Gene 567 (2015) 73–80

Contents lists available at ScienceDirect

Gene

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

Research paper

Identification of tissue-specific regulatory region in the zebrafish lamin A promoter

Ajay D. Verma, Veena K. Parnaik *

CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India

ARTICLE INFO

Article history:
Received 19 November 2014
Received in revised form 24 March 2015
Accepted 24 April 2015
Available online 25 April 2015

Keywords:
Lamin promoter
Lamin gene regulation
Lamin embryonic expression
Lamin tissue-specific expression
Lamin developmental regulation

ABSTRACT

Lamins are major structural proteins present in the nuclei of metazoan cells and contribute significantly to nuclear organization and function. The expression of different types of lamins is developmentally regulated and lamin A is detectable in most differentiated tissues. Although the proximal promoter of the mammalian lamin A gene has been characterized, the tissue-specific regulatory elements of the gene have not been identified. In this study, we have cloned and functionally characterized a 2.99 kb segment upstream of exon 1 in the zebrafish lamin A gene. This fragment was able to drive GFP expression in several tissues of the developing embryo at 14–72 h post fertilization in stable transgenic lines. Deletion fragments of the 2.99 kb promoter were analyzed by microinjection into zebrafish embryos in transient assays as well as by luciferase reporter assays in cultured cells. A minimal promoter segment of 1.24 kb conferred tissue-specific expression of GFP in the zebrafish embryo as well as in a myoblast cell line. An 86 bp fragment of this 1.24 kb segment was able to activate a heterologous promoter in myoblasts. Mutational analysis revealed the importance of muscle-specific regulatory motifs in the promoter. Our results have important implications for understanding the tissue-specific regulation and functions of the lamin A gene.

1. Introduction

Lamins are conserved type V intermediate filament proteins that form a fibrous network or lamina underlying the inner nuclear membrane which also extends throughout the interior of the nucleus. The lamina plays an essential role in maintaining the integrity of the nuclear envelope and is required for the spatial organization of various nuclear functions as well as organization of chromatin. Two major classes of lamins are found in most metazoan species: B-type lamins B1 and B2 that are coded by separate genes and are expressed in nearly all somatic cells, and A-type lamins A and C that are encoded by a single lamin A gene through alternative splicing and are detectable in several differentiated cell types. Additional splice variants of both types of lamins are expressed in germ cells. In vertebrates, A-type lamins are developmentally regulated and the onset of their expression in different tissues generally correlates with cellular differentiation (Broers et al., 2006; Dechat et al., 2008; Parnaik, 2008). Although the proximal promoter motifs that control lamin A expression have been well documented in the mammalian gene (Lin and Worman, 1993; Nakajima and Abe, 1995; Tiwari et al., 1998), there is very little information available on tissue-specific regulatory elements of the gene. Understanding the tissue-specific regulation of lamin A expression assumes importance in light of the discovery that different mutations in human lamin A cause a spectrum of tissue-specific diseases which mainly affect skeletal muscle, cardiac tissue, adipose and bone tissues and also cause premature aging disorders (Broers et al., 2006; Dechat et al., 2008; Parnaik, 2008; Parnaik et al., 2011; Butin-Israeli et al., 2012).

The zebrafish has emerged as a useful system for the study of vertebrate development, especially due to the ease of visualization of GFP-tagged reporters during embryogenesis in both stable and transient transgenic fish. Zebrafish has four genes that code for lamins A, B1, B2 and LIII. Zebrafish lamin A gene shows 63% identity and 76% similarity with the human lamin A gene. Based on its conserved gene structure and sequence similarity, zlamin A can be considered to be an orthologue of mammalian lamin A. Mutations in zlamin A have been reported to functionally simulate human premature aging disorders (Koshimizu et al., 2011).

In the present study, we have isolated a 2.99 kb region of the zebrafish lamin A gene upstream of the ATG translational start site which can drive GFP expression in several tissues of the developing embryo. By deletion analysis, we have identified a minimal promoter segment of 1.24 kb that confers tissue-specific expression of GFP in the zebrafish embryo as well as luciferase expression in the C2C12 mouse myoblast cell line. This segment harbors an 86 bp element that is able to activate a heterologous promoter in C2C12 cells and contains a muscle-specific regulatory motif.

Abbreviations: Dpf, days post fertilization; GFP, green fluorescence protein; hpf, hours post fertilization; MEF2C, myocyte-enhancing factor 2C; zlamin A/C, zebrafish lamin A/C.

* Corresponding author at: CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India.
E-mail address: veenap@ccmb.res.in (V.K. Parnaik).

http://dx.doi.org/10.1016/j.gene.2015.04.067
0378-1119/

2. Materials and methods

2.1. Cloning of lamin A putative promoter region from zebrafish

The zebrafish lamin A cDNA sequence (GI 23308640) was blasted to zebrafish genome sequence (Zv9 reference assembly) in NCBI blast (http://blast.ncbi.nlm.nih.gov/blast/Blast). It aligned to the sequence NC_007127.5 at *Danio rerio* strain Tubingen chromosome 16, Zv9. A 2.99 kb region upstream of the zlamin A translational ATG codon spanning from 32869654 bp to 32866666 bp was identified and amplified from genomic DNA by PCR using the primers and conditions listed in Table 1 (2.99 kb zLmnaA P). Deletion fragments of length 0.91, 1.16 and 2.20 kb were made by digestion with suitable restriction enzymes. Fragments of 1.24, 1.35, 1.41, 1.52 and 1.67 kb were generated by PCR using the primers and conditions listed in Table 1. Mutant constructs were made by PCR-based mutagenesis using the 1.67 kb lamin A promoter fragment as template, using the primers and conditions listed in Table 1. Single mutations were made in E box E5(m) at −1165 to −1160 (CAGCTG to GTGCGT), E6(m) at −1506 to −1501 (CATTTG to GTTTGT) and E7(m) at −1639 to −1634 (CATTTG to GTTTGT), followed by double and triple mutations. The promoter fragments were cloned into the Tol2-GFP vector (Urasaki et al., 2006) for analysis in zebrafish embryos or pGL3-Basic vector (Promega Corporation, Madison, WI) for analysis in cultured cells. The fragments spanning −1158/−1243 bp and −1204/−1289 bp were amplified by PCR and cloned into the pGL3-Promoter vector, upstream of the SV40 promoter. The sequences of all constructs were verified by automated DNA sequencing.

Table 1
List of PCR primers and reaction conditions.

Name of fragment	Primer sequences (5′–3′)	Reaction conditions
2.99 kb zLmnA P	Forward : ATCCAATTCATATTAA AGTGTTCA Reverse : GGTTGTCTGGAACTAC TGATACTA	94 °C − 1 min, 94 °C − 15 s, 56 °C − 25 s, 68 °C − 3 min, 68 °C − 10 min
1.67 kb zLmnA P	Forward : CTCTGGAAGTCAATGG TTACATGT Reverse : GGTTGTCTGGAACTAC TGATACTA	94 °C − 1 min, 94 °C − 15 s, 60 °C − 25 s, 68 °C − 2 min, 68 °C − 10 min
1.41 kb zLmnA P	Forward : AATTTACTGCTGTTCA AGCTGC Reverse : GGTTGTCTGGAACTAC TGATACTA	94 °C − 1 min, 94 °C − 15 s, 56 °C − 25 s, 68 °C − 2 min, 68 °C − 10 min
1.52 kb zLmnA P	Forward : TTTGTTGTAATTGTAT CATTTGTATT Reverse : GGTTGTCTGGAACTAC TGATACTA	94 °C − 1 min, 94 °C − 15 s, 58 °C − 25 s, 68 °C − 2 min, 68 °C − 10 min
1.29 kb zLmnA P	Forward : GACTTCACAAACTCCG CCTTC Reverse : GGTTGTCTGGAACTAC TGATACTA	94 °C − 1 min, 94 °C − 15 s, 58 °C − 25 s, 68 °C − 1.5 min, 68 °C − 10 min
1.24 kb zLmnA P	Forward : GAGGCGTGAGTTTCTG CCAGA Reverse : GGTTGTCTGGAACTAC TGATACTA	94 °C − 1 min, 94 °C − 15 s, 58 °C − 25 s, 68 °C − 1.5 min, 68 °C − 10 min
−1204/−1289	Forward : TGAGAGGCGTGAGTTT CTGCCAGAGATCAGAAGCGCGA GTCGCCTAGCCCGGGCTCGAGA TCT Reverse : ATGAAGAGAAGGAGGA GGAGGAGAAGGCGGAGTTTGTG AAGTCCACGCGTAAGAGCTCGGTA CC	94 °C − 1 min, 94 °C − 15 s, 64 °C − 25 s, 68 °C − 12 min, 68 °C − 10 min
−1158/−1243	Forward : ACACACTCTTTCAGCA GAGTTTCAGCGGGACACAGCAGCT GTACTAGCCCGGGCTCGAGATCT Reverse : GCTGCGACTCGCGCTT CTGATCTCTGGCAGAAACTCACGC CTCCACGCGTAAGAGCTCGGTACC	94 °C − 1 min, 94 °C − 15 s, 62 °C − 25 s, 68 °C − 12 min, 68 °C − 10 min
zLmnA P E7(m)	Forward : GTTACATGTTTGAAAT ATCTTGTTTGTTGTTCAACGGGAC AAAGG Reverse : CCTTTGTCCCGTTGAACA ACAAACAAGATATTTCAAACATGT AAC	94 °C − 1 min, 94 °C − 15 s, 56 °C − 25 s, 68 °C − 14 min, 68 °C − 10 min
zLmnA P E6(m)	Forward : TTATTATTTTGTTGTAAT TGTATGTTTGTTATTTTATTAAAA TAT Reverse : ATATTTTAATAAAATA ACAAACATACAATTACAACAAAAT AATAA	94 °C − 1 min, 94 °C − 15 s, 60 °C − 25 s, 68 °C − 14 min, 68 °C − 10 min
zLmnA P E5(m)	Forward : CAGAGTTTCAGCGGGA CACAGGTGCGTTAGGAGCTTCAAT AACC Reverse : GGTTATTGAAGCTCCT AACGCACCTGTGTCCCGCTGAAAC TCTG	94 °C − 1 min, 94 °C − 15 s, 59 °C − 25 s, 68 °C − 14 min, 68 °C − 10 min

2.2. Generation of transgenic zebrafish

Adult zebrafish (Tubingen strain) were maintained on a 14 h light/10 h dark cycle in a temperature controlled room at 28 ± 1 °C. Fish were fed with live brine shrimps twice daily and water was changed daily. Breeding and rearing of zebrafish were performed according to standard methods (Westerfield, 2000). To obtain Tol2 transposase mRNA, the pDB600 plasmid encoding the transposase (Balciunas et al., 2006) was linearized by Xba I digestion and transcribed using the T3 mMessage mMachine in vitro transcription kit (Ambion, Foster City, CA) according to the manufacturer's instructions. To generate transgenic larvae, 35 ng/μl of Tol2 transposase mRNA and 30 ng/μl of GFP construct were co-injected into 1-cell stage embryos as described (Fisher et al., 2006). Stable transgenic lines of GFP expressing zebrafish were established by standard breeding procedures. The embryos were staged by hours post fertilization (hpf) or days post fertilization (dpf) and also by morphological criteria as described (Westerfield, 2000). All animal experiments were conducted as per institutional guidelines.

2.3. Transient GFP reporter assays

For transient assays of GFP reporter expression, the GFP construct (50 ng/μl) was injected into 1-cell stage zebrafish embryos and the embryos were allowed to develop. At 2.5 dpf embryos were anesthetized by adding tricaine methanesulfonate (Sigma-Aldrich) (0.4% stock solution) to the embryo medium and imaged using a Leica M205 FA fluorescence stereo microscope (Leica Microsystems GmbH, Germany). GFP-positive tissues were counted manually for each embryo (0–5 fluorescent streaks per tissue were denoted as low intensity and 5–20 streaks were denoted as high intensity); tissues were identified by morphological criteria as described (Westerfield, 2000). Approximately 70–100 injected embryos that were positive for GFP fluorescence were imaged per construct and percent values were calculated for data presentation.

2.4. Confocal laser scanning fluorescence microscopy (CLSM)

Transgenic zebrafish embryos of different stages (14 hpf, 1 dpf, 2 dpf and 3 dpf) were dechorionated and anesthetized. The anesthetized embryos were imaged using a Leica SP8 confocal microscope (Leica Microsystems GmbH, Germany) with a 20× objective. Confocal images were taken using the laser channel 488 nm. While capturing a tile scan of the embryo, the pinhole was set to 2 airy units and the scanning speed was 300 Hz with a step size of 1.9 μm in the *z*-direction. The images were processed using LASAF software.

2.5. Cell culture and luciferase promoter assay

C2C12 mouse skeletal myoblasts were maintained at subconfluent densities in DMEM supplemented with 20% FBS. For lamin A promoter analysis, deletion and mutated fragments were cloned into pGL3-Basic vector, which contains the firefly luciferase gene as a reporter but does not contain any eukaryotic promoter or enhancer elements. For