

Eukaryotic Gene Expression: Basics & Benefits

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Lecture 6

**Eukaryotic Transcription factors:
Transcription Activation Domains**

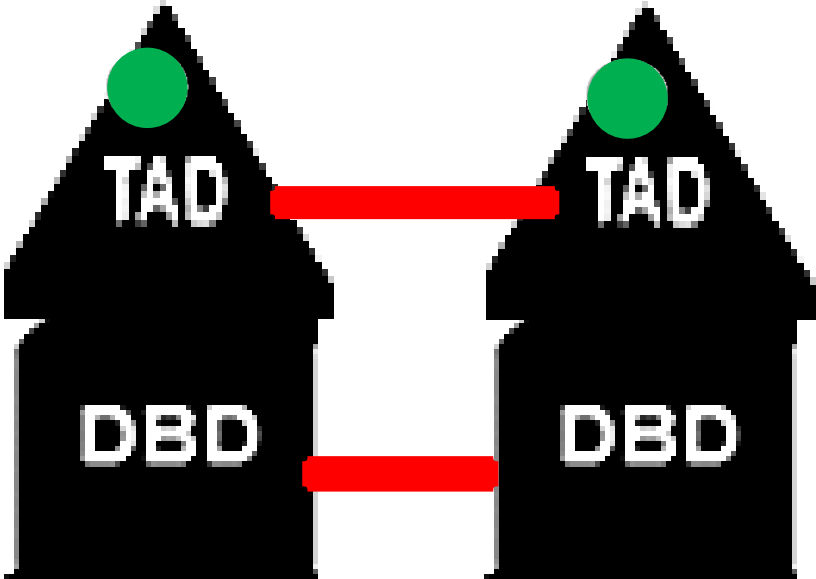
Major functional domains of eukaryotic transcription factor

DNA binding domain (DBD)

Transcription Activation domain (TAD) ←

Dimerization domain

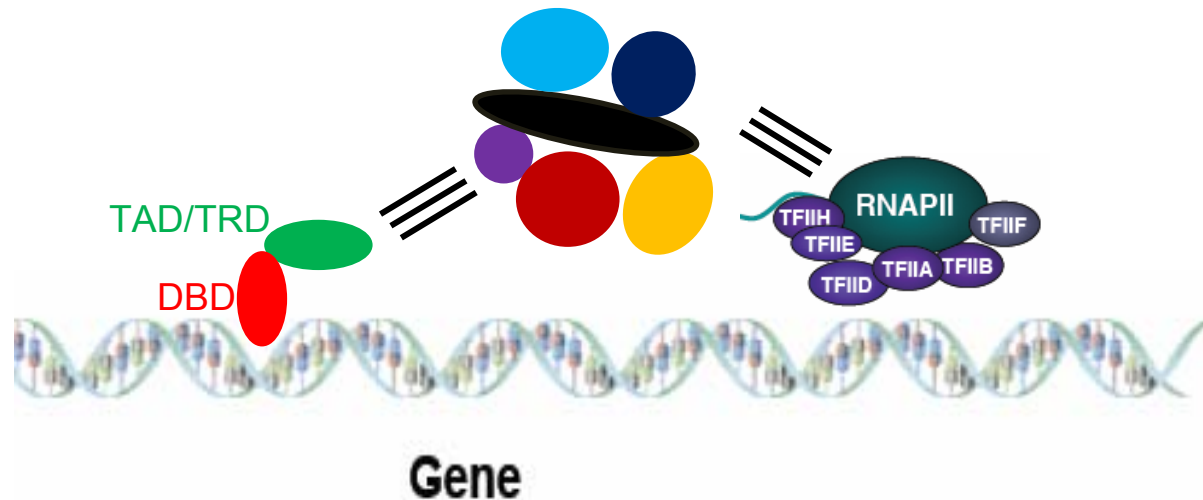
Effector binding domain for binding to an effector molecule



Transcriptional activation domains (TADs) are regions of a transcription factor which in conjunction with a DNA binding domain can activate transcription from a promoter by contacting transcriptional machinery (general transcription factors + RNA Polymerase) either directly or through other proteins known as **co-activators**.

Similarly, **Transcriptional repressor domains (TRDs)** are regions of a transcription factor which in conjunction with a DNA binding domain can repress transcription from a promoter by contacting transcriptional machinery (general transcription factors + RNA Polymerase) either directly or through other proteins known as **co-repressors**.

Understanding the mechanism by which TADs/TRDs function is rather complicated because of the fact that the eukaryotic basal transcription machinery consists of > 50 proteins.



To understand the mechanism by which TADs function, certain transcription factors are used as model proteins. These include:

- GAL4, GCN4, HAP1 etc., in yeast cells ,
- Steroid hormone receptors, heat shock transcription factors, NFκB etc., in mammalian cells,
- viral proteins such as herpes virus activator VP16, HIV TAT etc.

Domain swap experiments

moving domains among proteins, proving that DBD and TADs are separable domains and they can function independently.



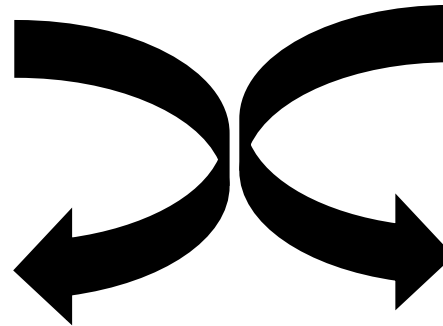
DNA binding domain of a prokaryotic transcription factor

Transactivation domains of eukaryotic transcription factors

LEXA DBD

GCN4 TAD

GAL4 TAD



LEXA DBD

GCN4 DBD

LEXA DBD

GAL4 TAD

Transcription Activation Domains

- ◆ Acidic domains
- ◆ Glutamine-rich domains
- ◆ Proline-rich domains

Acidic TADs

Also called “acid blobs” or “negative noodles”

Rich in acidic amino acids (DDD, EEE)

Exists in many transcription activation domains:

- **yeast GCN4 and GAL4**
- **mammalian glucocorticoid receptor**
- **herpes virus activator VP16 domains.**

A series of experiments by Mark Ptashne's group demonstrated in the 1990's that short peptides which form an **amphipathic helix**, with acidic residues along one surface and hydrophobic residues along another, can function as TADs

When fused to the DBD of GAL4, these peptides efficiently activated transcription from promoters containing GAL4 binding sites.

Giniger E, Ptashne M(1987) Nature (London) 330:670–672

Ma J, Ptashne M(1987) Cell 51:113–119

Gill G, Sadowski I, Ptashne M(1990) Proc Natl Acad Sci USA 87:2127-2131

Glutamine-rich TADs

Rich in glutamine (QQQXXXQQQ)

the proportion of glutamine residues seems to be more important than overall structure.

Exists in the general transcription factor SP1.

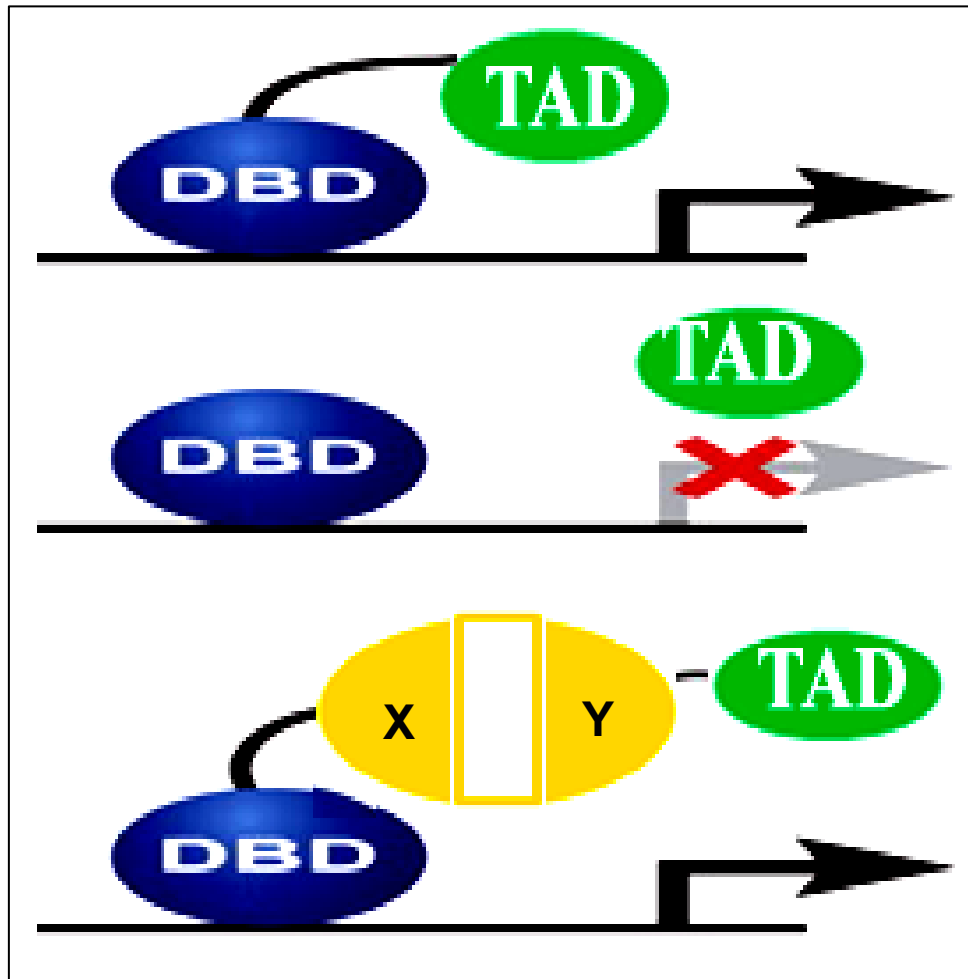
Proline-rich TADs

Proline-rich (PPPXXXPPP)

continuous run of proline residues can activate transcription

Exists in transcription factors c-jun, AP2 and Oct-2.

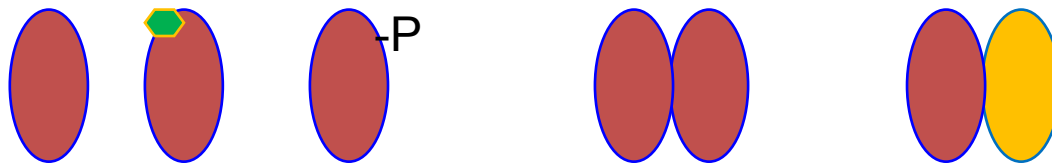
Isoleucine-rich TADs (IIXXII)



**Yeast two hybrid system
to identify protein-protein interactions**

Modulation of TAD function

TAD function is modulated by small effector molecules, post-translational modifications, dimerization etc.



Cell free transcription studies carried out with purified RNA polymerase II and general transcription factors indicated that transcription activation by certain transcription factors require additional proteins.

in vitro transcription reactions assembled from *partially purified* basal transcription factors and RNA polymerase II:

when assaying basal transcription (no activator) in vitro, recombinant TBP can substitute for TFIID

However, activator-dependent transcription required TFIID and recombinant TBP cannot substitute for TFIID when assaying activator-dependent transcription in vitro.

Certain components of TFIID are essential for activator-dependent transactivation.

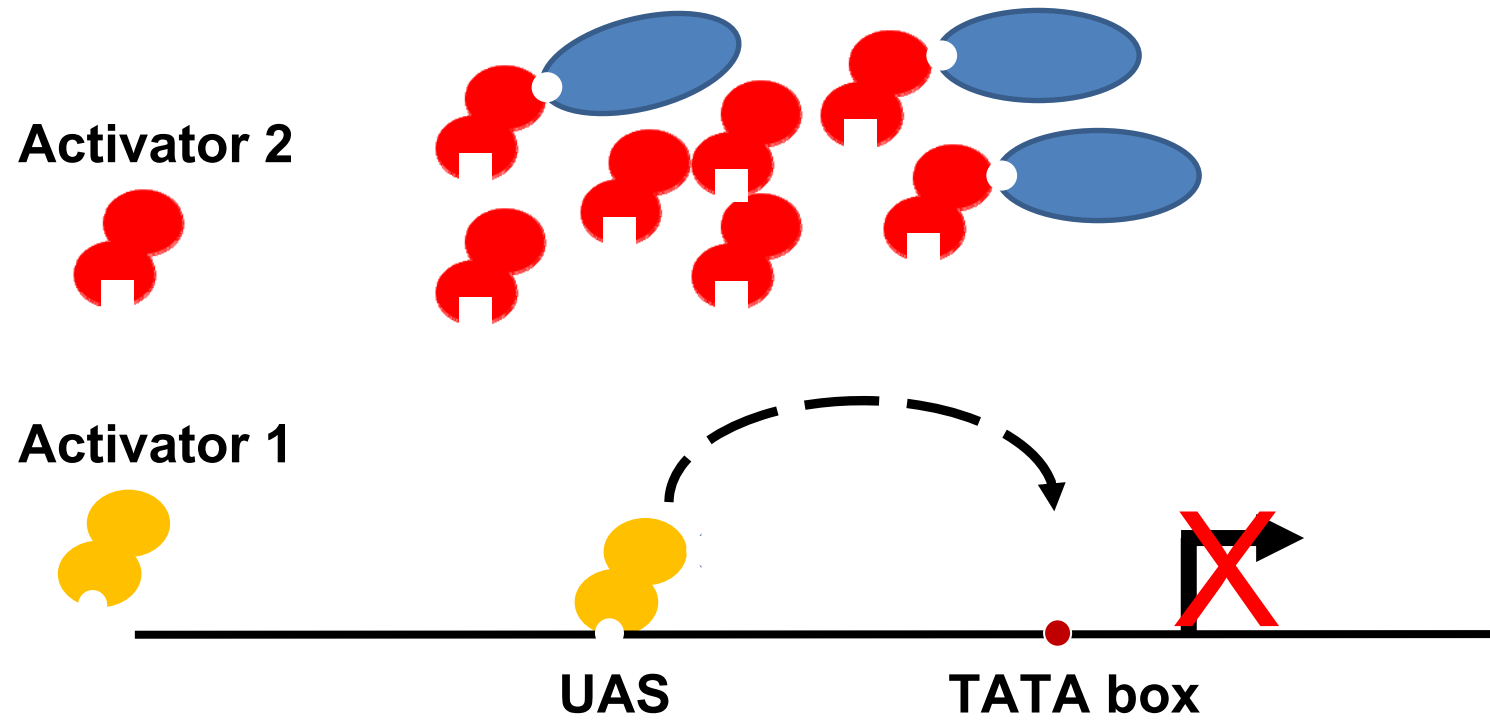
In fact, many of the TAFs in the TFIID complex interact with activators and thus function as coactivators.

Different transcriptional activators may require different coactivators.

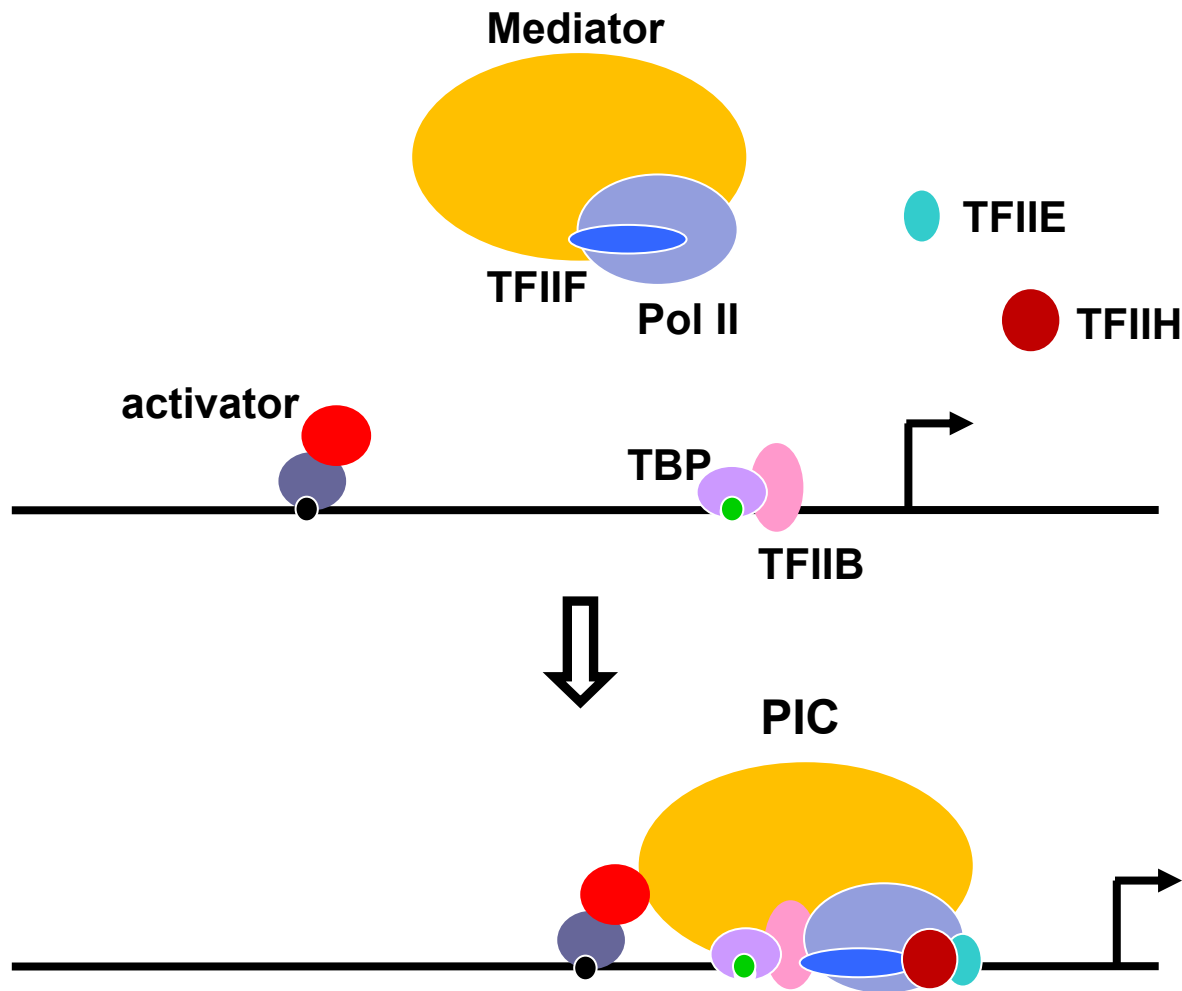
Eukaryotic activators do not bind to RNA pol II polymerase and therefore do not directly recruit RNA polymerase to promoters.

Activators through their TADs may indirectly recruit RNA polymerase by recruiting factors (**co-activators**) that serve as a physical bridge between activator and polymerase.

Activator interference / squelching



Yeast MEDIATOR as a co-activator



Mediator is an essential component of the RNA polymerase II general transcriptional machinery.

It plays a crucial part in the activation and repression of eukaryotic mRNA synthesis.

The *Saccharomyces cerevisiae* Mediator was the first to be identified and is a high molecular mass complex composed of >20 distinct subunits.

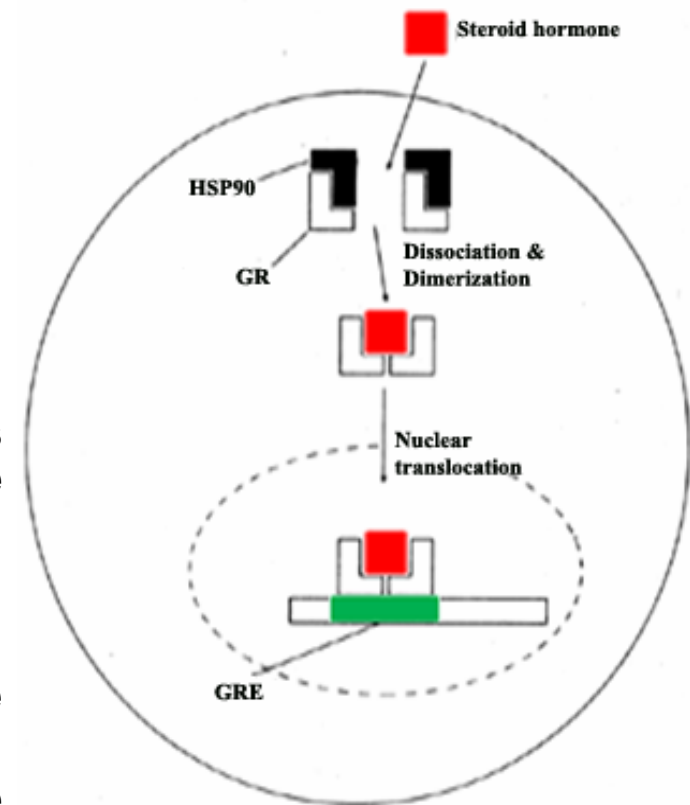
1. Constitutive transcription factors:SP1
2. Hormonal regulation:steroid hormone receptors
3. Regulation by phosphorylation:STAT proteins
4. Transcription elongation:HIV Tat
5. Cell determination:myoD
6. Embryonic development:homeodomain proteins

SP1

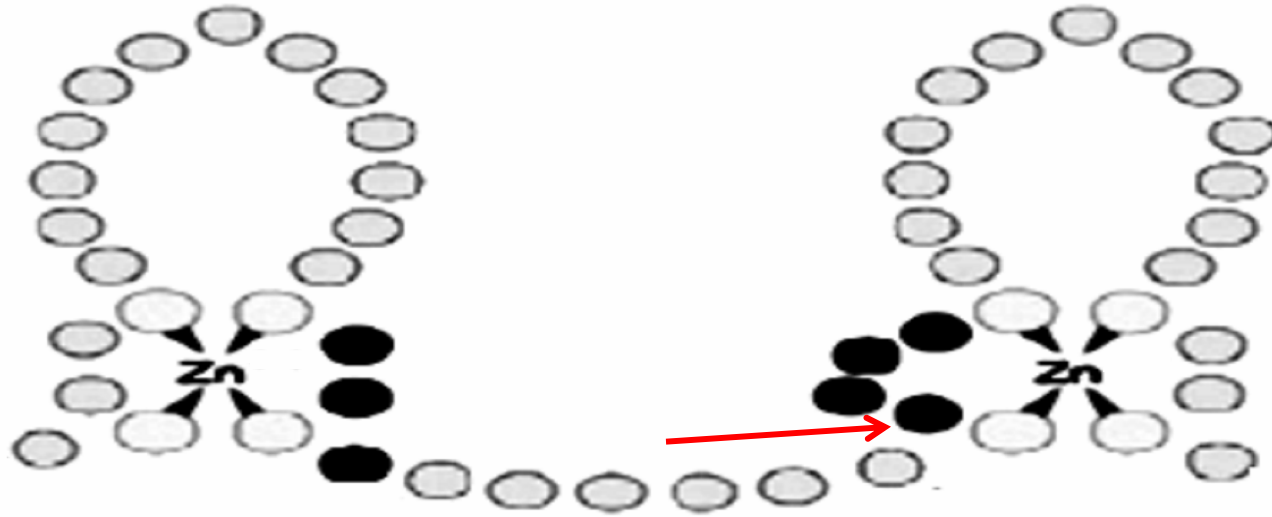
SP1 contains two glutamine-rich TADs which interact with TAF_{II}110, thus regulating the basal transcription complex.

steroid hormone receptors

- Many transcription factors are activated by hormones which are secreted by one cell type and transmit a signal to a different cell type.
- **steroid hormones: lipid soluble** and can diffuse through cell membranes to interact with transcription factors called steroid hormone receptors.
- In the **absence** of steroid hormone, the receptor is bound to an inhibitor (hsp90), and located in the cytoplasm.
- In the **presence** of steroid hormone,
 1. **the hormone binds to the receptor** and releases the receptor from the inhibitor,
 2. **receptor dimerization and translocation** to the nucleus.
 3. **receptor interaction its specific DNA-binding sequence**
(response element) via its DNA-binding domain, activating the target gene.



Dimerization domain of Glucocorticoid Receptor (GR)



At position 458 of the GR DBD, mutation of alanine to threonine results in a GR that is defective in dimerization.

Regulation by phosphorylation: STAT proteins

Certain hormones **bind** to cell-surface receptors and **pass a signal** to proteins within the cell through signal transduction.

In a signal transduction pathway, a specific ligand binds to an extracellular domain of a specific cell surface receptor and this binding brings about an allosteric change in the intracellular domain of receptor leading to activation of a series of protein kinases ultimately resulting in the activation of a transcription factor.

Signal transduction often involves protein phosphorylation.

Example: Interferon- γ induces phosphorylation of a transcription factor called STAT1 α through activation of the intracellular kinase called Janus activated kinase(JAK).

1. **Unphosphorylated STAT1 α protein:** exists as a monomer in the cell cytoplasm and has no transcriptional activity.
2. **Phosphorylated STAT1 α** at a specific tyrosine residue forms a homodimer which moves into the nucleus to activate the expression of target genes whose promoter regions contain a consensus DNA-binding motif

Transcription elongation:HIV Tat

- Human immunodeficiency virus (HIV) encodes an transcriptional activator protein called **Tat**, which is required for productive HIV gene expression.
- Tat binds to an RNA stem-loop structure called **TAR**, which is present in the 5'-UTR of all HIV RNAs just after the HIV transcription start site, to regulate the level of transcription elongation.
- In the absence of Tat, the HIV transcripts terminate prematurely due to poor processivity of the RNA Pol \square transcription complex.
- **Tat binds to TAR** on one transcript in a complex together with cellular RNA-binding factors. This protein-RNA complex may loop backwards and interact with the new transcription initiation complex which is assembled at the promoter.
- This interaction results in the **activation of the kinase activity of TFIIF**, leading to phosphorylation of the carboxyl-terminal domain (CTD) of RNA Pol \square , making the polymerase a processive enzyme to read through the HIV transcription unit, leading to the productive synthesis of HIV proteins.

Cell differentiation:myoD

- myoD was identified as a gene to regulate gene expression in cell determination, commanding cells to form muscle.
- MyoD protein has been shown to **activate muscle-specific gene expression directly**. Overexpression of myoD can turn fibroblasts into muscle-like cells which express muscle-specific genes and resemble myotomes.
- Four genes,myoD,myogenin, myf5 and mrf4 have been shown to have the ability to convert fibroblasts into muscle. The encoded proteins are all members of the **helix-loop-helix** transcription factor family.
- These proteins are regulated by an inhibitor called **Id** that lacks a DNA-binding domain, but contains the HLH dimerization domain.
- Id protein can bind to MyoD and related proteins, but the resulting heterodimers cannot bind DNA, and hence cannot regulate transcription

Embryonic development: homeodomain proteins

- The homeobox, encodes the **helix-turn-helix** DNA binding motif called the homeodomain.
- Homeotic genes of *Drosophila* are responsible for the correct specification of body parts.
- For example, mutation of one of the homeotic genes, **Antennapedia**, causes the fly to form a leg where the antenna should be.
- conserved between a wide range of eukaryotes.
- important in mammalian development.