Clockwork Genes: Discoveries in Biological Time
2000 Holiday Lectures on Science
Chapter List

Lecture One
Biology in Four Dimensions
Joseph S. Takahashi, Ph.D.

Lecture Two
Unwinding Clock Genetics
Michael Rosbash, Ph.D.

1. Start of Lecture Two
2. Introduction by HHMI Vice President Dr. Peter Bruns
3. Introductory interview with Dr. Michael Rosbash
4. How much of what we are is inherited? Genetics as a tool to search for disease mechanisms
5. Pioneering the use of the fruit fly *Drosophila* as a genetic tool
6. Examples of mutants in *Drosophila*
7. General insect behavior: Waggle dance of the honeybee
8. Similarity of cellular structures and genome between humans and *Drosophila*
9. Behavior in fruit flies: Courtship song
10. Behavior in fruit flies: Learning and memory
11. Behavior in fruit flies: Circadian rhythm assayed by time of eclosion
12. *period* gene (*per*) located on the X chromosome
13. Animation: Activity pattern of circadian mutant
14. Apparatus for measuring fly activity
15. Activity patterns of *per-short* flies
16. Do flies actually sleep?
17. Q&A: Are there fundamental differences between diurnal and nocturnal animals?
18. Q&A: Are there many natural variations in the fruit fly?
19. Q&A: Have you tried to change the clock gene?
20. Q&A: What are the characteristics of humans with a biological clock mutation?
21. Q&A: Are clock mutations inherited?
22. Q&A: Does temperature affect circadian rhythm in flies?
23. Q&A: Is the clock mutation dominant, semi-dominant, or something else?
24. Q&A: Is the human *period* gene located in the X chromosome?
25. Beginning of the molecular era: Cloning of the *period* gene
26. Finding the exact location of mutation in *per* mutants by using transgenic flies
27. *per* gene organization
28. Antibody against *per* protein (PER) reveals where *per* gene is expressed
29. Negative feedback loop as a mechanism for oscillating *per* RNA levels
30. Animation: Negative feedback loop of the *per* gene
31. Measurements of oscillations by using a *per*-luciferase transgenic fly
32. Summary of Lecture Two
33. Q&A: How does PER control the fly's behavior?
34. Q&A: What affects biological clocks more: genes or environment?
35. Q&A: Is circadian rhythm affected by shortening of telomeres?
36. Q&A: What do fruit flies and humans have in common?
37. Q&A: What kind of protein is PER and what does it do?
38. Q&A: How does X inactivation affect the *per* gene on the fly's X chromosome?
39. Q&A: Does aging affect the circadian clock?
40. Closing remarks by HHMI Vice President Dr. Peter Bruns

Lecture Three
PERfect TIMing
Michael Rosbash, Ph.D.

1. Start of Lecture Three
2. Introduction by HHMI President Dr. Thomas Cech
3. Introductory interview with Dr. Michael Rosbash
4. Summary of the fruit fly's circadian clock
5. Using attached-X-chromosome screening to find *per* gene
6. Other approaches for finding new clock genes
7. *Drosophila* DNA microarray used to assay RNA expression levels
8. Currently known *Drosophila* clock genes
9. Central pacemaker genes: *clock* and *cycle*
10. Animation: Negative feedback by *period* protein (PER)
11. *timeless* protein (TIM) and PER levels in a day
12. TIM is degraded by light and stabilizes PER
13. Animation: TIM causes PER feedback to be affected by daylight
14. Q&A: Can you affect the master clock by affecting the clock in the liver?
15. Q&A: How does light degrade the protein?
16. Q&A: How many different RNAs do we know of?
17. Q&A: Does light-related protein degradation happen in all cells?
18. Q&A: Why is it necessary for two proteins to control the circadian rhythm?
19. Q&A: Do any other external factors affect the system?
20. The role of the kinase Doubletime (DBT)
21. Cryptochromes: The light-sensitive protein as a candidate for circadian light sensor
22. Are transgenic flies that have a double dose of the *chryptochrome* gene sensitive to light?
23. *cryptochrome* mutant showing no circadian oscillations
24. Constant light does not suppress the circadian rhythm of *cryptochrome* mutants
25. Animation: DBT and *cryptochrome* protein's effects on circadian rhythms
26. DBT mutant strain showing a long period
27. Animation: DBT mutant kinase makes circadian period longer
28. Description of some output genes, particularly *pdf*
29. Experiment indicating the lateral neurons as the *Drosophila* pacemaker
30. Summary of the *Drosophila* clock system
31. Q&A: Have you substituted heat or other cues for light?
32. Q&A: How does a circadian clock sustain the 24-hour cycle in the absence of light?
33. Q&A: Do the pacemaker genes have a similar sequence to a mammalian gene?
34. Closing remarks by HHMI President Dr. Thomas Cech

Lecture Four
The Mammalian Timekeeper
Joseph S. Takahashi, Ph.D.

1. Start of Lecture Four
2. Introduction by HHMI Senior Program Officer Dr. Dennis Liu
3. Introductory interview with Dr. Joseph Takahashi
4. Review: Animation of mammalian circadian neuroanatomy
5. Genetic approaches in mice; the model mammalian system
6. Mutagenesis in mice
7. Genetic screens to isolate circadian clock mutants
8. Genetic analysis of the circadian clock mutant
9. Phenotype of an SCN neuron from a circadian clock mutant
10. Impact of the Human Genome Project on clock research
11. Positional-cloning analysis to map the *Clock* gene
12. Using positional cloning and transgenic mice together
13. Map of the *Clock* gene region
14. Structure of the *Clock* protein (CLOCK)
15. Q&A: What would you do after understanding the mechanism of the biological clock?
16. Q&A: Can you phase-shift a mammal with blue light?
17. Q&A: Have you done overexpression experiments with *Clock*?
18. Q&A: What experiments do you use to see the difference between mutant and wild-type genes?
19. Q&A: What other kinds of mutations are there in the *Clock* gene?
20. Function of CLOCK
21. Nine different proteins related to circadian clock genes
22. *Cryptochrome 1* and *2* genes
23. Animation: Role of *Period (Per)* and *Cryptochrome (Cry)* genes
24. *tau* mutant in the golden hamster
25. Identifying the gene involved in the *tau* mutant
26. Biochemical basis of *tau* mutation on *Casein kinase 1, epsilon* gene
27. Animation: Role of *Casein kinase* gene
28. The effect of *tau* mutation on the *Period* gene's expression level
29. Animation: *tau* mutation causes early buildup of negative feedback proteins
30. Summary and comparison of mammalian and fly clocks
31. Video: Interview on how *per* and other genes relate to humans
32. A human sleep disorder related to circadian clock
33. Summary of the four lectures
34. Q&A: How do *Cryptochrome* double mutants behave in light/dark cycles?
35. Q&A: Does temperature affect the circadian rhythm in mammals?
36. Closing remarks by HHMI President Dr. Thomas Cech