GENE REGULATION IN EUKARYOTES

Course: Molecular Biology (02022312)
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Reference:
Watson et al. Molecular biology of the gene, 6th ed. 2008,
Chap 17 pp. 589-632;

Lec # 12 Wed 04.04.2012

Chap 17

Transcriptional Regulation in Eukaryotes

- Transcription in eukaryotes is more elaborate than in prokaryotes and include additional complexities like:
- Nucleosomes and their modifiers
- More regulators and more extensive regulatory sequences
- The expansion of regulatory sequence in eukaryotes reflects the more extensive signal integration found in those organisms

The regulatory elements of Bacterial, Yeast & Human Genes

The figure illustrates the increasing complexity of regulatory sequences from as simple bacterial gene controlled by a Repressor to a human gene controlled by multiple Activators & Repressors.

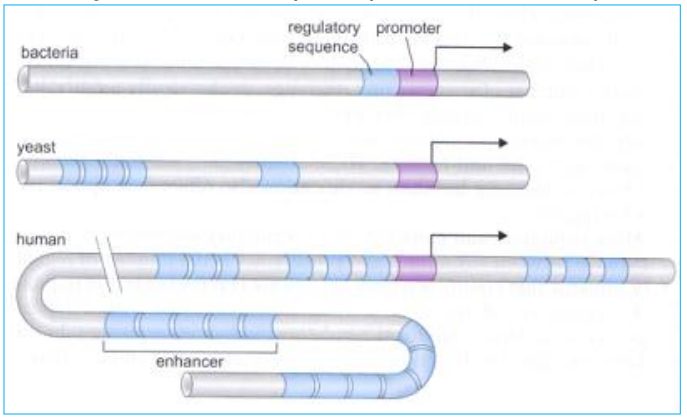


Fig 17-1, p. 500

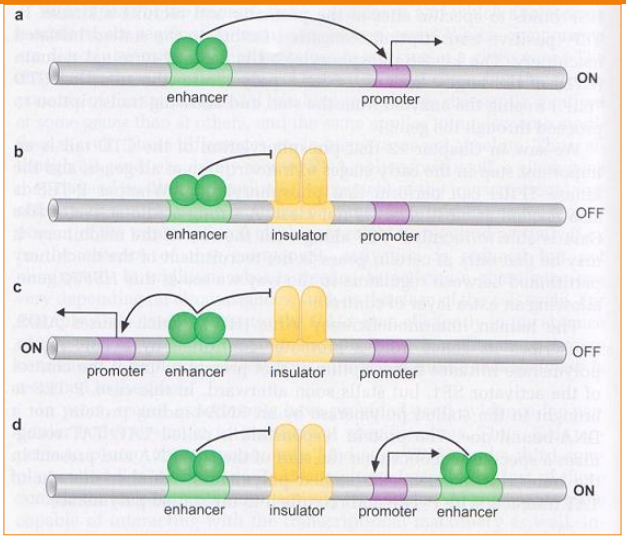
Enhancers

- In eukatyotes (multicellular) regulatory sequences can spread thousands of nts from promoter- both upstream and downstream- and can be made of tens of regulator binding sites.
- Often these regulator binding sites are grouped in units called "enhancers"
- Enhancers: bind regulators responsible for activating the gene at a give time and place
- Enhancers : example of "action at a distance"

Enhancers & insulators

- Activation at a distance raises another problem: When activators are bound at an enhancer, there may be several genes within range of this activator, yet a given enhancer typically regulates only one gene??
- How the effect of an activator/enhancer is blocked from affecting other genes located in the range of this activator/enhancer?
- Transcriptional silencing: propagation of certain repressing histone modifications over stretches of chromatin

Insulators block activation by enhancers



Conserved mechanisms of transcriptional regulation from yeast to mammals

- Activators have separate DNA-binding & activating functions:
- Example: activator Gal4 binds UAS in the presence of galactose> activates genes for galactose metabolism

Gal4 bound to its site on DNA

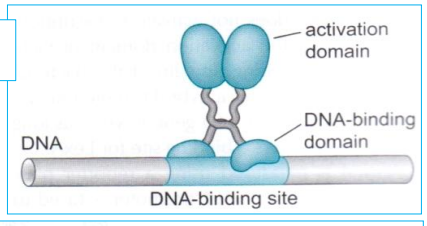


Fig 17-2

Regulatory sequence of yeast GAL1 gene. UAS contains 4 binding sites, each binds a dimer of Gal4 & activates GAL1 1000-fold

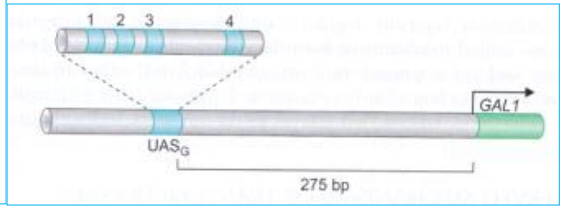
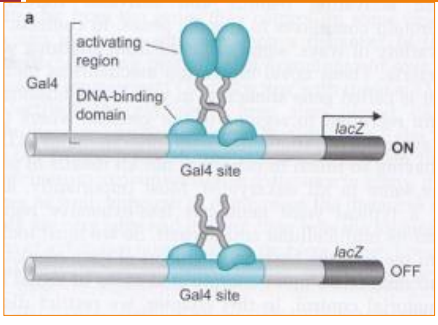
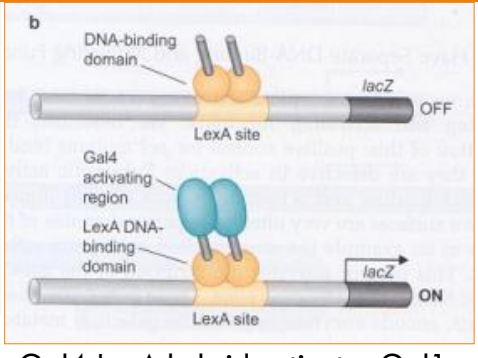


Fig 17-3

Domain swap experiment





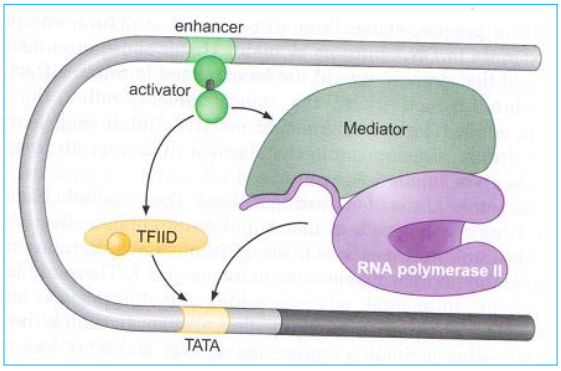
Gal4-LexA hybrid activates Gal1 promoter placed upstream of bacterial LacZ.
LexA: bacterial repressor
Gla4: activator

Eukaryotic Activators recruit the transcriptional machinery to the gene

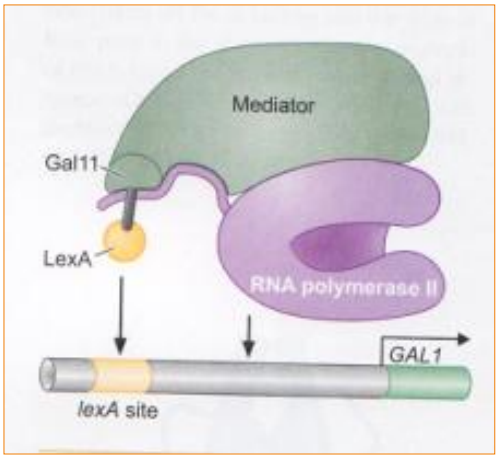
- Eukaryotic activators recruit proteins to the promoter and rarely, if ever, interact with RNA Pol directly, How?
  - Activators recruit proteins required for RNA Pol to bind promoter
  - Activators recruit nucleosome modifiers
  - Activators recruit factors required for RNA Pol to initiate and elongate
- Many of the transcriptional machinery proteins are recruited to promoter and come in preformed complexes like Mediator & TFIID
- Activators interact with these complexes & recruit them to the promoter

Activation of transcription initiation in eukaryotes by recruitment of transcription machinery

- A single activator is recruiting Mediator (bound to RNA Pol) and TFIID

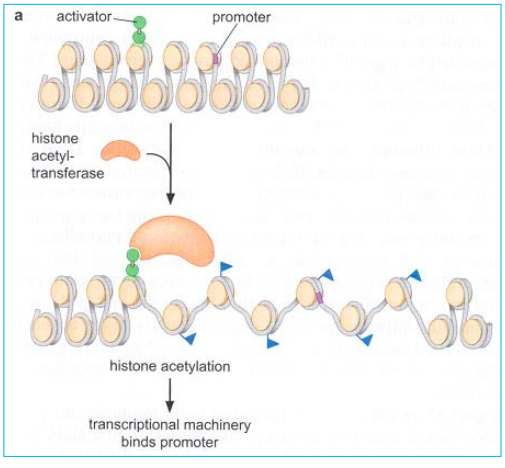


Activation of transcription through direct tethering of Mediator to DNA

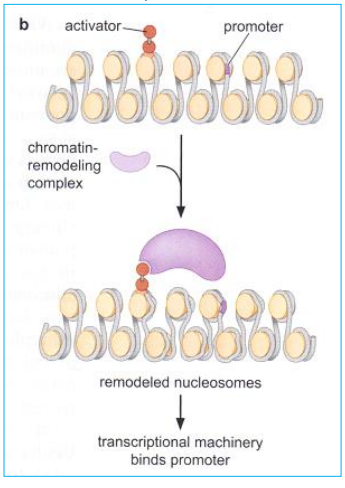


Activator recruit Nucleosome modifiers

Activator recruiting Histone Acetyltransferase (HATs)



Activator recruiting nucleosome remodeler SWI/SNF



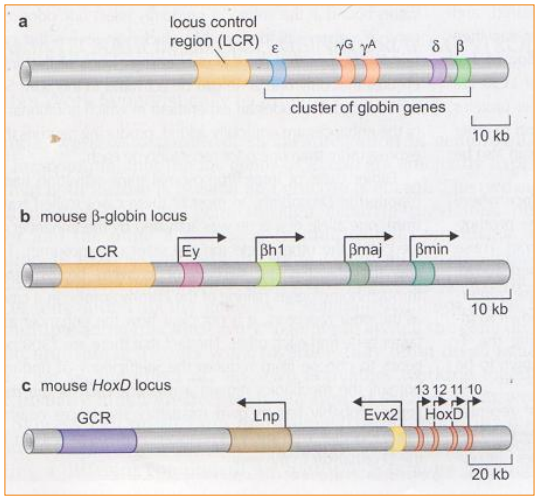
Activators recruit an additional factor needed for efficient initiation or elongation at some promoters

- Example: HSP70 in Drosophila is controlled by 2 activators working together, GAGA-binding factors & HSF
- In response to heat shock, the HSF binds to promoter & recruite kinase P-TEF (positive transcription elongation factor)> phosphorylates CTD of RNA Pol II

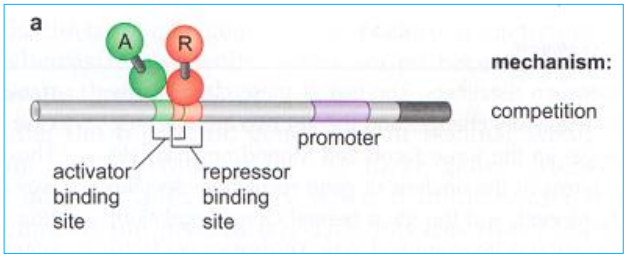
Appropriate regulation of some groups of genes require locus control regions

- Humans have 5 different globin genes? These genes are not expressed at the same time, rather at different stages of development starting with epsilon, gamma followed by delta and beta:
- How is their expression is regulated?
- B-globin gene has 2 enhancers: one upstream and one downstream??
- Locus control region: a group of regulatory elements collectively called LCR is found 30-50 kb upstream of whole cluster of globin genes

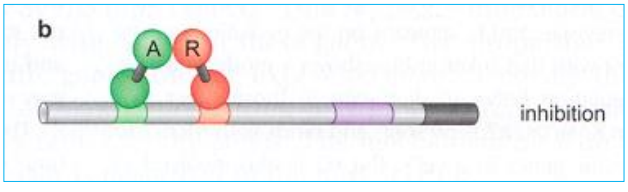
Regulation by LCRs



Transcriptional Repressors: ways in which eukaryotic repressors work

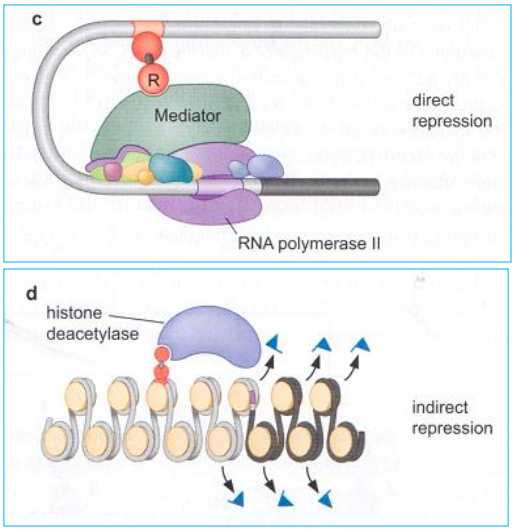


Repressor competes with Activator for a site on DNA



Repressor interact with Activator & block its activating region

Transcriptional Repressors: ways in which eukaryotic repressors work

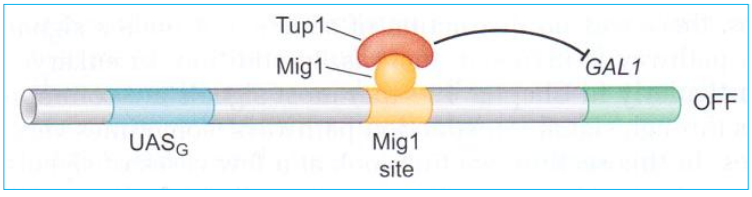


Repressor binds upstream of a gene and repress transcriptional machinery

Repressor recruits histone modifiers that inhibit transcription

Repression of GAL1 gene in yeast

- Mig1 repressor repress GAL1 genes in absence of galactose
- Mig1 binds to Mig1 site on DNA and recruits Tup1 repressing complex
- Tup1 mediates repression by recruiting Histone Deacetylase & also probably by contacting & inhibiting transcriptional initiation



Chap 18

Regulatory RNAs

- The role of regulatory RNAs in gene regulation arises from two sources:
  - The discovery of microRNAs in early 1990s
  - The discovery of the phenomenon of RNA interference in late 1990s

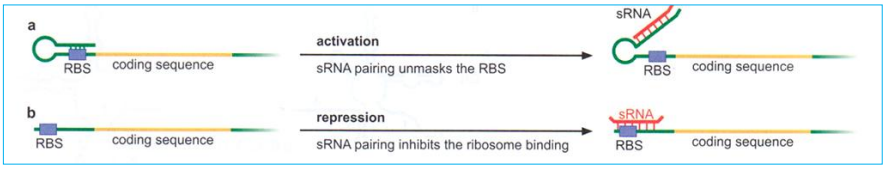
Chap 18

Regulatory RNAs

- Regulation of transcription by RNAs in bacteria
- Example: 6S RNA binds to the σ70 subunit of RNA Pol & down-regulates transcription from many σ70 promoters
- Bacterial small RNAs called sRNAs (80-110 nts). E. coli has >100 sRNAs. The sRNAs work by base pairing with target mRNAs & directing mRNA destruction, inhibiting translation or even in some cases stimulate translation
- Binding of sRNA to its target mRNA is aided by bacterial protein Hfq>>
- Example: E. coli 81-nt RybB RNA, form a double-strand stretch of heteroduplex with its target mRNA & is recognized by RNase E

Regulation of transcription by RNAs in bacteria

- Activation & repression of translation by sRNA



RNA interference is a major regulatory mechanism in eukaryotes

- Several types of very short RNAs repress or silence expression of genes with homology to those short RNAs> this silencing is called RNA interference (RNAi)
- These short RNAs are generated by special enzymes from longer double-stranded RNAs or various origins
- RNAi has been adapted for use as a powerful experimental tool providing means for "turning off' expression of any specific genes
- Small interfernce RNAs (siRNA): produced artificially or in vivo from dsRNA precursors
- microRNA (miRNA): derived from precursor RNAs encoded by genes expressed in cells where those miRNAs have specific regulatory functions

RNAi silencing

siRNA inhbit expression of homologous target genes by:

(1) Trigger destruction of mRNA

(2) Inhibit translation of mRNA

(3) Induce chromatin modification within the target gene & thus silence transcription

(4) Amplification Of inhibitory signal

RISC: RNA-induced silencing complex