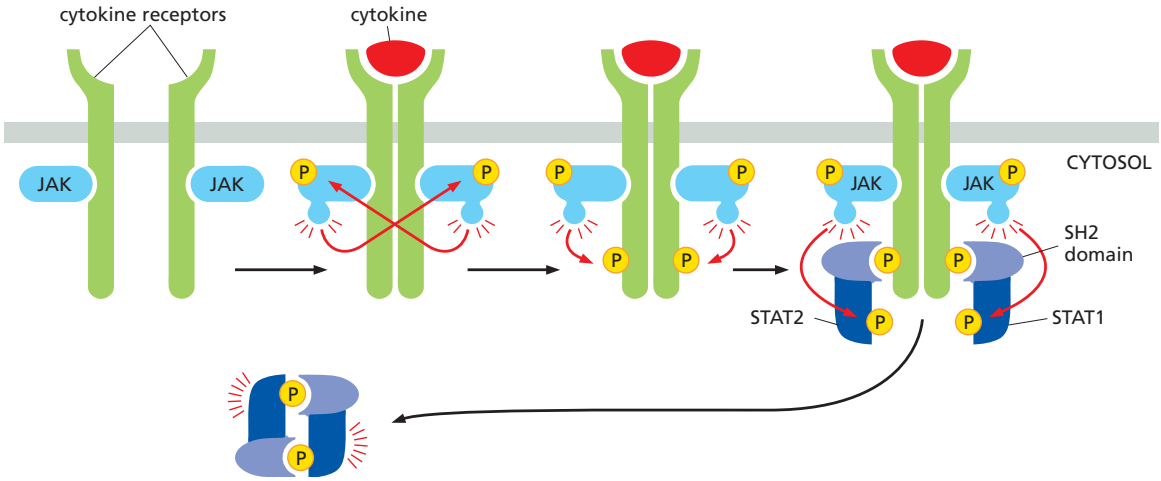
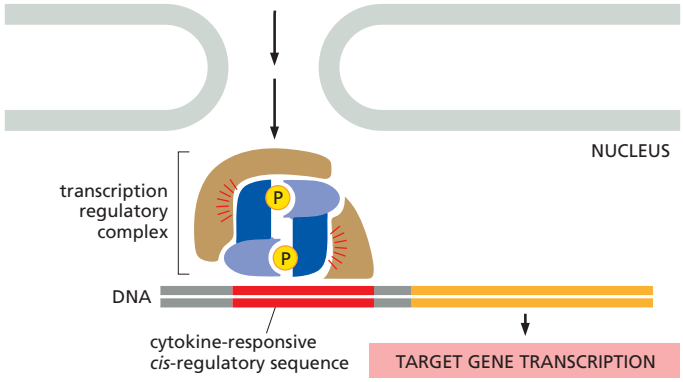
Answers Cell signaling Part 3

1A) A decrease in *IFNB* mRNA levels suggests that a negative feedback loop has been activated. This could be due to degradation of RIG-I, CARDIF, IRF3 or another protein in the pathway, mediated by the proteasome for example. Another mechanism could be sequestration of one of the signaling proteins to another cellular compartment. Another mechanism could be that a target gene of IRF3 will be produced, and the protein product of that gene is an inhibitory protein of this pathway. This protein could, for example, bind to IRF3 in the nucleus, and pull it back to the cytoplasm as a way of inactivation.

1B) The model looks like the following one from the course:





JAK: the kinase bound to the interferon-beta receptor.
STAT1/2: the transcription factor subunits recruited to the phosphorylated receptor, themselves phosphorylated by JAK, enabling their dimerization, nuclear translocation, DNA binding and modulated target gene transcription.

2A) Paracrine signaling

2B) The signaling pathway has become constitutively active. 1) Cells started making the ligand TNFa and signal in an autocrine fashion. 2) There is a mutation in a positive signaling molecule, constitutively activating the pathway 3) A negative regulator is no longer functional, rendering the pathway constitutively active

3A) *APC* is a tumor suppressor gene. The normal function of the APC protein is to inhibit b- catenin, by helping to retain it in the cytoplasm, promoting its degradation through the proteasome. When APC is not functioning due to complete loss-of-function, b-catenin becomes free to accumulate in the cell, part of it translocating to the nucleus to drive transcription of target genes, even in the absence of a Wnt signal. In contrast, one wild type, functional copy of APC is sufficient to prevent b-catenin accumulation.

3B) Mutations on *CTNNB1* in tumor cells typically affect the protein b-catenin in a way that it becomes resistant to degradation. For example, because there is a short deletion in the gene, in the region coding for the two amino acids that are phosphorylated by GSK3 and CK1 in the APC-containing complex. This creates a stable protein, which accumulates in the tumor cell similarly to the situation seen in (**3A**). *CTNNB1* is a proto-oncogene: its mutations found in tumors are gain-of-function mutations.

4) You would want to remove the area of the protein interacting with the complex retaining AR in the cytosol. Once this interaction is stopped the truncated protein is free to move to the nucleus. You cannot remove the nuclear localization signal, if this is removed the protein will stay in the cytoplasm.

5A) Notch has become inactive.

5B) This is an example of lateral inhibition. The ligand (DLL) is expressed in the goblet cells and notch receptor is expressed on the enterocytes.

6)
A: Notch
B: Wnt proteins
C: Wnt/β-catenin pathway

D: Hedgehog proteins
E: Cubitus interruptus (Ci)
F: NFκB proteins
G: Steroid hormone

7) The modifications of cholesterol to make steroid hormones increase the hydrophilicity of the molecules by removing the hydrocarbon tail and by introducing polar groups. These modifications make the molecules sufficiently hydrophilic to diffuse from their carrier molecules in the blood- stream to cells, but not so hydrophilic as to prevent their crossing the plasma membrane to enter cells. By contrast, cholesterol is so hydrophobic that it normally spends all its time in the membrane. A lipid that is virtually insoluble in water could not serve as a hormone because it could not move readily from one cell to another via the extracellular fluid.

8)
In both cases the signaling pathways themselves are rapid. When the pathway modifies a protein that is already present in the cell, its activity is changed immediately, leading to a rapid response. When the pathway modifies gene expression, however, there will be a delay corresponding to the time it takes for the mRNA and protein to be made and for the cellular levels of the protein to be altered sufficiently to invoke a response, which would usually take an hour or more.

9)
A: False. Although some signaling pathways activate latent gene regulatory proteins by regulated proteolysis, others control their activity by phosphorylation.

B: True. Notch carries both its functions—cell-surface receptor and latent gene regulator—in one polypeptide chain. When activated by a ligand such as Delta, its cytoplasmic tail is cleaved of, enters the nucleus, and activates gene expression.

C: True. Activated NFκB increases expression of the IκBα gene, and IκBα then binds to NFκB and inactivates it, thereby shutting of the response. If the initial activating signal persists, then additional cycles of NFκB activation and inactivation may follow.

10) The ligand would no longer closely to the membrane as the hydrophobic lipid modifications would be removed. It would freely diffuse into the extracellular milieu. Most likely the interaction with the LRP/Frizzled receptors will also be affected and it will not bind with high affinity.