The Krüppel-like Factor Epiprofin Is Expressed by Epithelium of Developing Teeth, Hair Follicles, and Limb Buds and Promotes Cell Proliferation*

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We identified a cDNA clone for epiprofin, which is preferentially expressed in teeth, by differential hybridization using DNA microarrays from an embryonic day 19.5 mouse molar cDNA library. Sequence analysis revealed that this cDNA encodes a member of the Krüppel-like factor family containing three characteristic C_H2-type zinc finger motifs. The full-length cDNA was obtained by the 5’ Cap capture method. Except for its 5’-terminal sequence, the epiprofin mRNA sequence is almost identical to the predicted sequence of Krüppel-like factor 14/Sp6 (specificity protein 6), which was previously identified in expressed sequence tag data bases and GenBank™ by an Sp1 zinc finger DNA-binding domain search (Scoby, S., Gabant, P., Van Reeth, T., Hertveldt, V., Drezé, P. L., Van Vooren, P., Riviere, M., Szpirer, J., and Szpirer, C. (2000) Genomics 70, 93–101). This sequence difference is due to differences in the assignment of the location of exon 1. In situ hybridization revealed that epiprofin mRNA is expressed by proliferating dental epithelium, differentiated odontoblast, and also hair follicle matrix epithelium. In addition, whole mount in situ hybridization showed transient expression of epiprofin mRNA in cells of the apical ectodermal ridge in developing limbs and the posterior neuropore. Transfection of an epiprofin expression vector revealed that this molecule is localized in the nucleus and promotes cell proliferation. Thus, epiprofin is a highly cell- and tissue-specific nuclear protein expressed primarily by proliferating epithelial cells of teeth, hair follicles, and limbs that may function in the development of these tissues by regulating cell growth.

Many vertebrate organs begin their development by inductive interactions between epithelium and mesenchyme. Tooth development is a classic example of this process and provides a useful experimental system for understanding the molecular mechanisms of organogenesis (2, 3). Mouse molar tooth development is initiated at embryonic day (E) 11.5,1 when the oral epithelium thickens and invaginates into the underlying neural crest-derived mesenchyme (4). Continuation of this invagination process results in the formation of epithelial tooth buds at E13.5. Ectomesenchymal cells surrounding the bud form the dental papilla, which later develop into dentin-secreting odontoblasts and the tooth pulp. After the bud stage, the tooth germ progresses to the cap and bell stages, and epithelial cells differentiate into enamel-secreting ameloblasts. All of these stages can be seen simultaneously along incisors of rodents, which continuously grow and erupt throughout life (5). Dental epithelium differentiates into ameloblasts through several distinct stages: 1) the presecretory stage, 2) the secretory stage, 3) the early maturation stage, and 4) the late maturation stage (6). At the presecretory stage, dental epithelium proliferates. At the secretory stage, the cells stop proliferating and differentiate into ameloblasts, which secrete enamel matrix proteins including amelogenin, ameloblastin, enamelin, and tuftelin. During the maturation stage, the enamel matrix is almost completely replaced by calcium and phosphorous, and ameloblasts eventually undergo apoptosis (7).

The Sp/KLF family consists of more than 21 proteins in humans and 17 in mice, and this family has unique features including a DNA-binding domain with three tandem C_H2-type (Krüppel-like) zinc finger motifs at the C terminus and a transcriptional regulatory domain at the N terminus (8–10). This protein family can be divided into several classes by its sequence and functional similarities, including an Sp1-like subgroup and two KLF subgroups. Some of these proteins, such as Sp1 and BTEB1/KLF9, are ubiquitously expressed (11, 12), whereas others, such as EKLF/KLF1 and LKLF/KLF2, are expressed in a tissue-specific manner (13, 14). KLF13 and KLF14/Sp6 were identified by screening a mouse expressed sequence tag data base using the Sp1 zinc finger domain as a probe and were reported to be ubiquitous by RT-PCR analysis (1). Sp/KLF protein factors regulate a wide range of cellular functions including cell growth, differentiation, apoptosis, and tumor formation. Although the sequence of the zinc finger domain is highly conserved and binds many GC-rich sequences in vitro, the N-terminal regulatory domain is considerably di-

1 The abbreviations used are: En, embryonic day n; KLF, Krüppel-like factor; RT, reverse transcription; GFP, green fluorescent protein; PBS, phosphate-buffered saline; BrdU, 5-bromo-2’-deoxyuridine; Sp, specificity protein.
verse within the family. Each factor can act as either an activator, or repressor, or both, and they are thought to interact with different promoters and/or with other coregulators. Some Sp/KLF family proteins have been shown to play essential roles in tooth and skin formation. For example, Sp6 knockout mice revealed growth retardation and defective tooth enamel formation caused by the lack of the enamel matrix proteins amelogenin and ameloblastin (15). KLF4 is encoded by the Sp6/KLF14 gene and has nearly the same sequence as the previously predicted sequence for Sp6/KLF14 except for a difference in the N terminus. Overexpression of epiprofin promoted cell proliferation in cell culture, suggesting that epiprofin regulates epithelial growth and thereby plays a role in the development of these tissues.

MATERIALS AND METHODS

Cloning of Epiprofin cDNA—A mouse E19.5 molar tooth cDNA library was constructed and screened by differential hybridization using DNA microarrays. The coding sequence of epiprofin was subcloned into the GFP vectors (GFP) fusion proteins were generated in the expression vectors (pM20-GFP) (Invitrogen). The sequence for the full-length epiprofin was obtained using MetaMorph 3.5 software. Sequence analysis revealed that the Sp6/KPL14 gene and has nearly the same sequence as the previously predicted sequence for Sp6/KPL14 except for a difference in the N terminus. Overexpression of epiprofin promoted cell proliferation in cell culture, suggesting that epiprofin regulates epithelial growth and thereby plays a role in the development of these tissues.

RESULTS

Cell Proliferation Assay—COS7 cells and primary dental epithelial cells were used for proliferation assays. Primary dental epithelial cells were prepared from molars of newborn mice. After molars were digested by 0.1% collagenase, 0.05% trypsin, 0.5 mM EDTA for 10 min, dental epithelium was separated from dental mesenchyme. Dental epithelium was further treated with 0.1% collagenase, 0.5 mM EDTA for 15 min and then cultured in keratinocyte-SFM medium (Invitrogen) supplemented with epidermal growth factor and bovine pituitary extract for 7 days to remove contaminating mesenchymal cells before transfection. The cells transfected with pTKLF/Myc, pTKLF-Zn/Myc, or empty vector were seeded at a density of 5.0 × 10^5 cells/well in 24-well plates and maintained in Dulbecco's modified Eagle's medium with 5% fetal bovine serum for COS7 cells and keratinocyte-SFM medium (Invitrogen) supplemented with epidermal growth factor and bovine pituitary extract for dental epithelial cells. The cell numbers were counted under a microscope. The cell proliferation assays were also performed by incorporation of 5-bromo-2'-deoxyuridine (BrDU). Three days after transfection, the cells were incubated with 10 μM BrDU and 10% fetal bovine serum for 1 h. The cells were fixed with 4% paraformaldehyde/PBS overnight, dehydrated in methanol, and kept at -20°C until analyzed. Whole molar RNA in situ hybridization was carried out according to Nieto et al. (20).

For cell proliferation assays used BrDU in embryonic dental tissue, BrDU (50 mg/kg of body weight) was diluted in PBS and intraperitoneally injected into timed pregnant mice. The animals were sacrificed 2 h later. Immunohistochemistry was performed according to the instructions provided by the manufacturer. The color reaction was performed by the ABC method (Vector) followed by diaminobenzidine and H2O2 at room temperature. The sections were counterstained with eosin.

Eighteen-day (E18) mouse brains were fixed in 4% paraformaldehyde/PBS overnight, dehydrated in methanol, and kept at -20°C until analyzed. Whole molar RNA in situ hybridization was carried out according to Audrezet et al. (20).

RESULTS

cDNA and Gene Structure of Epiprofin (Sp6/KLF14)—DNA microarrays containing about 10,000 cDNA clones from a mouse E19.5 molar cDNA library were differentially hybrid-
ized with mRNA from E19.5 molars versus body. About 200 clones showed preferential hybridization to molar mRNA. Sequencing of these clones identified seven clones that matched sequences in the expressed sequence tag data base. One of the seven clones corresponded to the 3' portion of Sp6/KLF14 cDNA. We named this protein epiprofin, because its expression

FIG. 1. Gene structure and coding sequence of epiprofin. A, gene structure of epiprofin. The mouse gene sequence (NCBI GenBank™ accession number AL686664; Celera Genomics data base identification number CG13224) was compared with the full-length cDNA for epiprofin (NCBI GenBank™ accession number AY338955). The solid boxes represent exons. The location of a TATA box is shown. Exon 1 encodes the 5'-untranslated sequence, and exon 2 encodes the entire epiprofin protein sequence. Donor and acceptor sequences are shown (small letters for exon sequences, and large letters for the intron sequence). The broken box and dotted line represent the previously predicted exon 1 and promoter region of the Sp6/KLF14 gene (1). B, the promoter sequence and exon 1 and 2 sequences with translated amino acids. Bold letters indicate a TATA sequence, an ATG translation initiation codon, and proline residues. Underlining indicates cysteine and histidine residues in the Kruppel zinc finger domain. C, the previously predicted 5'-flanking sequence and 5' coding sequence (GenBank™ accession number AJ275988) (1). The underlined sequence corresponds to the dotted line in A. The underlined 5' amino acid sequence of the proposed Sp6 and KLF14 differs from that of epiprofin.
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is primarily restricted to epithelium in certain tissues as described below. The coding sequence of Sp6/KLF14 cDNA was previously predicted on the basis of composite sequences from the mouse gene and expressed sequence tags (1). To obtain the 5′ end of epiprofin cDNA to compare it with Sp6/KLF14 cDNA, we performed 5′ Cap rapid amplification of cDNA ends PCR using mouse E18 molar RNA with specific extension primers for epiprofin and cloned into the pCR4-TOPO vector. All of the extended clones picked for sequencing showed an identical 5′ end. The 5′ 48-bp sequence of epiprofin mRNA differed from the 5′ sequence of the presumptive full-length Sp6/KLF14 mRNA, but the remaining 3′ sequence corresponding to exon 2 was identical except for a few silent base substitutions (Fig. 1, A and B). Comparison of the epiprofin mRNA sequence and the gene for Sp6/KLF14 from NCBI GenBankTM (accession number AL606664) and Celera Genomics data bases (ID CG13224) revealed that the 42-bp sequence is located 7.5 kb upstream from exon 2 and likely represents exon 1, which is flanked by sequences characteristic for the Cap signal at the 5′ side and splicing site at the 3′ side (Fig. 1, A and B). A TATA box is located at position −25 bp, but there is no CAAAT motif in this region. The 5′ 280-bp region containing the 180-bp promoter, 42-bp exon 1, and intron 1 showed a substantial sequence homology (80% identity) in mice and humans. The sequence immediately upstream of exon 1 showed the conserved Cap motif for transcription initiation, and the boundary of exons 1 and 2 contained the consensus donor and acceptor sequences (Fig. 1, A and B). Transfection with a reporter gene construct containing the −200 to +30 bp promoter and SV40 enhancer confirmed that this region has basal promoter activity (data not shown). The presumptive exon 1 sequence of the Sp6/KLF14 gene previously proposed by computer analysis shows poor sequence homology (40% identity) between mice and humans, and this sequence is located about 2.2 kb upstream of exon 2 (dotted line and broken box in Fig. 1A). The presumptive promoter immediately adjacent to this sequence shows no significant sequence homology between mice and humans. Fig. 1C shows the reported presumptive 5′ sequence for Sp6/KLF14 with underlined nucleotide and amino acid sequences different from these of epiprofin (nucleotide sequence including the presumptive promoter and 5′ coding sequences and the N-terminal amino acid sequence) (1).

The 42-bp exon 1 contains an ATG at +35, and exon 2 also has an ATG at +95. The first ATG is not in a favorable context for the initiation of protein translation according to Kozak (21) and is followed by a premature termination codon (Fig. 1B). By contrast, the second ATG in exon 2 is adjacent to the consensus Kozak sequence. It is therefore likely that major mRNA translation initiates at this second ATG codon. This prediction was confirmed by GFP fusion protein expression analysis in which the second ATG was capable of initiating translation (see Fig. 8). The second ATG is followed by a sequence with an open reading frame for 376 amino acids plus a 2.2-kb 3′ noncoding sequence containing the consensus polyadenylation signal (Fig. 1B). The predicted protein sequence contains three contiguous zinc finger motifs present near the C-terminal end and two potential nuclear localization signals PDGGKKK (amino acid residues 245–251) and PGGKGKR (amino acid residues 360–366) (Fig. 1B). The zinc finger domain has a high degree of
homology with Sp3, Sp7/Osterix, Sp1, GKLF (KLF4), EKLF (KLF1), LKLF (KLF2), and BKLF (KLF3), with the highest with Sp3 and Sp7/Osterix (~80% identity). In contrast to the zinc finger domain, the N-terminal domain of epiprofin (Sp6/ KLF14) shows no homology to other known proteins.

Expression of Epiprofin mRNA According to RT-PCR and Northern Blot—To study tissue-specific expression, we performed RT-PCR and Northern blotting (Fig. 2). RT-PCR analysis revealed that epiprofin-specific primers produced a 517-bp product with RNA from newborn mouse molars and incisors (Fig. 2 A). The primers also produced a faint 517-bp band with skin RNA but not with other tissue RNA. Primers for ameloblastin and glyceraldehyde-3-phosphate dehydrogenase were used as positive controls for tooth-specific and ubiquitous mRNA, respectively. Northern blotting showed a single 3.6-kb mRNA band for epiprofin in molars but not in other tissues (Fig. 2 B). Ameloblastin mRNA is expressed only in molars, and another control, β-actin mRNA, is expressed in all tissues. Thus, expression of epiprofin mRNA is highly tissue-specific. This conclusion is in contrast to the previous report of ubiquitous expression of Sp6/KLF14 (1).

Tissue-specific Expression of Epiprofin by in Situ Hybridization Analysis—We next analyzed expression patterns of epiprofin mRNA in developing mouse mandible by in situ hybridization (Fig. 3). Epiprofin mRNA was first detected at E11.5 in the dental epithelium of the first branchial arch and was continuously expressed in molars and incisors (E12.5–14.5). To identify which cell types express epiprofin mRNA, we performed in situ hybridization with sections of molars at various stages and compared its expression with cell proliferation by BrdU assays (Fig. 4). The expression of epiprofin mRNA was observed in E12.5, E14.5, and E17.5 inner dental epithelium but not other parts of dental tissues (Fig. 4 A–D). At E19.5 (Fig. 4 F) and later (data not shown), epiprofin mRNA is expressed in the inner enamel epithelium (preameloblast/ameloblasts). Epiprofin mRNA is expressed weakly in differentiated odontoblasts (Fig. 4 H). In newborn incisors, epiprofin mRNA is expressed by the dental epithelium of the cervical loop starting from the early stage to the secretory stage of ameloblasts (Fig. 5 B). This is in contrast to amelogenin mRNA, which is predominantly expressed at the secretory stages of ameloblasts but not at the

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**Fig. 4.** Comparative analysis of cell proliferation (A–D) and expression of epiprofin (E–H) in sections of mouse embryo first molars at the bud/cap stage E12.5 (A and E), cap stage E14.5 (B and F), early bell stage E17.5 (C and G), and late bell stage E19.5 (D and H). BrdU is incorporated in the nuclei of epithelium and dental mesenchyme (dm) cells in bud, cap, and early bell stages (A–C). Note the absence of BrdU incorporation and epiprofin expression in the enamel knot (ek). Epiprofin transcripts are restricted to the inner dental epithelium (ide, E–G). At the late bell stage (E19.5), proliferative cells are found in the preameloblast (pam), intermediate stratum (is), and the preodontoblast and subodontoblast layers (arrowsheads) (D). Epiprofin labeling is seen in preameloblasts and preodontoblasts (od) (G). ds, dental sac; ode, outer dental epithelium; sr, stellate reticulum; oe, oral epithelium. The dashed lines indicate the borders between dental epithelium and dental mesenchyme.

**Fig. 5.** In situ hybridization in incisors of newborn mice. A and D, control with a sense probe for epiprofin. B and E, expression of epiprofin mRNA. E, magnified boxed area in B. The arrow in B indicates the expression of epiprofin mRNA in the dental epithelium of the cervical loop. The arrow in E indicates the expression of epiprofin mRNA in differentiating odontoblasts. C and F, expression of amelogenin mRNA. E, magnified boxed area in C. Amelogenin mRNA is expressed by secretory ameloblasts but not in the cervical loop.
presecretory stage (Fig. 5C). Epiprofin mRNA is also expressed in mesenchymal odontoblasts of incisors (Fig. 5E), whereas amelogenin mRNA is not expressed in those cells (Fig. 5F). E18.5 mouse head sections showed the expression of epiprofin mRNA not only in molars and incisors but also in hair follicles (Fig. 6, A and C). The expression was observed in the proliferating epithelium of the inner root sheath in the matrix of hair follicles (Fig. 6D).

To investigate further the expression of epiprofin mRNA in other organs at early stages, we performed whole mount in situ hybridization of mouse embryos (Fig. 7). We also found that epiprofin mRNA is expressed transiently in caudal neuropore
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at E8.5 and the ectodermal ridge of limb buds starting from E9.5 and ending at E13.5. In E12.5 limb buds, epiprofin was restricted to the apical ectodermal ridge of the digits. At E14.5 epiprofin mRNA was not detectable in limbs.

Nuclear Localization of Epiprofin—Epiprofin has two potential nuclear localization signals that are located upstream and in the middle of the zinc finger domain. To test the cellular localization of epiprofin, COS7 cells were transfected with two GFP fusion expression vectors, pGFP-TKLF and pTKLF-GFP, where GFP was attached to either the N- or C-terminal end of epiprofin. As seen in Fig. 8, control GFP alone was localized throughout the cell. In contrast, GFP-TKLF fusion proteins were localized in the nuclei, and no difference was observed between N- and C-terminal epiprofin-GFP fusion proteins (Fig. 8).

Promotion of Cell Proliferation by Epiprofin—Because epiprofin is expressed by proliferating epithelium, the epiprofin protein may be involved in the regulation of cell growth. Therefore, we examined whether epiprofin promotes DNA synthesis by transfecting the epiprofin-Myc expression vector into COS7 cells. The number of BrdU-positive cells increased significantly 3 days after transfection with the full-length epiprofin vectors, and the nuclei of these cells were also stained with antibody to Myc, which was used as a tag sequence for recombinant epiprofin (Fig. 9). Transfection of the N-terminally truncated vector containing the zinc finger domain but lacking the N-terminal region showed no increase in the numbers of BrdU-positive cells, similar to the control empty vector. We next quantified cell numbers in a time course after transfection (Fig. 10). Full-length epiprofin enhanced cell numbers by greater than 2-fold within 4 days after transfection, whereas N-terminal truncated epiprofin did not. This finding is consistent with the results of BrdU incorporation analysis (compare Figs. 9 and 10). Similar to COS7 cells, we found that the full-length epiprofin but not the truncated epiprofin promotes cell proliferation of primary dental epithelial cells that express endogenous epiprofin. These results suggest that epiprofin may be a positive regulator of cell proliferation.

DISCUSSION

Although epiprofin cDNA was isolated from a molar tooth germ cDNA library by differential screening, it was encoded by a gene that had previously been reported to code for Sp6/KLF14, a new member of the Krüppel-like protein family identified by a zinc finger motif search through GenBank™ (1). The full-length epiprofin cDNA is different in its 5' sequence from the proposed 5' sequence of the mouse Sp6/KLF14 cDNA derived from composites of two expressed sequence tags and the mouse gene. This difference is due to misprediction of the location of presumptive exon 1 in the gene by computer analysis. The location of this predicted sequence is actually within intron 1 about 2.5 kb upstream of exon 2. The real exon 1 is located 5 kb further upstream of this sequence. The exon 1 sequence matches the 42-bp 5' sequence of epiprofin cDNA and is flanked by consensus Cap and splicing signals. The 5'-flanking and exon 1 sequences are highly conserved between mouse and human. Because primary cells from the tissues expressing epiprofin are difficult to obtain and no reliable cell lines are available, we cannot examine tissue-specific promoter activity of the 5' sequence by transfection assays. However, a reporter gene construct with SV40 enhancer demonstrates promoter activity of the 5'-flanking sequence of the epiprofin gene in NIH3T3 cells, confirming the 5' end of the gene. Because the ATG in exon 1 is followed by a premature termination codon, exon 1 encodes an untranslated sequence. Exon 2 encodes the entire coding sequence and 3'-untranslated sequence. We prepared a set of primers to examine potential transcript variants of the gene, but we did not find any evidence of alternative splicing that would result in producing a variant containing the 5' 48-bp of the proposed Sp6/KLF14 transcript (data not shown).
effective. Sp3 is apparently required for synthesis of enamel matrix proteins. Transcripts for Sp4 have been detected throughout the central nervous system of the mouse as well as in other tissues, including the dental papilla and dental sac of developing teeth (25). Osterix/Sp7 is expressed in all developing bone and tooth, and it is essential for osteoblast differentiation and bone formation (23). The role of Osterix/Sp7 in tooth development is not known. Expression of epiprofin is predominantly in proliferating dental epithelium but not in the late differentiation stage of ameloblasts. Epiprofin/Sp6/KLF14 shows strong sequence homology in the zinc finger domain to other Sp proteins. It binds to G/C-rich DNA sequences sharing common binding characteristics to the Sp/Krüppel family proteins (data not shown). However, the proline-rich N-terminal region, the presumptive activation domain of epiprofin, does not show similarity with other family proteins. This regulatory domain may consequently provide unique functions in tooth development distinct from other Sp proteins.

We found in transfection assays that epiprofin promotes cell proliferation in both non-epiprofin-expressing COS7 cells and epiprofin-expressing dental epithelial cells. This growth-promoting activity of epiprofin is consistent with the expression of epiprofin in proliferating epithelial cells in developing teeth, limbs, and hair follicles. Thus, epiprofin likely regulates cell growth in these tissues. KLF4/GKLF/EZEF and KLF5/BTEB2/IKLF are known to regulate cell proliferation in an opposite manner (26–31). KLF4 is expressed in differentiating epithelial cells of intestine and skin, and it is associated with growth arrest and is required for the barrier function of the skin (16, 32). In contrast, KLF5 is enriched in basal keratinocytes and cells of the matrix and inner root sheath and is known to promote cell proliferation (28). Although both epiprofin and KLF5 are expressed in the matrix of hair follicles and both promote cell proliferation, epiprofin is restricted to the matrix cells and is not present in other hair follicle cells and epidermal cells (27). Although the reason for the redundancy of epiprofin and KLF5 in the matrix is not clear, it is possible that these protein factors might promote cell differentiation through different signaling pathways for effective cell growth and its regulation. It is also conceivable that their activity may be regulated by post-translational modification (28, 33). The identification of the mechanism by which epiprofin promotes cell proliferation may clarify a distinct role in hair follicle formation.

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REFERENCES
1. Scohy, S., Gabant, P., Van Reeth, T., Hertveldt, V., Dreze, P. L., Van Vooren, P., Riviere, M., Szpirer, J., and Szpirer, C. (2000) Genomics 70, 93–101
2. Aberg, T., Wozney, J., and Thesleff, I. (1997) Dev. Dyn. 210, 383–396
3. Jernvall, J., and Thesleff, I. (2000) Mech. Dev. 92, 19–29
4. Cohn, S. A. (1957) Am. J. Anat. 101, 220–295
5. Leblond, C. P., and Warshawsky, H. (1979) J. Dent. Res. 58, 950–975
6. Warshawsky, H., and Smith, C. E. (1982) Anat. Rec. 207, 423–436
7. Smith, C. E. (1984) Annu. Rev. Cell Biol. 10, 289–300
8. Turner, C. A., Jr., Mack, D. H., and Davis, M. M. (1994) Cell 77, 297–306
9. Phillips, S., and Suske, G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10692–10696
10. Kaczynski, J., Cook, T., and Urrutia, R. (2003) Genome Biol. 4, 206
11. Kadonaga, J. T., Carner, K. R., Masiarz, F. R., and Tjian, R. (1987) Cell 51,
Epithelial-specific Krüppel-like Factor Epiprofin

12. Imataka, H., Sogawa, K., Yasumoto, K., Kikuchi, Y., Sasano, K., Kobayashi, A., Hayami, M., and Fuji-Kuriyama, Y. (1992) EMBO J. 11, 3663–3671

13. Miller, I. J., and Bieker, J. J. (1993) Mol. Cell. Biol. 13, 2776–2786

14. Anderson, K. P., Kern, C. B., Crable, S. C., and Lingrel, J. B. (1995) Mol. Cell. Biol. 15, 5957–5965

15. Bouwman, P., Gollner, H., Elsasser, H. P., Eckhoff, G., Karis, A., Grosveld, F., Philipsen, S., and Suske, G. (2000) EMBO J. 19, 655–661

16. Segre, J. A., Bauer, C., and Fuchs, E. (1999) Nat. Genet. 22, 356–360

17. Shalon, D., Smith, S. J., and Brown, P. O. (1996) Genome Res. 6, 639–645

18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Extraction, Purification and Analysis of Messenger RNA from Eukaryotic Cells: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

19. Hirota, S., Ino, A., Morii, E., Wanaka, A., Tehyama, M., Kitamura, Y., and Nomura, S. (1992) Brain Res. Mol. Brain Res. 15, 47–54

20. Nieto, M. A., Patel, K., and Wilkinson, D. G. (1996) Methods Cell Biol. 51, 219–225

21. Kosak, M. (1989) J. Cell Biol. 108, 229–241

22. Black, A. R., Black, J. D., and Azizkhan-Clifford, J. (2001) J. Cell. Physiol. 188, 143–160

23. Nakashima, K., Zhou, X., Kunkel, G., Zhang, Z., Deng, J. M., Bebringer, R. R., and de Crombrugghe, B. (2002) Cell 108, 17–29

24. Marin, M., Karis, A., Visser, P., Grosveld, F., and Philipsen, S. (1997) Cell 89, 619–628

25. Supp, D. M., Witte, D. P., Branford, W. W., Smith, E. P., and Potter, S. S. (1996) Dev. Biol. 176, 284–299

26. Chen, X., Johns, D. C., Geiman, D. E., Marban, E., Dang, D. T., Hamlin, G., Sun, R., and Yang, V. W. (2001) J. Biol. Chem. 276, 30425–30428

27. Sun, R., Chen, X., and Yang, V. W. (2001) J. Biol. Chem. 276, 6897–6900

28. Sur, I., Unden, A. B., and Toftgard, R. (2002) Eur. J. Cell Biol. 81, 323–334

29. Dang, D. T., Mahatan, C. S., Dang, L. H., Agboola, I. A., and Yang, V. W. (2001) Oncogene 20, 4884–4890

30. Dang, D. T., Zhao, W., Mahatan, C. S., Geiman, D. E., and Yang, V. W. (2002) Nucleic Acids Res. 30, 2736–2741

31. Yoon, H. S., Chen, X., and Yang, V. W. (2003) J. Biol. Chem. 278, 2101–2105

32. Jaubert, J., Cheng, J., and Segre, J. A. (2003) Development 130, 2767–2777

33. Zhang, Z., and Teng, C. T. (2003) Nucleic Acids Res. 31, 2196–2208