

Review

A Median Third Eye: Pineal Gland Retraces Evolution of Vertebrate Photoreceptive Organs[†]

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Received 24 February 2006; accepted 10 June 2006; published online 13 June 2006 DOI: 10.1562/2006-02-24-IR-813

ABSTRACT

In many vertebrates, the pineal gland serves as a photoreceptive neuroendocrine organ. Morphological and functional similarities between the pineal and retinal photoreceptor cells indicate their close evolutionary relationship, and hence the comparative studies on the pineal gland and the retina are the keys to deciphering the evolutionary traces of the vertebrate photoreceptive organs. Several studies have suggested common genetic and molecular mechanisms responsible for their similarities, but largely unknown are those underlying pineal-specific development and physiological functions. Recent studies have identified several *cis*-acting DNA elements that participate in transcriptional control of the pineal-specific genes. Genetic approaches in the zebrafish have also contributed to elucidating the genetic network regulating the pineal development and neurogenesis. These efforts toward elucidating the molecular instrumentation intrinsic to the pineal gland, back to back with those to the retina, should lead to a comprehensive understanding of the evolutionary history of the vertebrate photoreceptive structures. This article summarizes the current status of research on these topics.

INTRODUCTION

The evolutionary processes for the structure/function of various organs in living organisms have been of great interest in molecular, developmental and evolutionary biology. Among these organs, the eyes have attracted particular attention owing to their structural varieties albeit all serving to detect light. A promising approach to cutting this Gordian knot in biology is a comparative analysis of molecular characteristics of the eyes among species. Indeed, a series of genetic studies have demonstrated that a highly conserved transcription factor Pax6 serves as a master control gene for the eye morphogenesis in both vertebrate and invertebrate species (1). This finding strongly supports the idea that various types of the eyes found in the animal kingdom have a

common evolutionary origin, in spite of their significant anatomical dissimilarities. A comparison between the eyes of phylogenetically distant animals, for instance, between the vertebrate camera eye and the insect compound eye, is helpful to reconstruct a hypothetical primitive structure of their common ancestral eye that could have emerged at a very early stage of the evolution. On the other hand, such a comparison provides little information about how the primitive eye has evolved into the modern sophisticated eyes present in various animal taxa.

An insightful inspection of these processes could be accomplished by a comparative analysis between the eyes or eye-like structures that exhibit distinct but sufficiently comparable designs with each other. The extra-ocular photoreceptive structures present in various vertebrate species (2) offer an unparalleled opportunity to explore the unique evolution and diversification of the photoreceptive structures including the camera eye. The most prominent and well-developed extra-ocular photoreceptor is the pineal gland (also referred to as the pineal organ or the epiphysis), an organ that has developed only among vertebrates and retains photoreceptive function in many nonmammalian species. Molecular and phenotypic evidence indicates a close evolutionary relationship between the pineal gland and the neural retina of the eye, particularly within their photoreceptor cell lines. The photosensitive pineal gland of some vertebrate species has a considerably simpler structure that possibly reflects an ancient form of the vertebrate retina, providing an excellent model for comparative developmental studies.

In this review, we focus on recent progress in understanding genetic variation of the pineal photoreceptor cells, regulation of pineal-specific gene expression and development of the pineal gland. On the basis of these molecular data, we discuss a potential application of a cell-type homology approach to the comparative evolutionary studies between the pineal gland and the retina.

INTERSPECIFIC VARIATION OF THE PINEAL GLAND

Similar to the optic vesicle, the pineal gland evaginates from the roof of the diencephalon during development (3). The pineal gland in the adult brain is a midline structure that

[†]This invited paper is part of the Symposium-in-Print: Photobiology in Asia.

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exhibits highly variable shapes among vertebrate species, and is often associated with an accessory organ such as the parapineal organ in lampreys and fishes, the frontal organ in amphibians, and the parietal eye in lacertilian reptiles (3). A well-conserved physiological function of the pineal gland is to rhythmically produce melatonin, an indoleamine hormone that is involved in regulation of biological rhythms (4,5). In mammals, the pineal gland is no longer photosensitive but serves as a neuroendocrine organ, in which the activity of melatonin synthesis is regulated by external neural inputs from the sympathetic nerves. In contrast to the mammalian counterpart, the pineal gland of the other vertebrate classes, such as lampreys, fishes, amphibians, reptiles and birds generally retains photosensitivity, and plays an active role as the photosensory organ in some species. The pineal gland of several animals also contains an intrinsic circadian oscillator, which is entrained to environmental light–dark cycles due to the endogenous photic input pathway (4). These three components of the clock system, *i.e.* the circadian oscillator, the photic input and the melatonin output machineries, all co-exist in individual pineal cells of the chicken and possibly in those of some other vertebrate species (6–8).

The functional transformation of the pineal gland from the photosensory to neuroendocrine organ is closely correlated with changes in its cellular composition and cell morphology (4,9). In lampreys, fishes and amphibians, the pineal photoreceptor cells are endowed with well-developed lamellar outer segments that closely resemble those of their retinal photoreceptor cells. The light signals captured in the pineal photoreceptor cells are not only transduced into intracellular signals regulating melatonin production, but also transmitted to secondary neurons through synaptic contacts within the pineal gland. These secondary afferent neurons, also called the projection neurons, innervate several areas of the central brain, similar to the retinal ganglion cells (10,11). The pineal photoreceptive cells of reptiles and birds are often called modified photoreceptor cells, which possess regressed outer segments with the lamellar structures degenerated to various degrees. The secondary neurons in the pineal gland are less abundant among these species, possibly as a consequence of reduced photosensory function requiring afferent outputs. Finally, the light-insensitive pinealocytes in the mature mammalian pineal gland lack pronounced outer segment-like structures, although they are regarded as a cellular homolog of the pineal photoreceptor cells of the other vertebrates.

The phylogenetic variation in the photoreceptor-related cells of the pineal gland is generally interpreted as a gradual transformation that has occurred in a single cell lineage during the evolution of vertebrates (Fig. 1a) (4). However, Ekström and Meissl (9) recently proposed a new hypothesis that the pineal gland of vertebrates commonly harbors the repertoire of photoreceptor-related cells, and the observed interspecies variation of the cellular composition should originate from ontogenetic changes in cell-fate restriction from progenitor cells during the evolution of vertebrates (Fig. 1b). These two hypotheses are not mutually exclusive, and relative contribution of the two mechanisms remains to be elucidated by further detailed studies, in particular, on the molecular and developmental features of various pineal cell types.

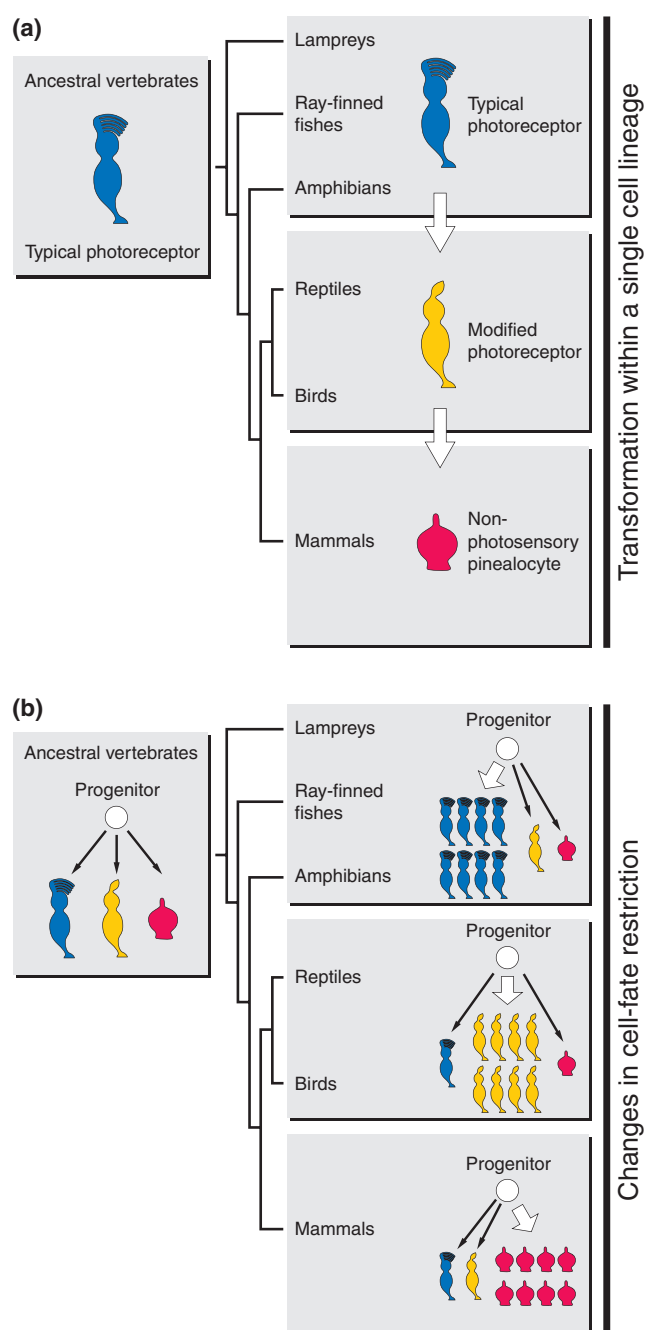


Figure 1. Two hypotheses accounting for the dynamic evolutionary changes of vertebrate pineal cells. (a) Successive regression of photoreceptor phenotype within a single cell lineage. (b) Changes in cell-fate restriction from a common progenitor during development.

MOLECULAR SIMILARITY AND DIVERSITY BETWEEN PINEAL AND RETINAL PHOTORECEPTOR CELLS

In addition to the morphological similarities, the molecular kinship between the pineal and retinal photoreceptor cells underpins their close evolutionary relationship. The pineal cells of the chicken and fishes contain a series of components found in the retinal phototransduction pathway, such as

opsins, transducin (12–14), cGMP-phosphodiesterase (15), cGMP-gated cation channel (16) and interphotoreceptor retinoid-binding protein (14,17). These observations suggest that the pineal photoreceptor cells share a similar phototransduction mechanism with the retinal photoreceptor cells (18). Interestingly, expression of several phototransduction genes is retained in the light-insensitive mammalian pinealocytes (19). This fact supports the idea that the mammalian pinealocytes have evolved from photoreceptor cells (Fig. 1), although the physiological relevance of the remnant expression of these genes remains unclear.

In contrast to these genes expressed in both the pineal and the retinal photoreceptor cells, a subset of genes that are expressed selectively in either of the two organs reflect their independent evolutionary paths. Chicken pinopsin with a blue-light sensitivity was the first example of pineal-specific opsin that is closely related to but distant from the retinal opsins (20,21), and pinopsin has a chimeric biochemical property between the rod and cone visual pigments (22). In the chicken, pineal opsin(s) is functionally coupled with two different types of G-proteins, G_{i1} (13) and G_{i11} (23), which mediate acute inhibition of melatonin synthesis and entrainment of the intrinsic circadian oscillator, respectively. The pineal expression of pinopsin gene has also been found in other birds (24,25) and reptiles (25,26), but is very weak or undetectable in the other vertebrate classes (25). Another variant form of the pineal-specific opsin is exo-rhodopsin (Exorh) that was originally identified in the pineal gland of the zebrafish (27). This animal is unique in that it possesses two duplicated rhodopsin genes, *exorh* and retinal *rhodopsin*, which are expressed selectively in the pineal photoreceptor cells and the retinal rods, respectively (27). Phylogenetic analyses indicated the occurrence of *exorh* gene early in the ray-finned fish lineage (27,28), and pineal-specific expression of an *exorh* ortholog (extra-retinal rod-like opsin) has also been demonstrated in the Atlantic salmon (28). These observations, together with the fact that the rhodopsin-like immunoreactivities have been detected in the pineal gland of various teleost species (9,29,30), strongly suggest that teleosts generally have exo-rhodopsin gene that is expressed in a pineal-specific manner. In contrast to these pineal-specific opsins, several opsins such as red (20,26,27,31), green (26,32), blue (26) and ultraviolet (26,32) cone opsins are detected in both the pineal gland and the retina. Expression of the multiple opsin genes within a single pineal gland is consistent with immunohistochemical studies demonstrating heterogeneity of the pineal photoreceptor cells in terms of the opsin distribution (9,24,33,34), while the majority of the pineal photoreceptor cells of the chicken (24) and the zebrafish (27) express pineal-specific opsins.

Recent studies also identified novel members of non-visual type opsins possessing more peculiar features in the pineal gland of some vertebrate species. The lamprey pineal gland and parapineal organ express parapinopsin, which shows a reversible photoreaction with its stable photoproduct, an interesting feature common to those of invertebrate rhodopsins (35). In the chicken, both the pineal gland and the retina express melanopsin (36,37), which is remarkably distant from the vertebrate canonical opsins in molecular phylogeny, possibly suggesting an additional aspect of their molecular similarity.

REGULATION OF PINEAL-SPECIFIC GENE EXPRESSION

Tissue- and cell type-specific control of gene expression is expected to play a pivotal role in generating the molecular basis for physiological characteristics that are similar or different between the pineal and retinal photoreceptor cells. One of the key regulators responsible for both pineal and retinal photoreceptor cell-specific gene expression is cone rod homeobox (Crx), an Otx-related homeodomain transcription factor that binds to bicoid-type TAATCC recognition sequences (38,39). In the retina, Crx transactivates photoreceptor cell-specific genes including opsin genes, and regulates photoreceptor cell differentiation (38–40). Both genetic (40) and *in vitro* (41) studies in rodents demonstrated that Crx also participates in the transcriptional control of several pineal (-specific) genes such as *arylalkylamine N-acetyl transferase (aanat)*. Similarly, circadian gene expression in the zebrafish pineal gland requires Otx5, which is closely related to Crx (17). These findings suggest that Crx/Otx5 serves as a common genetic instruction that contributes to the overall similarity between the pineal and retinal photoreceptor cells.

Although little is known about the molecular mechanisms directing specificity difference between the pineal gland and the retina, recent studies identified several *cis*-acting DNA elements that are involved in regulation of pineal-specific gene expression. A detailed mutation analysis of the zebrafish *exorh* promoter demonstrated that the pineal-specific activity of this promoter requires not only putative Crx/Otx binding sites, but also a 12-bp sequence (TGACCCCAATCT) designated PIPE, an abbreviation of pineal expression-promoting element (42). Chimeric rhodopsin promoters appended with the PIPE sequence in a foreign position can induce the gene expression in the pineal gland in addition to the retinal photoreceptor cells. These findings suggest that Otx5 and uncharacterized PIPE-binding protein(s) constitute a combinatorial code for the pineal photoreceptor cell-specificity.

Another pineal gene, zebrafish *aanat2* (43) appears to be transactivated synergistically by a combinatorial action of Otx5 and BMAL/CLOCK heterodimer via PRDM, an abbreviation of pineal-restrictive downstream module that comprises both Crx/Otx-binding sites and a functional CACGTG E-box for BMAL/CLOCK-binding (44–46). Because no circadian variation is observed for *otx5* mRNA levels in the zebrafish pineal gland, circadian regulated genes including *aanat2* is likely under the coordinated control of Otx5 and temporal regulatory factors such as BMAL/CLOCK heterodimer.

Another important regulator for the pineal gene expression is environmental light signal, which is transduced intracellularly and eventually inputs to a DNA regulatory sequence, a light-responsive element abbreviated as LRE. A 18-bp sequence (TGGCACGTGGGGGTTCCTC) present in the promoter of chicken pinopsin gene mediates apparently light-stimulated expression and hence it represents LREs, but interestingly, the sequence contributes to transcriptional repression in the dark (47,48). Similar to the zebrafish PRDM, the pinopsin LRE comprises a CACGTG E-box (underlined in the 18-bp sequence) that constitutes the core sequence required for the light-dependent gene regulation (48). The expression

Chx10 directs specification of bipolar cells (60), while amacrine cell-fate specification requires a different combination of transcription factors, bHLH-type NeuroD/Math3 and homeodomain-type Pax6/Six3 (58). It is therefore likely that particular combinations of bHLH and homeodomain transcription factors control the cell-fate specification in the pineal neurogenesis as well.

EVOLUTION OF VERTEBRATE PHOTORECEPTIVE ORGANS

Diversification of neuronal subtypes should have been a critical factor that defines the evolution of the retina. The existing vertebrate retina is composed of six classes of neurons organized into three cellular layers. Among the retinal neurons, rod and cone photoreceptor cells are generally thought to share a common evolutionary origin, a ciliary photoreceptor precursor, and hence these two classes are sometimes combined into a single one. On the other hand, the origins of the other retinal neurons, *i.e.* bipolar, horizontal, amacrine and ganglion cells remain obscure. A recent attempt to address retinal cell-type homologies within and among animal species led to the hypothesis that the ganglion, amacrine and horizontal cells in the vertebrate retina could be traced back to a common evolutionary precursor (Fig. 4) (61). This hypothesis is supported by a number of similarities of molecules that operate in their developmental processes and physiological functions (61). For example, expression of Pax6 at the later developmental stage is detected in the ganglion, amacrine and horizontal cells but not in the rod, cone and bipolar cells of the vertebrate retina (61,62). More interestingly, the former three neurons probably share a common origin with rhabdomeric photoreceptor cells found in invertebrate retinas (61). This evolutionary scenario implies that the vertebrate retina is composed of at least two types of evolutionary distant neuronal classes, the ciliary and rhabdomeric cells (Fig. 4), and in fact it appears that these two types of photoreceptor cells had already coexisted in the

common ancestor of the deuterostomes and the protostomes (63). A rhabdomeric type opsin, melanopsin, is expressed in the ganglion, amacrine and horizontal cells of some vertebrate species (64–68), providing a supportive evidence for their evolutionary kinship.

The cell-type analysis described above for the retinal neurons could be applied to the pineal neurons, and such a comparative point of view should provide valuable clues to further understanding of the evolutionary processes of the vertebrate photoreceptive organs. Striking similarities between the pineal and retinal photoreceptor cells indicate that they have diverged from a common precursor of the ciliary cell type. Even in the highly transformed state of the mammalian pineal gland, the pinealocyte represents characteristics of the ciliary type retinal photoreceptor cell such that they both produce melatonin (69–71), express a subset of genes for phototransduction components (19), and share developmental regulation depending on Crx/Otx-related transcription factors (40,56). On the other hand, the pineal projection neuron possibly belongs to the rhabdomeric cell type, because it expresses Pax6 at the later stage of the neuronal differentiation just like the retinal ganglion, horizontal and amacrine cells do (Fig. 3) (10). This idea may be supported by the functional similarity between the pineal projection neuron and the retinal ganglion cell, both of which project their axons to the central areas of the brain (10,11). Such parallelism delineates a fundamental composition of the cell lineages common to the pineal gland and the retina, raising the possibility that a common ancestor of the two organs had already consisted of two distantly related cellular components, ciliary and rhabdomeric precursors (Fig. 4). One of the open questions in the cell-type homologies is the enigmatic origin of the retinal bipolar cells (61), which relay neural signals from the photoreceptor cells to the ganglion cells (Fig. 4). Because recruitment of the bipolar cells appears to be a key evolutionary step in order for the vertebrate retina to establish the highly ordered structure of the three-layered neural network, it is worthwhile to unravel

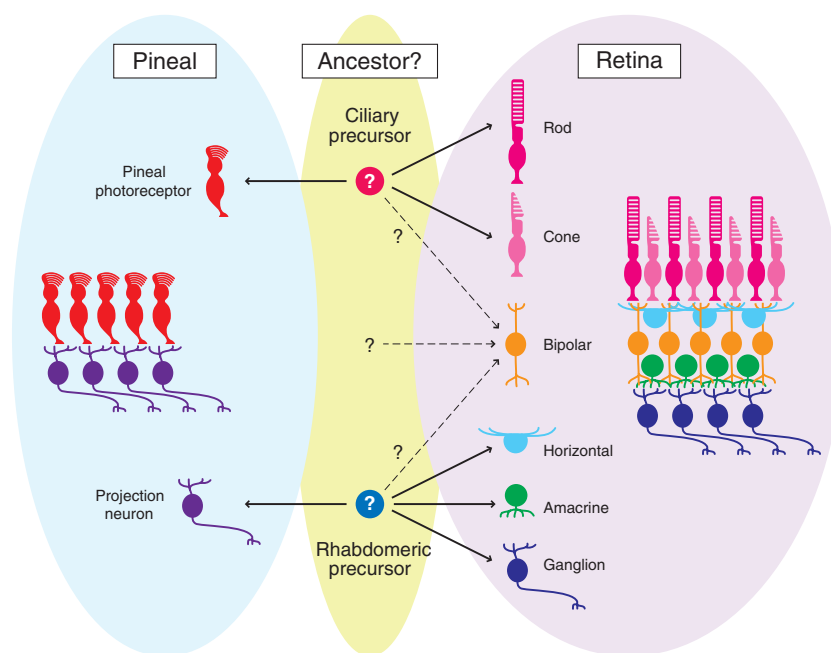


Figure 4. Parallelism of neuronal subtypes between the two typical photoreceptive organs in vertebrates, the retina and the pineal gland.

the origin of the bipolar cells by comparative developmental studies between the retina and the pineal gland, the latter of which lacks a recognizable cellular counterpart to the bipolar cells. At present, only limited information is available as to the molecular characteristics of the pineal cells, preventing a more detailed comparison of the cell-type homologies between the pineal and retinal neurons. Validation and refinement of the evolutionary model require further elucidation of the molecular mechanisms underlying the pineal-specific gene expression, developmental process and physiological functions.

Finally, understanding the mechanism for the pineal neurogenesis, in which both the ciliary and rhabdomeric type cells are generated from the same progenitor cells in a relatively simple manner (Fig. 3), would shed light on the evolution of the developmental processes that integrated the two distantly related cell-types into a single photoreceptive organ. Important clues to the issue may also be obtained by comparative studies on more primitive photoreceptor systems found in cephalochordates (72) and in the deep brain of some vertebrate species (73–76). The analyses of the cell-type homologies and the developmental mechanisms of these systems will ultimately provide a comprehensive and detailed view of the evolution and diversification of the vertebrate photoreceptive structures.

CONCLUDING REMARKS

The molecular analysis of the pineal gland provides a unique opportunity to investigate the evolutionary history of vertebrate photoreceptive organs, which has long attracted great enthusiasm of biologists since Darwin's time. The molecular mechanisms for the pineal development and cell-type specification are just beginning to be elucidated, and considerable progress will certainly be made in the near future. In addition to the molecular comparison among the retinal, pineal and other primitive photoreceptors, comparative studies of the pineal gland itself among species will provide helpful hints as to the mechanisms underlying dynamic evolution of the brain functions in vertebrates. In the zebrafish, the pineal complex has also been studied as a model for the formation of left-right asymmetry in the brain (77). These recent studies represent a trend of pineal research beyond the traditional way of analysis on the circadian clock and photoendocrine mechanisms.

Acknowledgements—This work is supported in part by a research grant from Human Frontier Science Program, and by Grants-in-Aid from the Japanese Ministry of Education, Science, Sports, and Culture.

REFERENCES

- Gehring, W. J. and K. Ikeo (1999) *Pax 6*: Mastering eye morphogenesis and eye evolution. *Trends Genet.* **15**, 371–377.
- Kojima, D. and Y. Fukada (1999) Non-visual photoreception by a variety of vertebrate opsins. *Novartis Found Symp.* **224**, 265–282.
- Oksche, A. (1965) Survey of the development and comparative morphology of the pineal organ. *Prog. Brain Res.* **10**, 3–29.
- Falcón, J. (1999) Cellular circadian clocks in the pineal. *Prog. Neurobiol.* **58**, 121–162.
- Klein, D. C. (2004) The 2004 Aschoff/Pittendrigh lecture: Theory of the origin of the pineal gland—a tale of conflict and resolution. *J. Biol. Rhythms* **19**, 264–279.
- Nakahara, K., N. Murakami, T. Nasu, H. Kuroda and T. Murakami (1997) Individual pineal cells in chick possess photoreceptive, circadian clock and melatonin-synthesizing capacities in vitro. *Brain Res.* **774**, 242–245.
- Natesan, A., L. Geetha and M. Zatz (2002) Rhythm and soul in the avian pineal. *Cell Tissue Res.* **309**, 35–45.
- Okano, T. and Y. Fukada (2003) Chicktacking pineal clock. *J. Biochem.* **134**, 791–797.
- Ekström, P. and H. Meissl (2003) Evolution of photosensory pineal organs in new light: The fate of neuroendocrine photoreceptors. *Phil. Trans. R. Soc. Lond. B* **358**, 1679–1700.
- Masai, I., C.-P. Heisenberg, K. A. Barth, R. Macdonald, S. Adamek and S. W. Wilson (1997) *floating head* and *masterblind* regulate neuronal patterning in the roof of the forebrain. *Neuron* **18**, 43–57.
- Ekström, P. and H. Meissl (1997) The pineal organ of teleost fishes. *Rev. Fish Biol. Fish.* **7**, 199–284.
- Okano, T., K. Yamazaki, T. Kasahara and Y. Fukada (1997) Molecular cloning of heterotrimeric G-protein α -subunits in chicken pineal gland. *J. Mol. Evol.* **44**, S91–S97.
- Kasahara, T., T. Okano, T. Yoshikawa, K. Yamazaki and Y. Fukada (2000) Rod-type transducin α -subunit mediates a photo-transduction pathway in the chicken pineal gland. *J. Neurochem.* **75**, 217–224.
- Shen, Y.-C. and P. A. Raymond (2004) Zebrafish *cone-rod (crx)* homeobox gene promotes retinogenesis. *Dev. Biol.* **269**, 237–251.
- Morin, F., C. Lugnier, J. Kameni and P. Voisin (2001) Expression and role of phosphodiesterase 6 in the chicken pineal gland. *J. Neurochem.* **78**, 88–99.
- Decressac, S., A. Grechez-Cassiau, J. Lenfant, J. Falcón and P. Bois (2002) Cloning, localization and functional properties of a cGMP-gated channel in photoreceptor cells from fish pineal gland. *J. Pineal Res.* **33**, 225–233.
- Gamse, J. T., Y.-C. Shen, C. Thisse, B. Thisse, P. A. Raymond, M. E. Halpern and J. O. Liang (2002) *Otx5* regulates genes that show circadian expression in the zebrafish pineal complex. *Nat. Genet.* **30**, 117–121.
- Meissl, H. (1997) Photic regulation of pineal function. Analogies between retinal and pineal photoreception. *Biol. Cell* **89**, 549–554.
- Blackshaw, S. and S. H. Snyder (1997) Developmental expression pattern of phototransduction components in mammalian pineal implies a light-sensing function. *J. Neurosci.* **17**, 8074–8082.
- Okano, T., T. Yoshizawa and Y. Fukada (1994) Pinopsin is a chicken pineal photoreceptive molecule. *Nature* **372**, 94–97.
- Max, M., P. J. McKinnon, K. J. Seidenman, R. K. Barrett, M. L. Applebury, J. S. Takahashi and R. F. Margolskee (1995) Pineal opsin: A nonvisual opsin expressed in chick pineal. *Science* **267**, 1502–1506.
- Nakamura, A., D. Kojima, H. Imai, A. Terakita, T. Okano, Y. Shichida and Y. Fukada (1999) Chimeric nature of pinopsin between rod and cone visual pigments. *Biochemistry* **38**, 14738–14745.
- Kasahara, T., T. Okano, T. Haga and Y. Fukada (2002) Opsin- G_{11} -mediated signaling pathway for photic entrainment of the chicken pineal circadian clock. *J. Neurosci.* **22**, 7321–7325.
- Okano, T., Y. Takanaka, A. Nakamura, K. Hirunagi, A. Adachi, S. Ebihara and Y. Fukada (1997) Immunocytochemical identification of pinopsin in pineal glands of chicken and pigeon. *Brain Res. Mol. Brain Res.* **50**, 190–196.
- Vigh, B., P. Röhlich, T. Görös, M. J. Manzano e Silva, Á. Szél, Z. Fejér and I. Vigh-Teichmann (1998) The pineal organ as a folded retina: Immunocytochemical localization of opsins. *Biol. Cell* **90**, 653–659.
- Kawamura, S. and S. Yokoyama (1997) Expression of visual and nonvisual opsins in American chameleon. *Vision Res.* **37**, 1867–1871.
- Mano, H., D. Kojima and Y. Fukada (1999) Exo-rhodopsin: A novel rhodopsin expressed in the zebrafish pineal gland. *Brain Res. Mol. Brain Res.* **73**, 110–118.
- Philp, A. R., J. Bellingham, J.-M. Garcia-Fernandez and R. G. Foster (2000) A novel rod-like opsin isolated from the extra-retinal photoreceptors of teleost fish. *FEBS Lett.* **468**, 181–188.
- Vigh-Teichmann, I., H.-W. Korf, A. Oksche and B. Vigh (1982) Opsin-immunoreactive outer segments and acetylcholinesterase-

- positive neurons in the pineal complex of *Phoxinus phoxinus* (Teleostei, Cyprinidae). *Cell Tissue Res.* **227**, 351–369.
30. Vigh-Teichmann, I., H.-W. Korf, F. Nürnberger, A. Oksche, B. Vigh and R. Olsson (1983) Opsin-immunoreactive outer segments in the pineal and parapineal organs of the lamprey (*Lampetra fluviatilis*), the eel (*Anguilla anguilla*), and the rainbow trout (*Salmo gairdneri*). *Cell Tissue Res.* **230**, 289–307.
 31. Robinson, J., E. A. Schmitt and J. E. Dowling (1995) Temporal and spatial patterns of opsin gene expression in zebrafish (*Danio rerio*). *Vis. Neurosci.* **12**, 895–906.
 32. Forsell, J., P. Ekström, I. N. Flamarique and B. Holmqvist (2001) Expression of pineal ultraviolet- and green-like opsins in the pineal organ and retina of teleosts. *J. Exp. Biol.* **204**, 2517–2525.
 33. Tamotsu, S., T. Oishi, K. Nakao, Y. Fukada, Y. Shichida, T. Yoshizawa and Y. Morita (1994) Localization of iodopsin and rod-opsin immunoreactivity in the retina and pineal complex of the river lamprey, *Lampetra japonica*. *Cell Tissue Res.* **278**, 1–10.
 34. Masuda, H., T. Oishi, M. Ohtani, M. Michinome, Y. Fukada, Y. Shichida and T. Yoshizawa (1994) Visual pigments in the pineal complex of the Japanese quail, Japanese grass lizard and bullfrog: Immunocytochemistry and HPLC analysis. *Tissue Cell* **26**, 101–113.
 35. Koyanagi, M., E. Kawano, Y. Kinugawa, T. Oishi, Y. Shichida, S. Tamotsu and A. Terakita (2004) Bistable UV pigment in the lamprey pineal. *Proc. Natl. Acad. Sci. USA* **101**, 6687–6691.
 36. Chaurasia, S. S., M. D. Rollag, G. Jiang, W. P. Hayes, R. Haque, A. Natesan, M. Zatz, G. Tosini, C. Liu, H.-W. Korf, P. M. Iuvone and I. Provencio (2005) Molecular cloning, localization and circadian expression of chicken melanopsin (*Opn4*): Differential regulation of expression in pineal and retinal cell types. *J. Neurochem.* **92**, 158–170.
 37. Bailey, M. J. and V. M. Cassone (2005) Melanopsin expression in the chick retina and pineal gland. *Brain Res. Mol. Brain Res.* **134**, 345–348.
 38. Furukawa, T., E. M. Morrow and C. L. Cepko (1997) *Crx*, a novel *otx*-like homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* **91**, 531–541.
 39. Chen, S., Q.-L. Wang, Z. Nie, H. Sun, G. Lennon, N. G. Copeland, D. J. Gilbert, N. A. Jenkins and D. J. Zack (1997) *Crx*, a novel *Otx*-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron* **19**, 1017–1030.
 40. Furukawa, T., E. M. Morrow, T. Li, F. C. Davis and C. L. Cepko (1999) Retinopathy and attenuated circadian entrainment in *Crx*-deficient mice. *Nat. Genet.* **23**, 466–470.
 41. Li, X., S. Chen, Q. Wang, D. J. Zack, S. H. Snyder and J. Borjigin (1998) A pineal/regulatory element (PIRE) mediates transactivation by the pineal/regulatory-specific transcription factor *CRX*. *Proc. Natl. Acad. Sci. USA* **95**, 1876–1881.
 42. Asaoka, Y., H. Mano, D. Kojima and Y. Fukada (2002) Pineal expression-promoting element (PIPE), a *cis*-acting element, directs pineal-specific gene expression in zebrafish. *Proc. Natl. Acad. Sci. USA* **99**, 15456–15461.
 43. Gothilf, Y., S. L. Coon, R. Toyama, A. Chitnis, M. A. A. Nambodiri and D. C. Klein (1999) Zebrafish serotonin *N*-acetyltransferase-2: marker for development of pineal photoreceptors and circadian clock function. *Endocrinology* **140**, 4895–4903.
 44. Gothilf, Y., R. Toyama, S. L. Coon, S.-J. Du, I. B. Dawid and D. C. Klein (2002) Pineal-specific expression of green fluorescent protein under the control of the serotonin-*N*-acetyltransferase gene regulatory regions in transgenic zebrafish. *Dev. Dyn.* **225**, 241–249.
 45. Appelbaum, L., R. Toyama, I. B. Dawid, D. C. Klein, R. Baler and Y. Gothilf (2004) Zebrafish serotonin-*N*-acetyltransferase-2 gene regulation: Pineal-restrictive downstream module contains a functional E-box and three photoreceptor conserved elements. *Mol. Endocrinol.* **18**, 1210–1221.
 46. Appelbaum, L., A. Anzulovich, R. Baler and Y. Gothilf (2005) Homeobox-clock protein interaction in zebrafish. A shared mechanism for pineal-specific and circadian gene expression. *J. Biol. Chem.* **280**, 11544–11551.
 47. Takanaka, Y., T. Okano, M. Iigo and Y. Fukada (1998) Light-dependent expression of pinopsin gene in chicken pineal gland. *J. Neurochem.* **70**, 908–913.
 48. Takanaka, Y., T. Okano, K. Yamamoto and Y. Fukada (2002) A negative regulatory element required for light-dependent *pinopsin* gene expression. *J. Neurosci.* **22**, 4357–4363.
 49. Talbot, W. S., B. Trevarrow, M. E. Halpern, A. E. Melby, G. Farr, J. H. Postlethwait, T. Jowett, C. B. Kimmel and D. Kimelman (1995) A homeobox gene essential for zebrafish notochord development. *Nature* **378**, 150–157.
 50. Heisenberg, C.-P., C. Houart, M. Takeuchi, G.-J. Rauch, N. Young, P. Coutinho, I. Masai, L. Caneparo, M. L. Concha, R. Geisler, T. C. Dale, S. W. Wilson and D. L. Stemple (2001) A mutation in the Gsk3-binding domain of zebrafish *Masterblind*/*Axin1* leads to a fate transformation of telencephalon and eyes to diencephalon. *Genes Dev.* **15**, 1427–1434.
 51. van de Water, S., M. van de Wetering, J. Joore, J. Esseling, R. Bink, H. Clevers and D. Zivkovic (2001) Ectopic Wnt signal determines the eyeless phenotype of zebrafish *masterblind* mutant. *Development* **128**, 3877–3888.
 52. Barth, K. A., Y. Kishimoto, K. B. Rohr, C. Seydler, S. Schulte-Merker and S. W. Wilson (1999) Bmp activity establishes a gradient of positional information throughout the entire neural plate. *Development* **126**, 4977–4987.
 53. Cau, E. and S. W. Wilson (2003) *Ash1a* and *Neurogenin1* function downstream of Floating head to regulate epiphyseal neurogenesis. *Development* **130**, 2455–2466.
 54. Itoh, M., C.-H. Kim, G. Palardy, T. Oda, Y.-J. Jiang, D. Maust, S.-Y. Yeo, K. Lorick, G. J. Wright, L. Ariza-McNaughton, A. M. Weissman, J. Lewis, S. C. Chandrasekharappa and A. B. Chitnis (2003) Mind bomb is a ubiquitin ligase that is essential for efficient activation of Notch signaling by Delta. *Dev. Cell* **4**, 67–82.
 55. Schier, A. F., S. C. F. Neuhauss, M. Harvey, J. Malicki, L. Solnica-Krezel, D. Y. R. Stainier, F. Zwartkruis, S. Abdelilah, D. L. Stemple, Z. Rangini, H. Yang and W. Driever (1996) Mutations affecting the development of the embryonic zebrafish brain. *Development* **123**, 165–178.
 56. Nishida A., A. Furukawa, C. Koike, Y. Tano, S. Aizawa, I. Matsuo and T. Furukawa (2003) *Otx2* homeobox gene controls retinal photoreceptor cell fate and pineal gland development. *Nat. Neurosci.* **6**, 1255–1263.
 57. Hong S.-K., C.-H. Kim, K.-W. Yoo, H.-S. Kim, T. Kudoh, I. B. Dawid and T.-L. Huh (2002) Isolation and expression of a novel neuron-specific *onecut* homeobox gene in zebrafish. *Mech. Dev.* **112**, 199–202.
 58. Inoue, T., M. Hojo, Y. Bessho, Y. Tano, J. E. Lee and R. Kageyama (2002) *Math3* and *NeuroD* regulate amacrine cell fate specification in the retina. *Development* **129**, 831–842.
 59. Hatakeyama, J. and R. Kageyama (2004) Retinal cell fate determination and bHLH factors. *Semin. Cell Dev. Biol.* **15**, 83–89.
 60. Hatakeyama, J., K. Tomita, T. Inoue and R. Kageyama (2001) Roles of homeobox and bHLH genes in specification of a retinal cell type. *Development* **128**, 1313–1322.
 61. Arendt, D. (2003) Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563–571.
 62. de Melo, J., X. Qiu, G. Du, L. Cristante and D. D. Eisenstat (2003) *Dlx1*, *Dlx2*, *Pax6*, *Brn3b*, and *Chx10* homeobox gene expression defines the retinal ganglion and inner nuclear layers of the developing and adult mouse retina. *J. Comp. Neurol.* **461**, 187–204.
 63. Arendt, D. and J. Wittbrodt (2001) Reconstructing the eyes of Urbilateria. *Phil. Trans. R. Soc. Lond. B* **356**, 1545–1563.
 64. Provencio, I., G. Jiang, W. J. De Grip, W. P. Hayes and M. D. Rollag (1998) Melanopsin: An opsin in melanophores, brain, and eye. *Proc. Natl. Acad. Sci. USA* **95**, 340–345.
 65. Provencio, I., I. R. Rodriguez, G. Jiang, W. P. Hayes, E. F. Moreira and M. D. Rollag (2000) A novel human opsin in the inner retina. *J. Neurosci.* **20**, 600–605.
 66. Bellingham, J., D. Whitmore, A. R. Philp, D. J. Wells and R. G. Foster (2002) Zebrafish melanopsin: Isolation, tissue localisation and phylogenetic position. *Brain Res. Mol. Brain Res.* **107**, 128–136.
 67. Drivenes, Ø., A. M. Søviknes, L. O. E. Ebbesson, A. Fjose, H.-C. Seo and J. V. Helvik (2003) Isolation and characterization of two teleost melanopsin genes and their differential expression within the inner retina and brain. *J. Comp. Neurol.* **456**, 84–93.

68. Tomonari, S., A. Takagi, S. Akamatsu, S. Noji and H. Ohuchi (2005) A non-canonical photopigment, melanopsin, is expressed in the differentiating ganglion, horizontal, and bipolar cells of the chicken retina. *Dev. Dyn.* **234**, 783–790.
69. Cahill, G. M. and J. C. Besharse (1992) Light-sensitive melatonin synthesis by *Xenopus* photoreceptors after destruction of the inner retina. *Vis. Neurosci.* **8**, 487–490.
70. Cahill, G. M. and J. C. Besharse (1993) Circadian clock functions localized in *Xenopus* retinal photoreceptors. *Neuron* **10**, 573–577.
71. Iuvone, P. M., G. Tosini, N. Pozdeyev, R. Haque, D. C. Klein and S. S. Chaurasia (2005) Circadian clocks, clock networks, arylalkylamine *N*-acetyltransferase, and melatonin in the retina. *Prog. Retin. Eye Res.* **24**, 433–456.
72. Lacalli, T. C. (2004) Sensory systems in amphioxus: A window on the ancestral chordate condition. *Brain Behav. Evol.* **64**, 148–162.
73. Wada, Y., T. Okano, A. Adachi, S. Ebihara and Y. Fukada (1998) Identification of rhodopsin in the pigeon deep brain. *FEBS Lett.* **424**, 53–56.
74. Wada, Y., T. Okano and Y. Fukada (2000) Phototransduction molecules in the pigeon deep brain. *J. Comp. Neurol.* **428**, 138–144.
75. Yoshikawa, T., T. Okano, T. Oishi and Y. Fukada (1998) A deep brain photoreceptive molecule in the toad hypothalamus. *FEBS Lett.* **424**, 69–72.
76. Kojima, D., H. Mano and Y. Fukada (2000) Vertebrate ancient-*long* opsin: A green-sensitive photoreceptive molecule present in zebrafish deep brain and retinal horizontal cells. *J. Neurosci.* **20**, 2845–2851.
77. Halpern, M. E., J. O. Liang and J. T. Gamse (2003) Leaning to the left: Laterality in the zebrafish forebrain. *Trends Neurosci.* **26**, 308–313.
78. Swaroop, A., J. Xu, H. Pawar, A. Jackson, C. Skolnick and N. Agarwal (1992) A conserved retina-specific gene encodes a basic motif/leucine zipper domain. *Proc. Natl. Acad. Sci. USA* **89**, 266–270.
79. Mears, A. J., M. Kondo, P. K. Swain, Y. Takada, R. A. Bush, T. L. Saunders, P. A. Sieving and A. Swaroop (2001) *Nrl* is required for rod photoreceptor development. *Nat. Genet.* **29**, 447–452.
80. Rehemtulla, A., R. Warwar, R. Kumar, X. Ji, D. J. Zack and A. Swaroop (1996) The basic motif-leucine zipper transcription factor *Nrl* can positively regulate rhodopsin gene expression. *Proc. Natl. Acad. Sci. USA* **93**, 191–195.
81. Mitton, K. P., P. K. Swain, S. Chen, S. Xu, D. J. Zack and A. Swaroop (2000) The leucine zipper of *NRL* interacts with the *CRX* homeodomain. A possible mechanism of transcriptional synergy in rhodopsin regulation. *J. Biol. Chem.* **275**, 29794–29799.