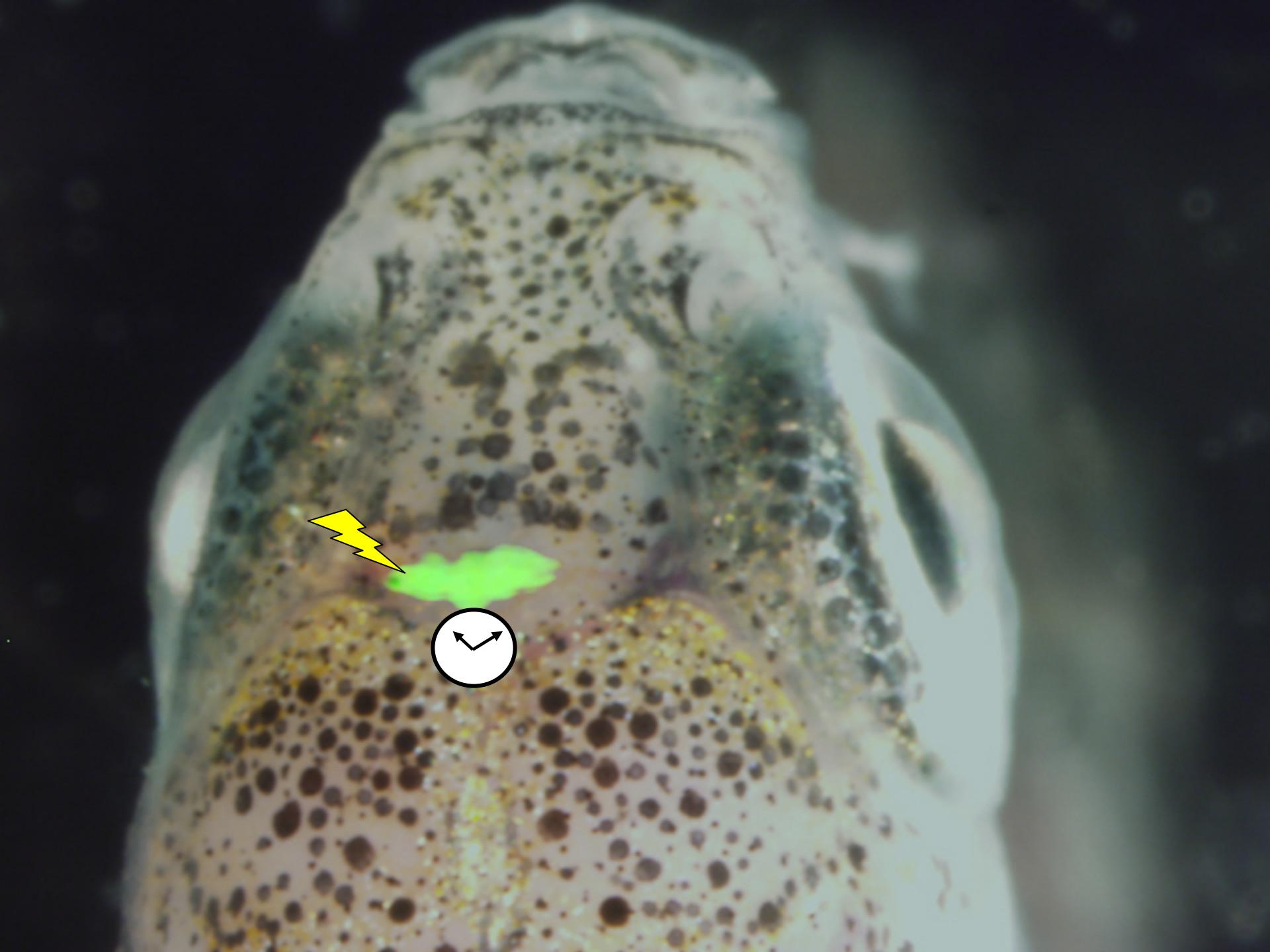
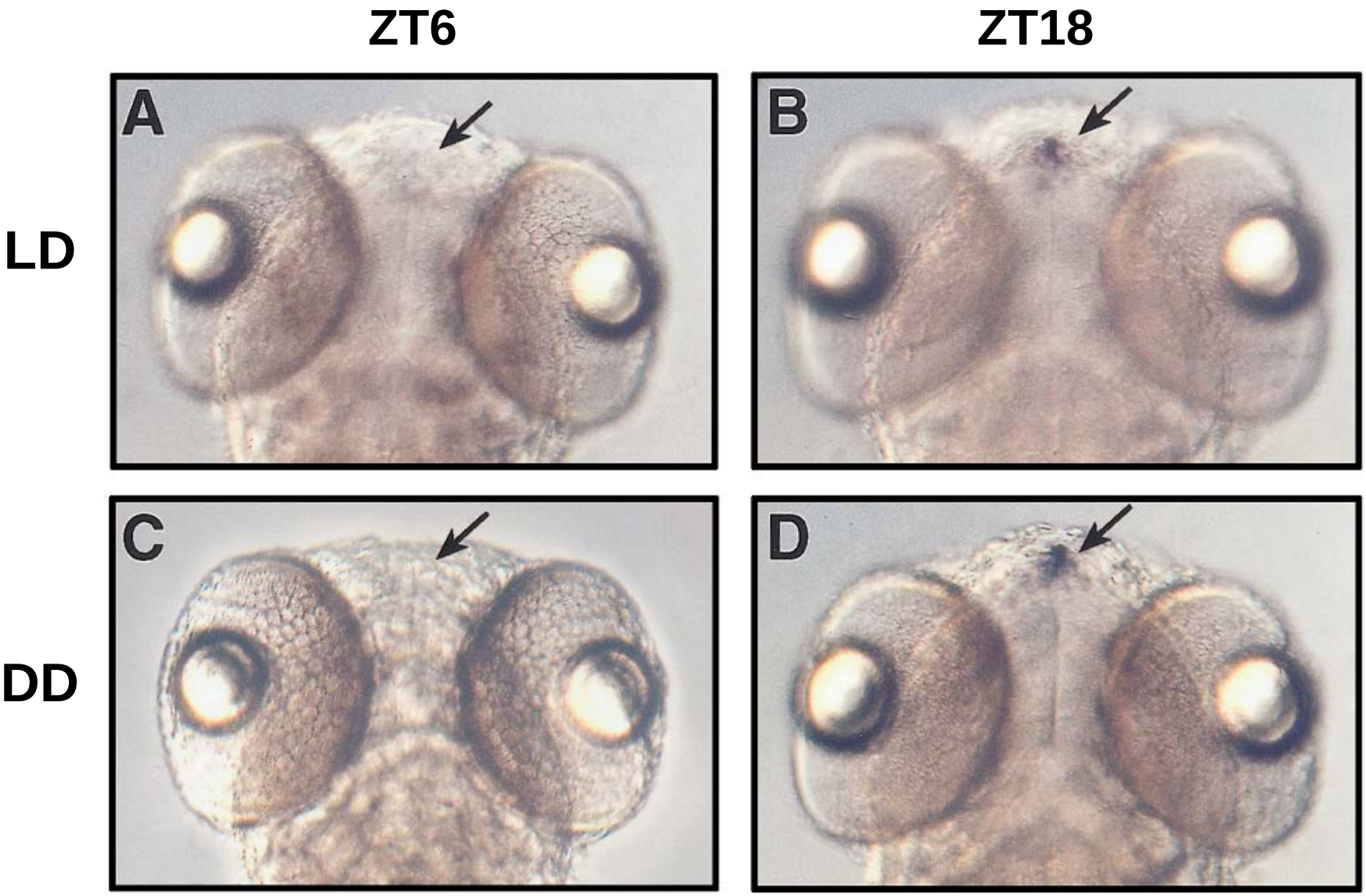


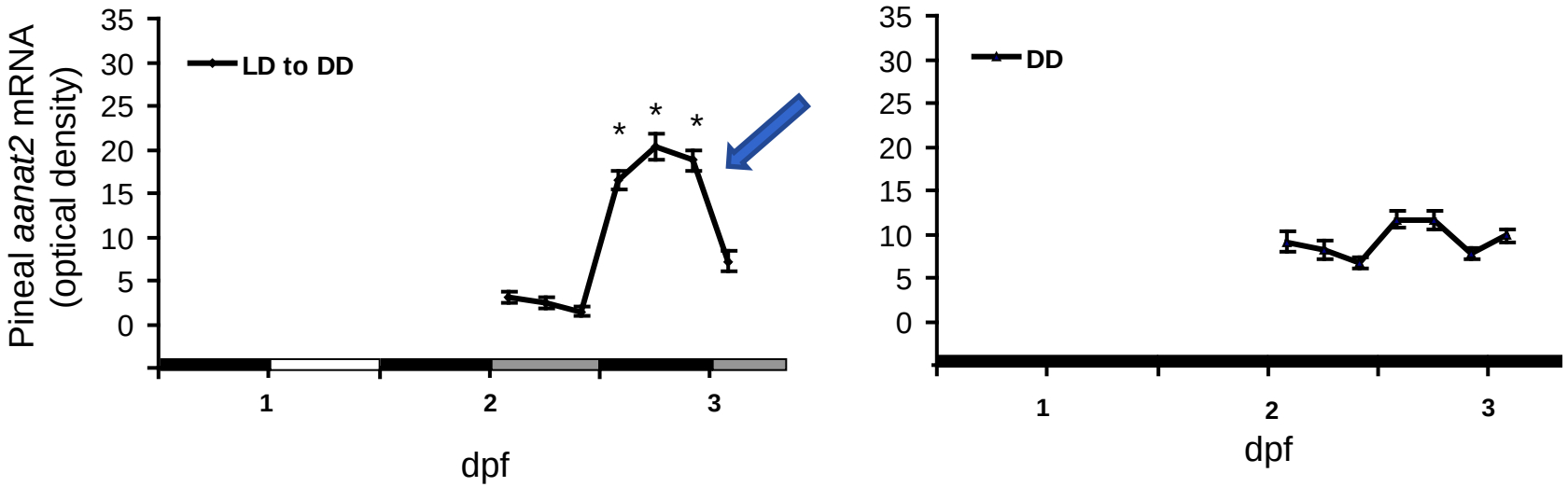
Pineal-specific expression of *aanat2* mRNA:
Whole mount ISH in a 22 hr embryo

Gothilf et al., 1999

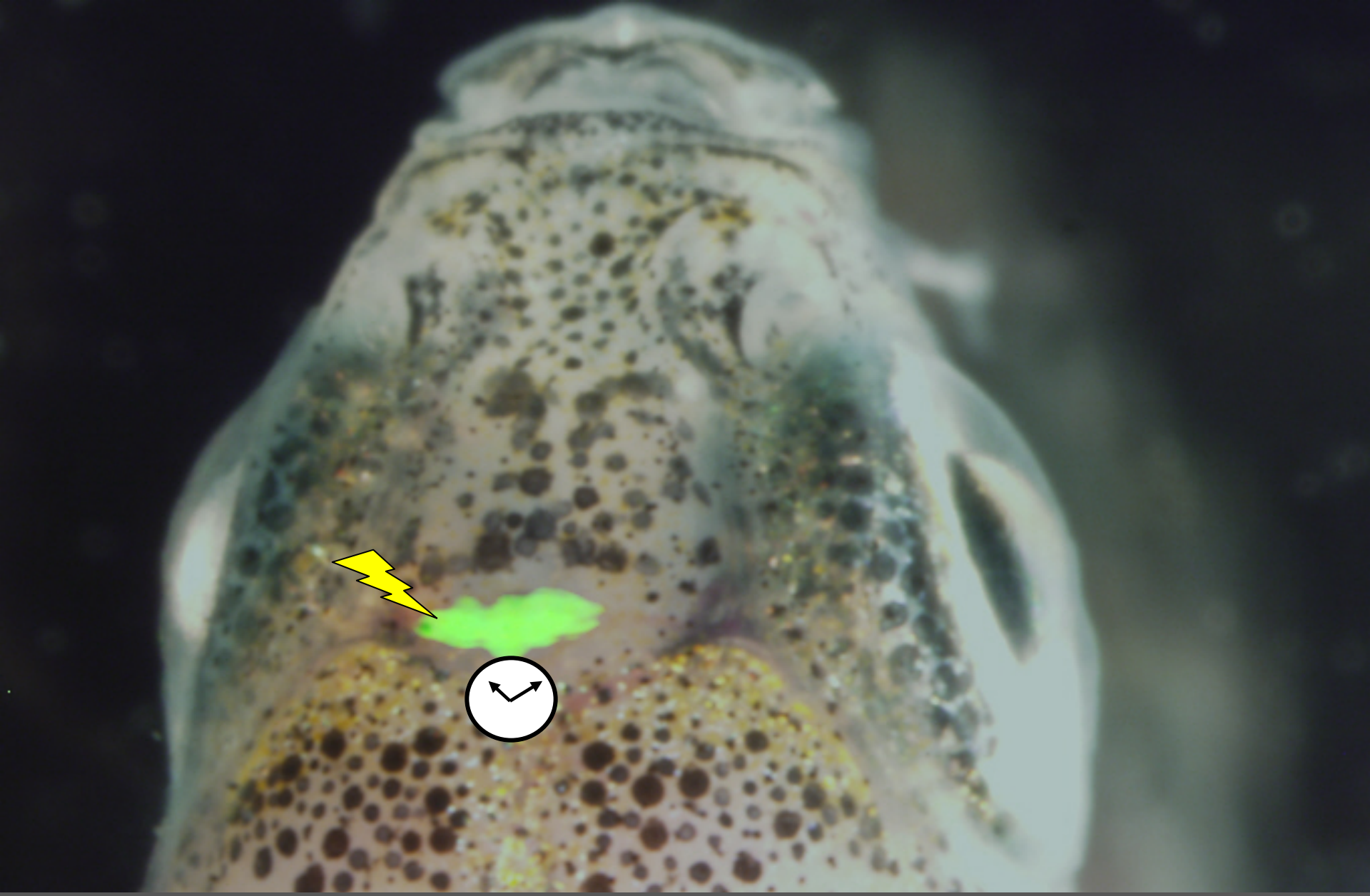


Gothilf et al., 1999

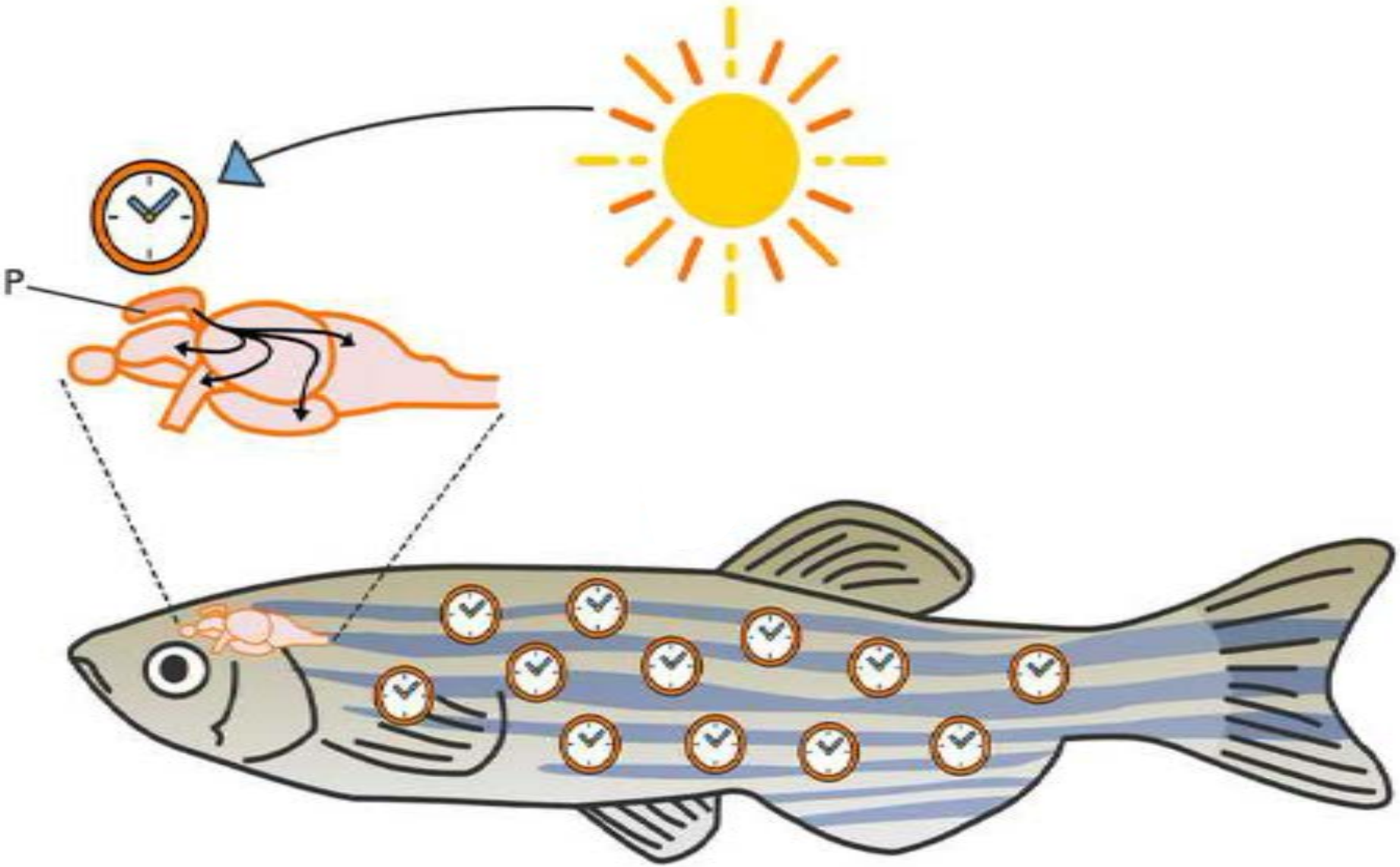
Onset of the clock-controlled *aanat2* rhythm: Light pulse is required



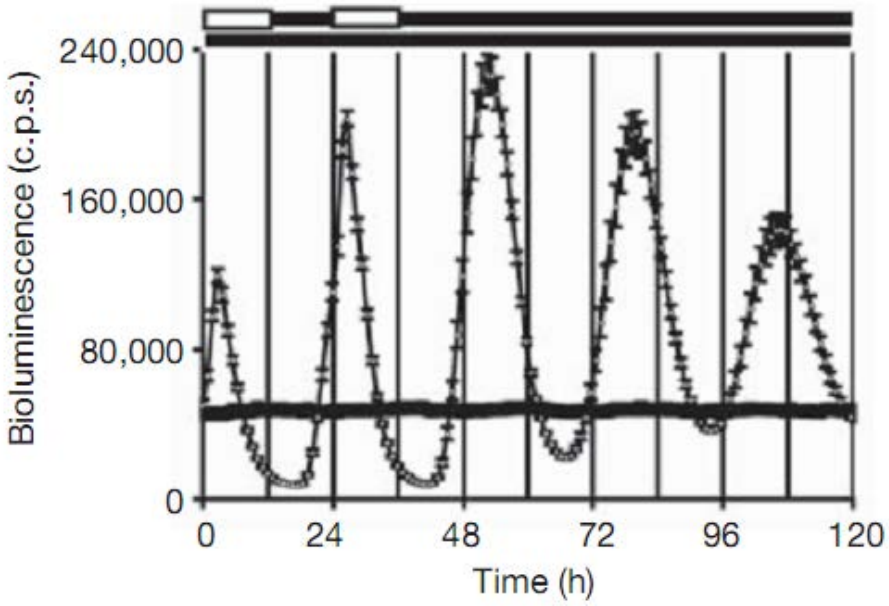
Ziv et al., 2005

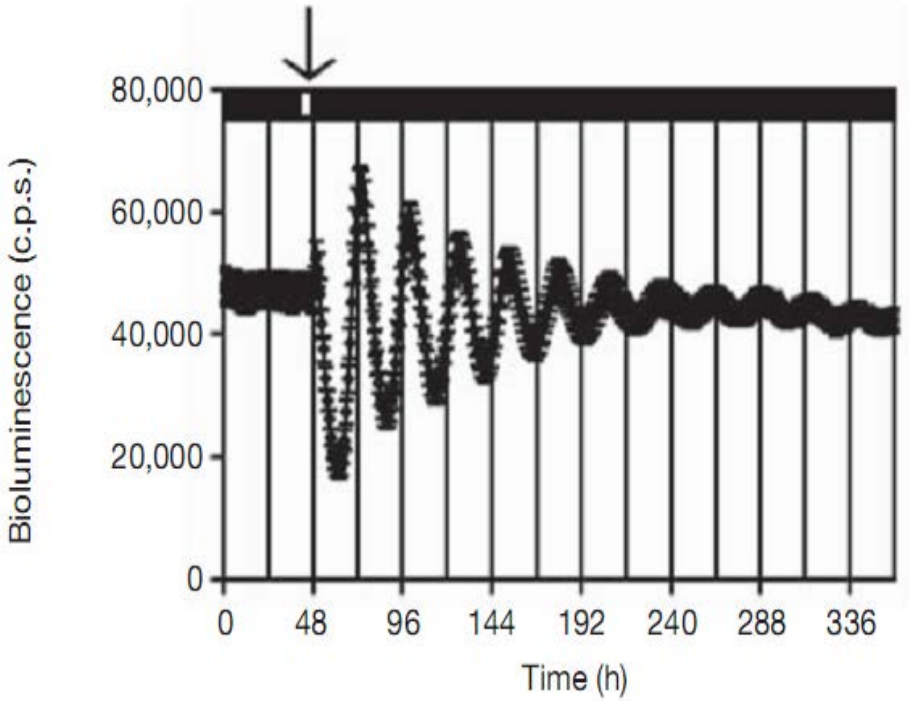


Traditional view: In fish, the pineal gland is the central circadian clock organ


P

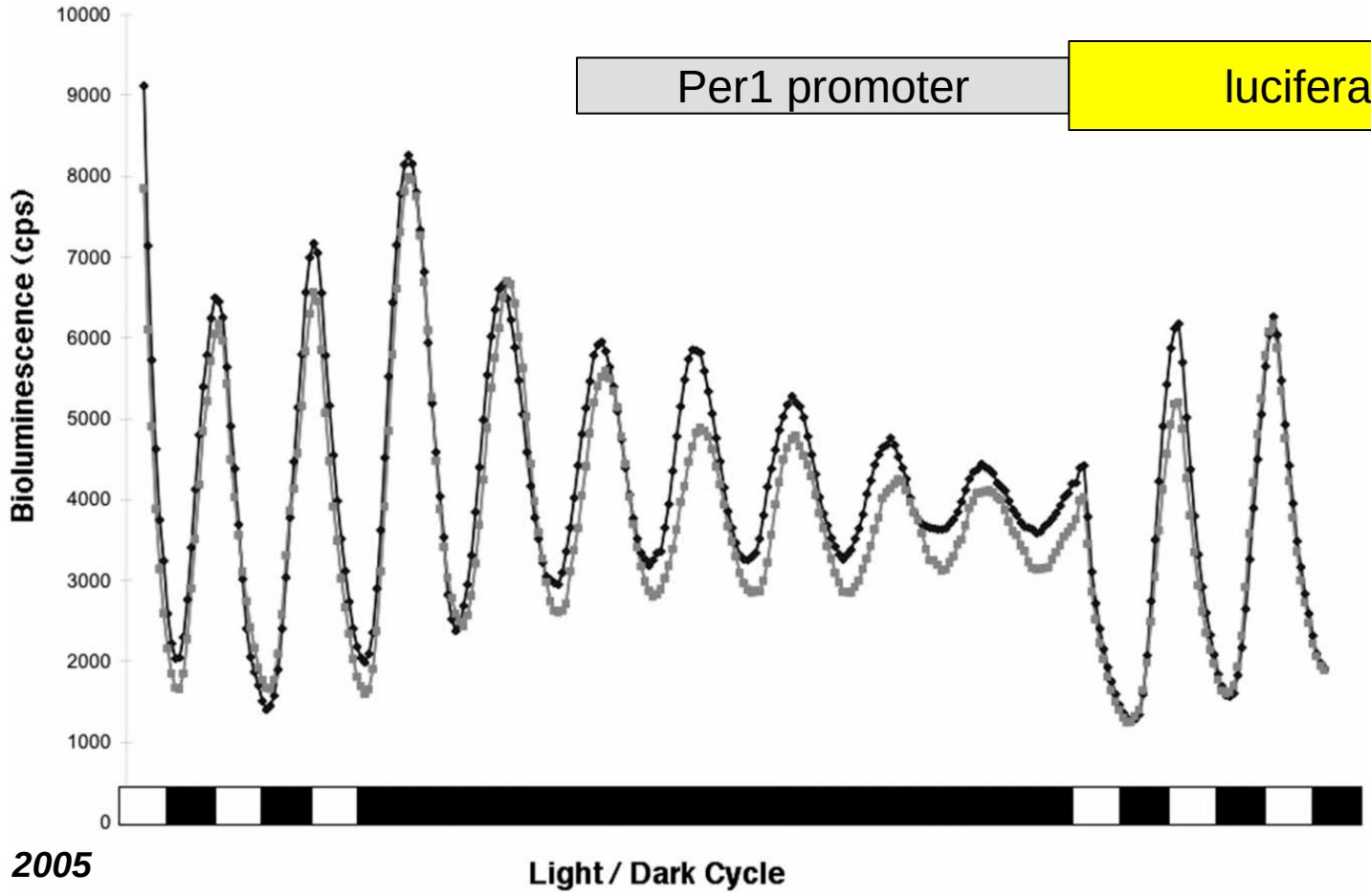
Per1 promoter | luciferase





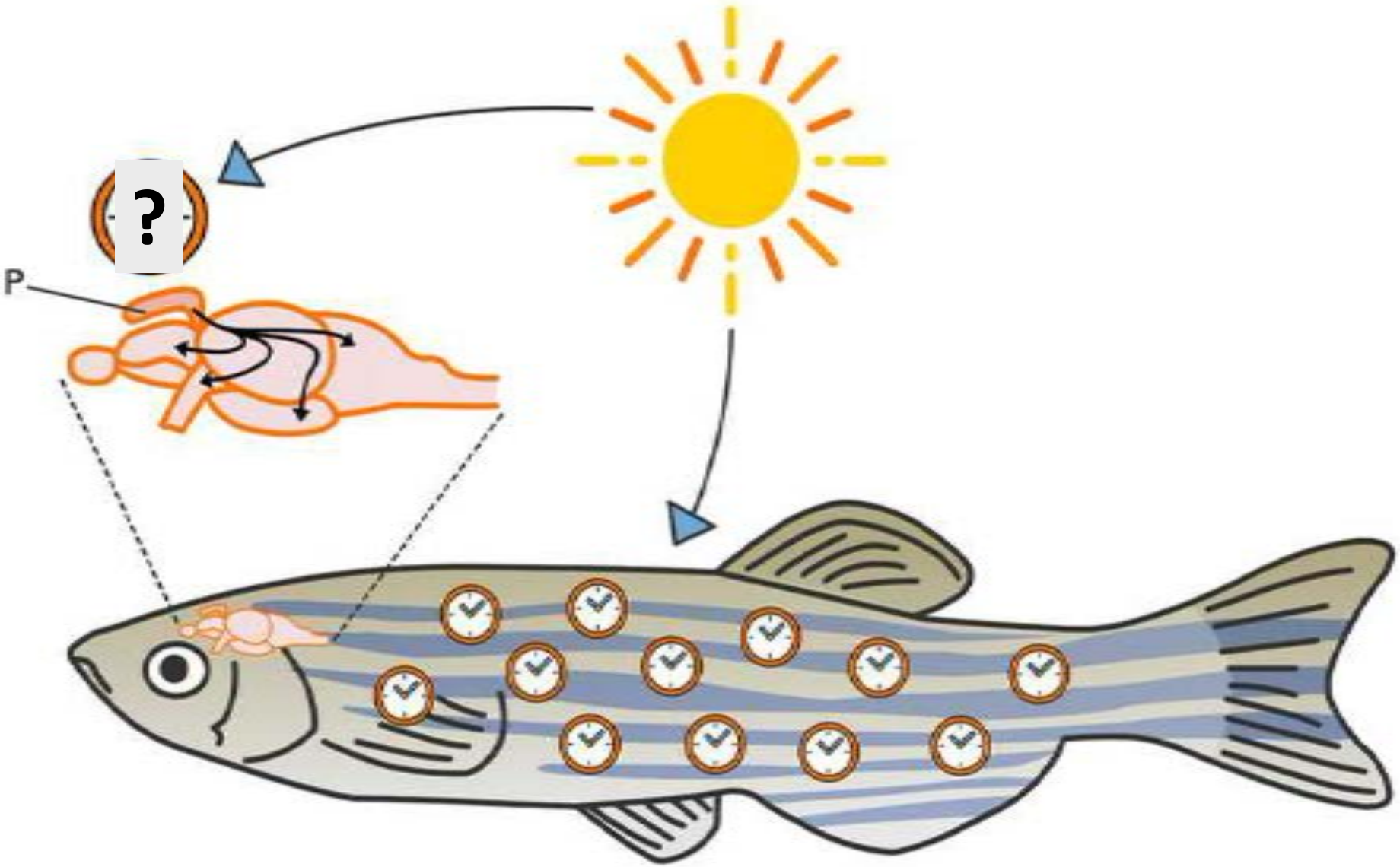
Carr and Whitmore 2005

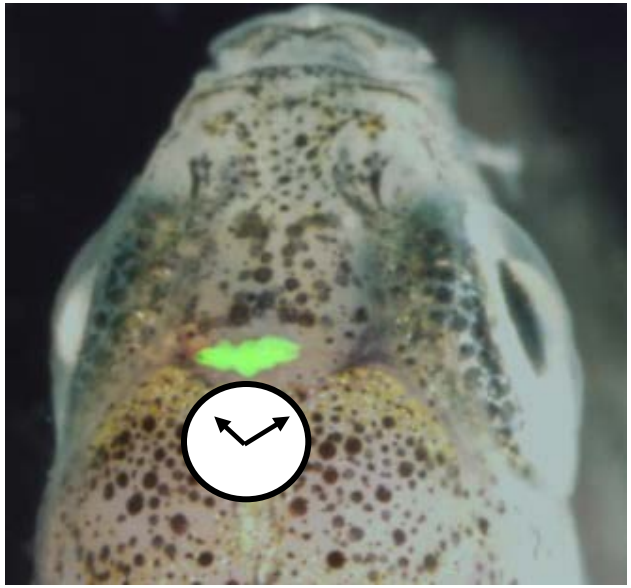
Luminescent measurements from zebrafish PAC2 cells transfected with *period1-luciferase*



Tamai et al., 2005

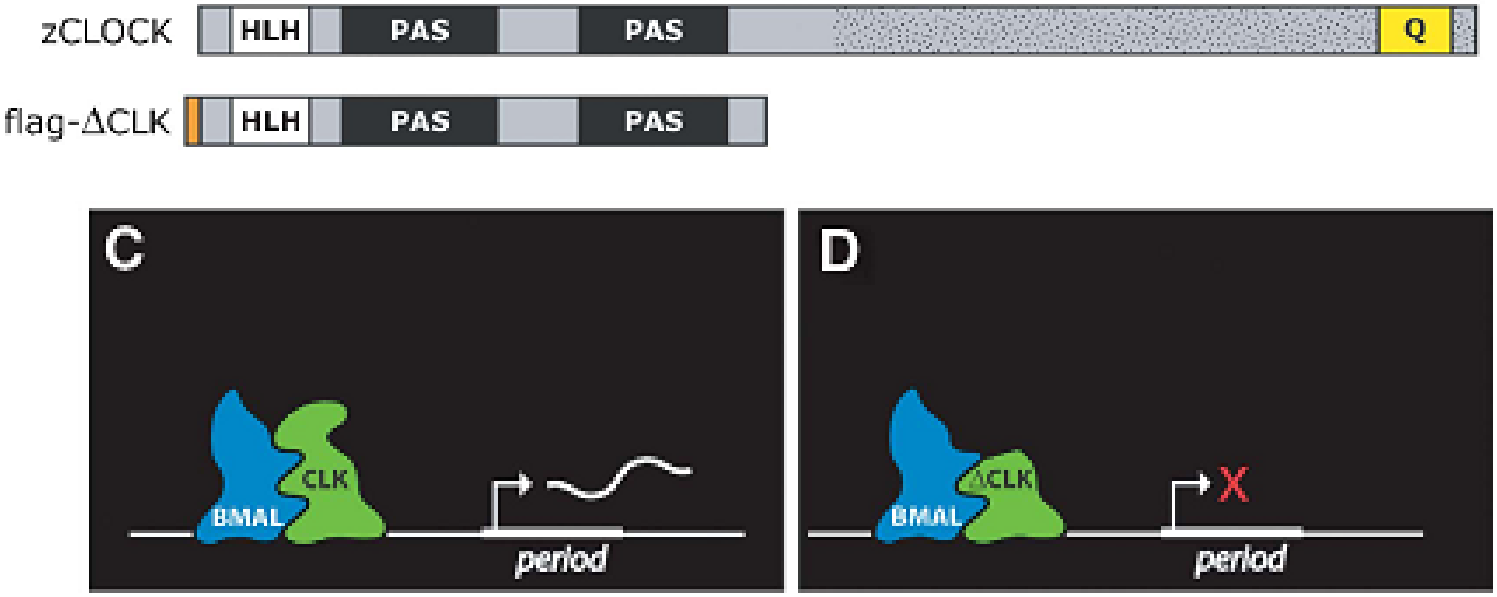
In fish, the circadian clock system is de-centralized


?
P

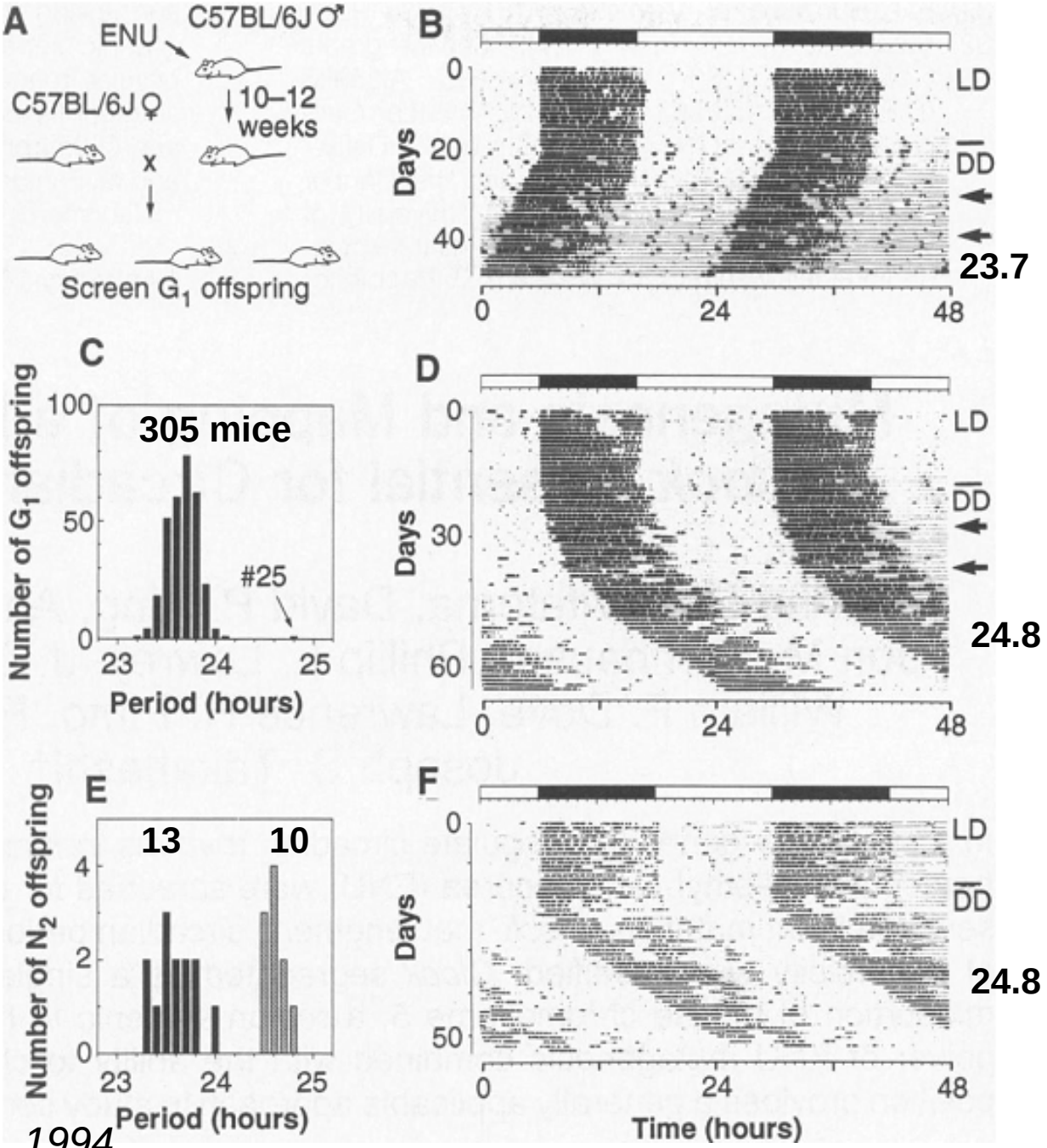


- Genetic ablation of the pineal gland
- *Aanat2* knockout – no melatonin
- Stop the clock in the pineal gland

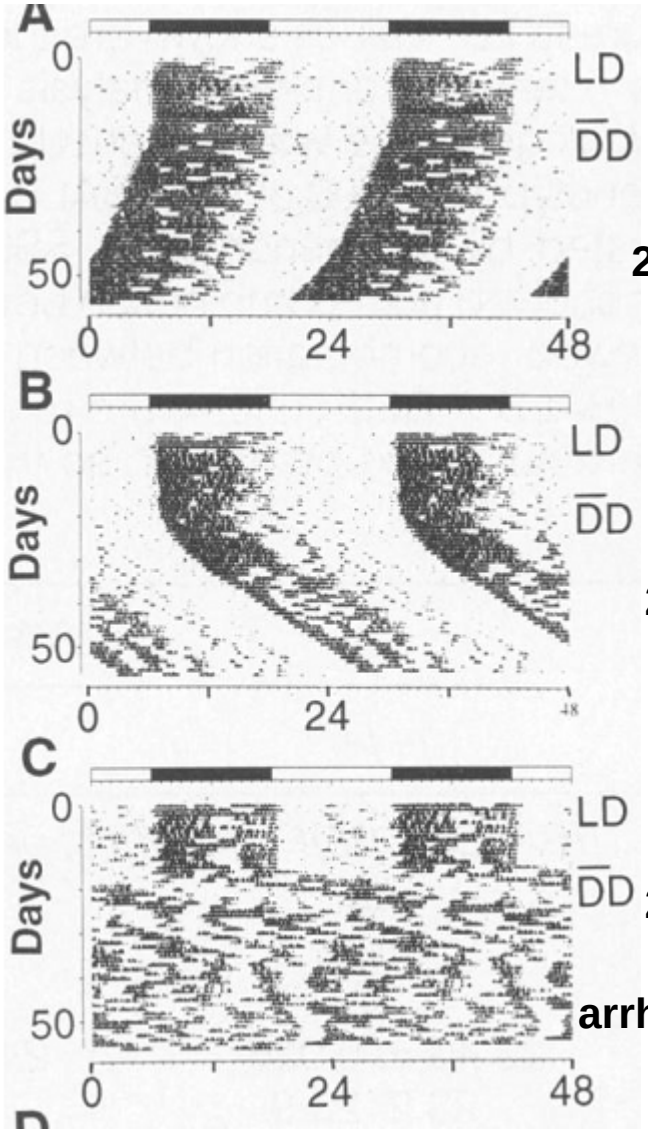
Generation of a transgenic fish line with impaired pineal gland clock function



Pineal-specific promoter



Vitaterna et al., 1994



+/+ **23.6**

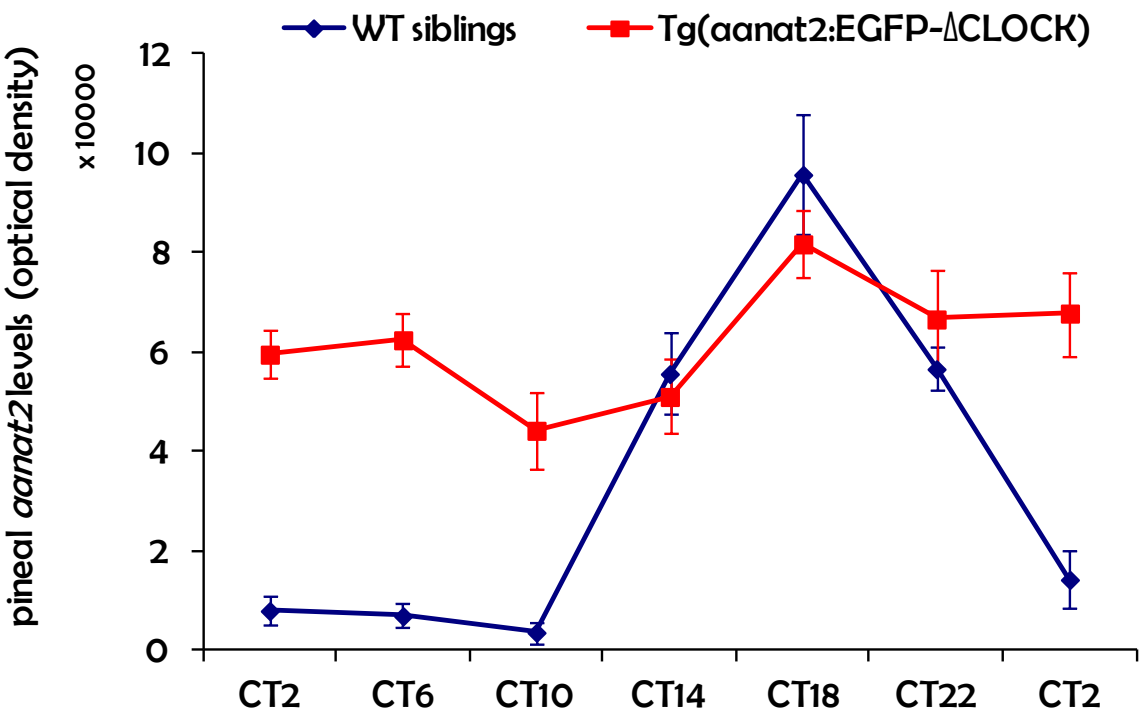
+/- **24.8**

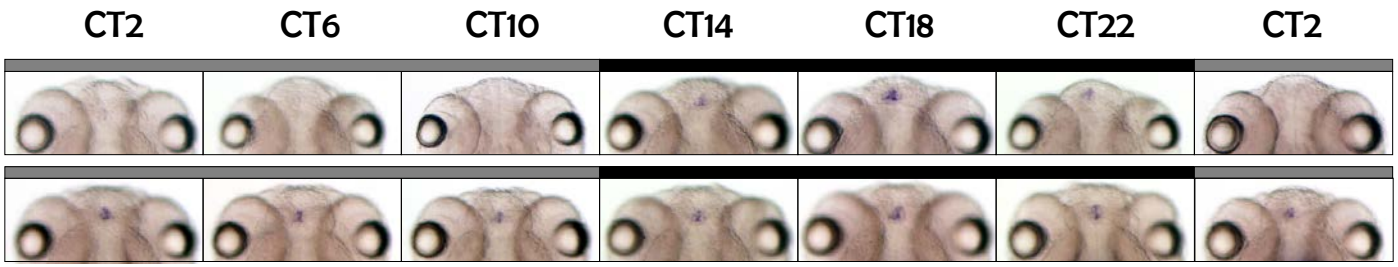
-/- **27.1**

arrhythmic

Vitaterna et al., 1994

Tg(*aanat2*:EGFP-ΔCLOCK) larvae display arrhythmic pineal *aanat2* mRNA expression

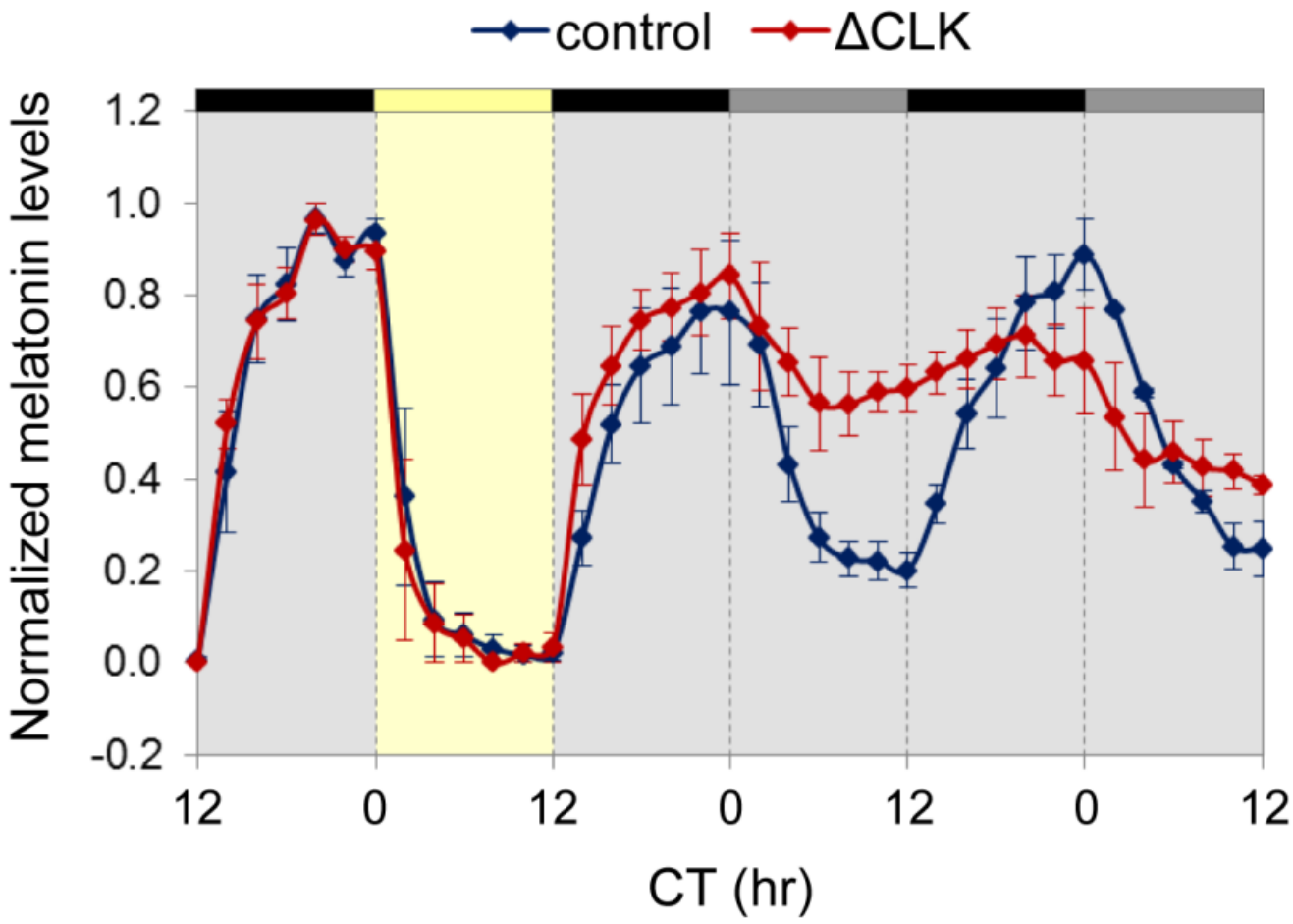




WT siblings

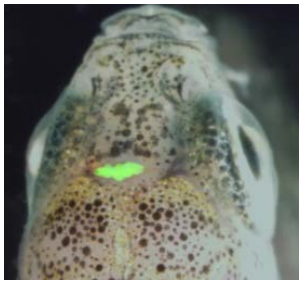
Tg(*aanat2*:EGFP-ΔCLOCK)

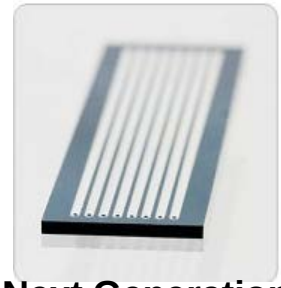
ΔCLK pineal glands display arrhythmic melatonin production under constant dark



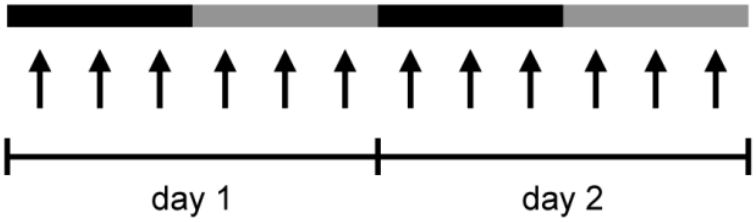
Ben-Moshe Livne et al., 2016

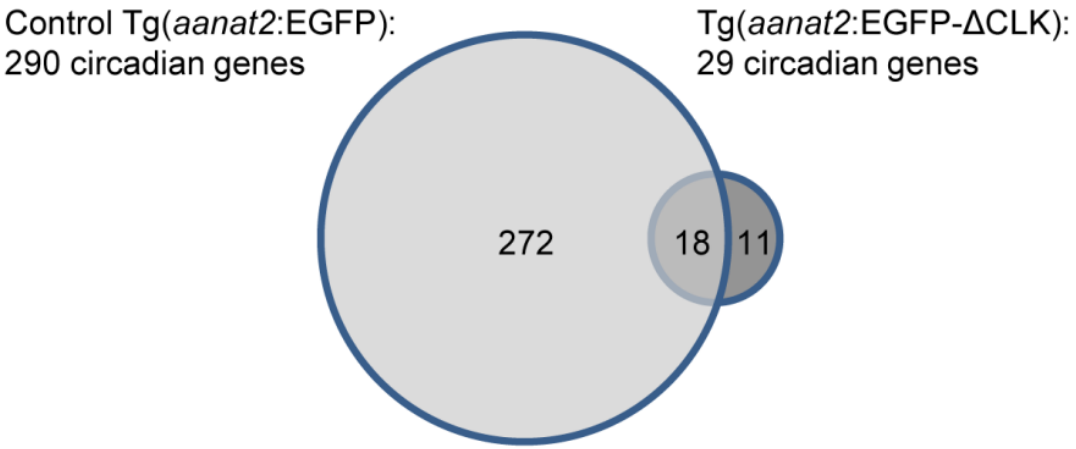
Most rhythmic genes lost their rhythm in ΔCLK pineal glands



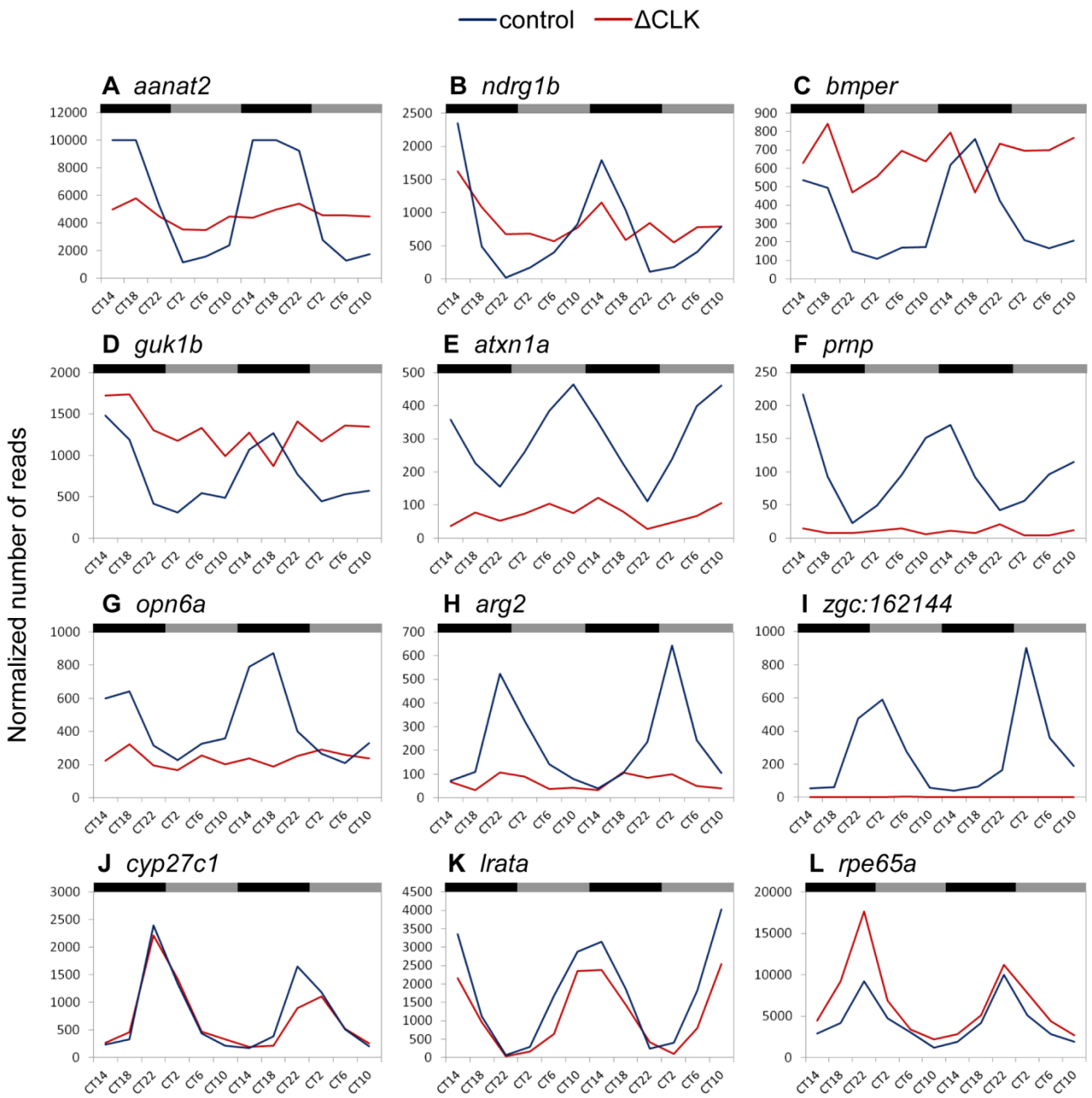


Next Generation Sequencing



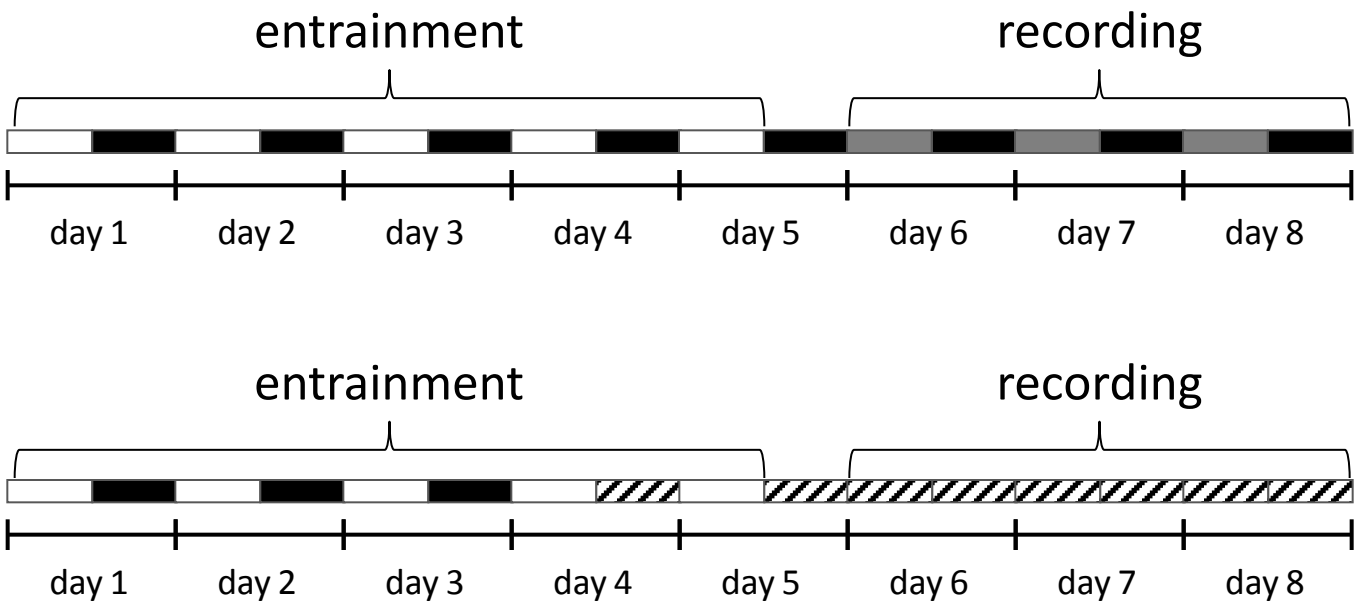


Ben-Moshe Livne et al., 2016



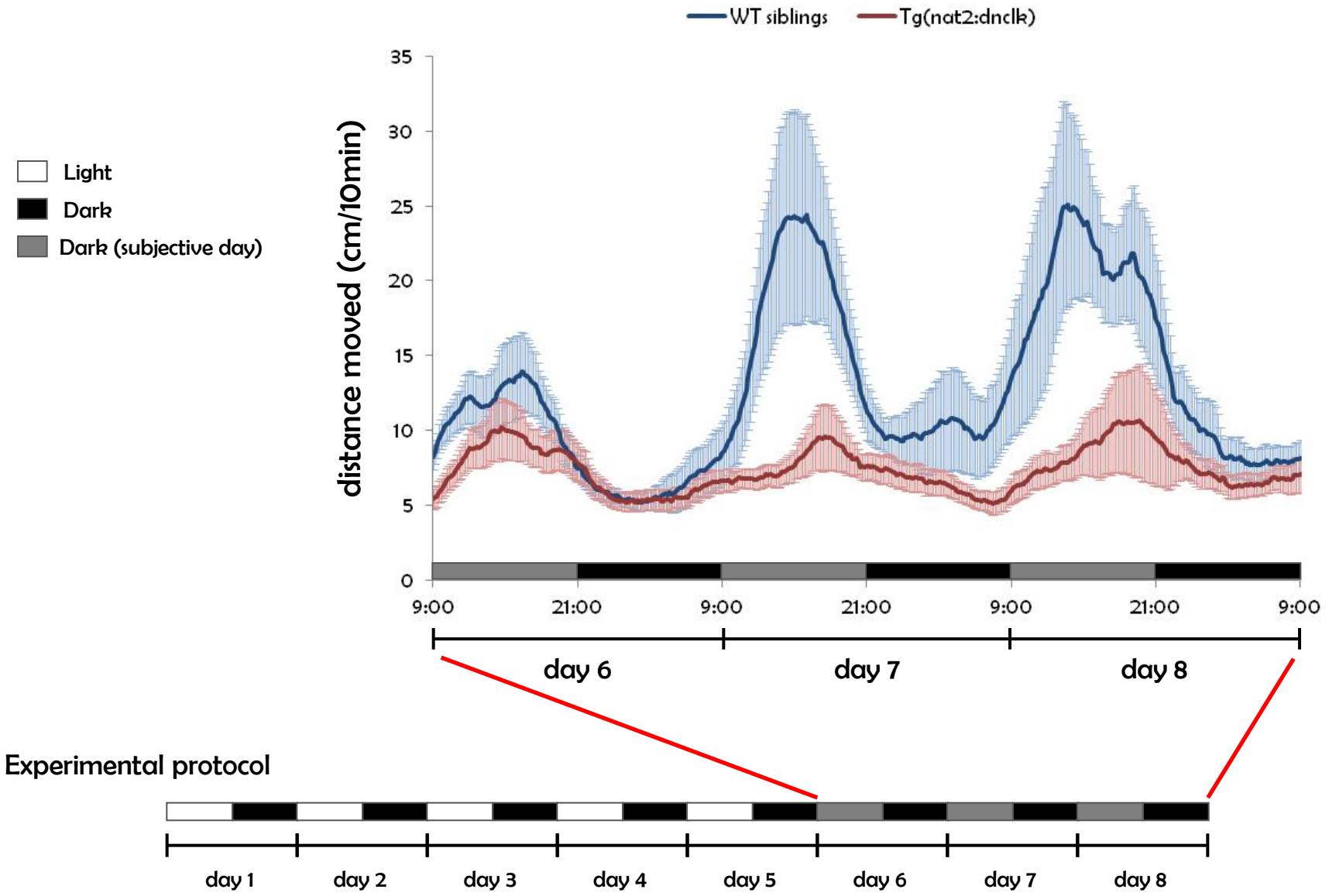
Ben-Moshe Livne et al., 2016

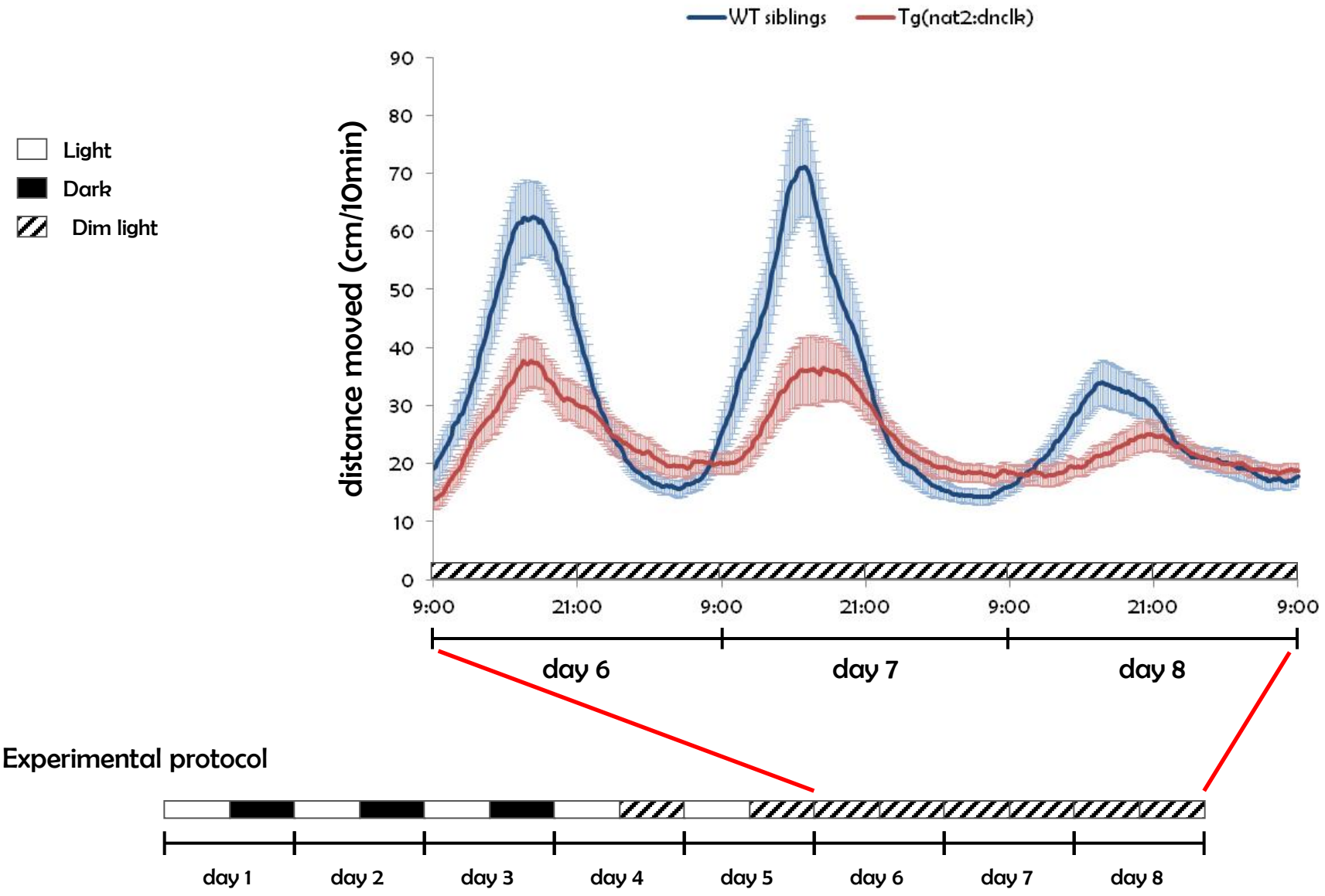
Experimental protocols

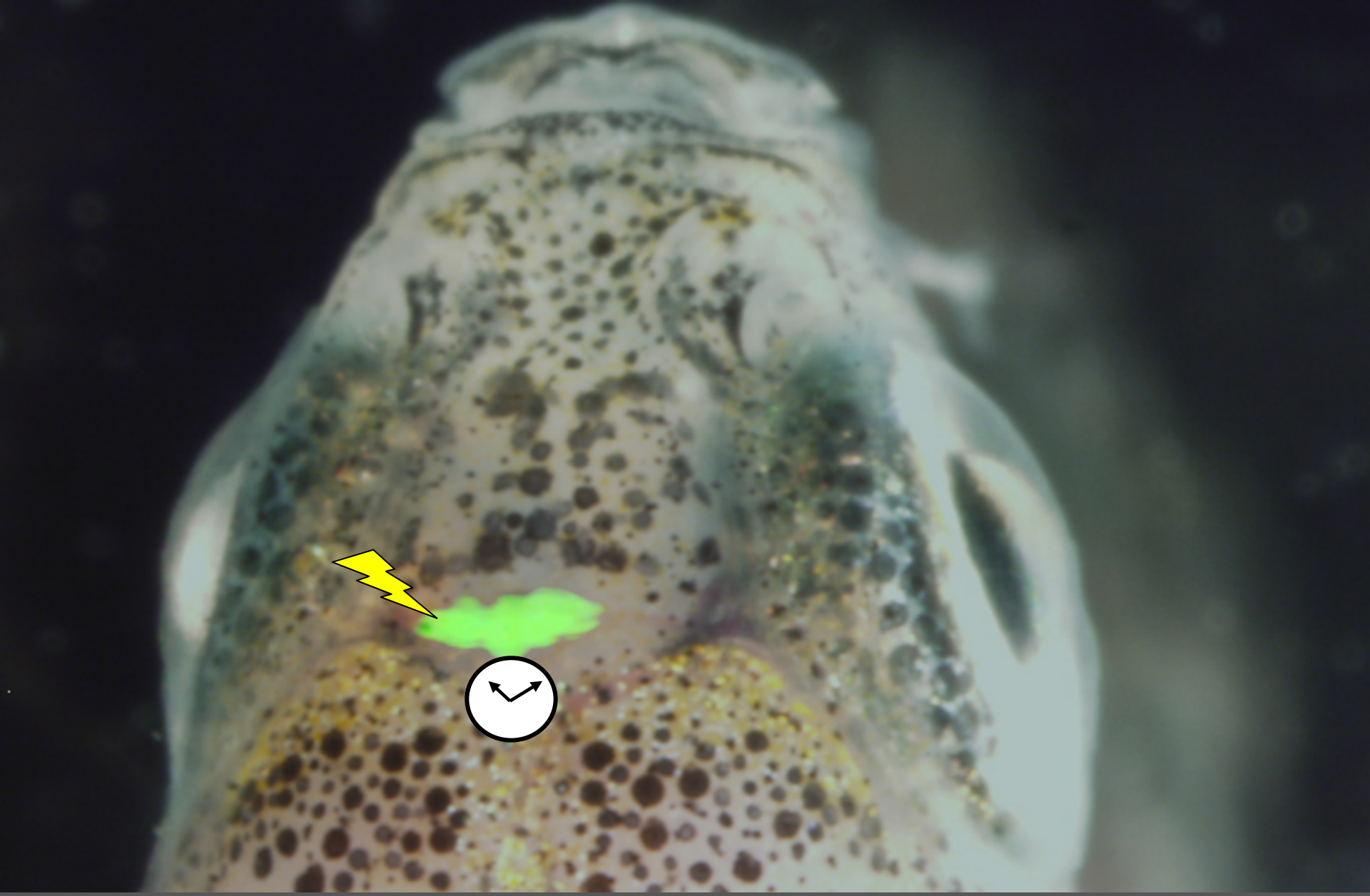


Ben-Moshe Livne et al., 2016

Blockage of the pineal clock leads to reduced amplitudes of rhythmic locomotor activity




WT siblings
Tg(nat2:dnclk)
Light
Dark
Dim light
distance moved (cm/10min)
90
80
70
60
50
40
30
20
10
0
9:00
21:00
9:00
21:00
9:00
21:00
9:00
day 6
day 7
day 8
Experimental protocol
day 1
day 2
day 3
day 4
day 5
day 6
day 7
day 8



The pineal molecular clock plays a key role in modulating behavioral circadian rhythms

Pineal transcriptome analysis – rhythmic genes

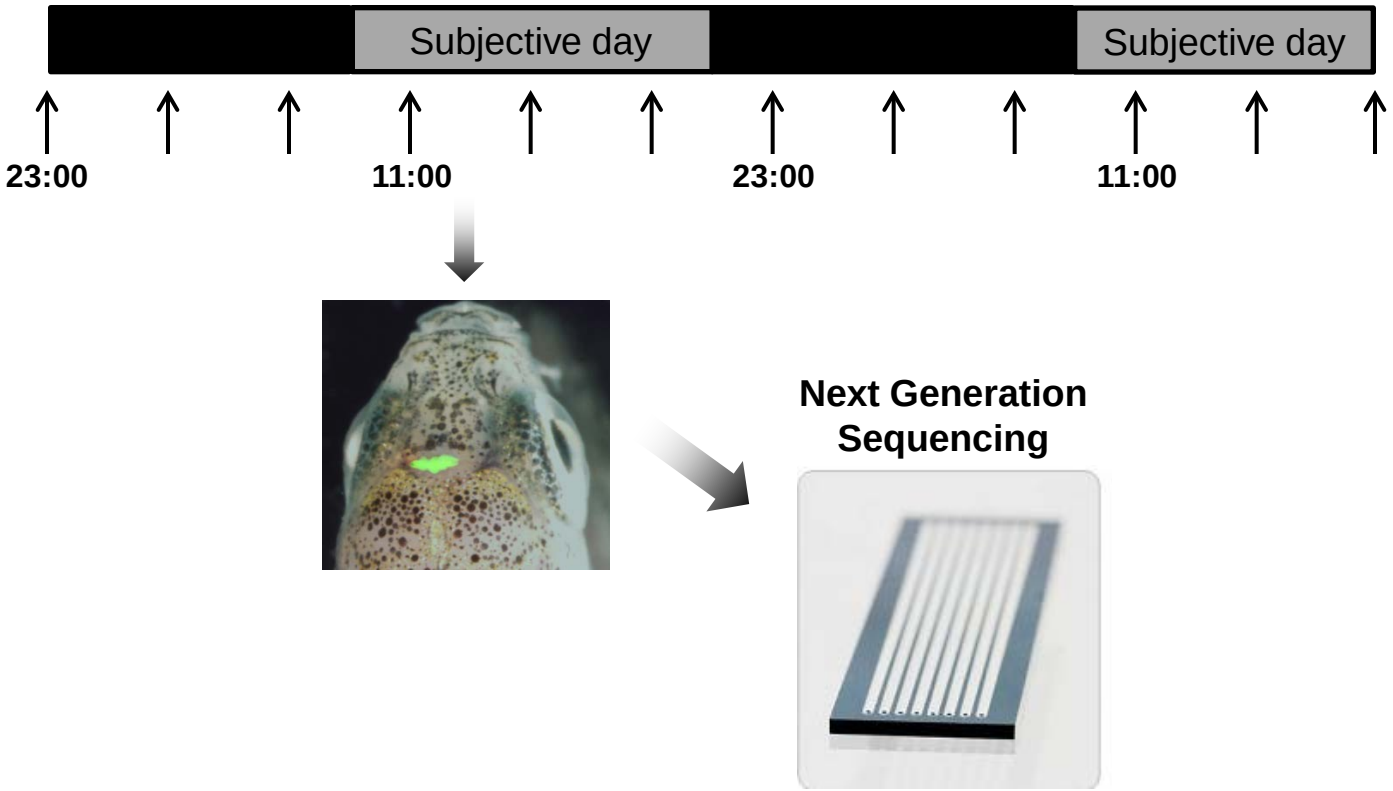
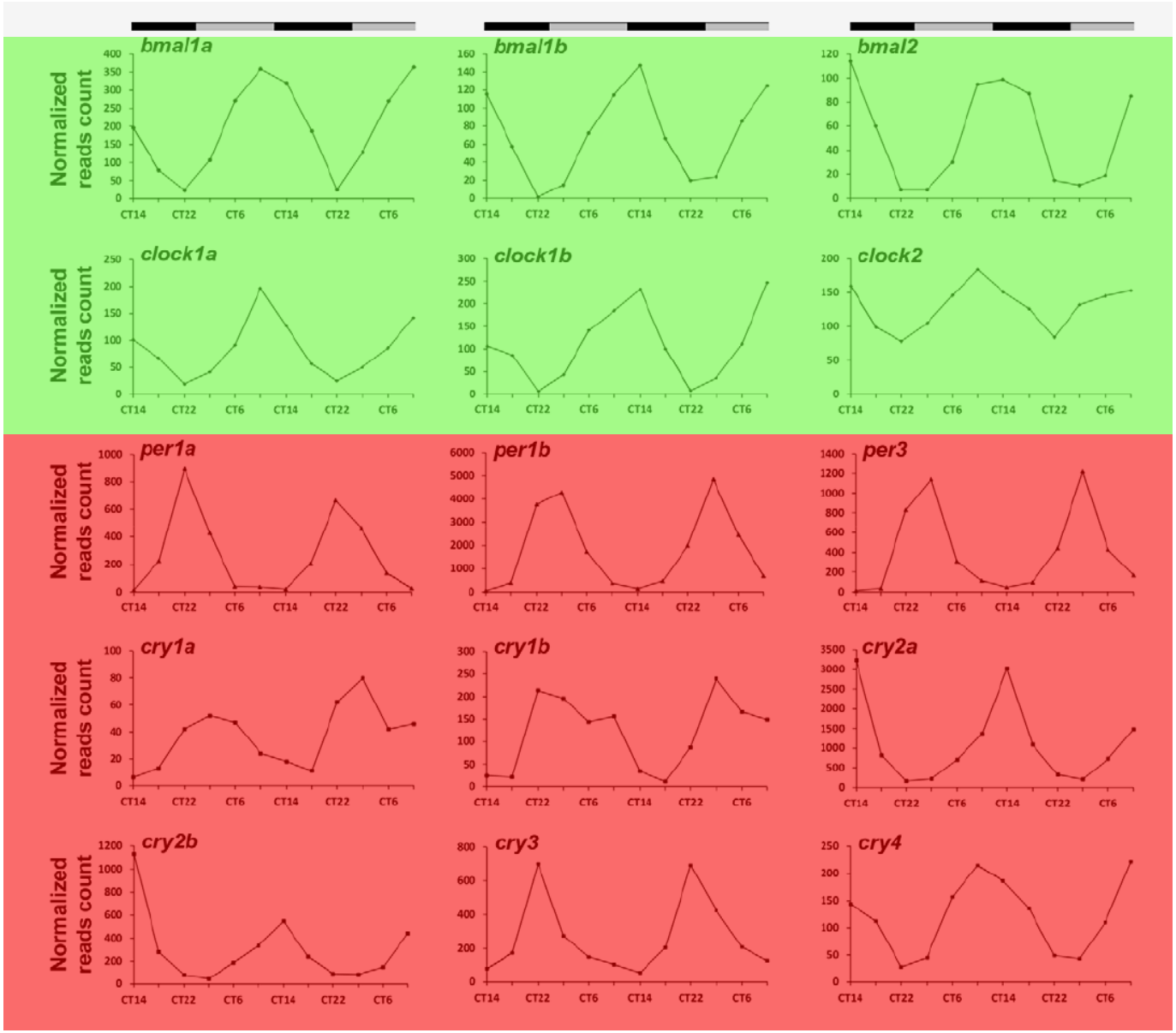


Table S2: List of circadian RefSeq genes using RNA-seq

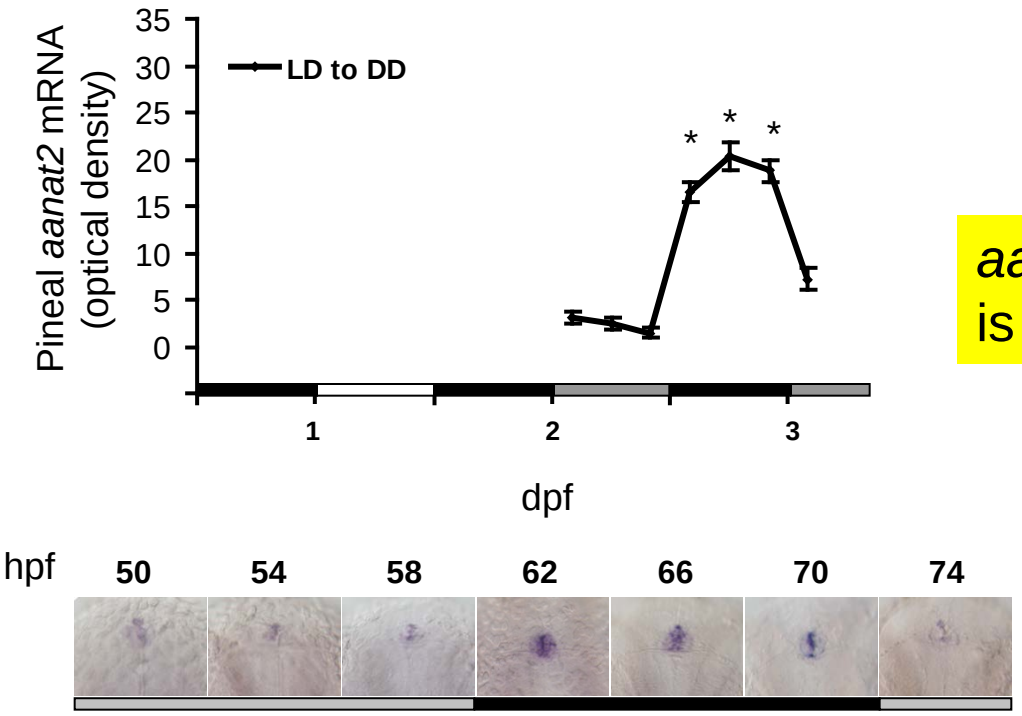
Number	RefSeq ID	Gene symbol	g-factor
1	NM_1002642	*camk2n2*	0.9380
2	NM_1197058	*nfil3-5 (e4bp4-5)*	0.9269
3	NM_131791	*cry2a*	0.9229
4	NM_131451	*irbp*	0.9151
5	NM_213439	*mid1ip1*	0.9147
6	NM_131792	*cry2b*	0.9130
7	NM_1113337	*cyp27c1*	0.9112
8	NM_131578	*arntl2 (bmal2)*	0.9102
9	NM_212584	*ctsl1a*	0.9079
10	NM_212439	*per1b*	0.9074
11	NM_1045055	*si:ch211-132b12.7*	0.9051
12	NM_1030183	*per1a*	0.8949
13	NM_1044826	*atxn1a*	0.8927
14	NM_1002369	*zgc:92502*	0.8906
15	NM_200139	*cyp2p6*	0.8902
16	NM_201158	*aldh4a1*	0.8897
17	NM_1197060	*dbpa*	0.8892
18	NM_1039107	*bhlhe41 (dec2)*	0.8889
19	NM_205729	*nr1d1 (reverba)*	0.8871
20	NM_200751	*rpe65a*	0.8864
21	NM_130957	*clock1a*	0.8851
22	NM_1077323	*zgc:154001*	0.8845
23	NM_1114579	*fam131a*	0.8835
24	NM_200829	*camk1gb*	0.8814
25	NM_213523	*krt15*	0.8810
26	NM_1042720	*atp7a*	0.8805
27	NM_1013555	*zgc:113006*	0.8777
28	NM_131584	*per3*	0.8762
29	NM_199871	*mat1a*	0.8744
30	NM_1082979	*zgc:162144*	0.8737
31	NM_199567	*tgif1*	0.8722
32	NM_131065	*nr1d2b (reverbb2)*	0.8717
33	NM_131571	*smad3a*	0.8709
34	NM_1110486	*zgc:158292*	0.8687
35	NM_212723	*mknk2a*	0.8686
36	NM_1098177	*myh9a*	0.8679
37	NM_199611	*arg2*	0.8669
38	NM_1079657	*zgc:158340*	0.8665
39	NM_1199134	*ptprb*	0.8650
40	NM_131787	*cry4*	0.8642
41	NM_201217	*nop56*	0.8632
42	NM_1197065	*nfil3-2 (e4bp4-2)*	0.8630
43	NM_213387	*pgk1*	0.8624
44	NM_1166205	*hip1*	0.8615
45	NM_1006090	*fam73a*	0.8607
46	NM_201067	*rorab*	0.8600
47	NM_1044834	*si:dkey-239i20.4*	0.8598
48	NM_1080022	*mylipb*	0.8586
49	NM_213495	*cul1b*	0.8581
50	NM_131411	*aanat2*	0.8565
51	NM_212822	*pik3r2*	0.8565
52	NM_201493	*nucb2a*	0.8552
53	NM_1004595	*utp3*	0.8518
54	NM_1044846	*slain2*	0.8507
55	NM_194377	*aldoaa*	0.8507
56	NM_199624	*her8a*	0.8497
57	NM_205759	*zgc:77395*	0.8487
58	NM_1110477	*zgc:162232*	0.8463
59	NM_201149	*cdc14aa*	0.8452
60	NM_1127308	*dtx1*	0.8441
61	NM_205686	*cry-dash*	0.8423
62	NM_131786	*cry3*	0.8420
63	NM_1007445	*phyhd1*	0.8410
64	NM_199609	*dhrs9*	0.8403
65	NM_205586	*prnp*	0.8403
66	NM_1045208	*letm1*	0.8345
67	NM_1082901	*si:ch211-197n10.4*	0.8333
68	NM_199649	*zfp36l1b*	0.8318
69	NM_201116	*sh3gl2*	0.8313
70	NM_1008620	*tsta3*	0.8312
71	NM_212682	*clic1*	0.8312
72	NM_198915	*rbm19*	0.8307
73	NM_1082819	*rorca*	0.8267
74	NM_1007372	*gsto2*	0.8259
75	NM_199873	*ldhd*	0.8258
76	NM_1204131	*lrata*	0.8251
77	NM_1201566	*usp54a*	0.8234
78	NM_213528	*ng36*	0.8232
79	NM_201083	*fus*	0.8227
80	NM_178295	*clock1b*	0.8220
81	NM_213112	*rabggtb*	0.8204
82	NM_1005400	*cthl*	0.8200
83	NM_1002297	*gnl3*	0.8197
84	NM_1105524	*ttll6*	0.8195
85	NM_1080204	*cyp11c1*	0.8193
86	NM_1126461	*etv5a*	0.8188
87	NM_213157	*slc25a33*	0.8178
88	NM_200610	*dopey2*	0.8172
89	NM_1077580	*dcaf12*	0.8152
90	NM_131373	*gfap*	0.8147
91	NM_1037379	*sept4a*	0.8131
92	NM_1020730	*zgc:109949*	0.8129
93	NM_200644	*nat10*	0.8129
94	NM_131801	*odc1*	0.8129
95	NM_199583	*csnk1da*	0.8123
96	NM_201202	*phc2b*	0.8111
97	NM_1039808	*slc2a1a*	0.8101
98	NM_1166332	*nlgn3a*	0.8098
99	NM_1111150	*bms1l*	0.8098
100	NM_1017881	*sqrdl*	0.8097
101	NM_1004562	*fth1b*	0.8095
102	NM_1020647	*acbd5a*	0.8089
103	NM_1014296	*mat2ab*	0.8088
104	NM_205724	*fbxo25*	0.8045
105	NM_1128538	*cdc14ab*	0.8039
106	NM_213010	*acadm*	0.8031
107	NM_1002649	*aclya*	0.8013
108	NM_1089417	*pfkmb*	0.8001
109	NM_1077253	*gnl1*	0.7995
110	NM_200567	*zgc:65987*	0.7992
111	NM_1006043	*ctsz*	0.7979
112	NM_199969	*mdh1b*	0.7972
113	NM_1040393	*zgc:136817*	0.7957
114	NM_212679	*bhlhe40 (dec1)*	0.7957
115	NM_1017753	*asb5*	0.7955
116	NM_1123011	*zgc:158426*	0.7955
117	NM_200172	*mycb*	0.7953
118	NM_131247	*ldhba*	0.7948
119	NM_1114583	*abcb4*	0.7946
120	NM_198363	*tob1a*	0.7940
121	NM_1135142	*zgc:194398*	0.7926
122	NM_1190816	*slc1a8b*	0.7921
123	NM_201326	*hspa9*	0.7889
124	NM_152949	*ccna2*	0.7882
125	NM_1082877	*ppat*	0.7874
126	NM_1077584	*zgc:152911*	0.7871
127	NM_1044892	*cspg5b*	0.7870
128	NM_1077731	*zgc:154093*	0.7870
129	NM_1024111	*pcdh1g11*	0.7869
130	NM_212714	*poldip2*	0.7868
131	NM_131799	*bcdo2l*	0.7867
132	NM_1200020	*si:ch1073-204j4.2*	0.7861
133	NM_1199680	*si:dkey-77a20.7*	0.7837
134	NM_1080989	*dennd4a*	0.7835
135	NM_1033591	*zgc:112334*	0.7830
136	NM_201025	*pgm1*	0.7815
137	NM_1003588	*zgc:101040*	0.7815
138	NM_1098485	*pgm2l1*	0.7807
139	NM_1013475	*bccip*	0.7807
140	NM_178436	*mcm5*	0.7806
141	NM_1098777	*sap130a*	0.7804
142	NM_131094	*tcf3*	0.7800
143	NM_1204257	*si:ch73-9j13.1*	0.7795
144	NM_212619	*cd9a*	0.7794
145	NM_1190305	*slc1a2a*	0.7787
146	NM_214748	*rpf2*	0.7779
147	NM_199333	*pkm2a*	0.7777
148	NM_201143	*pik3r3*	0.7775
149	NM_1083015	*acap3b*	0.7767
150	NM_1045207	*asap2a*	0.7766

Circadian expression pattern of clock genes in the zebrafish pineal gland.

RNA-seq analysis



What goes up, must come down



aanat2 mRNA is unstable

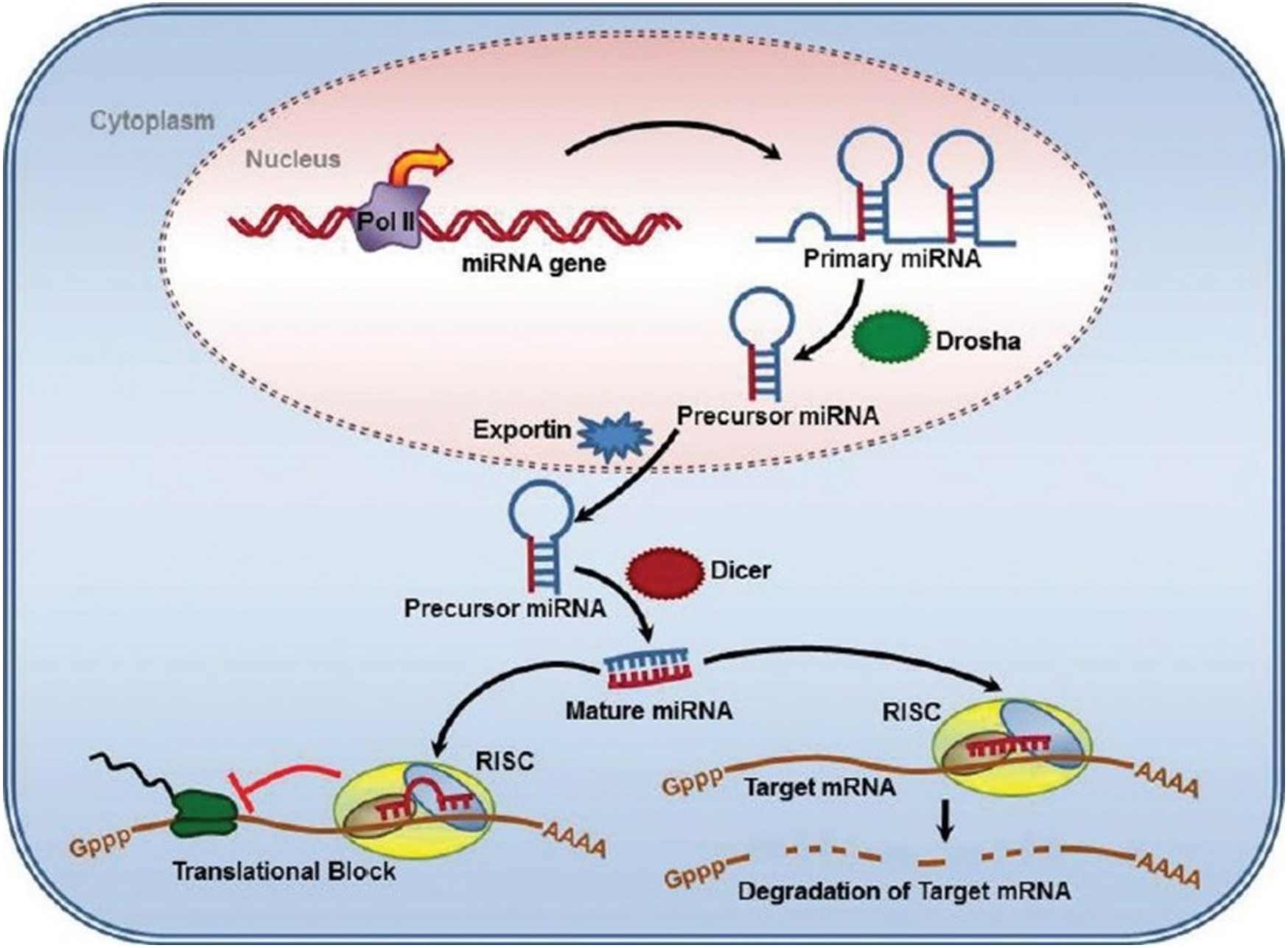
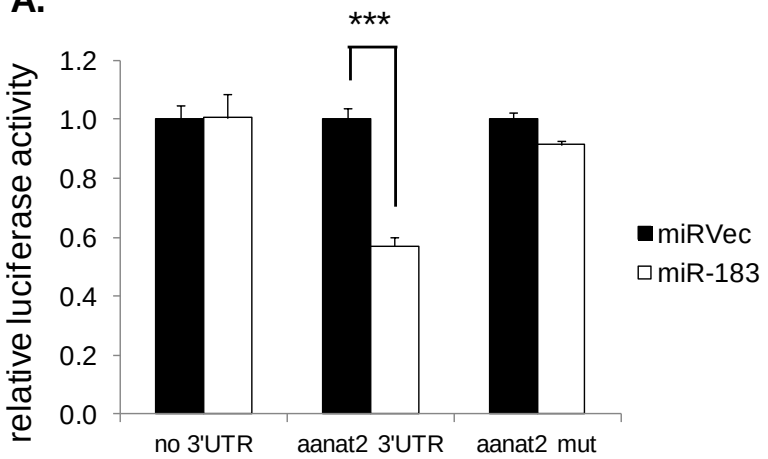

Cytoplasm
Nucleus
Pol II
miRNA gene
Primary miRNA
Drosha
Precursor miRNA
Exportin
Precursor miRNA
Dicer
Mature miRNA
RISC
Gppp
AAAA
Translational Block
RISC
Gppp
Target mRNA
AAAA
Gppp
AAAA
Degradation of Target mRNA

Table S5. Pineal-enhanced miRNAs

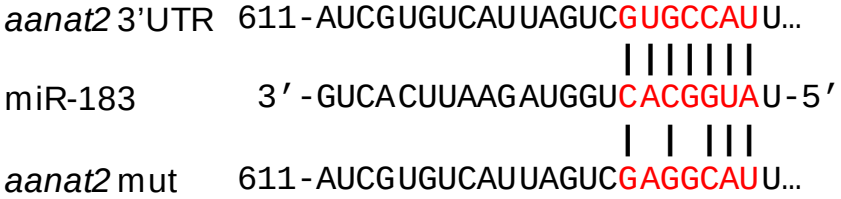
No.	miRNA	Pineal	Brain	Pineal/brain
1	dre-mir-182†	5791266	2008	2884.5
2	dre-mir-726†	15520	12	1342.0
3	dre-mir-96†	4422	5	908.0
4	dre-mir-183†	142119	178	799.6
5	dre-mir-204-2†	1918	38	50.4
6	dre-mir-733	46	2	25.2
7	dre-mir-27c*	280	32	8.7
8	dre-mir-725	486	64	7.6
9	dre-mir-92b	255088	41586	6.1
10	dre-mir-203a	390	80	4.9
11	dre-mir-30e-2*	11600	2685	4.3
12	dre-mir-460	628	147	4.3
13	dre-mir-1388*†	3010	749	4.0
14	dre-mir-738†	1546	393	3.9
15	dre-mir-214	439	116	3.8
16	dre-mir-203b†	1532	411	3.7
17	dre-mir-455	1050	284	3.7
18	dre-mir-2185	140	39	3.6
19	dre-mir-222b	714	205	3.5
20	dre-mir-25†	13940	4168	3.3
21	dre-mir-92a-1	56077	17172	3.3
22	dre-mir-155	74	23	3.2
23	dre-mir-462†	18524	6122	3.0
24	dre-mir-2188*	917	309	3.0
25	dre-mir-125b-2†	408	159	2.6
26	dre-let-7f	42828	16888	2.5
27	dre-mir-735†	929	368	2.5
28	dre-mir-731†	2293	961	2.4
29	dre-mir-2184	2333	1009	2.3
30	dre-mir-205	881	381	2.3
31	dre-mir-1388	848	394	2.2
32	dre-mir-27c†	2518	1227	2.1

† Identified also as light-induced in the pineal gland

A.



B.



Pineal transcriptome analysis – light induced genes

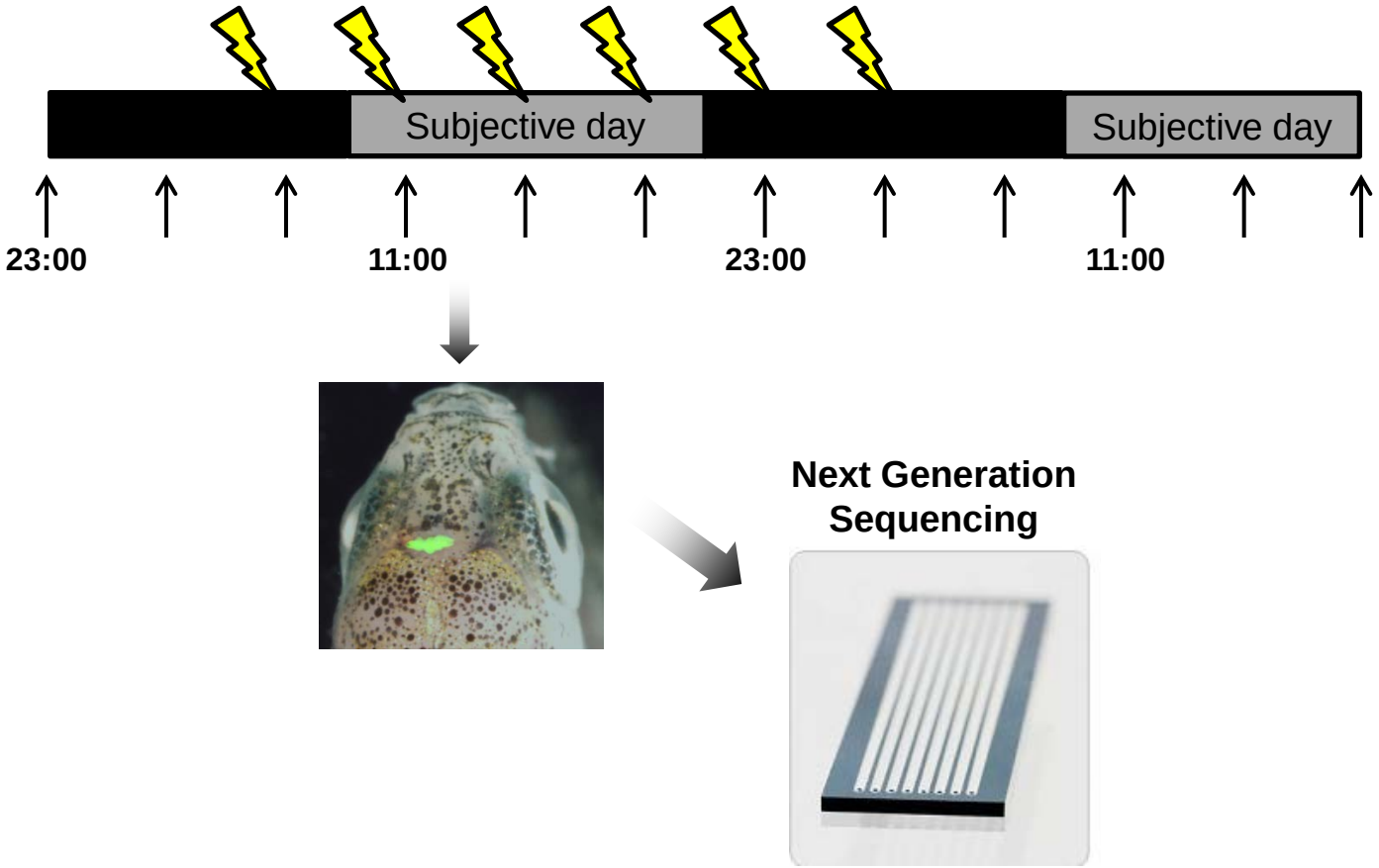


Table 1. Pineal gland light-induced genes detected by mRNA-seq

No.	Gene symbol	RefSeq ID	L/D fold	No.	Gene symbol	RefSeq ID	L/D fold
1	*pfkfb4l*	NM_198816	5.09	47	*rtn4rl2a*	NM_203479	2.28
2	*rtn4rl2b*	NM_203483	4.92	48	*vsnl1a*	NM_001007366	2.26
3	*nr4a3*	NM_001172629	4.81	49	*si:ch211-195b13.1*	NM_001077302	2.25
4	*pvalb7*	NM_205574	4.81	50	*insig1*	NM_199869	2.23
5	*nr1d1 (reverba)*	NM_205729	4.79	51	*hif1al*	NM_200405	2.22
6	*stmn4l*	NM_001002563	4.61	52	*znf238*	NM_001082952	2.21
7	*tagln2*	NM_201576	4.51	53	*stk35*	NM_001077616	2.2
8	*nppcl*	NM_001161341	4.38	54	*eng2a*	NM_131044	2.2
9	*mibp*	NM_131693	4.26	55	*bactin1*	NM_131031	2.17
10	*zgc:122979*	NM_001037574	4.09	56	*ifrd1*	NM_001076555	2.12
11	*fkbp5*	NM_213149	3.99	57	*hivep2*	NM_001030164	2.1
12	*jun*	NM_199987	3.77	58	*pik3r3b*	NM_201143	2.09
13	*bhlhe40 (dec1)*	NM_212679	3.6	59	*trim9*	NM_205563	2.05
14	*lonrf1l*	NM_001277234	3.59	60	*hsd11b2*	NM_212720	2.05
15	*osbpl7*	NM_001005927	3.54	61	*nfil3-5 (e4bp4-5)*	NM_001197058	2.03
16	*cnn2*	NM_213349	3.41	62	*glulb*	NM_182866	2.02
17	*nfil3-6 (e4bp4-6)*	NM_001002218	3.36	63	*zgc:63637*	NM_213365	2.02
18	*klf9*	NM_001128729	3.35	64	*dennd4a*	NM_001080989	2.01
19	*itm2cb*	NM_199980	3.32	65	*ppm1nb*	NM_001103117	2
20	*gabrb2*	NM_001024387	3.29	66	*coch*	NM_001003823	1.99
21	*per1a*	NM_001030183	3.29	67	*camk4*	NM_001017607	1.96
22	*slc20a1a*	NM_213179	3.28	68	*camk2n1*	NM_001145089	1.96
23	*tuft1a*	NM_001080001	3.1	69	*snap25a*	NM_131435	1.95
24	*per2*	NM_182857	3.07	70	*midn*	NM_207052	1.95
25	*cry1a*	NM_001077297	3	71	*chac1*	NM_001110126	1.94
26	*tmem178*	NM_001076640	2.99	72	*ucp2*	NM_131176	1.91
27	*nfil3-2 (e4bp4-2)*	NM_001197065	2.97	73	*scn8aa*	NM_131628	1.91
28	*camk2d2*	NM_001002542	2.89	74	*cyp27c1*	NM_001113337	1.9
29	*dbpb (dbp2)*	NM_001197062	2.87	75	*nrxn1a*	NM_001080021	1.78
30	*sptlc2b*	NM_001114741	2.75	76	*myl9b*	NM_213212	1.74
31	*pim3*	NM_001034978	2.71	77	*rpe65a*	NM_200751	1.73
32	*tgfbi*	NM_182862	2.7	78	*slc17a7*	NM_001098755	1.68
33	*jund*	NM_001128342	2.57	79	*tef1*	NM_131400	1.67
34	*bhlhe41 (dec2)*	NM_001039107	2.54	80	*grk7a*	NM_001031841	1.6
35	*mid1ip1*	NM_213439	2.54	81	*nr1d2b (reverbb2)*	NM_131065	1.6
36	*npas4a*	NM_001045321	2.52	82	*neurod*	NM_130978	1.58
37	*gfap*	NM_131373	2.51	83	*cplx4a*	NM_001077300	1.55
38	*speg*	NM_001007109	2.5	84	*stmn2a*	NM_001005923	1.54
39	*cry3*	NM_131786	2.48	85	*atp1a1b*	NM_131690	1.53
40	*nr1d2a (reverbb1)*	NM_001130592	2.45	86	*atp1b1a*	NM_131668	1.45
41	*abcf2*	NM_201315	2.4	87	*diras1*	NM_199831	1.44
42	*zgc:154093*	NM_001077731	2.36	88	*stxbp1*	NM_001025182	1.43
43	*arg2*	NM_199611	2.34	89	*atp2b2*	NM_001123238	1.41
44	*c1qtnf4*	NM_001017702	2.33	90	*gpm6aa*	NM_213200	1.4
45	*camk2b1*	NM_001271393	2.29	91	*gpm6ab*	NM_214687	1.37
46	*zgc:153615*	NM_001077791	2.28	92	*zic2a*	NM_131558	1.28

Per2

Cry1a

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Per2

Cry1a

Per1a

Cry3

Dec1

Dec2

Reverba

Reverbb1

Reverbb2

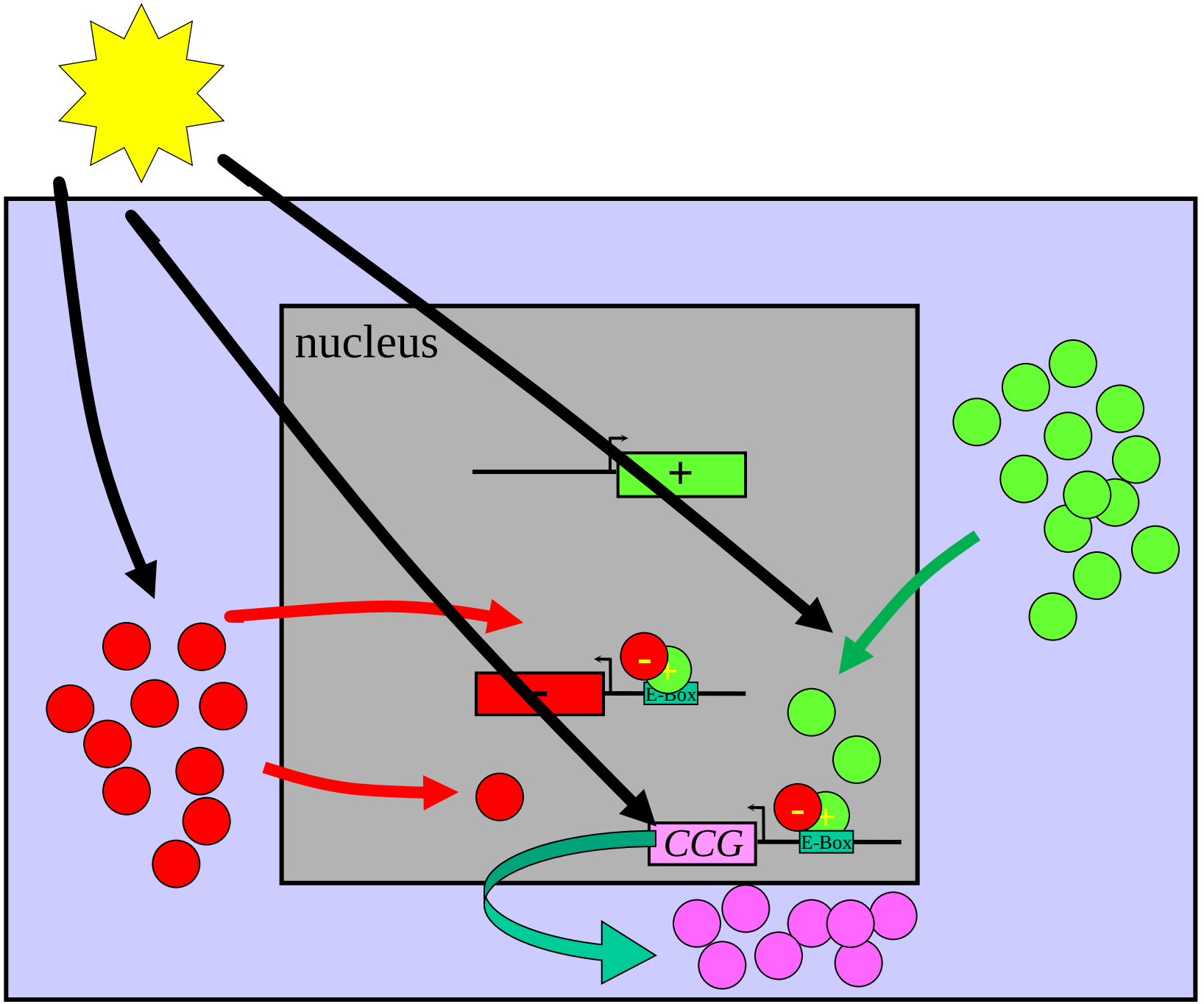
Tef1

Dbp2

E4bp4-2

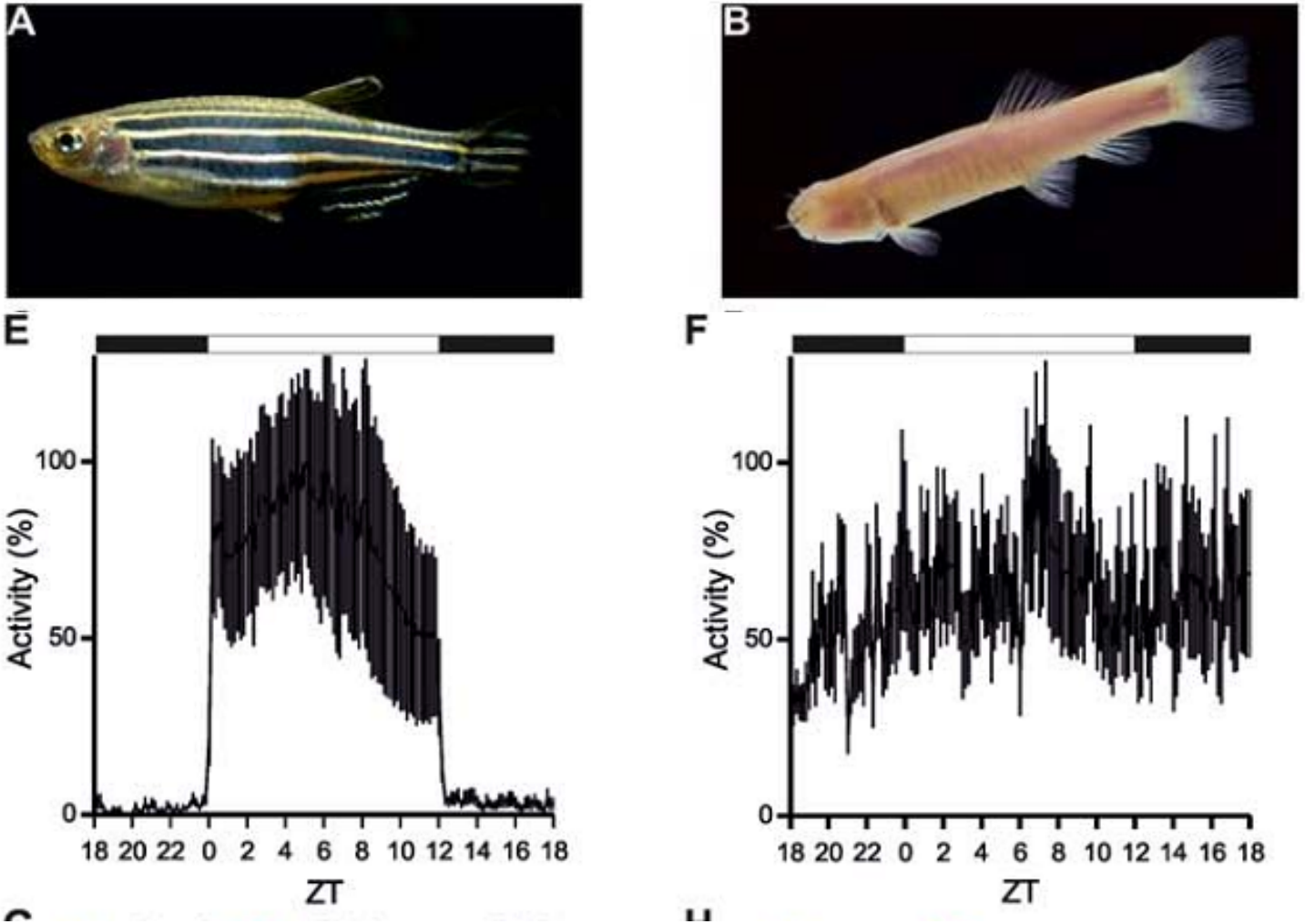
E4bp4-5

E4bp4-6


nucleus
+
-
+
E-Box
-
+
E-Box
CCG

How do peripheral clocks detect light?

Studies in blind cave fish



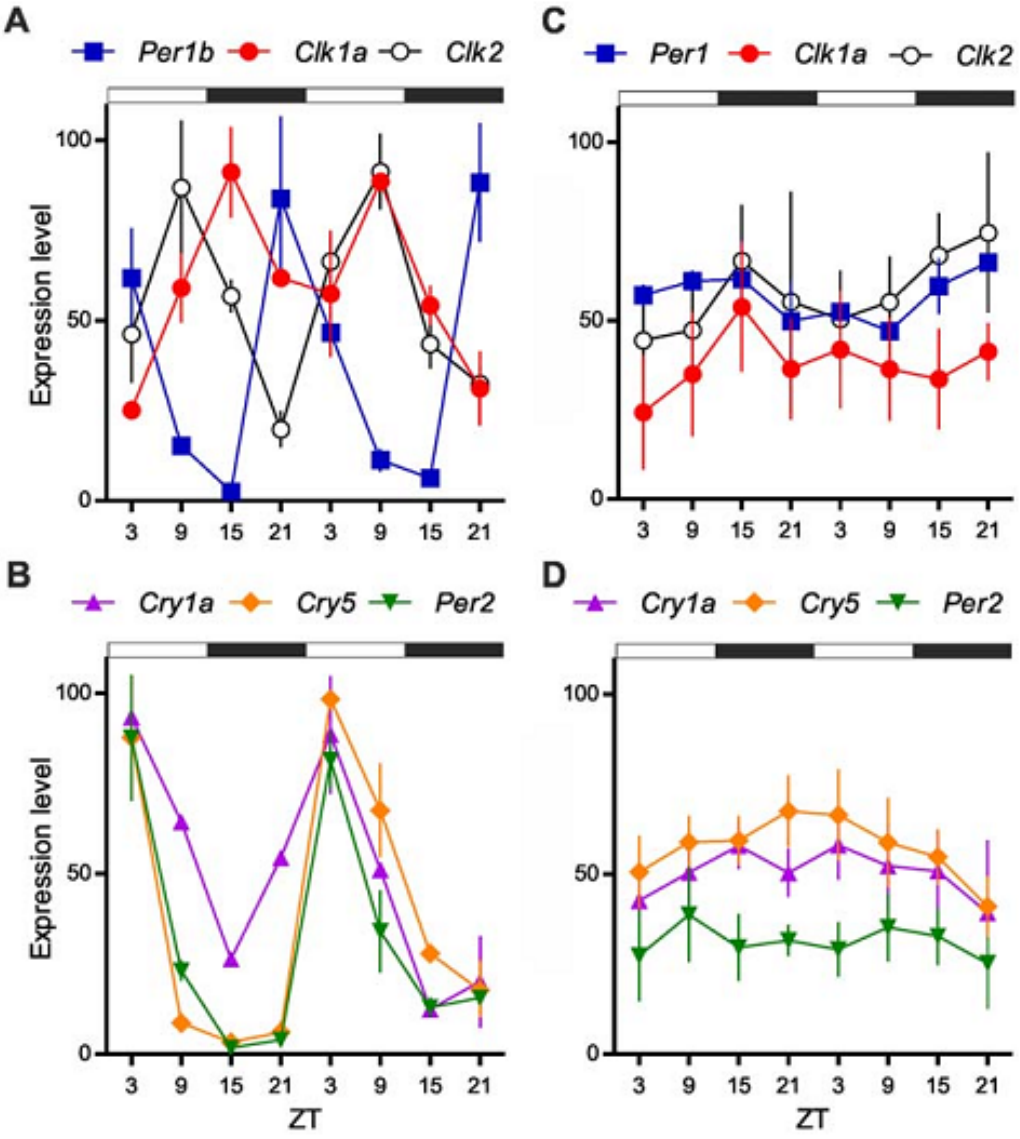
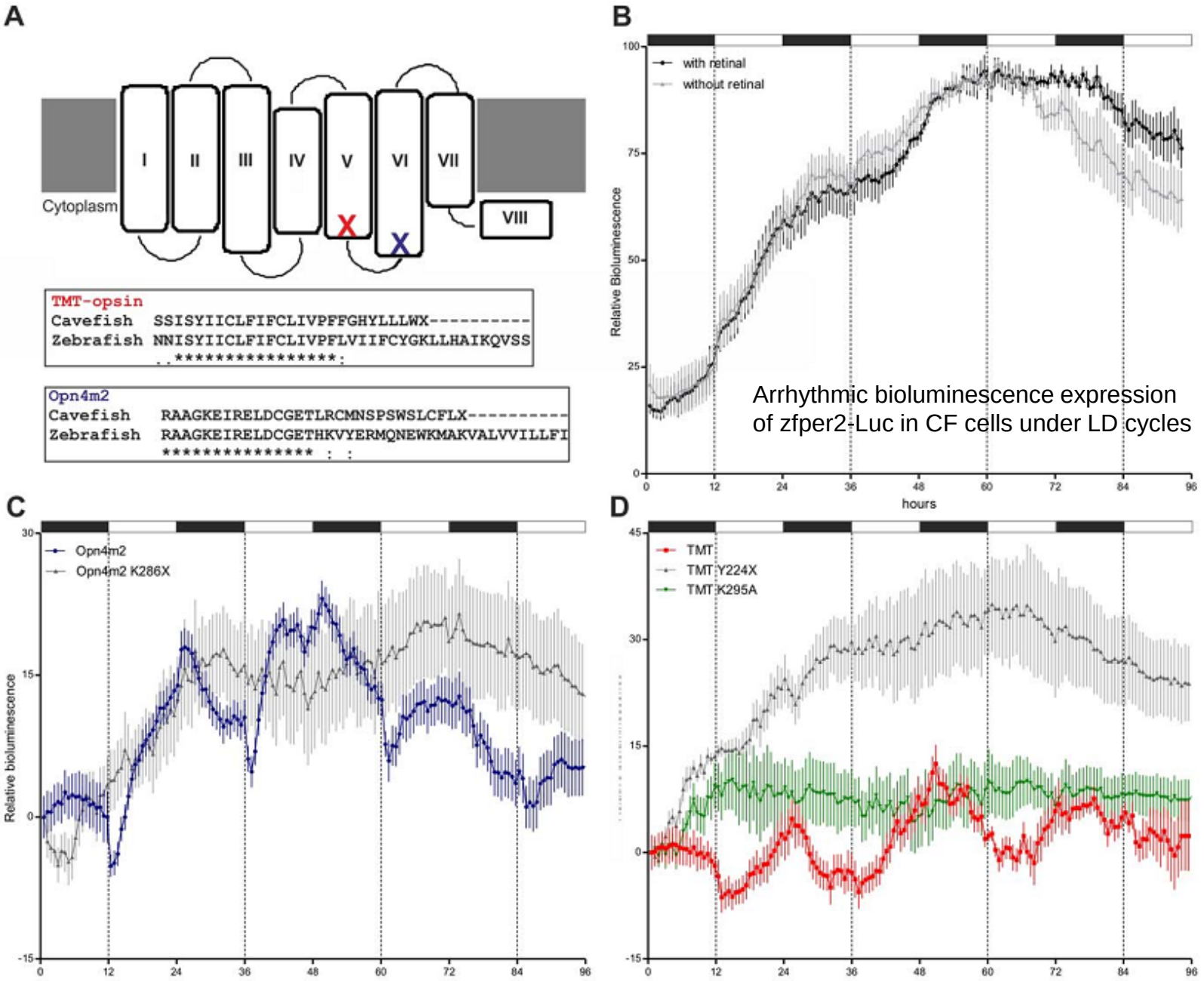
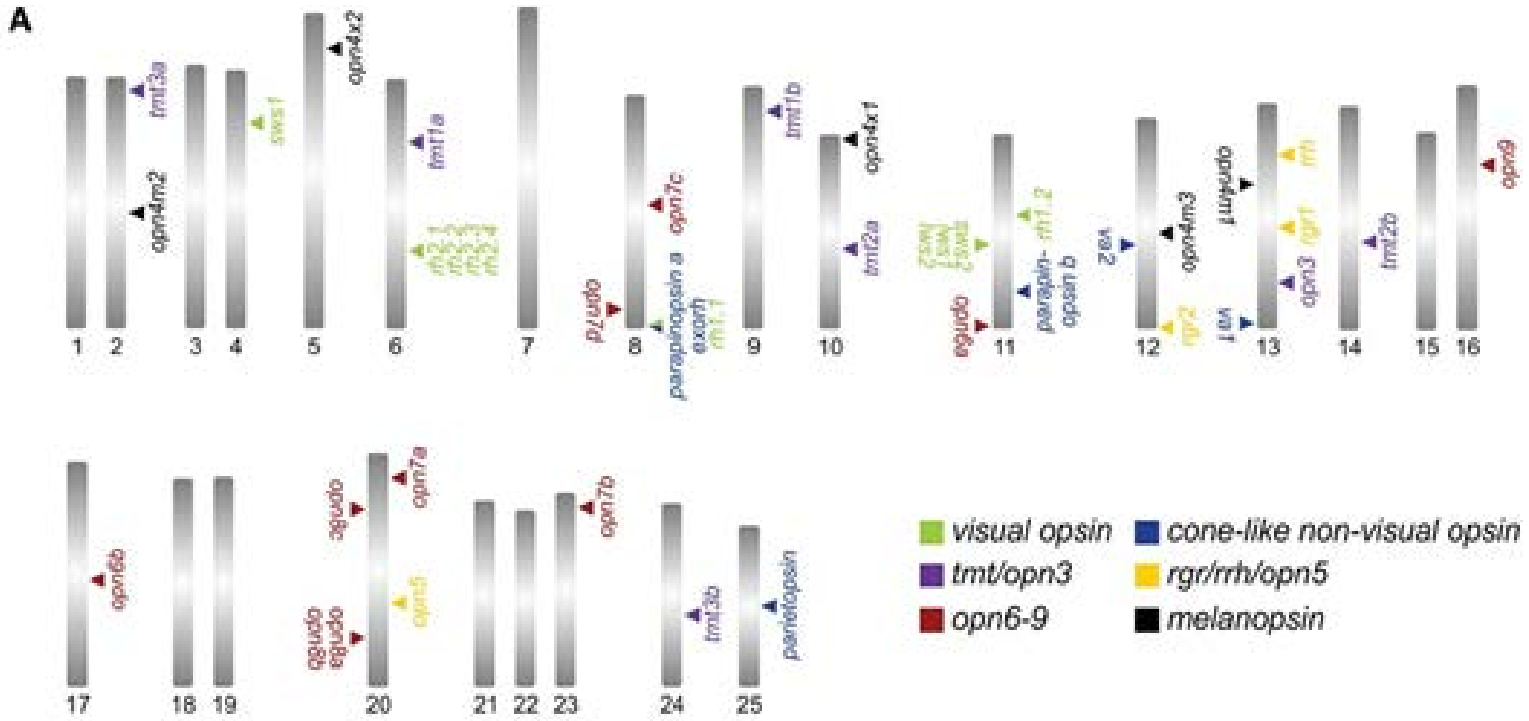


Figure 2. Lack of rhythmic clock gene expression in *P. andruzzii* cell lines exposed to LD cycles. Quantitative RT-PCR analysis of clock and light-regulated clock gene expression in zebrafish (A,B) and cavefish (CF; C,D) cell lines. The presentation, color code for the plotted



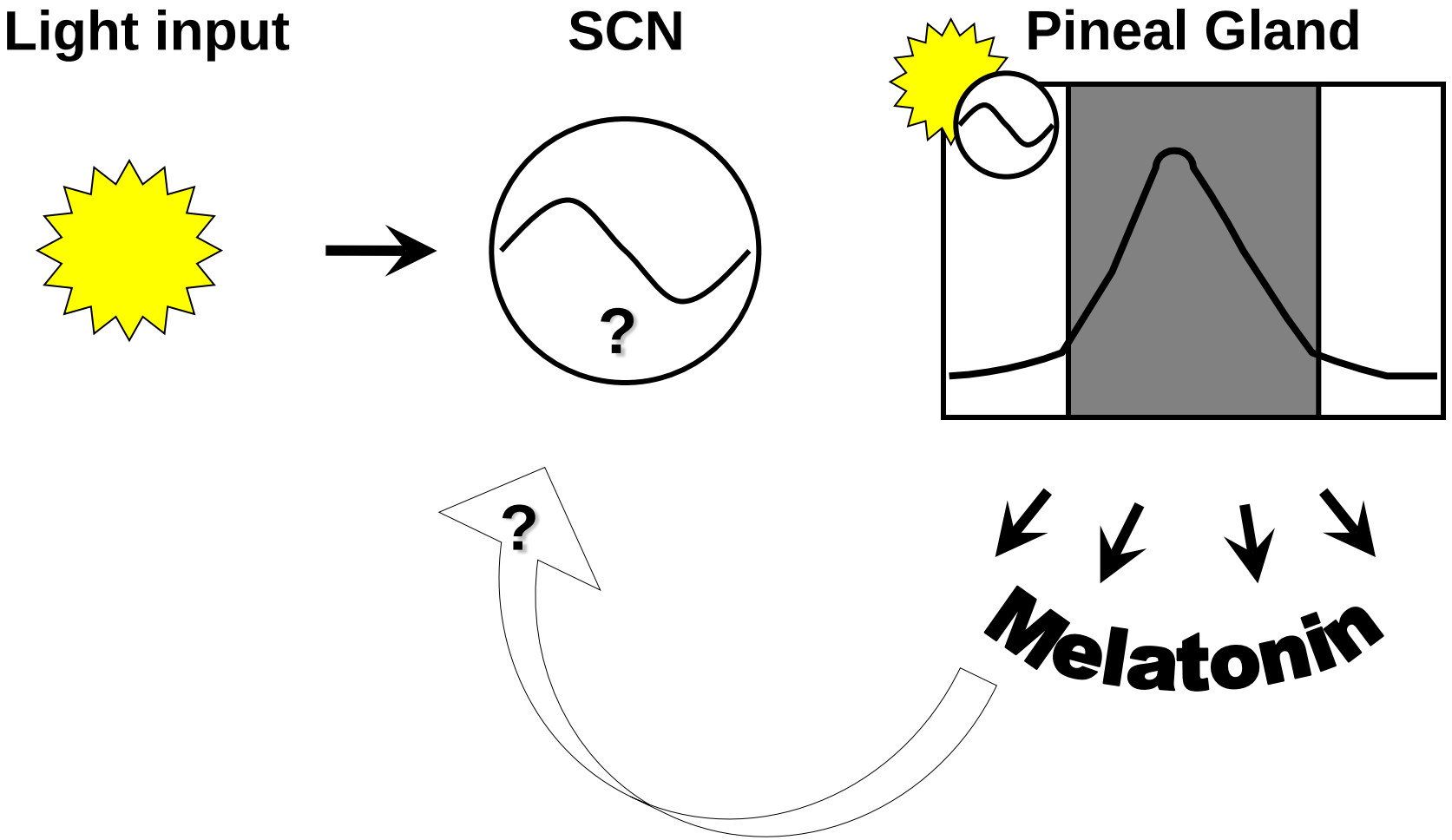
Light-induced expression of zfper2-Luc in CF cells is rescued by co-transfection with zebrafish Opn4m2 (C) and TMT-opsin (D)



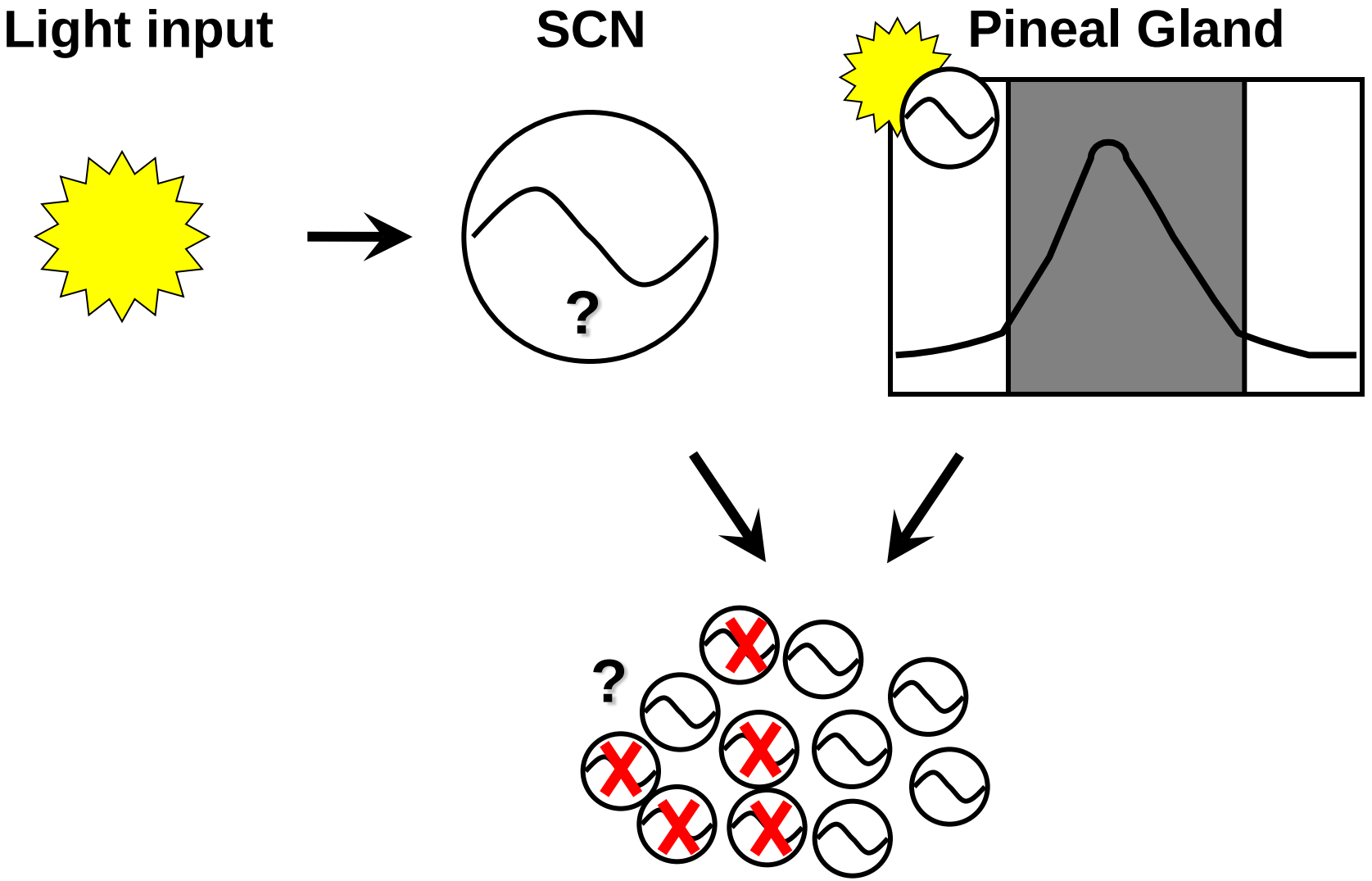
Davis et al., 2015

The ability of single cells to detect light and trigger light-dark cycles may represent an evolutionary ancient capability that precedes the centralization of circadian regulation by discrete brain nuclei. *Fernandes et al., 2013*

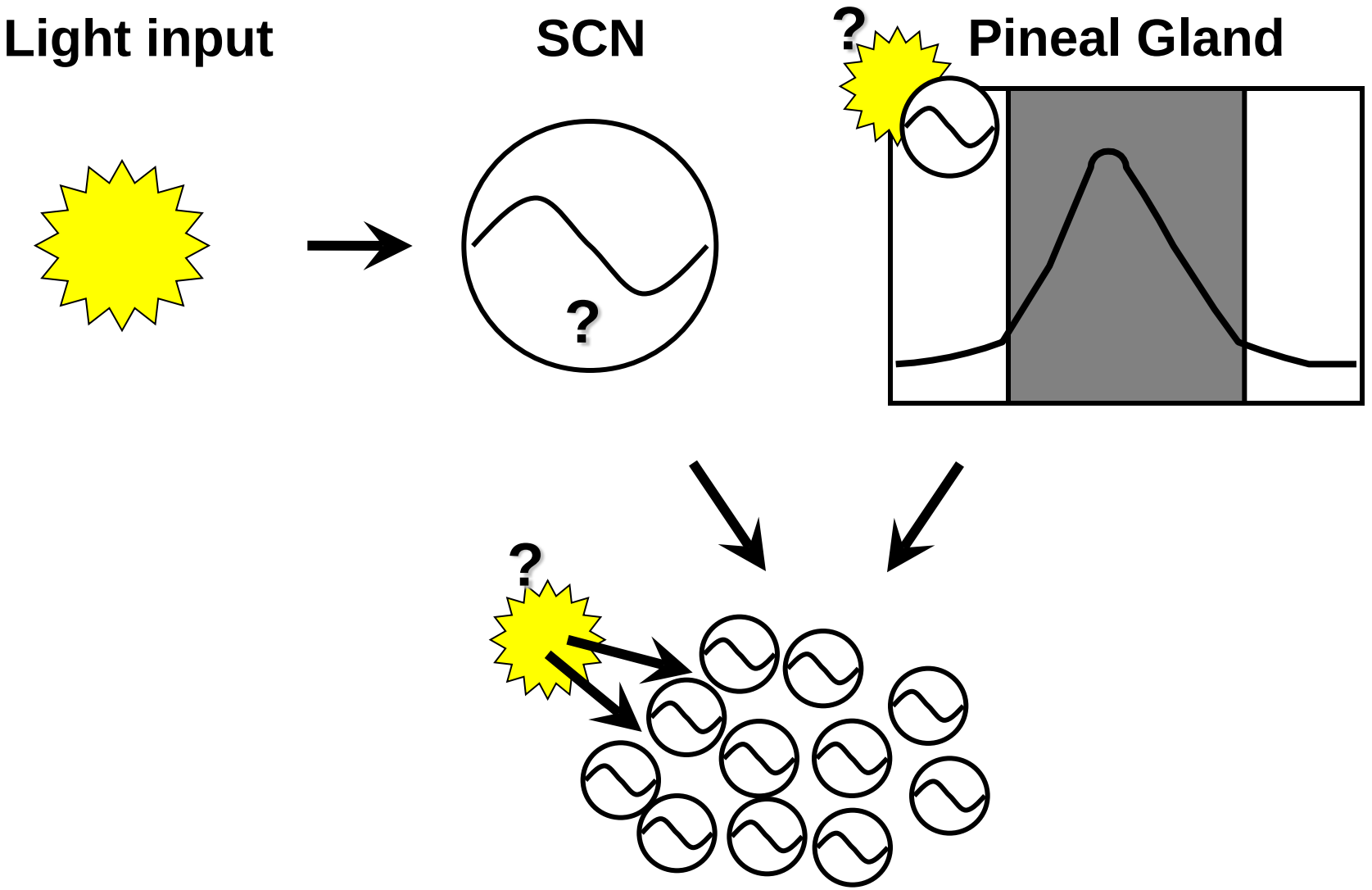
Questions: what are the melatonin targets?
Is there an SCN in fish?

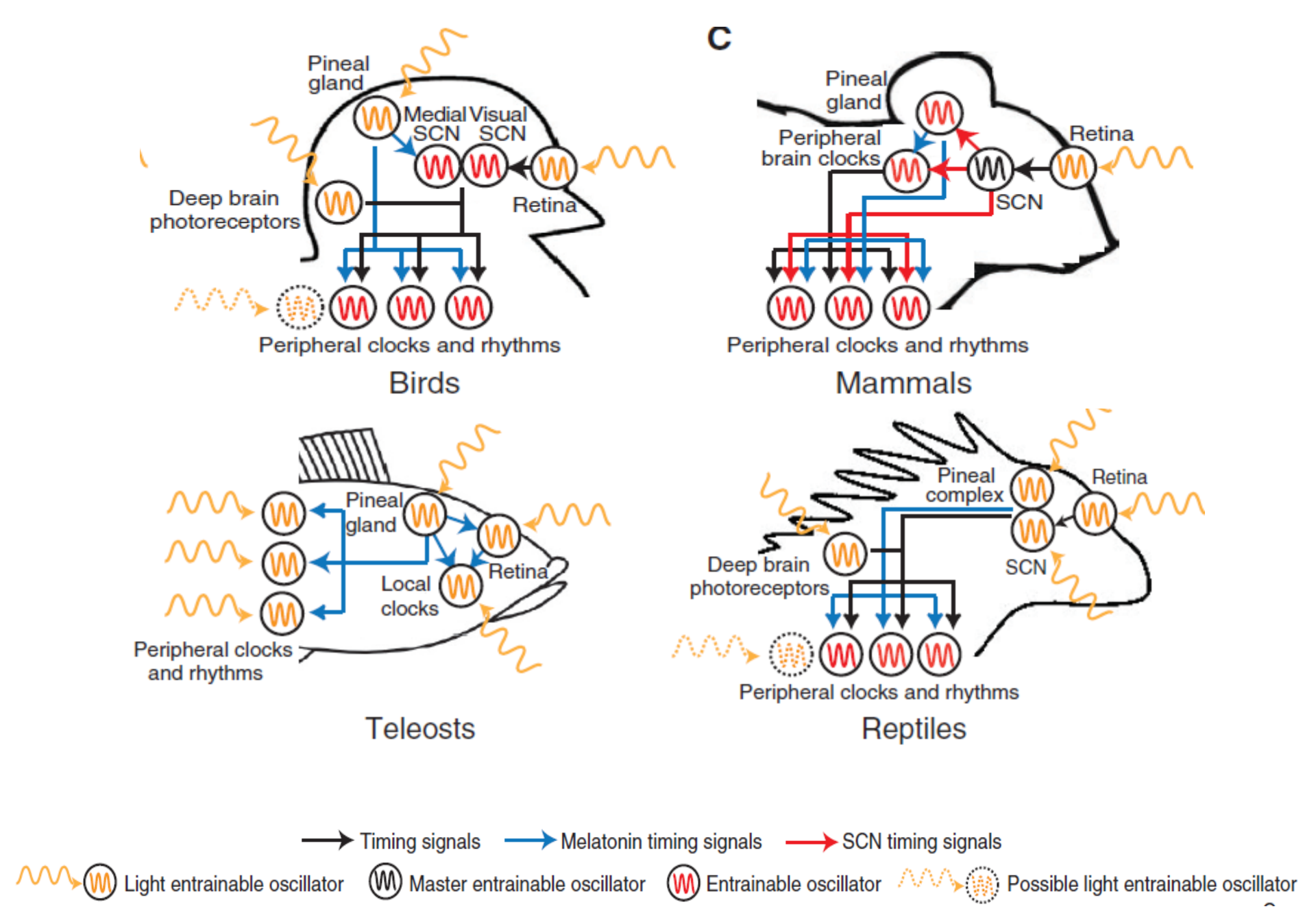


Questions: are peripheral clocks regulated?
What is their role?



Questions: what are the differences between light-entrainment of central vs. peripheral clocks?





Patton and Hastings 2018