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Analysis of amino acids essential for an interaction between glucocorticoid receptor and a heterdimerization partner

Steroid hormones of the glucocorticoid class regulate homeostasis, stress, the acute phase response, and several other functions. Specifically, glucocorticoid hormone is involved in regulation of ?-fibrinogen production, which plays a major role in blood clotting. Glucocorticoids act by binding to glucocorticoid receptor (GR), an intracellular receptor protein. Glucocorticoid binding enables GR to be transported to the nucleus, where GR binds to DNA, influencing gene expression. The classical DNA binding site for GR is a glucocorticoid response element (GRE) with the sequence 5? GGTACAnnnTGTTCT 3?. However, to regulate the ?-fibrinogen gene in frog liver cells, GR interacts with another protein, Xenopus Glucocorticoid Receptor Accessory Factor (XGRAF), to form a heterodimer. This complex binds to an upstream position on the ?-fibrinogen gene that possesses a non-classical recognition site, composed of an XGRAF binding site (italicized) adjacent to a downstream half GRE, 5? GAGTTAA TGTTCC 3?. It has been shown that heterodimer binding to this site increases transcription of the ?-fibrinogen gene in response to hormone treatment in liver cells. Formation of the XGRAF:GR complex is expected to rely on interactions between specific amino acids in both proteins. To examine which amino acids within GR are essential for that dimerization interaction, stretches of amino acids in GR have been substituted with analogous regions from other proteins in the steroid receptor/nuclear receptor family. A closely related protein, androgen receptor (AR), was the initial source of the amino acid sequences. The GR/AR hybrids were still able to heterodimerize with XGRAF. Based on those data, a more distantly related protein, called Daf-12, from the nematode C. elegans was chosen as the new amino acid sequence source. If complex formation with XGRAF is disrupted in the GR/Daf-12 hybrids, it would indicate that the specific amino acids on GR, substituted with Daf-12, may be involved in direct protein-protein interactions between GR and XGRAF.