BICH 107 lecture - Transcription in Eukaryotes – Oct. 16, 2003

1) Eukaryotic promoters and enhancers
2) Transcription factors
3) Transcription factors involved in bone formation: CBFA1, Osterix
4) Structures of DNA-binding domains of some transcription factors

Introduction
Recall central dogma:

DNA ----------→ RNA ----------→ protein

replication transcription translation

Transcription is first step in gene expression, and therefore the most likely place to regulate gene expression (although not the only possible point of regulation)

1) Eukaryotic promoters and enhancers: general function
Genes and promoters: promoters are defined as region of DNA that directs transcription of a gene - provides information regarding where RNA synthesis begins and how efficient it will be.

Promoters consist of small segments of DNA organized in a specific fashion - each segment is called a transcriptional control element, or element (PP slide 1)

2) Transcription factors
These elements are binding sites for some transcription factors - Some element/transcription factor pairs are found in many different promoters; others are found in only a limited subset of promoters.
Transcription factors are proteins that control transcription of genes

A complicated set of transcription factors binds to DNA elements and assemble together with other transcription factors that do not bind DNA - form a transcription complex that brings RNA polymerase - Then the gene is transcribed (PP slide 1)

The promoter is divided into elements that are sufficient for a basal level of transcription and elements that increase (or decrease) transcription - the latter elements compose so-called enhancers.
Transcription is usually regulated via these enhancers and the transcription factors that bind to them.

3) Transcription factors involved in bone formation: CBFA1, Osterix
A transcription factor (CBFA1) that is expressed specifically in pre-osteoblasts, cells that produce proteins present in bone (PP slide 2)

- targets (binding sites) for CBFA1 are in the promoters for genes encoding osteocalcin and type I collagen, proteins that are produced in bone cells

Show transgenic vs. normal mouse with knockout in gene encoding CBFA1 -
transparency: transgenic mouse defective in bone formation (osteogenesis)
blue dye stains cartilage; red dye stains calcified bone
In humans, lack of expression of one allele of CBFA1 results in a disease called cleidocranial dysplasia (CCD), a skeletal disorder. (PP slide 3)
Clinical features of CCD: delayed ossification of cranial bones; hypoplasia (delayed development) of clavicle

Another transcription factor is critical for bone formation – Osterix (Osx) (PP slide 4)
knock-out of osterix gene in transgenic mouse results in similar phenotype as the CBFA1 knock-out – show figure of skeletons from late-stage embryo and newborn. (PP slide 5)

Osx acts downstream of CBFA1 in the differentiation pathway for osteoblasts – show figure (PP slide 6) – Because, transgenic mice without Osx still produce CBFA1, but transgenic mice without CBFA1 do not produce Osx.

4) Structures of DNA-binding domains of some transcription factors
During the last two decades we have learned a great deal about how these proteins bind DNA - many detailed structures have been determined using X-ray crystallography and NMR spectroscopy

Show some examples:
VMD program - a fairly simple and user-friendly program; downloaded for free by academic users; then you can download coordinates for any protein whose 3D structure has been solved to use with this program

helix-turn-helix protein (POU domain of Oct-1 transcription factor) -
structure solved of a portion of a regulator protein, Oct-1, that is found in many eukaryotic promoters (enhancers) - the so-called POU domain
opens in wireframe representation
color structure – DNA is white, protein is colored
show space-fill representation (VDW) – there is no open space in the protein
change to ribbon representation in order to pick out secondary structure of the protein
show alpha helix fitting into major groove in two places

zinc finger protein (Zif268) - a zinc finger is 30 aa piece of a protein that recognizes about 3 bp of specific DNA sequence - a very common structural motif, named because a single zinc ion is included in each zinc finger - hundreds of transcription factors contain zinc fingers
3 Zn fingers for Zif268 that wrap around DNA
show alpha helices and beta sheets in licorice representation
alpha helix fits into major groove of DNA and recognizes specific base-pairs

TATA-binding protein (TBP) -
binds TATA box, a control element found in many eukaryotic promoters
DNA in white, protein in yellow and purple

differentiate alpha helices and beta sheets
unusual aspects about this protein is that it binds DNA in minor groove via beta sheets and causes severe bend in DNA while splaying open of minor groove

Once the transcription factor binds its specific sequence, or transcription element, in the promoter or enhancer, then it must "send its signal" to another protein, usually by binding to that protein or set of proteins. How that happens is the subject of much research in this field.