Smarcd3 Regulates the Timing of Zebrafish Myogenesis Onset*

Received for publication, October 16, 2007, and in revised form, November 9, 2007 Published, JBC Papers in Press, December 3, 2007, DOI 10.1074/jbc.M708594200

Haruki Ochi, Stefan Hans¹, and Monte Westerfield²

From the Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403-1254

A cascade of signaling events triggers myogenesis in vertebrates. Although studies of zebrafish indicate that fibroblast growth factor (Fgf), Hedgehog (Hh), and the T-box transcription factors, No tail (Ntl) and T-box gene 16 (Tbx16), regulate myogenesis, the hierarchy of these factors has not been determined. Recently, another transcriptional cofactor, Smarcd3, a subunit of the SWI/SNF chromatin-remodeling complex, has been shown to be required for heart muscle formation in mouse. The vertebrate SWI/SNF complex consists of 7–13 subunits, and it contributes to the function of transcriptional activators by opposing chromatin-dependent repression of transcription (13). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The initial step in muscle formation is induction of myogenesis in a small group of cells that subsequently proliferate and differentiate into various muscle cell types. Numerous factors have been implicated in the initial induction of myogenesis, but the precise hierarchy of the genetic pathway leading to the onset of myogenesis is still incompletely understood. We have studied the early steps in myogenesis using zebrafish, an excellent system to analyze the genetic control of induction and specification at the blastula stages. In zebrafish, Fgf expression commences during blastula stages, whereas myogenesis, as indicated by myod expression, does not begin until much later during mid-gastrula stages. Smarcd3 expression, on the other hand, becomes enriched in the marginal zone just prior to the beginning of myod expression. Overexpression of smarcd3 shifts the onset of myod and myf5 expression earlier, and myod and myf5 expression in adaxial cells, the earliest muscle precursors, requires Smarcd3, indicating that Smarcd3 is the limiting factor that regulates the onset of myogenesis. Smarcd3 physically interacts with Ntl, and Smarcd3 overexpression fails to rescue myod expression in ntl mutants, demonstrating that function of Smarcd3 depends on Ntl activity. We propose a model in which cooperative activity of Fgf, Ntl, and Smarcd3 is required for the onset of myogenesis, with Smarcd3 serving as the primary regulator of the timing of myogenesis onset.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2 and Table S1.

1 To whom correspondence should be addressed: Institute of Neuroscience, University of Oregon, Eugene, OR 97403-1254. E-mail: monte@uoneuro.uoregon.edu.

2 The on-line version of this article contains supplemental Figs. S1 and S2 and Table S1.

3 The abbreviations used are: Fgf, fibroblast growth factor; Hh, Hedgehog; MO, morpholino oligonucleotide; Ntl, No tail; Tbx16, T-box gene 16.

© 2008 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.
contains one of the two ATPases, Smarca4 (previously BRG1) or Smarca2 (BRM), that are functional homologs of yeast SWI2 (12). Recent studies have shown that the SWI/SNF complex interacts with tissue-specific transcription factors that operate during development (13, 14), suggesting that these transcription factors function in the context of DNA that is assembled into higher-order chromatin structures. During muscle development, Smarca4 promotes Myod, Mef2D, and Myogenin-mediated muscle differentiation (15, 16). Smarc3d (previously Baf60c) was purified as a component of the human SWI/SNF complex by immunoaffinity purification with an antibody directed against Smarca4 (17). Northern blot analysis revealed that Smarc3d is expressed in heart and skeletal muscle (17). More recent analysis showed that Smarc3d regulates heart development by recruiting Smarca4 to Tbx5 (14). Thus, several chromatin-remodeling factors seem to be essential for the activity of tissue-specific transcription factors in developing muscle, and one or more of them may serve as the proximal regulator of the onset of myogenesis.

Consistent with our previous studies, we show that Fgf and Ntl induce myod and myf5 expression independently of Hh activity. To explore how Fgf and Ntl regulate the onset of myod expression, we studied the function of smarcad4 and smarc3d in early myogenesis. We cloned the zebrafish duplicates, smarc3dα and smarc3dβ, and find that although smarcad4 is ubiquitously expressed throughout gastrulation, smarc3dβ mRNA becomes enriched in the marginal zone at mid-gastrula stages when myod expression begins. Later smarc3dβ is expressed in the notochord. Functional analysis of Smarc3dβ reveals that the onset of myod and myf5 expression can be shifted to earlier developmental stages by precocious expression of Smarc3dβ, and induction of myod and myf5 expression disappears in embryos depleted of Smarc3dβ by morpholino oligonucleotide (MO) injection. Furthermore, Smarc3dβ interacts physically with Ntl and fails to induce myod in ntl mutant embryos, demonstrating that the function of Smarc3dβ depends on Ntl. We propose a model in which cooperative activity of Fgf, Ntl, and Smarc3d leads to the onset of myogenesis in mesoderm, with Smarc3dβ serving as the primary regulator of the timing of myogenesis onset.

**EXPERIMENTAL PROCEDURES**

**Animals**—Embryos were obtained from the University of Oregon zebrafish facility, produced as previously described (18) using the markers: myod, myf5, myogenin (2), ntl (22), tbx16 (spt) (23), fgf8 (4), and sprouty2 (24). Probes were synthesized using SP6 RNA polymerase or T7 RNA polymerase from the pCS2-smarc3dβ plasmid linearized by KpnI. Dominant negative smarc4 was synthesized using SP6 RNA polymerase from pcS2-DNxBrg1 (smarca4) plasmid linearized by NotI (13).

**In Situ mRNA Hybridization**—The in situ labeling was performed as previously described (18) using the markers: myod, myf5, myogenin (2), ntl (22), tbx16 (spt) (23), fgf8 (4), and sprouty2 (24). Probes were synthesized using SP6 RNA polymerase or T7 RNA polymerase. Embryos processed for whole-mount in situ hybridization were photographed using a Leica MZ12/FIII microscope and Axioam digital camera.

**Microinjection**—Microinjection was performed using published procedures (18). smarc3dβ-MO was directed to the translation start site or splice acceptor sites (Gene Tools, LLC); smarc3dβ-UTR, TTCCCTCCGTTCCTCCTGCCTTT-TTG; smarc3dβ-exon 2, GCCAGGTGCGTAAGAAAAATA-ATGAC; smarc3dβ-exon 4, CCACTCGAATCTCGGAAAAAAGG; smarc3dβ-exon 8, ACTGAGGAGGCTGCA-CACAGGGACAC.

**SU5402 and Cyclopamine Treatments**—SU5402 (25) (Calbiochem) was dissolved in dimethyl sulfoxide (Me₂SO) and cyclopamine (26) was dissolved in 95% ethanol (EtOH). Embryos in their chorions were treated with 30 μM SU5402 or 100 μM cyclopamine from the 40% epiboly stage to Bud stage or somite stages. Treatments were performed in 12-well plates, 40 embryos per well, in 1 ml of fish water. No effects were observed by exposure to Me₂SO or EtOH vehicle alone at the same concentration as used for the experimental treatments. Treated embryos were collected and fixed in 4% paraformaldehyde in phosphate-buffered saline, and processed for in situ hybridization.

**In Vitro Translation, Co-immunoprecipitation, and Western Blot Analysis**—The PCR product of ntl was cloned into pcS2-MT (pcS2-myc-ntl) and pcCMV-3Tag (Stratagene) (pcCMV-flag-ntl). smarc3dβ was cloned into pcCMV-3Tag (pcCMV-flag-smarcd3b). Proteins were produced by in vitro translation using the TxT coupled reticulocyte lysate system (Promega). After translation, 20 μl of Myc-NTL containing lysate was added to 20 μl of FLAG-Smarcd3b containing lysate and incubated for 1 h at 30 °C for binding. Dilution buffer (360 μl of 20 mM HEPES at pH 7.6, 150 mM NaCl, 0.2% Triton X-100, 2 mM EDTA) supplemented with Protease inhibitor and anti-Myc antibody-conjugated beads (Sigma) were added and incubated overnight at 4 °C. Beads were collected and washed four times with dilution buffer. Bound proteins were analyzed by Western blot using anti-FLAG M2 antibody (Sigma).
RESULTS

Induction of Muscle Precursors by Fgf and Ntl Is Independent of Hh Activity—Numerous studies have shown that Fgf signaling, Ntl, Tbx16, and Hh signaling are required for muscle development in zebrafish. hh expression starts just before myod expression, whereas fgf8 and ntl are expressed earlier, prior to gastrulation (Fig. 1A). Recently, we showed that slow muscle precursors, the adaxial cells, form independently of Hh signaling (3). Consistent with these previous observations, myod expression in adaxial cells and myf5 expression in paraxial mesoderm are unaffected in embryos treated with the Hh inhibitor, cyclopamine, from 40% epiboly stage to Bud stage (Fig. 1, H–N), whereas myod expression is completely suppressed in embryos treated with cyclopamine from Bud stage to 8 somite stage (Fig. 1N). Thus, Hh activity is required for maintenance rather than induction of myogenic gene expression. In contrast, embryos treated with the Fgf receptor (Fgfr) inhibitor, SU5402, from the 40% epiboly stage to Bud stage lack myod and myf5 expression (Fig. 1, B–G and N). We confirmed that ntl and tbx16 expression disappears in embryos treated with the Fgfr inhibitor, whereas snaila expression remains in the paraxial mesoderm (data not shown). Thus, Fgf and Hh have distinct roles in the formation of slow muscle precursors. Furthermore, we find myod expression is expanded in embryos overexpressing fgf8 and ntl and the same expanded myod expression is observed in embryos overexpressing fgf8 and ntl that are treated with the Hh inhibitor (Fig. 1, O–T). Thus, Fgf and Ntl induce muscle precursors and this induction is independent of Hh activity.

Ubiquitously Expressed Smarca4 Participates in Early Myogenesis—There is a long delay between when fgf and tbx gene expression begins in the blastoderm margin, and when
Smarcd3 and Ntl Regulate Myogenesis

FIGURE 2. smarcd3b expression is enriched in the marginal zone just before myod expression begins. A–D, Smarca4 participates in early myogenesis. A–C, smarca4 transcripts are ubiquitously distributed during the gastrula period. D, smarca4 expression disappears from the paraxial mesoderm, but remains in the anterior region at the 3-somite stage (arrow). E–H, Dominant negative Smarca4 suppresses myod expression. E and F, 75% epiboly stage. G and H, bud stage embryos. E and G, control embryos. F and H, DN-smarca4 injected embryos (200 ng/μl, 10/40, 18/27 injected embryos, respectively). E and F, dotted lines indicate the blastoderm margin. I–M, smarcd3b and ntl are co-expressed in the marginal zone and notochord. Expression of smarcd3b (blue) and ntl (red) at the 70% epiboly stage (I) and 90% epiboly stage (J). K and L, expression of smarcd3b (K) and ntl (L) in parasagittal sections of tail bud region. M, 8-somite stage. Arrows indicate regions where smarcd3b and ntl are co-expressed and the arrowhead indicates cells that express ntl but not smarcd3b. N–Q, overlap of smarcd3b, ntl, and myod expression domains. N, higher magnification of I. O, expression of myod (blue) and ntl (red). P, higher magnification of O. Arrows indicate double-labeled cells and arrowheads indicate cells expressing ntl alone. Q, diagram summarizing the expression of ntl, smarcd3b, and myod at the 75% epiboly stage. The area expressing all three genes is colored yellow. I, J, and M–P, flat-mount embryos. Scale bar: A–H, 200 μm; I, J, and M, 125 μm; O, 100 μm; K, L, N, and P, 50 μm.

Smarca4, containing an ATPase domain that is essential for ATP-dependent nucleosome remodeling, and Smarcd3 that is expressed in skeletal muscle in mouse (17). A recent study showed that Smarcd3 regulates the transcriptional activity of Tbx5, a T-box transcription factor, by recruiting Smarca4 to Tbx5 (14). Thus, Smarca4 and Smarcd3 are good candidates for regulating myogenesis.

To learn whether Smarca4 may be the factor that controls the onset of myod expression, we examined the expression of smarca4 and whether Smarca4 regulates myod expression. Dominant negative Smarca4 slightly reduces myod expression (Fig. 2, E–H). Because smarca4 is ubiquitously distributed throughout the embryo during gastrula stages (Fig. 2, A–D) and is known to regulate several other developmental processes, such as neurogenesis (13), it is an unlikely candidate for specific regulation of myogenesis onset. So, we then identified and cloned two zebrafish smarcd3 genes (supplemental Fig. S1).

smarcd3b Expression Is Enriched in Mesodermal Cells Just Before myod Expression Begins—Zebrafish smarcd3b transcripts are maternally supplied and ubiquitously distributed until the 50% epiboly stage (data not shown). Later, smarcd3b expression becomes enriched in mesodermal cells (Fig. 2I, arrows). As development proceeds, smarcd3b transcripts appear in the tail bud region (Fig. 2J and K) and in the notochord (Fig. 2M). Transcripts from smarcd3a, the duplicate gene, are also supplied maternally. However, unlike smarcd3b, smarcd3a transcripts are ubiquitously distributed until segmentation stages, and then only later, are restricted to somites (data not shown). Thus, Smarcd3b is a good candidate to act together with Ntl as a regulator of myogenesis. Double labeling for smarcd3b and ntl expression indicates that they are co-expressed in the marginal zone during late gastrula stages and in the notochord during segmentation stages (Fig. 2, I–M). We also find that cells co-express smarcd3b, ntl, and myod by the 75% epiboly stage (Fig. 2, N–Q, arrows). Together, these results indicate that zebrafish smarcd3b is expressed with ntl in muscle precursor cells. Because Fgf signaling regulates both ntl and myod expression and myogenesis (8), it is possible that Fgf signaling also regulates mesodermal expression of smarcd3b. We find that mesoder-
Smarcd3 and Ntl Regulate Myogenesis

Smarcd3b depletion suppresses early myogenesis—To learn whether Smarcd3b is required for the onset of myogenesis, we examined myod and myf5 expression in embryos injected with splice blocking MO. We confirmed that injection of MO results in the production of aberrantly spliced smarcd3b transcripts (supplemental Fig. S2). Although myod expression is apparent by the 75% epiboly stage in control embryos, we do not observe myod expression in smarcd3b-MO-injected embryos (Fig. 4, A and B), and myod expression is still missing at Bud stage (Fig. 4, C and D). myf5 expression is also blocked in smarcd3b-MO injected embryos (Fig. 4, E and F, bracket). In contrast, ntl, shha, tbx16, and snai1a are expressed normally in smarcd3b-MO injected embryos (Fig. 4, G–L, data not shown), suggesting that Smarcd3b function is not required for formation of the notochord and paraxial mesoderm. Although smarcd3b-MO suppresses early myogenesis, muscle formation eventually recovers (supplemental Table S1), suggesting that some other Smarcd, such as Smarcd3a expressed in somites, may provide later support for myogenesis. Thus, Smarcd3b regulates the initial onset of myogenesis.

Overexpression of Smarcd3b Shifts the Onset of myod and myf5 Expression to Earlier Developmental Times—We tested whether Smarcd3b regulates the timing of myod and myf5 expression. The onset of myod and myf5 expression normally occurs at the 70–75% epiboly stage, and myod and myf5 expression is undetectable earlier at the 50% epiboly stage (Fig. 4, M and T). In contrast to fgf8 and ntl injection (Fig. 4, N, O, and T), smarcd3b significantly increases myod expression in the dorsal blastoderm margin (Fig. 4, P and T), where the endogenous fgf8 and ntl transcripts are present. We find that myod expression can be induced by Smarcd3b as early as the 30% epiboly stage (Fig. 4, S and T). Parasagittal sections reveal that the myod expression induced by Smarcd3b is restricted to the mesendodermal layer (Fig. 4Q). Although smarcd3b mRNA slightly increases the number of muscle pioneers, there are no apparent differences in the number of slow muscle cells (supplemental Table S1). These results indicate that Smarcd3b is the limiting factor that regulates the onset of myod expression and that expressing Smarcd3 earlier than normal leads to premature induction of myod and myf5 expression in the mesendoderm.

Function of Smarcd3b Depends on Ntl—To analyze the position of Smarcd3 within the genetic pathway that regulates muscle development, we examined whether Smarcd3b can induce myod expression in ntl and fgf8 mutants or in embryos with disrupted Hh signaling. As previously reported, myod expression is absent at Bud stage in ntl mutants (Fig. 5C) (2) and we do not detect myod expression in smarcd3b-injected ntl mutants (Fig. 5, A–D). In addition, injection of smarcd3b mRNA fails to induce myod in fgf8 mutant embryos (Fig. 5, E and F). These results indicate that function of Smarcd3b depends on Ntl and Fgf activity. In contrast, injection of smarcd3b mRNA shifts myod expression earlier in cyclopamine-treated embryos (Fig. 5H), as in wild-type embryos (Fig. 5G), indicating that Hh activity is not required for the function of Smarcd3b.

Our observation that Smarcd3b depends on Ntl activity, together with previous work indicating that mouse Smarcd3 recruits Smarca4 to Tbx5 (14), suggests that Smarcd3b may interact, either directly or indirectly, with Ntl. Proteins were produced by in vitro translation using the reticulocyte lysate. We find that FLAG-tagged Smarcd3b co-immunoprecipitates with myc-tagged Ntl (Fig. 5J), indicating that zebrafish Smarcd3b interacts with Ntl. We also find that Ntl binds to Smarca4. However, we could not observe that Smarcd3b enhances this interaction (Fig. 5J). We also confirmed that Ntl directly binds to the ½ T-site of the myod promoter (Fig. 5K) (27). Taken together, our results suggest that Fgf, Ntl, Smarcd3b, and Smarca4 act together to regulate the onset of myogenesis in zebrafish.

DISCUSSION

The Time Lag between the Onset of fgf and ntl Expression and the Onset of myod and myf5 Expression Indicates Missing Factors That Regulate Myogenesis—Precise timing of induction, specification, and differentiation of particular cell types is critical for organogenesis. Sequential activation and repression of genes is a common mechanism used to regulate developmental timing. During zebrafish myogenesis, myod and myf5 are initially expressed by cells adjacent to the notochord (2). Signal transduction pathways, such as Fgf and Hh, and the transcription factor Ntl are known to regulate myod and myf5 expression during skeletal muscle development. Because these molecules participate in numerous other aspects of development, however, their specific roles in the induction of
muscle cells are still unclear. We demonstrate that Fgf and Ntl regulate the formation of slow muscle precursors independently of Hh activity. However, there is a long delay between blastula stages, when \(fgf\) and \(tbx\) gene expression begins in the blastoderm margin, and late gastrula stages when muscle precursors first express \(myod\). This gap suggested that additional factors control the timing of the onset of myogenesis. Our results demonstrate that Smarcd3 proteins fulfill this role. Smarcd3b expression appears in the marginal region just prior to the onset of myogenesis, Smac3b is necessary for the onset of myogenesis and expressing Smac3b in the marginal zone at earlier developmental stages concomitantly shifts myogenesis earlier.

**Hh Signaling in Muscle Development**—In zebrafish, \(hh\) gene expression begins just before the onset of \(myod\) expression, and overexpression of \(shha\) mRNA is sufficient to induce \(myod\) expression in paraxial mesoderm (2). Moreover, the \(Myf5\) epaxial somite enhancer is Shh-dependent and directly regulated by Gli in mouse (28, 29). Hence, Hh signaling has been considered to be a good candidate for regulating the induction of myogenesis (30). On the other hand, however, we previously showed that slow muscle precursors form independently of Hh signaling (3), and our results presented here further demonstrate that Hh is not involved in the initial induction of \(myod\) expression. Instead, we find that Hh signaling is required for maintenance of \(myod\) expression after muscle precursor cells converge toward the midline and join the adaxial cell layer next to the notochord. Consistent with this interpretation, analysis of sonic you (\(shha\)) mutants revealed defects in maintenance of \(myf5\) and \(myod\) expression and in timely terminal differentiation of muscle cells in zebrafish (31); and, in mouse, Shh is required for Myf5-dependent epaxial muscle determination (32). Thus, Hh signaling functions primarily in specification and differentiation of muscle cells rather than in induction of myogenesis.

**The Chromatin-remodeling Factor, Smac3d, and Ntl Cooperatively Regulate the Onset of Myogenesis**—Genes are packaged into chromatin structures that can modulate transcriptional activation and repression. The SWI/SNF complex facilitates the functions of transcriptional activators by altering chromatin structure (33). During myogenesis in mammals, Smarca4, a SWI/SNF component, is required for MyoD, Mef2D, and Myogenin-mediated muscle differentiation (15, 16). However, Smarca4 also regulates retinal and neural
crest development in zebrafish (34, 35), neurogenesis in Xenopus (13), and surface ectoderm and fetal epidermal keratinocyte formation in mouse (36). Thus, Smarca4 functions not only in muscle development but also in numerous other developmental processes.

Most chromatin remodeling factors control expression of large numbers of genes and are typically expressed ubiquitously, although Smarcd2 is strongly expressed in pancreas and Smarcd3 is specifically expressed in heart and skeletal muscle of mouse (17). Hence, Smarcd2 and Smarcd3 are unusual; their restricted expression patterns suggest that some chromatin remodeling factors may act in a tissue-specific or target-specific manner. Consistent with this interpretation, we find that knockdown of Smarcd3b activity reduces myod and myf5 expression without affecting expression of other well known markers of paraxial mesoderm or notochord (Fig. 4). Thus, Smarcd3b controls the expression of a restricted subset of genes in a restricted set of tissues. Vertebrate SWI/SNF complexes contain one of the two ATPases, Smarca2 (BRM) or Smarca4 (BRG1), plus a variable subset of the Brg-associated factors, including Smarcd. Together, these results indicate that various combinations of Brg-associated factors may provide the specificity of action required to regulate the subsets of genes required for development of different cell types, tissues, and organs.

We envisage a mechanism that can explain how Fgf and Ntl regulate the onset of myogenesis (Fig. 5L). During the late blastula period, Fgf and Ntl in the marginal zone promote mesoderm formation (37, 38). smaca4 is ubiquitously expressed throughout the embryo during blastula and gastrula stages. Smarcd3b enhances the interaction between Smaca4 and tissue-specific transcription factors, whereas the Smac3 protein fails to interact directly with Smaca4 (14). By midgastrula stage, Smarcd3b accumu-
Smarcd3 and Ntl Regulate Myogenesis

lates in the marginal zone and, together with Ntl and Smaca4, activates myod and myf5 transcription.

Acknowledgments—We thank Bruce Draper for helpful suggestions, Kunio Yasuda for sharing SU5402, and Seongjin Seo and Kriste L. Kroll for pCS2-DN-xbrg1 (smarca4) and pCS2-myc-xbrg1.

REFERENCES

1. Ochi, H., and Westerfield, M. (2007) Dev. Growth Differ. 49, 1–11
2. Weinberg, E. S., Allende, M. L., Kelly, C. S., Abdelhamid, A., Murakami, T., Andermann, P., Doerre, O. G., Grunwald, D. J., and Riggelman, B. (1996) Development 122, 271–280
3. Hirsinger, E., Stellabotte, F., Devoto, S. H., and Westerfield, M. (2004) Dev. Biol. 275, 143–157
4. Reifers, F., Bohli, H., Walsh, E. C., Crossley, P. H., Stainier, D. Y., and Relaix, F. (2003) Science 298, 4211–4222
5. Griffin, K. J., Hamond, C. L., and Hughes, S. M. (2005) Development 132, 4211–4222
6. Amacher, S. L., Draper, B. W., Summers, B. R., and Kimmel, C. B. (2002) Development 129, 3311–3323
7. Griffin, K., Patient, R., and Holder, N. (1995) Development 121, 2983–2994
8. Du, S. J., Devoto, S. H., Westerfield, M., and Moon, R. T. (1997) J. Cell Biol. 139, 145–156
9. Wolff, C., Roy, S., and Ingham, P. W. (2003) Curr. Biol. 13, 1169–1181
10. Krauss, S., Concordet, J. P., and Ingham, P. W. (1993) Cell 75, 1431–1444
11. Martens, J. A., and Winston, F. (2003) Curr. Opin. Genet. Dev. 13, 136–142
12. Seo, S., Richardson, G. A., and Kroll, K. L. (2005) Development 132, 105–115
13. Li, J., Takeuchi, J. K., Von Both, I., Walls, J. R., McAuliffe, F., Adamson, S. L., Henkelman, R. M., Wrana, J. L., Rossant, J., and Bruneau, B. G. (2004) Nature 432, 107–112
14. de la Serna, I. L., Carlson, K. A., and Imbalzano, A. N. (2001) Nat. Genet. 27, 187–190
15. Obikawa, Y., Marfella, C. G., and Imbalzano, A. N. (2006) EMBO J. 25, 490–501
16. Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B. R., and Crabtree, G. R. (1996) Genes Dev. 10, 2117–2130
17. Westerfield, M. (2000) The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (Brachydanio rerio), University of Oregon Press, Eugene, OR
18. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., and Schilling, T. F. (1995) Dev. Dyn. 203, 253–310
19. Halpern, M. E., Ho, R. K., Walker, C., and Kimmel, C. B. (1993) Cell 75, 99–111
20. Hans, S., Christison, J., Liu, D., and Westerfield, M. (2007) BMC Dev. Biol. 7, 5
21. Schulte-Merker, S., van Eeden, F. J., Halpern, M. E., Kimmel, C. B., and Nusslein-Volhard, C. (1994) Development 120, 1009–1015
22. Griffin, K. J., Amacher, S. L., Kimmel, C. B., and Kimelman, D. (1998) Development 125, 3379–3388
23. Furthauer, M., Van Celst, J., Thissie, C., and Thissie, B. (2004) Development 131, 2853–2864
24. Mohammadi, M., McMahon, G., Sun, L., Tang, C., Hirth, P., Yeh, B. K., Hubbard, S. R., and Schlessinger, J. (1997) Science 276, 955–960
25. Cooper, M. K., Porter, J. A., Young, K. E., and Beachy, P. A. (1998) Science 280, 1603–1607
26. Yang, H. W., Kurok, J. L., Lee, N. H., Piao, H. Y., Fletcher, C. D., Kanki, J. P., and Look, A. T. (2004) Cancer Res. 64, 7256–7262
27. Gustafsson, M. K., Pan, H., Pinney, D. F., Liu, Y., Lewandowski, A., Epstein, D. J., and Emerson, C. P., Jr. (2002) Genes Dev. 16, 114–126
28. Teboul, L., Summerbell, D., and Rigby, P. W. (2003) Genes Dev. 17, 2870–2874
29. Pownall, M. E., Gustafsson, M. K., and Emerson, C. P., Jr. (2002) Annu. Rev. Cell Dev. Biol. 18, 747–783
30. Coutelle, O., Blagden, C. S., Hampson, R., Halai, C., Rigby, P. W., and Hughes, S. M. (2001) Dev. Biol. 236, 136–150
31. Borycki, A. G., Brunk, B., Tajbakhsh, S., Buckingham, M., Chiang, C., and Emerson, C. P., Jr. (1999) Development 126, 4053–4063
32. Sudarsanam, P., and Winston, F. (2000) Trends Genet. 16, 345–351
33. Gregg, R. G., Willer, G. B., Fadool, J. M., Dowling, J. E., and Link, B. A. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 6535–6540
34. Eroglu, B., Wang, G., Tu, N., Sun, X., and Mivechi, N. F. (2006) Dev. Dyn. 235, 2722–2735
35. Indra, A. K., Dupe, V., Bornert, J. M., Messaddeq, N., Yaniv, M., Mark, M., Chambon, P., and Metzger, D. (2005) Development 132, 4533–4544
36. Griffin, K. J., and Kimelman, D. (2003) Dev. Biol. 264, 456–466
37. Draper, B. W., Stock, D. W., and Kimmel, C. B. (2003) Development 130, 4639–4654