

## MCB 52: POST-MIDTERM STUDY GUIDE

## LECTURE 25: EUKARYOTIC GENE REGULATION

Goal: To understand some of the basic principles of gene control in higher cells

## Objectives:

1. Define the terms Transcription factor, Activator, and a negative transcriptional regulator.

Some transcription sites for activators can be relatively far from the promoter. In such cases, the binding site is known as an enhancer. Enhancers are regulatory sequences located thousands or even tens of thousands of base pairs away from a promoter. Enhancers can be located either upstream or downstream of a promoter. Activators work from near and far by recruiting the transcription machinery.

2. Explain how an inducible regulatory protein, Gal4, can be an example of gene control universality and modularity.

The gal4 gene cluster of yeast is historically equivalent to the lactose operon of *Escherichia coli*. It is one of the genetic systems in which the principles of eukaryotic gene regulation has emerged. The Gal4 regulatory protein governs transcription of genes involved in galactose utilization such as *GAL1*.

The enhancer contains the Gal4 binding sites for the Gal4 protein called Gal4p. The Gal4 binding site is partially palindromic (conserved sites are *ATGTGT*). Gal4 binds as a dimer. It is possible to create a yeast with genes under Gal4 control by synthetically over-expressing Gal4 corresponding to the Gal4 binding site is sufficient to confer Gal4-dependent expression of a heterologous gene in yeast.

Universality of eukaryotic transcriptional activation: Gal4 activates transcription in any eukaryotic cell that contains an artificial promoter with a TATA box and the Gal4 binding sequence.

Experiment: Imagine a human cell growing in culture contains a gene of interest (such as a reporter gene) with a TATA box and several copies of the Gal4 upstream activating sequence (UAS) located upstream of the gene of interest. Add the Gal4 gene with inducible expression. Once Gal4 expression is induced, it will bind and activate expression of the gene of interest.

The idea that a yeast activator can be active in a human cell demonstrates that the mechanism of gene activation can be conserved from yeast to humans. The interface between the Gal4 dimer and the transcription machinery must be conserved in yeast, plants, and humans.

Modularity of eukaryotic transcription factors: Gal4 is modular, consisting of a DNA-binding domain and an actuator domain. The DNA-binding domain plays no role other than to tether the actuator domain to DNA. Modularity can be demonstrated by the construction of a hybrid protein consisting of a DNA-binding domain from the Gal4p and the activation domain of Gal4.

Experiment: case 1) use a reporter gene, lacZ, with a Gal4 binding site and Gal4 protein  $\rightarrow$  it should be expression of lacZ, case 2) if there's only a DNA binding domain and no activation domain  $\rightarrow$  no expression. In case 3) replace the Gal4 binding site with an operator, there will be

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