Plasmid 74 (2014) 1–8

Contents lists available at ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

Short Communication

Construction and validation of a novel dual reporter vector for studying mammalian bidirectional promoters

Véronique Léjard, Emmanuelle Rebours, Cédric Meersseman, Dominique Rocha *

INRA, UMR1313, Unité Génétique Animale et Biologie Intégrative, Domaine de Vilvert, F-78352 Jouy-en-Josas, France
AgroParisTech, UMR1313, Unité Génétique Animale et Biologie Intégrative, Domaine de Vilvert, F-78352 Jouy-en-Josas, France

ARTICLE INFO

Article history:
Received 3 April 2014
Accepted 8 May 2014
Available online 21 May 2014
Communicated by Grzegorz Wegrzyn

Keywords:
Bidirectional promoter
Fluorescent proteins
Flow cytometry
Reporter gene

ABSTRACT

Regulation of gene expression plays important role in cellular functions. With the development of sequencing techniques, more and more genomes are available and genome-wide analyses of genomic structures that may affect gene expression regulation are now possible. Analyses of several genomes have found a class of regulatory regions that contain elements that initiate transcription of two different genes positioned with a head-to-head arrangement in two opposite directions. These regulatory regions are known as bidirectional promoters. Although bidirectional promoters have been known for years, recent genome-scale studies have shown that the regulation of the expression of up to 10% of the genes are controlled by bidirectional promoters. These findings are based mostly on computational work and only a limited number of putative bidirectional promoters have been experimentally validated. Developing methods to study bidirectional promoters will allow researchers to understand how these regions are regulated and the roles that divergent transcription plays in the expression of genes. Here, we have developed a novel dual-fluorescence reporter gene vector to study the transcriptional output of mammalian bidirectional promoters. We demonstrate that this vector is capable of expressing reporter genes under the control of bidirectional promoters, using the known human *OSGEP/APEX* bidirectional promoter.

1. Introduction

Regulation of gene expression plays important role in cellular functions. With the development of sequencing techniques, more and more eukaryotic genomes are available and genome-wide analyses of genomic structures that may affect gene expression regulation are now possible. Computational analyses of many genomes have identified a class of regulatory regions that contain elements that initiate transcription of two different genes positioned with a head-to-head (5′–5′) orientation on opposite strands of the DNA. These regulatory regions often have fewer than 1000 base pairs separating the transcription start sites of the two genes. The regulatory regions with this arrangement of gene pairs have previously been termed "bidirectional promoters" (Adachi and Lieber, 2002; Takai and Jones, 2004; Trinklein et al., 2004). Bidirectional promoters have been known for years (e.g. Williams and Fried, 1986); however recent genome-scale studies have shown that the regulation of the expression of up to 10% of the genes is controlled by bidirectional promoters (Trinklein et al., 2004). Bidirectional promoters are a common feature within not only the human genome but are also present

* Corresponding author at: UMR1313, Unité de Génétique Animale et Biologie Intégrative, Centre INRA de Jouy-en-Josas, Domaine de Vilvert, F-78352 Jouy-en-Josas cedex, France. Fax: +33 1 34 65 24 78.
E-mail address: dominique.rocha@jouy.inra.fr (D. Rocha).

http://dx.doi.org/10.1016/j.plasmid.2014.05.001
0147-619X/

in many other genomes, including yeast, plants, invertebrates and vertebrates (Adachi and Lieber, 2002; Herr and Harris, 2004; Takai and Jones, 2004; Trinklein et al., 2004; Koyanagi et al., 2005; Engström et al., 2006; Li et al., 2006; Yang and Elnitski, 2008a,b; Yang et al., 2008; Piontkivska et al., 2009; Wang et al., 2009; Woo and Li, 2011), suggesting an important biological significance for this regulatory configuration.

It has been shown that some bidirectional promoters, such as those of the histone genes, regulate the transcription of pair of genes that need coordinated expression to maintain stoichiometric relationships (*e.g.* Maxson et al., 1983), or regulate the coexpression of genes that function in the same biological pathway (e.g. Momota et al., 1998.

Despite substantial interest in bidirectional promoters, the functional mechanisms underlying the activation of bidirectional promoters are currently not well characterized and only a limited number of putative bidirectional promoters have been experimentally validated (Trinklein et al., 2004).

The head-to-head arrangement and the small distance between transcription start sites is not sufficient to prove that the sequence between these start sites contains a true bidirectional promoter. Indeed, two short unidirectional promoters could regulate the expression of the two divergent genes. To determine whether the predicted eukaryotic bidirectional promoters function bidirectionally, their ability to initiate transcription in both directions is tested in a transient transfection assay. Each bidirectional promoter is cloned into a reporter vector (for example a luciferase reporter vector) in the two possible orientations. Cells are then transfected with the two different constructs and the activity of each promoter in both directions is measured. Some dual reporter vectors allowing the assay of putative bidirectional promoter in both directions simultaneously have also been reported (Ueda et al., 2006; Zanotto et al., 2007; Christophe-Hobertus and Christophe, 2010; Polson et al., 2011). These directional transcriptional assays are based on the measurement of luciferase and/or alkaline phosphatase activities and therefore require cell treatments before the assays.

The development of a novel dual reporter vector using genes coding for two different fluorescent proteins will allow an easier and simpler assay of bidirectional promoter activity. In addition this new vector will allow to study *in vivo* the activity of these regulatory regions. We present here the construction of a novel dual color fluorescent reporter gene vector to study the transcriptional output of mammalian bidirectional promoters. We demonstrate that this vector is capable of expressing reporter genes under the control of bidirectional promoters, using a known bidirectional promoter between the human O-sialoglycoprotein endopeptidase gene (*OSGEP*) and *APEX* genes (Seki et al., 2002).

O-Sialoglycoprotein endopeptidase (EC 3.4.24.57) is a unique enzyme that specifically cleaves the polypeptide backbone of membrane glycoproteins that contain clusters of O-linked sialoglycans (Abdullah et al., 1992). The human *OSGEP* gene is located in the chromosome region 14q11.2-q12 and lies immediately adjacent to the *APEX* gene, which encodes APEX nuclease, in a "head-to-head" orientation. APEX nuclease (also called HAP1, APE, REF-1 and APE1) is a multifunctional DNA repair enzyme that exhibits AP endonuclease, 3′-to-5′ exonuclease, DNA 3′-repair diesterase, DNA 3′-phosphatase and RNase H activities (Seki et al., 1992; Walker et al., 1994). It is also known to be a redox factor (REF-1), stimulating DNA-binding activity of several transcription factors (AP-1, Myb, p53, CREB and ATF) (Evans et al., 2000). The promoters of the *OSGEP* and *APEX* genes may overlap in view of the proximity of the two genes. In addition, Northern blot analysis showed ubiquitous and similar expression patterns for both genes. In order to study the regulation of *OSGEP* and *APEX* gene expression, Seki and collaborators analyzed the ~900 bp-long spacer sequence between the two genes, by luciferase assay in HeLa cells and have showed that this sequence has a bidirectional promoter activity (Seki et al., 2002).

Our novel dual color fluorescent reporter gene vector will allow researchers to study the transcriptional output of mammalian bidirectional promoters and to understand how these regions are regulated.

2. Materials and methods

2.1. Bidirectional expression vector preparation

The pBI-CMV2 vector (Clontech) expressing a Green Fluorescent Protein reporter gene from the *Aequorea coerulescens* jellyfish (herein referred as AcGFP1) was digested with *Bam*HI and *Not*I restriction enzymes (New England Biolabs) and gel-purified with Qiagen MinElute Gel Extraction kit (Qiagen). The pDsRed-Monomer vector (Clontech), which expresses a monomer mutant form of a Red Fluorescent Protein reporter gene from the *Discosoma striata* reef coral (herein referred to as DsRed monomer), was digested with *Bam*HI and *Not*I restriction enzymes. The 705 bp-long fragment encoding DsRed monomer was gel purified with the Qiagen MinElute Gel Extraction kit and DNA concentration estimated using a NanoDrop 1000 spectrophotometer (Thermo Scientific). The fragment was then ligated using T4 DNA ligase (New England Biolabs) to the *Bam*HI/*Not*I digested pBI-CMV2 vector, at a 3:1 ratio. *Escherichia coli* DH5α competent cells (Invitrogen) were transformed with the ligation product. Ten clones were then amplified and plasmids were purified with the Pureyield Plasmid Miniprep System DNA purification kit (Promega). Positive clones were identified by digesting plasmid DNA with *BstE*II or *Bgl*II (New England Biolabs). Digestion products were then visualized after gel electrophoresis on a 1% agarose gel. Clones carrying the right inserts gave a 3455/1041 bp bands or 2574/1922 bp bands with *BstE*II or *Bgl*II, respectively. The plasmids of two positive clones were then sequenced bidirectionally (Eurofins). One clone containing the right plasmid construct was then chosen. The fragment containing PminCMV2, PminCMV1 and an enhancer was removed by digesting the vector with *Eco*RI and *Bam*HI. After gel purification, the digested vector was blunt-ended with Klenow enzyme (New England Biolabs) and then self-ligated using T4 DNA ligase. The newly constructed vector (pBiP0) contains the two fluorescent protein reporter genes in opposite orientations.