Kidney International, Vol. 41 (1992), pp. 550–553

The Egr transcription factor family: From signal transduction to kidney differentiation

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Extracellular "signals" in the form of neurotransmitters, growth factors, hormones, and matrix are known to be key modulators of cellular phenotype. These agents lead to the generation of second messenger signals in the plasma membrane and cytosol. In turn, these biochemical events modulate the expression of a set of so-called immediate-early genes (IEG), whose induction does not require *de novo* protein synthesis. Several years ago, we and others identified several IEGs [reviewed in 1 and 2]. Of particular interest to our laboratory has been a subset of IEGs that encode transcription factors, since as such they might: (1) be the targets for second messenger events, and (2) activate or repress the transcription of critical genes required to effect a particular cellular phenotype. Thus, immediate-early transcription factors (IETF) should couple short-term responses in the form of second messenger events to long-term changes in gene expression instrumental in altering phenotype.

The current status of the IETFs whose genes have been cloned is shown in Table 1. c-*fos*, identified as the cellular homolog of the v-*fos* oncogene present in two viruses causing osteosarcomas, was discovered to be an IEG in serum stimulated fibroblasts in 1984 [3]. At that time, it was not known that *fos* was a transcription factor that forms heterodimeric complexes with c-*jun*. c-*jun* was likewise first identified as the cellular homolog of the v-*jun* transforming gene and only through homology with part of the yeast GCN4 transcription factor was it suspected to be a transcription factor itself. The best characterized of the Egr family of IETFs is Egr-1 (early growth response gene-1). Egr-1 (also known as Zif 268, Tis 8, NGFI-A, and Krox 24) (references are in Table 1) was isolated as a serum inducible IEG in quiescent fibroblasts (G_0-G_1 transition) utilizing a differential screening protocol [4, 5]. The gene is induced by mitogen stimulation in every mammalian cell type tested including B cells, T cells, kidney mesangial, glomerular and tubular epithelial cells, hepatocytes and endothelial cells. It was also discovered as a nerve growth factor inducible gene in PC12 pheochromocytoma cells, a physiologic context in which mitotic cells convert to a non-mitotic state [5, 6] and as a gene induced by the protein kinase C activator TPA [4, 7]. The cDNA structure predicts a protein whose carboxy terminus contains three zinc fingers of the cysteine$_2$-histidine$_2$ type, first identified in the Xenopus transcription factor TFIIIA. Table 2 summarizes this work on Egr-1.

During the last few years, a dozen or so transcription factor genes have been isolated. Through the generation of chimeric proteins, it has become clear that transcription factors are essentially modular in design, with different regions involved in various functional roles, namely DNA binding, dimerization and transcriptional activation/repression [8]. The families of IETFs illustrate these ideas nicely (Table 1). The *fos* and *jun* families all contain basic DNA binding regions and form heterodimers via their so-called leucine zipper domains. Similarly, the Egr family contains highly related proteins in the DNA binding zinc finger domain but which differ significantly outside this region [9]. This review will focus on the Egr family of transcription factors. For a more comprehensive account, the reader is referred to a recent review on this subject [1]. Here, emphasis is placed on recent data and on providing a perspective on future directions.

Several studies have started identifying the events—from cell surface to second messengers—which modulate Egr-1 expression. Identification of the "upstream" or "proximal" events can be done either in the context of mitogenesis or in other situations in which Egr-1 mRNA levels change (Table 2). The following comments will be restricted to cell proliferation and to studies conducted primarily in fibroblasts. Multiple kinases regulate Egr-1 expression [10]. For example, it has been known that activation of the PDGF receptor or the EGF receptor by their cognate ligands leads to Egr-1 induction [10, 11]. Recently, v-*src* has been shown to regulate Egr-1 independent of protein kinase C [12, 13]. Using co-transfection analysis of cloned Egr-1 promoter chloramphenicol acetyl transferase (CAT) constructs and a temperature-sensitive v-*src* vector, one can define the so-called *cis* elements in the Egr-1 promoter that are targets for v-*src* kinase activity. In this manner, it has been shown that the multiple serum response elements (SREs) present in the Egr-1 promoter serve this function. These elements were first defined in the c-*fos* promoter by genetic mutational means and can confer serum responsiveness to a heterologous gene. More recently, v-*raf*, a serine-threonine kinase whose activation results from the convergence of diverse cell surface signals, has been found to lead to Egr-1 induction. Furthermore, a dominant negative mutant of v-*raf* will ablate the v-*src* induction of Egr-1, suggesting that v-*src* stimulates Egr-1 via v-*raf* (S. Qureshi, V.P.S., D. Foster, unpublished data). Even if Egr-1 served as

Table 1. Immediate-early transcription factor families

Name	Transcription factor motif	Refs.
Fos (c-*fos*, fra-1, *fos*-B)	Basic DNA binding domain and leucine zipper	3, 23, 24
Jun (c-*jun*, *jun*-B, *jun*-D)	Basic DNA binding domain and leucine zipper	25, 26, 27, 28, 29
Egr (Egr-1, 2, 3, 4)	$Cysteine_2$-$histidine_2$ zinc fingers	1, 4, 5, 6, 7, 9, 30, 31, 32
nur 77	Cys_4 zinc finger of steroid hormone receptor subtype	33, 34

Current status of immediate-early transcription factor (IETF) families. Names of independent isolates are as follows: Egr-1 = Zif 268 = Krox 24 = Tis 8 = NGFI-A; Egr-1 = Krox 20; nur 77 = NGFI-B = N 10 = Tis 1.

Table 2. Signals modulating Egr-1 expression

- Induction by multiple second messenger pathways in the context of a mitogenic stimulus in all mammalian cell types tested to date
- Induction by non-mitogenic stimuli
  - — neuronal excitation
  - — hypertrophic responses
  - — differentiation
    - — PC12 pheochromocytoma cells
    - — embryonal carcinoma
  - — ischemic injury
  - — X-irradiation
  - — developmental regulation
    - — bone/skeletal muscle

Mitogenic as well as many non-mitogenic signals regulate Egr-1 expression in mammalian cells. References are given in [1].

nothing more than just a target marker, these types of studies are helping to define the circuitry among second messenger systems.

Since Egr-1 induction lies at the convergence of so many regulators of mitogenesis, it is natural to ask whether Egr-1 is itself a proto-oncogene. The situation is highly analogous to that for c-*fos* and c-*jun* since Egr-1, c-*fos* and c-*jun* are IETFs, and the latter two are known oncogenic agents. This question has turned out to be a difficult one to answer. Early experiments in fibroblasts utilizing Egr-1 expression vectors driven by viral long terminal repeats (LTRs) so as to provide strong constitutive expression did not show a phenotype: namely, morphologic alterations, changes in growth characteristics, diminished serum requirements, anchorage independence, etc. In some of these cells, high levels of Egr-1 mRNA and protein were noted. However, the over-expressed Egr-1 protein appears to be *non-functional*, as measured by its inability to activate a test reporter plasmid containing multiple Egr-1 binding sites (sequences of the form 5′-GCGGGGGCG-3′ known to bind the Egr-1 protein) [9, 14] placed upstream of a minimal promoter and CAT (I. Drummond, X. Cao and V.P. Sukhatme, unpublished data). Needed is a non-leaky inducible system to circumvent this problem and work is ongoing in this direction.

The opposite tack—attempts at abrogating Egr-1 activity—may well prove to be more useful in defining a phenotype. In this regard, the use of antisense methodology—either as oligonucleotides or in the form of stable inducible vectors—is currently under investigation. The latter technique when applied to c-*fos* has resulted in a remarkable and reversible inhibition of cell growth [15, 16]. Another approach is also available to inhibit Egr-1 activity: nature has designed its own *inhibitor* of Egr-1 activity in the form of a tumor suppressor gene, one whose absence is likely to be responsible for the genesis of Wilms' tumor.

To understand the Egr-Wilms' tumor connection, a small digression is in order. A recent interest in our laboratory is on kidney development. Essentially, we are trying to identify genes whose expression is modulated during the conversion of metanephric mesenchymal cells into epithelium. We have taken the viewpoint, based on our interest in transcription factors, that it would be particularly exciting to define a cascade or hierarchy of these regulatory proteins that occur during this transition. For this purpose, one can contemplate two approaches. The first is to utilize known motifs that occur in transcription factors [8] to screen for the presence of kidney specific transcription factors; the second is to use a strategy in which one picks a target kidney specific gene, tries to identify its promoter and ultimately works backward in developmental time defining the transcription factors regulating expression of this kidney specific gene. We are currently attempting both of these approaches, but here we will focus on the former. We have isolated several clones from a kidney cDNA library that contain zinc finger motifs (S. Patwardhan, A. Aplin and V.P. Sukhatme, unpublished data). Among this set was in fact the candidate Wilms' tumor gene (WT1) [17, 18], cloned by reverse genetic means. This gene has an interesting structure: it predicts a protein with four zinc fingers with a 60 to 70% amino acid sequence similarity in three out of its four zinc fingers to those of Egr-1. An antibody, raised by F. Rauscher and his colleagues against the zinc finger domain of the Wilms' tumor protein reacts against an 80 kd protein in addition to the 50 kd Wilms' tumor protein. The 80 kd band corresponds to Egr-1. This result is very exciting because it suggests that WT1 and Egr-1 might bind to a common DNA sequence. Indeed, this hypothesis proves to be correct [19]. Next, to determine whether this result was interesting biochemistry or whether there was some in vivo correlate, we asked what would happen if both the Wilms' tumor gene product and Egr-1 were present in the same cell. Such a situation would occur in metanephric mesenchymal cells since Egr-1 expression is modulated in all proliferating cells, and recent studies have shown that Wilms' tumor expression coincides with the beginning of condensation of metanephric mesenchymal cells [20]. In collaboration with the Rauscher laboratory, we found that the Wilms' tumor protein is able to repress transcription through an Egr-1 binding site. In other words, transfection of an Egr-1 expression plasmid results in activation from a reporter plasmid containing Egr-1 binding sites, but as increasing amounts of a construct which expresses WT1 are added, there is an abrogation of the activation by Egr-1. This result is significant because it indicates that Egr-1 and WT1 form a binary on/off system. Furthermore, it was found that the Wilms' tumor protein also represses transcription of the Egr-1 promoter itself (Fig. 1).

The last part of this story is an unfinished one. Is there biological significance to what we have found? We have made a guess: namely, that there exists a physiologic target for these two proteins and that this target is the insulin-like growth factor-II (IGF-II) gene (Fig. 1). Our thinking is as follows: first,

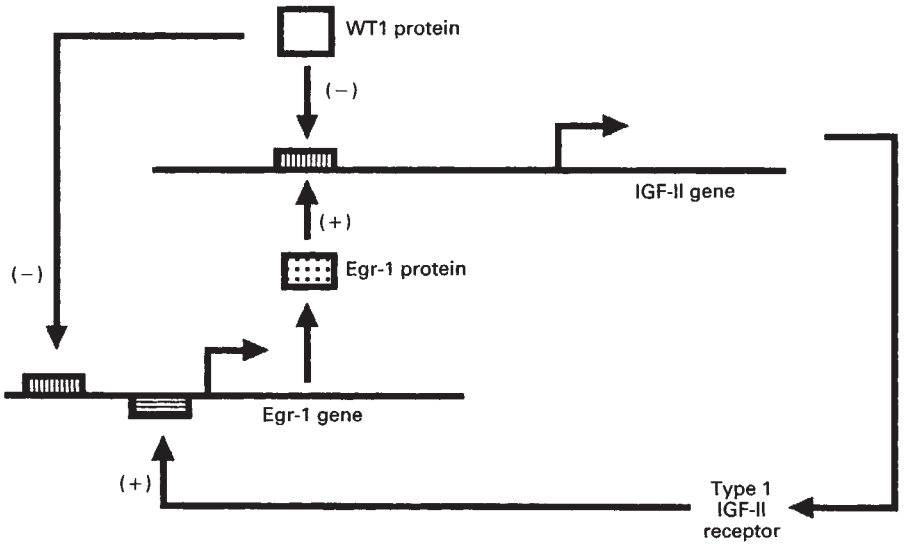

Fig. 1. *Speculative model for interaction between the Wilms' tumor protein (WT1), Egr-1 and insulin-like growth factor-II (IGF-II) gene.* The box with vertical lines in the IGF-II and Egr-1 promoters designates an Egr-1 binding site. The box with horizontal lines in the Egr-1 promoter is an element (?SRE) mediating activation of Egr-1 gene transcription as a consequence of mitogenic signalling by IGF-II. Activation and repression of transcription are shown with (+) and (−) symbols, respectively.

it has been known that IGF-II levels are high, 10- to 100 fold elevated in Wilms' tumor samples compared with normal kidney [21, 22]; second, IGF-II is a known mitogen; third, levels of IGF-II decrease as kidney differentiation proceeds and levels of WT1 increase during conversion of metanephric mesenchyme into epithelia [20]; and finally, the IGF-II promoter (which is quite complex) contains multiple sites for Egr-1/WT1 binding (I. Drummond and V.P. Sukhatme, unpublished data). Given the data that WT1 can act as a repressor and Egr-1 as an activator along with the collective information above and the expected fact that Egr-1 is upregulated every time a cell receives a mitogenic signal, we have a potentially exciting model for a key regulatory event in kidney differentiation (Fig. 1). Essentially the model is that of an autocrine circuit, a circuit that is broken during differentiation as WT1 levels are upregulated. Therefore, as differentiation proceeds, WT1 levels rise, IGF-II transcription is down regulated and growth comes to a stop. In certain Wilms' tumor patients, WT1 is functionally inactivated and growth continues. This is surely a simplistic model, but is possibly a first step. We are currently testing part of this model using promoter regions of IGF-II to see if in fact WT1 will repress IGF-II expression. This illustration of the curious intersection of our studies on Egr-1 with the Wilms' tumor story is worth mentioning for two reasons: first, I thought it would be of interest, especially in this forum on kidney development in mesangial cells, to show how a ubiquitous factor such as Egr-1 can in fact interact with a tissue specific factor and give rise to a rather tissue specific interaction and, second, it may just happen that it is the kidney that will provide us a phenotype in which to investigate the effects of overexpression or knocking out of Egr-1.

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