SHH signaling mediated by a prechordal and brain enhancer controls forebrain organization

Tomoko Sagai,a,b, Takanori Amano,a,c,d, Akiteru Maeno,a,e, Rieko Ajima,f,g, and Toshihiko Shiroishi1a,d,h

A n early event of organization of the vertebrate central nervous system is the inductive action of the axial mesoderm on differentiation of the neural ectoderm (1, 2). An anterior part of the axial mesoderm referred to as the prechordal plate (PrCP) is crucial for formation of the forebrain (3–5), which consists of 2 subdivisions, the telencephalon and diencephalon. Sonic hedgehog (SHH) is a major signaling molecule that promotes regionalization of the embryo brain along the anteroposterior axis (6) as well as the dorsoventral axis (9–12). SHH is expressed throughout the axial mesoderm, including the PrCP and the notochord. Surgical removal of the PrCP from chick, mouse, and amphibian embryos revealed that prechordal SHH expression is necessary for differentiation and growth of the forebrain, suggesting that the PrCP is an early organizing center for brain development (4, 13–15). SHH protein produced in the PrCP is secreted dorsally to induce SHH expression in the ventral midline of the forebrain (6). Transition of the signal from the prechordal SHH to the neuronal secondary source of SHH is an essential event in the cascade of brain formation (6, 13).

Six brain enhancers for SHH, named SBE1 to SBE6, have been identified in the genomic region spanning the SHH and Lmbr1 coding sequences (7, 16–19). Two of these, SBE1 and SBE5, located in an intron of SHH and Lmbr1, direct SHH expression in the ventral midline of the posterior forebrain and midbrain, respectively (18, 20). A screen for enhancers upstream of the SHH coding sequence uncovered a cluster of forebrain enhancers, SBE2, SBE3, and SBE4. When a transgenic LacZ reporter is flanked by SBE2 and SBE3, the enhancers drive reporter expression in the anterior diencephalon and the anterior portion of the telencephalon, respectively, while SBE4 drives the transgenic reporter expression in both diencephalon and telencephalon (17). These nested expressions driven by the 3 forebrain enhancers recapitulate the endogenous expression of SHH in the forebrain (17). Although the enhancers that direct neuronal SHH expression in telencephalon and diencephalon have been identified, and some of the upstream transcription factors (TFs) for these enhancers have been elucidated (21, 22), the entire spatiotemporal regulation of SHH is not yet fully understood. In particular, enhancer(s) that regulate SHH expression in the axial mesoderm including the PrCP remain to be elucidated. Recent genome-wide screenings around the SHH locus suggested the presence of 4 notochord enhancers in the vicinity of the known forebrain enhancers and in more-upstream regions of the SHH locus (23).

In the present study, we identified a forebrain enhancer in the vicinity of the forebrain enhancer cluster, and named it SBE7. It directs SHH expression not only in the ventral midline of the

Significance

Identification of enhancers responsible for tissue-specific expression is essential for an in-depth understanding of developmental gene regulation and of the etiology of congenital malformation caused by defective gene function. SHH is indispensable for forebrain and hypothalamic development, and its deletion causes severe craniofacial defects observed in human holoprosencephaly (HPE). SHH signaling starts with SHH expression in the prechordal plate (PrCP), and this SHH then induces SHH signaling in the forebrain. Despite its importance, no enhancer responsible for SHH expression in the PrCP has yet been identified. We report here a brain enhancer, named SBE7. Mice lacking SBE7 lost SHH expression in both the PrCP and the ventral midline of the forebrain, exhibiting developmental defects similar to HPE.
forebrain but also in the PrCP. When SBE7 was eliminated from the mouse genome, Shh expression in both the anterior forebrain and the PrCP was significantly reduced. As a consequence, the mutant embryos exhibited severe malformation of diencephalon-derived brain structures such as the hypothalamus and the midline structure of the forebrain, which are similar to a mild form of human holoprosencephaly (HPE) (24). Our results showed that the identified enhancer is essential to induce Shh expression in the PrCP, and, in turn, to induce secondary Shh expression in the rostral and ventral midline of the forebrain overlaying the PrCP, and that SBE7-mediated SHH signaling from the PrCP is crucial for development of the rostral and midline structures of the forebrain and hypothalamus.

Results

A Brain Enhancer in the Shh Regulatory Region. The University of California, Santa Cruz (UCSC) public database provides epigenetic information on active enhancer markers (http://genome.ucsc.edu) (25, 26). Chromatin immunoprecipitation sequencing (ChIP-seq) data for the mouse embryonic brain at E14.5 using histone H3K4me1 and H3K27ac antibodies revealed crowded signal peaks in a region between the H3K4me1 and H3K27ac antibodies revealed crowded signal peaks data for the mouse embryonic brain at E14.5 using histone (25, 26). Chromatin immunoprecipitation sequencing (ChIP-seq) information on active enhancer markers (http://genome.ucsc.edu/) California, Santa Cruz (UCSC) public database provides epigenetic

![Image](https://example.com/image.png)

**Fig. 1.** Identification of a forebrain enhancer. (A) Genomic locations of the 6 known forebrain enhancers in the Shh regulatory region. SBE1 and SBE5 are intrinsic enhancers in the Shh and Lmbr1 genes, respectively. The other 4 enhancers are located in the intergenic region. (B) ChIP-seq data on the embryonic brain at E14.5 with anti-H3K27ac and anti-H3K4me1 antibodies uploaded in the UCSC genome browser. Several signal peaks were found upstream of the Shh locus. (Lower) 6 plots showing an enlarged view of the cluster harboring 3 forebrain enhancers, shaded in 2 first (Upper) plots. (Bottom) 4 plots showing phylogenetically conserved regions detected by PhyloP and PhastCons. (C–G) Transgenic reporter expression driven by e1, e2, e3, e4, and SBE2 genome fragments at E10.5. The number of LacZ-positive embryos among the total number of embryos carrying a transgene is indicated (Bottom Right). (H) A sagittal section of the e2 transgenic brain. Di, diencephalon; Mb, midbrain; Nc, notochord; Lu, lung; Us, upper stomach; Rp, Rathke’s pouch; I, infundibulum. (Scale bar, 0.5 mm.)
In contrast, no reproducible expression of LacZ was observed in the SBE2-LacZ transgenic mouse at the same stage, suggesting that SBE2-LacZ alone fails to direct endogenous Shh expression after E8.5 (SI Appendix, Fig. S3). Given that SBE7 drives reporter expression at early stages in the mouse embryo, we investigated expression at earlier embryonic stages. From the early bud stage (E7.5) to the late head fold stage (E7.75), reporter expression was observed in the caudal axial mesoderm and node (Fig. 2 D–H). Moreover, 2 of the LacZ-positive embryos showed the prechordal expression of LacZ. The SBE7 regulatory activity recapitulated the endogenous expression at early stages (27).

SBE7 Is Essential for Shh Expression in the Prechordal Plate and Ventral Midline of the Forebrain. To directly examine the role of SBE7 in Shh regulation and forebrain development, we first eliminated SBE7 alone from the mouse genome using the CRISPR-Cas9 system (Fig. 3A and SI Appendix, Fig. S4A), and established 2 SBE7 deletion mouse lines (Δ7). Endogenous Shh expression was detected in both the PrCP and the ventral midline of the forebrain of wild-type embryos at E8.5 (Fig. 3B and D). In the Δ7 homozygote, Shh expression clearly diminished in the PrCP and the overlying neuroepithelium, suggesting that SBE7 is essential as a prechondral enhancer for Shh expression (Fig. 3C and E). To elucidate the demarcation of Shh regulation between SBE7 and other forebrain enhancers including SBE2, we further generated 2 mouse lines with deletions in the genomic region around the forebrain enhancer cluster. One line, named ΔFC, had a 120-kb deletion including all of the forebrain enhancers, and the other, named Δ234, had a 118-kb deletion encompassing SBE2, SBE3, and SBE4, but retained SBE7 (Fig. 3A and SI Appendix, Fig. S4B and C). The Δ7 and ΔFC homozygous embryos showed abrogation of Shh expression in the telencephalon and the rostral diencephalon (Fig. 3G and H). These 2 deletion mutant lines retained endogenous Shh expression in the ventral midbrain and the caudal diencephalon including the zona limitans intratralamica (ZLI), although SBE7 can drive reporter expression in these regions. Although Δ234 embryos also showed a down-regulation of Shh in the telencephalon, a detectable level of the Shh expression was shown in the rostral diencephalon (Fig. 3I and SI Appendix, Fig. S5B and D).

Elimination of SBE7 Causes Severe Morphological Defects in the Ventral Midline of the Forebrain. Homozygotes of Δ7 died within 2 d after birth (SI Appendix, Table S1), whereas heterozygotes of Δ7 were viable and did not show any outward abnormalities. To establish whether downstream regulation of Shh in the forebrain impacts normal brain formation, we performed a 3D morphological analysis of E16.5 mouse brains using X-ray microcomputed tomography (micro-CT). A 3D reconstruction of micro-CT images revealed that homozygotes of Δ7 and ΔFC showed facial hypoplasia with reduced binocular distance, nostril obstruction, loss of philtrum, and cleft lip (Fig. 4 and SI Appendix, Fig. S6). These phenotypes are typical midline defects observed in HPE. In contrast, facial structures of the Δ234 mouse were virtually normal at E16.5.

Virtual sections of the CT images revealed that brains of the Δ7 and ΔFC homozygotes had expanded third and lateral ventricles, with deformation of the septum and the midline of the hypothalamus (Fig. 4 and SI Appendix, Fig. S7A and B). Furthermore, we observed absence or hypoplasia of the olfactory bulb and the pituitary gland in both mutants. In facial organs, the Δ7 and ΔFC homozygous embryos showed a thin nasal septum, a short nasal cavity, and abnormally shaped eyes with absence of the optic chiasma. The Δ7 homozygous embryo (SI Appendix, Table S2). Three out of 8 Δ7 homozygous embryos showed the severe phenotype, nearly equivalent to that of the ΔFC homozygous embryo. The remaining 5 showed no abnormality in the brain septum, the lateral ventricles, the nasal septum, or the olfactory bulb (SI Appendix, Table S2). In contrast, Δ234 homozygous embryos showed little abnormality in the brain and facial structures, but their third ventricle was slightly expanded (Fig. 4 and SI Appendix, Fig. S7C and Table S2).

Since the Δ234 homozygote was viable after birth and showed far less severe abnormality in craniofacial development at E16.5, we further analyzed the phenotype of adult Δ234 mice (n = 3) (SI Appendix, Fig. S8). The Δ234 homozygote had a significantly smaller body size than the heterozygote and wild-type mouse at 1 mo of age (SI Appendix, Fig. S8A). Micro-CT analysis revealed that the Δ234 homozygote has a hypoplastic pituitary gland and a small nasal septum (SI Appendix, Fig. S8B and C). Therefore, one or a combination of the 3 enhancers, SBE2, SBE3, and SBE4 (or perhaps some unknown regulatory element residing within the 118-kb deletion), probably provides the Shh regulation required for pituitary and nasal development.

SIX3 Is Not Required for SBE7-Mediated Shh Regulation in the PrCP or the Hypothalamus. It is known from previous studies that SIX3, SOX2, and SOX3 are upstream TFs of the Shh expression
through SBE2 binding (21, 22). Inactivation of Six3 results in a failure to activate Shh expression in the ventral forebrain, and consequently causes HPE (28). To test the possibility that SIX3 and SOX2 and SOX3 also regulates SBE7 activity, we first searched for potential TF-binding sites in the SBE7 sequence by using the JASPAR database (29). We could not find typical SIX3-binding sites but found 3 potential sites for SIX1 and SIX2 and one binding site for SOX2 and SOX3 (SI Appendix, Fig. S9). We introduced a dysfunctional mutation into all 3 potential SIX-binding sites or the SOX2 and SOX3 binding site, respectively. Transgenic reporter assays revealed that the SBE7-driven reporter expression was not affected by mutations in these binding sites (SI Appendix, Fig. S9A and B). In addition, the Six3 expression level was unchanged in the Δ7 homozygote embryo (SI Appendix, Fig. S9C). SBE7 is unlikely regulated by direct binding of SIX3, and thereby may not be involved in the feedback regulation between Shh and Six3 in the forebrain (SI Appendix, Fig. S10). To functionally dissect SBE7, we generated 2 deletion constructs of SBE7 and carried out transgenic reporter assay (SI Appendix, Fig. S9A). A 514-bp SBE7 construct drove reporter expression similar to that driven by the full-length SBE7 (SI Appendix, Fig. S9B), whereas a 486-bp SBE7 construct did not show specific expression in the forebrain (SI Appendix, Fig. S9B). The result suggested that a 190-bp fragment at the right side of the 514-bp SBE7 construct, which contains an evolutionary conserved sequence block across mammals (SI Appendix, Fig. S9A), is required for regulatory activity in the forebrain. To understand interactions between SBE7 and HPE- and SOD-causative genes other than Six3, Sox2, and Sox3, we sought potential TF-binding sites for ZIC2, TGIF, HESX1, and OTX2 within the 190 bp. Although a binding site for HESX1 was found in the 190 bp, abolishment of the binding site by introducing a mutation did not influence the reporter expression in transgenic mice (SI Appendix, Fig. S9A and B).

**Discussion**

SBE7 Is an Enhancer That Regulates Shh Expression in the Diencephalon, Mesencephalon, and Prechordal Plate. SBE7 is located within the forebrain enhancer cluster, and turns out to possess a broad regulatory activity from the diencephalon to mesencephalon (SI Appendix, Fig. S11). We note that SBE7 directs Shh expression in the PrCP, which is the most anterior tip of the axial mesoderm, as well as in the diencephalon. A deletion of SBE7 strikingly diminished Shh expression in the PrCP, but not in the notochord; this indicates that SBE7 is indispensable for Shh regulation in the PrCP, and that there is another enhancer for Shh expression in the notochord. SHH protein produced in the PrCP is secreted dorsally and induces an Shh-expressing cell population at the rostral and ventral midline of the neuroepithelium (6, 13, 30). This induced cell population acts as the secondary signaling center for forebrain organization. Indeed, the single deletion of SBE7 resulted in abrogation of Shh expression in
the midline of the diencephalon overlying the PrCP, leading to marked morphological defects in the ventral midline of the forebrain, and eventually developmental anomalies of the hypothalamus.

In contrast to the phenotype of SBE7 deficiency, the brain of Δ234 exhibited far less severe morphological anomalies (Fig. 4). Likewise, it is known that a mutant with a deletion of SBE1, which directs Shh expression in the mesencephalon and in the posterior diencephalon including ZLI, shows no obvious defects in brain formation, although Shh was down-regulated in a part of the mesencephalon and diencephalon (20). Loss of SBE1 may be compensated by one or more redundant enhancers including SBE5 (18). Taking these observations together, it appears that the forebrain enhancers including SBE7 have redundant Shh-regulating activity. Notably, SBE7 is necessary for the normal expression of Shh in the ventral diencephalon. It is possible that the SHH protein produced by SBE7 in the PrCP, the first signaling center, is indispensable for induction of Shh in the forebrain. Alternatively, SBE7 may also play a pivotal role in induction of Shh in the forebrain cooperating with SBE2. In the present study, we could not dissect the enhancer function of SBE7 into the prechordal- and diencephalic-specific activity. The necessity of diencephalic function of SBE7 for the normal brain development is still unclear.

**SBE7 Deficiency Models Semilobar-Type HPE.** Abrogation of Shh expression in the PrCP and the overlying diencephalon caused morphological defects in the midline brain and facial structures, including the hypothalamus, septum, optic chiasma, pituitary, olfactory bulb, and nasal septum (Fig. 4 and SI Appendix, Fig. S6), which are also observed in HPE (3, 13) and partially in septooptic dysplasia (SOD) (31). HPE is a midline defect with incomplete separation of the brain hemispheres, and is classified into alobar, semilobar, and lobar types according to the degree of severity. In the alobar type, there is no brain division at all, with one eye, a tubular nose, and cleft lip. The most serious symptom is known as cyclopia (3, 4). Many genetic factors are involved in the onset of HPE. Mutations in Shh, ZIC2, TGIF, and SIX3 are listed as genetic causalities of HPE (32–36). It is well known that Six3 regulates Shh expression through its direct binding to one of the forebrain enhancers, SBE2 (21). The common features of SOD are abnormal structures along the midline of the brain, optic nerve, pituitary, septum pellucidum, and corpus callosum (31, 37). Mutations of HESX1, OTX2, SOX2, and SOX3 are known to cause SOD (38). Mouse mutants generated by conditional gene targeting with Cre drivers of SBE1 and SBE2 (22) displayed abrogated Shh expression specifically in the ventral midline of the rostral diencephalon, including the hypothalamus primordium, while leaving Shh expression in the ventral midline of the diencephalon intact (41, 42). All of these mutants showed defects in growth of the hypothalamus and in specification of the basal portion of the forebrain (43). Notably, the mutant mouse lacking Shh expression by SBE2-mediated Cre recombination is reported as a model of SOD, in which Shh regulation by the Sox21 family members Sox2 and Sox3 is impaired specifically in the ventral diencephalon (22). The homozygous SBE7 deletion mutant shows cleft lip, orbital hypertelorism (narrow space between the eyes), and no philtrum, in addition to defects in the pituitary, optic nerve, and midline forebrain that are commonly found in SOD. It is most likely that Shh down-regulation in the ventral midline of the forebrain is at least one of the common etiologies of HPE and SOD. Considering the broader spectrum of the SOD model and SBE7-deficient mice, the phenotypic difference between HPE and SOD may be due to a spatiotemporal difference in Shh down-regulation: Early and prechordal down-regulation is responsible for the incomplete separation of the 2 cerebral hemispheres in HPE, and late and diencephalic down-regulation is responsible for the hypothalamic and midline forebrain defects in SOD. Taking all of these observations into account, it is appropriate to classify the phenotype observed in the SBE7-deficient mutant as semilobar-type HPE (24, 44). It will be intriguing to test whether familial semilobar-type HPE patients have mutations in their SBE7 sequences.

Homozygotes of Δ7 lost Shh expression in the PrCP and the overlying diencephalon (Fig. 3), and Δ7 and ΔFC homozygotes developed semilobar-type HPE, but not alobar-type HPE (Fig. 4). This result is not consistent with a previous report that about half of the mice whose PrCP was surgically removed developed alobar-type HPE (13). Large phenotypic variability is a marked feature of HPE and mouse models of HPE. Multiple genetic factors are involved in HPE pathogenesis, and different genetic backgrounds in human patients and mouse mutant lines may explain the penetrance of HPE and its phenotypic variability (24, 35). Several Six3 conditional mouse mutants exhibited semilobar-type HPE in the genetic background of C57BL/6 strain, but a far more severe form of HPE...
was observed in embryos with mixed genetic backgrounds (28). Given that the genetic background of Δ7 and ΔFC is C57BL/6, even more severe phenotypes may be seen when the deletion is placed in a mixed genetic background. Alternatively, the PrCP may provide a signaling molecule other than SHH that is required for normal forebrain and facial development. For instance, BMP7 secreted from the PrCP is involved in positioning of the hypothalamus along anteroposterior axis in the diencephalon, in combination with SHH signaling (6, 45). Therefore, it is possible that another unknown factor(s) is responsible for proper development of the ventral midline of the rostral diencephalon. Notably, the Δ7 homozygote retains Shh expression in the notochord, suggesting at least one other enhancer to regulate the Shh expression in the axial mesoderm posterior to the PrCP. Thus, we cannot rule out the possibility that an unknown enhancer has activity to regulate the Shh expression at the anterior or posterior tip of the PrCP, which is necessary for normal development of the ventral midline of the rostral diencephalon.

**Context-Dependent Regulation of Shh by Brain Enhancers.** Six3 knockout mice show down-regulation of Shh in the ventral diencephalon and human HPE-like defects in the brain (21, 28). Considering that SHH signaling is necessary for the maintenance of Six3 expression, there is a positive feedback loop between Shh and Six3 that is crucial for normal development of the hypothalamus (28). Shh expression in the diencephalon is down-regulated in a compound mutant of Sox2 and Sox3 (22), whereas the Shh expression is up-regulated by deficiency of Tbx3, which is a negative regulator for Sox2 (46). A ChIP study revealed that SOX2 binds to a Six3 forebrain enhancer located upstream of the Six3 locus (47). Furthermore, compound knockout of Sox2 and Sox3 markedly down-regulated Six3 expression in the diencephalon (47). These findings suggest that SOX2 and SOX3 are TFs to link the feedback loop between Shh and Six3. Since SIX3 and SOX2 bind directly to SBE2 (21, 22), SBE2 is a key mediator of Shh regulation by SIX3 and SOXs in the diencephalon. During brain development, the expression domain of Shh at the midline of ventral brain becomes bilateral at E10.5 onward (17). T-box TFs are involved in lateralization of the medial Shh expression through direct binding to SBE2, which is an intrinsic brain enhancer of Shh, or are involved in blocking SOX2 activity to suppress the Shh expression at the ventral midline (46, 48). In this study, we did not find any evidence that SIX3 or SOX proteins act as TFs in the regulation of Shh through SBE7 in forebrain development. In fact, SBE7 itself did not reproduce the lateralized expression of Shh, probably due to the absence of functional SOX2 binding sites within the sequence. Therefore, our results indicate that Shh expression in the forebrain is regulated in 2 distinct contexts, one mediated by SBE2 and SBE2 and the other by SBE7. SBE7 mainly contributes to the early prechoral expression of Shh and the subsequent induction of neural Shh at the midline of forebrain. SBE2 plays a central role in the diencephalic expression of Shh, especially in the mediolateral transition of Shh to form bilateral stripes at later stages, which is necessary for the growth and differentiation of bilateral hypothalamic areas (17, 21, 22, 46). The 2 modes of enhancer function may act in a coordinated fashion for regulatory dynamics of Shh in the mouse forebrain.

**Materials and Methods**

The sequences of SBE7 and e4 were ascribed to DNA Data Bank of Japan with Accession Numbers LC461025 and LC461026, respectively. An extended description of the materials and methods is presented in **SI Appendix, SI Materials and Methods**.

Animal experiments in this study were performed in accordance with the approved guidelines by the Animal Care and Use Committee of National Institute of Genetics (NIG).

**Acknowledgments.** We thank Dr. A. McMahon for the Shh probes, and Dr. P. Gruss for the Six3 probe. We thank the ENCODE Consortium and Dr. B. Ren laboratory for the Encyclopedia of DNA Elements (ENCODE) dataset. We thank the Mouse Resource Supporting Unit at NIG. We thank H. Nakazawa and N. Yamatani for embryonic manipulation for generation of transcription factor mutants and mouse embryos. Y. Sakakibara for whole-mount in situ hybridization at early embryonic stage. This work was supported by KAKENHI (Grants-in-Aid for Scientific Research) Grants JP24224702 and JP15H04212 from JSPS (Japan Society for the Promotion of Science).

1. A. M. Rowan, C. D. Stern, K. G. Storey, Axial mesendoderm refines rostralcaudal pattern in the chick nervous system. Development 126, 2921–2934 (1999).
2. Y. Tanabe, T. M. Jessell, Diversity and pattern in the developing spinal cord. Science 274, 1115–1122 (1996).
3. C. Chiang et al., Cyclopa and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383, 407–413 (1996).
4. E. M. Pera, M. Kessel, Patterning of the chick forebrain anlage by the prechordal plate. Development 124, 4153–4162 (1997).
5. K. Shimamura, J. L. Rubenstein, Inductive interactions direct early regionalization of the mouse forebrain. Development 124, 2709–2718 (1997).
6. J. K. Dale et al., Cooperation of BMP7 and SHH in the induction of forebrain ventral midline cells by prechordal mesoderm. Cell 90, 257–269 (1997).
7. D. J. Epstein, A. P. McMahon, A. L. Joyner, Regionalization of Sonic hedgehog transcription along the anteroposterior axis of the mouse central nervous system is regulated by Hnf1-dependent and -independent mechanisms. Development 126, 281–292 (1999).
8. W. Ye, K. Shimamura, J. L. Rubenstein, M. A. Hynes, A. Rosenthal, FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. Cell 93, 755–766 (1998).
9. J. Ericson, J. Muhr, T. M. Jessell, T. Edlund, Sonic hedgehog: A common signal for ventral patterning along the caudorostral axis of the neural tube. Int. J. Dev. Biol. 39, 809–816 (1995).
10. J. Ericson et al., Sonic hedgehog induces the differentiation of ventral forebrain neurons: A common signal for ventral patterning within the neural tube. Cell 81, 747– 756 (1995).
11. E. Marti, D. A. Bumcrot, R. Takada, A. P. McMahon, Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants. Nature 375, 322–325 (1995).
12. R. Krumlauf et al., Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis. Cell 81, 445–455 (1995).
13. K. Aoto et al., Mouse Shh is required for prechordal plate maintenance during brain and craniofacial morphogenesis. Dev. Biol. 327, 106–120 (2009).
14. H. Li, L. C. Tien, L. Wen, J. Y. Wu, Y. Rao, A single morphogenetic field gives rise to two retina primordia under the influence of the prechordal plate. Development 124, 603–615 (1997).
15. V. A. Schneider, M. Merola, Spatially distinct head and heart inducers within the Xenopus organizer region. Curr. Biol. 9, 800–809 (1999).
16. N. S. Benabdallah et al., SBE6: A novel long-range enhancer involved in driving sonic hedgehog expression in neural progenitor cells. Open Biol. 6, 160197 (2016).
17. V. Yeong, A functional screen for sonic hedgehog regulatory elements across a 1 Mb interval identifies long-range ventral forebrain enhancers. Development 133, 761–772 (2006).
18. Y. Yao et al., cis-regulatory architecture of a brain signaling center predates the origin of chordates. Nat. Genet. 48, 575–580 (2016).
19. N. Tsukiji, T. Amano, T. Shiroishi, A novel regulatory element for Shh expression in the lung and gut of mouse embryos. Mech. Dev. 131, 127–136 (2014).
20. Y. Yeong et al., Spatial and temporal requirements for sonic hedgehog in the regulation of thalamic interneuron identity. Development 138, 531–541 (2011).
21. V. Yeong et al., Regulation of a remote Shh forebrain enhancer by the SIX3 homeobox protein. Nat. Genet. 40, 1348–1353 (2008).
22. L. Zhao et al., Disruption of SoxB1-dependent Sonic hedgehog expression in the hypothalamus causes septo-optic dysplasia. Dev. Cell 22, 585–596 (2012).
23. O. Symmons et al., The Shh topological domain facilitates the action of remote enhancers by reducing the effects of genomic distances. Dev. Cell 39, 529–542 (2016).
24. T. C. Winter, A. M. Kennedy, P. J. Woodward, Holoprosencephaly: A survey of the mouse model, with embryology and fetal imaging. Radiographics 35, 275–290 (2015).
25. W. J. Kent et al., The human genome browser at UCSC. Genome Res. 12, 996–1006 (2002).
26. E. P. Consortium, ENCODE Project Consortium, An integrated encyclopedia of DNA elements in the human genome. Nature 489, 51–74 (2012).
27. Y. Escherlich et al., Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 75, 1417–1430 (1993).
28. X. Geng et al., Haploinsufficiency of Six3 fails to activate Sonic hedgehog expression in the ventral forebrain and causes holoprosencephaly. Dev. Cell 15, 236–247 (2008).
29. A. Khan et al., JASPAR 2018: Update of the open-access database of transcription factor binding profiles and its web framework. Nucleic Acids Res. 46, D260–D266 (2018).
30. K. Ohyama, P. Ellis, S. Kimura, M. Placzek, Directed differentiation of neural cells to hypothalamic dopaminergic neurons. Development 132, 5185–5197 (2005).
31. D. Kelberman, M. T. Dattani, Septo-optic dysplasia—Novel insights into the aetiology. Horm. Res. 69, 257–265 (2008).
32. R. Houtmeyers et al., Zic2 mutation causes holoprosencephaly via disruption of NODAL signalling. Hum. Mol. Genet. 25, 3946–3959 (2016).
33. L. Nanni, R. L. Schelper, M. T. Muenke, Molecular genetics of holoprosencephaly. Front. Biosci. S, D334–D342 (2000).
34. A. D. Paulussen et al., The unfolding clinical spectrum of holoprosencephaly due to mutations in SHH, ZIC2, SIX3 and TGFIR genes. Eur. J. Hum. Genet. 18, 999–1005 (2010).
35. E. Roessler, P. Hu, M. Muenke, Holoprosencephaly in the genomics era. Am. J. Med. Genet. C. Semin. Med. Genet. 178, 165–174 (2018).
36. K. Taniguchi, A. E. Anderson, A. E. Sutherland, D. Wotton, Loss of Tgif function causes holoprosencephaly by disrupting the SHH signaling pathway. PLoS Genet. 8, e1002524 (2012).
37. E. A. Webb, M. T. Dattani, Septo-optic dysplasia. Eur. J. Hum. Genet. 18, 393–397 (2010).
38. D. Kelberman, M. T. Dattani, Genetics of septo-optic dysplasia. Pflügers Arch. 459, 407–415 (2007).
39. T. Zhao, X. Zhou, N. Szabó, M. Leitges, G. Álvarez-Bolado, Foxb1-driven Cre expression in somites and the neuroepithelium of diencephalon, brainstem, and spinal cord. Genesis 45, 781–787 (2007).
40. Q. Xu, M. Tam, S. A. Anderson, Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. J. Comp. Neurol. 506, 16–29 (2008).
41. T. Shimogori et al., A genomic atlas of mouse hypothalamic development. Nat. Neurosci. 13, 767–775 (2010).
42. N. E. Szabó et al., Role of neuroepithelial Sonic hedgehog in hypothalamic patterning. J. Neurosci. 29, 6989–7002 (2009).
43. S. Blaes, N. Szabó, R. Haddad-Tovoli, X. Zhou, G. Álvarez-Bolado, Sonic hedgehog signaling in the development of the mouse hypothalamus. Front. Neuroanat. 8, 156 (2015).
44. X. Geng, S. Acosta, O. Lagutin, H. J. Gil, G. Olivier, Six3 dosage mediates the pathogenesis of holoprosencephaly. Development 143, 4462–4473 (2016).
45. L. Manning et al., Regional morphogenesis in the hypothalamus: A BMP-Tbx2 pathway co-ordinates fate and proliferation through Shh downregulation. Dev. Cell 15, 873–885 (2008).
46. M. O. Trowe et al., Inhibition of Sox2-dependent activation of Shh in the ventral diencephalon by Tbx3 is required for formation of the neurohypophysis. Development 140, 2299–2309 (2013).
47. B. Lee et al., Genomic code for Sox2 binding uncovers its regulatory role in Six3 activation in the forebrain. Dev. Biol. 381, 491–501 (2013).
48. Y. Jeong, D. J. Epstein, Distinct regulators of Shh transcription in the floor plate and notochord indicate separate origins for these tissues in the mouse node. Development 130, 3891–3902 (2003).