

Molecular Cloning and Characterization of the Human *PED/PEA-15* Gene Promoter Reveal Antagonistic Regulation by Hepatocyte Nuclear Factor 4 α and Chicken Ovalbumin Upstream Promoter Transcription Factor II[§]

Received for publication, May 21, 2008, and in revised form, August 27, 2008. Published, JBC Papers in Press, September 2, 2008, DOI 10.1074/jbc.M803895200

Paola Ungaro^{‡§1}, Raffaele Teperino^{‡§1}, Paola Mirra^{‡§}, Angela Cassese^{‡§}, Francesca Fiory^{‡§}, Giuseppe Perruolo^{‡§}, Claudia Miele^{‡§}, Markku Laakso[¶], Pietro Formisano^{‡§}, and Francesco Beguinot^{‡§2}

From the [‡]Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università di Napoli "Federico II", 80131 Naples, Italy, the [§]Istituto di Endocrinologia e Oncologia Sperimentale Gaetano Salvatore, Consiglio Nazionale delle Ricerche, 80131 Naples, Italy, and the [¶]Department of Medicine, University of Kuopio and Kuopio University Hospital, 70210 Kuopio, Finland

Overexpression of the *ped/pea-15* gene in mice impairs glucose tolerance and leads to diabetes in conjunction with high fat diet treatment. *PED/PEA-15* is also overexpressed in type 2 diabetics as well as in euglycemic offspring from these subjects. The cause(s) of this abnormality remains unclear. In the present work we have cloned and localized the promoter region of the human *PED/PEA-15* gene within the first 230 bp of the 5' -flanking region. A cis-acting regulatory element located between -320 and -335 bps upstream the *PED/PEA-15* gene transcriptional start site (+1) is recognized by both the hepatocyte nuclear factor 4 α (HNF-4 α) and the chicken ovalbumin upstream promoter transcription factor II (COUP-TFII), two members of the steroid/thyroid superfamily of transcription factors, both of which are involved in the control of lipid and glucose homeostasis. HNF-4 α represses *PED/PEA-15* expression in HeLa cells, whereas COUP-TFII activates its expression. In hepatocytes, the activation of *PED/PEA-15* gene transcription is paralleled by the establishment of a partially dedifferentiated phenotype accompanied by a reduction in mRNA levels encoded by genes normally expressed during liver development. Cotransfection of HeLa cells with a reporter construct containing the *PED/PEA-15* response element and various combinations of HNF-4 α and COUP-TFII expression vectors indicated that COUP-TFII antagonizes the repression of the *PED/PEA-15* gene by HNF-4 α . Thus, at least in part, transcription of the *PED/PEA-15* gene *in vivo* is dependent upon the intracellular balance of these positive and negative regulatory factors. Abnormalities in HNF-4 α and COUP-TFII balance might have important consequences on glucose tolerance in humans.

Phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes (*PED/PEA-15*)³ is a cytosolic phosphoprotein widely expressed in different tissues and highly conserved in mammals (1–4). It binds to and modulates the function of a number of signaling proteins and effectors. *PED/PEA-15* binds several pro- and anti-apoptotic proteins thereby exerting a broad anti-apoptotic function (5–9). It also controls mitogenic signaling by binding extracellular-regulated kinases (ERKs) and anchoring ERKs to the cytoplasm (10). Indeed, changes in *PED/PEA-15* expression play an important role in tumor development and sensitivity to anti-neoplastic agents (11, 12). *PED/PEA-15* binds to phospholipase D, enhancing its stability and increasing intracellular diacylglycerol levels (13, 14). This effect, in turn, activates classical protein kinase C isoforms and generates resistance to insulin action on glucose metabolism in peripheral tissues. Protein kinase C dysregulation by *PED/PEA-15* also impairs glucose-stimulated insulin secretion in β cells in mice (14, 15).

PED/PEA-15 gene maps on human chromosome 1q21–22 (4) and is overexpressed in type 2 diabetics as well as in the euglycemic offspring from these individuals. Interestingly, in these same subjects, *PED/PEA-15* levels correlate with insulin resistance (4, 16). *PED/PEA-15* cellular levels are regulated by ubiquitinylation and proteasomal degradation (17). However, run-on experiments in cultured cells from type 2 diabetic subjects demonstrated that, at least in part, the overexpression observed in these subjects is caused by transcriptional abnormalities (4). The molecular details responsible for these abnormalities and the mechanisms responsible for *PED/PEA-15* gene regulation are still unclear.

Hepatocyte nuclear factor-4 α (HNF-4 α) and the chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) are two members of the steroid/thyroid superfamily of transcription factors involved in the control of glucose homeostasis (18–20). Studies in mice in which the early lethal phenotype is circumvented have revealed that HNF-4 α is essential for

* This work was supported, in part, by European Community FP6 EUGENE2 Contract LSHM-CT-2004-512013, FP7 PREPROBEDIA Grant 201681, and by grants from the European Foundation for the Study of Diabetes (EFSD)/Lilly, the Associazione Italiana per la Ricerca sul Cancro, and the Ministero dell'Università e della Ricerca Scientifica. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[§] The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Table 1.

¹ Both authors contributed equally to this work.

² To whom correspondence should be addressed: Istituto di Endocrinologia e Oncologia Sperimentale Gaetano Salvatore, CNR, Via S. Pansini, 5, 80131 Naples, Italy. Tel.: 39-081-7463248; E-mail: beguinot@unina.it.

³ The abbreviations used are: *PED/PEA-15*, phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes; HNF-4 α , hepatocyte nuclear factor 4 α ; COUP-TFII, chicken ovalbumin upstream promoter transcription factor II; RT, reverse transcription; HRE, HNF-4 α response element; shRNA, short hairpin RNA; PIPES, piperazine-*N,N'*-bis(2-ethanesulfonic acid); ChIP, chromatin immunoprecipitation; RE, response element.