

# Regulation of Gene Expression and Adipogenesis by PPAR- $\gamma$

## Student Worksheet

Carefully read the following information and complete the **ACTIONS** in the boxes below.

**Introduction:** All of us, even the most lean, have some body fat, which is called adipose tissue or white fat. Cells in the adipose tissue, or **adipocytes**, are important for absorbing the fat molecules that circulate in the bloodstream (from the diet) and storing these fats in a special cellular organelle called the **lipid droplet**.



When energy is being consumed, for example, during extended periods of aerobic activity, part of the stored fat is released back into circulation so that muscle cells and other tissues can use it as fuel to produce energy and maintain activity. In return, if your fuel consumption (carbohydrates, fats and proteins) is greater than the amount of energy you are using, the excess fuel will be converted to fat and stored in adipocytes. To store fats, the lipid droplets in adipocytes become larger and the whole cell changes in size. Furthermore, the numbers of adipocytes in fat tissue can increase through a process of **adipogenesis**, whereby precursor cells are recruited to differentiate into new, smaller fat cells. These new fat cells formed through adipogenesis are more efficient in absorbing and storing fats. At the molecular level, this process of adipogenesis is regulated by a transcription factor called **Peroxisome Proliferator-Activated Receptor-Gamma (PPAR- $\gamma$ )**.

**Overview:** **PPAR- $\gamma$  (Peroxisome Proliferator-Activated Receptor-Gamma)** is a protein that acts as an intracellular sensor and receptor for lipids within the nucleus. When fatty acids (FA) are found in high levels in the bloodstream, for example after a meal, some of these FAs reach the cell nucleus and bind to a PPAR- $\gamma$ . Once FA binds to PPAR- $\gamma$ , this complex binds to DNA and regulates the transcription of multiple genes involved in the differentiation of precursor cells into adipose cells (adipogenesis) and the regulation of fat content within adipocytes. This transcription factor, PPAR- $\gamma$  is found in the nucleus of most cells of the body, but is found in the highest concentration in adipose tissue, macrophages and colon cells. The model depicted in Figure 1 shows more detail about how PPAR- $\gamma$  activity is regulated.

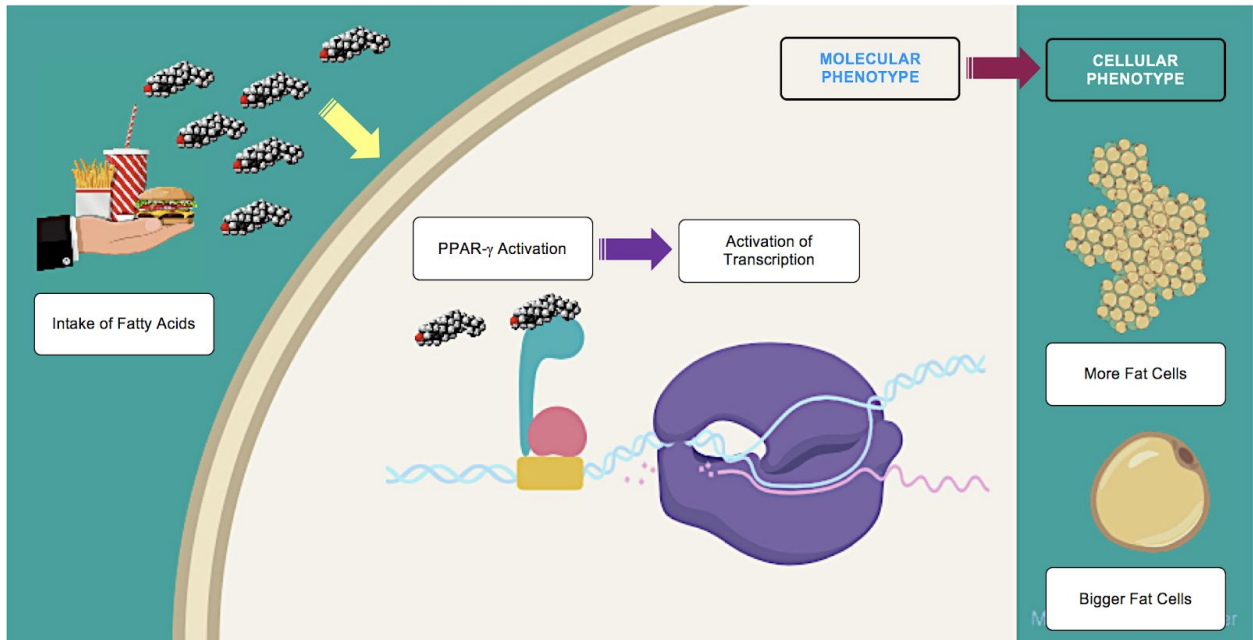


Figure 1. Activation of PPAR- $\gamma$  after intake of fatty acids and its effects at the molecular and cellular level.

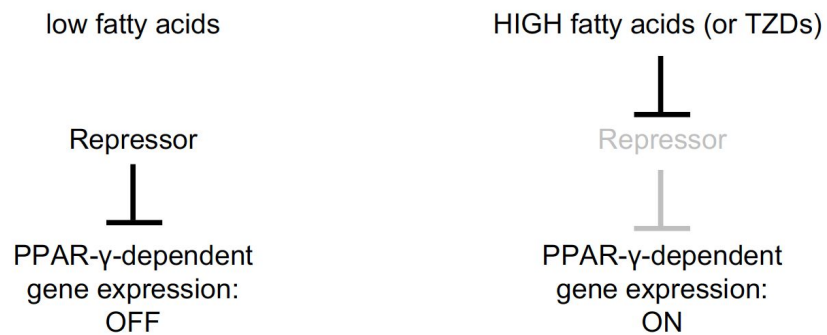


Figure 2. Regulation of gene expression by PPAR- $\gamma$  through *negative inhibition*. Note: standard biological symbols are employed in figures. Pointed arrows,  $\rightarrow$ , indicate positive effect or turning on. Blunt arrows,  $\perp$ , indicate negative effect or turning something off. In this case, turning off a repressor has a positive effect on PPAR- $\gamma$  activity. To see these interactions at play, please watch the following two videos. The videos are crucial to understanding the regulation of gene expression in this system and doing well on this exercise.

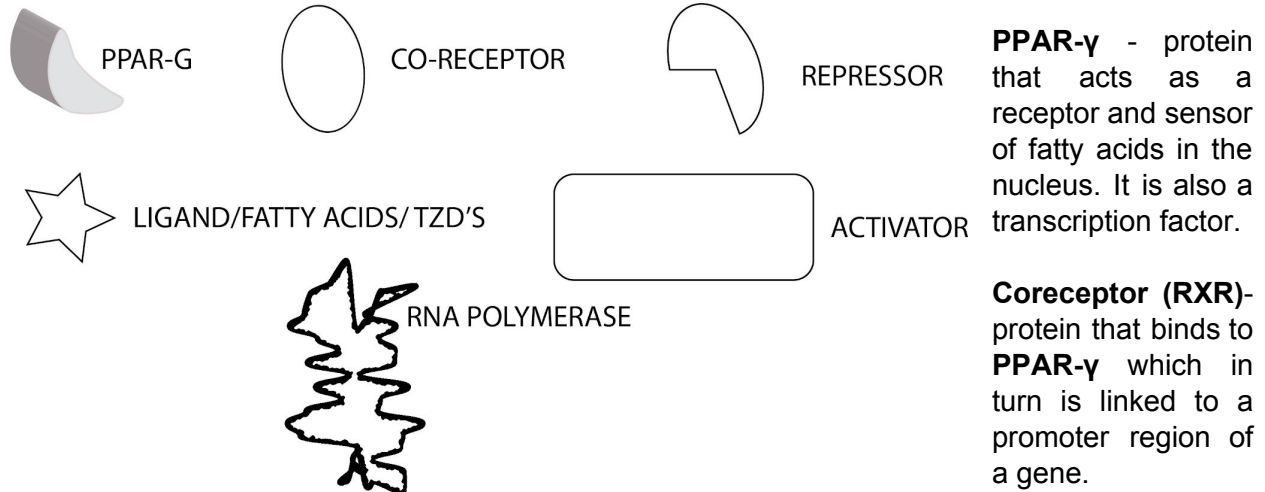
**ACTION:** Watch these videos

<https://www.hhmi.org/biointeractive/dna-transcription-basic-detail>

<https://www.hhmi.org/biointeractive/ppar-gamma-activation-fat-cell>

**ACTION:** Color code this legend

Choose one color or pattern for proteins, and another color or pattern for DNA, and a third color or pattern for the ligands (drug or PUFA).



**Repressor:** Protein that prevents the RNA polymerase from transcribing a gene.

**Activator:** When the repressor is released from **PPAR- $\gamma$** , the activating proteins bind freely. Activator binding to DNA recruits RNA polymerase and begins the transcription of a gene.

**RNA Polymerase:** Enzyme that transcribes DNA into mRNA.

## LIGANDS

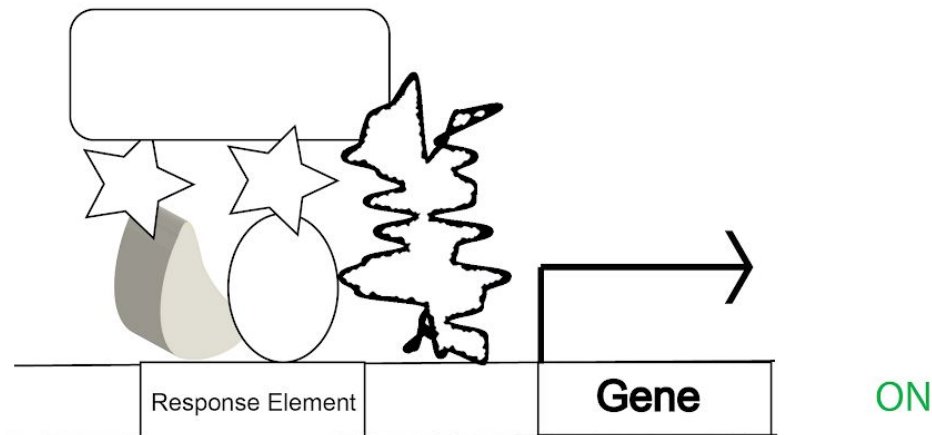
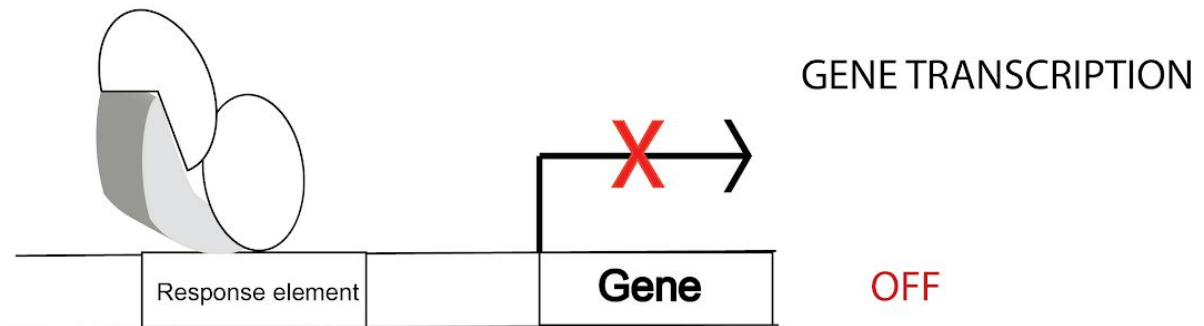
**PUFA or Polyunsaturated fatty acids** : Endogenous ligands for the activation of **PPAR- $\gamma$** .

**TZD (drug):** Synthetic agonists that activate conformational changes in **PPAR- $\gamma$**  and, in turn, improve insulin signaling. TZD drugs are used as a treatment for type 2 diabetes mellitus, because PPAR- $\gamma$  activity can indirectly lead to improved insulin sensitivity.

Shown below is a model of **PPAR- $\gamma$**  bound to the promoter region of a gene.

- Under conditions of low levels of fatty acids in the bloodstream, a repressor binds to PPAR- $\gamma$ , blocking the activity of RNA polymerase and gene transcription does not occur.
- When fatty acid levels are elevated, the fatty acids bind to PPAR- $\gamma$ , causing dissociation of the repressor. Activator proteins then bind to the response element and consequently RNA polymerase binds and starts transcription
- **PPAR- $\gamma$  Response Element (DNA binding site for PPAR- $\gamma$ )** is shown below. This specific site in the promoter region of several genes is where PPAR- $\gamma$  binds to (Sequence in DNA: AGGTCAxAGGTCA).

**ACTION:** Color the following large diagram using the legend of the previous ACTION.



**ACTION:** Answer the following questions and fill in the Scenario Table below

1. **A gene:**
  - a. Is composed of mRNA
  - b. Is synonymous with a chromosome.
  - c. Is a specific segment of nucleotides in DNA.
  - d. Contains only the nucleotides needed to synthesize a protein.
  
2. **Which of the following is a single-stranded molecule that contains the information for the assembly of a specific protein?**
  - a. Transfer RNA
  - b. Messenger RNA
  - c. Ribosomal RNA
  
3. **A transcription factor is:**
  - a. An activated gene
  - b. An activated mRNA
  - c. A molecule of intercellular communication.
  - d. A protein that binds to DNA and regulates mRNA synthesis
  
4. **The general structural and functional differences between a neuron and an adipocyte within an individual are the result of differences between:**
  - a. the DNA in a cell (the genome) is different between adipocytes and nerve cells.
  - b. the amount of certain genes between the fat and nerve cells.
  - c. the presence of certain proteins in the adipocytes and not in the neurons.
  
5. **If the DNA is in the nucleus and the proteins are synthesized in the cytoplasm, what is the CORRECT mechanism that allows the transfer of information in eukaryotic cells?**
  - a. Messenger RNA is transcribed from a single gene and transfers information from the DNA in the nucleus to the cytoplasm where protein synthesis occurs.
  - b. Transfer RNA takes the information from the DNA directly to the ribosomes in the cytoplasm, where protein synthesis occurs.
  - c. The DNA of a single gene is replicated in the nucleus and transferred to the cytoplasm where it serves as a guide for the synthesis of a protein.

### Scenario Table:

SCENARIO	EXPRESSION (ON/OFF)	PHENOTYPE AT CELLULAR LEVEL
REPRESSOR BOUND IN FAT CELL NUCLEUS		
LIGAND BOUND TO REPRESSOR IN FAT CELL NUCLEUS		
LIGAND PRESENT IN MUSCLE CELL NUCLEUS		
LIGAND PRESENT IN FAT CELL NUCLEUS WITH MUTATED PPAR- $\gamma$ RESPONSE ELEMENT		

### Questions for Group Discussion:

Under what conditions are PPAR- $\gamma$  responsive genes turned off?

What proteins are bound to the responsive element?

Are there lipids or TZDs in the cell's environment?

Under what conditions are PPAR- $\gamma$  responsive genes turned on?

What proteins are bound to the responsive element?

Are there lipids or TZDs in the cell's environment?

What do some lipids and TZDs have in common?