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Hormone-regulated v-*rel* estrogen receptor fusion protein: reversible induction of cell transformation and cellular gene expression

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We describe the construction of a v-*rel* estrogen receptor fusion protein (v-*rel*ER) which allows the regulation of v-*rel* oncoprotein activity by hormone. In the presence of estrogen, v-*rel*ER readily transformed primary chicken fibroblasts and bone marrow cells *in vitro*. In both cell types, v-*rel*-specific transformation was critically dependent on the presence of estrogen or the estrogen agonist 4-hydroxytamoxifen (OHT). Withdrawal of estrogen or application of an estrogen antagonist, ICI164,384 (ICI) caused a reversal of the transformed phenotype. We also demonstrate that the v-*rel*ER protein binds to NF-κB sites in an estrogen-dependent manner, thereby showing that sequence-specific DNA binding of v-*rel*ER is critical for the activation of its transforming capacity. In transient transfection experiments, we failed to demonstrate a clear repressor or activator function of the v-*rel* moiety in v-*rel*ER. However, in v-*rel*ER-transformed bone marrow cells, estrogen and OHT induced elevated mRNA levels of two cellular genes whose expression is constitutive and high in v-*rel*-transformed cells. These results suggest that v-*rel* might exert part of its activity as an activator of *rel*-responsive genes.

Key words: estrogen receptor/NF-κB/oncogene/transformation/v-*rel*

Introduction

The v-*rel* oncogene was first identified as the transforming component of the avian retrovirus REV-T (for review see Rice and Gilden, 1988, Gilmore, 1991). The gene encodes a protein of 59 000 Daltons which localizes in both the cytoplasm and the nucleus and forms complexes with several other cellular proteins including p68[c-rel] (Morrison *et al.*, 1989, Gilmore, 1991 and references therein). The function of v-*rel* was unknown until the demonstration that it was homologous in part to members of the transcription factor family NF-κB (for reviews see Gilmore, 1990, 1991, Baeuerle, 1991, Blank *et al.*, 1992). This growing family of proteins includes the p50, p49 and p65 subunits of NF-κB, the *Drosophila* morphogen dorsal, and the *Lyt-10* gene product (Neri *et al.*, 1991). Each of these proteins has been shown to bind DNA and appears to be involved in positive and negative regulation of gene expression (for reviews see Baeuerle, 1991, Gilmore, 1991; Blank *et al.*, 1992).

While a variant of the p65 subunit of NF-κB has been shown to transform rat embryo fibroblasts and induce tumors in athymic nude mice when overexpressed (Narayanan *et al.*, 1992), v-*rel* remains the sole naturally occurring oncogenic member of this family. We and others have shown previously that v-*rel* induces a set of characteristic transformation-specific changes in chicken embryo fibroblasts (CEFs; Morrison *et al.*, 1991 and references therein). In addition, we have determined that the hematopoietic target cell transformed by v-*rel* exhibits properties of an early progenitor cell that expresses markers of the myeloid, lymphoid and erythroid lineages (Morrison *et al.*, 1991).

The mechanism by which v-*rel* brings about oncogenic transformation remains unclear, but was suggested to be the result of aberrant gene expression induced by v-*rel*. It has been suggested that v-*rel* transforms by inhibiting the normal activity of c-*rel* (or of other members of the NF-κB/*rel*/dorsal transcription factor family) in a dominant negative fashion (reviewed in Gilmore, 1991 and Forrest and Curran, 1992). Additionally, several groups have demonstrated that v-*rel* represses gene expression in transient transactivation assays (Ballard *et al.*, 1990; Inoue *et al.*, 1991, Richardson and Gilmore, 1991; McDonnell *et al.*, 1992) leading again to the hypothesis that v-*rel* represses the transcription of genes involved in the regulation of growth control. However, these genes have yet to be identified.

We have chosen to approach the identification of *rel*-regulated genes by constructing an inducible form of v-*rel* whose activity can be modulated by hormone. In this approach, first described by Picard *et al.* (1988), the human estrogen receptor hormone binding domain (ER) is fused in frame to the protein of interest, rendering that protein's activity hormone-inducible. Several proteins, including the *myc*, *fos* and *myb* oncogene products (Eilers *et al.*, 1989; Superti-Furga *et al.*, 1991; Burk and Klempnauer, 1991) have been fused to this ER domain and shown to function in a hormone-inducible fashion. Thus, this approach should allow one to control and examine the biological and biochemical activities of the v-*rel* oncoprotein and in addition to search for *rel*-regulated genes.

In this report, we describe the construction and characterization of a v-*rel*ER fusion protein. In the presence of estrogen, v-*rel*ER transforms both avian fibroblasts and primary bone marrow cells, while in the absence of hormone v-*rel*ER is nontransforming in both cell types. Estrogen also induces v-*rel*ER to bind to DNA in a sequence-specific fashion, thereby correlating DNA binding with the activation of its transforming potential. Finally, we have identified two cellular genes whose transcription is upregulated in a hormone dependent fashion. NF-κB/*rel* sites present in the promoter regions of these two genes are bound by v-*rel*ER in a hormone-dependent manner in electrophoretic mobility shift assays. It is therefore possible that v-*rel*ER (and perhaps also v-*rel*) may exert its specific effects on gene expression

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