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Analysis of the Hormone-dependent Regulation of a JunD-Estrogen Receptor Chimera*

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The modular ligand-binding domains of steroid receptors have been widely used to generate protein chimeras that are ligand dependent for activity. In a similar manner, we generated a series of conditionally active JunD and c-Fos proteins by fusing their carboxyl (COOH)-terminal ends with a COOH-terminal fragment of the human estrogen receptor (ER) that contains the ligand-binding domain. JunD-ER (DER) and Fos-ER (FER) chimeras with an intact leucine zipper and basic region exhibit hormone-dependent activation of activator protein-1-directed transcription in transient expression assays. One of these fusions, DER, has been examined in detail to determine its mechanism of action. Results from immunoprecipitation experiments with extracts from *DER* and *Fos* transfected cells demonstrate that Fos and DER readily form heterodimer complexes. Surprisingly, the formation of Fos:DER heterodimers, and possibly DER homodimers, is estrogen-independent. However, gel shift assays clearly demonstrate that DNA binding to AP1 sites by Fos:DER heterodimers or DER homodimers is estrogen-dependent. Moreover, in the absence of estrogen, the DER protein is an effective inhibitor of Fos-mediated transactivation, and this effect is reversed by the presence of estrogen. Our results indicate that the DER protein is a direct, hormone-reversible inhibitor of Fos and that estrogen controls the conditional positive or dominant negative activities of DER at the level of DNA binding to AP1 sites. Accordingly, clonally derived fibroblast cell lines that stably express the DER protein exhibit reduced entry into the S phase of the cell cycle when quiescent cells are serum stimulated in the absence of estrogen. This is in contrast to the estrogen-treated controls. These results support the hypothesis that AP1 is important for cell cycle progression and provide a unique approach for examining the role of AP1 in this process.

Members of the Jun and Fos families of oncoproteins are the essential components of the dimeric transcription factor activator protein-1 (AP1).[1] The three members of the *jun* gene family, *c-jun*, *junB*, and *junD*, all undergo similar, but not identical, regulation and encode proteins that share a high degree of amino acid sequence identity (1). They are also similar in their interaction with c-Fos and Fos-related proteins (2). Jun:Jun or Jun:Fos dimer complexes bind to DNA at AP1 sites and regulate the expression of target genes. Since *jun* and *fos* family members are rapidly induced in response to extracellular growth signals, they are classified as immediate early response genes. Expression of these two gene families is an integral part of a well-orchestrated cascade of events leading to cellular proliferation (1 and 3,and references therein). Since Jun proteins are important participants in the cellular growth response, further studies analyzing the direct effects of these proteins on gene expression and cell growth would be greatly facilitated by a well characterized inducible expression system.

One approach for creating an inducible system focuses on placing the activity of a protein under the control of the ligand-binding domain of a steroid hormone receptor such as the glucocorticoid receptor (GR) or the estrogen receptor (ER). Steroid receptor fusion proteins have been created with proteins such as E1A (4), c-Myc (5), the CCAAT/enhancer-binding protein (6), MyoD (7), p53 (8), and c-Fos (9, 10). In each of these cases, the fusion protein appeared to be devoid of biological activity when expressed in cells in the absence of hormone, whereas its appropriate wild type (WT) function was restored in the presence of hormone.

We explored the possibility that the activities of WT and mutated Jun proteins could be regulated post-translationally by fusion with the ER. A JunD chimeric molecule was created by appending a truncated human ER sequence to its 3′ end resulting in the JunD-ER (DER) fusion. Analysis of the DER fusion protein shows that it is estrogen dependent for the activation of transcription from AP1 reporter constructs. In addition, a detailed analysis of the mechanism by which estrogen regulates the DER protein was performed. The chimera forms heterodimers with WT Fos proteins but does not readily form heterodimers with WT Jun proteins. However, neither the weak interactions between DER and WT Jun nor the strong interactions between DER and Fos are regulated by estrogen.

In contrast, DNA binding by DER homodimers or DER:Fos heterodimers is completely estrogen-dependent. Because of this novel mechanism of action, the DER fusion protein is an effective dominant negative protein that complexes with Fos and reversibly inhibits its DNA binding activity. In addition, cell clones that stably express the DER protein exhibit a reduced capacity to enter S phase from a growth arrested state in the absence of estrogen, a result that is consistent with the hypothesis that AP1 is important for normal cell cycle progression.

While a number of recombinant chimeras between steroid

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[1] The abbreviations used are: AP1, activator protein-1; GR, glucocorticoid receptor; ER, estrogen receptor; WT, wild type; PCR, polymerase chain reaction; CAT, chloramphenicol acetyl transferase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; TR, thyroid hormone receptor, RAR, retinoic acid receptor.