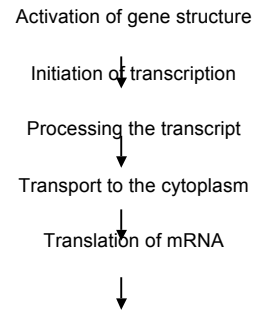
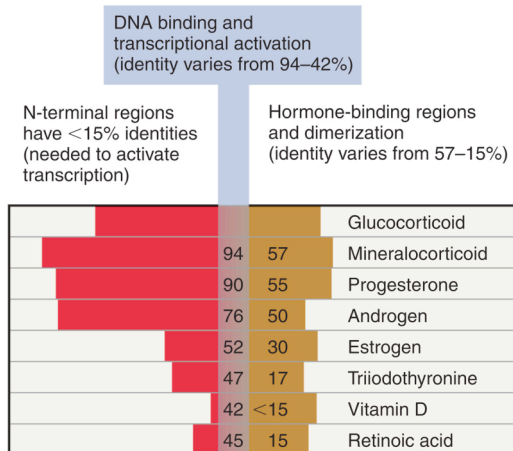


Chapter 25: Regulating Eukaryotic Transcription

- At least 5 potential gene expression control points



Ligand-gated receptors share structural features



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The Ligand Responsive Activators

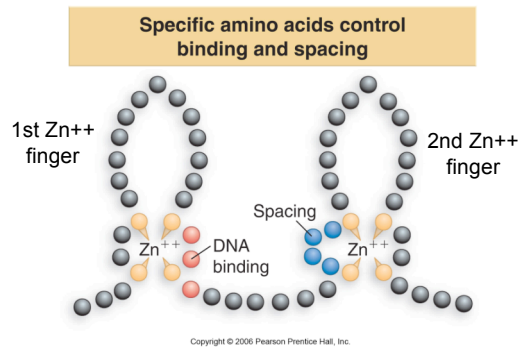
- Superfamily of Gene Regulators
- All receptors have independent domains for DNA binding and Hormone binding in the same relative locations
 - with an individual N-terminal region
 - conserved DNA-binding region
 - and a C-terminal hormone-binding region
- Includes the
 - Steroid hormone receptors
 - Thyroid hormone receptors
 - Retinoic Acid receptors

25.9

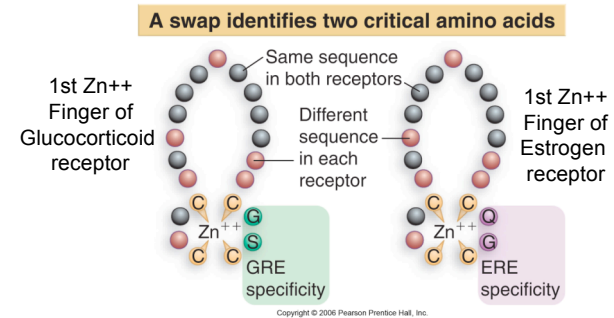
Zinc Fingers of Steroid Receptors Use a Combinatorial Code

- The DNA-binding domain of a steroid receptor is a type of zinc finger that has Cys but not His residues.
 - Cys₂/Cys₂ Zn⁺⁺ finger
- Usually, steroid receptors each have two zinc fingers.
- The receptor forms a dimer - each subunit binds to a half site in DNA.
- The first zinc finger recognizes the sequence of the half site.
- The second zinc finger is responsible for dimerization, which determines the distance between the subunits.

The sequence of the half site is recognized by the first zinc finger.
 The second zinc finger is responsible for dimerization, which determines the distance between the subunits.



Discrimination between GRE and ERE target sequences is determined by two amino acids at the base of the first zinc finger in the receptor.



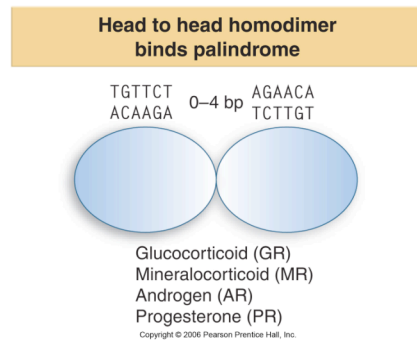
25.9 Zinc Fingers of Steroid Receptors Use a Combinatorial Code

- A receptor recognizes its response element by the orientation and spacing of the half sites.
 - A steroid response element consists of two short half sites that may be palindromic or directly repeated.
- Subunit separation in the receptor determines the recognition of spacing in the response element.

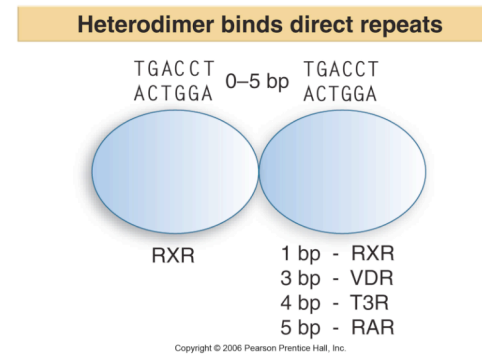
25.9 Zinc Fingers of Steroid Receptors Use a Combinatorial Code

- Some steroid receptors function as homodimers but others form heterodimers.
- Homodimers recognize palindromic response elements
- Heterodimers recognize response elements with directly repeated half sites.
- These are the only two types of half sites.

Response elements formed from the palindromic half site TGTTCT are recognized by several different receptors depending on the spacing between the half sites.



Response elements with the direct repeat TGACCT are recognized by several different receptor heterodimers depending on the spacing between the half sites.

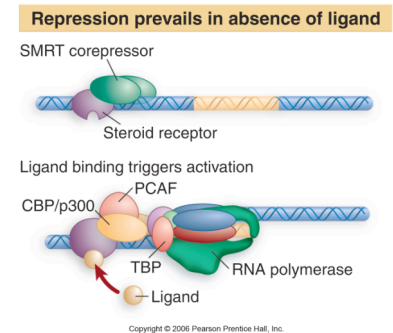


25.10

Steroid Receptor Binding to the Response Element Is Activated by Ligand Binding

- All receptors in the steroid receptor superfamily are ligand dependent activators of transcription
 - I.e. - only binds DNA when ligand is bound to receptor
- Binding of ligand to the C-terminal domain of an activator increases the affinity of the DNA-binding domain for its specific target site in DNA.
 - I.e. - only binds DNA when ligand is bound to receptor
- Some are also able to repress transcription
 - E.g. - TR and RAR in the form of heterodimers with RXR
- How?

- The steroid receptors TR and RAR bind the SMRT corepressor in the absence of ligand.
- The promoter is not expressed.
- When SMRT is displaced by binding of ligand,
 - the receptor binds a coactivator complex.
 - activation of transcription by the basal apparatus.



25.11 Homeodomains Bind Related Targets in DNA

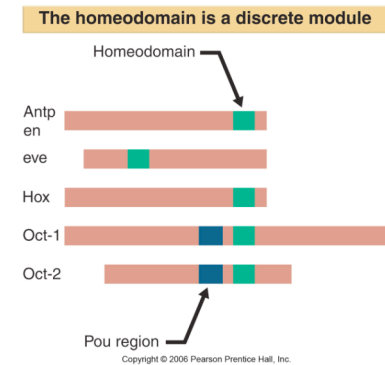
- The homeodomain is a DNA-binding domain of 60 amino acids that has three α -helices.
- The C-terminal α -helix-3 is 17 amino acids and binds in the major groove of DNA.
- The N-terminal arm of the homeodomain projects into the minor groove of DNA.
- Proteins containing homeodomains may be either activators or repressors of transcription.

The homeodomain is a module of 60 amino acids

- The homeodomain of the *Antennapedia* gene represents the major group of genes containing homeoboxes in *Drosophila*;
 - *engrailed (en)* represents another type of homeotic gene;
 - the mammalian factors Oct1 and Oct2 represent a distantly related group of transcription factors (POU homeodomain factors).
- The homeodomain is conventionally numbered from 1 to 60.
- It starts with the N-terminal arm, and the three helical regions occupy residues 10-22, 28-38, and 42-58.
- Amino acids in red are conserved in all three examples.

The homeodomain may be the sole DNA-binding motif in a transcription factor or may be combined with other motifs.

It represents a discrete (60 residue) part of the protein.

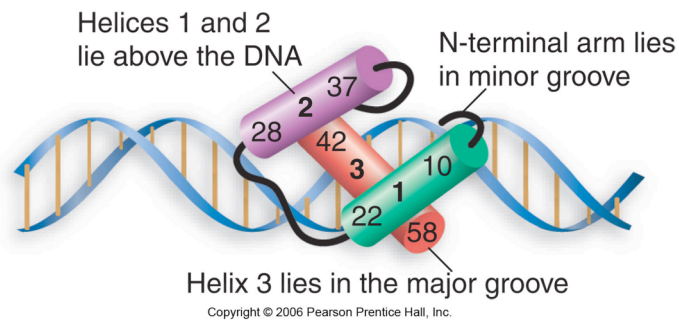


The homeodomain is a module of 60 amino acids

	1	N-terminal arm	10	Helix 1	20
En	Glu	Lys Arg Pro Arg Thr Ala	Phe Ser Ser Glu Gln Leu Ala Arg	Leu Lys Arg Glu Phe	Asn Glu
Antp	Arg	Lys Arg Gly Arg Gln Thr Tyr Thr	Arg Tyr Gln Thr Leu Glu Leu	Glu Lys Glu Phe	His Phe
Oct2	Arg	Lys Lys Arg Thr Ser	Ile Glu Thr Asn Val Arg Phe Ala	Leu Glu Lys Ser	Phe Leu Ala
	30	Helix 2	40		
En	Asn	Arg Tyr Leu Thr	Glu Arg Arg Arg Glu Glu	Leu Ser Ser Glu	Leu Gly Leu
Antp	Asn	Arg Tyr Leu Thr	Arg Arg Arg Arg Ile Glu	Ile Ala His Ala	Leu Cys Leu
Oct2	Asn	Glu Lys Pro Thr	Ser Glu Glu Ile Leu Leu	Ile Ala Glu Gln	Leu His Met
	41	50	Helix 3	60	
En	Asn	Glu Ala Gln Ile Lys Ile Trp Phe	Gln Asn Lys Arg Ala	Lys Ile Lys Lys Ser	Asn
Antp	Thr	Glu Arg Gln Ile Lys Ile Trp Phe	Gln Asn Arg Arg Met	Lys Trp Lys Lys Glu	Asn
Oct2	Glu	Lys Glu Val Ile Arg Val Trp Phe	Cys Asn Arg Arg Gln Lys	Glu Lys Arg Ile	Asn

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The homeodomain has 3 α -helices



25.12 Helix-Loop-Helix Proteins Interact by Combinatorial Association

- A **bHLH protein** has a basic sequence adjacent to the HLH motif that is responsible for binding to DNA.
- Class A bHLH proteins are ubiquitously expressed.
- Class B bHLH proteins are tissue specific.
- A class B protein usually forms a heterodimer with a class A protein.

25.12 Helix-Loop-Helix Proteins Interact by Combinatorial Association

- The **helix-loop-helix (HLH)** motif is
 - responsible for dimerization of a class of transcription factors called HLH proteins.
- Helix-loop-helix proteins have
 - a motif of 40–50 amino acids that comprises two amphipathic α -helices of 15–16 residues separated by a loop.
- The helices are responsible for dimer formation

All HLH proteins have regions corresponding to helix 1 and helix 2, separated by a loop of 10-24 residues.

Basic HLH proteins have a region with conserved positive charges immediately adjacent to helix 1.

HLH proteins have two helical regions

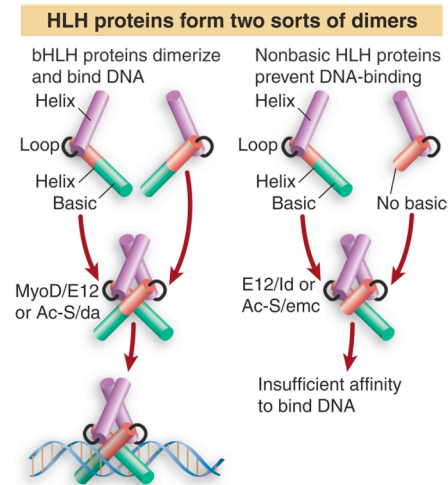
MyoD	Ala Asp Arg Arg Lys Ala Ala Thr Met Arg Gln Arg Arg Arg	Basic region 6 conserved residues are absent from Id
Id	Arg Leu Pro Ala Leu Leu Asp Gln Glu Glu Val Asn Val Leu	
MyoD	Leu Ser Lys Val Asn Gln Ala Phe Gln Thr Leu Lys Arg Cys Thr	Helix 1 Conserved residues are found in both MyoD and Id
Id	Leu Tyr Asp Met Asn Gly Cys Tyr Ser Arg Leu Lys Gln Leu Val	
MyoD	Lys Val Gln Ile Leu Arg Asn Ala Ile Arg Tyr Ile Gln Gly Leu Glu	Helix 2
Id	Lys Val Gln Ile Leu Glu His Val Ile Asp Tyr Ile Arg Asp Leu Glu	

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25.12

Helix-Loop-Helix Proteins Interact by Combinatorial Association

- A **bHLH** protein has a basic sequence adjacent to the HLH motif that is responsible for binding to DNA.
- HLH proteins that lack the basic region prevent a bHLH partner in a heterodimer from binding to DNA.
- Each pair of HLH protein dimers has a particular ability to activate or repress transcription
- Gene expression can therefore be regulated by controlling the availability of particular HLH and bHLH proteins

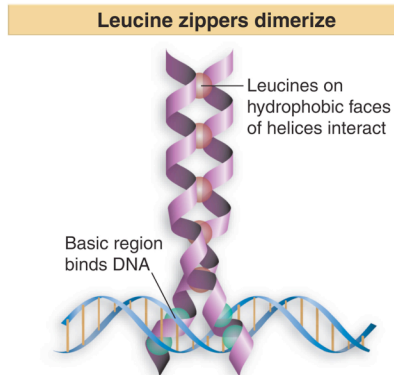


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25.13 Leucine Zippers Are Involved in Dimer Formation

- The **leucine zipper** is a dimerization motif that is found in a class of transcription factors.
- A **bZIP** protein has a basic DNA-binding region adjacent to a leucine zipper dimerization motif.
- Dimerization between proteins with leucine zippers forms the bZIP motif in which the two basic regions symmetrically bind inverted repeats in DNA.

Leucine zipper or bZIP protein dimers bind inverted repeats with no separation between the repeats



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