Melatonin is synthesized in two vertebrate tissues, the pineal gland and the retina (1–5). Production of melatonin is almost always under control of a circadian clock, with the highest levels occurring at night (6–10). The pineal gland produces circulating melatonin, which serves as an endocrine signal of the night period. This signal of light in turn is important in circadian and seasonal physiology (11, 12). In contrast, retinal melatonin functions as a paracrine signal within the retina to enhance retinal functioning under low light conditions. This is done by shifting the positions of the rods and cones and regulating the turnover rates of the photoreceptive apparatus of the rods and cones and surrounding pigment epithelium (5, 13, 14).

The enzyme which controls the circadian pattern of melatonin synthesis (serotonin → N-acetylsertotonin → melatonin) is serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase, AANAT; EC 2.3.1.87) is the first enzyme in the conversion of serotonin to melatonin. Large changes in AANAT activity play an important role in the daily rhythms in melatonin production. Although a single AANAT gene has been found in mammals and the chicken, we have now identified two AANAT genes in fish. These genes are designated AANAT-1 and AANAT-2; all known AANATs belong to the AANAT-1 subfamily. Pike AANAT-1 is nearly exclusively expressed in the retina and AANAT-2 in the pineal gland. The abundance of each mRNA changes on a circadian basis, with retinal AANAT-1 mRNA peaking in late afternoon and pineal AANAT-2 mRNA peaking 6 h later. The pike AANAT-1 and AANAT-2 enzymes (66% identical amino acids) exhibit marked differences in their affinity for serotonin, relative affinity for indolemethyamines versus phenylethylamines and temperature-activity relationships. Two AANAT genes also exist in another fish, the trout. The evolution of two AANATs may represent a strategy to optimally meet tissue-related requirements for synthesis of melatonin: pineal melatonin serves an endocrine role and retinal melatonin plays a paracrine role.

Melatonin is synthesized in two vertebrate tissues, the pineal gland and the retina (1–5). Production of melatonin is almost always under control of a circadian clock, with the highest levels occurring at night (6–10). The pineal gland produces circulating melatonin, which serves as an endocrine signal of the night period. This signal of light in turn is important in circadian and seasonal physiology (11, 12). In contrast, retinal melatonin functions as a paracrine signal within the retina to enhance retinal functioning under low light conditions. This is done by shifting the positions of the rods and cones and regulating the turnover rates of the photoreceptive apparatus of the rods and cones and surrounding pigment epithelium (5, 13, 14).

The enzyme which controls the circadian pattern of melatonin synthesis (serotonin → N-acetylsertotonin → melatonin) is serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase; AANAT; EC 2.3.1.87) (15). Rhythms in AANAT activity in most vertebrates are driven by circadian clocks; light acts to reset and entrain these clocks. Light also acts independently via a downstream mechanism to turn off AANAT activity by initiating AANAT proteolysis (16).

It has been generally thought that a single AANAT gene is expressed in both the pineal gland and retina. AANATs from various species are characterized by three highly conserved peptide sequences (C/c-1, D/c-1, and D/c-2; see Fig. 1) that have been proposed to be involved in arylalkylamine binding (15, 17).

The single AANAT gene hypothesis is consistent with the results from studies in mammals and the chicken, but not with those from studies in teleosts (bony fishes). Biochemical investigations of the Northern pike indicate that pineal and retinal AANAT have distinctly different affinities toward arylalkylamine substrates and different temperature-activity relationships (18). Furthermore, mRNA analyses in both pike and trout indicate that a trout pineal-derived AANAT probe does not detect retinal AANAT mRNA (19). These observations are reconciled by the results presented here demonstrating the existence of a second AANAT gene in teleosts.

MATERIALS AND METHODS

cDNA Synthesis, Reverse Transcriptase PCR, and Genomic PCR—
RNA was extracted from pike, trout, or sturgeon pineal glands and retinae as described below. cDNA was synthesized on oligo(dT) magnetic beads (Dynal) as described previously (20). PCR was conducted in a 50-µl volume containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 µM dNTPs, 500 nM primers, and 0.5 units of ExTaq DNA polymerase (Takara). The cycling regime was as follows: 94 °C for 5 min; 30–35 cycles of annealing at 55–60 °C (55 °C was used for primers from a species different from the cDNA; 60 °C was used when primers and DNA were from the same species) and extension at 72 °C for 1 min; and final extension at 72 °C for 9 min. Conditions for amplification of genomic DNA were the same as for cDNA, except the annealing temperature was 58 °C and 50 ng of DNA was used as template. PCR products were cloned into pGEM-T-Easy (Promega) for sequencing.

Determination of the Sequence of AANAT cDNAs—The initial step leading to identification of two pike AANATs (pAANATs) involved reverse transcriptase-PCR generation of fragments of AANAT from sturgeon and trout pineal cDNA. A 179-bp sturgeon fragment (clone S12) was obtained using the sheep-based primers 562 and 563 (Table I). A 138-bp trout fragment (clone T149) was then obtained using primer 562 and primer S02R, which was based on the sequence of the sturgeon clone S12. A longer trout fragment (clone T3; 184 bp) was obtained using primers T01R (based on trout clone T149) and D07L (a degenerate primer based on mammalian AANATs). Trout clone T3 overlapped with the sturgeon clone S12 by 78 bp and was 63% identical within the overlap region. Probes derived from these clones generated different patterns when used in Northern blot analysis: the sturgeon pineal-based probe (S12) detected multiple bands of mRNA in the trout and pike pineal gland and only a single band in the retina from these species, whereas the trout pineal-based probe (T3) only detected mRNA.
in the pine and trout pineal gland. Based on this, we hypothesized that two AANAT genes are expressed in trout and pine, with the one corresponding to S12 expressed preferentially in the teleost retina and the second corresponding to T3 expressed preferentially in the teleost pineal gland.

Sturgeon AANAT-derived primers S03L and S02R were used with pike retinal cDNA to obtain clone 11/15; primers derived from clone 11/15 were used to obtain a full-length sequence from pike retinal cDNA by 3'-RACE and 5'-RACE (see below). Trout AANAT-derived primers T04L and T05R were used with pike pineal cDNA to obtain clone 2/6; primers derived from clone 2/6 were used to obtain a full-length sequence from pike pineal mRNA by 3'-RACE and 5'-RACE.

3'-RACE was performed using pike AANAT-specific nested primers in conjunction with oligo-dT primers (GAATTCGACCTCGAGAAGC). The initial round of PCR consisted of 10 cycles with the first specific primer only at 60 °C annealing temperature, followed by the addition of oligo-dT primers and continuing the PCR at 50 °C annealing temperature for 5 cycles then at 60 °C for 20 cycles. An aliquot of this first reaction was used as a template for nested PCR employing 30 cycles at 60 °C annealing temperature, followed by the addition of oligo-dT primers at the indicated concentrations and 0.5 mM [3H]acetyltryptamine, [3H]acetylserotonin or [3H]acetyltyramine, a sample of the N-acetylcoenzyme A (C) moiety in 40-μl final volume of 100 mM sodium phosphate buffer (pH 6.8) at 37 °C for 20 min, unless otherwise indicated. Radiolabeled products were separated from 1 [3H]acetylcoenzyme A and quantitated by one of two methods. To isolate and measure N-[3H]acetyctryptamine, N-[3H]acetyl-5-methoxytryptamine, or N-[3H]acetylphenylethylamine, the assay mixtures were extracted into chloroform; the chloroform was taken to dryness; and the residue was redissolved in scintillation fluid. To isolate and measure N-[3H]acetyctryptamine or N-[3H]acetylserotonin, a sample of the assay was mixed with ethanol containing the appropriate authentic carrier, the mixture was subjected to thin layer chromatography, products were located, corresponding gel was removed and product was extracted into scintillation fluid. Radioactivity was determined by routine methods.

### RESULTS

#### Sequence Analysis

The deduced amino acid sequences of the two pAANAT clones are 66% identical and 76% similar (Fig. 1A). Both sequences are ~65–75% identical to known mammalian and avian AANAT clones (Fig. 1B). Both contain known conserved features of AANATs including two putative protein kinase A phosphorylation sites, a conserved putative ubiquitination site (K9), a histidine-rich stretch of residues proposed to be important in catalysis (residues 113–128) and two regions, described as motifs A and B, which characterize members of a large acetyltransferase superfamily referred to as the A/B or GNAT superfamily (15, 17, 22).

Clone pRX50, from the trout pineal gland, contains the three highly conserved peptides (C/c-1, D/c-1, and D/c-2) found in all previously published AANATs (17); this clone and the known AANATs will be designated numbered for the first round.

Clone pRXX36, from the pike pineal gland, differs at 6 of 36 amino acids in these sequences. This clone, along with the trout pineal AANAT cloned here, will be considered to represent a second AANAT subfamily, designated AANAT-2. This segregation is also consistent with the results of computer-assisted phyloge-
Two AANAT Genes in Teleosts

**A**

**Fig. 1. Sequence analysis of pike AANAT-1 and AANAT-2. A, alignment of pAANAT-1 and pAANAT-2 deduced amino acid sequences.** It is not clear which of the two methionines within the first four residues of pAANAT-2 is the preferred start of translation. Residues linked by vertical lines are identical; vertical dots indicate similarity. B, alignment of pAANATs and published AANATs (GenBank™ accession numbers: human, U40347; rat, U38306; sheep, U29663; cow, AD000742; chicken, U46502; quail, AF007068; trout AANAT-1, AB007294). The two motifs that identify proteins as members of the motif A/B superfamily of acetyltransferases and the three highly conserved regions (C/c-1, C/c-2) which distinguish members of the AANAT subfamilies are indicated. pka, putative cyclic nucleotide-dependent kinase site. The consensus sequences for AANAT-1 and AANAT-2 match all known sequences. **Capitalized** residues in individual AANAT-1 and AANAT-2 sequences match the consensus. The consensus for AANAT-1/-2 indicates residues identical between all AANAT-1 and AANAT-2 sequences. The last 35 amino acids of trout AANAT-1 have been omitted for clarity. C, the organization of pAANAT-1 and pAANAT-2 genes, compared with the human AANAT (hAANAT-1) gene (25). Dark boxes indicate the ORF.

**B**

**Tissue Distribution**

Northern blot analysis reveals that pAANAT-1 and pAANAT-2 exhibit strikingly different expression patterns. pAANAT-1 is expressed at high levels only in the retina and pAANAT-2 only in the pineal gland. A trace amount of a 1.6-kilobase pAANAT-2-positive transcript is also found in the ovary. Northern blot analysis did not detect expression of either gene in other tissues. Day/night differences in expression also occur in retina and pineal gland (discussed below).

pAANAT-2 is present in the pineal gland as 2.1- and 2.8-kilobase transcripts; a single 2.2-kilobase pAANAT-1 transcript is found in the retina. The relative amounts of the two pAANAT-2 transcripts varied between samples for unexplained reasons not linked to experimental history or sample preparation, as judged from the actin mRNA signal. Although Northern blot analysis failed to reveal expression of pAANAT-1

**C**

netic analyses (ClustalW and Phylib) which cluster all AANAT-1 s on a separate branch from pAANAT-2.

Analysis of genomic sequences indicates that the locations of the intron/exon junctions in the ORF of both pAANAT genes are identical to that of human, sheep, and chicken AANAT-1 (Fig. 1C, and Ref. 15). Genomic Southern blot analysis confirmed that each gene was present as a single copy and did not co-migrate following digestion with five different restriction enzymes. This and the above results indicate that it is highly likely that AANAT-1 and AANAT-2 evolved from a common ancestor rather than resulting from convergent evolution or alternative splicing.

---

2 E. Koonin (National Institutes of Health), personal communication.

3 S. L. Coon and D. C. Klein, unpublished results.
Two AANAT Genes in Teleosts

Fig. 2. Tissue distribution of pAANAT-1 and pAANAT-2. Tissues were removed from an adult pike at 2400 h and frozen in liquid nitrogen. Total RNA was extracted, and each lane was loaded with 20 µg. The same blot was probed sequentially for both pAANAT-1 and pAANAT-2 and then for β-actin. Similar results were obtained with tissues taken at midday and also with a second series of midnight and midday tissues. Molecular size markers are indicated to the left, kb, kilobases.

in the pineal gland or of pAANAT-2 in the retina, reverse transcriptase PCR analysis detected trace levels of pAANAT-2 mRNA in the retina and of pAANAT-1 mRNA in the pineal gland.3

**Kinetic Characterization of Recombinant AANAT-1 and AANAT-2 Proteins**

**Temperature Dependence**—Temperature-activity relationships of the recombinant enzymes were different. Maximal activity with 10 mM tryptamine for pAANAT-1 occurred at 37 °C and that for pAANAT-2 at 18 °C. The temperature profile of pAANAT-1 corresponds to that reported for pike retinal homogenates and that of pAANAT-2 to pike pineal homogenates (Ref. 18; Fig. 3A, inset), consistent with the strong expression of pAANAT-1 in the retina and of pAANAT-2 in the pineal gland (Fig. 2). It is interesting that recombinant pAANAT-1 and pAANAT-2 enzymes exhibit significant levels of activity at 0 to 4 °C, as is also true of the native enzymes (18).

**Substrate Preferences**—The affinities of pAANAT-1 and of pAANAT-2 for tryptamine differ by more than 2 orders of magnitude (pAANAT-1, apparent K_m ~ 0.02 mM; pAANAT-2, apparent K_m ~ 7 mM) (Fig. 3B). An almost identical pattern exists between AANATs from pineal gland homogenates (Ref. 18; Fig. 3B, inset). The apparent K_m values for serotonin are also very different between pAANAT-1 (~0.02 mM) and pAANAT-2 (~3 mM); however, whereas pAANAT-1 acetylates tryptamine and serotonin with very similar affinities, pAANAT-2 acetylates serotonin about twice as well as tryptamine (Fig. 3C).

pAANAT-1 and pAANAT-2 also have different relative affinities for indoleethylamines versus phenylethylamines: pAANAT-1 does not appear to differentiate between indoleethylamines (serotonin, methoxytryptamine, and tryptamine) and phenylethylamines (tyramine and phenylethylamine). In contrast, pAANAT-2 has almost no ability to acetylate phenylethylamines (Fig. 3C).

The substrate-product formation relationships of pAANAT-1 and pAANAT-2 also differ in that only pAANAT-1 generates a pattern where acetylation is inhibited at substrate concentrations above 0.3 mM with indoleethylamine substrates; this same pattern is observed at 4 °C, as well as 37 °C. Neither enzyme exhibits such a pattern with phenylethylamines. We failed to obtain evidence for product inhibition of pAANAT-1 with up to 1 mM N-acetylsertotonin or melatonin.3 This suggests that substrate inhibition is likely to explain the observed pattern.

**Circadian Pattern of Abundance of Retinal pAANAT-1 and Pineal pAANAT-2**

The abundance of AANAT mRNA transcripts changes on a 24-h basis in both the retina and pineal gland in animals maintained in a lighting environment providing 12 h of light and 12 h of darkness (Fig. 4). The amplitude of the retinal AANAT-1 mRNA rhythm is >20-fold and that of pineal pAANAT-2 mRNA is >8-fold. The phase of the rhythms differs, with the retinal AANAT-1 mRNA rhythm peaking at dusk, 6 h prior to the middark period peak in pineal AANAT-2 mRNA.

Daily rhythms in the abundance of retinal pAANAT-1 mRNA and pineal pAANAT-2 mRNA persist under constant lighting conditions of dim red light or constant light, indicating they are not dependent upon light/dark transitions but are controlled by an endogenous circadian clock.

**DISCUSSION**

Two AANAT Genes Explain Paradoxical Results in Pike—
The existence of a second AANAT gene in pike resolves two paradoxical observations regarding AANAT in pike retina and pineal gland (18, 19). First, the report that AANAT mRNA was undetectable in the pike retina is explained by the finding that the trout pineal-derived probe used in those studies was AANAT-2-selective and did not detect pAANAT-1 under the conditions used, in part reflecting only a 68% bp match. As reported here, pike retina preferentially expresses AANAT-1; AANAT-2 is not detectable in the retina by Northern blot analysis.

The second paradox explained by these findings is that the kinetics of AANAT in the pike pineal gland and retina are different. This is explained by the dominant expression of pAANAT-1 in the retina and pAANAT-2 in the pineal gland, and the inherent differences in the proteins encoded by pAANAT-1 and pAANAT-2. This is supported by the remarkable similarity of the published kinetic characteristics of pike retinal AANAT and recombinant pAANAT-1 and that of pineal AANAT and recombinant pAANAT-2. It is noteworthy that retinal AANAT activity and recombinant pAANAT-1 appear to have nearly identical temperature dependence and substrate specificity characteristics, as did pineal AANAT activity and recombinant pAANAT-2, despite the fact that native enzymes were studied in crude pineal or retinal homogenates and fusion proteins were studied in bacterial extracts. The remarkable similarities observed between recombinant pAANAT-1 and retinal AANAT versus recombinant pAANAT-2 and pineal AANAT provide strong indications that the differences seen are valid reflections of the AANAT proteins and are not caused by other proteins or factors in the assay. Further investigations with pure preparations of the expressed proteins are required to fully characterize and provide a molecular explanation for these differences.

**Functional Significance of Two AANAT Genes**—It is not unreasonable to consider that the retinal/pineal expression pattern of pAANAT-1 and pAANAT-2 reflects a fine-tuning of AANAT’s regulatory role in melatonin synthesis in each tissue. As indicated above, rhythmic changes in melatonin synthesis are regulated by changes in AANAT activity. Recent studies have established that AANAT activity is modulated through control of enzyme protein levels by regulated proteolysis (16). With this type of regulation, changes in enzyme protein will be most effectively converted into changes in product formation if the K_m value approximates the substrate concentration. This is consistent with our finding that pAANAT-1 is expressed in the retina, which typically has a relatively low concentration of serotonin and that pAANAT-2 is expressed in the pineal gland,
which contains one of the highest concentrations of serotonin (29). The low $K_m$ of pAANAT-1 appears to best support melatonin synthesis if very low levels of serotonin are available, as is the case in the retina. This level of synthesis appears to be sufficient for local demands, and the higher $K_m$ of pAANAT-2 in the pineal gland is consistent with the higher concentration of serotonin there and the role of the pineal gland as a factory for circulating melatonin.

There may also be functional significance to the differences in substrate specificity between the proteins encoded by pAANAT-1 and pAANAT-2. The former acetylates indoleethylamines and phenylethylamines equally well, whereas the latter exhibits strong preference for indoleethylamines. This weak affinity of pAANAT-2 for phenylethylamines eliminates or minimizes acetylation as a factor influencing phenylethylamine metabolism in the pineal gland. Likewise, the strong affinity of pAANAT-1 for phenylethylamines suggests the enzyme might function in the retina to control phenylethylamine levels. This is of interest because 3,4-dihydroxyphenylethylamine (dopamine) is an important retinal neurotransmitter (5, 9).

The remarkable activity-temperature dependence difference between these two enzymes raises two questions: one being the molecular basis of the difference, and the second being the physiological significance. It has been suggested that molecular adaptation of closely related enzymes in different fish species to different physiological temperatures can be achieved through modification of flexibility and mobility within the molecule (30). The recent publication of the crystal structure of ovine AANAT-1 may help determine whether sequence differences between pAANAT-1 and pAANAT-2 could result in sim-
The physiological significance is difficult to explain but may relate to the fact that the Northern pike is a cold water fish; the steep rise in pAANAT-2 activity within the range of seasonal temperatures may be important in integrating environmental parameters.

Two AANAT Genes—Exception or the Rule?—The retinal/pineal expression pattern of AANAT-1 and AANAT-2 seen in pike is also present in trout because trout AANAT-1 was previously cloned from retina (28), and AANAT-2 was cloned from the trout pineal gland in this study. However, the strict pattern of expression of AANAT-1 in the retina and of AANAT-2 in the pineal gland is not conserved within or across classes. For example, AANAT-1 is expressed at high levels in both the retina and pineal gland of the chicken and some mammals (22, 25–27), and AANAT-2 is expressed at high levels in both the pineal gland and retina of zebrafish (19). Accordingly, species-to-species variations in tissue expression of AANAT-1 or AANAT-2 might reflect unique species- and tissue-dependent requirements for acetylation of serotonin and perhaps other arylalkylamines. Such biochemical and genetic variation is consistent with the anatomical and molecular diversity of these tissues (15, 32–34) and the important phototransduction roles the pineal gland and retina play in survival.

The question of whether AANAT-2 is expressed in non-teleosts remains open at this time. Efforts to clone chicken and human AANAT-2 s have been unsuccessful so far. Based on the degree of difference between pAANAT-1 and AANAT-2, it is not unreasonably that pAANAT-1 and pAANAT-2 existed prior to the emergence of teleosts; both might have been present in a vertebrate ancestor common to both teleosts and higher vertebrates. However, it is also possible that AANAT-2 appeared only after fish emerged from the teleost lineage that led to higher vertebrates.

Circadian Patterns of Expression of pAANAT-1 and pAANAT-2—The circadian expression of pAANAT-1 and pAANAT-2 is consistent with previous reports indicating that there are rhythms in AANAT mRNA in the chicken retina and in the pineal glands of the rat, pike, and chicken. This appears to reflect the role of endogenous clocks which determines that melatonin synthesis can be significantly elevated only during the night and not during the day. This improves the reliability of melatonin as an indicator of the night period. The clock is reset and modulated by light. However, as indicated in the Introduction, the clock is not the only mechanism controlling AANAT activity. An additional control mechanism involves light acting downstream of the clock to suppress AANAT activity; this apparently involves light-initiated proteasomal proteolysis of AANAT protein (16). This provides a mechanism through which the system can respond immediately to changes in photoperiod length that are not anticipated by the endogenous clock. This is of special significance in considering the importance of the different phasing of the rhythms of retinal AANAT-1 and pineal AANAT-2 mRNAs.

We suspect that the earlier peak in retinal AANAT-1 mRNA may reflect a requirement for maximal melatonin production early in the night period, perhaps to optimally promote dark adaptation. As a result, as soon as light levels fall below a threshold and retinal AANAT-1 proteolysis ceases, retinal
AANAT activity and melatonin production will increase immediately.

The later peak in pineal melatonin is consistent with the observation that in most species the circulating melatonin signal is broad and peaks in the middle of the dark period; it provides a hormonal signal that is proportional in length to the duration of the night period. Light participates by tailoring the rhythms in AANAT-1 mRNA in the rat pineal gland and retina (35), both of which are generated by endogenous oscillators.

The molecular basis of the different phasing of the retinal pAANAT-1 and pineal pAANAT-2 is not clear. It might reflect tissue-related differences in clock function, in the links between the clock and the AANAT genes, or in regulatory elements in the AANAT-1 and AANAT-2 promoters. Accordingly, continued analysis of this system will provide valuable insights into molecular aspects of clock function and the relationship of clocks to clock-driven genes.

Final Comment—The presence of a second AANAT gene in the pike provides evidence of intricate and divergent strategies for regulating melatonin production in the pike retina and pineal gland, and also provides unexpected opportunities for further investigation. For example, determination of the three-dimensional differences between pAANAT-1 and pAANAT-2 will help understand the molecular basis of kinetic differences, as will side-by-side determination of effects of site-directed mutagenesis on three-dimensional structure and kinetic activity. In addition, analysis of promoter elements in pAANAT-1 and pAANAT-2 will enhance our understanding of the molecular basis of tissue specificity and circadian-timed gene expression.

REFERENCES
1. Axelrod, J. (1974) Science 184, 1341–1348
2. Klein, D. C. (1986) in Photoperiodism, Melatonin and the Pineal (Evered, D., and Clark, S., eds) pp. 38–56. Pitman, London, Ciba Foundation Symposium 117
3. Skene, D. (1992) Biochem. Soc. Trans. 20, 312–315
4. Zawilska, J. B., and Nowak, J. Z. (1992) Neurochem. Int. 20, 23–36
5. Morgan, I. G., and Boelen, M. K. (1996) Visual Neurosci. 13, 399–409
6. Klein, D. C., and Weller, J. L. (1970) Science 169, 1093–1095
7. D’Istria, M., Monteleone, P., Serino, L., and Chieffi, G. (1994) Gen. Comp. Endocrinol. 96, 6–11
8. Cahill, G. M., and Besharse, J. C. (1995) Prog. Retinal Eye Res. 14, 267–291
9. Iuvone, P. M. (1986) in Retinal Degeneration and Regeneration (Kato, S., Osborne, N. N., and Tamai, M., eds) pp. 3–13. Kugler Publications, New York
10. Tosini, G., and Menaker, M. (1996) Science 272, 419–421
11. Karsch, F. J., Wolford, C. J., Malpaux, B., Robinson, J. E., and Wayne N. L. (1991) in Suprachiasmatic Nucleus: The Mind’s Clock (Klein, D. C., Moore, R. Y., and Reppert, S. M., eds) pp. 217–232. Oxford Press, New York
12. Arendt, J. (1995) Melatonin and the Mammalian Pineal Gland, pp. 201–285. Chapman & Hall, London
13. Besharse, J. C., and Dunis, D. A. (1983) Science 219, 1341–1343
14. Iuvone, P. M. (1986) in Neurobiology and Clinical Aspects of the Outer Retina (Diemuer, M. B. A., Arther, S., and Vailera, S., eds) pp. 25–55. Chapman & Hall, London
15. Klein, D. C., Coon, S. L., Roseboom, P. H., Weller, J. L., Bernard, M., Gastel, J. A., Zatz, M., Iuvone, M., Rodriguez, J. B., Begay, V., Faleim, J., Cahill, G., Cassone, V. M., and Baler, R. (1997) Recent Prog. Horm. Res. 52, 307–327
16. Gastel, J. A., Roseboom, P. H., Rinaldi, P. A., Weller, J. L., and Klein, D. C. (1995) Science 270, 1358–1360
17. Klein, D. C., Baler, R., Roseboom, P. H., Weller, J. L., Bernard, M., Gastel, J. A., Zatz, M., Iuvone, M., Begay, V., Faleim, J., Cahill, G., Cassone, V. M., and Coon, S. L. (1998) in Handbook of Behavioral State Control: Cellular and Molecular Mechanisms (Lydic, R., and Baghdoyan, H., eds) pp. 45–59. CRC Press, Boca Raton, FL
18. Faleim, J., Boliert, V., and Collin, P. (1996) Eur. J. Physiol. 412, 386–393
19. Begay, V., Faleim, J., Cahill, G. M., Klein, D. C., and Coon, S. L. (1998) Endocrinology 139, 905–912
20. Rodriguez, I. R., Mazuruk, K., Schoen, T. J., and Chader, G. J. (1994) J. Biol. Chem. 269, 31969–31977
21. Devereaux, J., Haselber, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395
22. Coon, S. L., Roseboom, P. H., Baler, R., Weller, J. H., Nambodiri, M. A. A., Konin, E. V., and Klein, D. C. (1980) Science 201, 1681–1683
23. Roseboom, P. H., Coon, S. L., Baler, R., McCune, S. K., Weller, J. L., and Klein, D. C. (1996) Endocrinology 137, 3033–3045
24. Borjigin, J., Wang, M. M., and Snyder, S. H. (1995) Nature 378, 783–785
25. Coon S. L., Mazuruk, K., Bernard, M., Roseboom, P. H., Klein, D. C., and Rodriguez, I. R. (1996) Genomics 34, 76–84
26. Klein, D. C., Roseboom, P. H., and Coon, S. L. (1996) Trends Endocrinol. Metab. 7, 109–112
27. Bernard, M., Iuvone, P. M., Cassone, V. M., Roseboom, P. H., Coon, S. L., and Klein, D. C. (1997) J. Neurochem. 68, 213–224
28. Minzawa, K., Igo, M., Suzuki, H., Yashiro, Y., Gen, K., Kituchi, K., Okans, T., Fukada, Y., and Aida, R. (1998) Zool. Sci. 15, 345–351
29. O’Brien, P. J., and Klein, D. C. (1986) Pineal and Retinal Relationships, Academic Press, Orlando, FL
30. Walls, G. L. (1942) J. Biol. Chem. 146, 109–112
31. Hickman, A. B., Klein, D. C., and Dyda, F. (1999) in Neurobiology and Clinical Aspects of the Outer Retina (Diemuer, M. B. A., Arther, S., and Vailera, S., eds) pp. 25–55. Chapman & Hall, London
32. Cassone, V. M., and Baler, R. (1997) Recent Prog. Horm. Res. 52, 307–327
33. Klein, D. C., Coon, S. L., Roseboom, P. H., Weller, J. L., Bernard, M., Gastel, J. A., Zatz, M., Iuvone, M., Rodriguez, J. B., Begay, V., Faleim, J., Cahill, G., Cassone, V. M., and Baler, R. (1997) Recent Prog. Horm. Res. 52, 307–327
34. Roseboom, P. H., Coon, S. L., Baler, R., McCune, S. K., Weller, J. L., and Klein, D. C. (1996) Endocrinology 137, 3033–3045
35. Borjigin, J., Wang, M. M., and Snyder, S. H. (1995) Nature 378, 783–785
36. Coon S. L., Mazuruk, K., Bernard, M., Roseboom, P. H., Klein, D. C., and Rodriguez, I. R. (1996) Genomics 34, 76–84
37. Klein, D. C., Roseboom, P. H., and Coon, S. L. (1996) Trends Endocrinol. Metab. 7, 106–112
38. Bernard, M., Iuvone, P. M., Cassone, V. M., Roseboom, P. H., Coon, S. L., and Klein, D. C. (1997) J. Neurochem. 68, 213–224
39. Minzawa, K., Igo, M., Suzuki, H., Yashiro, Y., Gen, K., Kituchi, K., Okans, T., Fukada, Y., and Aida, R. (1998) Zool. Sci. 15, 345–351
40. O’Brien, P. J., and Klein, D. C. (1986) Pineal and Retinal Relationships, Academic Press, Orlando, FL
41. Fields, P. A., and Sumero, G. N. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 11476–11481
42. Hickman, A. B., Klein, D. C., and Dyda, F. (1999) Mol. Cell 3, 23–32
43. Walls, G. L. (1942) The Vertebrate Eye and its Adaptive Radiation, Hafner Publ. Co., NY (postprint in 1967)
44. Oksche, A. (1965) Proc. Brain Res. 10, 3–28
45. Soni, B. G., Philp, A. R., Foster, R. G., and Knox, B. E. (1998) Nature 394, 27–28
46. Sakamoto, K., and Ishida, N. (1998) Neurosci. Lett. 245, 113–116