REGULATORY MECHANISMS OF TRANSCRIPTION FACTOR FUNCTION

• Protein synthesized
• Protein phosphorylated
• Ligand binding
• Release inhibitor
• Change partner, etc
### Transcription factors are activated in several ways

<table>
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<tr>
<th>Inactive Condition</th>
<th>Activation mechanism</th>
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<tr>
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<td>Membrane-bound protein</td>
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</table>
Body plan is constructed through interactions of the developmentally regulated homeotic gene expression

posterior late                  anterior early
low RA response                high RA response

trunk                           hindbrain

Figure 1. Organization of the Hox and HOM-C homeotic complexes. The organization of the mouse Hox and Drosophila HOM-C homeobox clusters is shown. There are four separate complexes in vertebrates labeled HoxA, HoxB, HoxC, and HoxD; the vertical alignment of the mouse and human genes signifies that these genes share a high degree of sequence identity and are related by duplication and divergence. The genes within a vertical column are collectively termed a paralogous group, numbered 1 to 13, moving 3' to 5'. The complexes are oriented so that the genes expressed in the most anterior regions are to the right.

Transcription control of the *Hox* genes: insight into the colinearity mechanism


correlation between linear arrangement along the chromosome and timing of transcriptional regulation

WT MUT WT MUT WT MUT
Activation of TRX following G-Protein Coupled Receptor GPCR Ligand Binding

Figure 2 | Genomic organization of human members of the CREB family of transcription factors. The consensus
CREB  (cyclic-AMP Responsive Elements Binding Protein)

Structure of the CREB basic region/leucine zipper domain (amino acids 285–339) (bZIP) bound to the somatostatin CRE (TGACGTCA)
Phosphorylation of CREB at Ser133 Induces Complex Formation With an α-Helical Domain in CBP
Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators

Richard I. Morimoto

Department of Biochemistry, Molecular Biology, and Cell Biology, Rice Institute for Biomedical Research, Northwestern University, Evanston, Illinois 60208 USA

Figure 1. Conditions that induce the heat shock response. Heat shock gene expression represented here by the activation of HSF and binding to HSE results in the elevated expression of HSPs such as Hsp70. The regulatory conditions are represented by environmental and physiological stress and nonstressful conditions, including cell growth and development and pathophysiological states.
Figure 2. General structural and regulatory features of HSFs. Schematic representation of HSF1 structural motifs corresponding to the DNA-binding domain, hydrophobic heptad repeats (HR-A/B and HR-C), the carboxy-terminal transcriptional activation domain, and the negative regulatory domains that influence HSF1 activity. The relative positions of these domains in HSF1 are indicated by the amino acid residues. Shown below is a schematic of the intramolecular negatively regulated monomer that, upon stress exposure, is activated to form homotrimers with DNA-binding activity.
The Heat Shock Transcription Factor DBD (wHTH)

cgcctcGAAtgTTCgcGAAa -46 hsp70

Figure 1. Structure of the heat shock transcription factor (HSF) DNA-binding domain. (A) The central helix-turn-helix motif (red) is composed of α-helix2 and α-helix3, where the latter is the DNA recognition helix. The structure was modeled using the Ribbons program from the coordinates of NMR structure of the Drosophila DBD (Visscher et al. 1994a). The loop
The Heat Shock Factor Family Across Species

Table 1. Characterization of HSFs across species

<table>
<thead>
<tr>
<th>Organisms Homology Expression</th>
<th>HSF1</th>
<th>HSF2</th>
<th>HSF3</th>
<th>HSF4&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Human, mouse, chicken 92% between species ubiquitous</td>
<td>human, mouse, chicken 92% between species ubiquitous</td>
<td>chicken ubiquitous</td>
<td>human tissue-specific heart, skeletal muscle, brain</td>
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<tr>
<th>In vivo conditions</th>
<th>37°C</th>
<th>42°C</th>
<th>37°C</th>
<th>42°C</th>
<th>Hemin/MG-132&lt;sup&gt;b&lt;/sup&gt;</th>
<th>37°C</th>
<th>45°C</th>
<th>37°C&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>127</td>
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<td>55</td>
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<tr>
<td>native [kD]</td>
<td>70</td>
<td>85</td>
<td>72</td>
<td>72</td>
<td></td>
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<td>denatured [kD]</td>
<td>C/N</td>
<td>N</td>
<td>C and N</td>
<td>C and N</td>
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<td>C</td>
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<td>Subcellular localization</td>
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<td>T</td>
<td>D</td>
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<td>Oligomeric state</td>
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<td>inducible phosphorylation</td>
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<sup>a</sup>Analysis based on transient or stably transfected cells.
<sup>b</sup>Hemin, an iron-containing protein, is an inducer of erythroid differentiation in human K562 cells. MG-132 is a peptide aldehyde inhibitor of the ubiquitin-dependent proteasome.
<sup>c</sup>DNA-binding activity is lost in vitro upon heat shock.

Canonical HSE (multiple adjacent pentanucleotide motifs)

cgcctcGAAhgTTCgcGAAa -46 hsp70
Regulation of the HS Response and the HSF Cycle

- Inert monomer in the cytoplasm and nucleus
- Stress induced DNA binding and TA potential (hyperphosphorylation)
- Trimerization
- HSF binding protein (HSBP1) negatively regulates trimer
- Dissociation of trimers
- Generation of unactive monomers upon binding of chaperones Hsp70 and Hdj-1
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The Nuclear Pore Complex is the Gateway That Regulates the Two-Way Traffic Between the Nucleus and the Rest of the Cell.
The Nuclear Pore Complex is the Gateway That Regulates the Two-Way Traffic Between the Nucleus and the Rest of the Cell

Figure 1 The nuclear pore complex. Rout et al.¹
Nucleocytoplasmic Trafficking: Nucleopore Complex and Karyopherins

Schematic illustration of the nuclear protein import cycle. Importin-β binds cargo with an NLS in the cytoplasm via the importin-α adaptor. The cargo–carrier complex then translocates through an NPC and is
Regulation of FoxO by Nuclear Shuttling in Response to AKT Mediated Phosphorylation

Winged-helix family of trx factors are involved in development, metabolism, cell differentiation

Fox=Forkhead box (100AA)

Phosphorylation stimulates nuclear export (NES) and prevents nuclear import (NLS)
The activity of FoxO is tightly regulated by post-translational modifications including phosphorylation, acetylation and ubiquitination.
Multiple Levels of Control of NFAT Signaling

A. General Features of NFAT Signaling

B. Structural Features of NFATc Proteins

**CsA**

**TCR, IGF, EGF**

**inhibition**

**induction**

IL2, IL3, IL4, GMCSF, TNF
Cooperative Binding of Two Unrelated Transcription Factors to Neighboring Sites

NF-AT (REL homology domain), AP-1 (Fos-Jun) cooperatively bind a composite DNA site (ARRE from IL-2 promoter)

Chen L. and Harrison SC. Nature 392:42-48
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*Note: The diagram shows various mechanisms by which transcription factors are activated, including protein synthesis, phosphorylation, dephosphorylation, ligand binding, and release by inhibitors.*
Hormone Dependent Gene Activation by a Homodimeric or Heterodimeric Nuclear Hormone Receptor
Conformational Change of the LBD of Two Related Nuclear Hormone Receptors Upon Ligand Binding
The NFκB Paradigm: Signal Induced Degradation of a Cytosolic Inhibitor Proteins Which Activates the TF

Phosphorylation dependent release by inhibitor
The NFκB/REL Family and IκB Proteins

NF-κB p50 homodimer bound to DNA
Müller CW, Harrisson SG and Verdine GL. Nature 373:311-317
The NFκB Paradigm: Signal Induced Degradation of a Cytosolic Inhibitor Proteins Which Activates the TF: Today!

TRX factors such as NFκB are final effectors of various cellular signaling cascades

Helix-Loop-Helix Domains

A. E box TF 5′CANNTG3′

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Figure 3. Amino Acid Sequence Relationships between E12, E47, and a Variety of Other Proteins


(B) Amino acid identities in E12, E47, and daughterless are indicated. Identical amino acids are shaded. Brackets indicate the homology region shown in Figure 3A.

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Figure 4. Helical Wheel Analysis of the Conserved Amino Acid Sequence of E12, c-myc, MyoD, and achaete-scute

The amino acid sequence of the conserved region of E12, MyoD, c-myc, and T5 achaete-scute (left to right) is displayed on a helical wheel. The hydrophobic residues are presented in bold, and are located in both helices on one side of the helix. Note the complete conservation of some of the hydrophobic residues of the different proteins. In contrast, the predominantly polar residues on the other side of the helix are not conserved. The hydrophobic surface of both helices is indicated by a shaded box.
Structural Classes Helix-Loop-Helix Domains - the DBD is Separated by Nonhelical Loops from the Leu-Zipper Region (Coiled-Coil)

T. Kadesch Cell Growth and Diff. 4:49 (1993)
bHLH Factors Activity is Modulated by Partner Exchange

bHLH dimers in which both subunits have a basic region can bind DNA; a dimer in which a subunit lacks the basic region (eg Id) cannot bind the promoter regulatory element.
Cholesterol and Triglyceride Synthesis Pathways

SREBP-2
- ATP-citrate lyase
- Acetoacetyl CoA thiolase
- Acetyl CoA synthase
- Acetyl CoA carboxylase
- Malonyl CoA
- HMG CoA synthase
- HMG CoA reductase
- Malic enzyme
- Mevalonate kinase
- Mevalonate PP decarboxylase
- GPP synthase
- IPP isomerase
- FPP synthase
- Squalene synthase
- Squalene epoxidase
- Lanosterol synthase
- CYP51
- Lathosterol oxidase
- DHCRC

SREBP-1c
- NADPH
- Malate
- Glucose-6-P
- PGDH
- 6-P-glucuronate
- Fatty acyl CoA
- GPAT
- Monoacylglycerol 3-phosphate
- Cholesterol receptor
- Triacylglycerides and phospholipids

NADPH
- Saturated fatty acids
- Monounsaturated fatty acids
- Fatty acyl CoA desaturase
- Stearoyl CoA desaturase
- Long chain fatty acyl elongase

SCAP
- N
- HMG-CoA Reductase

NPC1
- N
- Patched
Domain Structure of SREBP’s Membrane Bound TF
(sterol regulatory element (SRE) binding protein - 5’PyCAPyNPyCAPy3’)

Figure 1. Domain Structures of Human SREBP-1a and SREBP-2
The sequence of SREBP-1c (not shown) is identical to that of SREBP-1a except for a shortened NH2-terminal acidic domain (24 amino acids in SREBP-1c versus 42 amino acids in SREBP-1a).
Sterol Dependent Two-Steps Proteolytic Cleavage of TF
The Sterol Response: Cleavage to Release the Active TF

Figure 3. Immunofluorescence of SREBP-2 in Cultured Fibroblasts

Brown M. Goldstein JL. Cell 89 (1997)
The tubby gene is mainly expressed in the hypothalamus and is involved in feeding behaviour control. Mice bearing a tubby null allele develop adult onset of obesity.

Tubby contains both a DBD and a AD; it is localized in the cytoplasm, near the plasma membrane (unusual for a TF!)

Tubby is bound tightly to PIP2 in the plasma membrane and Go or Gq-coupled receptors (which activate phospholipase C) activate Tubby by activating PIP2 hydrolysis and the consequent release of Tubby into the nucleus.
Domain Structure of LEF-1/TCF proteins. Depicted are the protein domains of LEF-1, the mammalian TCF homologs (TCF-1, -3, and -4) and the Drosophila orthologue dTCF. The region of highest homology between LEF-1/TCF family members is the HMG DNA-binding domain, and the amino-terminal β-catenin-binding domain. LEF-1/TCF family members are more divergent in the region between the β-catenin-binding domain and the DNA-binding domain, which in LEF-1 mediates interactions with ALY and in TCF-1 mediates interactions with Groucho. dCBP apparently binds to the HMG domain but acetylates a lysine (K25) in the amino terminus of dTCF.

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<tr>
<th>Protein</th>
<th>β-catenin binding domain</th>
<th>Context dependent activation domain</th>
<th>HMG domain</th>
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<tr>
<td>mLEF-1</td>
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<td>Interaction with β-catenin</td>
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<td>Interactions with ALY</td>
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<td>Interaction with DNA and CBP</td>
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<td>55-176-299-372-511</td>
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<td>Interactions with Groucho</td>
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<tr>
<td>dTCF</td>
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<td>271-366</td>
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K25 (site of acetylation by CBP)
The canonical Wnt–β-catenin pathway