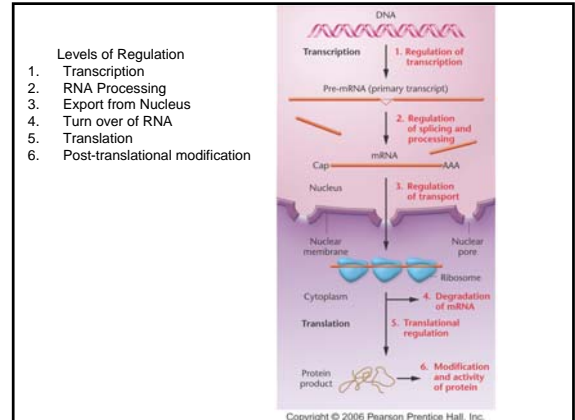


Eukaryotic Gene Regulation

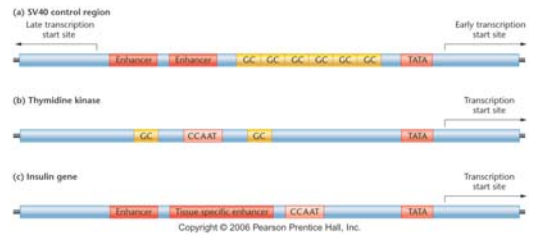
1. Levels of Regulation
2. Cis and Trans Elements
3. Analysis Cis/Trans Elements
4. Mechanisms of Activation/Repression
 - A. Chromatin Modification and remodeling
 - B. Interaction with preinitiation complex
5. Example of Regulation
 - A. Yeast Gal System
 - B. Steroid Regulation



Cis Elements

- Types of cis elements - two issues
 - Distance from start site
 - Proximal elements – within 200 bp of start
 - Distal elements – farther than 200 bp of start
 - Effect of Regulation
 - Positive elements
 - Negative elements
- Proximal Cis elements – positive effect
 - TATA Box
 - GC Box GGGCCG
 - CAAT Box
- Distal Cis elements
 - Enhancer – positive elements
 - Simple and Complex Enhancers
 - Silencer – negative elements

Modular Promoter Concept



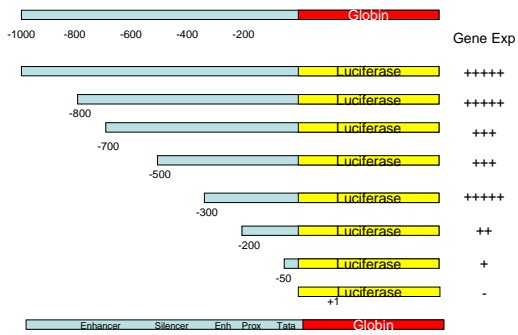
Transfactors

- Activators proteins that bind positive cis elements and increase rate of transcription
 - Examples
 - Proximal Elements
 - TBP – binds tata box
 - SP1 – binds GC box
 - CBP – binds CAAT box
 - Enhancers
 - GAL4
 - Estrogen Receptor
- Repressors bind negative cis elements and decrease rate of transcription
- Coactivators and co repressors – proteins that bind activators and repressors and participate in regulation of expression.

Identification of Cis and Trans Elements.

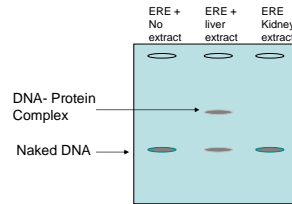
- Cis elements - Promoter Deletion Analysis
- Trans factors – mobility shift assay

Promoter Deletion Experiment



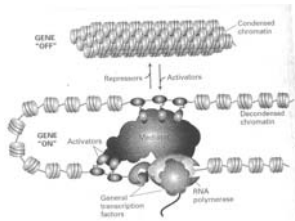
Mobility Shift Assay

1. Prepare Nuclear Extract from tissues
2. Isolate DNA
3. PCR Promoter Element (Estrogen Receptor Element) - Label (radioactive ^{32}P)
3. Electrophoresis with and without incubation with nuclear extracts

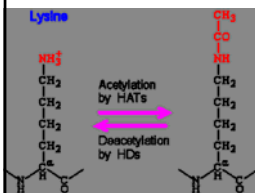


Mechanism of Activation

- Chromatin
 - Chemical Modification
 - Chromatin Remodeling Complex
- Promote Assembly of preinitiation complex
 - Direct interaction with TAF's and General Transcription Factors
 - Interaction with Mediator

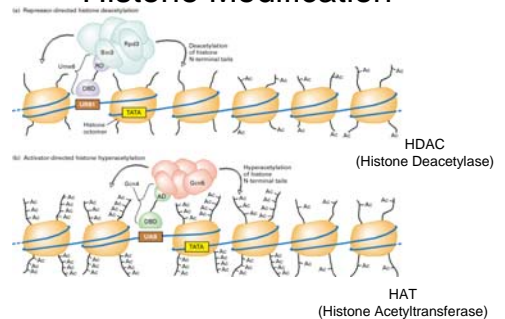


Histone Acetylation

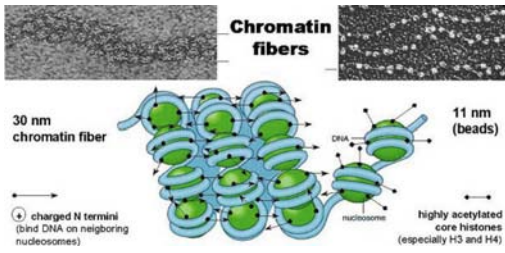


1. Direct modification of chromatin structure
2. Target for chromatin binding proteins
 - Second genetic code
 - bromodomain

Histone Modification

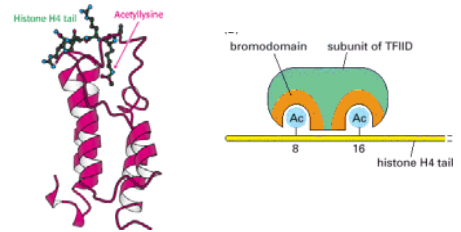


Effect of Histone Acetylation on Chromatin Structure

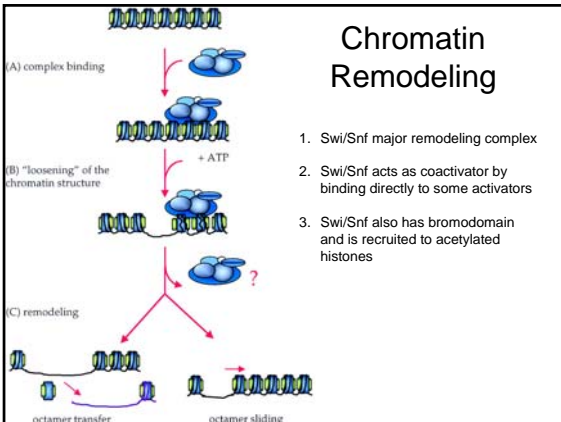


<http://edoc.hu-berlin.de/dissertationen/geissenhoerner-ange-2004-07-13/HTML/chapter1.html>

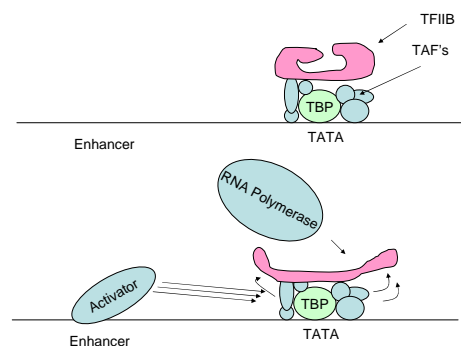
Bromodomain



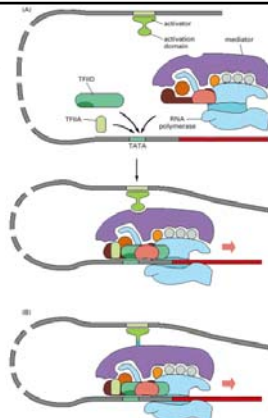
Chromatin Remodeling



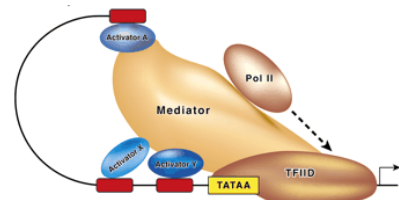
Activators interact with TAF's



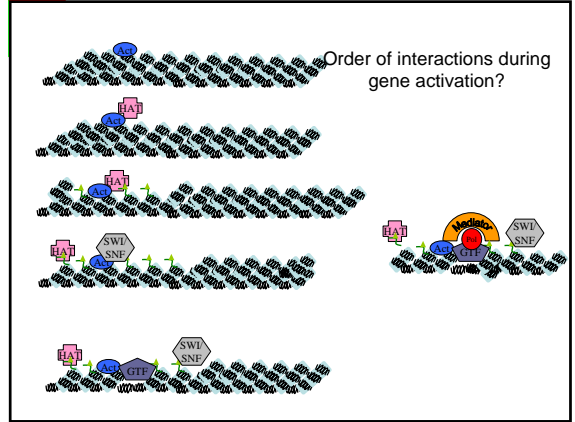
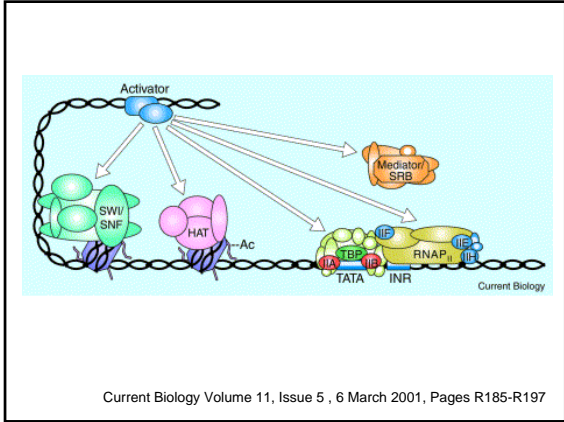
Interaction with Mediator



Multiple Activators and Mediator



Journal of Cell Science 116, 3667-3675 (2003)

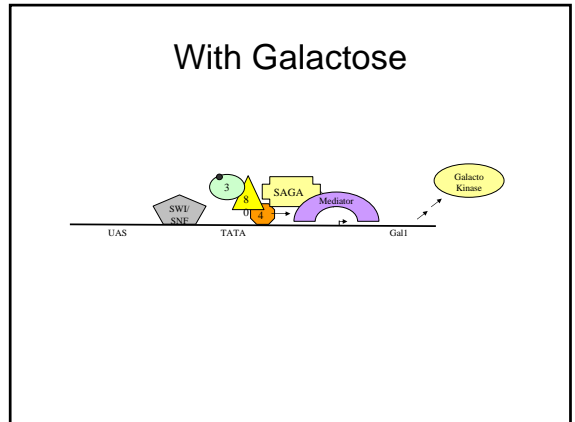
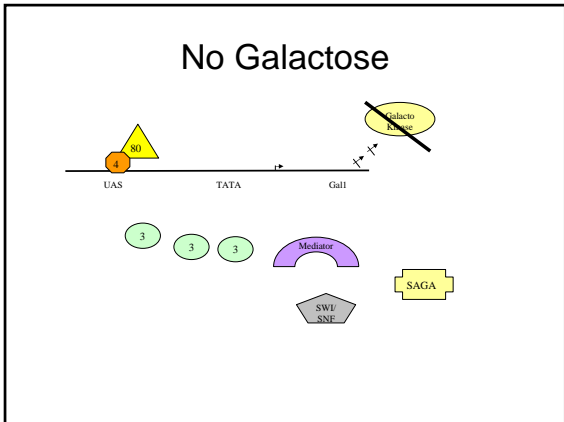
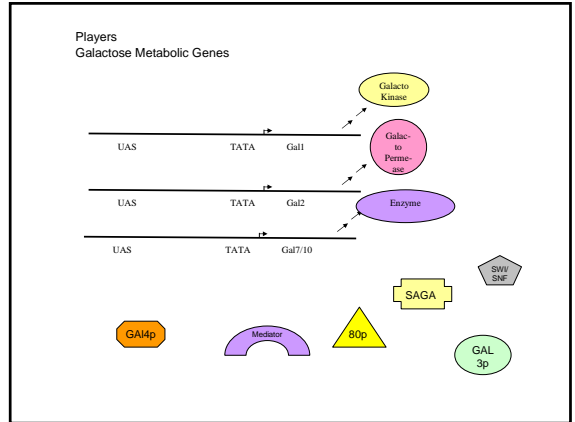


Gal Genes of Yeast

Yeast Gal System
 Yeast Genes for Galactose metabolism induced by Galactose
 Genes for Metabolizing Galactose
 gal2 -- Galactopermease
 gal1 -- Galactokinase
 gal7 and Gal 10 two enzymes that convert Phoso-galactose to Phoso- glucose

Regulatory Machinery
Cis-Elements
 TATA box – found 5' to all gal genes.
 UASgal - enhancer essential for up regulation of gal1,2,7,10. - found 5' to gal1,2,7,10 tata boxes

Trans Factors
 gal4 - GAL4p activator that binds UAS
 gal80 - GAL80p binds and represses GAL4p
 gal3 - GAL3p when bound to galactose binds and represses GAL80p
 SAGA – Complex recruited by GAL4p – has HAT activity
 Swi/Snf – Chromatin Remodeling Complex
 Mediator – recruited by GAL4p - complex associated with RNA polymerase II



Estrogen Regulation of vitellogenin Gene

Players

Example Gene
Vitellogenin gene

Cis Elements
ERE

Transfactor
Estrogen Receptor
HSP90

Coactivator
CBP/P300

Corepressor
NuCoR

Estrogen regulation of vitellogenin gene

Without estrogen

Estrogen receptor is in a complex with HSP 90 in cytosol
ERE is empty - no activation of vitellogenin

With estrogen

Estrogen binds estrogen receptor
Estrogen receptor undergoes allosteric change
Estrogen receptor release HSP90, exposes NLS and goes into nucleus
Estrogen receptor binds to ERE
Estrogen receptor recruits CBP
CBP is co-activator that acetylates histones, recruits mediator and interacts with GTFs.

With tamoxifen

Tamoxifen is estrogen agonist
Tamoxifen binds estrogen receptor
Estrogen receptor release HSP 90
Estrogen receptor moves into nucleus and binds ERE
Estrogen receptor with tamoxifen recruits NuCoR
NuCoR is nuclear co-repressor deacetylates histones

Cancer implications of tamoxifen

Huntingtons disease cause by Huntingtin polyglutamine tract binds CBP
Flies with Huntingtons disease live longer treated with inhibitor of deacetylase. Why?