Biochemical and Biophysical Research Communications 442 (2013) 189–194

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Zinc finger protein 219-like (ZNF219L) and Sox9a regulate *synuclein-γ2* (*sncgb*) expression in the developing notochord of zebrafish

Huang-Wei Lien [a,1], Chung-Hsiang Yang [b,1], Chia-Hsiung Cheng [c], Yung-Feng Liao [d], Yu-San Han [a,*], Chang-Jen Huang [b,*]

[a] Institute of Fisheries Sciences, National Taiwan University, Taipei 106, Taiwan
[b] Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan
[c] Department of Biochemistry, Taipei Medical University, Taipei 110, Taiwan
[d] Institute of Cellular and Organismic Biology, Academia Sinica, Taipei 115, Taiwan

ARTICLE INFO

Article history:
Received 6 November 2013
Available online 20 November 2013

Keywords:
Synuclein-γ2 (*sncgb*)
ZNF219-like (ZNF219L)
Sox9a
Notochord
Zebrafish

ABSTRACT

Zebrafish *synuclein-γ2* (*sncgb*) has been reported to be expressed specifically in the notochord. However, the mechanism by which the *sncgb* gene promoter is regulated has not been described. In this paper, we demonstrate that Zinc finger protein 219-like (ZNF219L) and sox9a are involved in the regulation of *sncgb* gene expression. Furthermore, we observed that over-expression of both ZNF219L and Sox9a resulted in increased *sncgb* expression. In addition, ZNF219L is physically associated with Sox9a, and simultaneous morpholino knockdown of *znf219L* and *sox9a* caused a synergistic decrease of *sncgb* expression in the notochord. Taken together, our results reveal that coordination of ZNF219L with Sox9a is involved in the regulation of notochord-specific expression of *sncgb*.

1. Introduction

The first member of the synuclein family was isolated from the purified cholinergic synaptic vesicles of the Pacific electric ray, *Torpedo californica* [1]. In humans, the synuclein family consists of α-, β-, and γ-synuclein genes (*SNCA*, *SNCB*, and *SNCG*, respectively). Misfolded α-synuclein protein has been reported to be involved in the pathological accumulation of intraneuronal protein aggregates (Lewy bodies and Lewy neurites), thereby leading to cell dysfunction and cell death in the pathogenesis of Parkinson's disease (PD) [2,3]. Furthermore, β-synuclein acts as a regulator of α-synuclein-induced neurotoxicity [4]. Although there is no clear evidence that γ-synuclein is involved in neurodegenerative diseases, γ-synuclein has been reported to be overexpressed in breast carcinomas and ovarian cancer [5].

In contrast to humans, the synuclein family in zebrafish consists of β-, γ1-, and γ2-synuclein genes (*sncb*, *sncga*, and *sncgb*, respectively). Early expression of zebrafish *sncb* begins at the trigeminal placode, before extending to the ventral diencephalon, olfactory placode, ventral tegmentum, and spinal cord neurons. *Sncga*, on the other hand, is expressed in cells of the nervous system, including hindbrain neurons, cranial ganglia, and retinal cells; simultaneous knockdown of *sncb* and *sncga* was reported to decrease spontaneous motor activity [6]. Expression of *sncgb* is significantly different from that of *sncga*; *sncgb* transcription is restricted to the notochord throughout embryogenesis, from the 13 somite-stage to 2 days post-fertilization (dpf) [7]. However, like human *SNCG*, the function and regulatory processes of *sncgb* are still unknown.

Zinc finger protein 219 (ZNF219) is a transcription factor partner of SOX9, required for the regulation of mammalian chondrocyte differentiation [8–10]. We previously reported that the *zinc finger 219-like* (*znf219L*) gene is required for regulating the expression of the collagen type 2 alpha 1a (*col2a1a*) gene in the zebrafish notochord [11]. We cloned the zebrafish ZNF219L gene based on mammalian ZNF219, which contains nine C2H2-type zinc finger domains. We further demonstrated that zebrafish ZNF219 recognizes the GGGGG motifs in the *col2a1a* promoter through its sixth and ninth zinc finger domains, thereby up-regulating promoter activity in a luciferase assay. In addition, morpholino knockdown of *znf219L* decreased endogenous expression of *col2a1a* in the notochord [11]. Based on these findings, we hypothesized that ZNF219L may regulate the expression of notochord-related genes during early developmental stages.

* Corresponding authors. Fax: +886 2 3366 5864 (Y.-S. Han). Address: Institute of Biological Chemistry, Academia Sinica, 128 Academia Rd., Sec. 2, Taipei 115, Taiwan. Fax: +886 2 2788 9759 (C.-J. Huang).
E-mail addresses: yshan@ntu.edu.tw (Y.-S. Han), cjibc@gate.sinica.edu.tw, brian2940b@yahoo.com.tw (C.-J. Huang).
[1] These authors contributed equally to this work.

0006-291X
http://dx.doi.org/10.1016/j.bbrc.2013.11.042

In this study, we demonstrate that *sncgb* is also a notochord-specific target gene of zebrafish ZNF219L. We confirm that ZNF219L activates *sncgb* expression in the notochord via binding to a GGGGG motif in the *sncgb* promoter. In addition, we demonstrate that ZNF219L forms a complex with Sox9a, and is required for the regulation of *sncgb* expression in the notochord. Knockdown of *znf219L* and *sox9a* resulted in a synergistic decrease of endogenous *sncgb* expression in the notochord. Collectively, these results indicate that coordination of ZNF219L and Sox9a regulates *sncgb* gene expression in the notochord.

2. Materials and methods

2.1. Zebrafish care

Zebrafish embryos were raised at 28.5 °C, and different developmental stages were determined based on criteria described in the *Zebrafish Book* [12]. All animal procedures were approved by Academia Sinica Institutional Animal Care and Utilization Committee (ASIACUC) (protocol #10-12-114).

2.2. Morpholino oligonucleotide (MO) injection

Antisense MOs were designed and obtained from Gene Tools (Philomath, OR, USA). MO sequences were as follows: zebrafish *znf219L*-MO, 5′-GTC TAT GCC ATG CTT CAC TTC CTT G-3′; *sox9a*-MO, 5′-AAT GAA TTA CTC ACC TCC AAA GTT T-3′; control-MO, 5′-CCT CTT ACC TCA GTT ACA ATT TAT A-3′. The MOs were diluted to produce 24 μg/μl injection stocks, which were stored at −20 °C prior to use.

2.3. Whole-mount in situ hybridization

Digoxigenin-labeled RNA probes (Roche, Penzberg, Germany) were generated by *in vitro* transcription of linearized pGEM-T-easy plasmid (Promega) carrying the 3′-UTR of the appropriate zebrafish gene. To synthesize digoxigenin-labeled (Roche, Penzberg, Germany) antisense RNA probes, pGEM-T easy-*sncgb* 3′-UTR was linearized with *Pst* I and transcribed with T7 RNA polymerase. Whole-mount *in situ* hybridization was performed as previously described [13,14].

2.4. Cell culture

Carp fin (CF) epithelioid cells [15] were maintained at 27 °C in Leibovitz's L-15 media supplemented with 10% fetal bovine serum. CF cells were transfected as previously described [16]. NIH/3T3 cells (ATCC CRL-1658; Manassas, VA, USA) were cultured in a humidified atmosphere of 5% CO_2 at 37 °C, in high-glucose Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA).

2.5. Luciferase reporter assay

Luciferase reporter gene assays were performed using pGL3 Luciferase Reporter Vectors (Promega) containing a modified coding region for firefly (*Photinus pyralis*) luciferase driven by the zebrafish *sncgb* 2.0-kb proximal promoter or 2.0-kb mut (in which the ZNF219L binding site was changed from GGGGG to GAAAG by PCR mutagenesis). The following primers were used to amplify the zebrafish *sncgb* 2.0-kb proximal and 2.0-kb mut promoter: forward primer: 5′-GGC ATA AAT CCA TGT ATG CGT CAA AAC GGC-3′; reverse primer: 5′-CTG GAT CTG GTG CTC GTC TAT AGC TGG-3′. The luciferase reporter constructs were transfected into CF epithelioid cells. One microgram of promoter DNA and 0.5 mg of pSV-β-galactosidase were co-transfected into CF epithelioid cells using the PolyJet *In Vitro* DNA Transfection Reagent (SignaGen Laboratories, Ijamsville, MD, USA). Transfections were performed using 12-well plates, and approximately 1×10^5 cells were seeded 1 day before transfection. Cells were lysed 2 days after transfection (by which time cells had grown to 90% confluency), and luciferase activity was determined using a luminometer (Promega), according to the manufacturer's protocol. Transfection efficiency was normalized by determining the activity of β-galactosidase activity in the cell lysates. All presented data are the results of three independent experiments, and were statistically analyzed by one-way ANOVA. Data represent means ± s.d.

2.6. Western blot

Western blot was performed by incubating membranes with anti-HA monoclonal antibody (1:3000; Santa Cruz, CA) at 4 °C overnight. Signals were detected using an enhanced chemiluminescence (ECL) kit (NEN Life Science Products, MA).

2.7. Oligonucleotide precipitation assay

NIH 3T3 cells were lysed in lysis buffer [20 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM EGTA, 1.5 mM $MgCl_2$, 10% glycerol, 1% Triton X-100, 10 mg/ml leupeptin, 1 mM PMSF, 0.2 mM sodium orthovanadate] for use in the *in vitro* binding assay. The lysates were pre-incubated with streptavidin-agarose beads for 3 h, and then incubated for 16 h with 1 mg of poly(dI-dC) and 1 mg of a biotinylated double-stranded oligonucleotide probe. The biotinylated double-stranded oligonucleotide probe contained the ZNF219L binding element present in the *sncgb* gene promoter, and was generated using the following primer pair: BS sense primer: 5′-GGC AAA AAG GGG AGG GGG TGG ATG GGT TTG-3′; anti-sense primer: 5′-CAA ACC CAT CCA CCC CCT CCC CTT TTT GCC-3′.

Table 1
Primers used for PCR amplification of cDNA encoding full-length and deletion mutations of zebrafish *znf219L*.

Gene	Sequences of forward (F) and reverse (R) primers
znf219L	HindIII-F, 5′-AAA AGC TTA TGG ATT CCC CAC CAG AAT GTA TGC TG-3′
	KpnI-R, 5′-GGG GTA CCC TAT TCC CCC AAG ACA CCT TCC TCC TC-3′
znf219L-Δ1	HindIII-F, 5′-AAA AGC TTCCA GAA ACT CCC GTC CCA CAA ATG TCT-3′
	KpnI-R, 5′-GGG GTA CCC TAT TCC CCC AAG ACA CCT TCC TCC TC-3′
znf219L-Δ2	HindIII-F, 5′-AAA AGC TTCAT CAG CAT CCT TCA TCA C-3′
	KpnI-R, 5′-GGG GTA CCC TAT TCC CCC AAG ACA CCT TCC TCC TC-3′
znf219L-Δ3	HindIII-F, 5′-AAA AGC TTA TGG ATT CCC CAC CAG AAT GTA TGC TG-3′
	KpnI-R, 5′-GGG GTA CCT GGA CAT GCC CAC TCT GGC AC-3′
znf219L-Δ4	HindIII-F, 5′-AAA AGC TTC GTG AGC AAA GGA ATG CTA TGG CA-3′
	KpnI-R, 5′-GGG GTA CCC TAT TCC CCC AAG ACA CCT TCC TCC TC-3′
znf219L-Δ5	HindIII-F, 5′-AAA AGC TTA TGG ATT CCC CAC CAG AAT GTA TGC TG-3′
	KpnI-R, 5′-GGG GTA CCT GCC ATA GCA TTC CTT TGC TCA CG-3′
znf219L-Δ6	HindIII-F, 5′-AAA AGC TTA TGG ATT CCC CAC CAG AAT GTA TGC TG-3′
	KpnI-R, 5′-GGG GTA CCG TTA CCC ATT TCT GCA ACC TG-3′

2.8. In vitro binding assay using recombinant His-tagged Sox9a protein and ZNF219L

The amplification product containing the *sox9* coding region was subcloned into the pQE30 vector (QIAGEN), and the construct was transformed into the JM109 *Escherichia coli* strain. The resulting His-tagged Sox9 protein was purified with Ni-NTA magnetic agarose beads (BIOMAN), according to the manufacturer's protocol. The immobilized His-tagged Sox9 protein was used for the binding assay with ZNF219L. Association of Sox9 with HA-tagged Znf219 mutants was determined by immunoblotting with anti-HA antibody, following precipitation of cell lysates with anti-His antibody. PCR was performed using gene-specific primers, as shown in Table 1.

3. Results

3.1. Zinc finger protein 219-like (ZNF219L) is required for regulation of sncgb promoter activity in vitro and in vivo

A ZNF219L binding motif (GGGGG) was identified within the 2.0-kb promoter region at the 5′ end of the zebrafish *sncgb* gene (genomic sequences were obtained from the NCBI Genbank database NM_001020652), suggesting that ZNF219L may regulate expression of this gene. Luciferase reporter assays were subsequently performed to confirm whether ZNF219L regulates *sncgb* promoter activity. Plasmids containing a 2.0-kb region of the *sncgb* promoter with either wild type (GGGGG) or mutant (GAAAG) binding motifs were used to drive luciferase expression. ZNF219L overexpression enhanced luciferase activity of wild type, but not mutant, constructs (Fig. 1A). Binding of ZNF219L to the *sncgb* 2.0-kb promoter was confirmed using *in vitro* pull-down assays; biotin-labeled oligonucleotide probes containing the *sncgb* 2.0-kb promoter sequence with the wild type binding motif pulled down ZNF219L (Fig. 1B).

We previously reported that *znf219L* knockdown decreased expression of notochord-specific genes [17], and therefore hypothesized that *znf219L* knockdown may also affect *sncgb* expression. Zebrafish embryos were injected with z*nf219L* morpholino (MO), and collected at 24 h post-fertilization (hpf) for analysis by whole mount *in situ* hybridization using an *sncgb* probe. Knockdown of *znf219L* resulted in a decrease in *sncgb* expression specifically in the notochord, as compared to embryos injected with control-MO (Fig. 1C).

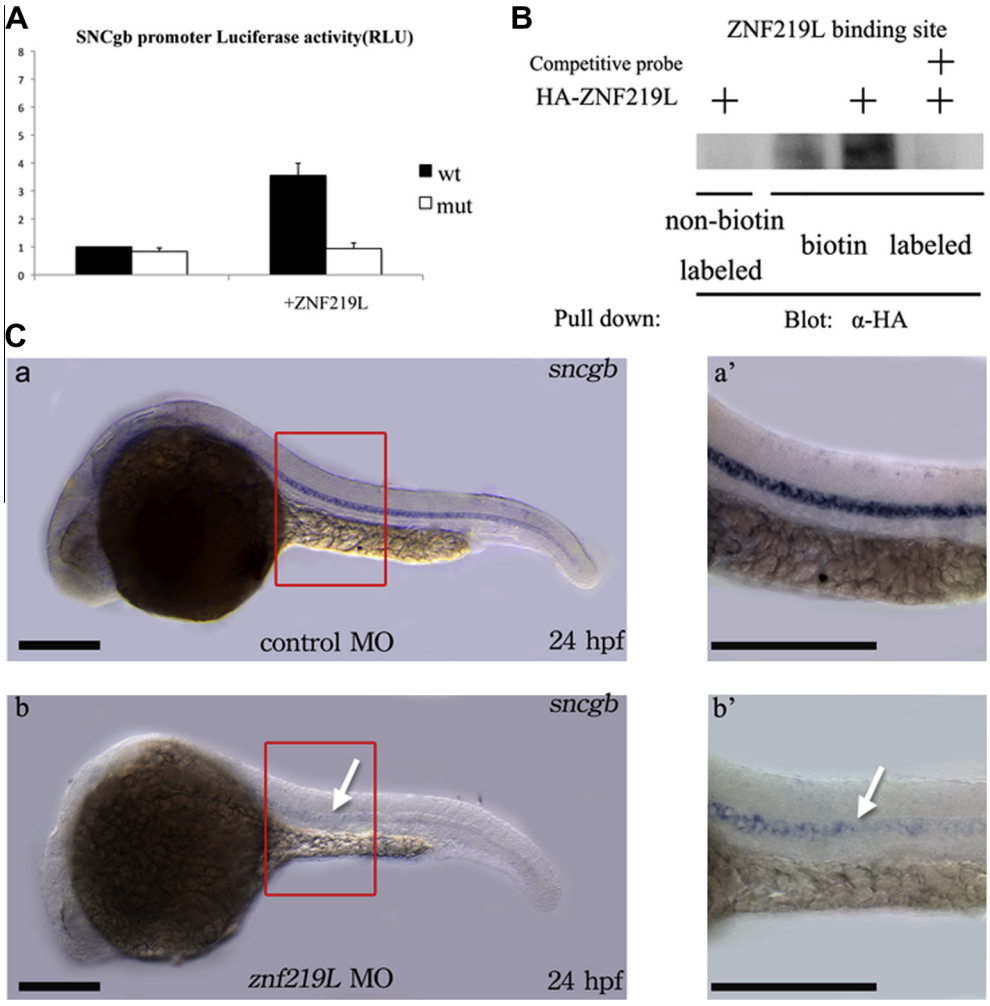

Fig. 1. ZNF219L binds the *sncgb* proximal promoter and affects *sncgb* expression in the notochord. (A) The effect of ZNF219L on *sncgb* promoter activity was studied in grass carp CF cells. Cells were co-transfected with a ZNF219L expression vector and *sncgb* gene 2.0-kb proximal promoter luciferase constructs containing either wild type (GGGGG) or mutant (GAAAG) ZNF219L binding sites. Luciferase activity was determined 2 days after transfection. Data represent the mean ± s.d. (n = 3). (B) ZNF219L binds to the GGGGG motif in the *sncgb* proximal promoter. NIH3T3 cells were transfected with empty or HA-ZNF219L vector, and then incubated with biotinylated oligonucleotides containing the GGGGG motif of the *sncgb* gene promoter. The probes were precipitated with streptavidin agarose beads, and association of ZNF219L protein with the oligonucleotides was demonstrated by immunoblotting with anti-HA antibody. (C) One-cell stage embryos were injected with *znf219L* or control morpholino (MO). At 24 hpf, morphants were subjected to *in situ* hybridization with an *sncgb*-3′ UTR probe. The endogenous *sncgb* signal was decreased in the notochord of *znf219L* morphants (panel b) as compared to that of control morphants at 24 hpf (panel a). The boxed regions are magnified in panels a′ and b′. White arrows indicate decreased expression of endogenous *sncgb* mRNA in the notochord. Scale bars = 100 μm.

3.2. Sox9a is required for regulation of sncgb promoter activity in vitro and in vivo

Two HMG consensus DNA sequences were also identified within the 2.0-kb promoter region at the 5′ end of the zebrafish *sncgb* gene, suggesting that Sox9 may regulate expression of *sncgb*. However, gene duplication in the zebrafish genome is common [18,19], and there are two *sox9* genes in zebrafish: *sox9a* and *sox9b*. These genes have been reported to be involved in the development of crest, otic placode, cartilage, and bone [20], and both Sox9a and Sox9b recognize HMG motifs [21]. Since Sox9a exerts a greater effect than Sox9b on the morphogenesis of condensations during cartilage differentiation [22] and *sox9b* knockdown has no significant effect on *sncgb* expression (data not shown), we focus here on the role of *sox9a*.

Luciferase reporter assays were subsequently performed to determine whether Sox9a regulates *sncgb* promoter activity. Plasmids containing a 2.0-kb region of the *sncgb* promoter with either wild type or mutant HMG-binding motifs were used to drive luciferase expression. Overexpression of Sox9a enhanced the luciferase activity of wild type reporters by about 5-fold, but had no effect on mutant constructs (Fig. 2A).

As for *znf219L*, knockdown of *sox9a* affected *sncgb* expression. Whole mount *in situ* hybridization using a *sncgb* probe revealed that expression of *sncgb* in the notochord of *sox9a* morphants was reduced, as compared to that in embryos injected with control-MO (Fig. 2B).

3.3. Zebrafish ZNF219L associates with Sox9a

To further understand the molecular interaction between ZNF219L and Sox9a, we performed an *in vitro* binding assay using recombinant His-tagged Sox9a protein and HA-tagged ZNF219L mutants lacking different C_2H_2 zinc fingers (Fig. 3A). Recombinant His-tagged Sox9a proteins were expressed in *E. coli*, and then purified and incubated with HA-tagged ZNF219L mutants. His-tagged Sox9a proteins were subsequently pulled down with nickel beads and subjected to Western blot using anti-HA. As shown in Fig. 3B, the association between Sox9a and ZNF219L was abolished when the sixth or ninth zinc finger domain was excluded, indicating that this region is required for interaction between these proteins.

3.4. Simultaneous knockdown of znf219L and sox9a results in a synergistic decrease of sncgb expression in the notochord

The association between ZNF219L and Sox9a suggests that these proteins may synergistically regulate *sncgb* expression. This hypothesis was tested using luciferase reporters driven by a region of the *sncgb* promoter containing the ZNF219L and Sox9a binding motifs.

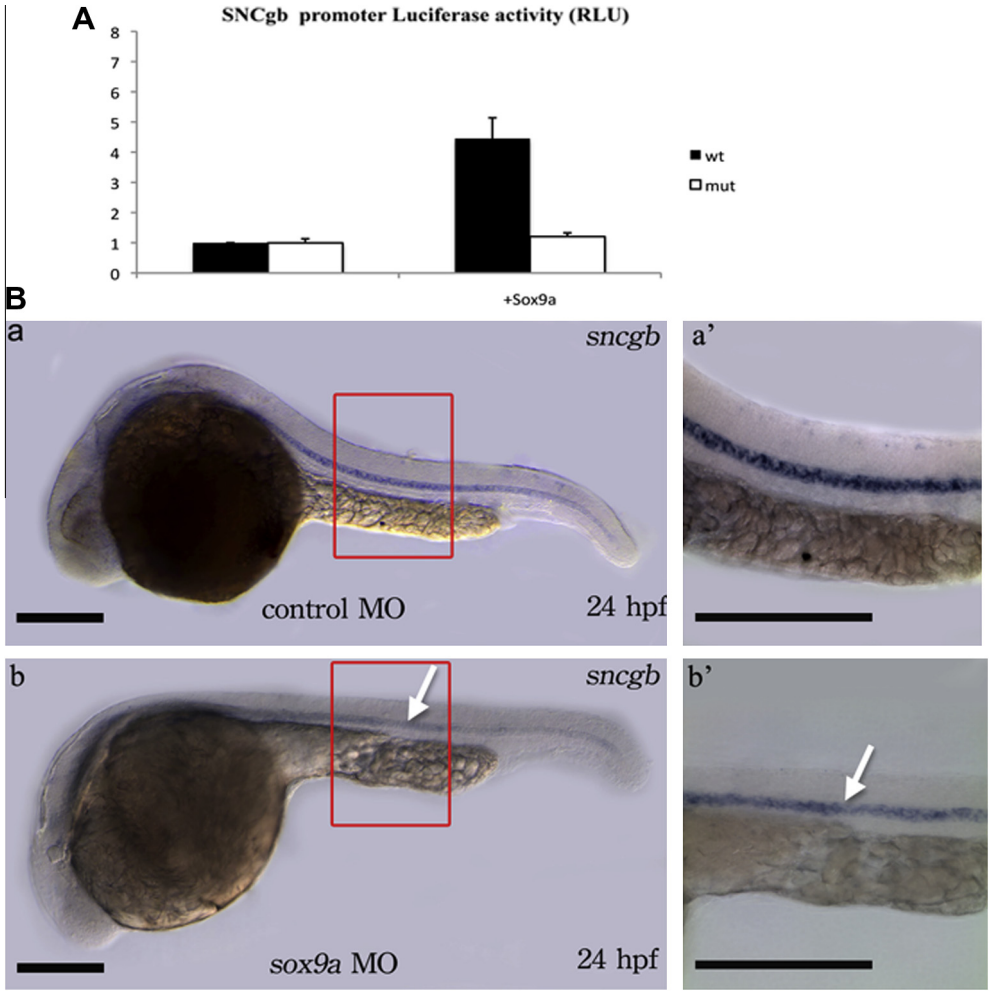

Fig. 2. Sox9a regulates the *sncgb* proximal promoter and affects *sncgb* expression in the notochord. (A) The effect of Sox9a on *sncgb* promoter activity was studied in grass carp CF cells. Cells were co-transfected with a Sox9a expression vector and *sncgb* gene 2.0-kb proximal promoter luciferase constructs containing either wild type or mutant Sox9a binding sites. Luciferase activity was determined 2 days after transfection. Data represent the mean ± s.d. ($n = 3$). (B) One-cell stage embryos were injected with *sox9a* or control morpholino (MO). At 24 hpf, morphants were subjected to *in situ* hybridization with an *sncgb*-3′ UTR probe. The endogenous *sncgb* signal was decreased in the notochord of *sox9a* morphants (panel b) as compared to that of control morphants at 24 hpf (panel a). The boxed regions are magnified in panels a′ and b′. White arrows indicate decreased expression of endogenous *sncgb* mRNA in the notochord. Scale bars = 100 μm.

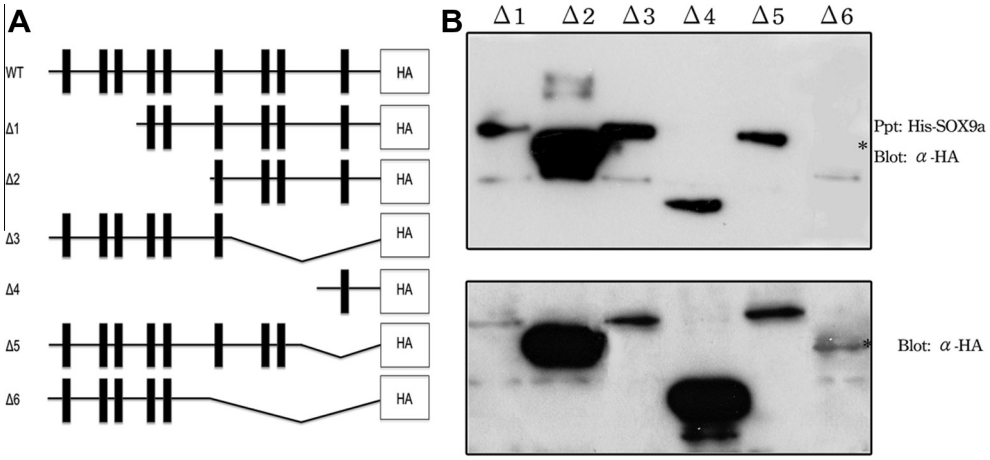

Fig. 3. ZNF219L physically interacts with Sox9a through its sixth and ninth zinc finger domains. (A) Schematic diagram of the six HA-tagged-ZNF219L mutants. The vertical bars indicate zinc finger domains. (B) Analysis of binding between Sox9a and ZNF219L mutants. Lysates of cells expressing ZNF219L mutants were precipitated with His-tagged Sox9a protein, and the precipitates were subjected to immunoblot with anti-HA antibody.

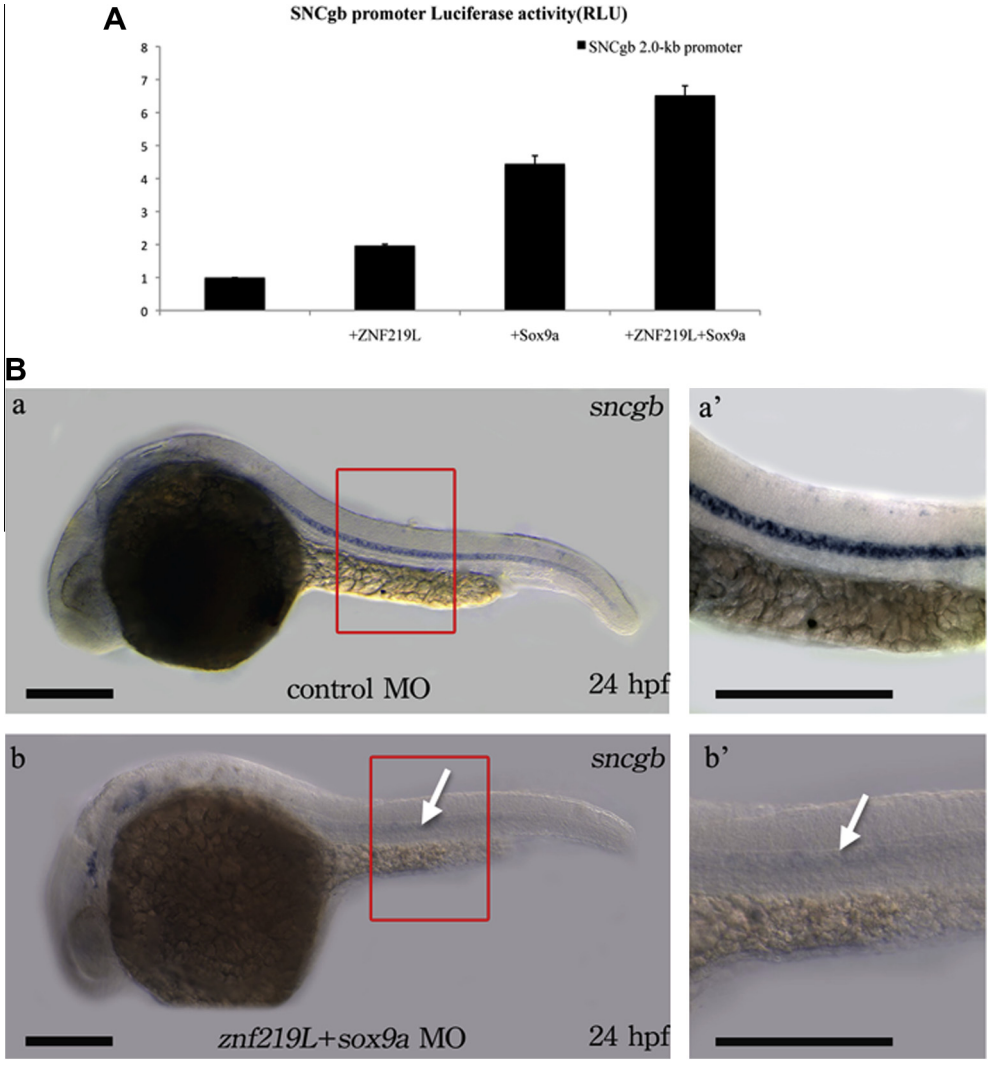

Fig. 4. Simultaneous knockdown of *znf219L* and *sox9a* results in a synergistic decrease of zebrafish *sncgb* expression in the notochord. (A) The synergistic effect of ZNF219L and Sox9a on *sncgb* promoter activity was studied in grass carp CF cells. Cells were co-transfected with Sox9a and ZNF219L expression vectors, and *sncgb* gene 2.0-kb proximal promoter luciferase constructs containing both ZNF219L and Sox9a binding sites. Luciferase activity was determined 2 days after transfection. Data represent the mean ± s.d. ($n = 3$). (B) One-cell stage embryos were injected with both *znf219L* and *sox9a*, or control morpholino (MO) alone. At 24 hpf, morphants were subjected to *in situ* hybridization with an *sncgb*-3′ UTR probe. The endogenous *sncgb* signal was synergistically decreased in the notochord of both *znf219L* and *sox9a* morphants (panel b) as compared to that of control morphants at 24 hpf (panel a). The boxed regions are magnified in panels a′ and b′. White arrows indicate decreased expression of endogenous *sncgb* mRNA in the notochord. Scale bars = 100 μm.

ZNF219L overexpression alone enhanced reporter activity by about 2-fold, whereas Sox9a overexpression increased it by about 4-fold. Simultaneous overexpression of both proteins, however, enhanced luciferase activity by about 6- to 7-fold (Fig. 4A). Therefore, ZNF219L and Sox9a have a synergistic effect on *sncgb* promoter activity.

Simultaneous morpholino knockdown of *znf219L* and *sox9a* caused a consistent and synergistic decrease in the expression of *sncgb* in the notochord, as compared to embryos injected with only one morpholino (Fig. 4B). Taken together, these results reveal that ZNF219L and Sox9a are involved in the regulation of notochord-specific expression of *sncgb*.

4. Discussion

In this study, we have established that Znf219L and Sox9a are both involved in regulating expression of *sncgb* specifically in the notochord. We report here that: (1) ZNF219L and SOX9a recognize their respective binding sites within, and induce activity of the *sncgb* promoter (Figs. 1A,B and 2A); (2) MO knockdown of *sox9a* consistently results in a decrease in *sncgb* expression in the notochord (Figs. 1C and 2B); (3) ZNF219L physically associates with Sox9a (Fig. 3); and (4) simultaneous knockdown of both *znf219L* and *sox9a* results in a synergistic decrease of *sncgb* expression and promoter activity. Overall, these findings indicate that zebrafish ZNF219L and Sox9a function coordinately in up-regulating *sncgb* expression in the notochord.

In human, α, β, and γ-synuclein are highly homologous proteins abundant in presynaptic terminals, and these proteins are thought to play a role in the regulation of neurotransmission [23]. However, as the expression pattern of zebrafish *sncgb* differs dramatically from that of its homologue *sncga*, it is possible that *sncgb* may play a role in a process other than neuronal dopamine homeostasis. Knockdown of *sncgb* did not cause any obvious defects in the development of zebrafish embryos (data not shown), and its function therefore remains unclear. Nevertheless, we have demonstrated that *sncgb* is a novel target gene of ZNF219L, specifically in the notochord. Furthermore, we have shown that ZNF219L associates with Sox9a, and that this interaction contributes to the regulation of *sncgb* expression. We suggest that *sncgb* may be involved in notochord development, possibly through a redundant pathway. Future studies are required to help delineate the role of the protein encoded by *sncgb*.

Acknowledgments

This study was supported by a Grant (NSC97-2313-B-001-002-MY3) from the National Science Council, Taipei, Taiwan. The AB wild type zebrafish were kindly provided by the Taiwan Zebrafish Core Facility at Academia Sinica (ZCAS), which is supported by the NSC (National Science Council, NSC 100-2321-B-001-030), Taiwan.

References

[1] L. Maroteaux, J.T. Campanelli, R.H. Scheller, Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal, J. Neurosci. 8 (1988) 2804–2815.
[2] S. George, N.L. Rey, N. Reichenbach, J.A. Steiner, P. Brundin, Alpha-synuclein: the long distance runner, Brain Pathol. 23 (2013) 350–357.
[3] C.W. Olanow, P. Brundin, Parkinson's disease and alpha synuclein: is Parkinson's disease a prion-like disorder? Mov. Disord. 28 (2013) 31–40.
[4] G. Taschenberger, J. Toloe, J. Tereshchenko, J. Akerboom, P. Wales, R. Benz, S. Becker, T. Outeiro, L. Looger, M. Bahr, M. Zweckstetter, S. Kugler, ss-Synuclein aggregates and induces neurodegeneration in dopaminergic neurons, Ann. Neurol. 74 (2013) 109–118.
[5] H. Ji, Y.E. Liu, T. Jia, M. Wang, J. Liu, G. Xiao, B.K. Joseph, C. Rosen, Y.E. Shi, Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing, Cancer Res. 57 (1997) 759–764.
[6] C. Milanese, J.J. Sager, Q. Bai, T.C. Farrell, J.R. Cannon, J.T. Greenamyre, E.A. Burton, Hypokinesia and reduced dopamine levels in zebrafish lacking beta- and gamma1-synucleins, J. Biol. Chem. 287 (2012) 2971–2983.
[7] Z. Sun, A.D. Gitler, Discovery and characterization of three novel synuclein genes in zebrafish, Dev. Dyn. 237 (2008) 2490–2495.
[8] T. Sakai, K. Hino, S. Wada, H. Maeda, Identification of the DNA binding specificity of the human ZNF219 protein and its function as a transcriptional repressor, DNA Res. 10 (2003) 155–165.
[9] T. Sakai, A. Toyoda, K. Hashimoto, H. Maeda, Isolation and characterization of a novel zinc finger gene, ZNF219, and mapping to the human chromosome 14q11 region, DNA Res. 7 (2000) 137–141.
[10] Y. Takigawa, K. Hata, S. Muramatsu, K. Amano, K. Ono, M. Wakabayashi, A. Matsuda, K. Takada, R. Nishimura, T. Yoneda, The transcription factor Znf219 regulates chondrocyte differentiation by assembling a transcription factory with Sox9, J. Cell Sci. 123 (2010) 3780–3788.
[11] H.W. Lien, C.H. Yang, C.H. Cheng, C.C. Hung, W.H. Liao, P.P. Hwang, Y.S. Han, C.J. Huang, A novel zinc finger protein 219-like (ZNF219L) is involved in the regulation of collagen type 2 alpha 1a (col2a1a) gene expression in zebrafish notochord, Int. J. Biol. Sci. 9 (2013) 872–886.
[12] M. Westerfield, The Zebrafish Book, fifth ed., A guide for the laboratory use of zebrafish (Danio rerio), Univ. of Oregon Press, Eugene, 2007.
[13] C. Thisse, B. Thisse, T.F. Schilling, J.H. Postlethwait, Structure of the zebrafish snail1 gene and its expression in wild-type, spadetail and no tail mutant embryos, Development 119 (1993) 1203–1215.
[14] C.Y. Chu, C.H. Cheng, G.D. Chen, Y.C. Chen, C.C. Hung, K.Y. Huang, C.J. Huang, The zebrafish erythropoietin: functional identification and biochemical characterization, FEBS Lett. 581 (2007) 4265–4271.
[15] J.H. Leu, S.J. Yan, T.F. Lee, C.M. Chou, S.T. Chen, P.P. Hwang, C.K. Chou, C.J. Huang, Complete genomic organization and promoter analysis of the round-spotted pufferfish JAK1, JAK2, JAK3, and TYK2 genes, DNA Cell Biol. 19 (2000) 431–446.
[16] M.S. Chang, G.D. Chang, J.H. Leu, F.L. Huang, C.K. Chou, C.J. Huang, T.B. Lo, Expression, characterization, and genomic structure of carp JAK1 kinase gene, DNA Cell Biol. 15 (1996) 827–844.
[17] H.W. Lien, C.H. Cheng, C.C. Hung, W.H. Liao, P.P. Hwang, Y.S. Han, C.J. Huang, A novel zinc finger protein 219-like (ZNF219L) is involved in the regulation of collagen type 2 alpha 1a (col2a1a) gene expression in zebrafish notochord, Int. J. Biol. Sci. 9 (2013) 872–886.
[18] A. Amores, A. Force, Y.L. Yan, L. Joly, C. Amemiya, A. Fritz, R.K. Ho, J. Langeland, V. Prince, Y.L. Wang, M. Westerfield, M. Ekker, J.H. Postlethwait, Zebrafish hox clusters and vertebrate genome evolution, Science 282 (1998) 1711–1714.
[19] K.Y. Huang, G.D. Chen, C.H. Cheng, K.Y. Liao, C.C. Hung, G.D. Chang, P.P. Hwang, S.Y. Lin, M.C. Tsai, K.H. Khoo, M.T. Lee, C.J. Huang, Phosphorylation of the zebrafish M6Ab at serine 263 contributes to filopodium formation in PC12 cells and neurite outgrowth in zebrafish embryos, PLoS ONE 6 (2011) e26461.
[20] Y.L. Yan, J. Willoughby, D. Liu, J.G. Crump, C. Wilson, C.T. Miller, A. Singer, C. Kimmel, M. Westerfield, J.H. Postlethwait, A pair of Sox: distinct and overlapping functions of zebrafish sox9 co-orthologs in craniofacial and pectoral fin development, Development 132 (2005) 1069–1083.
[21] E.F. Chiang, C.I. Pai, M. Wyatt, Y.L. Yan, J. Postlethwait, B. Chung, Two sox9 genes on duplicated zebrafish chromosomes: expression of similar transcription activators in distinct sites, Dev. Biol. 231 (2001) 149–163.
[22] Y.L. Yan, C.T. Miller, R.M. Nissen, A. Singer, D. Liu, A. Kirn, B. Draper, J. Willoughby, P.A. Morcos, A. Amsterdam, B.C. Chung, M. Westerfield, P. Haffter, N. Hopkins, C. Kimmel, J.H. Postlethwait, A zebrafish sox9 gene required for cartilage morphogenesis, Development 129 (2002) 5065–5079.
[23] S. Anwar, O. Peters, S. Millership, N. Ninkina, N. Doig, N. Connor-Robson, S. Threlfell, G. Kooner, R.M. Deacon, D.M. Bannerman, J.P. Bolam, S.S. Chandra, S.J. Cragg, R. Wade-Martins, V.L. Buchman, Functional alterations to the nigrostriatal system in mice lacking all three members of the synuclein family, J. Neurosci. 31 (2011) 7264–7274.