



Transgenic labeling of the zebrafish pronephric duct and tubules using a promoter from the *enpep* gene

Christoph Seiler^a, Michael Pack^{a,b,*}

^a Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

^b Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

ARTICLE INFO

Article history:

Received 10 June 2010

Received in revised form 12 October 2010

Accepted 15 October 2010

Available online 20 October 2010

Keywords:

Zebrafish

Pronephros

Kidney

Promoter

Development

Transgenic

Glutamyl aminopeptidase

Enpep

Aminopeptidase A

ABSTRACT

In recent years the zebrafish has become a popular model system to study organ development and disease. To facilitate these studies, genetic tools are required which allow to modify and manipulate gene expression in organs of interest. Here we describe a zebrafish 2 kb *glutamyl aminopeptidase (enpep)* promoter fragment, and show that it can drive gene expression specifically in the kidney during early and late development. We established a stable transgenic line using this promoter fragment that has specific GFP expression in pronephric ducts and tubules starting at 20 h post-fertilization.

© 2010 Elsevier B.V. All rights reserved.

Glutamyl aminopeptidase (*enpep*) is a small membrane bound metalloprotease that cleaves N-terminal aspartyl and glutamyl residues from peptides (Dijkman et al., 2006). Its best-known target is Angiotensin II (Ang II) a component of the angiotensin-renin system (RAS) (Cogolludo et al., 2005; Reaux et al., 2001). The RAS system plays a role in the regulation of blood pressure and is also important for the development of the mammalian kidney (Dijkman et al., 2006).

The zebrafish pronephric kidney forms in a stepwise fashion. The complete pronephric duct is present around 24 h post-fertilization (hpf), the tubules, which connect the duct to the glomerulus form slightly later at around 30hpf. The midline glomerulus derives from bilateral primordia that later fuse in the middle of the larvae. Glomerular filtrate can be detected beginning around 48 hpf (Drummond et al., 1998; Dressler, 1999; Drummond, 2003, 2000; Wingert et al., 2007).

Although the zebrafish only develops a pronephros and mesonephros, and not a metanephros, as do mammals, many of the pathways involving induction and differentiation of kidney

development are conserved with amniotes (Drummond, 2005; Dressler, 1999; Wingert et al., 2007). Furthermore mutations of the zebrafish orthologs of human genes responsible for polycystic kidney disease cause a similar phenotype in zebrafish larvae (Sun et al., 2004; Drummond, 2005; Low et al., 2006; Weber et al., 2008; Obara et al., 2006; Bahadori et al., 2003; Hostetter et al., 2003).

Here we describe a 2 kb (kilo base pairs) *glutamyl aminopeptidase* promoter sequence which can drive strong expression of GFP in the zebrafish pronephric duct and tubules but not the glomerulus. We established a line stably expressing GFP under control of this promoter (*Tg(enpep:GFP)*) and demonstrated persistent transgene activation in adult fish. These results will aid to study kidney development in zebrafish as well as genetic manipulation to influence kidney development and function.

1. Results

To find promoters that have the potential to drive expression in absorptive epithelia, such as is present in the intestine and kidney, we searched the zebrafish gene expression database (<http://zfin.org/cgi-bin/webdriver?Mlval=aa-xpatselect.apg> (Thisse and Thisse, 2004)) for genes showing strong expression restricted to these organs. One gene (zfin id: ch211-146m5.2, im:7152184, <http://zfin.org/cgi-bin/webdriver?Mlval=aa-fxallfigures.apg&>

* Corresponding author. Address: Associate Professor of Medicine and Cell and Developmental Biology, University of Pennsylvania School of Medicine, Rm 1212 BRB 2/3, 421 Curie Blvd, Philadelphia, PA, USA. Tel.: +1 215 573 4145; fax: +1 215 898 9871.

E-mail address: mpack@mail.med.upenn.edu (M. Pack).

OID = ZDB-PUB-040907-1&fxallfig_probe_zdb_id = ZDB-EST-050309-369) showed strong specific expression. BlastN analysis revealed that this gene is identical to a predicted gene with high similarity to *glutamyl aminopeptidase* (*enpep*) (XM_679675, also called *aminopeptidase A* or *differentiation antigen gp160*). A BlastX analysis using this DNA sequence showed that the translated protein was very similar to mouse and human ENPEP proteins (identities 60%, positives 76%, score = 1108 bits; identities 56%, positives 73%, score = 1067 bits, respectively). A TBLASTN with the human protein against the zebrafish nr database gave only good hits for the above sequences and a zebrafish gene predicted to be an ortholog of Aminopeptidase N, albeit with much lower degree of amino acid identity (34%). This suggests that XM_679675 is indeed the orthologue of human ENPEP.

To find the zebrafish *enpep* promoter we analyzed 2 kb sequence upstream of XM_679675 derived from the zebrafish genomic BAC clones zC20008 and zC146M5 sequenced by the Sanger center. We used the softberry FPROM program (<http://softberry.com>) to identify possible transcription start sites. This identified a possible promoter and TATA box located 108 bp (base pairs) and 136 bp upstream, respectively of the first bp of XM_679675. The XM_679675 sequence starts with an ATG, which is predicted to be the translational start. Accordingly, we chose the sequence 108 bp upstream of XM_679675 as the 3' end of the promoter (Fig. 1). However, a subsequent entry in the GenBank data base (accession number EB774757) led to the identification of a new predicted gene, XR_029637.2, that extended 200 bp in the 5' direction from XM_679675. Because we cannot be certain that additional transcribed sequence related to this gene will be subsequently identified, the transcription initiation site of the zebrafish *enpep* gene is not clearly defined at this time.

To test if the 2 kb fragment containing the presumptive *enpep* promoter could drive expression in the developing kidney and intestine we amplified it from genomic DNA and cloned it into the pT2KXIGΔin vector, replacing the original EF1p promoter (Kawakami and Shima, 1999; Kawakami et al., 1998). We thus created a GFP-reporter construct flanked by two Tol2 transposase sites. These sites greatly increase the integration rate into the zebrafish genome when co-injected with transposase mRNA (Kawakami, 2004; Kawakami et al., 2004). We injected this construct and the transposase mRNA into the first cell of newly fertilized zebrafish eggs. In most of the injected eggs we could see strong GFP expression in the pronephric duct and tubules starting around 28 hpf. This expression persisted throughout embryonic and larval development.

To see if the construct was stably integrated in the genome we grew the fish to adulthood and mated them with wild type fish. 5 out of 7 of the eggclays derived from these matings showed uniform expression throughout the pronephric tubules and duct in 10–20% of the embryos, consistent with mosaic germline integration of the transgene (Fig. 2). The expression started around 20 hpf and got stronger till 28 hpf. We could not detect expression in the intestine in any of the lines examined. One cross showed weak GFP expression in trunk muscle cells. Larvae of one of the informative crosses were raised to establish stable transgenic lines with GFP expression that was restricted to the kidney (*Tg(enpep:GFP)*).

We next asked if a shorter 1 kb promoter fragment derived from the larger 2 kb fragment could drive reporter gene expression

in the developing pronephros (Fig. 1). In contrast to the previous promoter, the F1 offspring of injected larvae had noticeable GFP expression in trunk and head in addition to the pronephros expression. We assume that the 1 kb promoter fragment lacks an inhibitory regulatory element which results in weak activation of the reporter in cells that do not normally express *enpep*.

To determine whether the 2 kb *enpep* promoter fragment was active in all cells of the pronephric duct and tubule, as well as the glomerulus, which is less easily identified in live embryos, we analyzed histological sections of transgenic larvae immunostained with antibodies that recognize GFP and laminin. As shown in Fig. 3 all cells of the pronephric tubules and ducts expressed GFP, we could also detect GFP expression in the cells resembling podocytes of the glomerulus that connect to the ducts. GFP expression was also detectable in the adult kidney in a comparable pattern as in the larvae. No GFP expression was detectable in the intestine at 3 or 6 dpf (days post fertilization) (Fig. 3).

2. Discussion

Here we show that a 2 kb promoter fragment from the *enpep* coding sequence can drive specific GFP expression in the pronephric tubules and ducts of zebrafish larvae. The expression in the pronephros recapitulates the *enpep* expression previously recorded by RNA in situ hybridization, with the exception of the intestinal epithelium. We assume that additional promoter elements needed to drive intestinal expression are not included in the 2 kb fragment we identified.

The transgenic line we established will help studying the development of the zebrafish pronephros in wild type and mutant larvae and will be useful for establishing zebrafish models of human kidney diseases. This line could also help to screen for pharmacological reagents and genes capable of modulation *enpep* expression in the kidney, thus influencing activation of Angiotensin II.

3. Material and method

3.1. Identifying the *enpep* upstream sequence

We used the Sanger BLAST server (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/d_rerio) to identify 5 kb upstream of XM_679675 from the clone zC20008 (acc.# CR559943, zC146M5: acc# BX005193). Tests for translated sequence were done using NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>) and TIGR (<http://compbio.dfci.harvard.edu/cgi-bin/tgi/Blast/index.cgi>) BLAST. For promoter prediction we used softberry fprom (<http://www.softberry.com/berry.phtml?topic=fprom&group=programs&subgroup=promoter>). The identified promoter sequence has the gene bank accession number HQ443696.

3.2. Amplification and cloning of the promoter

The 2 kb fragment was amplified from Tü genomic DNA with primer containing BamH1 and Xho1 adapters with Clontech Advantage 2 polymerase. The primer sequences are 5': AT-CTCGAG-CCTGG TGGAAAAGCGAACAAGAAAAT; 3': AT-GGATCC-AAGTCAGAACACT CTCTCCCTGCGAAC. The 5' primer for the 1 kb fragment is TGCAGTCT

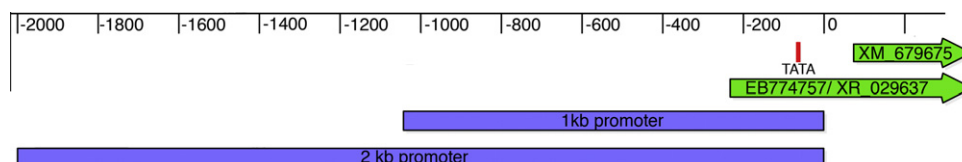


Fig. 1. Promoter organization of the predicted zebrafish *enpep* gene: Blue: 2 kb and 1 kb promoter constructs. Green: predicted *enpep* coding sequence, XM_679675 was the original synthetic construct, XR_029637.2 is a more recent prediction derived from the zebrafish genome sequencing project. Red: TATA box predicted by FPROM software.

Download English Version:

<https://daneshyari.com/en/article/8471107>

Download Persian Version:

<https://daneshyari.com/article/8471107>

[Daneshyari.com](https://daneshyari.com)