Biochemical and Biophysical Research Communications 384 (2009) 114–119

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

yap is required for the development of brain, eyes, and neural crest in zebrafish

Qiu Jiang [a,1], Dong Liu [a,1,2], Yuebo Gong [a], Yuexiang Wang [a], Shuna Sun [b], Yonghao Gui [b], Houyan Song [a,*]

[a] Department of Biochemistry and Molecular Biology, Shanghai Medical School and Key Laboratory of Molecular Medicine, Ministry of Education, Fudan University, Shanghai 200032, PR China
[b] Children's Hospital, Fudan University, Shanghai 200032, PR China

ARTICLE INFO

Article history:
Received 19 March 2009
Available online 22 April 2009

Keywords:
yap
Brain
Neural progenitor cells
Eyes
Branchial arches
Neural crest
Apoptosis
Zebrafish

ABSTRACT

The Yes-associated protein (YAP) is a small protein that binds to many transcription factors and modulates their activity. Bioinformatics analysis indicated that zebrafish Yap shares high identity with its orthologs in fruit fly, chicken, mouse, and human. Expression analysis revealed that maternal transcripts of *yap* are ubiquitous, and endogenous *yap* is chronologically expressed in the notochord, brain, eyes, branchial arches, and pectoral fins. Knockdown of *yap* causes distinct morphological defects in embryos, which display a small head with smaller eyes than normal and fewer cartilages in the branchial arches. Proneural and neuronal gene expression in *yap* morphant brain is significantly reduced. The expression of crestin is also markedly reduced in all recognizable arch-associated regions of *yap* morphants. Furthermore, TUNEL analysis revealed that there is a marked increase in cell death in *yap* morphant brain. In conclusion, zebrafish *yap* is required for the development of the brain, eyes, and neural crest during embryogenesis.

In vertebrate embryos, neurulation is a morphogenetic process that follows gastrulation and leads to the formation of the neural tube [1]. Neurogenesis can be subdivided into four processes, by analogy with neural induction: selection, specification, determination, and differentiation [2]. After the selection process, which is regulated by lateral inhibition involving the Delta/Notch signaling pathway, selected progenitor cells of neurons are allowed to express basic helix–loop–helix (bHLH) proneural genes, i.e., *neurog1* [3] and *olig2* [4]. The process of determination establishes the commitment of the neural progenitors to become neurons, even in the presence of signals that repress the development of neurons, and allows expression of early pan-neuron markers, i.e., *elavl3* and its family members [5,6]. The signaling pathways that regulate the number of neural progenitor cells are not only essential for achieving the proper size and composition of the nervous system during neurogenesis, but they are also likely to have participated in the expansion of brain size during evolution [7].

Recent studies in *Drosophila melanogaster* have delineated a novel signaling pathway, the Hippo pathway, which is extremely powerful in regulating growth owing to its ability simultaneously to inhibit cell proliferation and promote apoptosis [8–11]. This Hippo kinase cascade ultimately antagonizes the transcriptional coactivator Yki, which positively regulates the transcription of target genes involved in cell growth, proliferation, and survival [12]. The conservation of this system in evolution is underscored by the demonstration that the mammalian *YAP* (the *yki* homolog) can functionally rescue its *Drosophila* mutant *in vivo* [13,14]. Overexpression of YAP in the mouse liver increases liver size dramatically, and its overexpression in the intestine expands the progenitor pool [14,15]. In chick embryos, overexpression of YAP causes a marked expansion of the neural progenitor pool [16]. All these findings suggest that YAP has conserved functions in the regulation of cell proliferation and survival in vertebrates.

Previous studies using biochemical assays and cell culture model systems have shown that YAP binds to and/or coactivates several proteins and protein families, including Yes, Runx, EBP50, p73, TBX5, p53BP2, TEFs/TEADs, 14-3-3, ErbB4, Smad7, and hnRNPU [11,17]. However, the lack of a physiological assay system has made it difficult to investigate the function of YAP *in vivo*. Owing to its optical clarity, genetics, and ease of manipulation, the zebrafish may be an ideal model system in which to obtain a comprehensive understanding of YAP *in vivo*. In order to investigate *in vivo* roles for *yap*, we knocked down the *yap* in zebrafish embryos by using antisense morpholino oligonucleotides. Further studies indicated that *yap* is essential for the development of the brain, eyes, and neural crest during embryogenesis in zebrafish.

Materials and methods

Zebrafish strains and maintenance. Zebrafish (AB strain) embryos were collected and staged as previously described [18,19]. In order

* Corresponding author. Fax: +86 21 6403 3738.
E-mail address: hysong@shmu.edu.cn (H. Song).
[1] These authors contributed equally to this work.
[2] Present address: Department of Angiogenesis and Cardiovascular Pathology, Max Delbrueck Center for Molecular Medicine (MDC), Robert Roessle Strasse 10, 13125 Berlin, Germany.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.04.070

to block pigment formation in the embryos, 0.003% phenylthiourea (PTU) was added to the media at 18 hpf and replenished every 24 h thereafter. Embryos were fixed in 4% paraformaldehyde (PFA)–phosphate buffered saline (PBS) at 4 °C and stored in 100% methanol at −20 °C prior to *in situ* hybridization.

Sequencing and bioinformatics analysis of zebrafish Yap. Sequencing was carried out by Invitrogen Biotechnology Corporation (Shanghai). Multiple alignments and their graphic presentations were generated online with TCoffee (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi), and then analyzed by online specialized BLAST software with the BLOSUM62 matrix (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi).

Confirmation of yap-MO specificity and microinjection. A morpholino modified antisense oligonucleotide (*yap*-MO, Gene Tools, LLC) was designed against the 5′-terminal sequence around the putative start codon (5′-UTR) to block Yap translation. The sequence for *yap*-MO was 5′-CTCTTCTTTCTATCCAACAGAAACC-3′. A second MO was designed to control the microinjection (con-MO, standard control MO). The sequence for con-MO was 5′-CCTCTTACCTCAGTT ACAATTTATA-3′. A 350-bp fragment of the zebrafish *yap* cDNA corresponding to the 5′-UTR region (including the MO target region) was generated by PCR with specially designed primers (pN1-*yap*-F(EcoR1): 5′-CCGGAATTCCGGCAGTACAGTCACTACCAA TG-3′ pN1-*yap*-R(BamH1): 5′-CGCGGATCCGCGACTCCAGATAAAAG TAAAAC-3′). This PCR product was digested with BamH1 and EcoR1 (New England Biolaboratory, Inc.), gel-purified (QIAQuick gel extraction kit), and subcloned into the pEGFP-N1 plasmid (CLONTECH Laboratories, Inc.). The recombinant plasmid with an insert was identified by restriction digestion analysis, and sequence accuracy was confirmed by automated DNA sequencing. The MO (5–7.5 ng MO/embryo) or plasmid DNA (50–100 pg/embryo) was microinjected into embryos at the one to two cell stage.

Whole-mount in situ hybridization and histology. Whole-mount *in situ* hybridization experiments using the combination of digoxigenin-labeled antisense RNA probe and α-digoxigenin alkaline phosphatase-conjugated antibody (Roche) were performed as previously described [19]. For *yap*, *neurog1, olig2, elavl3*, *sox1a*, and *crestin* antisense probes, the NBT/BCIP coloring reactions proceeded at about 20 °C for 5 h, 2 h, 2 h, 0.5 h, 2 h, and 3 h, respectively. In order to section the embryos, the whole-mount *in situ* hybridized embryos were fixed in 4% PFA–PBS at 4 °C overnight. The embryos were then washed with PBST three times for 5 min each to remove the fixative reagents. This procedure was followed by a two-step embedment: one with 1.5% agarose–5% sucrose solution for adjustment of orientation, and the other with OCT compound (Tissue-Tek, SAKURA) for sectioning.

Whole-mount TUNEL (terminal deoxynucleotide transferase mediated dUTP nick-end labeling) staining. For whole-mount TUNEL staining, embryos were fixed in 4% PFA at 4 °C overnight, then rinsed in PBS before proceeding with TUNEL staining using the *in situ* Cell Death Detection kit (Roche) according to the manufacturer's instructions.

Cartilage staining. Larvae were fixed in 4% PFA at 96hpf and stained for cartilage with Alcian blue as described previously, except that embryos were not trypsinized [20].

Results

Sequence analysis of zebrafish Yap

Zebrafish Yap shares significant sequence similarities with representative proteins from the fruit fly, chicken, mouse, and human (31%, 59%, 76%, and 62% identity, respectively) (Fig. S1). Homology is especially high in the WW domains that are known to interact with PPXY-containing polypeptides [12,17,21–24], in the TEF/TEAD-binding (TB) domain that affects binding of TEF/TEAD to YAP family members [16,17,25,26], in the 14-3-3 binding site (containing the third HXRXXS motif) which has been identified as the consensus phosphorylation sequence for substrates of LATS/Ndr kinase (known to be a central player in the Hippo signaling pathway) [14,17,27,28], and in the proline-rich sequence that is implicated in the binding of YAP to the SH3 domain of the Yes proto-oncogene product and other signaling molecules [21].

Spatio-temporal expression of yap during embryogenesis

YAP/Yki has been identified in the fruit fly, chicken, mouse, and human, but there are fewer data concerning the measurement of the spatio-temporal expression pattern of *YAP*/*yki* mRNA during development. Therefore, we conducted a detailed analysis of the spatio-temporal regulation of vertebrate *yap* genes in zebrafish at a series of stages: 2-cell (0.75 hpf), 1k-cell (3 hpf), shield (6 hpf), 6-somite (12 hpf), 18-somite (18 hpf), prim-5 (24 hpf), prim-25 (36 hpf), long pec (48 hpf), and pec fin (60 hpf) (Fig. 1).

In situ hybridization with the *yap* antisense probe showed that *yap* transcripts were present in embryos at the 2-cell stage (Fig. 1A), the 512-cell stage (data not shown) and the 1k-cell stage (Fig. 1B). These data suggest the presence of *yap* maternal transcripts, because zygotic expression does not start until the mid-blastula transition (MBT). After the MBT, *yap* mRNA is persistently present in embryos until at least the long pec stage (Fig. 1H and H′). As development progresses, expression of *yap* is observed in conjunction with the development of several tissues and organs, mainly the notochord, brain, eyes, branchial arches, and pectoral fins (Fig. 1D–H′).

Characterization of the yap morphant phenotype

To determine the role of *yap* during embryogenesis in zebrafish, we knocked down *yap* using a morpholino (MO) designed against the 5′ untranslated region (*yap*-MO) of the zebrafish *yap* cDNA (Fig. 2I). To confirm the efficacy of the morpholino approach, *yap*-MO was co-injected with a green fluorescent protein (GFP) reporter containing the partial 5′ untranslated region and the start site sequence of *yap* (Fig. 2I). The *yap*-MO specifically knocked down the expression of GFP from this RNA transcript, as revealed at 7 hpf in 100% of embryos ($n = 67$) (Fig. 2IIC). In comparison, there was no detectable knockdown of GFP when co-injected with a random control morpholino ($n = 83$) (Fig. 2IIB). This revealed the ability of the *yap*-MO to inhibit the production of protein from its target sequence.

Although there appeared to be an overall delay in the development of age-matched morphants compared with the embryos injected with con-MO or wild-type embryos, the injection of *yap*-MO (5–7.5 ng/embryo) specifically affected brain and craniofacial development. The *yap* morphants at 50 hpf have a small head with smaller eyes (84%, $n = 93$), while embryos injected with con-MO were unaffected (Fig. 2IID–G). In addition, it was observed that the *yap* morphants displayed no tail and had cardiac edema.

Effects of yap-MO on the development of brain and eyes

The expression pattern of *yap* suggests a specific requirement for *yap* function in the development of the brain and eyes. To investigate the possibility that knockdown of *yap* affects neurogenesis, the expression pattern of the proneural genes *neurog1* and *olig2* were compared in *yap* morphant embryos and embryos injected with con-MO. At 24 hpf, expression of *neurog1* and *olig2* was strikingly reduced throughout the *yap* morphant brain, except in the forebrain region (Fig. 3A–D′), demonstrating that neurogenesis is significantly compromised by loss of *yap* function. In order to investigate the degree to which loss of *yap* function affects produc-

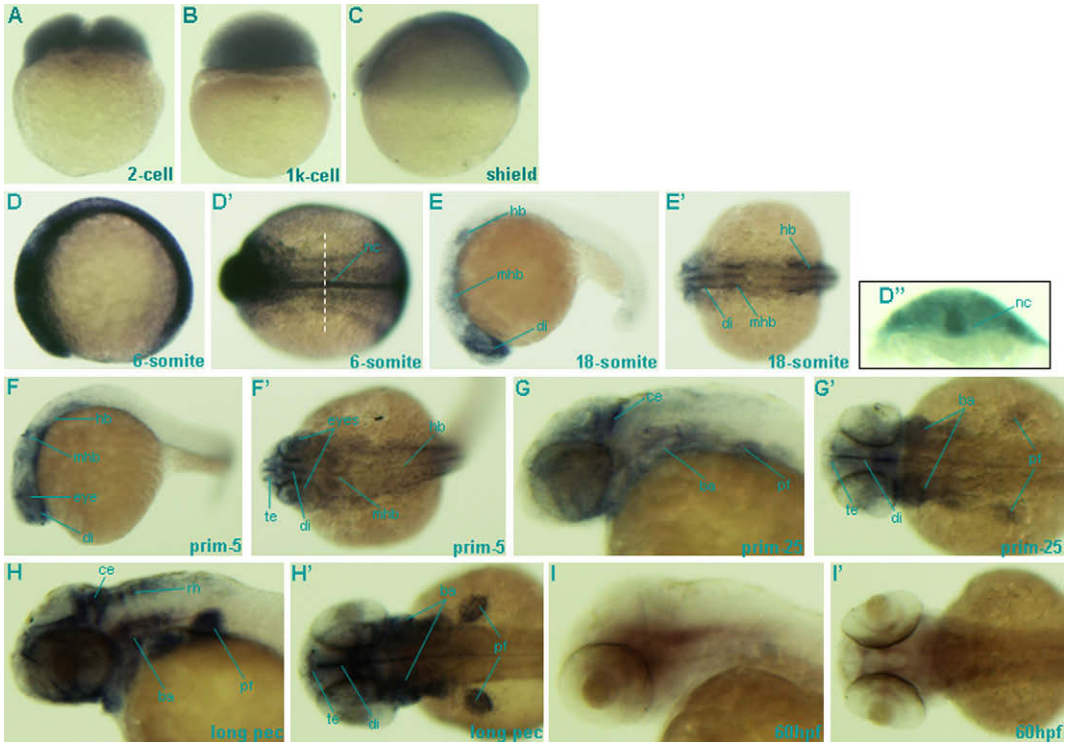

Fig. 1. Expression pattern of *yap* during zebrafish embryogenesis. (A– I) Lateral view. (D′–I′) Dorsal view. (D′′) Transverse section through the dashed line in D′. Stages are indicated bottom right. *Abbreviations:* ba, branchial arches; ce, cerebellum; di, diencephalon; hb, hindbrain; mhb, midbrain–hindbrain boundary; nc, notochord; pf, pectoral fin; rh, rhombomere; te, telencephalon.

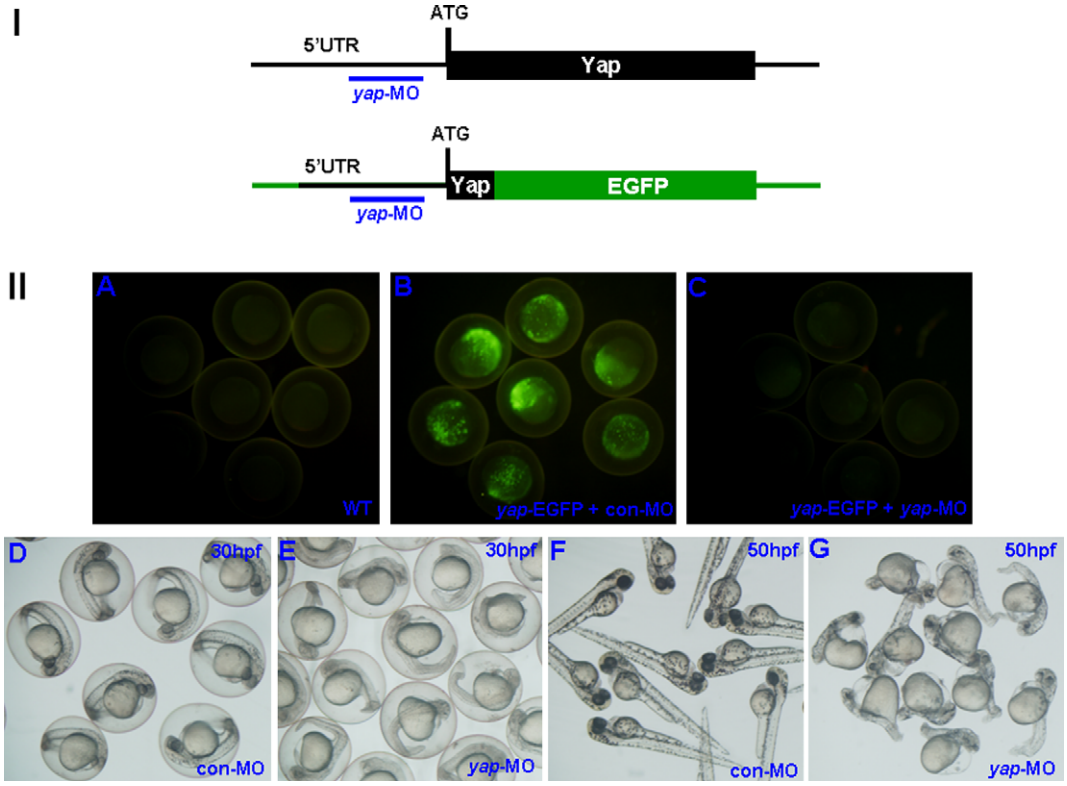

Fig. 2. Morpholino knockdown of zebrafish *yap*. (I) Schematic showing the position of morpholino oligos designed against a region of the 5′ UTR (*yap*-MO) of zebrafish *yap* mRNA. To test the ability of *yap*-MO to knock down expression of its target sequence, an EGFP reporter construct was produced. (IIA–C) Fluorescence microscopy images in 7 hpf zebrafish embryos. (IIB) Fluorescence microscopy image of zebrafish embryos co-injected with *yap*-GFP plasmid and con-MO. GFP expression (green) is detectable in a mosaic pattern throughout injected embryos. (IIC) Fluorescence microscopy image of zebrafish embryos co-injected with *yap*-GFP plasmid and *yap*-MO. Note the absence of detectable GFP expression in all embryos. (IID,G) Live embryos at 30 and 50 hpf. Injection of *yap*-MO (5–7.5 ng/embryo), but not con-MO, led to the phenotype of a small head with smaller eyes than normal. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

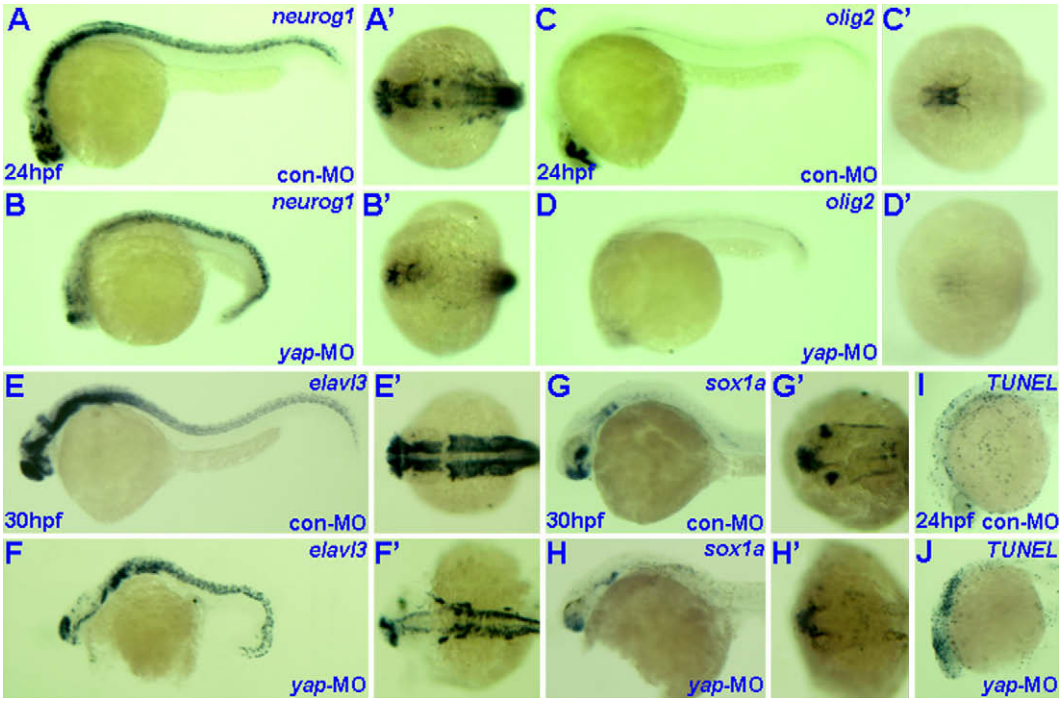

Fig. 3. Knockdown of *yap* affects brain and eyes. (A, A′, C, C′, E, E′, G, G′, and I) con-MO (5 ng)-injected embryos; (B, B′, D, D′, F, F′, H, H′, J) *yap*-MO (5 ng)-injected embryos. (A–J) Lateral view. (A′–H′) Anterior view. (A–D′) At 24 hpf, expression of the proneural genes *neurog1* and *olig2* were strikingly reduced throughout the *yap* morphant brain except in the forebrain region. (E,F′) At 30hpf, the number of *elavl3*-positive cells within the *yap* morphant brain was also significantly decreased except in the forebrain. (G,H′) Injection of *yap*-MO led to a smaller or even no lens field visualized with *sox1a*. (I,J) TUNEL assay indicated an increased number of apoptotic cells in the brain of embryos injected with *yap*-MO at 24 hpf.

tion of differentiated neurons in the brain, the distribution of cells expressing the pan-neuronal marker *elavl3* was compared in *yap* morphant embryos and embryos injected with con-MO at 30 hpf (Fig. 3E–F′). The number of *elavl3*-positive cells within the *yap* morphant brain was also significantly decreased, except in the forebrain. On the basis of the changes in the expression patterns of proneural/neuronal genes and the small eye phenotype of 50 hpf *yap* morphants, the expression pattern of a forebrain and lens marker *sox1a* was analyzed [29]. The expression domain of *sox1a* in the lens fields, but not in the forebrain field, was significantly reduced in the embryos injected with *yap*-MO (Fig. 3G–H').

The opacity in the brain and optic vesicle of *yap* morphants suggests a distinct tendency towards cell death (Fig. 2IID and E). The TUNEL assay revealed that *yap*-MO causes a marked increase in cell death in the brain (Fig. 3I and J).

Craniofacial cartilage defects in yap morphants

Embryos injected with *yap*-MO develop craniofacial abnormalities that are apparent at 96 hpf (Fig. 4). Alcian blue staining of cartilaginous structures showed that the shortened Meckel's cartilage and the ceratohyal arch resided more posteriorly in *yap* morphants than in embryos injected with con-MO, while other cartilaginous structures, including the ceratobranchial arches, were weakly stained or not visible at all (Fig. 4A–B′). In addition, in the most severely affected embryos, no signal for chondrogenesis was detected (data not shown).

The *crestin* gene is expressed by all neural crest cells (NCCs) [30,31], a subpopulation of which contributes to the branchial arches [32]. In *yap* morphant embryos, expression of *crestin* was markedly reduced at 30 hpf in all recognizable arch-associated regions (Fig. 4C–D′). In addition, a population of cranial NCCs was distributed abnormally in the dorsal anterior periocular mesenchyme, with few NCCs migrating to the eye field (Fig. 4C–D′).

Discussion

In this study, we investigated the evolutionary conservation of YAP/Yki/Yap in protein domains of the fruit fly, chicken, mouse, human, and zebrafish, and this is probably the basis of its functional consistency. Moreover, examination of the temporal and spatial expression pattern of zebrafish *yap* provides clues to the functions of the protein in development. Its expression pattern in zebrafish suggests that *yap* may be required for the development of brain, eyes, branchial arches and pectoral fins. Furthermore, we demonstrated that knockdown of *yap* with MOs gives rise to embryos with reduced eye and brain size and fewer cartilages in the branchial arches.

The role of yap in zebrafish brain neurogenesis

A loss-of-function study in mice has shown that YAP mutant mice are arrested at around E8.5 with widespread defects [33], and this prevented an assessment of the *in vivo* function of YAP during embryogenesis. Here, we have shown that knockdown of *yap* triggers apoptosis in the zebrafish brain. Consistent with our data, YAP has been shown to promote the survival of neural progenitor cells during neural tube development in chicks [16]. Moreover, recent studies have found that overexpression of YAP in the mouse liver and intestine induces several anti-apoptotic factors, including cIAP1, survivin, MCL1, and Bcl-XL [14,15]. Thus, we speculated that *yap* may also promote the survival of neural progenitor cells in the zebrafish brain. Identification of the direct target genes of Yap in brain neural progenitor cells is required to address this possibility.

The role of yap in the development of craniofacial cartilage

Neural crest-derived cells undergo a temporally and spatially stereotyped migration to a variety of embryonic locations. Ultimately, the neural crest gives rise to a variety of differentiated cell

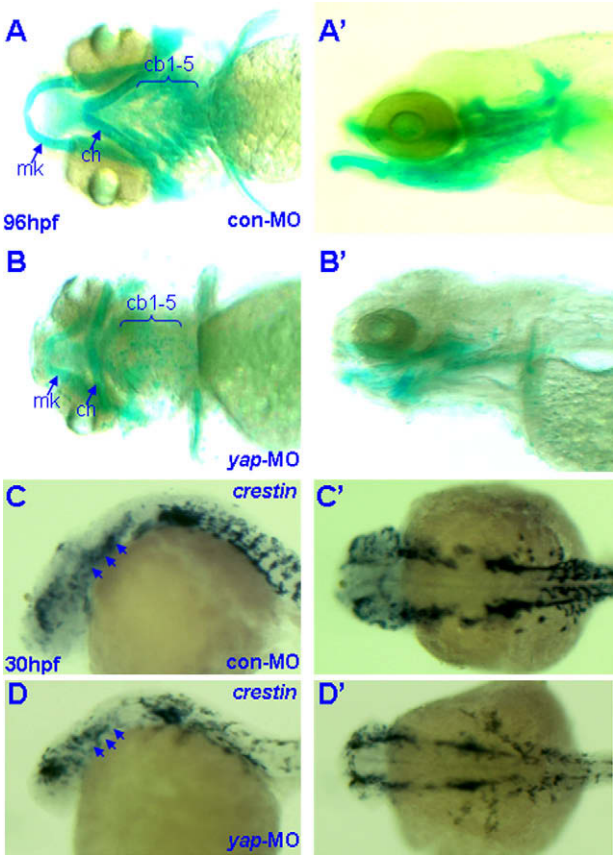

Fig. 4. Abnormal branchial arch development in *yap* morphants. Embryos injected with con-MO (5 ng) (A,A′) or *yap*-MO (5 ng) (B,B′) at 96 hpf, stained with Alcian blue to reveal craniofacial cartilages. Ventral (A,B) and lateral (A′,B′) views show that the shortened Meckel's cartilage and the ceratohyal arch resided more posteriorly in *yap* morphants than in embryos injected with con-MO, while other cartilaginous structures, including the ceratobranchial arches, were weakly stained or not visible at all. (C,D′) In *yap* morphants at 30hpf, expression of *crestin* was markedly reduced in all recognizable arch-associated regions, which are indicated by arrows. In addition, a population of cranial NCCs was distributed abnormally in the dorsal anterior periocular mesenchyme, with few NCCs migrating to the eye field. Abbreviations: cb1–5, ceratobranchial arches 1–5; ch, ceratohyal arch; mk, Meckel's cartilage.

types including peripheral neurons, glia, pigment cells, and elements of the craniofacial skeleton [30]. Reduced expression of *crestin* correlates with the severity of cartilage defects because NCCs in the arch regions give rise to cartilage elements, such as the ceratobranchial arches [34], which are absent in *yap* morphants at 30 hpf. Meanwhile, a population of cranial NCCs migrates abnormally to the dorsal anterior periocular mesenchyme, with few NCCs in the eye field. Consistent with the idea that the eye organizes the migration of NCCs [35], the cranial NCCs have significantly reduced migration rates and directionality in *yap* morphants.

Acknowledgments

We are grateful to Prof. Min Yu, Dr. Li Tan, and Jian Yang for constructive advice and critical reading of the manuscript, to other members from Song's lab for technical assistance. This work was supported by grants from '211' project to H.S., and National Natural Science Foundation of China to Y.W. (30600489).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.04.070.

References

[1] B. Schmitz, C. Papan, J.A. Campos-Ortega, Neurulation in the anterior trunk region of the zebrafish *Brachydanio rerio*, Rouxs Arch. Dev. Biol. 202 (1993) 250–259.
[2] S.I. Wilson, T. Edlund, Neural induction: toward a unifying mechanism, Nat. Neurosci. 4 (Suppl.) (2001) 1161–1168.
[3] P. Blader, N. Fischer, G. Gradwohl, F. Guillemot, U. Strahle, The activity of neurogenin1 is controlled by local cues in the zebrafish embryo, Development 124 (1997) 4557–4569.
[4] H.C. Park, A. Mehta, J.S. Richardson, B. Appel, Olig2 is required for zebrafish primary motor neuron and oligodendrocyte development, Dev. Biol. 248 (2002) 356–368.
[5] M.F. Marusich, H.M. Furneaux, P.D. Henion, J.A. Weston, Hu neuronal proteins are expressed in proliferating neurogenic cells, J. Neurobiol. 25 (1994) 143–155.
[6] C.H. Kim, E. Ueshima, O. Muraoka, H. Tanaka, S.Y. Yeo, T.L. Huh, N. Miki, Zebrafish elav/HuC homologue as a very early neuronal marker, Neurosci. Lett. 216 (1996) 109–112.
[7] P. Rakic, A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution, Trends Neurosci. 18 (1995) 383–388.
[8] B.A. Edgar, From cell structure to transcription: Hippo forges a new path, Cell 124 (2006) 267–273.
[9] K. Harvey, N. Tapon, The Salvador–Warts–Hippo pathway—an emerging tumour-suppressor network, Nat. Rev. Cancer 7 (2007) 182–191.
[10] D. Pan, Hippo signaling in organ size control, Genes Dev. 21 (2007) 886–897.
[11] L.J. Saucedo, B.A. Edgar, Filling out the Hippo pathway, Nat. Rev. Mol. Cell Biol. 8 (2007) 613–621.
[12] J. Huang, S. Wu, J. Barrera, K. Matthews, D. Pan, The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the *Drosophila* Homolog of YAP, Cell 122 (2005) 421–434.
[13] M. Overholtzer, J. Zhang, G.A. Smolen, B. Muir, W. Li, D.C. Sgroi, C.X. Deng, J.S. Brugge, D.A. Haber, Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon, Proc. Natl. Acad. Sci. USA 103 (2006) 12405–12410.
[14] J. Dong, G. Feldmann, J. Huang, S. Wu, N. Zhang, S.A. Comerford, M.F. Gayyed, R.A. Anders, A. Maitra, D. Pan, Elucidation of a universal size-control mechanism in *Drosophila* and mammals, Cell 130 (2007) 1120–1133.
[15] F.D. Camargo, S. Gokhale, J.B. Johnnidis, D. Fu, G.W. Bell, R. Jaenisch, T.R. Brummelkamp, YAP1 increases organ size and expands undifferentiated progenitor cells, Curr. Biol. 17 (2007) 1–7.
[16] X. Cao, S.L. Pfaff, F.H. Gage, YAP regulates neural progenitor cell number via the TEA domain transcription factor, Genes Dev. 22 (2008) 3320–3334.
[17] K. Wang, C. Degerny, M. Xu, X. Yang, YAP, TAZ, and Yorkie: a conserved family of signal-responsive transcriptional coregulators in animal development and human disease, Biochem. Cell Biol. 87 (2009) 77–91.
[18] C.B. Kimmel, W.W. Ballard, S.R. Kimmel, B. Ullmann, T.F. Schilling, Stages of embryonic development of the zebrafish, Dev. Dyn. 203 (1995) 253–310.
[19] M. Westerfield, The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Danio rerio*), University of Oregon Press, Oregon, 2000.
[20] T.F. Schilling, T. Piotrowski, H. Grandel, M. Brand, C.P. Heisenberg, Y.J. Jiang, D. Beuchle, M. Hammerschmidt, D.A. Kane, M.C. Mullins, F.J. Van Eeden, R.N. Kelsh, M. Furutani-Seiki, M. Granato, P. Haffter, J. Odenthal, R.M. Warga, T. Trowe, C. Nusslein-Volhard, Jaw and branchial arch mutants in zebrafish I: branchial arches, Development 123 (1996) 329–344.
[21] M. Sudol, P. Bork, A. Einbond, K. Kastury, T. Druck, M. Negrini, K. Huebner, D. Lehman, Characterization of the mammalian YAP (Yes-associated protein) gene and its role in defining a novel protein module, the WW domain, J. Biol. Chem. 270 (1995) 14733–14741.
[22] R. Yagi, L.F. Chen, K. Shigesada, Y. Murakami, Y. Ito, A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator, EMBO J. 18 (1999) 2551–2562.
[23] A. Komuro, M. Nagai, N.E. Navin, M. Sudol, WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus, J. Biol. Chem. 278 (2003) 33334–33341.
[24] R.I. Aqeilan, V. Donati, A. Palamarchuk, F. Trapasso, M. Kaou, Y. Pekarsky, M. Sudol, C.M. Croce, WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function, Cancer Res. 65 (2005) 6764–6772.
[25] A. Vassilev, K.J. Kaneko, H. Shu, Y. Zhao, M.L. DePamphilis, TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm, Genes Dev. 15 (2001) 1229–1241.
[26] S. Wu, Y. Liu, Y. Zheng, J. Dong, D. Pan, The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway, Dev. Cell 14 (2008) 388–398.
[27] B. Zhao, X. Wei, W. Li, R.S. Udan, Q. Yang, J. Kim, J. Xie, T. Ikenoue, J. Yu, L. Li, P. Zheng, K. Ye, A. Chinnaiyan, G. Halder, Z.C. Lai, K.L. Guan, Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control, Genes Dev. 21 (2007) 2747–2761.
[28] Y. Hao, A. Chun, K. Cheung, B. Rashidi, X. Yang, Tumor suppressor LATS1 is a negative regulator of oncogene YAP, J. Biol. Chem. 283 (2008) 5496–5509.
[29] Y. Okuda, H. Yoda, M. Uchikawa, M. Furutani-Seiki, H. Takeda, H. Kondoh, Y. Kamachi, Comparative genomic and expression analysis of group B1 sox genes

in zebrafish indicates their diversification during vertebrate evolution, Dev. Dyn. 235 (2006) 811–825.
[30] R. Luo, M. An, B.L. Arduini, P.D. Henion, Specific pan-neural crest expression of zebrafish Crestin throughout embryonic development, Dev. Dyn. 220 (2001) 169–174.
[31] M. An, R. Luo, P.D. Henion, Differentiation and maturation of zebrafish dorsal root and sympathetic ganglion neurons, J. Comp. Neurol. 446 (2002) 267–275.
[32] T.F. Schilling, C.B. Kimmel, Musculoskeletal patterning in the pharyngeal segments of the zebrafish embryo, Development 124 (1997) 2945–2960.
[33] E.M. Morin-Kensicki, B.N. Boone, M. Howell, J.R. Stonebraker, J. Teed, J.G. Alb, T.R. Magnuson, W. O'Neal, S.L. Milgram, Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65, Mol. Cell. Biol. 26 (2006) 77–87.
[34] T.F. Schilling, C.B. Kimmel, Segment and cell type lineage restrictions during pharyngeal arch development in the zebrafish embryo, Development 120 (1994) 483–494.
[35] T. Langenberg, A. Kahana, J.A. Wszalek, M.C. Halloran, The eye organizes neural crest cell migration, Dev. Dyn. 237 (2008) 1645–1652.