Structure and Promoter Analysis of Math3 Gene, a Mouse Homolog of Drosophila Proneural Gene aonal

NEURAL-SPECIFIC EXPRESSION BY DUAL PROMOTER ELEMENTS

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ath3, a vertebrate basic helix-loop-helix gene homologous to Drosophila proneural gene aonal, can directly convert non-neural cells into neurons with the anterior features. In the mouse, ath3 expression initially occurs widely in the developing nervous system and then gradually becomes restricted to the neural retina. Here, we characterized the genomic organization and promoter activity of mouse ath3 (Math3). Math3 gene consists of two exons separated by an 8-kilobase intron, and the whole protein-coding region is located in the second exon. Transcription starts at two sites, which are 75 nucleotides apart from each other, and there is no typical TATA box in the upstream region of either start site. Transient transfection analysis showed that the 5' region of Math3 can direct efficient expression in neuroblastoma cells but not in glioma or fibroblast cells. Deletion studies revealed that the proximal 193-base pair region, which contains the downstream transcription initiation site but not the upstream site, is essential for the Math3 promoter activity and can direct efficient expression in neuroblastoma cells. In contrast, retrovirus-mediated promoter analysis demonstrated that a region further upstream is additionally necessary for retinal expression. These results indicate that Math3 promoter contains two essential regulatory regions, the proximal 193-base pair region, which confers efficient neural-specific expression, and a region further upstream, required for retinal expression.

Recent studies demonstrated that vertebrate neuronal differentiation is controlled positively and negatively by multiple basic helix-loop-helix (bHLH)1 genes in an analogous way to Drosophila regulators (1, 2). For example, in Drosophila the bHLH genes of achaete-scute complex promote neuronal differentiation, whereas other bHLH genes, hairy and Enhancer of split, functionally antagonize achaete-scute complex and inhibit neuronal differentiation (3). Likewise, in mammals the bHLH gene Mash1, a mammalian homolog of Drosophila achaete-scute complex, promotes neuronal differentiation (4), whereas the bHLH gene Hes1, a mammalian homolog of Drosophila hairy and Enhancer of split, antagonizes Mash1 and inhibits neuronal differentiation (5, 6). Thus, the structures and functions of bHLH genes have been well conserved during evolution. Balance between these positive and negative bHLH genes is critical for normal neural development (4, 7, 8), and particularly, transcriptional regulation of these neural bHLH genes is very important because both ectopic expression and loss of expression cause severe abnormalities in the nervous system (4, 7–12).

Multiple neural bHLH genes homologous to Drosophila proneural gene aonal, which is essential for generation of photoreceptor and chordotonal organ neurons (13, 14), have been characterized from several vertebrate species (ath/neuroD/neurogenin family) (9–12, 15–22). Some of them are shown to promote neuronal differentiation in Xenopus (9–12). Among them, ath3 is unique because it is specifically expressed in the anterior neural tissues such as the forebrain, cranial ganglions, and retina and can generate neurons with the anterior features in Xenopus embryos (12). Thus, ath3 exhibits not only the anterior-specific expression but also the anterior-specific neurogenic activity. Furthermore, ath3 can induce expression of the photoreceptor-specific gene opsin (12), suggesting that ath3 may play an important role in retinal differentiation. In the mouse, ath3 expression occurs widely in the developing nervous system at early stages but then gradually becomes restricted to the anterior region like Xenopus ath3 (12). After birth, ath3 expression is detected only in the neural retina (12). Thus, ath3 shows two modes of the expression patterns during neural development; the initial general expression in the nervous system and the subsequent retina-specific expression.

In this study, to understand the molecular mechanism of neural-specific gene expression, we cloned mouse ath3 (Math3) gene and characterized its promoter activity. We found that the 5'-region of Math3 confers neural-specific gene expression. Furthermore, deletion study revealed that the proximal 193-bp region of Math3 promoter can direct efficient expression in neuroblastoma cells, whereas a region further upstream is necessary for retinal expression. Thus, these results suggest that the two modes of Math3 expression are controlled by two separate regulatory elements in the 5'-region of Math3 gene.

EXPERIMENTAL PROCEDURES

Isolation and Characterization of Math3 Gene—The mouse genomic library (Stratagene) was screened by hybridization with the Math3 cDNA as a probe. Nine clones were isolated from 9 × 106 plaques. The fragments hybridized positively were subcloned into pBluescript and subjected to sequence analysis.
For Southern blot analysis, the tail DNA was digested by restriction enzymes, electrophoresed on 0.7% agarose gel, and transferred to a nylon membrane filter. The $^{32}$P-labeled Math3 cDNA was hybridized at 65 °C in solution containing 0.23 SSC (13 SSC = 0.15 M NaCl, 0.015 M sodium citrate) and 0.1% SDS.

**Primer Extension and Reverse Transcription-mediated Polymerase Chain Reaction (PCR)**—
For the primer extension experiment, primer 4, 5'–CTCTTTCCCGGGGTCAGCTCCCGAGTAG–3' (corresponding to the region from 1175 to 1204), was labeled at the 5'-end, hybridized to the mouse retina poly(A) RNA, and subjected to reverse transcription, as described previously (23).

For the reverse transcription-mediated PCR, the following primers in addition to primer 4 were used; primer 1, 5'–ACACGCAGTGCGCAAAGCTGG–3' (220 to 21); primer 2, 5'–ACCTGGTCAGAGAAGCCTTG–3' (1120 to 1120); and primer 3, 5'–TGGTCAGCCAAAAC–3' (185 to 1110). Sets of primers 1 and 4, primers 2 and 4, and primers 3 and 4 were used to detect 224-, 204-, and 120-bp bands, respectively. After 30 cycles of reaction (94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min), the products were electrophoresed on 2% agarose gel.

**Transient Transfection Analysis**—
The reporter plasmids contained the firefly luciferase gene under the control of various lengths of Math3 promoter or the SV40 promoter. 1 μg of a reporter plasmid was transfected with 10 μl of LipofectAMINE reagent (Life Technologies, Inc.) into Neuro2a, NCB20 neuroblastoma brain hybrid cells, C6 glioma, or C3H10T1/2 cells, which were plated in 6-multiwell plates at the density of 2–4 x 10^5/well. 0.1 μg of the plasmid containing Renilla luciferase gene under the control of the herpes simplex virus thymidine kinase promoter (pHSVtk-RL) was also transfected as an internal standard to normalize the transfection efficiency. Medium was changed after incubation with the transfection complex at 37 °C for 6 h, and cells were further incubated at 37 °C. After 42–48 h, the cells were harvested, and the luciferase activity was measured.

**Retrovirus-mediated Promoter Analysis**—
For construction of pLNSZ, which directs lacZ expression from the SV40 promoter and neo expression from the upstream long terminal repeat, the bacterial lacZ reporter gene was ligated into the HindIII site of pLNSX (24). For the Math3 promoter constructs (pLNS2), the SV40 promoter region was removed from pLNSZ by BamHI and HindIII digestion, and various lengths of the Math3 promoter fragments were ligated into the BamHI and HindIII sites. Retrovirus was produced by transfecting the retroviral DNA constructs into the packaging cell line c2mp34 (a kind gift of Dr. Kazuhiro Ikenaka). Retrovirus solution was passed through a 0.45-μm filter and concentrated, as described previously (6). The viral titer was measured.

**Fig. 1. Structure of Math3 gene.** A, the nucleotide sequence of Math3 gene. The nucleotide sequence of the sense strand together with a part of the deduced amino acid sequence of Math3 is indicated. The upper- and lowercase letters represent the exon sequence and the flanking and intron sequences, respectively. The major alternative splicing site is indicated by an arrowhead. Two transcription initiation sites are shown by arrows. The E (caagtg) and N (cacag) boxes are underlined. B, the restriction map of Math3. The closed and open boxes represent the coding and noncoding regions, respectively. B, BgIII; H, HindII; P, PvuII.
The explants were fixed in 0.5% glutaraldehyde in phosphate-buffered saline at 4 °C for 30 min, stained with X-gal solution (1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside, 35 mM potassium ferricyanide, 35 mM potassium ferrocyanide, 2 mM MgCl₂, 0.02% Nonidet P-40, and 0.01% deoxycholate in phosphate-buffered saline), and incubated in 25% sucrose in phosphate-buffered saline at 4 °C overnight. The samples were frozen and sectioned at 10–16-μm thickness.

RESULTS

Structural Organization of Math3 Gene—To understand the molecular mechanism of neural-specific gene expression, we cloned Math3 gene. Nine overlapping genomic clones were isolated from 9 × 10⁵ plaques of a mouse genomic library by using the Math3 cDNA as a probe. Sequence comparison with the full-length Math3 cDNA revealed that Math3 gene encompassed a 12-kb region and consisted of two exons; the first exon contained only the 5'-noncoding region, whereas the second exon contained the whole protein-coding region (Fig. 1). The feature that the whole coding region is present in a single exon is also observed in Math1, Math2, and Mash1 genes (16, 17, 27), suggesting that these neural bHLH genes originated from a common ancestral gene. The two exons of Math3 were separated by an intron with the size of approximately 8 kb (Fig. 1B). Southern blot analysis using the tail DNA showed that the sizes of the hybridized DNA bands were identical to those of the cloned fragments (data not shown).

Previously, we isolated two types of Math3 cDNAs that differed only in the 5'-noncoding region; a 275-nucleotide 5'-noncoding region was deleted in the major species when compared with the minor one. This deleted portion corresponded to the region from the nucleotide residues 205–479 of Math3 gene (the first transcription initiation site is designated as the nucleotide residue +1; see below), indicating that the major species used the region upstream of the residue 205 as the first exon, whereas the minor species used the region extending to 479 as the first exon (Fig. 1A). In both cases, the exon-intron boundary conformed to the GT-AG rule (Fig. 1A).

The 3'-noncoding region was 2239 residues long, and the putative polyadenylation signal AATAAA was present at the residue 3698, which was 17 nucleotides upstream of the polyadenylation site (data not shown).

Determination of the Transcription Initiation Site—To determine the transcription initiation site, we first performed a primer extension experiment. The labeled antisense primer corresponding to the region from the nucleotide residue +175 to +204 was hybridized to retinal RNA (lane 1) or tRNA (lane 2) and subjected to reverse transcription. The bands specific to retinal RNA are indicated by arrows. The sequence ladder generated by the same primer with Math3 gene as a template was also electrophoresed to determine the position of the specific bands. PCR analysis. PCR was carried out with retinal RNA (lanes 1–3), the tail DNA (lanes 4–6), or retinal cDNA (lanes 7–9). Primers used for PCR are shown above each lane. All primer sets amplified the expected size of DNA from the tail DNA but not from retinal RNA. Only sets of primers 2 and 4 (lane 8) and primers 3 and 4 (lane 7) amplified the expected size of DNA from retinal cDNA, indicating that the region corresponding to primer 1 was not transcribed. The sizes of the molecular markers are indicated on the right. The positions of primers and two transcription start sites are shown at the bottom.

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Determination of the Transcription Initiation Site—To determine the transcription initiation site, we first performed a primer extension experiment. The labeled antisense primer corresponding to the region from the nucleotide residue +175 to +204 was hybridized to retinal RNA and subjected to reverse transcription. This analysis demonstrated two specific bands with the sizes of 204 and 129 nucleotides (Fig. 2A, lane 1, arrows), suggesting that transcription initiates at two sites, the nucleotide residues +1 and +76. In many promoters that lack the TATA box, transcription starts at multiple sites. Consistent with this notion, there was no typical TATA box in the upstream region of either transcription start site of Math3 gene (Fig. 1A). Instead, upstream of the first initiation site (+1) there was an AT-rich region (tttaaaa-caaaacaaaaa) at −35, and upstream of the second site (+76) there was a G-rich region (ggggaggagg) at +49, which could be recognized by Sp1.

To confirm that no transcription initiates in the further upstream region, we next carried out reverse transcription-mediated PCR with retinal RNA (Fig. 2B). Whereas a set of the primers 1 (corresponding to the region from −20 to −1) and 4 (+175 to +204) gave rise to no specific bands (lane 9), sets of primers 2 (+1 to +20) and 4 and of primers 3 (+85 to +104)
4 each generated a single band only after reverse transcription (compare lanes 1 and 2 with lanes 7 and 8). These results clearly showed that the region downstream from +1 was transcribed, but the further upstream region was not.

**Transcription from Math3 Promoter in Neural Cells**—To characterize the mechanism for neural-specific gene expression, the promoter activity of Math3 gene was examined by a transient transfection method. A reporter plasmid containing the luciferase gene under the control of the 5’-region of Math3 gene (from −2.8 kb to +196) was transfected into neuroblastoma, glioma, and fibroblast cell lines. The control SV40 promoter showed 30- to 40-fold activation in these cells when compared with the promoter-less construct. The activity of the SV40 promoter was designated as 100, and the relative activity of Math3 promoter (from −2.8 kb to +196) was determined. Each activity was the average of at least three independent experiments and was also normalized by the activity of cotransfected pHSVtk-RL.

We next tested the 5’ fragments that lacked the region from +5 to +196 but contained the first transcription initiation site. However, these fragments, including the one spanning from −2.8 kb to +4, did not show the promoter activity (Fig. 4, A and C), indicating that the first transcription initiation site was not functional in the absence of the downstream region. In addition, these results suggest that the region upstream of −1197, which significantly up-regulated the promoter activity, depended upon the proximal region from +5 to +196. Thus, the region from +5 to +196 that contained the second transcription initiation site was essential for the Math3 promoter activity.

To further narrow the essential regions, we made various deletions from the region between +4 and +196. However, whereas deletion from +4 to +31 retained a very weak activity, any further deletion led to almost complete loss of the promoter activity (Fig. 4, B and C). These results indicated that most of the region from +4 to +196 that consisted of the 72-bp upstream region, the second transcription initiation site (+76), and the 121-bp downstream regions were required for the promoter activity. Thus, the 193-bp region constituted a minimal promoter that was essential for Math3 expression in neural cells.

The low activity of Math3 promoter in non-neural cells could be due to the presence of a transcriptional repressor in non-neural cells. To test this possibility, various deletion constructs of Math3 promoter were transfected into C3H10T1/2 cells.
However, none of the deletion constructs showed up-regulation of the promoter activity (Fig. 4D), suggesting that there is no non-neural cell-specific repressor region in Math3 promoter. Thus, it is likely that the low activity of Math3 promoter in non-neural cells is due to the absence of transcriptional activators in such cells.

Transcription from Math3 Promoter in Retinal Cells—Math3 expression initially occurs in various regions of the developing nervous system but later becomes restricted to the neural retina (12). In the adult retina, Math3 is expressed at a high level in the outer region of the inner nuclear layer (INL), where bipolar and horizontal cells are present (12). To determine the promoter regions necessary for retinal cell type-specific expression, retrovirus-mediated promoter analysis was performed (28). We generated recombinant retroviruses that direct lacZ expression under the control of the SV40 promoter or various lengths of Math3 promoter (Fig. 5, A and D and Table I). The explants of the developing retina, known to well mimic the in vivo development (8, 25, 26), were prepared from mouse embryos at day 17.5 or 18.5 and infected with these retroviruses. Only mitotic cells are infected with retrovirus, and once infected, cells precisely transmit the virus genome to their daughter cells. After 14 days of culture, at which time neuronal differentiation was finished, the retinal explants were stained with X-gal. If the promoter was functional, virus-infected cells should become blue after X-gal staining. It has been shown that during the postnatal period, the newly differentiating cells are mostly rods (almost 80%) and bipolar cells (~10%) (29, 30).

As shown in Fig. 5, the SV40 promoter directed lacZ expression in various retinal cell types, such as rods and bipolar cells. More than 80% of the labeled cells were rods, which are located in the outer nuclear layer (ONL), and ~10% were bipolar cells, which are present in the INL (Fig. 5, B and C and Table I). The other retinal cell types that were labeled with X-gal constituted about 2% of the total infected cells. Thus, the ratios of these labeled cell types well reflected the cells that differentiate during this period, indicating that the SV40 promoter functioned well in all retinal cell types.

In contrast to the SV40 promoter, the region from −1197 to +196 of Math3 promoter directed lacZ expression specifically in the INL neurons, and no rods were labeled (Fig. 5, E and F and Table I). Furthermore, about 80% of the labeled cells were located in the outer region of the INL (Table I), where Math3 is mainly expressed (12). Therefore, the 5′-region of Math3 gene conferred the INL cell-specific expression, well mimicking neuronal type-specific Math3 expression. The region from −671 to +196 also directed the INL neuron-specific expression and, in addition, more than 80% of the labeled cells were present in the outer region of the INL (Table I). For this Math3 promoter activity in retinal neurons, the proximal 193-bp region was essential, because the 5′-region from −671 to +4 failed to induce lacZ expression (Table I). Interestingly, the region from +4 to +196, which was able to induce as efficient expression as the −671 to +196 promoter in neuroblastoma cells, did not
direct expression in retinal cells (Table I). Thus, the upstream region between −671 and +4, which was not essential for expression in neuroblastoma cells, was required for retinal cell type-specific expression. These results demonstrated that Math3 expression is controlled by at least two separate regions, the 193-bp minimal promoter required for neural expression and the upstream region essential for retinal cell type-specific expression.

**DISCUSSION**

**The Promoter Region of Math3 Directs Neural- and retinal-specific Expression**—In this study, we isolated and characterized Math3 gene and showed that the 5′-region of Math3 gene confers the cell type-specific expression. The 5′-region can direct efficient expression in neuroblastoma cells but not in other cell types. Interestingly, the proximal 193-bp region (from nucleotide residue +4 to +196), which consists of the 72-bp upstream region, the second transcription initiation site (+76), and the 121-bp 5′-noncoding region, which lacks the first transcription initiation site (+1), is sufficient for efficient expression in neuroblastoma cells. In addition, this 193-bp region is essential for Math3 expression, since the region further upstream that contains the first transcription initiation site but lacks the proximal 193-bp region cannot direct expression in neuroblastoma cells. Even the region from −2.8 kb to +5 did not show the promoter activity. Thus, the upstream regulatory region seems to depend upon the proximal 193-bp region.

Interestingly, this proximal 193-bp region cannot direct expression in retinal cells. A region further upstream, which is not essential for expression in neuroblastoma cells, is required for retinal expression, indicating that Math3 expression is controlled by at least two separate regions, the proximal 193-bp region and a region further upstream; addition of the region from −671 to +3 conferred the INL-specific expression in the retina. This retinal expression was mainly observed in the outer region of the INL, where Math3 is expressed at the highest level, suggesting that this upstream region contains the retinal cell type-specific regulatory element. During development, Math3 shows two different modes of expression: the initial wide distribution in the developing nervous system and later restriction to the subsets of the INL cells in the retina (12). Our results suggest that the initial wide distribution may be controlled by the proximal region, whereas the later retinal-specific expression may be regulated by the upstream region.

It is very important to identify the transcription factors that interact with the Math3 promoter elements. We previously found that neural-specific expression of the bHLH factor Hes5 is regulated by multiple repeats of GC-rich elements (23). A neural-specific factor interacts with these GC-rich elements and may be responsible for Hes5 expression (23). In the proximal 193-bp region of Math3 promoter, there is a GC-rich element similar to those of Hes5 promoter in the upstream region of the second transcription start site. Thus, it is possible that this GC-rich region may be responsible for neural-specific expression of Math3.

For retinal gene expression, it has been demonstrated that several transcription factors such as Pax6, Chx10, and Rx are essential; in the absence of Pax6 or Rx, eyes do not develop (31–33), and in the absence of Chx10, bipolar cells do not differentiate (34). Particularly, Math3 and Chx10 expressions are quite similar in the retina; expressions of both genes begin in progenitor cells at early stages of retinal development, become restricted to the INL at later stages, and continue in the INL in the adult (12, 35). Thus, Chx10 may regulate Math3 expression in the retina. However, coexpression of Chx10 did not up-regulate the Math3 promoter activity in neuroblastoma or fibroblast cells, thus suggesting that Chx10 may not di-

**TABLE I**

| Promoter | No. labeled cells in the ONL | No. labeled cells in the INL |
|----------|-----------------------------|-----------------------------|
| SV40     | 132 (88%)                   | 15 (10%)                    |
| Math3 (−1197 to +196) | 0 (0%)                     | 23 (82%)                    |
| Math3 (−671 to +196) | 0 (0%)                     | 35 (83%)                    |
| Math3 (+4 to +196) | 0 (0%)                     | 0 (0%)                      |
| Math3 (−671 to +4) | 0 (0%)                     | 0 (0%)                      |

*The average number of labeled cells per retina was determined. The relative ratios of each cell type is also shown in parentheses. At least three independent experiments were performed.*
rectly regulate Math3 expression.

In Math3 promoter, there is an E box sequence at –177, which is a potential Math3 target site. Thus, Math3 could up-regulate its own expression, as observed in the case of the muscle determination factor MyoD, which positively autoregulates its own expression by directly binding to the promoter (36). However, in transient transfection analysis with neuroblastoma and fibroblast cells, overexpression of Math3 failed to up-regulate the Math3 promoter activity, suggesting that Math3 does not positively autoregulate its expression or that factors required for Math3 function are missing in the cells that we used.

Negative Regulation and Retinal Development—We previously showed that continuous expression of Hes1 inhibits neuronal differentiation and that, conversely, Hes1-null mutation leads to up-regulation of Mash1 and premature neuronal differentiation in the retina (6–8). Thus, it is likely that Hes1 regulates the timing of differentiation by inhibiting Mash1 activity. These data raise the interesting possibility that Hes1 could also target to Math3 for inhibition of differentiation in the retina. For example, there is an N box sequence at –107 that can be recognized by Hes1. However, in transient transfection assay, Hes1 failed to repress Math3 promoter activity in neuroblastoma cells.2 Thus, Hes1 does not functionally antagonize Math3 at the transcriptional level, but it is still possible that Hes1 could inhibit the activity of Math3 at the protein level, because Hes1 can inhibit the activity of other bHLH factors such as Mash1 and MyoD through protein-protein interaction (5).

It was shown that activation of the membrane protein Notch also inhibits neuronal differentiation in the retina (37, 38), and it is suggested that Notch-induced suppression of differentiation requires induction of Hes1 (39). Similar to the case of Hes1, the active form of Notch failed to repress Math3 promoter activity in transient transfection assay.2

Math3 and Retinal Development—Characterization of the Math3 function is another important issue. In Xenopus, ath3 can induce retinal neuronal differentiation, and it is likely that Math3 also regulates retinal differentiation. Interestingly, Xenopus and mouse ath3, both, contain a possible phosphorylation site in the basic region, and in Xenopus, mutation of this site into Asp, which mimics the phosphorylation of this site, retains a general neurogenic activity but severely impairs the retinal differentiation activity (12). We speculate that in mice, ath3 activity is also regulated by phosphorylation of the basic region and that retinal differentiation may be induced by a nonphosphorylated form of Math3.

We previously showed that Math3 locus symbol: Atoh3) is located on chromosome 10 (40) and closely linked to eye blebs (eb) mutation, which shows eye anomalies (41). However, Southern blot analysis indicated that there is no major insertion or deletion in Math3 gene of eb mutant mice.7 Furthermore, there are many more defects in eb, including the kidney and limb, which are different from the regions expressing Math3. Therefore, the two genes Math3 and eb may be different. Now that the structure of Math3 was characterized, we can proceed to in vivo functional analysis such as loss-of-function assay in mice by targeted gene disruption.

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REFERENCES

1. Kageyama, R., Sasai, Y., Akazawa, C., Ishibashi, M., Takebayashi, K., Shimizu, C., Tomita, K., and Nakanishi, S. (1995) Crit. Rev. Neurobiol. 9, 177–188
2. Lee, J. E. (1997) Curr. Opin. Neurobiol. 7, 13–20
3. Jan, Y. N., and Jan, L. Y. (1993) Cell 75, 827–830
4. Guillemot, F., Lo, L.-C., Johnson, J. E., Auerbach, A., Anderson, D. J., and Joyner, A. L. (1993) Cell 75, 463–476
5. Sasai, Y., Kageyama, R., Tagaya, W., Shigemoto, R., and Nakanishi, S. (1992) Genes Dev. 6, 2620–2634
6. Ishibashi, M., Moriyoshi, K., Sasai, Y., Shiota, K., Nakanishi, S., and Kageyama, R. (1994) EMBO J. 13, 1769–1785
7. Ishibashi, M., Ang, S.-L., Shiota, K., Nakanishi, S., Guillemot, F., and Kageyama, R. (1995) Genes Dev. 9, 3136–3148
8. Tomita, K., Ishibashi, M., Nakahara, K., Ang, S.-L., Nakanishi, S., Guillemot, F., and Kageyama, R. (1996) Neuron 16, 723–734
9. Lee, J. E., Hollenberg, S. M., Snider, L., Turner, D. L., Lipnick, N., and Weintraub, H. (1995) Science 268, 836–844
10. Ma, Q., Kintner, C., and Anderson, D. J. (1996) Cell 87, 43–52
11. McCormick, M. B., Tamimi, R. M., Snider, L., Asakura, A., Bergstrom, D., and Tapscott, S. J. (1996) Mol. Cell. Biol. 16, 5792–5800
12. Takebayashi, K., Takahashi, S., Yokota, C., Tsuda, H., Nakanishi, S., Asahina, M., and Kageyama, R. (1997) EMBO J. 16, 384–395
13. Jarman, A. P., Grau, Y., Jan, L. Y., and Jan, Y. N. (1993) Cell 73, 1307–1321
14. Jarman, A. P., Grell, E. H., Ackerman, L., Jan, L. Y., and Jan, Y. N. (1994) Nature 369, 398–400
15. Bartholomai, A., and Nave, K.-A. (1994) Mech. Dev. 48, 217–228
16. Akazawa, C., Ishibashi, M., Shimizu, C., Nakanishi, S., and Kageyama, R. (1995) J. Biol. Chem. 270, 8720–8728
17. Shimizu, C., Akazawa, C., Nakanishi, S., and Kageyama, R. (1995) Eur. J. Biochem. 229, 239–248
18. Ben-Arie, N., McCall, A. E., Berkman, S., Eichele, G., Bellen, H. J., and Zoghbi, H. Y. (1996) Hum. Mol. Genet. 5, 1207–1216
19. Yasunami, M., Suzuki, K., Maruyama, H., Kawakami, H., Nagai, Y., Hijigawa, M., and Ohkubo, H. (1996) Biochem. Biophys. Res. Commun. 220, 754–758
20. Kume, H., Maruyama, K., Tomita, T., Iwatsubo, T., Saijo, T. C., and Obata, K. (1996) Biochem. Biophys. Res. Commun. 219, 526–530
21. Kawakami, H., Maruyama, H., Yasunami, M., Ohkubo, H., Harra, H., Saida, T., Nakanishi, S., and Nakamura, S. (1996) Biochem. Biophys. Res. Commun. 221, 199–204
22. Gradwohl, G., Fode, C., and Guillemot, F. (1996) Dev. Biol. 180, 227–241
23. Takebayashi, K., Akazawa, C., Nakanishi, S., and Kageyama, R. (1996) J. Biol. Chem. 270, 1342–1349
24. Miller, A. D., and Rosman, G. J. (1989) BioTechniques 7, 980–990
25. Caffo, A. B., Visser, H., Jansen, H. G., and Sanyal, S. (1989) Curr. Eye Res. 8, 1093–1092
26. Sparrow, J. R., Hicks, D., and Barnstable, C. J. (1990) Dev. Brain Res. 51, 69–84
27. Guillemot, F., and Joyner, A. L. (1993) Mech. Dev. 42, 171–185
28. Uenaka, K., Nakahara, K., Fujimoto, I., Kagawa, T., Ogawa, M., and Mikoshiba, K. (1992) New Biol. 4, 53–60
29. Turner, D. L., and Cepko, C. L. (1987) Nature 328, 131–136
30. Cepko, C. L., Austin, C. P., Yang, X., Alexiades, M., and Ezzeddine, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 589–593
31. Hill, R. E., Favor, J., Hogan, B. L. M., Tom, C. C. T., Saunders, G. F., Hansen, I. M., Prosser, J., Jordan, T., Hastie, N. D., and van Heeyningen, V. (1991) Nature 354, 522–525
32. Gruss, P., and Woltcher, C. (1992) Cell 69, 719–722
33. Mathers, P. H., Grinberg, A., Mahon, K. A., and Jamrich, M. (1997) Nature 387, 603–607
34. Burmeister, M., Novak, J., Liang, M.-Y., Basu, S., Ploder, L., Hawes, N. L., Viden, D., Hoover, F., Goldman, D., Calvisi, L., and Weintraub, H. (1989) Cell 58, 241–248
35. Dursky, R. I., Rapaport, D. H., and Harris, W. A. (1995) Nature 377, 487–496
36. Bao, Z.-Z., and Cepko, C. L. (1997) J. Neurosci. 17, 1425–1434
37. Jarriault, S., Brou, C., Legeat, F., Schroeter, E. H., Kopan, R., and A. (1995) Nature 377, 355–358
38. Isaka, F., Shimizu, C., Nakanishi, S., and Kageyama, R. (1996) Genomics 37, 400–402
39. Chapman, D. B., and Hummel, K. P. (1963) Mouse News Lett. 28, 32