

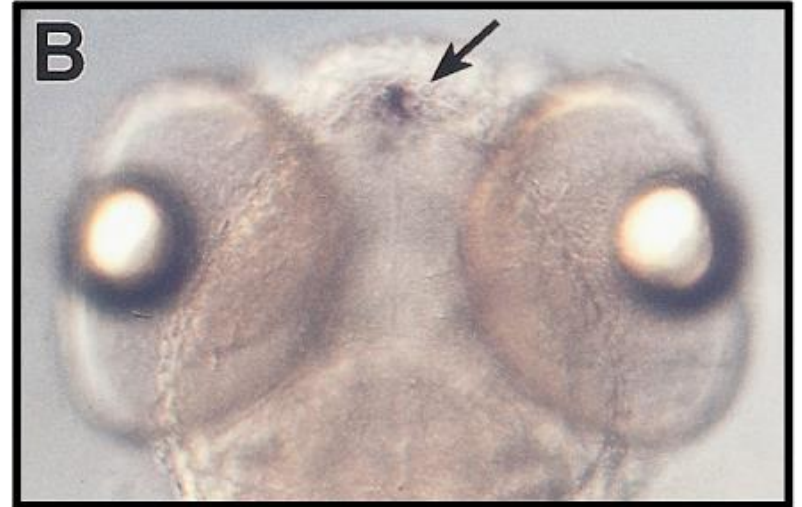
Pineal-specific expression of *aanat2* mRNA:
Whole mount ISH in a 22 hr embryo



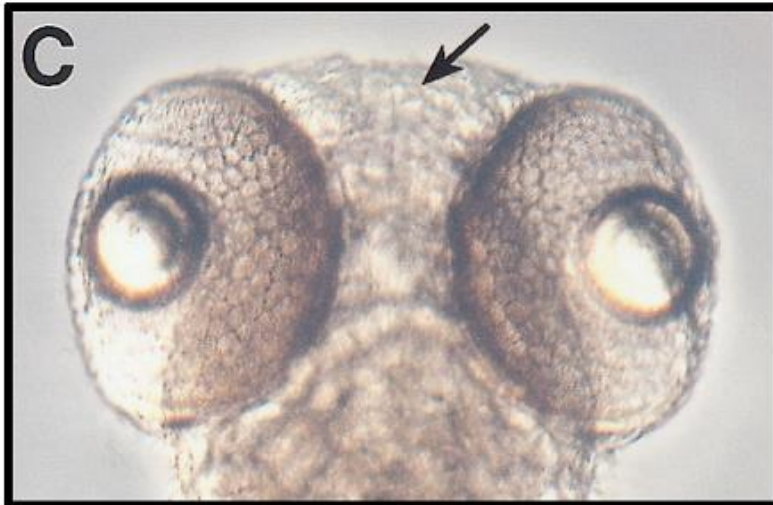
ZT6

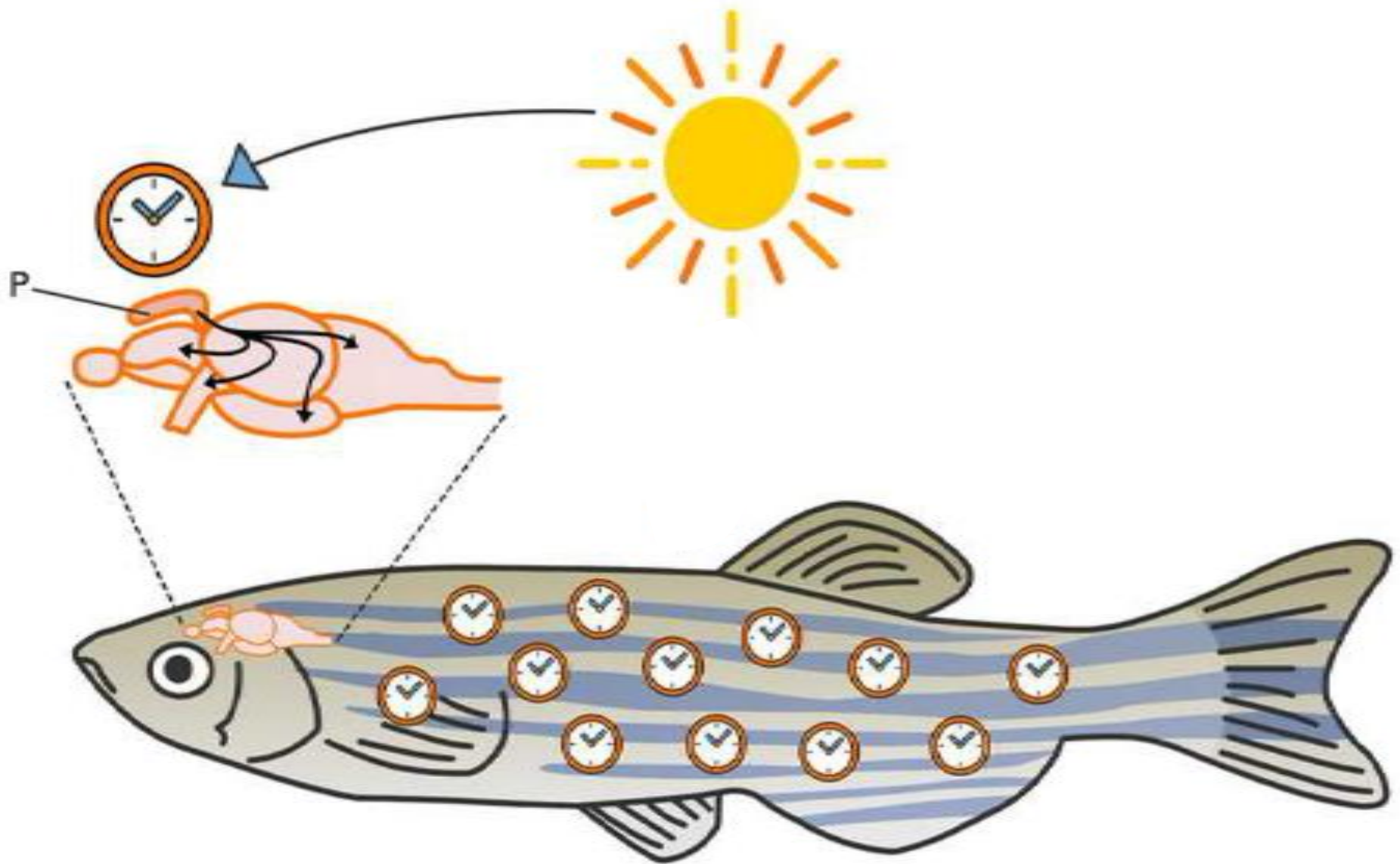
ZT18

LD



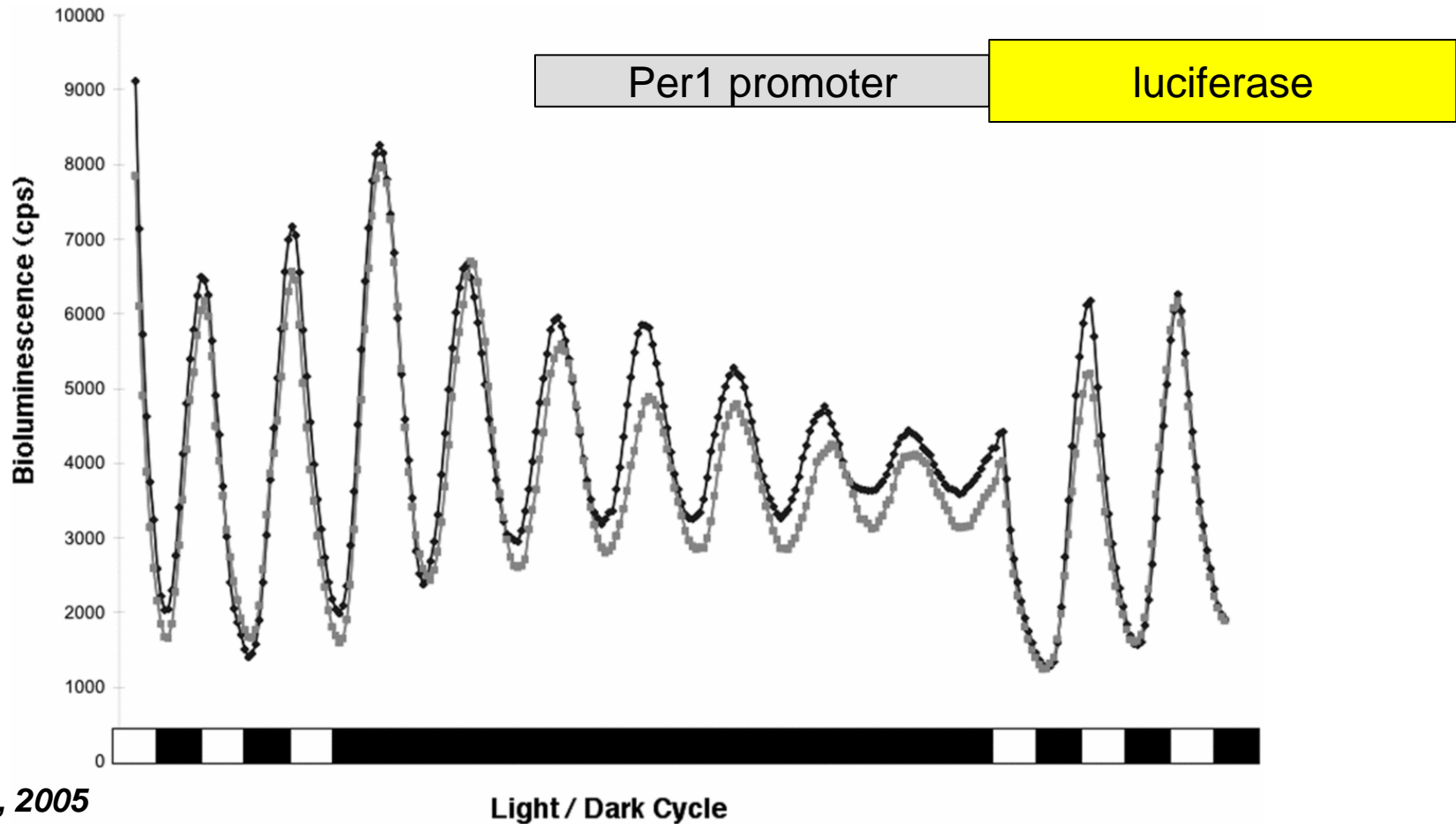
DD





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

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

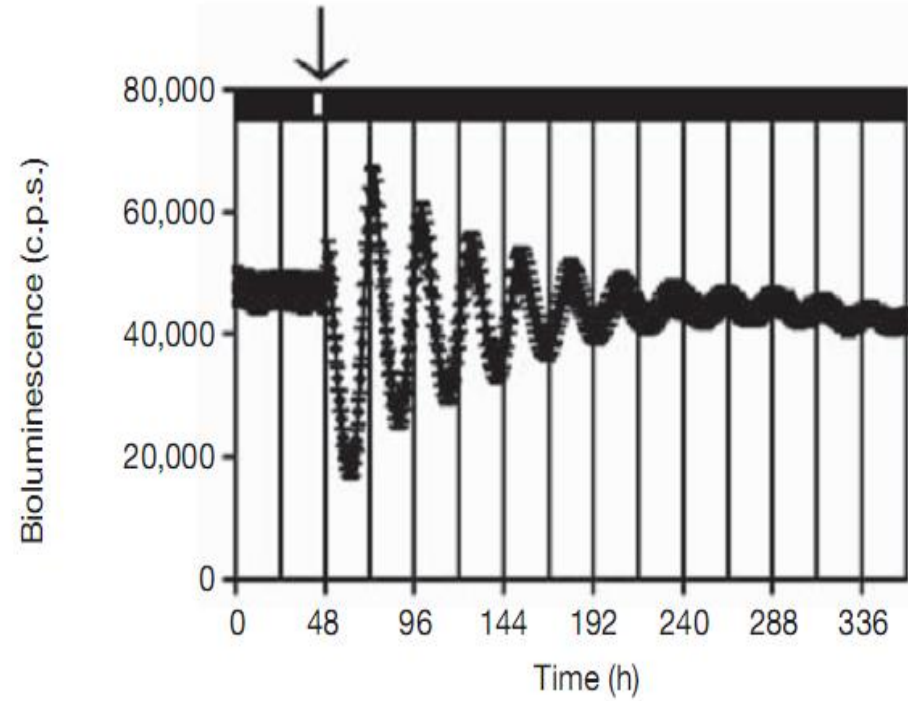
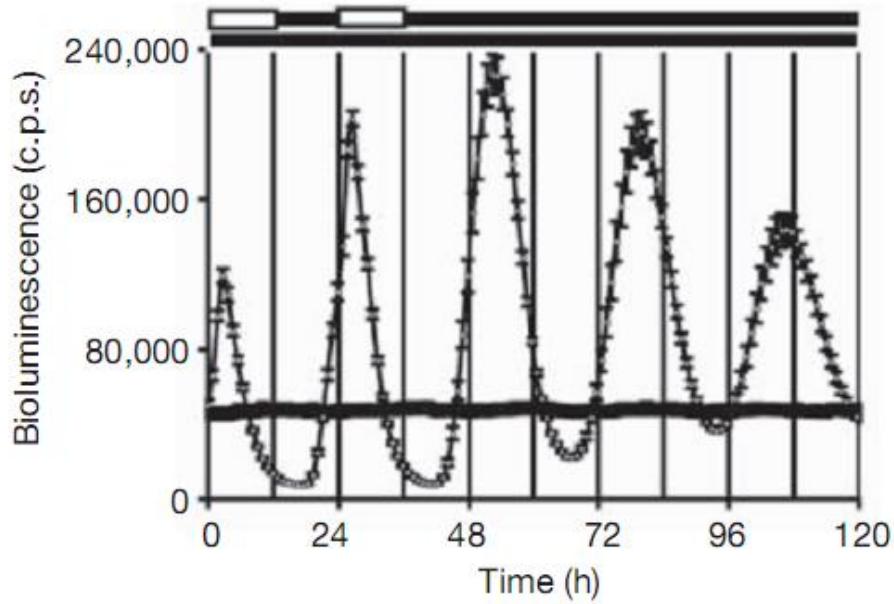


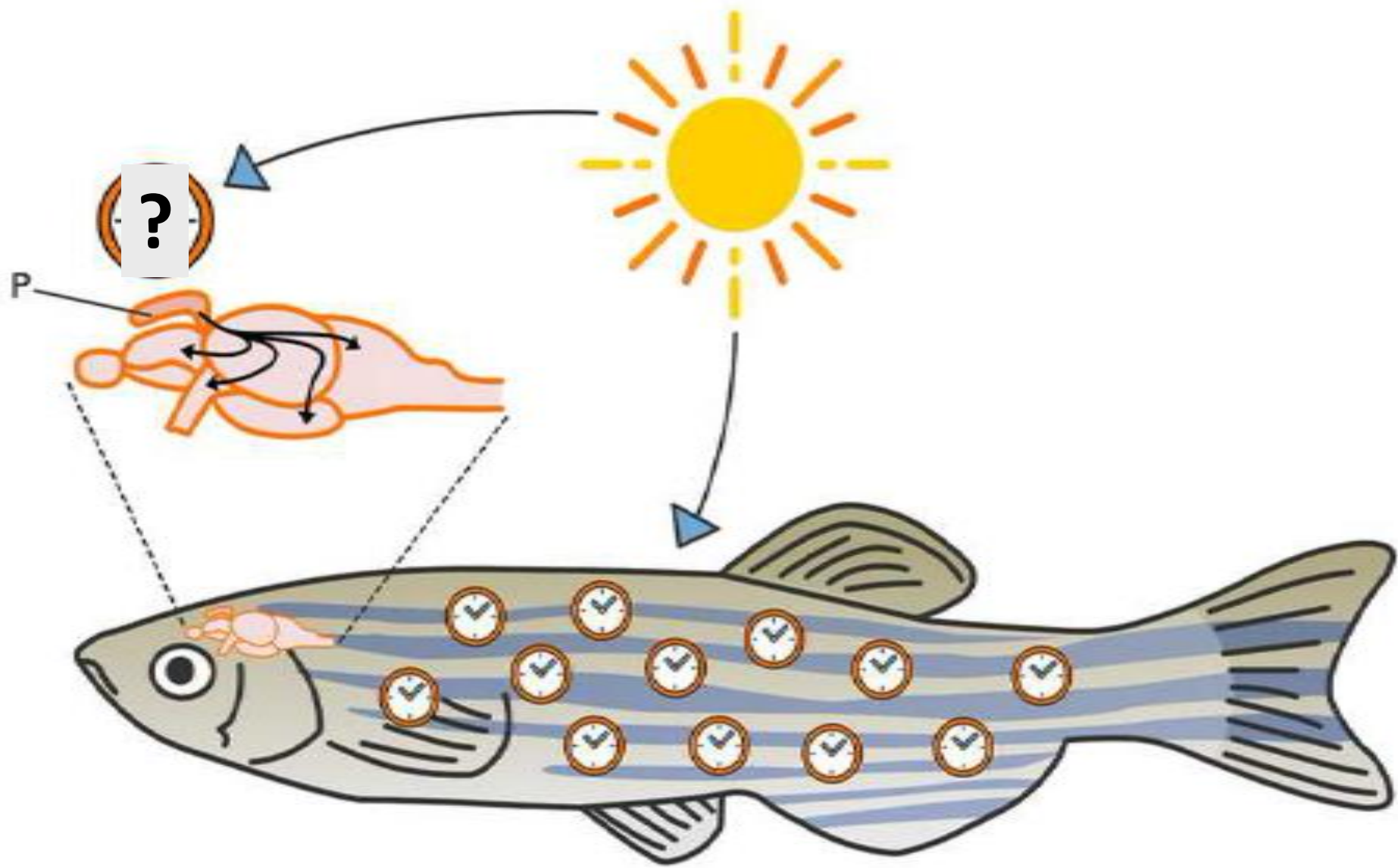
Tamai et al., 2005

In fish, the circadian clock system is de-centralized

Per1 promoter

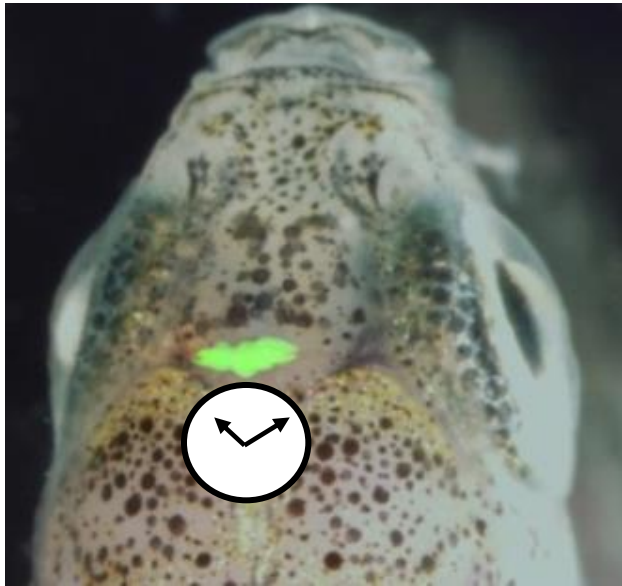
Luciferase





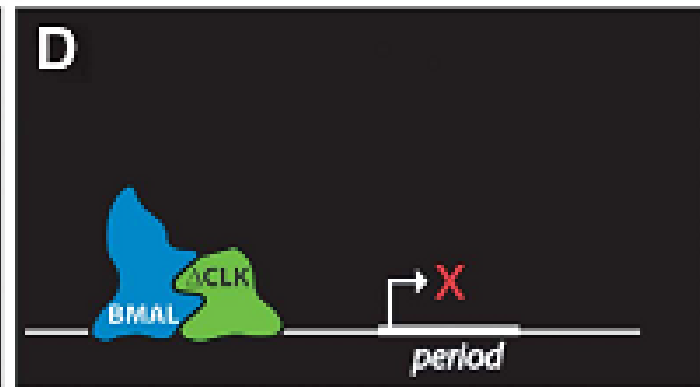
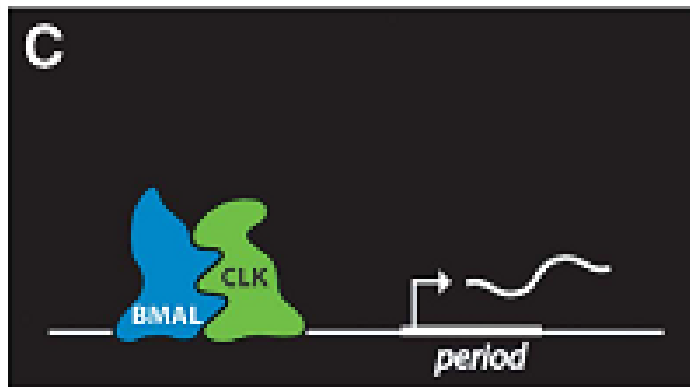
In fish, the circadian clock system is de-centralized

Really???



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

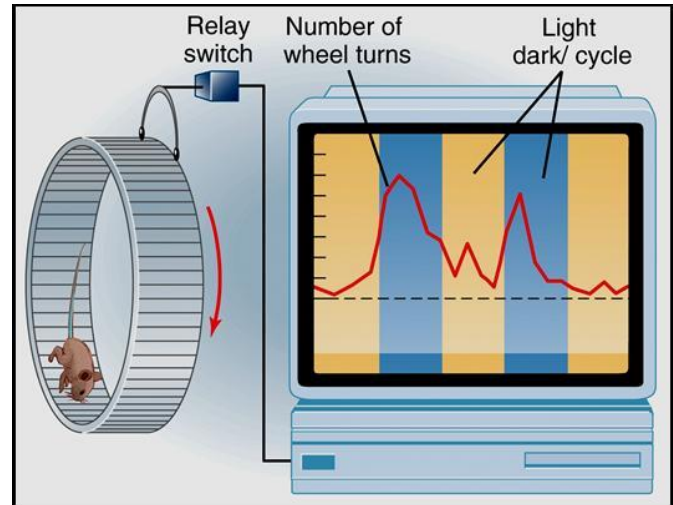
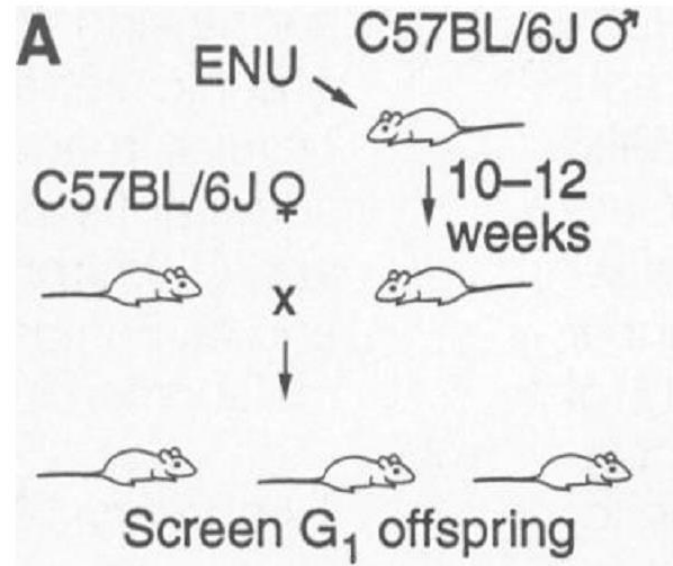
Generation of a transgenic fish line with impaired pineal gland clock function

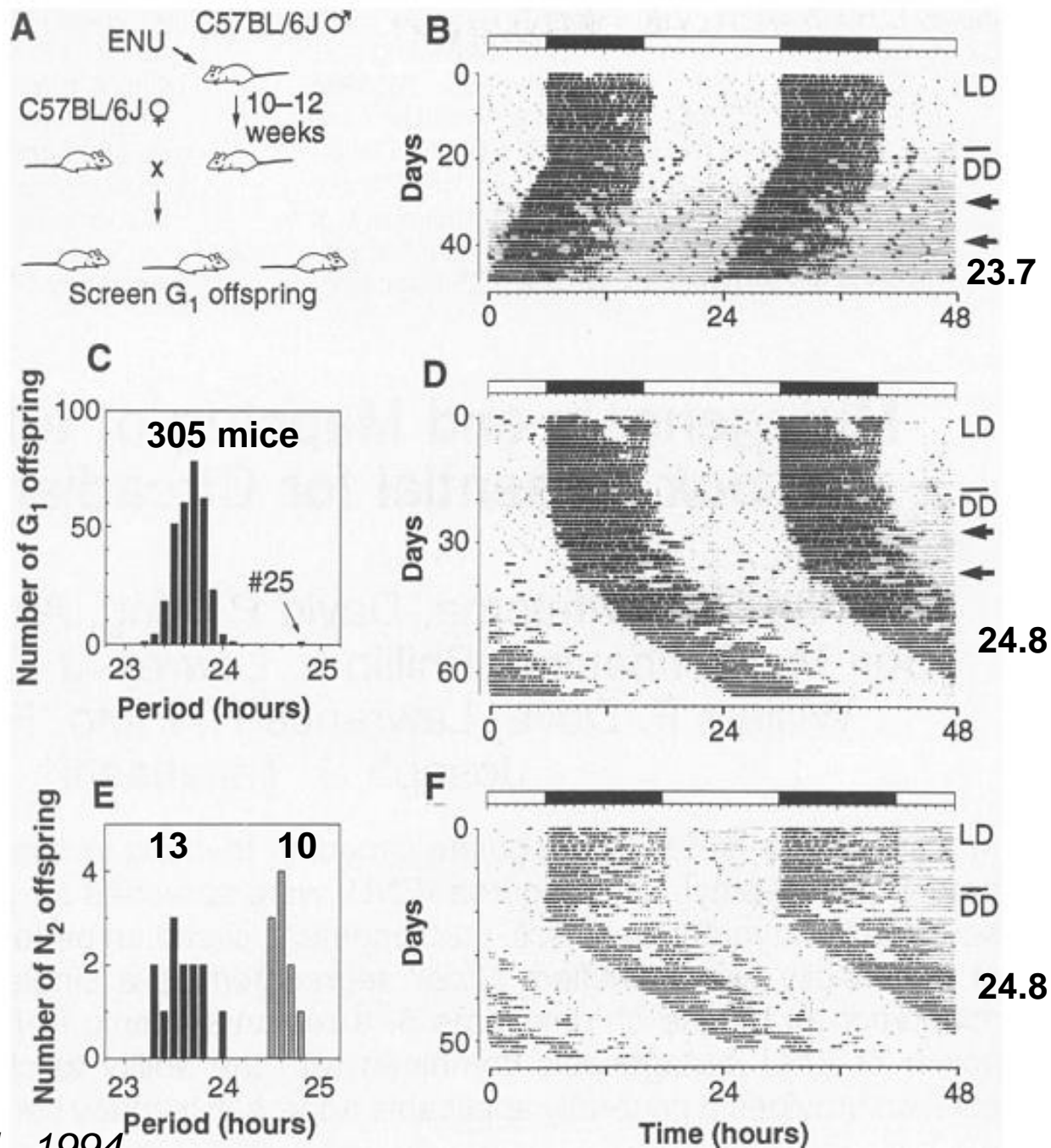


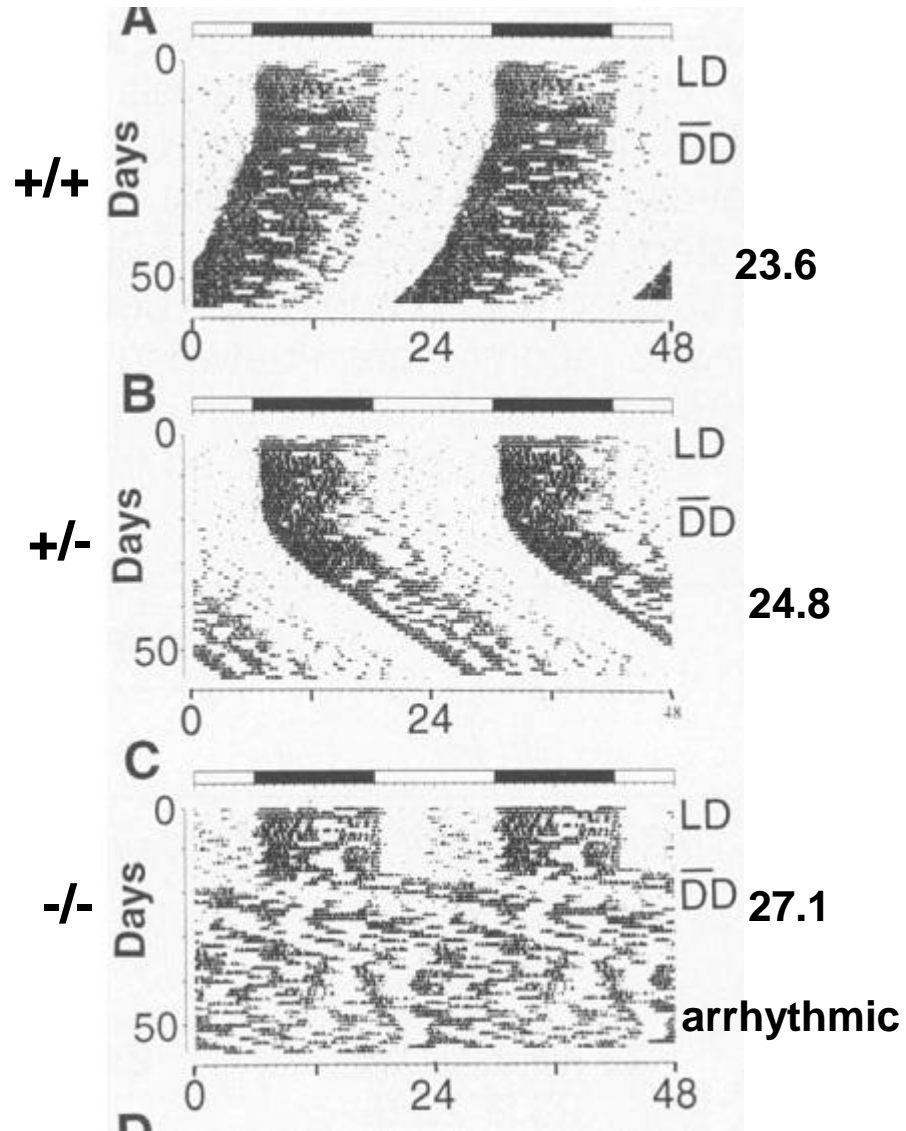
Pineal-specific promoter

An arrow points from the text 'Pineal-specific promoter' to the '*aanat2* promoter' region of the transgene construct.

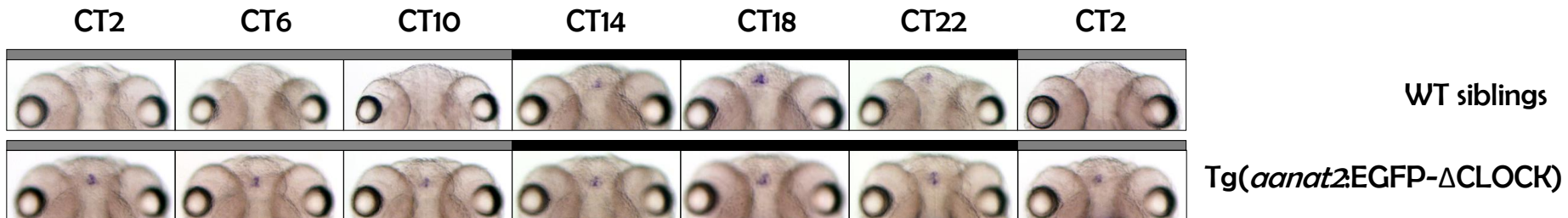
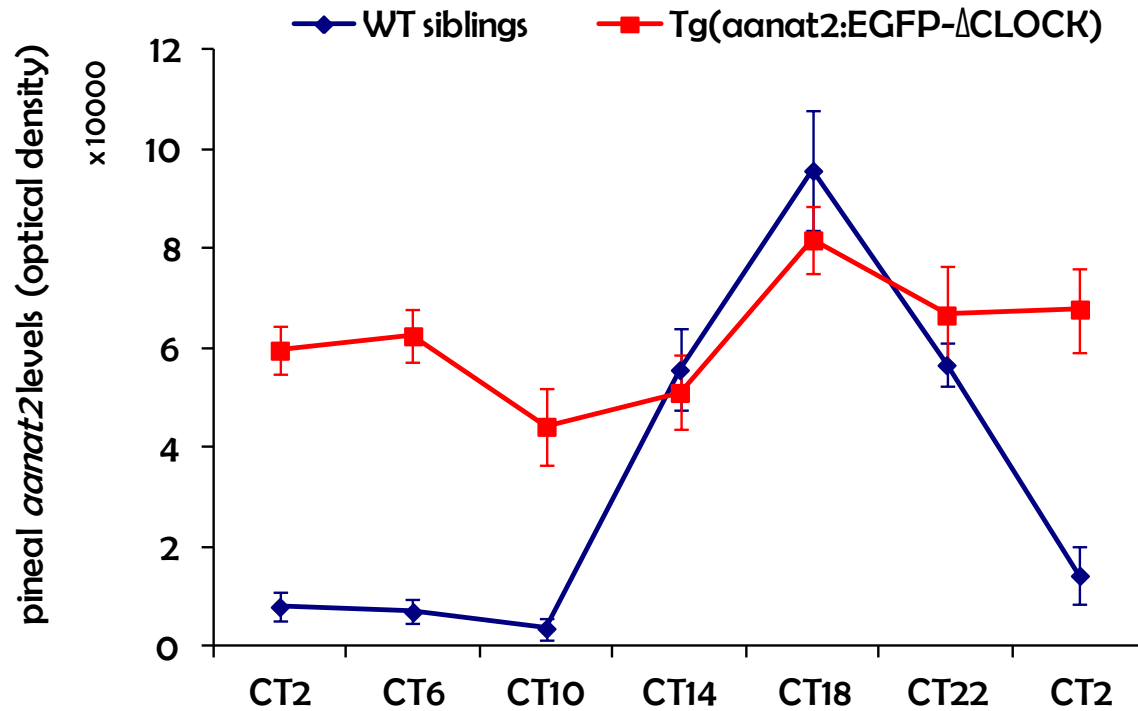
Searching for circadian clock mutant in mammals, *clock*



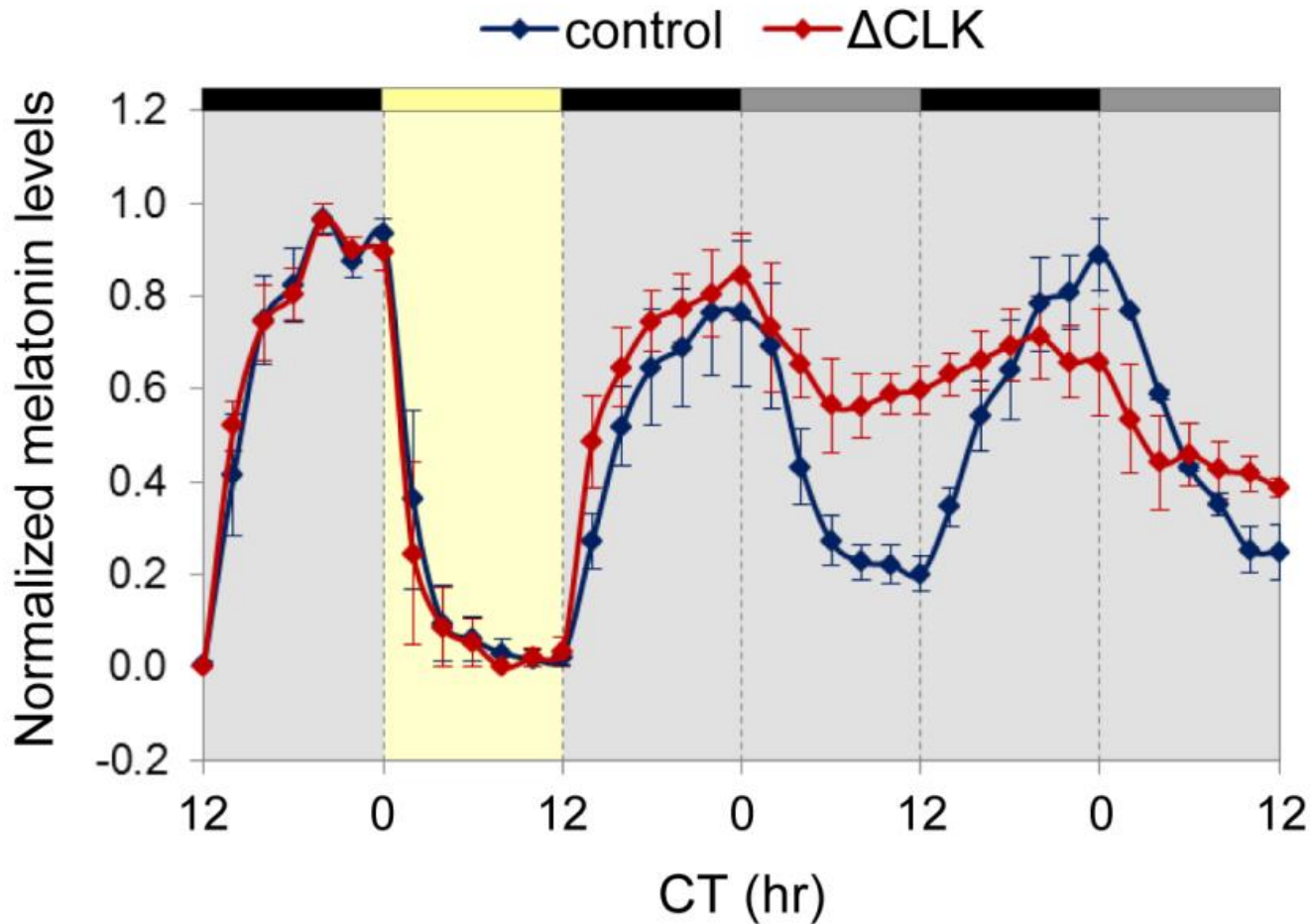




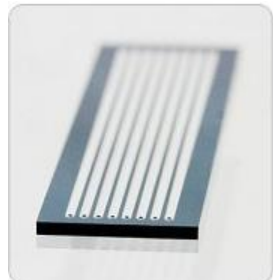
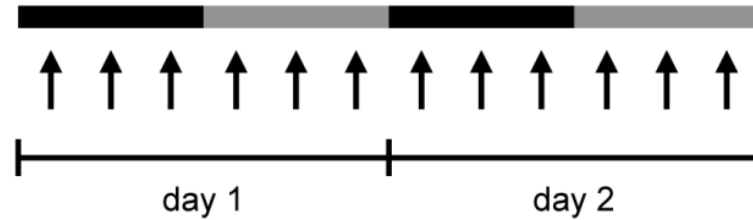
Tg(*aanat2*:EGFP- Δ CLOCK) larvae display arrhythmic pineal *aanat2* mRNA expression



Δ CLK pineal glands display arrhythmic melatonin production under constant dark



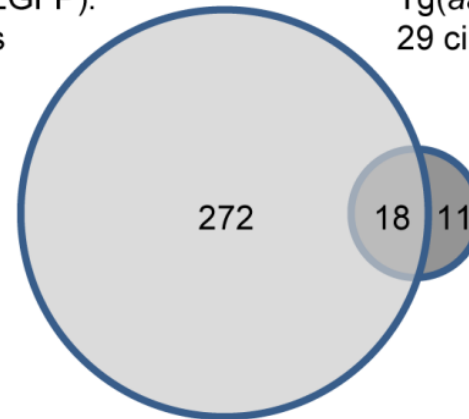
Most rhythmic genes lost their rhythm in Δ CLK pineal glands

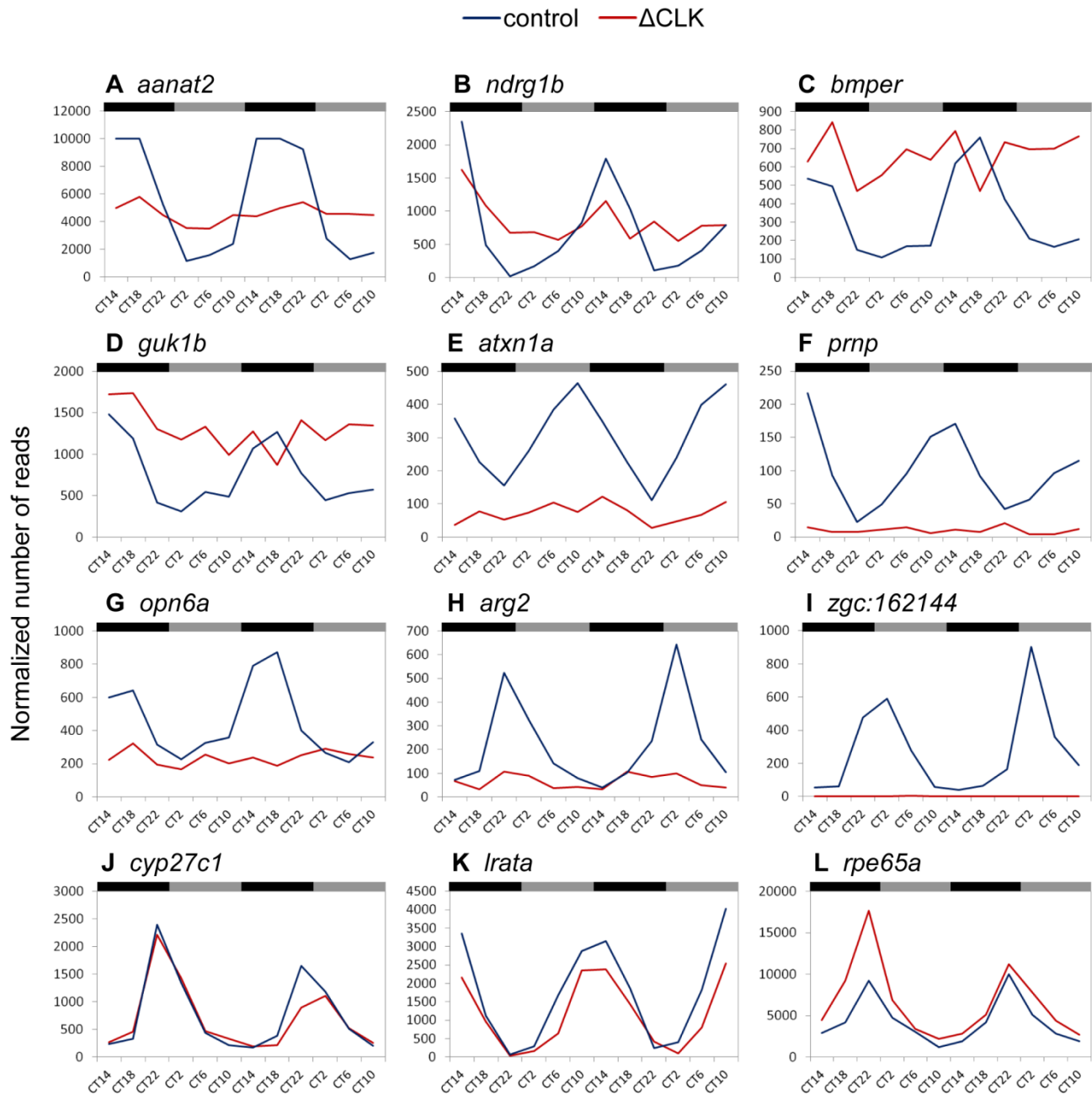


Next Generation Sequencing

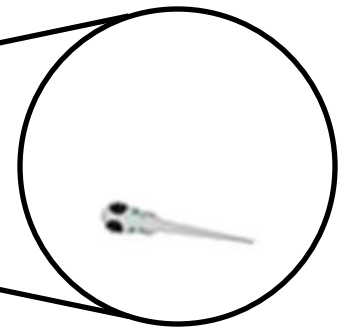
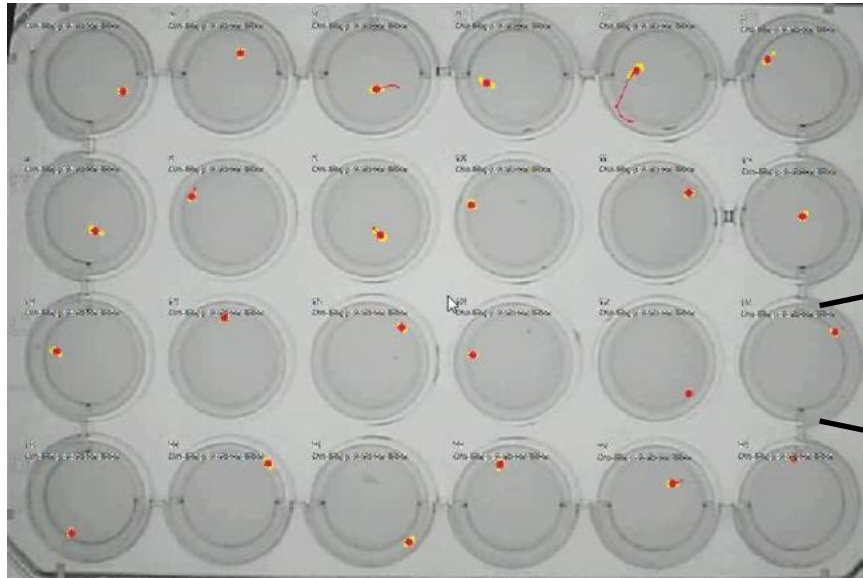
Control Tg(*aanat2*:EGFP):
290 circadian genes

Tg(*aanat2*:EGFP- Δ CLK):
29 circadian genes



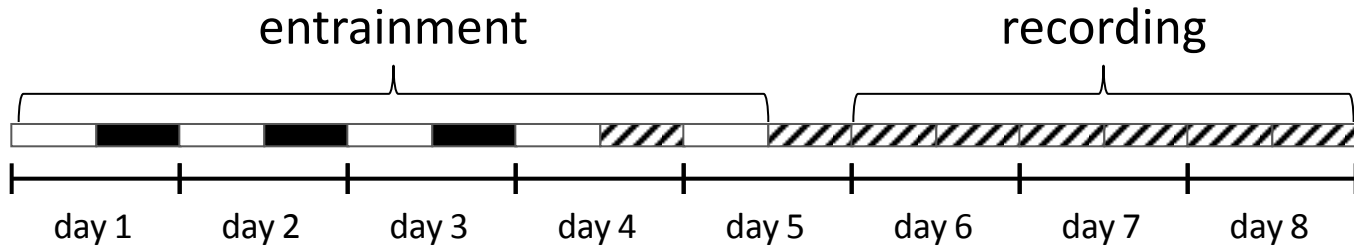
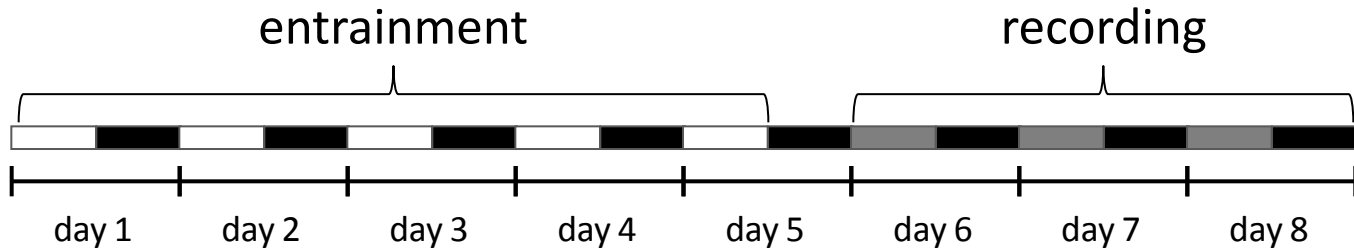


Monitoring locomotor activity of zebrafish larvae



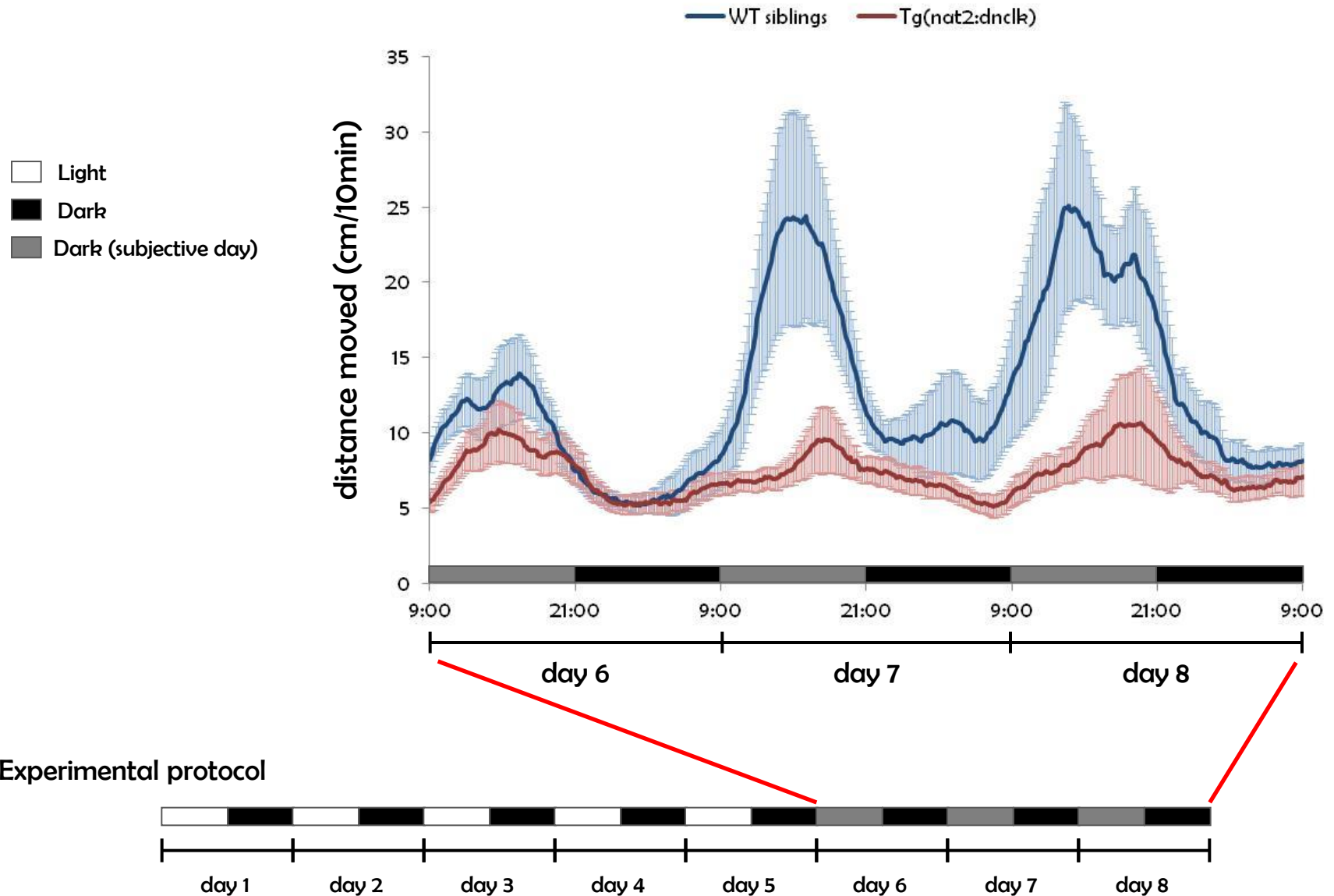
Danio Vision, Noldus

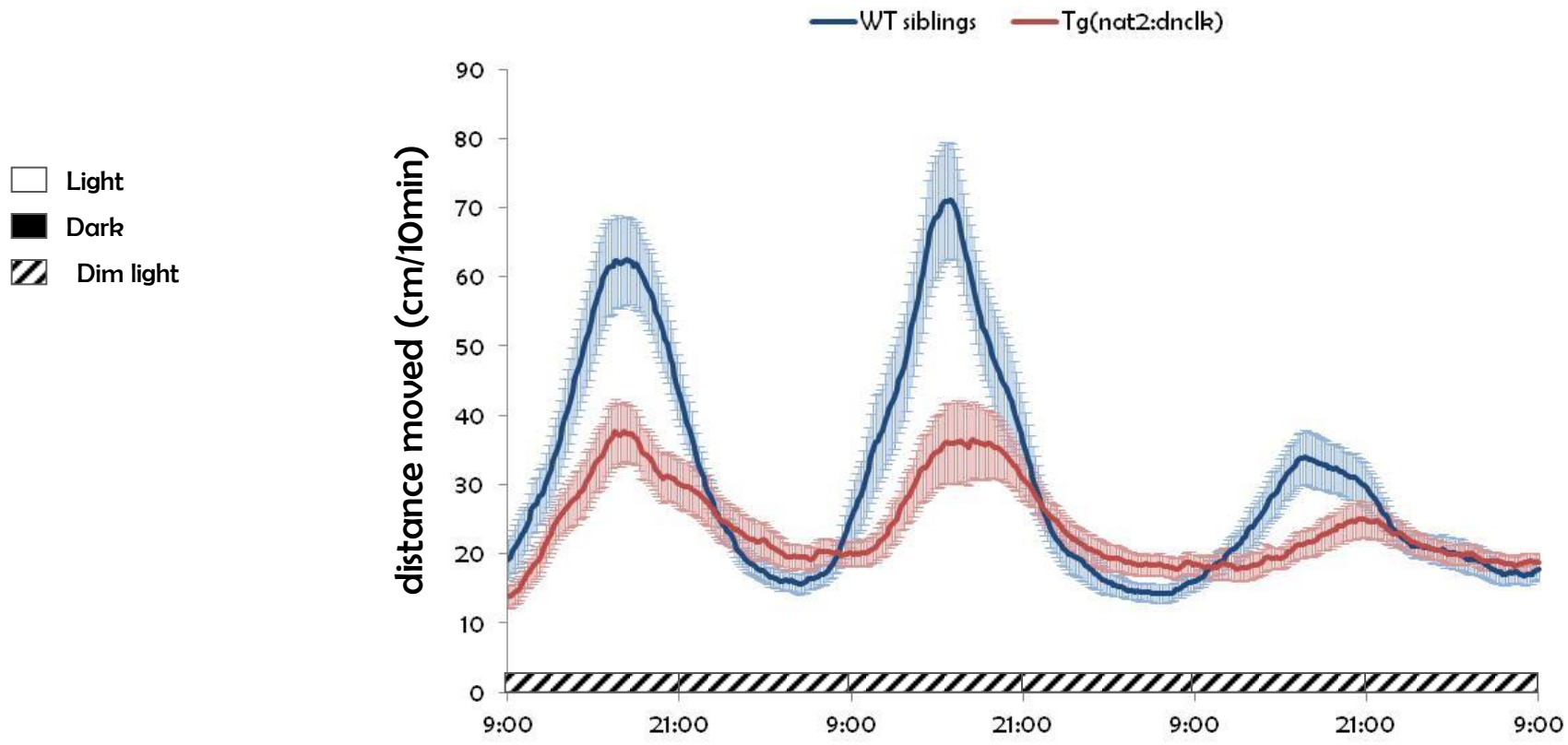
Experimental protocols



Ben-Moshe Livne et al., 2016

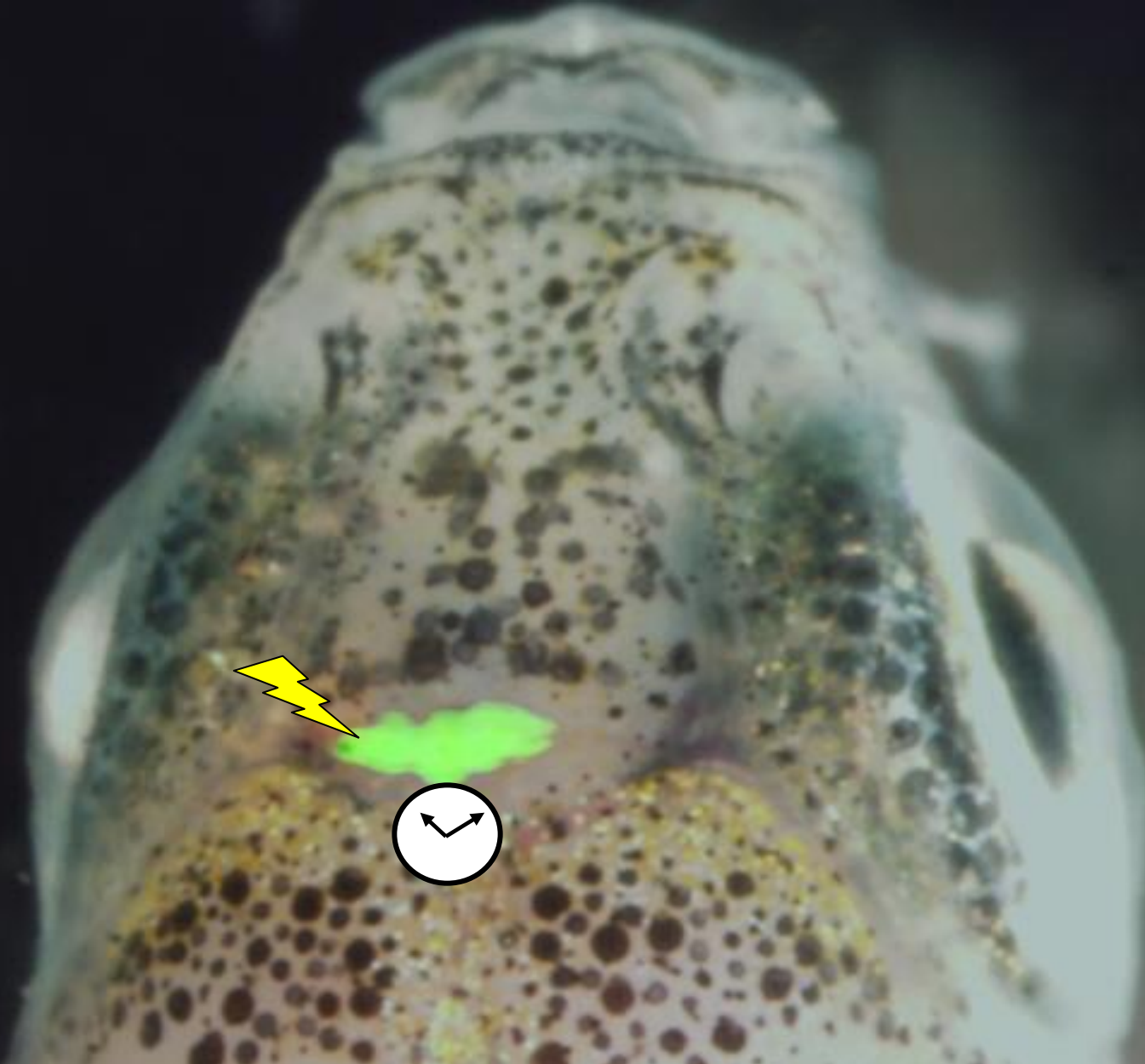
Blockage of the pineal clock leads to reduced amplitudes of rhythmic locomotor activity





Experimental protocol





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

**In fish, the pineal gland is a
central circadian clock organ.
But not the only one,
Where else?**



Pineal transcriptome analysis rhythmic genes

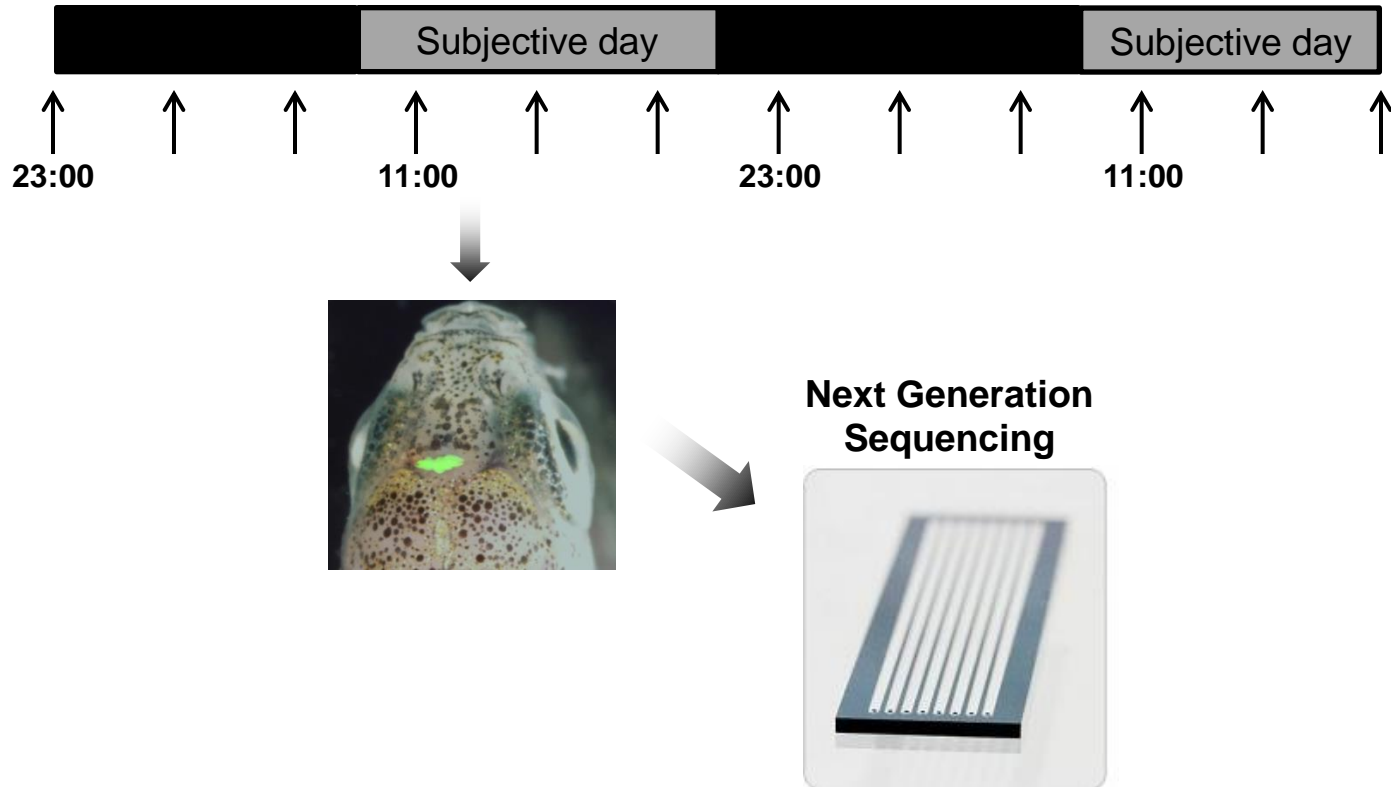


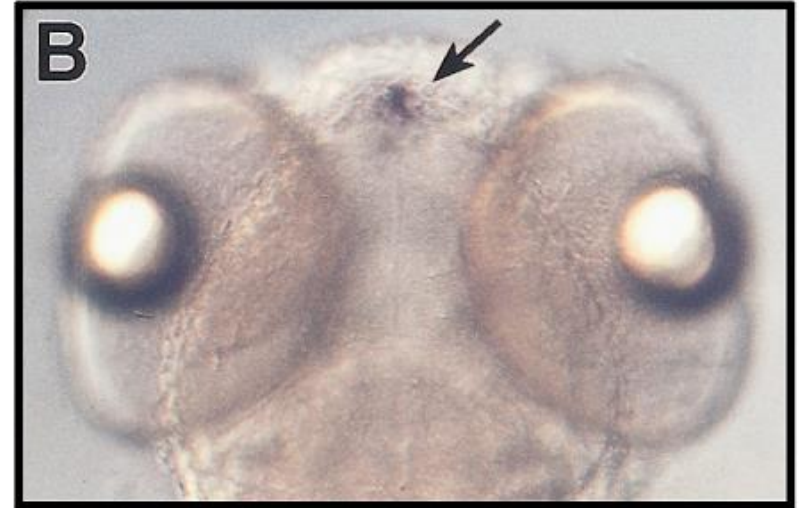
Table S2: List of circadian RefSeq genes using RNA-seq

Number	RefSeq ID	Gene symbol	g-factor
1	NM_1002642	<i>camk2n2</i>	0.9380
2	NM_1197058	<i>nfil3-5 (e4bp4-5)</i>	0.9269
3	NM_131791	<i>cry2a</i>	0.9229
4	NM_131451	<i>irbp</i>	0.9151
5	NM_213439	<i>mid1ip1</i>	0.9147
6	NM_131792	<i>cry2b</i>	0.9130
7	NM_1113337	<i>cyp27c1</i>	0.9112
8	NM_131578	<i>arnt12 (bmal2)</i>	0.9102
9	NM_212584	<i>ctsl1a</i>	0.9079
10	NM_212439	<i>per1b</i>	0.9074
11	NM_1045055	<i>si:ch211-132b12.7</i>	0.9051
12	NM_1030183	<i>per1a</i>	0.8949
13	NM_1044826	<i>atxn1a</i>	0.8927
14	NM_1002369	<i>zgc:92502</i>	0.8906
15	NM_200139	<i>cyp2p6</i>	0.8902
16	NM_201158	<i>aldh4a1</i>	0.8897
17	NM_1197060	<i>dbpa</i>	0.8892
18	NM_1039107	<i>bhlhe41 (dec2)</i>	0.8889
19	NM_205729	<i>nr1d1 (reverba)</i>	0.8871
20	NM_200751	<i>rpe65a</i>	0.8864
21	NM_130957	<i>clock1a</i>	0.8851
22	NM_1077323	<i>zgc:154001</i>	0.8845
23	NM_1114579	<i>fam131a</i>	0.8835
24	NM_200829	<i>camk1gb</i>	0.8814
25	NM_213523	<i>qrt15</i>	0.8810
26	NM_1042720	<i>atp7a</i>	0.8805
27	NM_1013555	<i>zgc:113006</i>	0.8777
28	NM_131584	<i>per3</i>	0.8762
29	NM_199871	<i>mat1a</i>	0.8744
30	NM_1082979	<i>zgc:162144</i>	0.8737
31	NM_199567	<i>tgif1</i>	0.8722
32	NM_131065	<i>nr1d2b (reverbb2)</i>	0.8717
33	NM_131571	<i>smad3a</i>	0.8709
34	NM_1110486	<i>zgc:158292</i>	0.8687
35	NM_212723	<i>mknk2a</i>	0.8686
36	NM_1098177	<i>myh9a</i>	0.8679
37	NM_199611	<i>arg2</i>	0.8669
38	NM_1079657	<i>zgc:158340</i>	0.8665
39	NM_1199134	<i>ptprb</i>	0.8650
40	NM_131787	<i>cry4</i>	0.8642
41	NM_201217	<i>nop56</i>	0.8632
42	NM_1197065	<i>nfil3-2 (e4bp4-2)</i>	0.8630
43	NM_213387	<i>pgk1</i>	0.8624
44	NM_1166205	<i>hip1</i>	0.8615
45	NM_1006090	<i>fam73a</i>	0.8607
46	NM_201067	<i>rorab</i>	0.8600
47	NM_1044834	<i>si:dkey-239j20.4</i>	0.8598
48	NM_1080022	<i>myl1pb</i>	0.8586
49	NM_213495	<i>cul1b</i>	0.8581
50	NM_131411	<i>aanat2</i>	0.8565
51	NM_212822	<i>pik3r2</i>	0.8565
52	NM_201493	<i>nucb2a</i>	0.8552
53	NM_1004595	<i>utp3</i>	0.8518
54	NM_1044846	<i>slain2</i>	0.8507
55	NM_194377	<i>aldoaa</i>	0.8507
56	NM_199624	<i>her8a</i>	0.8497
57	NM_205759	<i>zgc:77395</i>	0.8487
58	NM_1110477	<i>zgc:162232</i>	0.8463
59	NM_201149	<i>cdc14aa</i>	0.8452
60	NM_1127308	<i>dtx1</i>	0.8441
61	NM_205686	<i>cry-dash</i>	0.8423
62	NM_131786	<i>cry3</i>	0.8420
63	NM_1007445	<i>phyhd1</i>	0.8410
64	NM_199609	<i>dhrs9</i>	0.8403
65	NM_205586	<i>prnp</i>	0.8403
66	NM_1045208	<i>letm1</i>	0.8345
67	NM_1082901	<i>si:ch211-197n10.4</i>	0.8333
68	NM_199649	<i>zfp361b</i>	0.8318
69	NM_201116	<i>sh3gl2</i>	0.8313
70	NM_1008620	<i>tsta3</i>	0.8312
71	NM_212682	<i>clic1</i>	0.8312
72	NM_198915	<i>rbm19</i>	0.8307
73	NM_1082819	<i>rorca</i>	0.8267
74	NM_1007372	<i>gsto2</i>	0.8259
75	NM_199873	<i>ldhd</i>	0.8258
76	NM_1204131	<i>lrata</i>	0.8251
77	NM_1201566	<i>usp54a</i>	0.8234
78	NM_213528	<i>ng36</i>	0.8232
79	NM_201083	<i>fus</i>	0.8227
80	NM_178295	<i>clock1b</i>	0.8220
81	NM_213112	<i>rabggtb</i>	0.8204
82	NM_1005400	<i>ctlh</i>	0.8200
83	NM_1002297	<i>gnl3</i>	0.8197
84	NM_1105524	<i>ttl6</i>	0.8195
85	NM_1080204	<i>cyp11c1</i>	0.8193
86	NM_1126461	<i>etv5a</i>	0.8188
87	NM_213157	<i>slc25a33</i>	0.8178
88	NM_200610	<i>dopey2</i>	0.8172
89	NM_1077580	<i>dcaf12</i>	0.8152
90	NM_131373	<i>gfap</i>	0.8147
91	NM_1037379	<i>sept4a</i>	0.8131
92	NM_1020730	<i>zgc:109949</i>	0.8129
93	NM_200644	<i>nat10</i>	0.8129
94	NM_131801	<i>odc1</i>	0.8129
95	NM_199583	<i>csnk1da</i>	0.8123
96	NM_201202	<i>phc2b</i>	0.8111
97	NM_1039808	<i>slc2a1a</i>	0.8101
98	NM_1166332	<i>nlgn3a</i>	0.8098
99	NM_1111150	<i>bms1l</i>	0.8098
100	NM_1017881	<i>sqrdl</i>	0.8097
101	NM_1004562	<i>fth1b</i>	0.8095
102	NM_1020647	<i>acbd5a</i>	0.8089
103	NM_1014296	<i>mat2ab</i>	0.8088
104	NM_205724	<i>fbxo25</i>	0.8045
105	NM_1128538	<i>cdc14ab</i>	0.8039
106	NM_213010	<i>acadm</i>	0.8031
107	NM_1002649	<i>aclya</i>	0.8013
108	NM_1089417	<i>pfkmb</i>	0.8001
109	NM_1077253	<i>gnl1</i>	0.7995
110	NM_200567	<i>zgc:65987</i>	0.7992
111	NM_1006043	<i>ctsz</i>	0.7979
112	NM_199969	<i>mdh1b</i>	0.7972
113	NM_1040393	<i>zgc:136817</i>	0.7957
114	NM_212679	<i>bhlhe40 (dec1)</i>	0.7957
115	NM_1017753	<i>asb5</i>	0.7955
116	NM_1123011	<i>zgc:158426</i>	0.7955
117	NM_200172	<i>mycb</i>	0.7953
118	NM_131247	<i>ldhba</i>	0.7948
119	NM_1114583	<i>abcb4</i>	0.7946
120	NM_198363	<i>tob1a</i>	0.7940
121	NM_1135142	<i>zgc:194398</i>	0.7926
122	NM_1190816	<i>slc1a8b</i>	0.7921
123	NM_201326	<i>hspa9</i>	0.7889
124	NM_152949	<i>ccna2</i>	0.7882
125	NM_1082877	<i>ppat</i>	0.7874
126	NM_1077584	<i>zgc:152911</i>	0.7871
127	NM_1044892	<i>cspg5b</i>	0.7870
128	NM_1077731	<i>zgc:154093</i>	0.7870
129	NM_1024111	<i>pcdh1g11</i>	0.7869
130	NM_212714	<i>poldip2</i>	0.7868
131	NM_131799	<i>bcdo2l</i>	0.7867
132	NM_1200020	<i>si:ch1073-204j4.2</i>	0.7861
133	NM_1199680	<i>si:dkey-77a20.7</i>	0.7837
134	NM_1080989	<i>dennd4a</i>	0.7835
135	NM_1033591	<i>zgc:112334</i>	0.7830
136	NM_201025	<i>pgm1</i>	0.7815
137	NM_1003588	<i>zgc:101040</i>	0.7815
138	NM_1098485	<i>pgm2l1</i>	0.7807
139	NM_1013475	<i>bccip</i>	0.7807
140	NM_178436	<i>mcm5</i>	0.7806
141	NM_1098777	<i>sap130a</i>	0.7804
142	NM_131094	<i>tcf3</i>	0.7800
143	NM_1204257	<i>si:ch73-9j13.1</i>	0.7795
144	NM_212619	<i>cd9a</i>	0.7794
145	NM_1190305	<i>slc1a2a</i>	0.7787
146	NM_214748	<i>rpf2</i>	0.7779
147	NM_199333	<i>pkm2a</i>	0.7777
148	NM_201143	<i>pik3r3</i>	0.7775
149	NM_1083015	<i>acap3b</i>	0.7767
150	NM_1045207	<i>asap2a</i>	0.7766

ZT6

ZT18

LD



DD

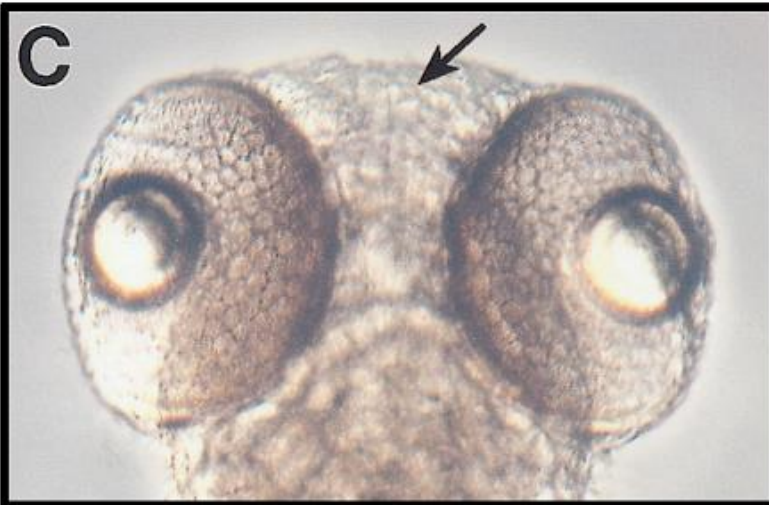
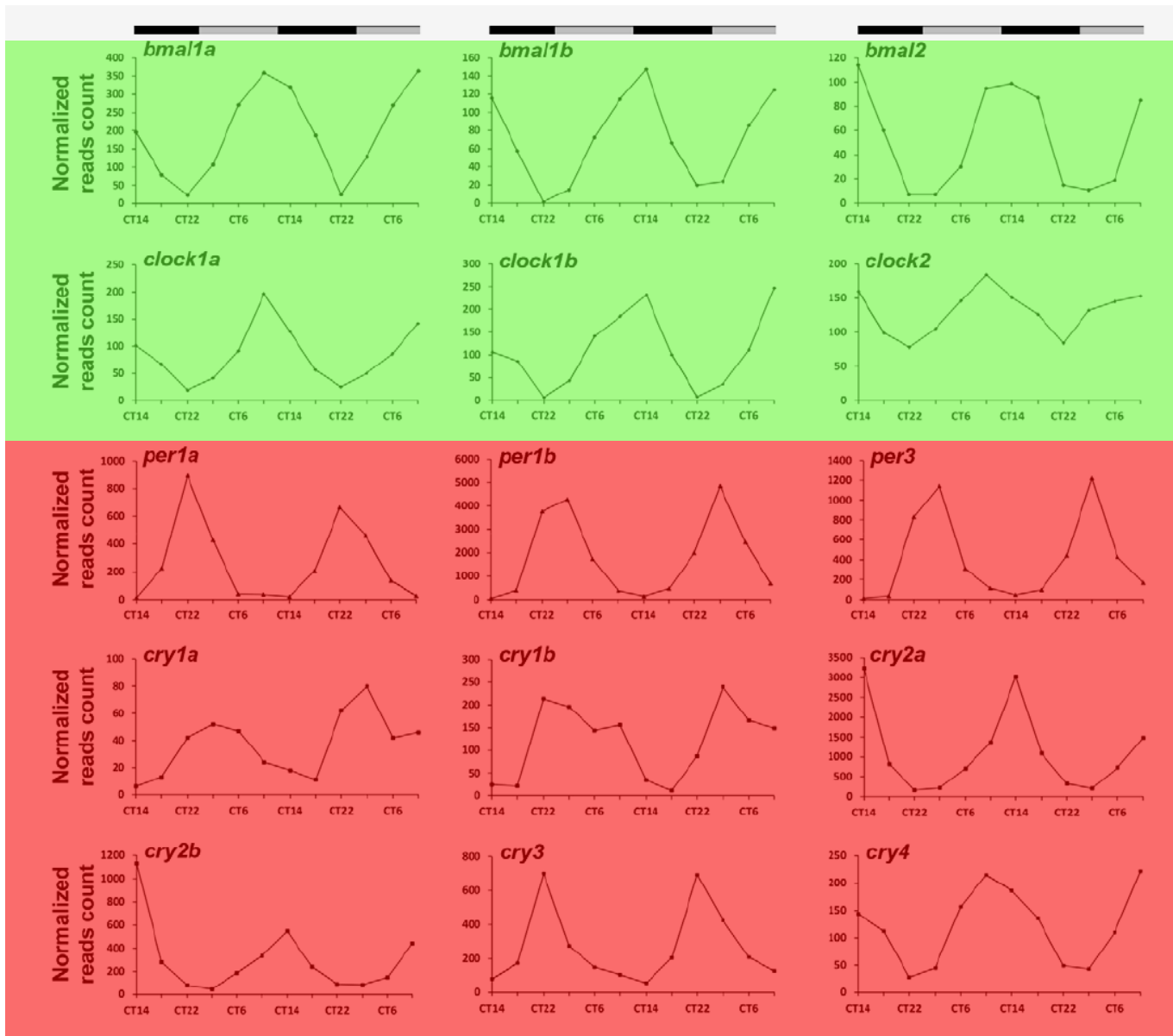


Table S2: List of circadian RefSeq genes using RNA-seq

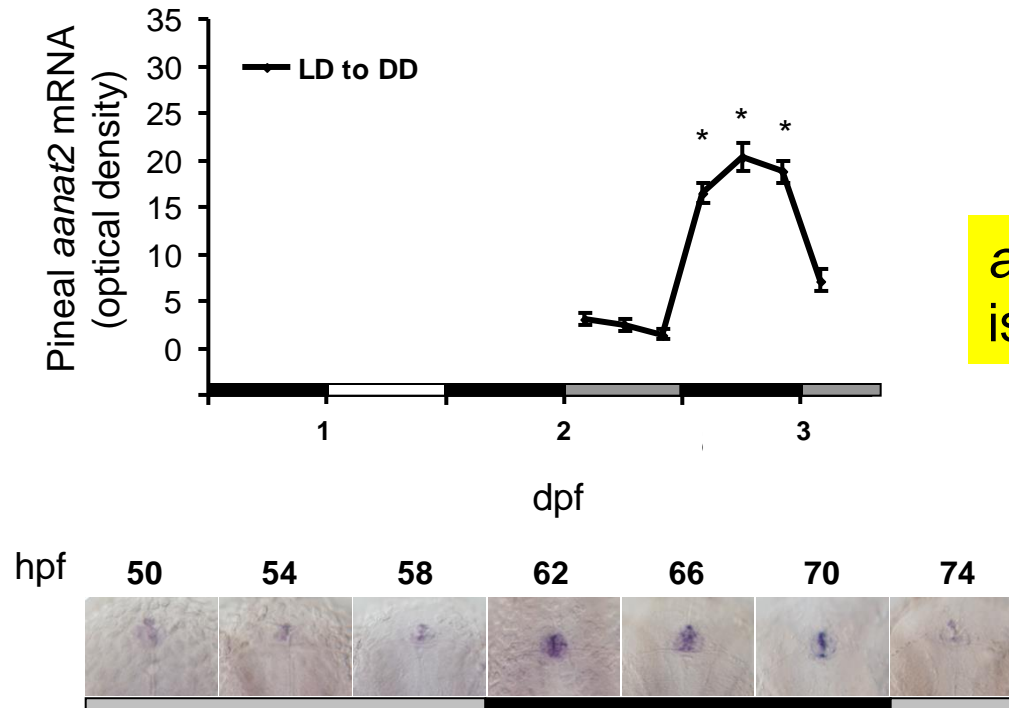
Number	RefSeq ID	Gene symbol	g-factor
1	NM_1002642	<i>camk2n2</i>	0.9380
2	NM_1197058	<i>nfil3-5 (e4bp4-5)</i>	0.9269
3	NM_131791	<i>cry2a</i>	0.9229
4	NM_131451	<i>irbp</i>	0.9151
5	NM_213439	<i>mid1ip1</i>	0.9147
6	NM_131792	<i>cry2b</i>	0.9130
7	NM_1113337	<i>cyp27c1</i>	0.9112
8	NM_131578	<i>arn12 (bmal2)</i>	0.9102
9	NM_212584	<i>ctsl1a</i>	0.9079
10	NM_212439	<i>per1b</i>	0.9074
11	NM_1045055	<i>si:ch211-132b12.7</i>	0.9051
12	NM_1030183	<i>per1a</i>	0.8949
13	NM_1044826	<i>atxn1a</i>	0.8927
14	NM_1002369	<i>zgc:92502</i>	0.8906
15	NM_200139	<i>cyp2p6</i>	0.8902
16	NM_201158	<i>aldh4a1</i>	0.8897
17	NM_1197060	<i>dbpa</i>	0.8892
18	NM_1039107	<i>bhlhe41 (dec2)</i>	0.8889
19	NM_205729	<i>nr1d1 (reverba)</i>	0.8871
20	NM_200751	<i>rpe65a</i>	0.8864
21	NM_130957	<i>clock1a</i>	0.8851
22	NM_1077323	<i>zgc:154001</i>	0.8845
23	NM_1114579	<i>fam131a</i>	0.8835
24	NM_200829	<i>camk1gb</i>	0.8814
25	NM_213523	<i>krt15</i>	0.8810
26	NM_1042720	<i>atp7a</i>	0.8805
27	NM_1013555	<i>zgc:113006</i>	0.8777
28	NM_131584	<i>per3</i>	0.8762
29	NM_199871	<i>mat1a</i>	0.8744
30	NM_1082979	<i>zgc:162144</i>	0.8737
31	NM_199567	<i>tgif1</i>	0.8722
32	NM_131065	<i>nr1d2b (reverbb2)</i>	0.8717
33	NM_131571	<i>smad3a</i>	0.8709
34	NM_1110486	<i>zgc:158292</i>	0.8687
35	NM_212723	<i>mknk2a</i>	0.8686
36	NM_1098177	<i>myh9a</i>	0.8679
37	NM_199611	<i>arg2</i>	0.8669
38	NM_1079657	<i>zgc:158340</i>	0.8665
39	NM_1199134	<i>ptprb</i>	0.8650
40	NM_131787	<i>cry4</i>	0.8642
41	NM_201217	<i>nop56</i>	0.8632
42	NM_1197065	<i>nfil3-2 (e4bp4-2)</i>	0.8630
43	NM_213387	<i>pgk1</i>	0.8624
44	NM_1166205	<i>hip1</i>	0.8615
45	NM_1006090	<i>fam73a</i>	0.8607
46	NM_201067	<i>rorab</i>	0.8600
47	NM_1044834	<i>si:dkey-239j20.4</i>	0.8598
48	NM_1080022	<i>myl1pb</i>	0.8586
49	NM_213495	<i>cul1b</i>	0.8581
50	NM_131411	<i>aanat2</i>	0.8565
51	NM_212822	<i>pik3r2</i>	0.8565
52	NM_201493	<i>nucb2a</i>	0.8552
53	NM_1004595	<i>utp3</i>	0.8518
54	NM_1044846	<i>slain2</i>	0.8507
55	NM_194377	<i>aldoaa</i>	0.8507
56	NM_199624	<i>her8a</i>	0.8497
57	NM_205759	<i>zgc:77395</i>	0.8487
58	NM_1110477	<i>zgc:162232</i>	0.8463
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60	NM_1127308	<i>dtx1</i>	0.8441
61	NM_205686	<i>cry-dash</i>	0.8423
62	NM_131786	<i>cry3</i>	0.8420
63	NM_1007445	<i>phyhd1</i>	0.8410
64	NM_199609	<i>dhhrs9</i>	0.8403
65	NM_205586	<i>prnp</i>	0.8403
66	NM_1045208	<i>letm1</i>	0.8345
67	NM_1082901	<i>si:ch211-197n10.4</i>	0.8333
68	NM_199649	<i>zfp3611b</i>	0.8318
69	NM_201116	<i>sh3gl2</i>	0.8313
70	NM_1008620	<i>tsta3</i>	0.8312
71	NM_212682	<i>clic1</i>	0.8312
72	NM_198915	<i>rbm19</i>	0.8307
73	NM_1082819	<i>rorca</i>	0.8267
74	NM_1007372	<i>gsto2</i>	0.8259
75	NM_199873	<i>ldhd</i>	0.8258
76	NM_1204131	<i>lrata</i>	0.8251
77	NM_1201566	<i>usp54a</i>	0.8234
78	NM_213528	<i>ng36</i>	0.8232
79	NM_201083	<i>fus</i>	0.8227
80	NM_178295	<i>clock1b</i>	0.8220
81	NM_213112	<i>rabggtb</i>	0.8204
82	NM_1005400	<i>ctlh</i>	0.8200
83	NM_1002297	<i>gnl3</i>	0.8197
84	NM_1105524	<i>ttl6</i>	0.8195
85	NM_1080204	<i>cyp11c1</i>	0.8193
86	NM_1126461	<i>etv5a</i>	0.8188
87	NM_213157	<i>slc25a33</i>	0.8178
88	NM_200610	<i>dopey2</i>	0.8172
89	NM_1077580	<i>dcaf12</i>	0.8152
90	NM_131373	<i>gfap</i>	0.8147
91	NM_1037379	<i>sept4a</i>	0.8131
92	NM_1020730	<i>zgc:109949</i>	0.8129
93	NM_200644	<i>nat10</i>	0.8129
94	NM_131801	<i>odc1</i>	0.8129
95	NM_199583	<i>csnk1da</i>	0.8123
96	NM_201202	<i>phc2b</i>	0.8111
97	NM_1039808	<i>slc2a1a</i>	0.8101
98	NM_1166332	<i>nlgn3a</i>	0.8098
99	NM_1111150	<i>bms1l</i>	0.8098
100	NM_1017881	<i>sqrdl</i>	0.8097
101	NM_1004562	<i>fth1b</i>	0.8095
102	NM_1020647	<i>acbd5a</i>	0.8089
103	NM_1014296	<i>mat2ab</i>	0.8088
104	NM_205724	<i>fbxo25</i>	0.8045
105	NM_1128538	<i>cdc14ab</i>	0.8039
106	NM_213010	<i>acadm</i>	0.8031
107	NM_1002649	<i>aclya</i>	0.8013
108	NM_1089417	<i>pfkmb</i>	0.8001
109	NM_1077253	<i>gnl1</i>	0.7995
110	NM_200567	<i>zgc:65987</i>	0.7992
111	NM_1006043	<i>ctsz</i>	0.7979
112	NM_199969	<i>mdh1b</i>	0.7972
113	NM_1040393	<i>zgc:136817</i>	0.7957
114	NM_212679	<i>bhlhe40 (dec1)</i>	0.7957
115	NM_1017753	<i>asb5</i>	0.7955
116	NM_1123011	<i>zgc:158426</i>	0.7955
117	NM_200172	<i>mycb</i>	0.7953
118	NM_131247	<i>ldhba</i>	0.7948
119	NM_1114583	<i>abcb4</i>	0.7946
120	NM_198363	<i>tob1a</i>	0.7940
121	NM_1135142	<i>zgc:194398</i>	0.7926
122	NM_1190816	<i>slc1a8b</i>	0.7921
123	NM_201326	<i>hspa9</i>	0.7889
124	NM_152949	<i>ccna2</i>	0.7882
125	NM_1082877	<i>ppat</i>	0.7874
126	NM_1077584	<i>zgc:152911</i>	0.7871
127	NM_1044892	<i>cspg5b</i>	0.7870
128	NM_1077731	<i>zgc:154093</i>	0.7870
129	NM_1024111	<i>pcdh1g11</i>	0.7869
130	NM_212714	<i>poldip2</i>	0.7868
131	NM_131799	<i>bcdo2l</i>	0.7867
132	NM_1200020	<i>si:ch1073-204j4.2</i>	0.7861
133	NM_1199680	<i>si:dkey-77a20.7</i>	0.7837
134	NM_1080989	<i>dennd4a</i>	0.7835
135	NM_1033591	<i>zgc:112334</i>	0.7830
136	NM_201025	<i>pgm1</i>	0.7815
137	NM_1003588	<i>zgc:101040</i>	0.7815
138	NM_1098485	<i>pgm2l1</i>	0.7807
139	NM_1013475	<i>bccip</i>	0.7807
140	NM_178436	<i>mcm5</i>	0.7806
141	NM_1098777	<i>sap130a</i>	0.7804
142	NM_131094	<i>tcf3</i>	0.7800
143	NM_1204257	<i>si:ch73-9j13.1</i>	0.7795
144	NM_212619	<i>cd9a</i>	0.7794
145	NM_1190305	<i>slc1a2a</i>	0.7787
146	NM_214748	<i>rpf2</i>	0.7779
147	NM_199333	<i>pkm2a</i>	0.7777
148	NM_201143	<i>pik3r3</i>	0.7775
149	NM_1083015	<i>acap3b</i>	0.7767
150	NM_1045207	<i>asap2a</i>	0.7766

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

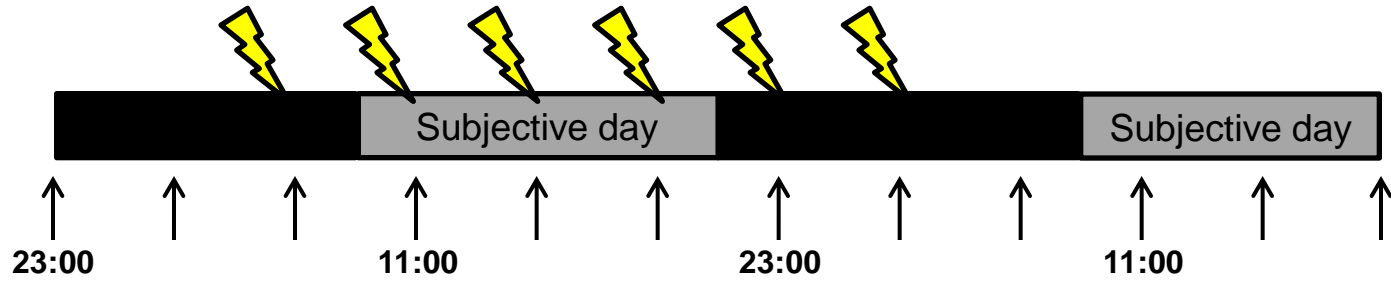
RNA-seq analysis



What goes up, must come down



Pineal transcriptome analysis light-induced genes



Next Generation
Sequencing

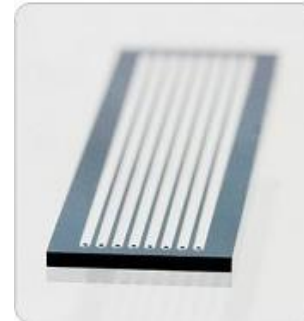
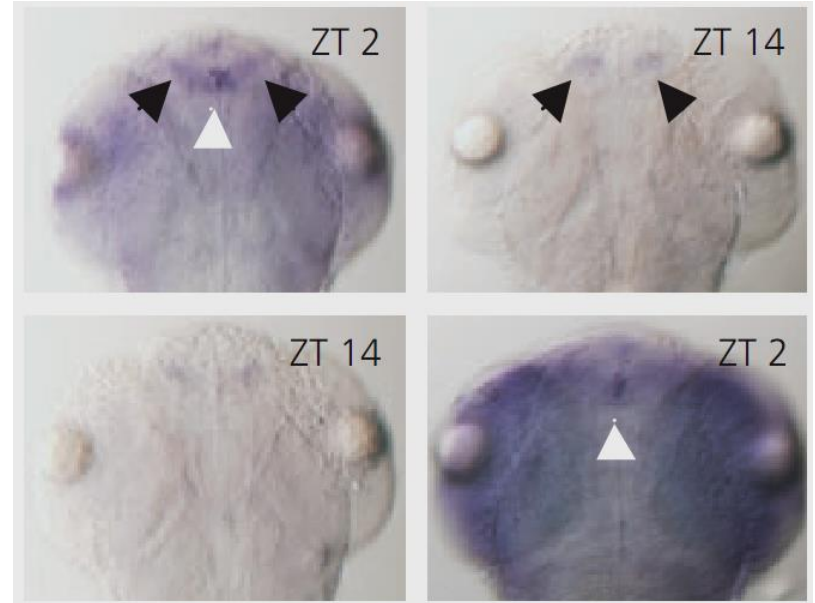
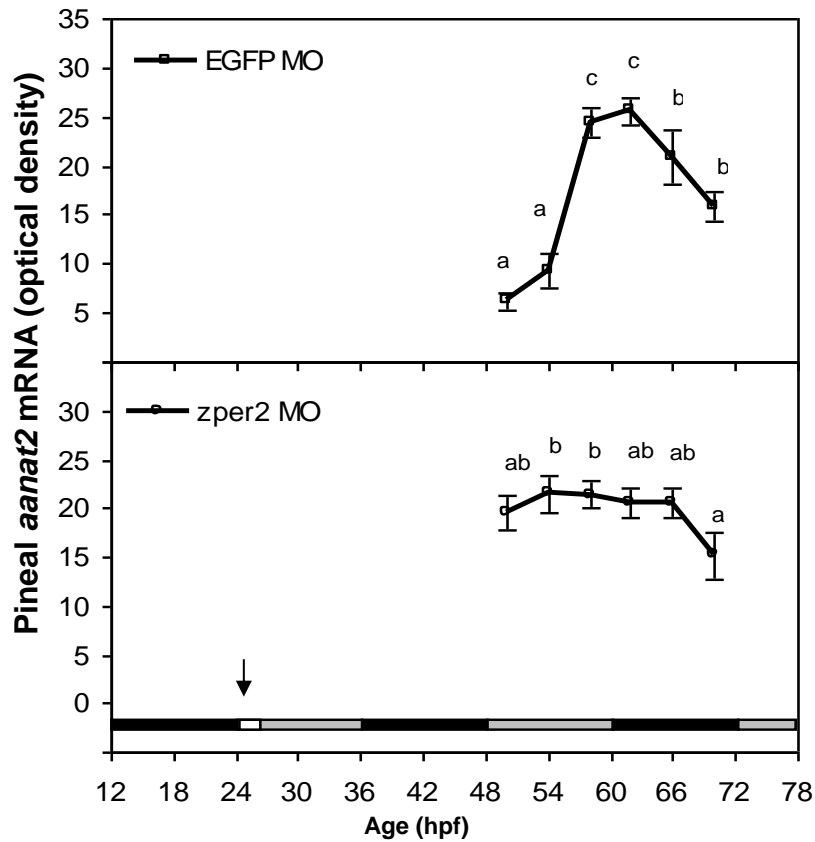


Table 1. Pineal gland light-induced genes detected by mRNA-seq

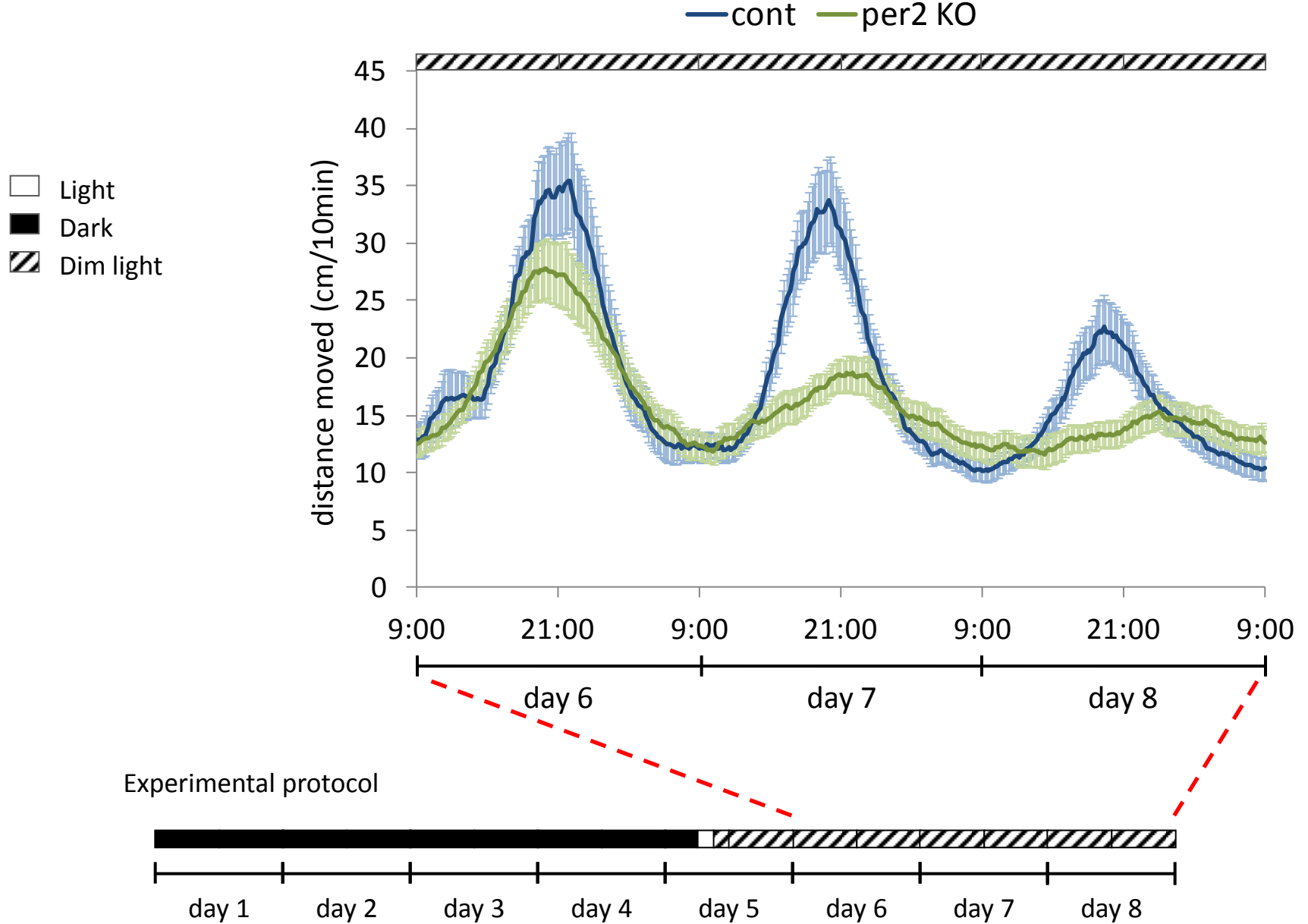
No.	Gene symbol	RefSeq ID	L/D fold	No.	Gene symbol	RefSeq ID	L/D fold
1	<i>pfkfb4l</i>	NM_198816	5.09	47	<i>rtn4rl2a</i>	NM_203479	2.28
2	<i>rtn4rl2b</i>	NM_203483	4.92	48	<i>vsnl1a</i>	NM_001007366	2.26
3	<i>nr4a3</i>	NM_001172629	4.81	49	<i>si:ch211-195b13.1</i>	NM_001077302	2.25
4	<i>pvalb7</i>	NM_205574	4.81	50	<i>insig1</i>	NM_199869	2.23
5	<i>nr1d1 (reverba)</i>	NM_205729	4.79	51	<i>hif1al</i>	NM_200405	2.22
6	<i>stmn4l</i>	NM_001002563	4.61	52	<i>znf238</i>	NM_001082952	2.21
7	<i>tagln2</i>	NM_201576	4.51	53	<i>stk35</i>	NM_001077616	2.2
8	<i>nppcl</i>	NM_001161341	4.38	54	<i>eng2a</i>	NM_131044	2.2
9	<i>mibp</i>	NM_131693	4.26	55	<i>bactin1</i>	NM_131031	2.17
10	<i>zgc:122979</i>	NM_001037574	4.09	56	<i>ifrd1</i>	NM_001076555	2.12
11	<i>fkbp5</i>	NM_213149	3.99	57	<i>hivep2</i>	NM_001030164	2.1
12	<i>jun</i>	NM_199987	3.77	58	<i>pik3r3b</i>	NM_201143	2.09
13	<i>bhlhe40 (dec1)</i>	NM_212679	3.6	59	<i>trim9</i>	NM_205563	2.05
14	<i>lonrf1l</i>	NM_001277234	3.59	60	<i>hsd11b2</i>	NM_212720	2.05
15	<i>osbpl7</i>	NM_001005927	3.54	61	<i>nfil3-5 (e4bp4-5)</i>	NM_001197058	2.03
16	<i>cnn2</i>	NM_213349	3.41	62	<i>glulb</i>	NM_182866	2.02
17	<i>nfil3-6 (e4bp4-6)</i>	NM_001002218	3.36	63	<i>zgc:63637</i>	NM_213365	2.02
18	<i>klf9</i>	NM_001128729	3.35	64	<i>dennd4a</i>	NM_001080989	2.01
19	<i>itm2cb</i>	NM_199980	3.32	65	<i>ppm1nb</i>	NM_001103117	2
20	<i>gabbr2</i>	NM_001024387	3.29	66	<i>coch</i>	NM_001003823	1.99
21	<i>per1a</i>	NM_001030183	3.29	67	<i>camk4</i>	NM_001017607	1.96
22	<i>slc20a1a</i>	NM_213179	3.28	68	<i>camk2n1</i>	NM_001145089	1.96
23	<i>tuft1a</i>	NM_001080001	3.1	69	<i>snap25a</i>	NM_131435	1.95
24	<i>per2</i>	NM_182857	3.07	70	<i>midn</i>	NM_207052	1.95
25	<i>cry1a</i>	NM_001077297	3	71	<i>chac1</i>	NM_001110126	1.94
26	<i>tmem178</i>	NM_001076640	2.99	72	<i>ucp2</i>	NM_131176	1.91
27	<i>nfil3-2 (e4bp4-2)</i>	NM_001197065	2.97	73	<i>scn8aa</i>	NM_131628	1.91
28	<i>camk2d2</i>	NM_001002542	2.89	74	<i>cyp27c1</i>	NM_001113337	1.9
29	<i>dbpb (dbp2)</i>	NM_001197062	2.87	75	<i>nrxn1a</i>	NM_001080021	1.78
30	<i>sptlc2b</i>	NM_001114741	2.75	76	<i>myl9b</i>	NM_213212	1.74
31	<i>pim3</i>	NM_001034978	2.71	77	<i>rpe65a</i>	NM_200751	1.73
32	<i>tgfb1</i>	NM_182862	2.7	78	<i>slc17a7</i>	NM_001098755	1.68
33	<i>jund</i>	NM_001128342	2.57	79	<i>tef1</i>	NM_131400	1.67
34	<i>bhlhe41 (dec2)</i>	NM_001039107	2.54	80	<i>grk7a</i>	NM_001031841	1.6
35	<i>mid1ip1</i>	NM_213439	2.54	81	<i>nr1d2b (reverbb2)</i>	NM_131065	1.6
36	<i>npas4a</i>	NM_001045321	2.52	82	<i>neurod</i>	NM_130978	1.58
37	<i>gfap</i>	NM_131373	2.51	83	<i>cplx4a</i>	NM_001077300	1.55
38	<i>speg</i>	NM_001007109	2.5	84	<i>stmn2a</i>	NM_001005923	1.54
39	<i>cry3</i>	NM_131786	2.48	85	<i>atp1a1b</i>	NM_131690	1.53
40	<i>nr1d2a (reverbb1)</i>	NM_001130592	2.45	86	<i>atp1b1a</i>	NM_131668	1.45
41	<i>abcf2</i>	NM_201315	2.4	87	<i>diras1</i>	NM_199831	1.44
42	<i>zgc:154093</i>	NM_001077731	2.36	88	<i>stxbp1</i>	NM_001025182	1.43
43	<i>arg2</i>	NM_199611	2.34	89	<i>atp2b2</i>	NM_001123238	1.41
44	<i>c1qtnf4</i>	NM_001017702	2.33	90	<i>gpm6aa</i>	NM_213200	1.4
45	<i>camk2b1</i>	NM_001271393	2.29	91	<i>gpm6ab</i>	NM_214687	1.37
46	<i>zgc:153615</i>	NM_001077791	2.28	92	<i>zic2a</i>	NM_131558	1.28

Per2
Cry1a

Light-induced *per2* is required for the onset of the pineal clock



per2 KO larvae display disrupted light-entrainment of locomotor activity rhythms



per2:EGFP transgenic line

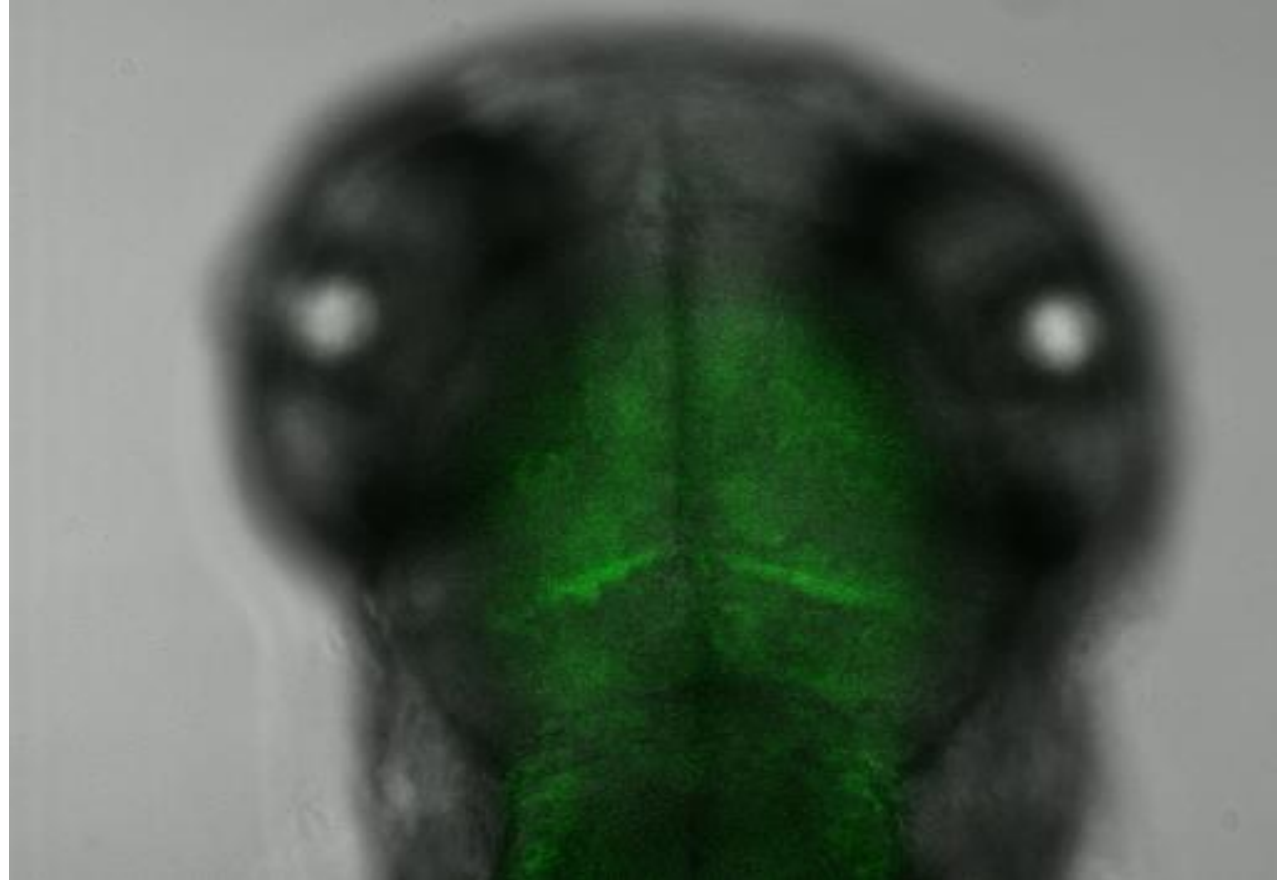
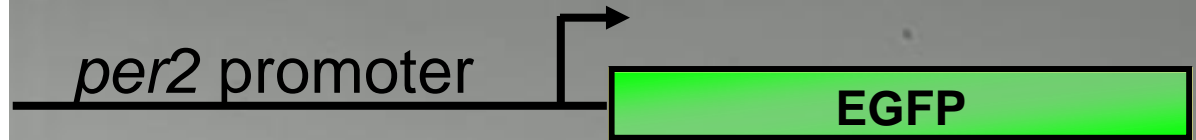
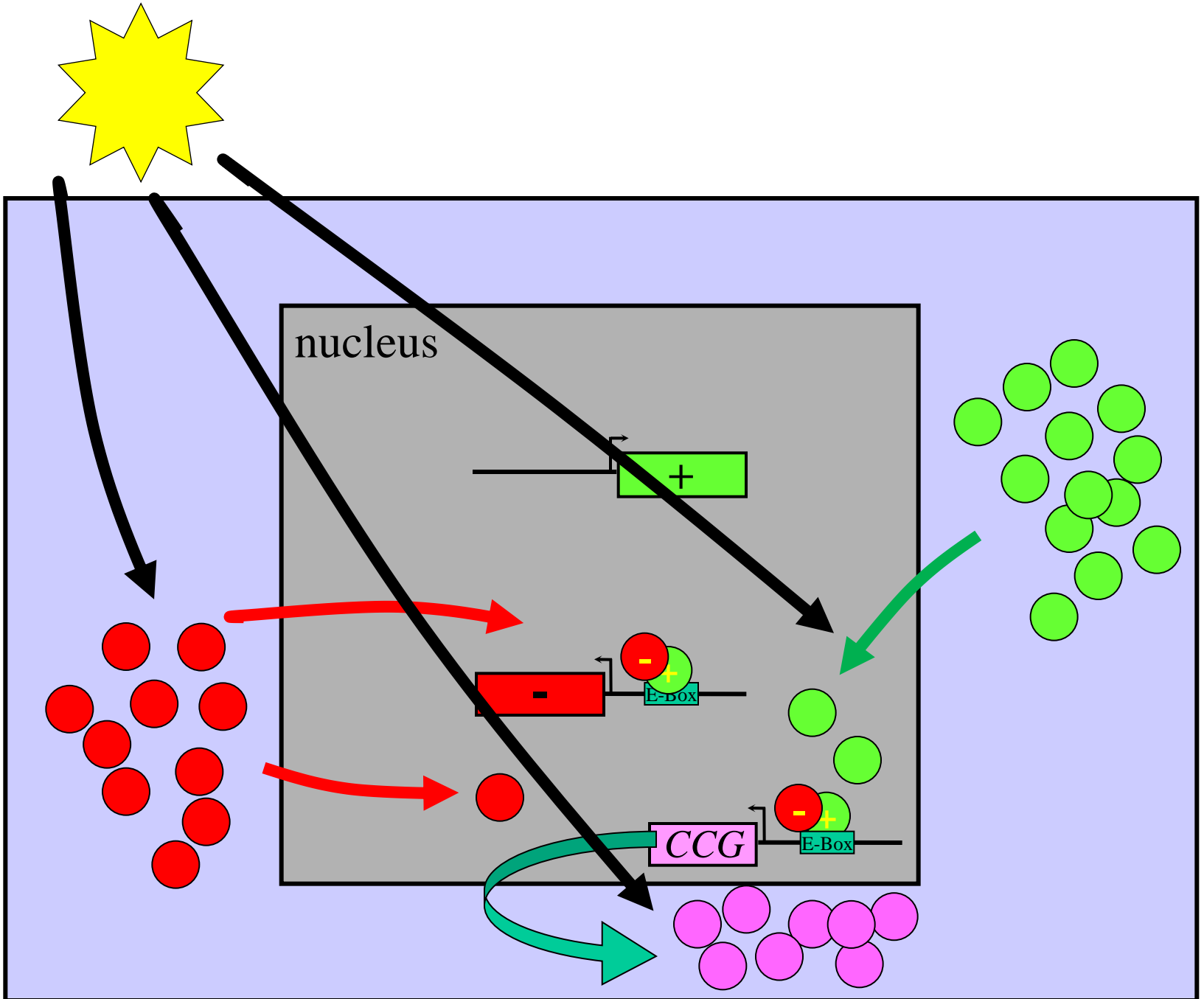


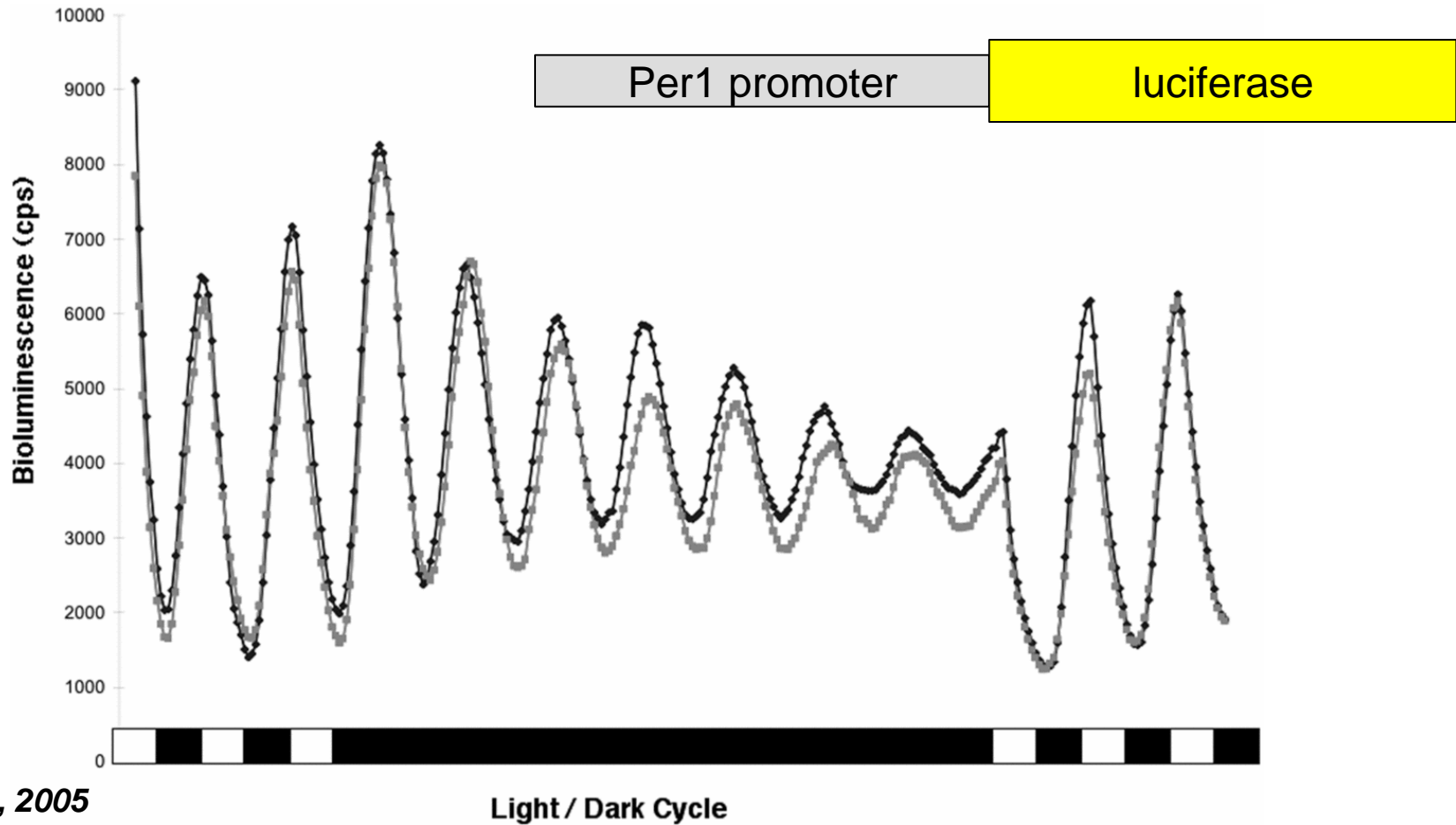
Table 1. Pineal gland light-induced genes detected by mRNA-seq

No.	Gene symbol	RefSeq ID	L/D fold	No.	Gene symbol	RefSeq ID	L/D fold
1	<i>pfkfb4l</i>	NM_198816	5.09	47	<i>rtn4r12a</i>	NM_203479	2.28
2	<i>rtn4r12b</i>	NM_203483	4.92	48	<i>vsnl1a</i>	NM_001007366	2.26
3	<i>nr4a3</i>	NM_001172629	4.81	49	<i>si:ch211-195b13.1</i>	NM_001077302	2.25
4	<i>pvalb7</i>	NM_205574	4.81	50	<i>insig1</i>	NM_199869	2.23
5	<i>nr1d1 (reverba)</i>	NM_205729	4.79	51	<i>hif1al</i>	NM_200405	2.22
6	<i>stmn4l</i>	NM_001002563	4.61	52	<i>znf238</i>	NM_001082952	2.21
7	<i>tagln2</i>	NM_201576	4.51	53	<i>stk35</i>	NM_001077616	2.2
8	<i>nppcl</i>	NM_001161341	4.38	54	<i>eng2a</i>	NM_131044	2.2
9	<i>mibp</i>	NM_131693	4.26	55	<i>bactin1</i>	NM_131031	2.17
10	<i>zgc:122979</i>	NM_001037574	4.09	56	<i>ifrd1</i>	NM_001076555	2.12
11	<i>fkbp5</i>	NM_213149	3.99	57	<i>hivep2</i>	NM_001030164	2.1
12	<i>jun</i>	NM_199987	3.77	58	<i>pik3r3b</i>	NM_201143	2.09
13	<i>bhlhe40 (dec1)</i>	NM_212679	3.6	59	<i>trim9</i>	NM_205563	2.05
14	<i>lonrf1l</i>	NM_001277234	3.59	60	<i>hsd11b2</i>	NM_212720	2.05
15	<i>osbpl7</i>	NM_001005927	3.54	61	<i>nfil3-5 (e4bp4-5)</i>	NM_001197058	2.03
16	<i>cnn2</i>	NM_213349	3.41	62	<i>glulb</i>	NM_182866	2.02
17	<i>nfil3-6 (e4bp4-6)</i>	NM_001002218	3.36	63	<i>zgc:63637</i>	NM_213365	2.02
18	<i>klf9</i>	NM_001128729	3.35	64	<i>dennd4a</i>	NM_001080989	2.01
19	<i>itm2cb</i>	NM_199980	3.32	65	<i>ppm1nb</i>	NM_001103117	2
20	<i>gabbr2</i>	NM_001024387	3.29	66	<i>coch</i>	NM_001003823	1.99
21	<i>per1a</i>	NM_001030183	3.29	67	<i>camk4</i>	NM_001017607	1.96
22	<i>slc20a1a</i>	NM_213179	3.28	68	<i>camk2n1</i>	NM_001145089	1.96
23	<i>tuft1a</i>	NM_001080001	3.1	69	<i>snap25a</i>	NM_131435	1.95
24	<i>per2</i>	NM_182857	3.07	70	<i>midn</i>	NM_207052	1.95
25	<i>cry1a</i>	NM_001077297	3	71	<i>chac1</i>	NM_001110126	1.94
26	<i>tmem178</i>	NM_001076640	2.99	72	<i>ucp2</i>	NM_131176	1.91
27	<i>nfil3-2 (e4bp4-2)</i>	NM_001197065	2.97	73	<i>scn8aa</i>	NM_131628	1.91
28	<i>camk2d2</i>	NM_001002542	2.89	74	<i>cyp27c1</i>	NM_001113337	1.9
29	<i>dbpb (dbp2)</i>	NM_001197062	2.87	75	<i>nrxn1a</i>	NM_001080021	1.78
30	<i>sptlc2b</i>	NM_001114741	2.75	76	<i>myl9b</i>	NM_213212	1.74
31	<i>pim3</i>	NM_001034978	2.71	77	<i>rpe65a</i>	NM_200751	1.73
32	<i>tgfb1</i>	NM_182862	2.7	78	<i>slc17a7</i>	NM_001098755	1.68
33	<i>jund</i>	NM_001128342	2.57	79	<i>tef1</i>	NM_131400	1.67
34	<i>bhlhe41 (dec2)</i>	NM_001039107	2.54	80	<i>grk7a</i>	NM_001031841	1.6
35	<i>mid1ip1</i>	NM_213439	2.54	81	<i>nr1d2b (reverbb2)</i>	NM_131065	1.6
36	<i>npas4a</i>	NM_001045321	2.52	82	<i>neurod</i>	NM_130978	1.58
37	<i>gfap</i>	NM_131373	2.51	83	<i>cplx4a</i>	NM_001077300	1.55
38	<i>speg</i>	NM_001007109	2.5	84	<i>stmn2a</i>	NM_001005923	1.54
39	<i>cry3</i>	NM_131786	2.48	85	<i>atp1a1b</i>	NM_131690	1.53
40	<i>nr1d2a (reverbb1)</i>	NM_001130592	2.45	86	<i>atp1b1a</i>	NM_131668	1.45
41	<i>abcf2</i>	NM_201315	2.4	87	<i>diras1</i>	NM_199831	1.44
42	<i>zgc:154093</i>	NM_001077731	2.36	88	<i>stxbp1</i>	NM_001025182	1.43
43	<i>arg2</i>	NM_199611	2.34	89	<i>atp2b2</i>	NM_001123238	1.41
44	<i>c1qtnf4</i>	NM_001017702	2.33	90	<i>gpm6aa</i>	NM_213200	1.4
45	<i>camk2b1</i>	NM_001271393	2.29	91	<i>gpm6ab</i>	NM_214687	1.37
46	<i>zgc:153615</i>	NM_001077791	2.28	92	<i>zic2a</i>	NM_131558	1.28

Per2
Cry1a
Per1a
Cry3
Dec1
Dec2
Reverba
Reverbb1
Reverbb2
Tef1
Dbp2
E4bp4-2
E4bp4-5
E4bp4-6



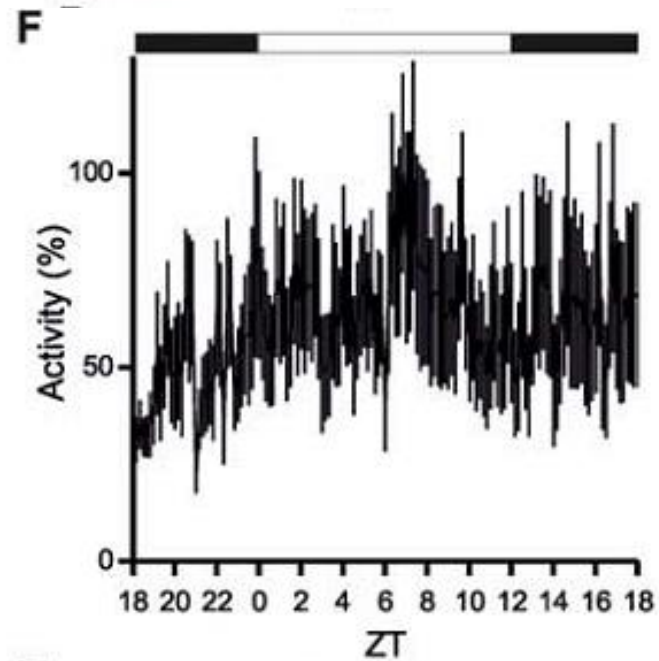
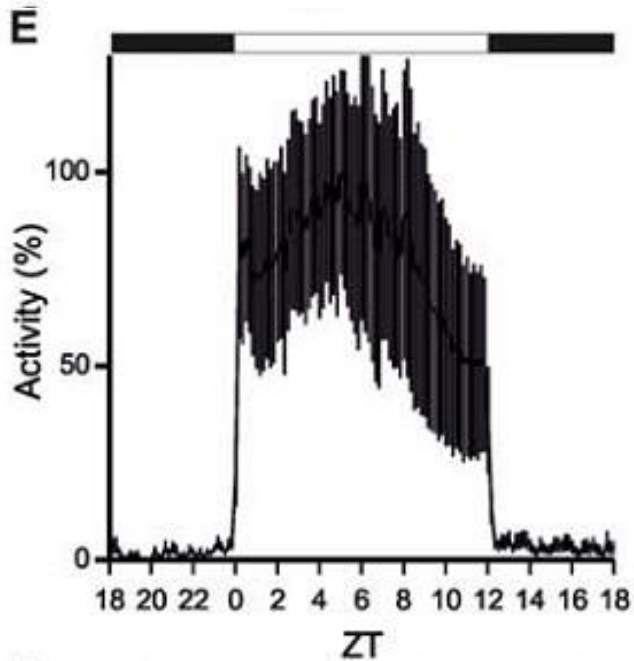
Luminescent measurements from zebrafish PAC2 cells transfected with *period1-luciferase*



Tamai et al., 2005

How do peripheral clocks detect light?

Studies in blind cave fish



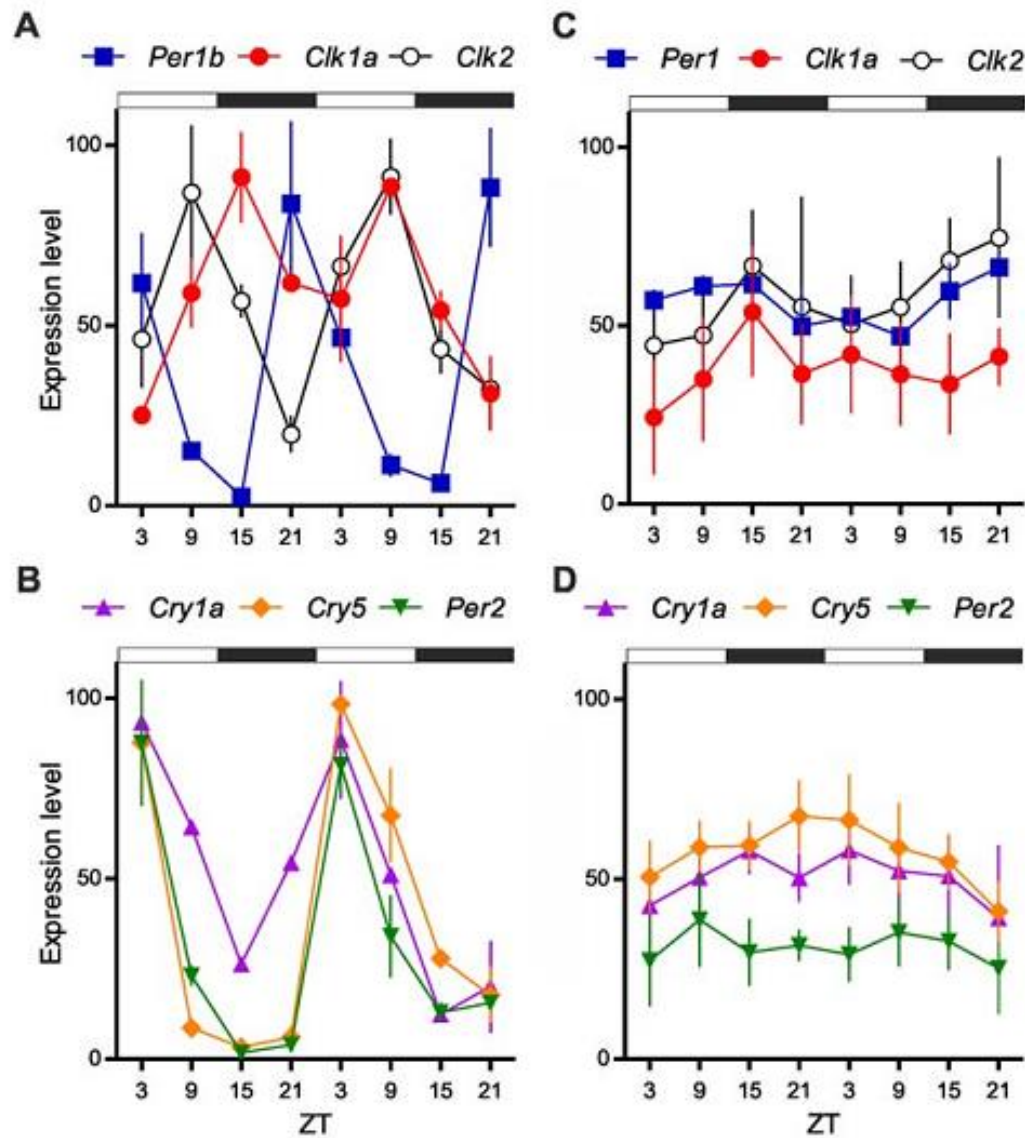
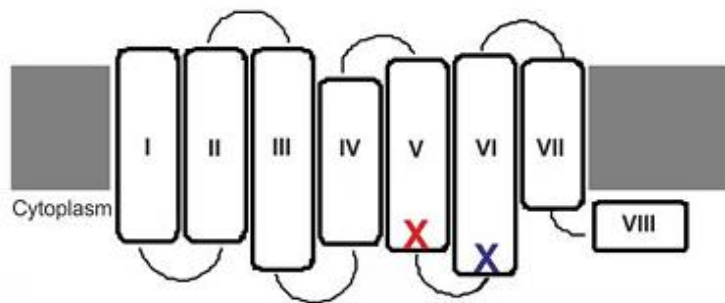


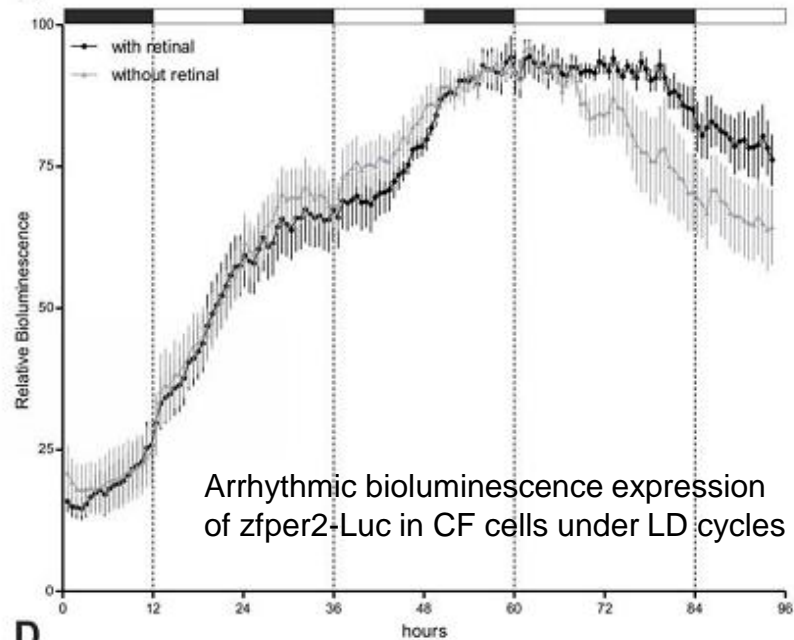
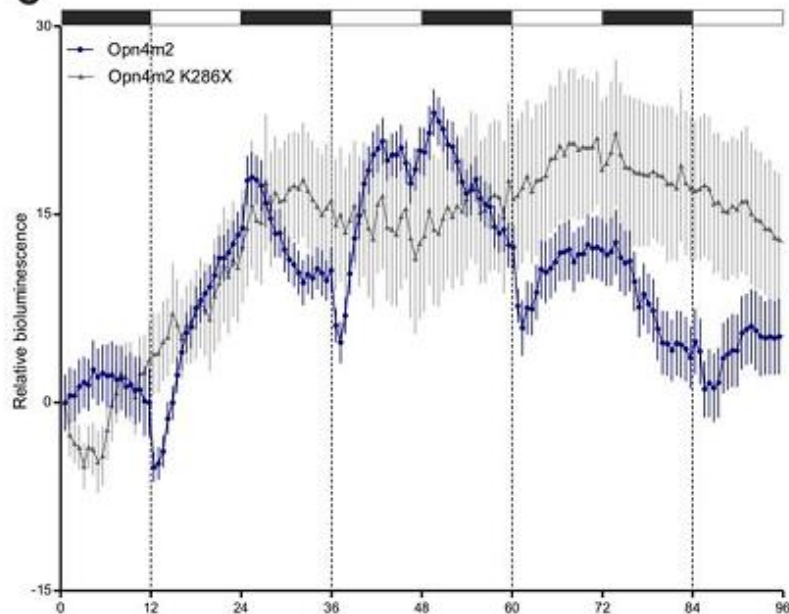
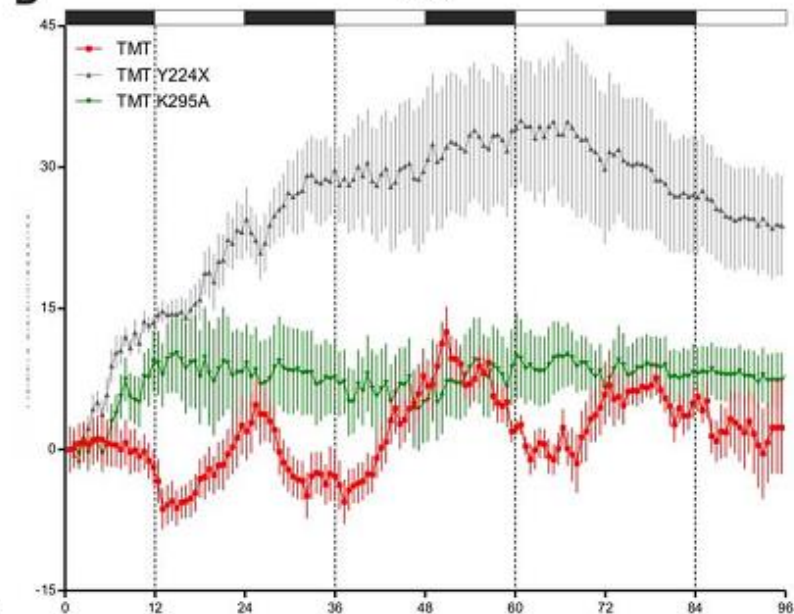
Figure 2. Lack of rhythmic clock gene expression in *P. andruzzii* cell lines exposed to LD cycles. Quantitative RT-PCR analysis of clock and light-regulated clock gene expression in zebrafish (A,B) and cavefish (CF; C,D) cell lines. The presentation, color code for the plotted

A**TMT-opsin**

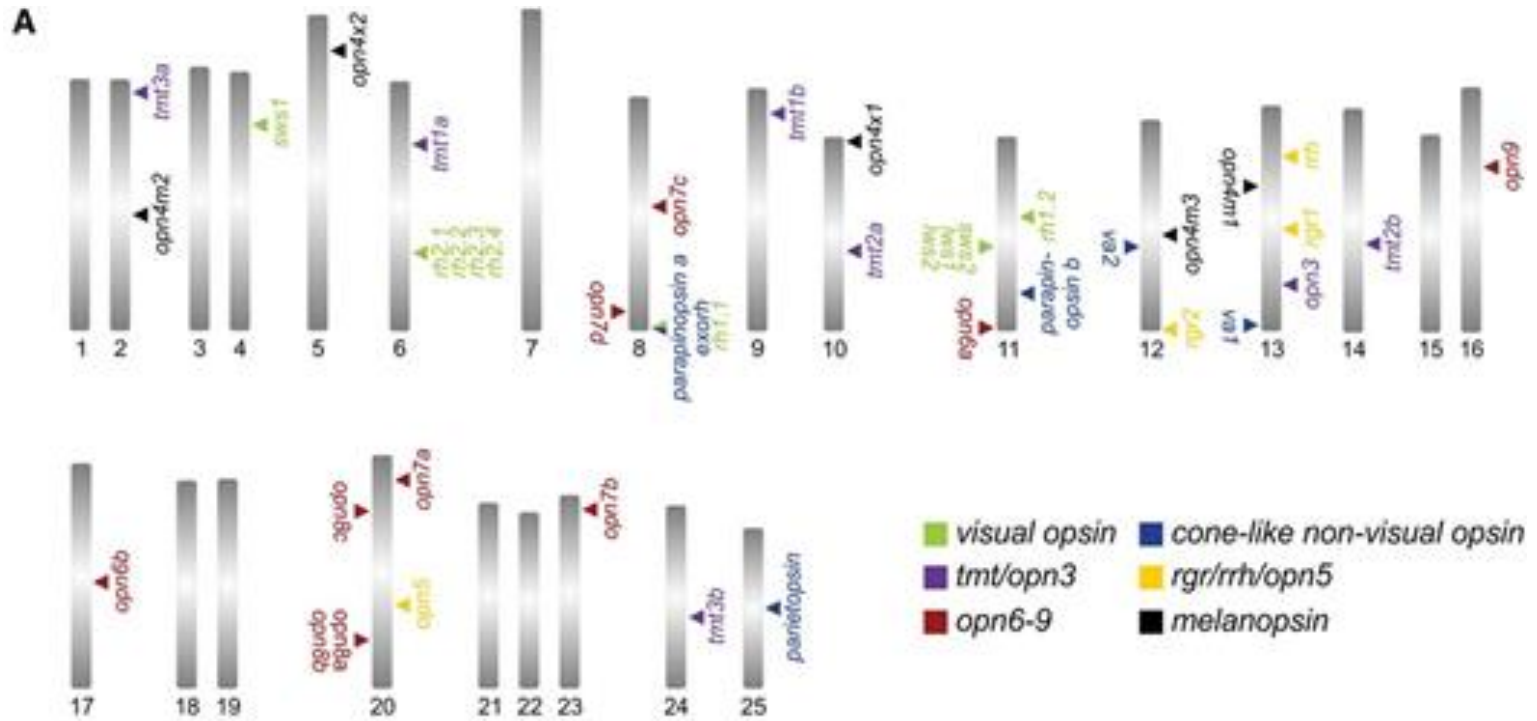
Cavefish SSISYIICLFIFCLIVPFFGHYLLWX-----
 Zebrafish NNISYIICLFIFCLIVPFLVIIFCYGKLLHAIKQVSS
 *****:

Opn4m2

Cavefish RAAGKEIRELDCGETLRMNSPSWSLCFLX-----
 Zebrafish RAAGKEIRELDCGETHKVYERMQNEWKMAKVALVVILFI
 ***** :

B**C****D**

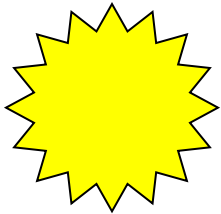
Light-induced expression of zper2-Luc in CF cells is rescued by co-transfection with zebrafish Opn4m2 (C) and TMT-opsin (D)



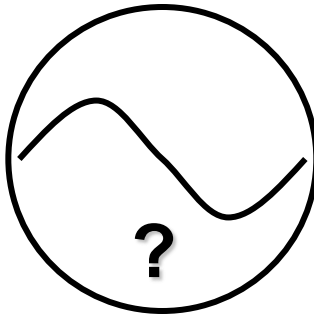
Davis et al., 2015

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*

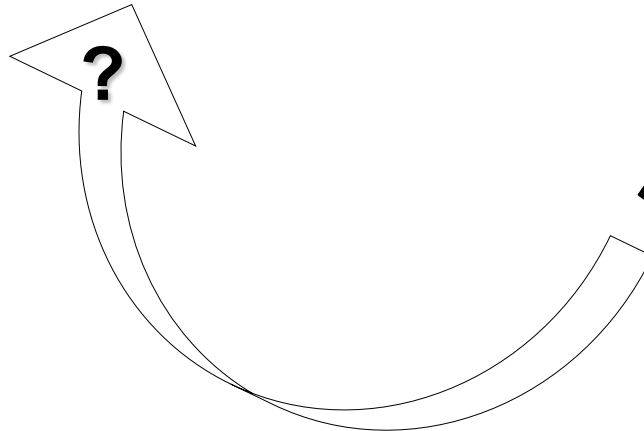
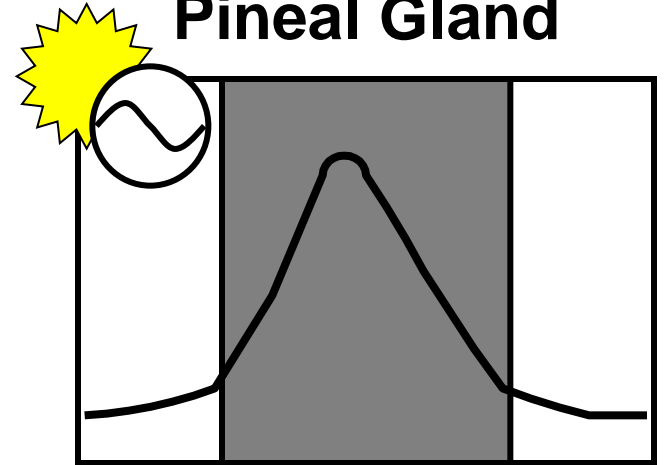
Light input



SCN

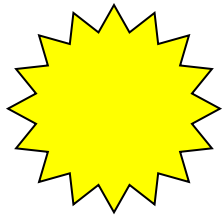


Pineal Gland

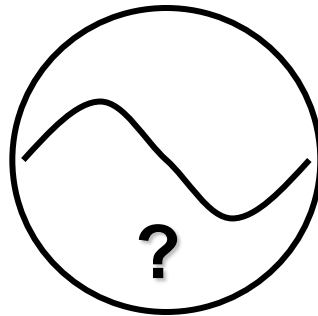


Melatonin

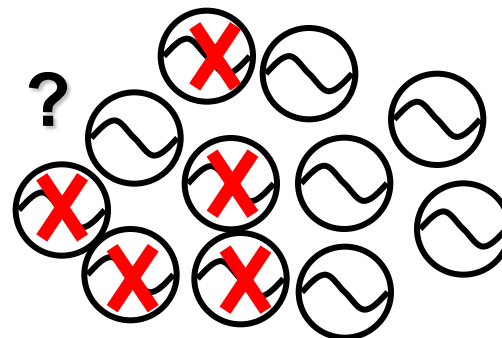
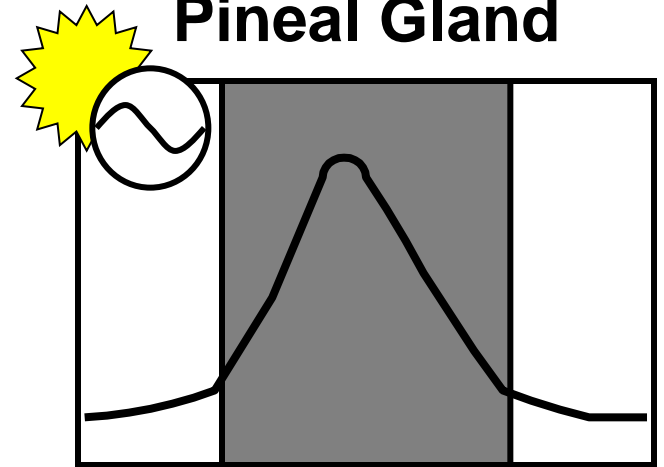
Light input



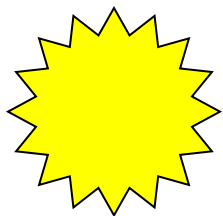
SCN



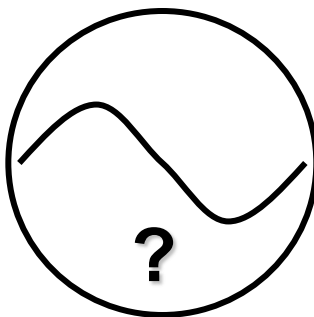
Pineal Gland



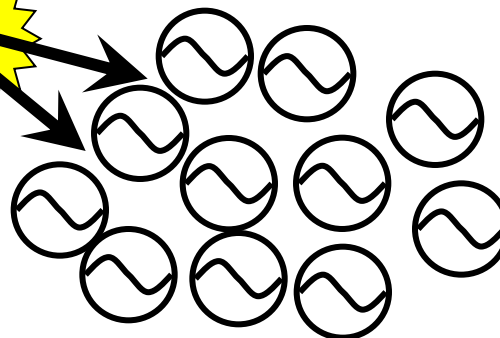
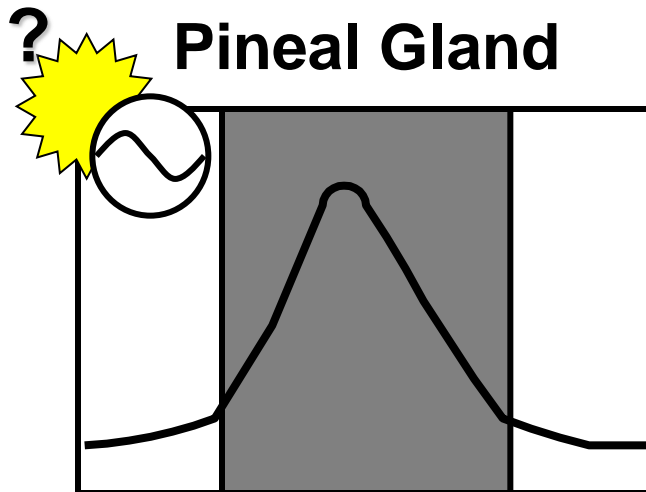
Light input



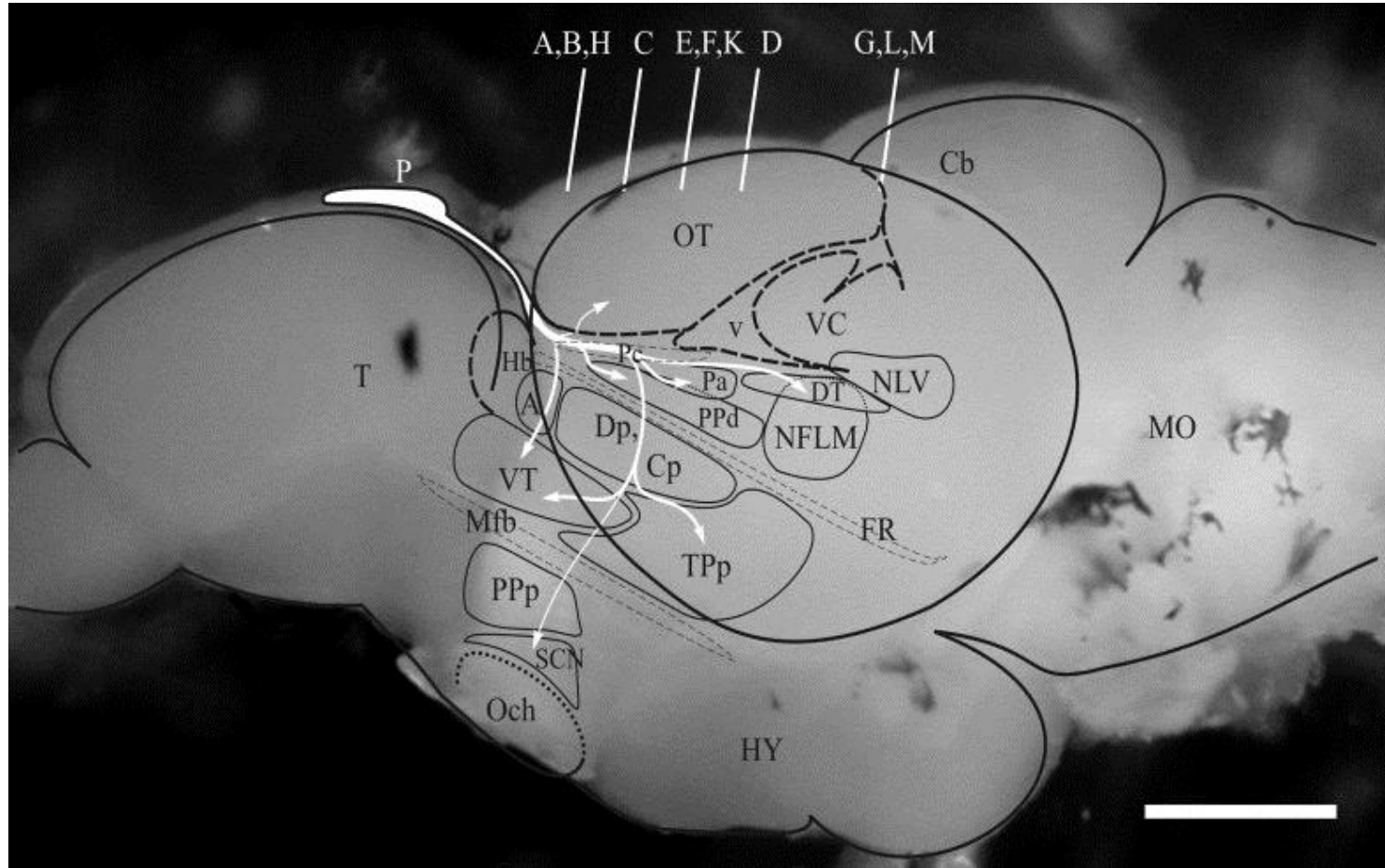
SCN



? Pineal Gland

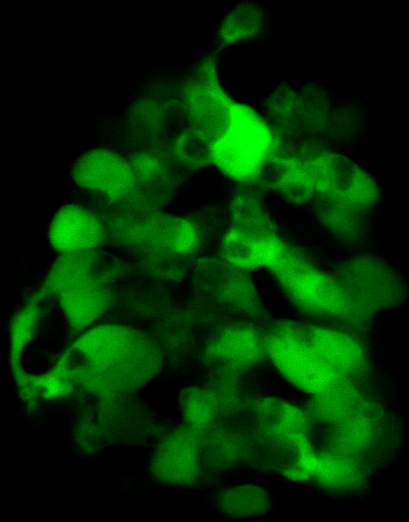


Pineal neuronal projections (Dil tract labeling)

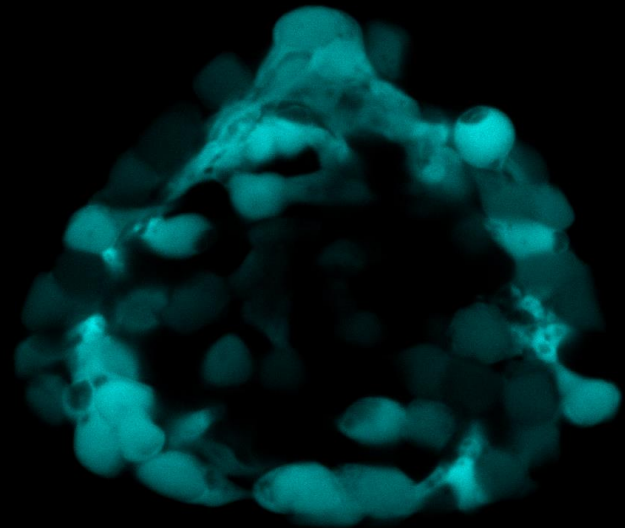


rostral hypothalamus, thalamus, pretectum, posterior tubercle and the mesencephalic tegmentum

Pineal photoreceptor cells vs. projection neurons

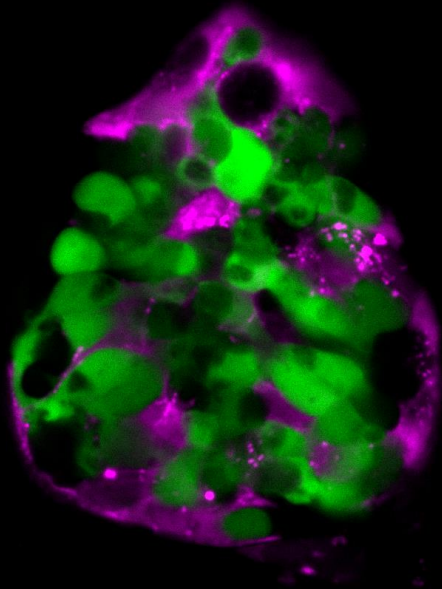


Photoreceptors
(*aanat2*)



Projection neurons
(*foxd3*)

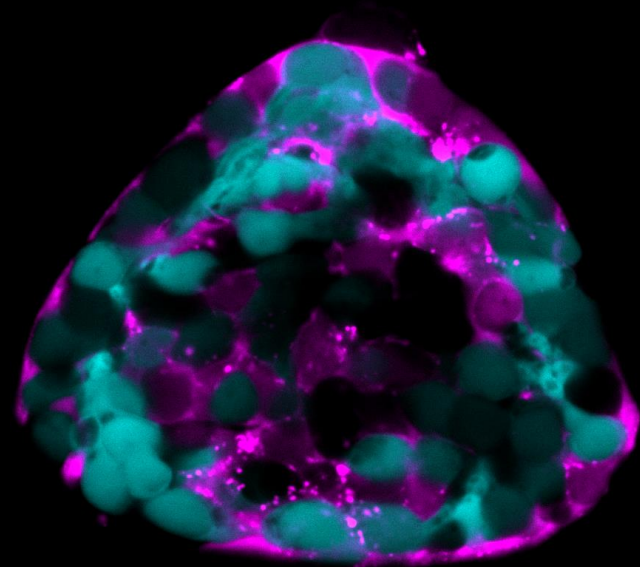
Pineal photoreceptor cells vs. projection neurons



10 μ m

Photoreceptors
(*aanat2*)

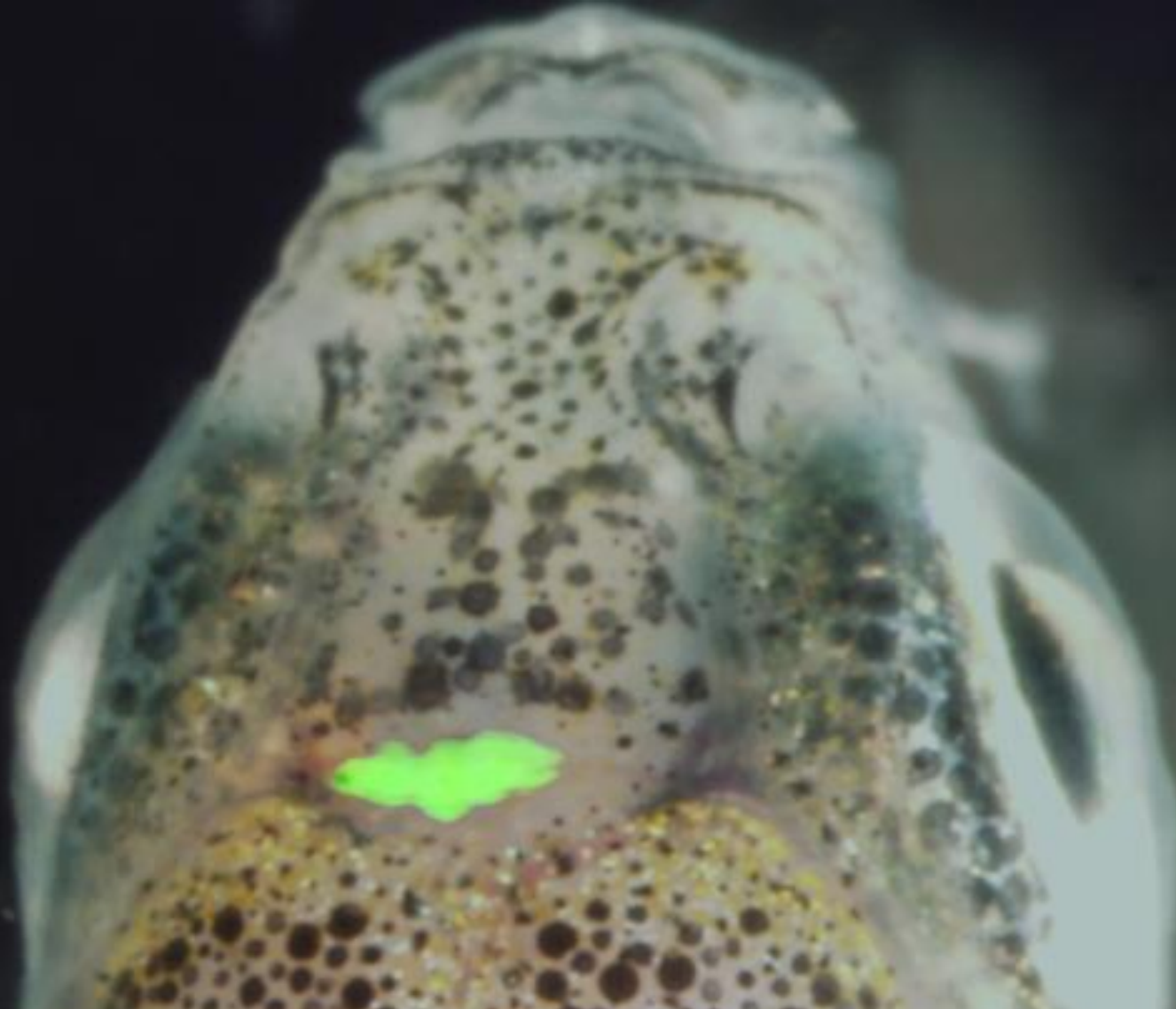
AgRP2



10 μ m

Projection neurons
(*foxd3*)

AgRP2



**Thank you for listening,
and, see you in Tel-Aviv**