

Regulation of Gene Expression and Adipogenesis by PPAR- γ

Instructor Guide

Relevance and goals of the module: This module aims to teach general biology students about the mechanism of regulation of gene expression in fat cells (adipocytes) by having students color or draw the components of a molecular "model" of a transcriptional complex. Specifically, the activity is meant to complement an HHMI BioInteractive video resource, "PPAR-gamma Activation in the Fat Cell" (<https://www.hhmi.org/biointeractive/ppar-gamma-activation-fat-cell>). The activity fits within the broader framework of first-year biology to help students understand the role of proteins, DNA and RNA in specialized cellular functions and also in a lecture on the central dogma of biology or the control of gene expression. It is expected that after completing this activity students can:

1. **Describe examples of regulation of gene expression** in eukaryotic cells at the level of DNA, RNA and protein.
2. **Compare and contrast the mechanisms of regulation** of the activation and repression of gene expression in eukaryotes.
3. **Draw the elements of a gene**, including the coding sequence and the regulatory elements.
4. **Understand that genes are differentially expressed** in tissues and at different times or under different environmental conditions.
5. **Give examples of the results of changes in regulation on gene expression.**

Background for Instructors:

Body Fat & Fat Metabolism: Everyone, even the slimmest amongst us, has body fat, which is called **adipose tissue** or white fat. Adipose tissue cells, or **adipocytes**, are important for absorbing fat molecules that circulate through the bloodstream after ingesting food and storing these fats in a special cellular organelle known as the **lipid droplet**. When energy is being utilized, 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 contrast, if our fuel (caloric) consumption is greater than the amount of energy that is being used, some of the excess calories will be converted into fat and be stored in these fat cells. As more fat is taken up, the lipid droplets in adipocytes become larger and the whole cell changes in size. Alternatively, fat tissue can expand by increasing the numbers of adipocytes through a recruitment process called **adipogenesis**. These new fat cells formed by from precursor cells are smaller, but more efficient at absorbing

and storing circulating fats. At the molecular level, this process of adipogenesis is regulated by a transcription factor called Peroxisome Proliferator-Activated Receptor Gamma (**PPAR- γ**).

PPAR- γ is a protein that acts as an intracellular sensor and receptor for lipids within the nucleus. When fatty acids (FA) levels in the bloodstream are high, for example after a meal, some of these FAs reach the cell nucleus and bind to a PPAR- γ . Once FAs bind to PPAR- γ , 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 transport and content within adipocytes. PPAR- γ activation also affects adipocyte secretion of several hormones that can modulate the effects of insulin on blood glucose regulation. One of these, **adiponectin**, has important effects on the sensitivity of target cells (muscle, fat) to insulin. The transcription factor, PPAR- γ 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.

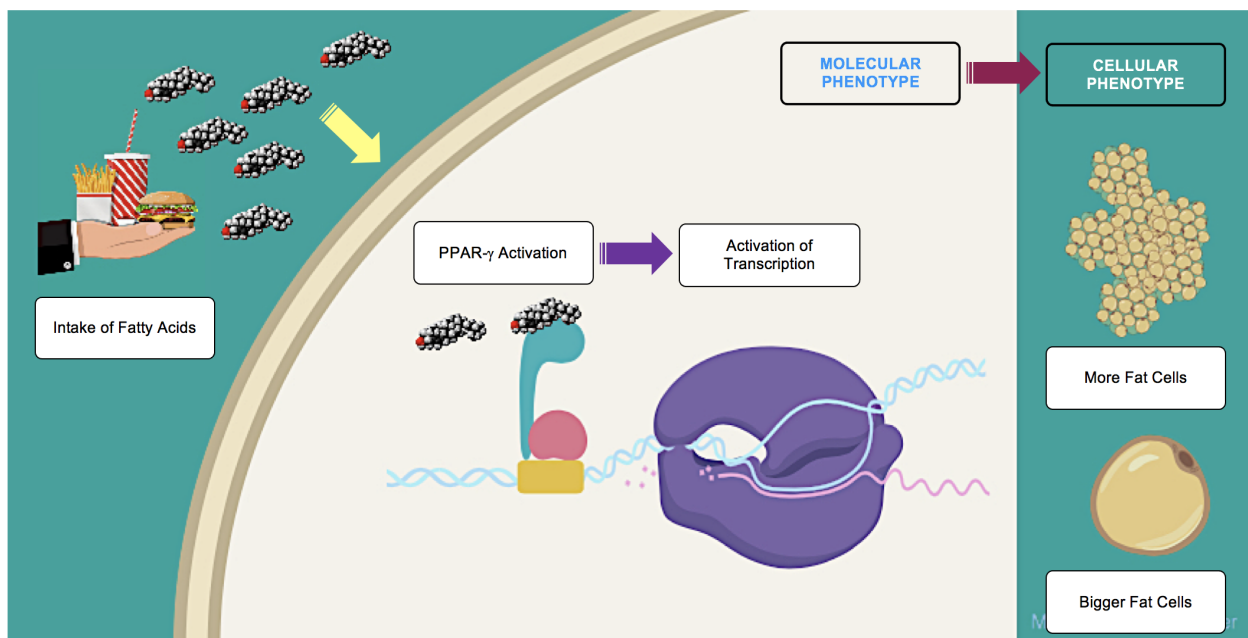


Figure 1. Activation of PPAR- γ after intake of fatty acids and its effects at the molecular and cellular level

Regulation of Gene Expression: A gene (one coding for a protein product) is an organized unit of DNA sequence which, in the presence of the appropriate signals, is transcribed into RNA and results in the formation of a functional protein product through the translation process. The regulation of transcription is mediated, in part, by regulatory proteins, including **transcription factors**, which bind to DNA and activate the expression of a gene by stimulating the activity of RNA polymerase (RNAPol). Similarly, there are factors that inhibit the expression of the gene by preventing the activity of RNAPol.

Regulation of PPAR- γ Activity: Under conditions of low FA levels in the nucleus, a repressor binds to PPAR- γ , blocking the activity of RNAPol and thus preventing the transcription of genes involved in adipogenesis. When the FA levels increase, they bind to PPAR- γ and cause the

dissociation of the repressor. This, in turn, allows subsequent binding of an activator protein followed by binding of RNAPol to the DNA and initiation of gene transcription. However, before PPAR-dependent gene expression can occur, PPAR- γ must also bind to another nuclear receptor called "retinoid X receptor" or RXR. After binding of the ligand, the PPAR- γ / RXR heterodimer recognizes and binds to specific DNA sequences termed **PPAR Response Elements (PPREs)** associated with transcriptional regulation of multiple, different genes. This type of regulation of gene expression in biological systems is known as negative regulation.

Pharmacological Regulation of PPAR- γ : The main effect of the regulation of PPAR- γ / RXR-dependent gene expression is an increase in fat storage (more adipocytes), thus decreasing the amount of fats present in the circulation. For this reason, the regulation of gene expression by PPAR- γ has been well studied, particularly since it has been shown that synthetic PPAR- γ ligands are an effective treatment for type 2 diabetes mellitus, a condition that affects millions of patients around the world. One of these classes of synthetic PPAR- γ ligands is a class of compounds called thiazolidinediones. Known as TZD, these synthetic agonists bind to PPAR- γ and stimulate PPAR- γ / RXR-dependent gene expression that leads to increased fat storage. In addition, PPAR- γ activation promotes release of an important adipocyte hormone called adiponectin. Adiponectin acts to increase the sensitivity of target cells to insulin, thus causing these cells to become more dependent on the oxidation of glucose for their energy requirements and decreasing the amount of glucose in circulation.

A model for the regulation of PPAR- γ activation is shown in Figure 2.

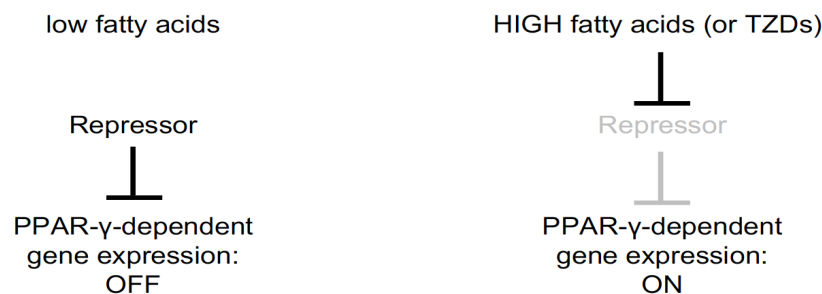


Figure 2. Regulation of gene expression by PPAR- γ through *negative inhibition*. Note: standard biological symbols are employed in figures. Pointed arrows, \rightarrow , indicate positive effect or turning on. Blunt arrows, \dashv , indicate negative effect or turning something off. In this case, to turn off a repressor (double negative) has a positive result.

Content knowledge requirement for students:

Before completing the activity, students should understand:

Minimum

- Structure and function of the four macromolecules that make up the cell
- General structure of the eukaryotic cell. Students need to know about lipid boundaries, the nucleus, DNA and RNA locations, and storage vesicles (termed fat droplets in this activity).

Recommended:

- Basic principles of how cells obtain energy and the different ways they can do it (oxidation of carbohydrates and fats)
- Cell signaling, including the concepts of ligand and receptor
- What is a gene?
- The structure and functional elements of a gene

Placement of the activity within the course of General Biology:

- In the unit of macromolecules to demonstrate macromolecule function:
 - **Proteins** as receptors and transcription factors
 - **Lipids** as signaling molecules and energy storage molecules
 - **DNA** as an information storage unit
 - **RNA** as a product of gene expression (transcription)
- In the cell signaling unit, as an example of nuclear receptors activated by ligands that diffuse freely through the plasma membrane and the nuclear envelope.
- In the transcription unit to demonstrate that gene expression differs from one cell to another and can be affected by environmental conditions.
- In the gene expression control unit to demonstrate how signaling molecules, transcription factors and even drugs affect the expression of specific genes for a phenotype.

Instructions for implementation:

In its entirety, the activity is designed to take 20 minutes of the class period, plus an additional 20 minutes that students will have to do before arriving to the classroom (read document + view videos). With modifications, the activity can last up to 50 minutes and/or be distributed among several classes.

BEFORE THE ACTIVITY:

1. Announce to the students that for the next class, they should bring colored pencils and / or markers of different colors.

ALTERNATIVE 1: If colored pencils or markers are not accessible, they can complete the activity with a pencil or pen in a single color and establish a legend with patterns to distinguish between components.

ALTERNATIVE 2: Provide students with colored pencils and/or markers of different colors.

ALTERNATIVE 3: Have students print out the worksheet and color the legend and gene transcription model before class.

2. Assign students to watch these videos to obtain background and context information necessary to complete the activity.

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

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

OPTIONAL:

<https://www.hhmi.org/biointeractive/obesity-related-health-problems>

<https://www.hhmi.org/biointeractive/signal-molecules-trigger-transcription-factors>

ALTERNATIVE: Show the videos at the beginning of the class. Each video lasts between 3-5 minutes. This alternative will add to the duration of the activity.

If assigned before class, instructors may wish to use cicker questions (or other polling methods) to test student preparedness (see sample questions below).

DAY OF ACTIVITY:

3. Give a pre-test to assess students' knowledge before carrying out the activity.
4. Distribute Student Activity and allow 10 minutes for students to complete the activity.
5. **OPTIONAL:** Give an extended period for peer discussion and/or group discussion. Perhaps ask students to write a brief description of the conditions when PPAR- γ genes are ON and OFF and discuss the scenarios in small groups or as a class.
6. Formative evaluation on the main concepts that are intended to be learned through this activity.

AFTER THE ACTIVITY:

7. Summative assessment by means of a short test or questions under examination.

SAMPLE ASSESSMENT QUESTIONS:

In the signal transduction cascade associated with adipose tissue and PPAR- γ receptor; which of the following is a false statement?

- a. Lipids bound to PPAR- γ cause the receptor to bind to DNA.
- b. More, bigger fat cells arise due to dietary fatty acid: PPAR- γ activation of genes.
- c. Receptor, coreceptor and repressor are all proteins.
- d. Some cell types lack the PPAR- γ receptor protein.
- e. PPAR- γ bound to lipids dimerizes and results in protein phosphorylation

In adipose tissue, dietary lipids bind PPAR- γ and

- a. A signal moves from the cells surface in a signal transduction pathway
- b. PPAR- γ binds DNA turning on specific genes
- c. Hormones are taken up by the lipid cells
- d. Protein:fat complexes accumulate making big, fat cells

SAMPLE CLICKER QUESTIONS BASED ON HHMI BIOINTERACTIVE VIDEO:

From the HHMI video on PPAR- γ and adiponectin secretion from fat cells, which of these was **not discussed** in relation to the regulation of PPAR- γ ?

- a. high fat diet
- b. increased size of fat cells
- c. decreased blood glucose concentration
- d. anti-diabetic medications on the "TZD" class

The TZD drug (anti-diabetic drug) seen described in the video activates PPAR- γ by:

- a. causing a change in the protein conformation (shape) of PPAR- γ
- b. causing release of a repressor molecule from PPAR- γ
- c. opening ion channels in the cell membrane
- d. both a and b are correct
- e. both b and c are correct

PPAR- γ , as a transcription factor, has all of these features **except**:

- a. it must be able to bind to a specific DNA sequence on some genes
- b. it is "off" or inactive due to binding of a repressor molecule
- c. it functions as an RNA polymerase to add nucleic acids to a growing mRNA molecule
- d. it functions in combination with another protein, referred to a co-receptor or "RXR"
- e. it is turned "on" when activator molecules are able to bind to it

SAMPLE QUESTIONS FOR GROUP DISCUSSION:

Foods rich in mono- or polyunsaturated fats (fish oil, nuts, avocados, olives) may help to increase circulating levels of adiponectin. Explain why.

In general, what does insulin do and why is insulin sensitivity of target cells important?

What do unsaturated fatty acids and TZD drugs have in common?