wnt pathway is active only in the presence of Wnt proteins. In cancer cells **(c)**, the Wnt pathway is active regardless of the presence or absence of Wnt proteins.

The β-catenin protein is regulated by binding with APC (adenomatous polyposis coli) in the cytoplasm, as well as several other proteins that make up a destruction complex, which leads to the phosphorylation of β-catenin, targeting β-catenin for degradation by a proteasome complex. In a normal cell, the β-catenin protein can be activated when a WNT protein binds to its receptor, preventing the assembly of the destruction complex. This regulation via the destruction complex is important for maintaining low levels of β-catenin in the absence of WNT signaling. The mutations in the β-catenin gene that are found in colon cancers result in the loss of one of the β-catenin protein phosphorylation sites, and therefore loss of this regulation. The β-catenin protein however still retains the binding sites for APC, TCF transcription factors, and E-cadherin, which is important for cell-to-cell adhesion. These β-catenin colon cancer mutations thus result in high levels and activity of β-catenin even in the absence of a WNT protein signal.

Summary of the WNT pathway. Reprinted from *Becker's World of the Cell* (9th edition, p. 800), by J Hardin, GP Bertoni, LJ Kleinsmith. Pearson Education. Copyright 2016 by Pearson, Inc. Reprinted with permission.

Appendix 2: Study Guide Materials

Hereditary Cancer Susceptibility

I. Learning Objectives

By the end of the week, you should able to:

• Diagram and explain the APC/WNT signaling pathway under various signaling conditions.

• Describe the interaction of APC and β-catenin in a normal cell.

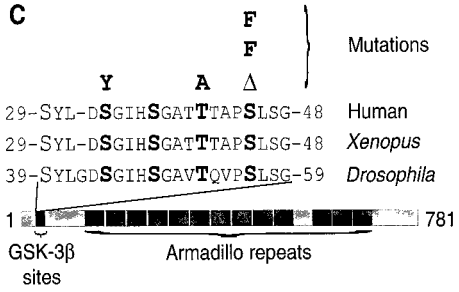
• Describe the effects of loss of APC function on β-catenin expression and function.

• Outline the potential effects of loss of APC on a cell or group of cells.

• Describe the potential effects of mutations in the CTNNB1 gene on β-catenin function and the signaling pathway.

• Compare and contrast the types of mutations and the results for the cell, of mutations in APC and CTNNB1 specifically and tumor suppressor genes and oncogenes in general.

II. Figure 3 from Morin el al 1997



(**C**) Schematic of b-catenin illustrating the Armadillo repeats (*24*) and negative regulatory domain. The residues in larger type fit the consensus sequence for GSK-3b phosphorylation (*20*) and those in bold have been demonstrated to affect down-regulation of b-catenin through GSK-3b phosphorylation in *Xenopus* embryos (*17*). The five mutations found in human colon cancers are indicated at the top. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Morin et al. http://www.sciencemag.org SCIENCE VOL. 275 21 MARCH 1997

III. Description of Figure in Study Guide

Year 0 (2012): No description

Year 1 (2013): **Figure 3C**. Shown here is a region of the β-catenin protein sequence, the mutations found in the colon cancer cell lines and the primary colon tumors (described in the text) are shown. Note that all of the mutations affect sites known to be phosphorylated by GSK-β.

Year 2 (2014): Same as Year 1

Year 3 (2015): **Figure 3C**. Shown here is a representation of the β-catenin protein sequence with the GSK3β phosphorylation site region enlarged to show the amino acid sequence. The mutations found in the colon cancer cell lines and the primary colon tumors (described in the text) are shown. Be sure you can relate this picture with the model of β-catenin shown earlier in the study guide.

Year 4 (2016): **Fig. 3C:** note that this is a linear representation of the β-catenin protein like the pictures I have shown of APC in class. Above the figure are shown the amino acid sequences from the GSK-3β region for 3 species indicating in bold the serine and threonine phosphorylation sites. Above that are indicated the position and amino acid encoded by the mutations described in the paper.

IV. Picture of Physical Model and Description in Study Guide

Year 0 (2012): No picture of models included.

Year 1 (2013): β-catenin: We will discuss this more in lecture and in this study guide. Review in your textbook.

Year 2 (2014): β-catenin: We will discuss this protein more in lecture and in this study guide. Review this in your textbook. Below is a picture of what the β-catenin protein looks like, based on crystal structure. Only the middle section of the protein could be crystalized. The N (beige) and C (purple) terminal regions of the protein are represented by wires. The purple and beige alternating sections represent the armadillo repeats (use Figure 3c in Morin et al. to orient yourself to the important parts of the protein). The orange squiggle is a small portion of the APC molecule showing its binding to β-catenin. We will use these models in lecture on Monday, so be prepared to identify the portions of the protein.

Year 3 (2015): (changes are underlined). **β-catenin**: We will discuss this protein more in lecture and in this study guide. Review this in your textbook. Below is a picture of a β-catenin protein model, based on crystal structure. <u>This "backbone" representation shows the peptide chain only, without the side chains (also called residues or R groups). Each kink on a chain represents the central α carbon of one amino acid.</u> Only the structures in the middle section of the protein could be crystalized. <u>The N (amino) region of the protein chain is represented by a beige wire and the C (carboxy) region is represented by a purple wire.</u> The purple and beige alternating α helix sections represent the armadillo repeats (use Figure 3c in Morin et al. to orient yourself to the important parts of the protein). The orange squiggle is a small portion of the APC molecule showing its binding to β-catenin. **<u>Be sure you can relate the protein shown in this photo with the representation of the protein in Figure 3C of Morin et al.</u>**

Appendix 3: Class Activities

Physical model activity

β-catenin models

- Identify which end is the N-terminus.
- Mark the approximate position of the GSK phosphorylation sites with one color marker.
- Mark the approximate position of one of the β-catenin mutations from the paper with another color marker.
- Look at β-catenin with either the TCF (green) or APC (orange) peptide and see how where and how they bind to β-catenin.

Questions

- What are the relationships of APC and TCF with respect to β-catenin and each other.
- Where are the phosphorylation sites relative to the TCF or APC binding sites?
- How would a mutation in the phosphorylation site affect β-catenin binding to APC or TCF?
- How would a mutation in or loss of the APC binding site affect β-catenin function?

Interactive WNT pathway activity

- Situation 1: Arrange the puzzle pieces to show the interaction of the various proteins in the ABSENCE of a WNT signal and in the presence of FUNCTIONAL APC
- Situation 2: Arrange the puzzle pieces to show what happens to the same proteins in the PRESENCE of WNT.
- Situation 3: Arrange the puzzle pieces to show what happens in a cell with a mutation in *APC* that was identified in a person with FAP. Would both alleles need to be mutated? What would be the effect of the mutation on APC?
- Situation 4: Arrange the puzzle pieces to show what would happen in the presence of a mutation in β-catenin as described in the Morin *et al.* paper. Would the mutation have to be present in both alleles of β-catenin? What would happen if a mutation deleted the APC binding site in β-catenin?

Compare situations 2, 3, and 4.

How are the consequences for the cell different?

Are any of these situations reversible?

Appendix D: Exam and Rubric

Parts that were removed are indicated by strikethrough and additions are underlined

Year 0-2

You want to generate a mouse model to study whether a mutation in the gene encoding β-catenin (*Ctnnb1*) can contribute to tumor development. Using transgenic technology, you can generate mice that express your mutated gene in colon stem cells after administration of a drug orally.

A. (3 points) Describe the mutation you would generate in the *Ctnnb1* gene encoding β-catenin.

B. (3 points) Does your mutation activate or inactivate the protein? What change in β-catenin function will result from the mutation? What KEY properties will remain intact in the mutant protein?

Year 3-4 (revised in year 3)

You want to generate a mouse model to study the effect of a mutation in the gene encoding β-catenin (*Ctnnb1*) in tumor development. Using transgenic technology, you can generate mice that express the mutated gene in colon stem cells under control of a promoter that is activated only when you treat the mice with a certain drug.

A. (2 points) Describe the mutation you would generate in the *Ctnnb1* gene encoding β-catenin. Be specific about where in the gene the mutation would be and what type of mutation it would be.

B. (1 points) ~~Does your mutation activate or inactivate the protein?~~ What key change(s) in β-catenin function will result from the mutation?

C. (3 points) What **three** key functions will remain intact in the mutant protein?

Basic understanding rubric:

9 points: All of these present (9 things)

- Missense or small in frame deletion
- Mutate one of the aa that are phosphorylated
- Protein will no longer be able to be phosphorylated
- Protein would no longer be degraded
- Increase in levels/activity/transcription or constitutive/unregulated activity
- Retains ability to drive transcription (If they say "*increase in transcription",* they get this point as well as the one above)
- Retain the ability to interact with TCFs
- Retain the ability to interact with APC
- Retain the ability to interact with E-cadherin

8 points: Missing one (8 things)

- Missense or small in frame deletion
- Mutate one of the aa that are phosphorylated
- Protein will no longer be able to be phosphorylated
- Protein would no longer be degraded
- Increase in levels/activity/transcription or constitutive/unregulated activity
- Retains ability to drive transcription (Ok if "*increase in transcription")*
- Retain the ability to interact with TCFs
- Retain the ability to interact with APC
- Retain the ability to interact with E-cadherin

7 points: Missing two- can't be in the same section; if missing two in the same section, score will be below 4 pts.

- Missense or small in frame deletion

- Mutate one of the aa that are phosphorylated
- Protein will no longer be able to be phosphorylated

- Protein would no longer be degraded
- Increase in levels/activity/transcription or constitutive/unregulated activity

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- Retains ability to drive transcription (Ok if "*increase in transcription")*
- Retain the ability to interact with TCFs

- Retain the ability to interact with APC

- Retain the ability to interact with E-cadherin

6 points: Missing three- can't be in the same section; if missing two in the same section, score will be below 4 pts.

- Missense or small in frame deletion

- Mutate one of the aa that are phosphorylated
- Protein will no longer be able to be phosphorylated

- Protein would no longer be degraded
- Increase in levels/activity/transcription or constitutive/unregulated activity

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- Retains ability to drive transcription (Ok if "*increase in transcription")*
- Retain the ability to interact with TCFs

- Retain the ability to interact with APC
- Retain the ability to interact with E-cadherin

5 points: Only these present (5 key things)

- Missense or small in frame deletion OR mutate one of aa that are phosphorylated
- Mutate one of the aa that are phosphorylated OR will no longer be able to be phosphorylated
- protein would no longer be degraded OR clear understanding that levels/activity increase
- retain the ability to interact with TCFs OR retains ability to drive transcription
- retain the ability to interact with APC OR E-cadherin

4 points: Only these present (4 key things)- basic understanding

- Missense or small in frame deletion OR mutate one of aa that are phosphorylated
- Mutate one of the aa that are phosphorylated OR will no longer be able to be phosphorylated
- Protein would no longer be degraded OR clear understanding that levels/activity increase
- Retain the ability to interact with TCFs OR retains ability to drive transcription

3 points: States mutate one of the aa that are phosphorylated OR will no longer be able to be phosphorylated and two of the following (missing 1)

- Missense or small in frame deletion
- Protein would no longer be degraded OR clear understanding that levels/activity increase
- Retain the ability to interact with TCFs OR retains ability to drive transcription

2 points: States mutate one of the aa that are phosphorylated OR will no longer be able to be phosphorylated and one of the following (missing 2)

- Missense or small in frame deletion
- protein would no longer be degraded OR clear understanding that levels/activity increase
- retain the ability to interact with TCFs OR retains ability to drive transcription

1 point: Mutate a phosphorylation site w/ no other info OR unclear mutation and some other correct info

0 points:

- Mutate APC (anything that will prevent APC from binding)
- (might still say that it binds TCF and drives transcription)

<u>Additional details: take note of whether students say:</u>

- Something about GSK binding/phosphorylating
- Something about the location of the mutation: at N-terminus, before armadillo repeats, near the beginning of the gene
- Something about the nucleus: translocate to nucleus, build up in nucleus, etc.