Glucocorticoid Receptor, Anti-Inflammation

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Nuclear Receptors (A.K.A Intracellular Receptors)

- Receptor signaling protein
  - Receptors are not membrane bound.
- Function as transcription factors that regulate gene expression in response to ligand binding
Nuclear Receptors (continued…)

- The glucocorticoid receptor is a steroid receptor.
- Steroid receptors are head-to-head homodimers, enabling them to bind to inverted repeat DNA sequences
  - Homodimer: A protein composed of two polypeptide chains that are identical in the order, number, and kind of their amino acid residues
- The ligands for steroid receptors are physiologic hormones derived from cholesterol
Glucocorticoid Receptor Induces an Anti-Inflammatory Response

Background information:

1. It is required for
   a. Lung development
   b. Carbohydrate metabolism in the liver
   c. Modulation of the inflammatory response
   d. Neuronal signaling in the brain

2. Glucocorticoids are steroid hormones that are synthesized in the adrenal glands and bind to the glucocorticoid receptor.
Molecular Structure of GR DNA-binding domain

- α-helical amino acids interacting with major groove nucleotides
- Pair of zinc atoms on top of the dimer that stabilizes the protein dimer interface
- Pair of zinc atoms in the middle that stabilize the α-helical region by optimizing amino acid contact with the DNA
- Notice this:

The orientation of the zinc finger $\alpha$-helices relative to the repeat DNA sequences

- The inverted repeat sequence must be at least partially **palindromic** to be recognized by a homodimer that binds in a **head-to-head** configuration
  - Palindrome: Same sequence of characters when read in either direction
    - Ex. madam, racecar
- GR DNA-binding domain interacts with a DNA region that contains the sequence $5'\text{-AGAACA}tga\text{TGTCTTCA-3'}$
  - Palindromic portion in uppercase letters
- **Fluticasone furoate:**
  Asthma drug derivative
- **Coregulatory binding site** is on the surface of the GR ligand-binding domain
  - Is in direct contact with two $\alpha$ helices that extend into hydrophobic pockets
- **Fluticasone:**
  - Potent anti-inflammatory drug
  - Higher affinity for GR than physiologic glucocorticoid cortisol
  - Making it highly effective for clinical use

Homodimer of GR ligand-binding domain in a complex with synthetic anti-inflammatory corticosteroid fluticasone furoate and an LXXLL motif-containing peptide
How do pharmaceutical glucocorticoids function as anti-inflammatory drugs?

- By directly or indirectly regulating the expression of genes encoding proteins that regulate the inflammatory response.
Indirectly

Directly
### Useful Abbreviations, Protein Names, and Their Functions in Nuclear Signal Transduction Systems

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Protein Name</th>
<th>Function</th>
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<tbody>
<tr>
<td>GR</td>
<td>Glucocorticoid Receptor</td>
<td>Nuclear receptor that binds steroid hormones</td>
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<tr>
<td>GRE</td>
<td>Glucocorticoid Response Element</td>
<td>DNA sequence where glucocorticoid receptors bind</td>
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<tr>
<td>Hsp90</td>
<td>Heat shock protein 90</td>
<td>Chaperonin protein that assist protein folding</td>
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1. Glucocorticoids cross the plasma membrane and bind to the **unliganded GR** protein. The unliganded GR protein resides in the **cytoplasm**.
   a. The unliganded GR protein forms part of a large inactive complex containing chaperonin proteins.
   b. Hsp90 (heat shock protein 90) is one of the most abundant chaperonin proteins in cells.
2. Ligand binding to GR results in **disassembly** of the GR-chaperonin protein complex
3. Translocation of GR into the nucleus.
- In the nucleus: Ligand-activated GR homodimers bind to DNA sequences called glucocorticoid response elements (GREs) in the regulatory region of the Annexin I gene.
- GR directly induces the expression of Annexin I
  - Annexin I gene: Anti-inflammatory protein that functions to inhibit prostaglandin synthesis
  - Prostaglandins are pro-inflammatory molecules.

Net Result: Reduced Inflammation!
- In the nucleus: Ligand-activated GR binds to the P65 subunit of NF\(_{κ}\)B heterodimer.
- GR sequesters the P65 subunit away from the cyclooxygenase-2 gene promoter (NF\(_{κ}\)B).
- This blocks the transcription regulatory functions of NF\(_{κ}\)B through protein-protein interactions, leading to reduced expression of cyclooxygenase-2.
  - Cyclooxygenase-2: Pro-inflammatory gene

** Net Result: **

**Reduced Inflammation!**

- ** Studies show that monomers of ligand activated GR, rather than homodimers, bind to the P65 subunit of NF\(_{κ}\)B.**


https://www.youtube.com/watch?v=sSP6QrTvPlo
