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Review

# Expression, signaling and function of Egr transcription factors in pancreatic β-cells and insulin-responsive tissues

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ABSTRACT

Egr-1 and the related zinc finger transcription factors Egr-2, Egr-3, and Egr-4 are stimulated by many extracellular signaling molecules and represent a convergence point for intracellular signaling cascades. Egr-1 expression is induced in insulinoma cells and pancreatic β-cells following stimulation with either glucose, or pregnenolone sulfate. Moreover, stimulation of Gαq and Gαs-coupled receptors enhances EGR-1 gene transcription. Functional studies revealed that Egr transcription factors control insulin biosynthesis via regulation of Pdx-1 expression. Glucose homeostasis and pancreatic islet size are regulated by Egr transcription factors, indicating that these proteins control central physiological parameters regulated by pancreatic β-cells. In addition, Egr-1 is an integral part of the insulin receptor signaling cascade in insulin-responsive tissues and influences insulin resistance.



**Contents**



## 1. Introduction

The family of Egr proteins encompasses four zinc finger transcription factors termed Egr-1, Egr-2, Egr-3, and Egr-4. All four Egr proteins exhibit a homologous domain structure, indicating that the functions of these proteins are – at least in part – similar. The best investigated Egr protein is Egr-1 (Sukhatme et al., 1988), also known as zif268, NGFI-A, Krox24, and TIS8 (Lim et al., 1987; Milbrandt, 1987; Christy et al., 1988; Lemaire et al., 1988). EGR-1 expression is induced by many environmental signals including growth factors, hormones, and neurotransmitters. Egr-1 function is regulated via its biosynthesis (Thiel and Cibelli, 2002; Rössler et al., 2006), in contrast to other stimulus-induced transcription factors that are activated either by nuclear translocation (NF-κB) or phosphorylation (CREB) (Barco and Kandel, 2006; Kaltschmidt et al., 2006). The EGR-1 gene as well as the genes encoding Egr-2, Egr-3, and Egr-4 function as a convergence points for distinct intracellular signaling cascades. As Egr-1 is the most widely analyzed protein of the Egr family of transcription factors, this review will mainly focus on results obtained in the study of EGR-1 expression and function.

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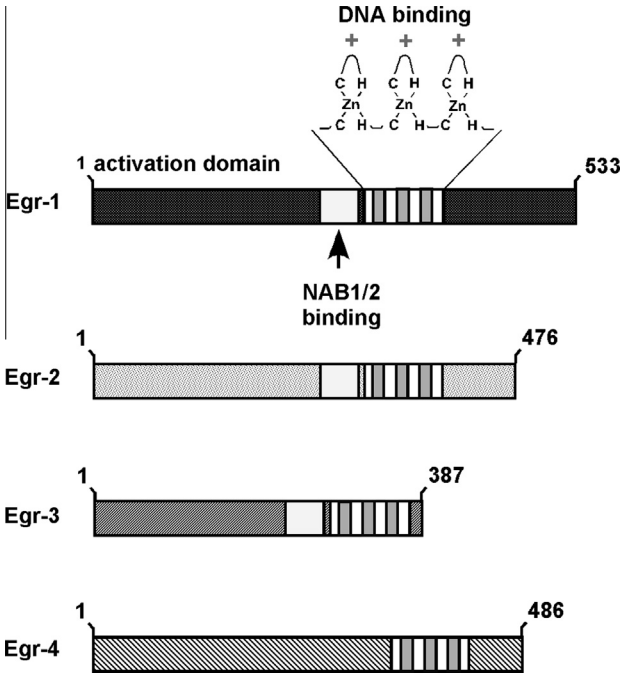


**Fig. 1.** Modular structure of the Egr zinc finger transcription factors. The Egr proteins contains an extented transcriptional activation domain on the N-terminus and a DNA binding domain, consisting of three zinc finger motifs. An inhibitory domain is located between the activation and DNA-binding domain of the Egr proteins Egr-1, Egr-2, and Egr-3 that functions as a binding site for the transcriptional corepressor proteins NAB1 and NAB2.

## 2. Modular structure of Egr transcription factors

The modular structure of the Egr transcription factors is depicted in Fig. 1. The DNA binding domains contain three zinc finger motifs that are very homologous and bind to identical GC-rich DNA recognition sites. The structure of a complex formed between these three zinc fingers of Egr-1 and its cognate DNA-binding site has been solved (Pavletich and Pabo, 1991) and has been subsequently used as a framework for understanding how zinc fingers recognize DNA (Jamieson et al., 1996; Greisman and Pabo, 1997; Elrod-Erickson and Pabo, 1999). Each zinc finger domain consists of an antiparallel β-sheet and an α-helix held together by a zinc atom and hydrophobic residues. Egr proteins preferentially bind to the GC-rich sequence 5′-GCGGGGGCG-3′ (Christy and Nathans, 1989; Nardelli et al., 1991; Patwardhan et al., 1991; Cao et al., 1993), that is very similar to the DNA binding domain of the transcription factor Sp1. In fact, the DNA binding sites of Egr-1 (GCG GGG GCG = A B A) and Sp1 (GGG GCG GGG = B A B) appear to be a rearrangement of one another (A B A versus B A B) (Kriwacki et al., 1992). In addition, the free solution structures of the $Cys_2$-$His_2$-zinc finger domains 2 and 3 of Sp1 are very similar to those of Egr-1 (Narayan et al., 1991). However, a detailed study showed that there are genuine Sp1/Sp3 or Egr-1 controlled target genes showing no cross-regulation of Sp1/Sp3 and Egr-1 through the same DNA-binding site (Al-Sarraj et al., 2005). However, composite GC-rich Sp1/Sp3/Egr-1 binding sites were identified in some genes where competition for a common DNA-binding site occurs. These composite sites have been detected in genes encoding platelet-derived growth factor (PDGF) A chain and B chains, adenosine deaminase, tissue factor, thrombospondin 1, monoamine oxidase B, ABCA2 transporter, $\beta_1$-adrenergic receptor, and angiotensin-converting enzyme (Ackerman et al., 1991; Shingu and Bornstein, 1994; Cui et al., 1996; Khachigian et al., 1995, 1996; Bahouth et al., 2002; Davis et al., 2003; Al-Sarraj et al., 2005).

An extensive transcriptional activation domain has been mapped to the N-terminus of Egr-1 (Thiel et al., 2000). An inhibitory domain between the activation domain and the DNA binding domain was identified in Egr-1, Egr-2, and Egr-3 that functions as a binding site for the two transcriptional co-factors NGFI-A binding proteins 1 and 2 (NAB1, NAB2) (Russo et al., 1995; Svaren et al., 1996). Both NAB1 and NAB2 block the biological activity of Egr-1 (Russo et al., 1995; Svaren et al., 1996; Thiel et al., 2000). A fusion protein consisting of NAB1 and a heterologous DNA-binding domain was shown to function as a transcriptional repressor (Thiel et al., 2000), indicating that NAB1 only needs to be recruited to transcription units, either by protein–protein or by DNA–protein interaction, to repress transcription. The discovery of the co-repressor proteins NAB1 and NAB2 produced a further level of complexity for the understanding of the function of Egr-1 because induction of transcription of the EGR-1 gene may have no biological effect when the transcriptional activator function of Egr-1 is neutralized by NAB1 or NAB2. The concentrations of both co-repressors in a particular cell is thus of extreme importance for Egr-1 function. In PC12 cells, for instance, overexpression of NAB2 inhibits nerve growth factor-induced differentiation (Qu et al., 1998). The expression of the NAB2 gene is controlled by Egr transcription factors, indicating that Egr proteins control their biological activities in a negative feedback loop via the synthesis of NAB2 (Ehrengruber et al., 2000; Buitrago et al., 2005; Kumbrik et al., 2005).

## 3. Expression of Egr-1 in insulinoma cells and pancreatic β-cells: role of L-type $Ca^{2+}$ channels and G protein-coupled receptors

Stimulation of pancreatic β-cells or insulinoma cells with glucose strongly activates the biosynthesis of Egr-1 (Frödin et al., 1995; Josefsen et al., 1999; Bernal-Mizrachi et al., 2000, 2001; Mayer and Thiel, 2009). Glucose is transported into the β-cells via GLUT2 transporters, phosphorylated to glucose-6-phosphate that is subsequently metabolized via glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation to increase the ATP concentration in β-cells. ATP induces a closure of the ATP-regulated potassium channels, $K_{ATP}$, leading to a depolarization of the plasma membrane, the opening of L-type voltage gated $Ca^{2+}$ channels, and the rapid influx of $Ca^{2+}$ ions into the cells (Fig. 2, signaling cascade #1 ). Accordingly, Egr-1 expression is also induced in insulinoma cells following treatment with tolbutamide, a compound that triggers a closure of $K_{ATP}$ in the plasma membrane, or by KCl that depolarizes the cell membrane (Mayer and Thiel, 2009; Mayer et al., 2011). In contrast, incubation of the cells with inhibitors of the L-type $Ca^{2+}$ channel prevented EGR-1 expression induced by either glucose, tolbutamide, or KCl (Mayer and Thiel, 2009). Recently, it was shown that insulin-secreting rat INS-1 insulinoma cells and pancreatic β-cells are responsive to stimulation with the neurosteroid pregnenolone sulfate, leading to a rapid influx of $Ca^{2+}$ ions into the cells (Wagner et al., 2008; Islam, 2011; Klose et al., 2011). A detailed analysis of the signaling cascade revealed that pregnenolone sulfate-induced Egr-1 expression in insulinoma cells involves both L-type $Ca^{2+}$ channels and transient receptor potential melastatin-3 (TRPM3) channels (Mayer et al., 2011) (Fig. 2, signaling cascade #2). Pregnenolone sulfate concentrations measured in plasma are in the nanomolar range, while micromolar concentration of pregnenolone sulfate are required to stimulate TRPM3 channels in pancreatic β-cells, suggesting that pregnenolone sulfate is not a physiological agonist of TRPM3 and may have only pharmacological relevance. Accordingly, it has been questioned that pregnenolone sulfate plays an important physiological role in insulin secretion (Colsoul et al., 2011; Thiel et al., 2013).