

BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 17 – Transcriptional Regulation in Eukaryotes

Compare complexity of regulatory elements between bacteria, yeast, humans

Enhancers

Insulators

Separable DNA-binding domain (DBD) and activating domain (AD) for activator proteins

Function and general structure of yeast Gal4 activator

Domain swap experiment to construct Gal4 AD/LexA DBD fusion protein – understand experiment and significance

Reporter genes: lacZ, firefly luciferase, GFP; why use reporter gene?

Understand two-hybrid assay using Gal4 DBD and AD fusions; significance?

Common types of DNA-binding domains: homeodomain, zinc fingers, leucine zipper, helix-loop-helix

Activation domains often unstructured; induced fit with target regulatory proteins

Recruitment by activator proteins

- general transcription factors (TAFs in TFIID, or TFIIB)
- mediator complex
- histone acetyltransferases
- chromatin remodeling complex (SWI/SNF)

Understand activator bypass experiment replacing Gal4 with LexA/Gal11 fusion protein

Examples of regulation of elongation: Activator recruitment of P-TEF to restart stalled RNAPII: *Drosophila* hsp70 promoter, HIV promoter + TAT protein

Synergistic effect of multiple activators – what does it mean? How does it work?

Modes by which cooperative binding of activators work at promoters

Know examples of cooperative binding: yeast HO promoter; enhanceosome at human beta-interferon enhancer

Know four general mechanisms how repressors can work

Example of repressor action: yeast gal1 gene with Mig1, Tup1

Signal transduction pathways – general concept and why important

Know basic aspects of JAK/STAT pathway

Know basic aspects of MAP kinase pathway

Two major ways activity of transcriptional activators controlled at end of signal transduction pathways

- unmasking transcriptional activating domain: examples with yeast Gal80/Gal4 proteins, and mammalian E2F activator bound by Rb protein
- regulation of transport to nucleus

Histone code hypothesis: what does it mean?

Transcriptional silencing; heterochromatin vs. euchromatin; examples of silencing in yeast; Sir2, Sir3, Sir4; Sir2 is HDAC

Correlation of DNA methylation with gene repression; 5-methylcytosine; binding of MeCP2 to methylated DNA; then MeCP2 recruits HDACs and chromatin remodelers

Gene imprinting is a good example of role of DNA methylation; know how mechanism works for mammalian imprinting of *Igf2* and *H19* genes

What is meant by epigenetic regulation? Example with DNA methylation

How are methylated DNA patterns inherited?

Reprogramming of mammalian somatic cells to pluripotent stem cells by adding four transcription factors (Oct4, Sox2, c-Myc, Klf4)