

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 term "enhancer" as it applies to eukaryotic gene control

Sometimes binding 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 in recruiting the transcription machinery.

2. Explain how the Gal4 regulatory protein illustrates two principles of gene control—modularity and universality

The galactose gene cluster of yeast is historically equivalent to the lactose operon of *E. coli*. It is one of the prototypical 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 consists of two binding sites for the activator called Gal4. The Gal4 binding site is partially palindromic (complementary sites on the strand). Gal4 binds as a dimer. It is possible to create a synthetic gene under Gal4 control; that is, a minimal sequence corresponding to the Gal4 binding site is sufficient to confer Gal4-dependent repression of a homologous 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 unicellular growing culture containing 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 mechanisms of gene activation are conserved from yeast to humans. The interface between the Gal4 activator 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 activation domain. The DNA-binding domain plays no role other than to tether the activation domain to DNA. Modularity can be demonstrated by the construction of a hybrid protein consisting of a DNA-binding domain from the yeast Gal4 and the activation domain of Gal3.

Experiment: case 1) use a reporter gene, lacZ, with a Gal4 binding site and Gal4 protein => there should be expression of lacZ; case 2) if there is only a DNA-binding domain and no activation domain => no expression; case 3) replace the Gal4 binding site with an operator, there will be

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