

Mechanism of activation

3 general types of activator domains

- Acidic
 - Amphipathic helix, acidic amino acids on one face
 - No consistent secondary or tertiary structure has been identified
- Glutamine-rich (Q-rich)
- Pro-rich (P-rich)

No correspondence between type of DBD and type of AD

- Examples of proteins with acidic AD
 - GAL4 (Zn₂Cys₆)
 - AP1 (bZIP)
 - VP16 (no DBD)
 - repressor (HTH)
- Examples of proteins with Q-rich AD
 - Sp1 (Zn finger)
 - Antp (homeodomain)
 - Oct (POU-homeo)

Lack of fixed structure in activator domains

- DBDs of transcription factors form discrete structures that can be analyzed by X-ray crystallography and NMR
- The ADs do **not** generate identifiable electron density in the crystallographic analysis.
- This indicates that they do **not** form discrete structures.
- One hypothesis is that the ADs are unstructured until they interact with their targets.
- This is an induced fit model.

Models for mechanism of activation

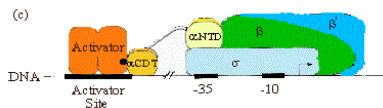
- Direct contact between an activator and RNA polymerase or GTF
- Indirect interactions
 - Adaptor
 - Mediator
 - Histone modifier complexes
 - Nucleosome remodelers
- No contact between enhancer bound proteins and the target promoter
 - Open a chromatin domain but not target a promoter
 - Linking via enhancer facilitators

Direct contact in activation

- Demonstrated in bacteria and yeast (some genes)
- Upstream activation sequences are adjacent to minimal promoters
- Examples
 - lambda repressor activates RNA polymerase at P_{RM}
 - cAMP-CAP activates RNA polymerase at *lac*. Direct contact between cAMP-CAP and the C-terminal domain of the alpha subunit of RNA polymerase

Suppression is strong evidence for direct contact

- Hypothesis: an AD makes direct contact with a component of the transcriptional apparatus
- Prediction: LOF mutations in the activation domain should be **suppressed** by appropriate mutations in that component.
- E.g. mutations in CAP can be suppressed by mutation in the σ subunit of RNA Pol.



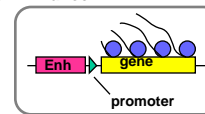
How do distal enhancers work?

Does activation require communication between an enhancer and a promoter?

- If so, expect
 - An effect on rate of transcription
 - Specific binding between activator or co-activator and the transcription complex
 - Mutations in target of binding should abolish activation
 - Find targets in suppressor screens
- If so, is it by looping vs. tracking?
 - Direct interaction?
 - Interact via another component?
 - Tracking?

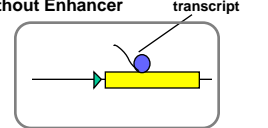
Some enhancers increase the rate of transcription initiation

With Enhancer



Polymerase density and amount of transcription increases in **all** cells in a population

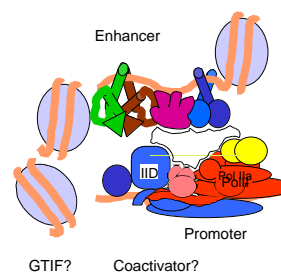
Without Enhancer

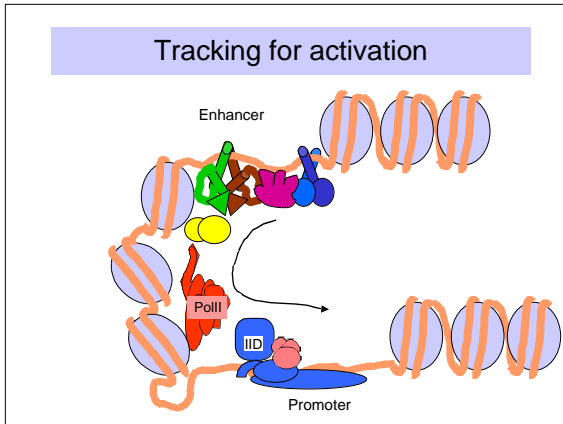


Looping vs. tracking

- Communication between enhancer and promoter can be via **direct** contact
 - Contact between proteins bound to **adjacent** sites
 - Contact between proteins at **distal** sites, with DNA between them **looped** out.
- Communication can be via **tracking**
 - Some component(s) of the transcriptional apparatus enter the chromosome at an enhancer and move along (track) until they act at a distal promoter.

Direct contact for activation



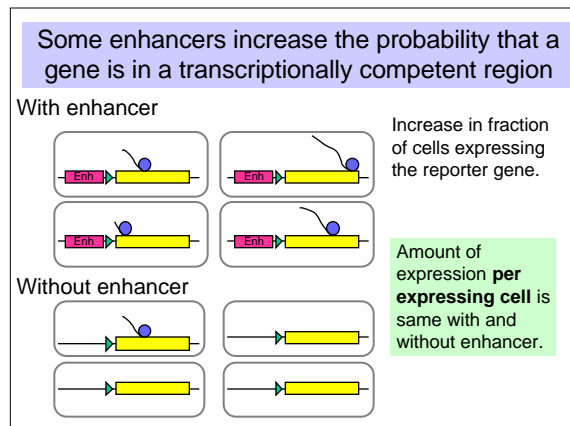


Interactions may be facilitated by DNA-bending proteins

- Many proteins that bind in the minor groove of DNA also bend the DNA.
 - TBP, YY1, HMG I(Y)
- interferon- gene enhancer
 - binding sites for 3 conventional txn factors
 - binding sites for HMG I(Y)
 - requires binding and bending of DNA by HMG I(Y) for activation by the other proteins bound to the enhancer.

Can activation occur without communication between an enhancer and a promoter?

- If so, expect no specific binding between activator and the transcription complex
- Possible models:
 - Open a chromatin domain so that it is more likely to be expressed
 - Affect probability that gene is in a transcriptionally competent region



Communication or not?

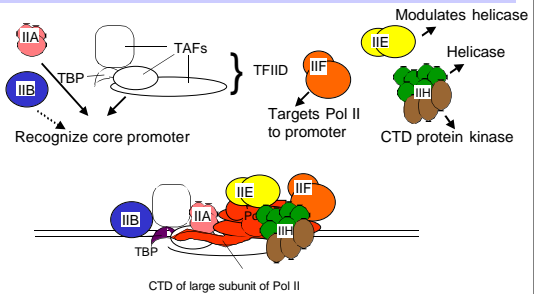
- An increase in rate of initiation by an enhancer can be explained by some kind of **communication** between the enhancer and the promoter
 - **Direct or indirect?**
- An increase in the probability that a gene is in a transcriptionally competent region does **not** require communication between the promoter and the enhancer.
 - It could be exerted by **making the chromatin structure in that domain accessible** to transcription factors in a greater fraction of cells.

Experiments to look for targets of activators

What proteins bind to the activation domain?

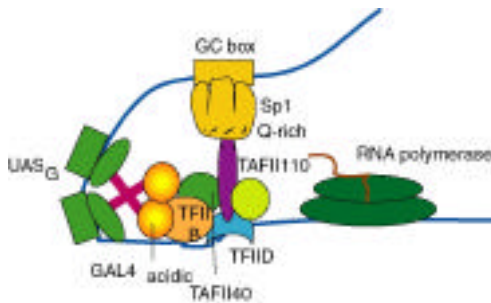
- Use **affinity chromatography**, with AD as the ligand
- Determine the nuclear proteins that bind specifically to that activation domain.
- Find that some GTFs, especially TAFs, bind to either acidic or Q-rich ADs
 - E.g. the acidic AD of VP16 will bind to TBP, TAFII40 and TFIIIB
 - Q-rich AD of Sp1 binds TAFII130

GTFs for RNA polymerase II



Many GTFs are possible targets for activators of transcription.

GTIFs can be targets of activation domains *in vitro*



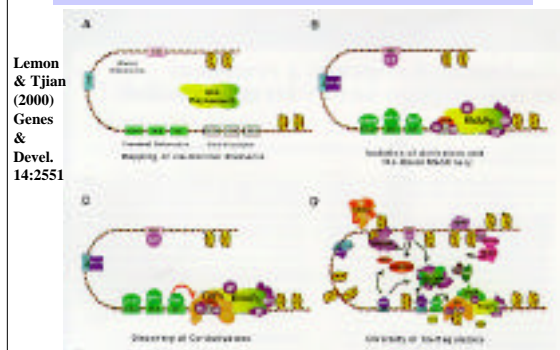
Are TAFs **required** for transcriptional activation?

- Construct conditional (ts) loss-of-function (LOF) alleles in genes for TAFs in yeast.
- Examine the level of expression of various target genes before and after temperature shift (active vs. inactive TAF).
- See that many genes are **still** activated in the **absence** of TAF function!
- TAFs are **not required** for **all** activation.
- TAFs **are** important - LOF alleles are lethal. Other functions include cell cycle progression.

Co-regulators

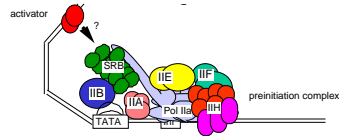
- Some sequence-specific activators (or repressors) do not regulate transcription by themselves.
 - Sp1 + **TBP** + RNA Pol II + other GTFs + promoter DNA: only basal transcription
- Co-activators and co-repressors are also needed
 - Sp1 + **TBP** + **TAFs** + RNA Pol II + other GTFs + promoter DNA: get activated transcription

Activators and Co-regulators



Mediator is a co-activator

- Yeast RNA Pol II does not respond to activators, but the RNA Pol II **holoenzyme** does respond to activators
 - **Mediator** (SRBs, Rgr1, Gal11, Med 1, 2, 6, 7, etc) is a type of co-activator



Some co-regulators work on chromatin

- Transcriptional activation *in vitro* from some promoters requires a chromatin template
- Some co-activators and co-repressors covalently modify histones and transcription factors:
 - Acetyl transferases
 - Deacetylases
 - Kinases, Methylases, ADP-ribosyltransferases
- Some co-activators use ATP hydrolysis to modify nucleosomes
 - SWI/SNF, ISWI, etc

Classes of co-regulators

- Class I: activator and repressor targets in polymerase and GTFs
 - TAFs, TFIID
- Adapters that bind to the activators
 - VP16, OCA-B
- Mediator
 - SRBs, etc. 3 in mammals: CRSP, SRC, NAT
- Complexes that covalently modify nucleosomal histones and transcription factors
 - HATS, HDACs, kinases, methyl transferases, etc
- Complexes that remodel chromatin in an ATP-dependent manner: SWI/SNF

Regulating the regulators

Regulation of activator proteins

- Tissue-specific synthesis of activator proteins
- Covalent modification
 - Phosphorylation of HSTF will activate it.
 - Phosphorylation of AP1 at some sites will activate it, at other sites will inhibit it.
- Active form of the transcription factor can be imported to the nucleus after dissociation of an inhibitor in cytoplasm
- Exchange heterodimeric partners

Example of steroid-hormone receptors

- In the absence of ligand (steroid), the receptor is in an inactive form in the cytoplasm.
 - Complexed with Hsp90 and other proteins.
- When steroid binds, Hsp90 dissociates, and the hormone-receptor complex is imported into the nucleus.
- In the nucleus, the hormone-receptor complex associates with an additional protein, binds to specific sites and activates target genes.