DNA Binding Domains: Structural Motifs

- Studies of known transcription factors have found several motifs of protein design to allow sequence-specific binding of DNA.
- We will cover only three of these motifs:
  - Zinc fingers
  - Homeodomains
  - Leucine Zipper (bZIP) & bHLH

Effector Domain

- May work by steric hindrance
- Usually, a region of protein interacts with RNA pol II or another transcription factor
- Some TFs have effector domains rich in acidic amino acids (GAL4)
- Some rich in glutamine (Sp1—two domains of 25% glutamine)
- Some rich in proline (CTF, nearly 25% pro)

Zinc Fingers

- Repeated elements of a 30-aa sequence containing cysteines, histidines or a combination in an orientation that coordinates a zinc ion in space.
- Finger-shaped loop of amino acids fits well into the major groove, binding several bp each

Zinc Fingers, continued

- There are usually several of these elements repeated in the structure of the protein. That provides for binding of larger sequence elements over a longer stretch of sequence.
- Consists of α-helix and β-sheet held together by zinc ion.
- Many types - includes glucocorticoid receptor

Zif268

- “Immediate-early” protein—one of the first genes activated upon mitogenic stimulation.
- Three Zn fingers, each a curled shape coordinated by a Zn ion.
- The 3 Zn fingers form a “C” shape that locks onto the DNA binding in the major groove.
- Target bases all lie on one strand—G-rich motif.
Yeast GAL4

- Controls expression of a group of genes metabolizing galactose in yeast.
- Each target gene contains a GAL4-binding site (called a UAS<sub>G</sub>),
- Binds to UAS<sub>G</sub> as a dimer.
- Requires two domains:
  - **DNA Binding domain**: two Zn<sup>2+</sup> ions coordinating a short α-helical region.
  - **Dimerization domain**: a parallel coiled coil of two α-helical regions.
Separation of GAL4 Domains

- Possible to separate DNA binding domain from transcription effector domain.
- GAL4 binds UAS$_2$ site and stimulates transcription in yeast.
- LexA is a prokaryotic repressor, binds lexA operator, inhibits transcription.
- Build chimeric proteins.

DNA Binding & Transcription Effector domains can be swapped

**Figure 12.16**

Endogenous GAL4 activates trxn.
LexA inhibits trxn.
LexA-GAL4 substitutes GAL4’s transcription activator domain & activates

Nuclear Receptors

- Class of Zn finger transcription factors that are also hormone receptors.
- Regulatory domain = ligand binding domain.
- Sex hormones, glucocorticoids, Vitamin D, thyroid hormone, retinoic acid.
- Exist in cytoplasm inactive, bound to hsp90.
- Some are always nuclear (thyroid hormone receptor)—bind same site and repress in absence of ligand.
Homeodomain (helix-turn-helix)

- Two short $\alpha$-helical regions that fit into the major groove of DNA separated by a non-helical region that provides a "bend" or turn.
- 180 bp (60 aa) conserved region (homeobox)
- Best example - homeodomain proteins involved in differentiation.
- Engrailed, antennapedia, ultrabithorax

Antennapedia Mutation

Leucine Zipper

- Two domains - LZ & DBD
- DNA Binding Domain (DBD) - contains basic amino acids & provides specificity.
- Leucine Zipper (LZ) - combines two polypeptide chains in correct shape for interacting with major groove.

LZ Domain

- $\alpha$-helical (about 3.5 aa/turn)
- leu each 7 amino acids - same face of helix
- Two subunits interact through LZ domains
- Subunits may be identical (homodimer) or different (heterodimer)
- Combinations allow diversity of activity.
“Coiled coil” interaction

Figure 12.13b

“Scissors Grip” Binding of DNA

Figure 12.14

Both LZ & Basic Region Required for Activity of DNA Binding Domain
- Without dimerization, basic region cannot interact with major groove.
- Since dimerization region (LZ) required for DNA binding function, it must be considered part of DNA binding domain, along with basic region.

Two Theories of Transcription Initiation
- Basal transcription factors cause a stepwise build-up of pre-initiation complex on DNA.
- Basal factors already bound to holoenzyme, bind DNA as a unit.
Regulation by recruitment of basal factors

- The herpesvirus factor VP16 was shown to bind TFIID through its acidic effector domain.
- GAL4 seems to interact with TFIIB.
  - Promoter constructed with Ad E4 sequences
  - UAS\(_{42}\) added
  - Attached to beads

Add Factors & Wash

- Add each factor, one at a time.
- Wash off unbound factor.
- Add next factor.
- Did bound first factor enable assembly with second factor?

GAL4 stimulates assembly of Basal Complex

- Presence of GAL4 in first step greatly facilitated assembly of transcription complex.
- Binding of TFIID, however, was not affected by GAL4 presence.
- Binding of TFIIB was enhanced by GAL4 presence.
- Lack of TFIIB limited transcription.
- TFIIB binding requires TFIID, is enhanced by transcription activator (here using an acidic region).

Multiple factors act at a distance in a coordinated fashion

- Each factor binds its own recognition sequence, which may be far away from start site.
- Complex forms by protein-protein interactions.
- Requires bending of dsDNA.

Four theories

- TF binds sequence and changes shape of DNA, possibly inducing supercoils.
- TF binds sequence then slides along DNA to find promoter complex.
- TF binds sequence, loops intervening sequences to interact directly with trxn complex.
- TF binds sequence, binds second sequence and tracks along DNA.
Looping proved by catenane construction

- Two plasmids linked together by catenation were able to interact to stimulate transcription.

Interferon-β promoter/enhancer

- IFNβ induced by viral infection.
- Four binding sites for HMG I(Y), a factor that bends DNA; required for binding by NFkB and ATF-2.
- NFkB induced by inflammatory responses, “unbends” DNA; interacts with many promoters.
- All factors must bind to form “enhanceosome” complex—requires coordinate regulation of all factors.

Mediators (co-activators)

- Non-DNA binding proteins that promote or interfere with transcription activation.
- Example is CBP (CREB-binding protein)
- CREB (cAMP response element binding protein), activated by phosphorylation by PKA in response to cAMP, binds CRE (cAMP response element).
- But CREB binds CRE even when not phosphorylated—how does it work?
- Phosphorylated CREB binds CBP, which in turn activates basal transcription complex.
CBP also found in MAP Kinase Cascade

- Addition of growth factors (mitogens) stimulates activation of mitogen-activated protein kinase (MAPK) by phosphorylation.
- Phospho-MAPK enters nucleus, phosphorylates factors like Sap-1a, jun.
- These TFs use CBP to mediate activation of promoters.
- CBP is also a histone acetyl transferase enzyme, can loosen histones on DNA.

Integration of Signaling Cascades

- Mitogen acts at extracellular receptor to stimulate receptor dimerization (activation) & activation of tyrosine kinase.
- Receptor monomers phosphorylate each other.
- Phosphotyrosines recognized by GRB2, an adaptor.
- GRB2 now binds the ras exchanger Sos.
- Sos replaces GDP bound to ras with GTP.
- Ras delivers Raf to cell membrane, where it phosphoylates MAPK.
- MAPKK phosphorylates MAPK, which translocates to nucleus.
- MAPK phosphorylates jun.
- Jun associates with fos to make AP-1.
- AP-1 binds an AP-1 site and stimulates transcription through interaction with CBP.

Signal transduction linked to transcription factors

- Each TF regulated by some signaling pathway using some mechanism (phosphorylation, ligand binding, release from cytoplasm...)
- TF bind sites on DNA (enhancers).
- Multiple TF/enhancer complexes interact by looping DNA.
- Multiple regulatory complexes coordinate binding and/or activation activities to integrate signaling.
- Some activating domains increase association of TFIIB, others TFIID.
- Affect efficiency of transcription initiation!