Clockwork Genes: Discoveries in Biological Time
Lecture Two—Unwinding Clock Genetics
Michael Rosbash, Ph.D.

1. Start of Lecture Two (00:15)

From the Howard Hughes Medical Institute, the 2000 Holiday Lectures on Science…This year's lectures, "Clockwork Genes: Discoveries in Biological Time," will be given by Dr. Michael Rosbash, Howard Hughes Medical Institute investigator at Brandeis University and Dr. Joseph S. Takahashi, Howard Hughes Medical Institute investigator at Northwestern University. The second lecture is titled "Unwinding Clock Genetics." And now, to introduce our program, Howard Hughes Medical Institute's vice president for grants and special programs, Dr. Peter Bruns.

2. Introduction by HHMI Vice President Dr. Peter Bruns (01:09)

Welcome back to the Howard Hughes Medical Institute and the Holiday Lectures 2000. I'm especially excited to be able to introduce this next lecture here. The institute has just recently appointed me as vice president for grants and special programs, and this lecture series is part of that show. And so it's a wonderful first assignment for me to introduce a lecture in this series. I've been professor of genetics at Cornell for many years doing the usual teaching and research, but for the last 10 years I've also been involved in a high school biology enhancement program which has been sponsored and really was started by this institute. And so this is a wonderful connection for me. This is a great time to be a geneticist. Professor Rosbash got into clocks by cloning the first gene from *Drosophila* involved in this whole process. That was a big deal, and that wasn't very long ago. Today, you can go to your computer and get sequences and clone genes really with the information right off the Internet. Just think what all of you will be able to do if you start a scientific career in genetics in just a few years from now. Dr. Rosbash will speak for about 40 minutes again with an interruption for questions partway through and then questions at the end. And so the lecture will in many ways run just the way the one we just saw did. We're going to start this off by introducing him with a short video

3. Introductory interview with Dr. Michael Rosbash (02:43)

I became interested in science in high school, but more math than science, and went to Caltech with the vague intention of becoming a mathematician, not knowing really what that was, of course. And I got turned on to biology. So my lab has two parts. Half of my lab or so works on problems in RNA, in RNA metabolism, and the other half, as you know, works on circadian rhythms and fruit flies. And the common denominator, such as it is, is genetics. We really are trying to understand how the clock works and what the molecules are, how they interact, how one really keeps time, and how the central pacemaker connects with light or the other major inputs from the environment, as well as how it connects to outputs, that is, the many things that it governs in physiology and behavior. And in rhythms we've done a number of things over the years: cloning rhythm genes, identifying rhythm genes, understanding how they work, the role of RNA metabolism and, more specifically, transcription in the process. So we've been, I think, respectable mainstream contributors to both of those fields for a long time. The aspect of human health that's most closely tied to what we do is sleep, and it is a major issue for human health. The community is becoming increasingly aware of this, not only for acute problems like sleep apnea, but for many, many people and the elderly, it's a big problem. If I were to guess, I would guess that as we learn more and more about the clock and discover more and more molecules, we will start to touch that area in what I hope will be a practically significant way. I have two goals for these lectures. One is that we're able to communicate to this high school audience what we do and how we do it, what we've learned about circadian rhythms and how clocks work. I think the more valuable goal is that these students are able to perceive some of the enthusiasm we have for our work and what it's like being a scientist -- what our personalities are like, how we go about doing what we do, why we enjoy it, and what kind of a life it is. Hello.

4. How much of what we are is inherited? Genetics as a tool to search for disease mechanisms. (05:27)

It's a pleasure and an honor to give these Holiday Lectures. I've spoken in this room for the past 11 years to my peers, and I can assure you that it's a welcome change to speak to a younger and more enthusiastic audience. Before launching into my talk about *Drosophila* genetics and its contribution to circadian rhythms, I want to say a few words about human genetics. So there are two features of genetics that are illustrated here, the first of which, on the left, demonstrated by those twins, is how much of who we are is inherited. So these identical twins, of course, have identical genes. And no one disputes the fact that these genes make a major contribution to their physical appearance, what I like to call "below the neck physiology." But what about aspects of their brain: their personality, their sense of humor, their likes and dislikes? How much is inherited due to their genes as opposed to their environment? That is, how much is due to nature and how much is due to nurture? So these two gentlemen, Mr. Levy and Mr. Newman, have different names because they were separated as infants and raised in two different families in two different parts of the country. And it is possible that the fact that they both chose to be firemen and many other similar aspects of their lives have come together and they met as grown-ups in their 40s is perhaps some indication that our genes not only control how tall we are and whether we have blond hair, but also aspects of our behavior. So the second feature of genetics and the one we're going to concentrate on today, pointed out on the right, is illustrated by a picture of a cell and a chloride channel that is indicated by CFTR, which is encoded by the gene that gives rise to cystic fibrosis, a devastating genetic illness. And so this cell and this channel and this gene illustrates the fact that genetics is also the search for disease mechanism, for therapy, and, more generally, for a basic understanding of biological mechanism. And it's that aspect of genetics that I want to concentrate on and that *Drosophila* has really contributed to.

5. Pioneering the use of the fruit fly *Drosophila* as a genetic tool (08:00)

So the next slide brings us to the world of *Drosophila*, the fruit fly, and its origins. As an experimental organism, *Drosophila* began in the laboratory of Thomas Hunt Morgan, the gentleman on the right, in about 1910. And he was incredibly camera-shy. And so one of his students, Alfred Sturtevant, hooked up a camera in the incubator and with a string managed to trigger the mechanism so he could actually take a picture of his boss. Morgan really began using fruit flies as a genetic tool, and two of his most illustrious students are shown here, Calvin Bridges and Alfred Sturtevant. Bridges actually did the first experiment that showed that genes were actually linked to chromosomes, that they moved together, and Sturtevant, as an undergraduate at Columbia University, produced the first genetic map and showed that genes were linked in a linear fashion on chromosomes. So a remarkable feat by an undergraduate, I'm sure you'll agree. And for this work in Morgan's laboratory, he was awarded the Nobel prize in 1933.

6. Examples of mutants in *Drosophila* (09:16)

So the first mutants that Morgan and his colleagues worked with were spontaneous mutants. They were found naturally in nature. This is the white mutant. And the eyes instead of being their natural red color are white, and they were used as markers in these original crosses. And illustrated here on the right is another *Drosophila* mutant called antennapedia in which instead of an antenna, which would normally be present at this position and is present over here on this side of the fly, there is a leg. Hence the term antennapedia. And this mutant was X-ray-induced and illustrates the fact that Herman Muller, another student of Morgan's, introduced mutagenesis as a tool in this field shortly after, while associated with Morgan, and continued to do that kind of work which increased dramatically the mutation rate and really produced the experimental strategy that exists to this day that we used in the eighties and nineties to study circadian rhythms, and that you'll hear about more in its application to mice from Joe. So this gene, antennapedia, has played a critical role in our understanding of developmental biology, the genes and proteins that are responsible for pattern formation.

7. General insect behavior: Waggle dance of the honeybee (10:41)

So preceding the role of *Drosophila* genetics in behavior is the field of insect behavior more generally. And for example, Von Frisch, a German scientist back in the thirties and forties, studied the behavior of bees, and he found out that bees, when they return from a food source, they undergo a dance called the waggle dance which illustrates to his colleagues, the rest of the bees in the hive, where that food source is. So the angle of the dance, this figure 8 dance that the bee undergoes, the straight run in this dance has an angle from the sun which indicates where the food source is with respect to the position of the sun, and the length of this straight run indicates approximately how far away the food source is. So insects have complex behavior and have been studied for a long time.

8. Similarity of cellular structures and genome between humans and Drosophila (11:40)

But what was unanticipated -- unappreciated, really -- both by classic geneticists and the insect behaviorists, was the extent to which many and perhaps most aspects of basic biology and even basic neurobiology was shared between distantly related organisms. So this is true morphologically. If one looks at the shape and structure of a *Drosophila* neuron shown here that is labeled with a fluorescent dye, that neuron is essentially indistinguishable from a neuron from a mouse or from a human. So a neuron really is a neuron is a neuron. And it is also true at the molecular level, this similarity between organisms. So as Joe mentioned, the genome projects that have been underway for several years, and in particular the *Drosophila* genome project which was completed earlier this year, has reinforced the fact that we are really all fellow travelers on this planet, all life on earth. Genes that are present in humans are present in flies, and many of these are even present in lowly single cell eukaryotes such as yeast. It turns out that the cloning of the *Drosophila* genome shows that at least 60% of human disease genes are also -- have clear *Drosophila* orthologs which means these diseases can be studied in fruit flies and animal models of the disease can be made with which therapies can be investigated. And finally, at the level of neuroscience and behavior, most neuronal signaling molecules and synapse proteins, proteins that actually contribute to how neurons function, are conserved between fruit flies and humans. And it's, of course, this aspect that I'm going to focus on.

9. Behavior in fruit flies: Courtship song (13:39)

So let me tell you a little bit about behavior in fruit flies. This is a picture of a male *Drosophila* who is courting a female, and that female is marked, in fact, with a benign mutation just so it can be recognized in subsequent crosses. Can anyone tell me what that mutation is? White eyes. You already have one, but you get one from my school. So it's almost like "Who's buried in Grant's tomb?" So this female is marked with white. And the male is extending his wing and singing a courtship song by vibrating his wing, which the female can hear and is part of the courtship ritual between males and females. I promised my wife I would practice this, so, dear, I'm doing it. So courtship is one aspect of *Drosophila* behavior that was begun by Seymour Benzer and his colleagues, and this picture comes from my colleague and friend and collaborator, Jeff Hall, at Brandeis who continues to study this particular problem.

10. Behavior in fruit flies: Learning and memory (14:51)

Now on the next slide is another aspect of behavior which also began in Seymour Benzer's lab when Bill Quinn was a postdoc there, and it speaks to the problem of learning and memory. Quinn and Benzer developed teaching machines in which fruit flies could be tested for learning. And they were exposed. They were put into the start tube, and then the separation piece here was raised so that the flies could then migrate in two directions and there would be different odors associated with moving to the left, to "a," or moving to the right, to "b," and one of those odors would be paired with a shock. And the insects would learn to avoid the odorant that had been previously paired with a shock, a form of learning which is a paradigm which is used also in higher organisms. And some of the genes that emerged from the study turn out to be very similar to genes which govern learning and memory in mammals. S

11. Behavior in fruit flies: Circadian rhythm assayed by time of eclosion (16:00)

So the third aspect of behavior that I want to come to, and of course this is now the subject of the rest of my talk, is circadian rhythms. So Ron Konopka, who was a student in Seymour Benzer's lab, decided to isolate single gene mutations which would impact circadian rhythms. And what they used as an assay was eclosion of the fruit fly from the pupal case. So fruit flies go through a complex developmental life cycle, and before they are adult, they're pupae in cocoons, effectively. And they emerge from these cocoons in a temporally-defined way. And they emerge preferentially at dawn, and then the number of flies that emerge subsequently is very low. And then the next day at dawn, a lot of flies emerge and then very few. And this rhythmic emergence constitutes a 24-hour cycle. And what Ron Konopka and Seymour isolated were 3 mutants which disrupted this normal 24-hour periodicity. They isolated a short period mutant in which the time distance between these emergence waves was 19 hours. They isolated a long period variant in which the distance was 29 hours, and they isolated an arrhythmic mutant which didn't have any periodicity at all. The flies emerged more or less willy-nilly with no apparent rhythmicity.

12. *period* gene (*per*) located on the X chromosome (17:31)

So all 3 of these mutants behaved as if they were allelic, that is as if all 3 of them were actually mutants of the same gene, and by that I mean that they all mapped to the same location on the X chromosome. This is an illustration of the banding pattern of salivary gland chromosomes which was described by Bridges, one of Morgan's students. And using genetic crosses, Konopka and Benzer were able to localize all 3 of these variants -- the arrhythmic per zero mutant, the per short mutant, and the per long mutant -- to this position, 3-b-1-2, on the X chromosome, very near our old friend white, which was used as a visible marker in these genetic crosses.

13. Animation: Activity pattern of circadian mutant (18:21)

So I now have an animation to give you a flavor for not only the emergence from the pupal case, but actually the locomotor activities which are also affected. So here is a fruit fly in a tube. And that fruit fly is jumping around, and on the actigram here, very similar to the actigrams that Joe showed, there is a morning peak of activity when the lights go on and there's a much larger evening peak of activity when the lights go off. And this constitutes the daily rhythms of the fruit fly in a light-dark cycle. Now if one assays the flies under constant conditions, constant darkness, very similar to the records that Joe showed for mice, the flies only have a very modest morning activity peak, but in the evening, the time when lights would have gone off, the flies have a very robust activity, and you'll notice that every day the activity record drifts slightly with respect to the day before, so these flies have an intrinsic rhythm of about 24 1/2 hours, every day about a half an hour longer. The per short fly, this variant here which has a 19-hour rhythm, it also has about one bout of activity per day, but notice that every day, that activity bout is about 5 hours earlier so that these -- these flies manifest a 19-hour rhythm and very different from what you see for the wild type. Yet these animals can entrain to the light-dark cycle so that if one assays these mutant flies in a light-dark cycle, now they show the same morning and evening peaks, but notice that the evening peak is advanced by about 3 or 4 hours with respect to the wild type light-dark cycle. So these flies entrain. They show 24-hour rhythms, so the external environment is dominant, but the evening activity peak is advanced.

14. Apparatus for measuring fly activity (20:25)

So if I could have a student volunteer here to give me a hand, I will show you how we actually do these measurements, how the fruit fly activity is measured. So this is more or less the equivalent of the running wheel, this is the insect biologist's running wheel, and each of these tubes is filled with a single fruit fly, and there is some food here and a cap and around -- just hold that so it doesn't wiggle -- and around each tube is a light source and a light receptor, and so every time the fly breaks a beam, breaks this beam, there is an event recorded on the actigram, and when the flies are active, there are lots of events that are recorded, and when they rest, there are very few events that are recorded, and the light beam is in the infrared, and the flies are blind in the infrared, so the light itself doesn't interfere with the experiment. Thanks. But...

15. Activity patterns of per-short flies (21:34)

So... So in summary, then, these actigrams show that the canton "s" fly has 24-hour rhythms in constant darkness, but the per short fly has 19-hour rhythms -- much, much shorter than what one would normally see. So...so it turns out, then, if I can relate this phenomenon to something that Joe talked about, these per short flies are equivalent to humans who have advanced phase sleep syndrome. That is, they have 24-hour rhythms, but their activity is shifted from the normal peak here at lights off by about 5 hours, and so they are really an insect equivalent of a human being whose cycle is shifted, a mutant human being whose cycle is shifted with respect to the rest of us.

16. Do flies actually sleep? (22:32)

And finally, let me point out that flies may actually sleep. That is, the question is, is there a connection between the sleep-wake cycle that you and I undergo and the period of relative inactivity that the flies experience? So in papers published quite recently by two groups, one, my Howard Hughes colleague Amita Sehgal, and also a group led by Paul Shaw at the Salk Institute, they did experiments which suggested that this rest period of flies is really biologically related to sleep in humans. Now, sleep in humans is a phenomenon which has been traditionally defined by our brain waves. So our brain does unusual things when we sleep and even does different things during the different stages of sleep, and this kind of an assay is impossible to bring to bear on a fruit fly, but you can ask the question, do some of the manifestations of sleep actually exist in flies? So, for example, when they're resting, if you poke a fruit fly, normally a fruit fly will respond very, very quickly to such a poke and jump or try to fly away, and when the flies are resting, they become rather insensitive to these kind of stimuli, much the same way that when we're sleeping, we don't respond at least as easily to a whisper or another kind of stimuli or a touch. And similarly, if one sleep deprives these fruit flies or, in this case, rest deprives the fruit flies so when they would normally be resting, if they're agitated so they can't rest, then the next day, instead of being very active here when they would normally be extremely active, the rest-deprived fruit flies show an increased level of rest as if they were trying to catch up on their sleep in the same way that the students sleep during the weekends to try to make up for the sleep deprivation that your 6 a.m. bus has caused during the week. So this suggests that there may be some connections between fruit flies and humans as far as sleep goes, and that indicates that this experimental animal may even be useful for trying to understand the genes and molecules that govern sleep -- very few of which have been identified to date.

17. Q&A: Are there fundamental differences between diurnal and nocturnal animals? (25:03)

And with that, I think I will take a question from the house.

My name is Vladimir Abramson from Poolesville High School, and I have to ask you, have you studied any other animals actually not active at night and active in the daytime just like humans, or have you actually studied only ones that are active at night like fruit flies and mice and those animals? Something about similarities between those

So the question relates to -- I think the question relates to, are there fundamental, if I may rephrase it, are there fundamental differences between the clocks between diurnal and nocturnal animals? And my studies have been restricted to fruit flies, but there is quite a bit known about what does happen to diurnal and nocturnal animals, and it appears that the switch is downstream of the actual clock so that the clocks in a diurnal animal and a nocturnal animal run the same with respect to external time, and there is a switch that's flipped, of course, because the activity pattern is switched but appears to be not in the clock. It appears to be something downstream of that because the clocks are identical with respect to their relationship to external time.

18. Q&A: Are there many natural variations in the fruit fly? (26:31)

So the next question is from Miami. Go ahead, Miami.

Hi. My name is Sam, and I'm in the B magnet at North Miami Beach, and my question was, how many natural variations are there in a fruit fly, and what might be the evolutionary consequences of these variations?

So there are lots of evolutionary variations in fruit flies. There is a whole field of people who go out into the wild and capture natural populations of fruit flies from different areas of the world, and to some extent those fruit flies have adapted to those different climates and environments with different traits. But, I think, with respect to circadian clocks, if I might refocus your question in that direction, there is one investigator that I know of, one colleague of ours named Bambos Kyriacou -- he actually has a much longer first name because he's Greek Cypriot, but I can't pronounce it -- and Bambos argues that, in fact, the clocks are subtly different when you collect flies from temperate zones or from zones in different parts of the world. That has to do with the light-dark cycle that exists in different places and believes that the clocks really are adaptive for the insects. So the next question is from Moscow. [speaks Russian] I learned how to say that at breakfast

My name is Stanislav with Chemical College. I would like to ask, how is biological clock reset when the organisms in the evolutional process are transiting from sexual proliferation to nonsexual reproduction in the biological sense of cell functionality?

A big part of science is being able to say, "I don't know." So that's my answer. I know absolutely nothing about the relationship between biological clocks and transitioning from sexual to asexual means of reproduction.

19. Q&A: Have you tried to change the clock gene? (28:40)

So next we have a question from the house. Yes?

Have you tried to synthetically change the gene that covers the circadian clock like you changed the gene that covers the eyes, that changes the eyes to white?

So you will -- the answer is yes. There's been a lot of work in my lab and in other circadian labs around the world in which altered genes have been transferred to -- genetically transferred to -- hosts, and they alter the properties of the clock, and I'm going to allude to one of these experiments in the next part of the lecture. So the simple answer is yes.

20. Q&A: What are the characteristics of humans with a biological clock mutation? (29:24)

So the next question is from Fox Chase Cancer Center. Go ahead, Fox Chase.

My name is Danny Greenburg, and I'm a senior at Abingdon High School, and I was wondering what are some of the characteristics that human mutants have concerning the biological clock?

So you're going to hear about a potential human mutant from Joe later in these presentations, but I think the expectation -- the expectation is that a human mutant, a human clock mutant, would be somebody who had sleep difficulty, or, at least, that's one of the most pronounced manifestations we could predict. So we would guess that a profound early bird or night owl, a lark or an owl, might be a clock mutant, which would entrain and be strictly on a 24-hour schedule like our mutant fruit flies, but would show phase advances or delays with respect to the rest of the world.

21. Q&A: Are clock mutations inherited? (30:21)

And next, we have another question from Moscow. Go ahead, Moscow.

Hello, my name is Julia. A change of genes in the 24-hour cycle, is it inherited? Are these mutations that take place in the embryonic stages of development?

So... [speaks Russian] How's that? "Excellent question." The guess... I don't see... It's almost certainly that these mutations are inherited from generation to generation, and they arise at some point in the population and then continue to be inherited much like most of the genetics that we -- that we're used to, thinking about genetic diseases and so forth and so on. So the guess would be these don't arise de novo in the embryo but actually have been there for a long time. Of course, they have to have arisen at some point in the population.

22. Q&A: Does temperature affect circadian rhythm in flies? (31:24)

Next, Miami. Go ahead, Miami.

Hello. My name is Nora. I'm a junior at North Miami Senior High. Does the temperature affect the rhythms in flies, and is it different from homeotherms?

Terrific question, and Joe alluded to the fact in his introduction that one of the hallmarks, one of the central tenets, of circadian research is this aspect of temperature compensation or temperature insensitivity. Remarkably, the period of circadian oscillations varies little, if at all, with external temperature so that fruit flies at 15 degrees and fruit flies at 29 degrees show almost no difference in period. So for the biochemically minded among you, the Q 10 is almost exactly 1.0, and we don't understand how this works. It makes sense because you want the clock not to be a thermometer. You want the clock to work at the same pace independent of whether it happens to be cold out, as today, or happens to be warm. But we don't understand how that's regulated, and the field as a whole is very interested in trying to solve that problem.

23. Q&A: Is the clock mutation dominant, semi-dominant, or something else? (32:36)

Next, a question from the house.

If you were to mate a wild type with a mutant, would this biological clock of the children be mixed, or would it choose one or the other, or would half of it be one and half of it be the other?

Also a terrific question. So a priori, you could get either, depending on whether the mutant was fully recessive or semi-dominant or dominant. It turns out, in general, the clock mutants -- at least the ones that we know to date, the dozen or so that exist -- most of them turn out to be semi-dominant so that the phenotype of the progeny children who have an allele from both parents turns out to be intermediate. And there are some interesting interpretations that accompany that fact.

24. Q&A: Is the human period gene located in the X chromosome? (33:27)

So the last question comes from East Lyme. Go ahead, East Lyme.

Hi. My name is Allison Grace, and I'm a junior at East Lyme High School, and I was wondering, that if a human version of the period gene exists, would it be located on the X chromosome, as the fruit fly's period, or is the location variable between organisms?

So the -- you'll hear from Joe some about the human version of the period gene, and it is not located on the X chromosome in humans. It is located on the X chromosome in fruit flies, and the X chromosome, in general, carries a lot of normal genes and plays little, if any, role in sexual determination. So we, for all intents and purposes, we can consider the X chromosome as a general gene-containing chromosome in flies, and it's on another chromosome in humans. So it is time to proceed and continue with the slides.

25. Beginning of the molecular era: Cloning of the period gene (34:27)

And so I will now tell you about the molecular era that began in the early-to-mid eighties when my laboratory, in collaboration with my colleague Jeff Hall, and also Mike Young's laboratory at The Rockefeller University, independently cloned the DNA which encodes the period gene, and the gene was then used in a gene transfer or rescue experiment in which a fruit fly that was arrhythmic, a per zero host fruit fly -- and here's an actigram from this fruit fly, and as I've shown you before, the activity of this animal is arrhythmic. It is an insomniac, which rests a little and is active, and rests a little and is active. And when this piece of DNA is inserted into this host animal, then the following activity pattern results in the progeny organisms, and a very good periodicity, which you can see by eye here, results. And so the animal is rhythmic and then rests, is active and then rests, is active and then rests with very good slightly longer than 24-hour periods. So that really began this molecular era for us. We knew we had cloned the gene and we knew it had biological activity in this kind of a gene transfer experiment.

26. Finding the exact location of mutation in per mutants by using transgenic flies (35:58)

So the next task was to try to identify the positions of the mutant alleles, that is, exactly where in the gene were the per "s," the per long and the per zero mutations. And you can almost imagine how one would go about trying to find these lesions. So first we did a biological experiment to try to mix and match pieces of DNA that came either from the wild type organism or that came from, in this case, for instance, in yellow, the per zero animal. And when we used per zero DNA, of course, we got no rescue because this piece of DNA is nonfunctional, as the genetics would tell us. But when we mixed the left half from per zero and the right half from per plus, we also got no rescue. But if we inserted, for instance, a little piece of per plus DNA in between these two segments of per zero DNA, then we could rescue with characteristic periodicity indicative of the per plus DNA. And similar experiments were done with per short DNA, for example, and these mix-and-match experiments localized the per short lesion to the left half of the gene here, as indicated by the short period of these resulting transgenic flies and also located the per short mutant to about the same region as the per plus -- as the per zero region.

27. per gene organization (37:29)

So sequencing the entire gene and constructing the gene organization that is the so-called exons and introns, the coding organization of the gene, allowed us to identify the start codon where protein synthesis began, the stop codon where protein synthesis ended, and the position of three single nucleotide changes which accounted for the per long, the per zero, and the per short mutations. These were missense mutations which changed the amino acid sequence of the protein and caused the protein to not function normally, but an intact protein was produced, and appealingly, the per zero-1 mutation was a stop codon, which truncated protein synthesis and produced no full-length protein. So this mutant really behaved as a null mutant, and the molecular biology was completely consistent with that, with those phenotypes, that is, that there really was no protein produced.

28. Antibody against per protein (PER) reveals where per gene is expressed (38:34)

So the gene, once you clone a gene, you then -- it then leads to reagents, which allow you to undertake a number of experiments to try to now go back to the organism and ask biological questions. So, for example, once we had cloned the gene, we could use the information to make antibodies so that we could recognize where the protein was expressed in the animal. And when we stained fruit flies with the antibodies against the period gene, we noticed that the gene was really widespread -- was expressed all over the animal, not absolutely everywhere, but in lots and lots of places. So these are the photoreceptor cells of the fruit fly eye, and those nuclei contain a lot of intense per protein staining, and there are cells, both neurons and glia, elsewhere in the brain which express the gene, and the gene is also expressed in a few particular locations that caught our attention for the following reason. Because these cells here are particularly intense locations of per gene expression so that one looks at how much staining one obtains in these cells -- it's much, much higher than one sees everywhere else -- and that clued us that we might really be -- we should pay attention to those particular cells. So, it turned out that those cells also, in studies by Charlotte Helfrich, those cells also contained a peptide, a neuropeptide called pigment dispersing factor, and that peptide localizes also to those intense staining cells. And that peptide had been known to be involved in circadian rhythm, because some animals -- crustaceans and other arthropods -- have natural sunglasses. And across their eyes, when the sun is high, pigment migrates, presumably to improve visual acuity when the sun is high, like you putting on sunglasses. And when the sun is less intense, that pigment migrates backwards. And this hormone, pigment disbursing factor, is responsible for that migration. And pigment dispersing factor activity is actually under the control of the circadian clock. And we might -- in the discussion period, someone might ask a question how do we actually know that?

29. Negative feedback loop as a mechanism for oscillating per RNA levels (41:07)

So, it also turns out that the gene leads to reagents, which can be used for biochemical studies. So Paul Hardin, when he was a postdoc in my lab, discovered that when one looked at per messenger RNA as a function of time, that that RNA underwent circadian oscillations essentially all over the animal. And, so, one was able to observe robust oscillations in the amount of RNA that was present in the animal, and when one looked at the per short mutant fly, which is only different from the wild type fly by the single mutation in the protein, then these oscillations occurred with the 20-hour periodicity consistent with the behavioral oscillations rather than the 24-hour periodicity which characterizes the wild type animal. And this observation led to a formulation of the original negative feedback loop. That is, the way that we think that the clocks operate in a molecular sense. And that is, that the period gene makes period protein, and that period protein migrates back into the nucleus and extinguishes its own expression. And it takes 24 hours for this molecular cycle to take place. And that is the basis -- or at least 10 years ago, when we first stumbled upon this, that is how we conceptualized the molecular oscillator that lies within each cell in the fruit flies. So, could I have the -- Could we have the animation, please?

30. Animation: Negative feedback loop of the per gene (42:46)

So, here's a figure of this. Here's the per gene, here's its promoter, there's a ribosome. And this gene is now active, illustrated by this glow, and the gene is producing messenger RNA, which is being turned into protein, into period protein, by the protein synthesis machinery. Some of those protein molecules are unstable, and they are degraded by the cellular machinery -- the pink ones -- and some of them are stable for reasons which we will come to tomorrow. And the stable proteins accumulate. And this protein build-up continues, the gene is active, RNA is made, protein is produced, and at some point, in the middle of the night, there's enough protein which has been produced, and that protein migrates into the nucleus, and the protein then acts as a repressor to turn off its own gene expression. And in the morning, when the sun comes up, these protein molecules start to turn over. They degrade and disappear over the course of several hours, leading to the turn-on of the gene, which begins the next cycle, the next production of RNA.

31. Measurements of oscillations by using a per-luciferase transgenic fly (44:01)

So if I could go back to the slides. So this is a picture of transgenic flies, which have been loaded with a gene which contains the period promoter plus a coating reagent from firefly luciferase. And this is, in fact, the technique that Joe alluded to earlier that's also been used subsequently for mice. This gene is put into these flies, and when the flies are fed with luciferin, they actually glow because they produce light from this gene. And because gene expression is oscillatory, the glow is actually oscillatory, and one can record the cyclical light production from these flies. Now, it turns out that you can take a leg from these transgenic flies, and you can put the leg in tissue culture, and the leg undergoes oscillations of light production. And if the lights are turned off, that oscillation damps over several days, but if the lights are turned back on, the oscillation repicks back up and in tune with the light-dark cycle that has -- that constitutes this new -- this new period. So, this illustrates several points, which connect to some points that Joe made earlier from mice. First of all, clocks are all over the animal. They're present in many different places. And these clocks are actually connected in flies independently to light because one can set the phase of the oscillation, even of peripheral organs, by exposing them to light. Recognize the difference between the back of the knee experiment that Joe alluded to earlier. So these really are connected to light.

32. Summary of Lecture Two (45:48)

So, in summary, let me say that transcriptional feedback is critical to all clocks. That is, this per oscillator has turned out to be a model which is applicable to the clocks that we know about present in all organisms from humans and mice down to photosynthetic bacteria. That's not to say that the period gene itself is relevant in all of these organisms, but the model of transcriptional feedback as a central element of the pacemaker is relevant to all these organisms. We know that light input is critical and that we need a signal transduction pathway; we need some way to transfer the light information that comes into these cells -- to these organisms -- to the clock machinery to reset the clock. And finally, these are cell-autonomous rhythm centers in flies. That is, these tissues independently keep time -- keep time using these clock proteins, and in the fruit fly, these rhythm centers are independently connected to the external world through light receptors, as evidenced by that experiment by Steve Kay and Jeff Hall, which put those organs in tissue culture.

33. Q&A: How does PER control the fly's behavior? (47:08)

So, that's the end of this lecture, and now I'd be delighted to take a question from the house. Yes.

I was curious as to what effect the circadian protein has on the biology of the fruit fly that allows for it to encode for action and rest periods.

So -- that's a terrific question. And on the whole, we know very little about the answer to that question. We've done one series of experiments which suggests that food and feeding is one aspect of the control. That is, why would you move around at some times of day rather than others? And it appears to be, in part, due to food searching, let's say. The other -- if I were to ask you what the other great biological driving force is, you would probably say sex -- food and sex, and we are trying to demonstrate that the clock actually contributes to mating behavior in some way, that the flies are synchronized to be interested in mating at the same times, but we haven't been able to show that yet.

34. Q&A: What affects biological clocks more: genes or environment? (48:18)

So, next question from Fox Chase. Go ahead, Fox Chase.

I'm Monique McClinton. I go to Abingdon Senior High, and I'm in 12th grade. And my question is: what affects our biological clocks more, genes or the environment?

So, what affects us more, genes or the environment -- I guess was the question. The guess -- the guess is, I would say both, but as far as humans are concerned, if we really had to place a bet down right now, based on what we know -- we know quite a bit about environmental effects on the clock because we know how important the light-dark cycle is, and even feeding schedules -- I should say, in experimental animals -- how important this is for synchronizing us with the environment. And we know from genetic experiments with these mutant animals that genes underlie this process, but we know relatively little about which genes might make us different, one from the other. So, I'd say we know a lot about the environmental influences. We have a good idea about the conceptual machine underneath through genetics but not much about which of those genes might make us different -- one from the other.

35. Q&A: Is circadian rhythm affected by shortening of telomeres? (49:34)

So, the next question is from Miami. Go ahead, Miami.

My name is Omar, and I go to Miami Northwestern Senior High. My question is: if there is a circadian rhythm -- circadian oscillations in other cells -- how are they affected, or are they affected at all, in the shortening of telomeres as a result of mitosis?

I should transfer that experiment to my boss, Dr. Cech. There has been -- who studies shortening of telomeres. There is... I think there is no known connection -- there's no known connection between the circadian clock and the shortening of telomeres, and, more generally, there is very little connection between the circadian clock and the cell cycle, the cellular clocks that impact on telomere shortening. And that's not to say that there isn't a connection, but it's probably pretty vague in most cases because we know that the circadian clocks are always bang-on 24 hours, for instance, and you can lengthen or shorten a cell cycle with changes in temperature very dramatically. So, cell cycles are temperature-sensitive, unlike the circadian clocks. So there isn't much of a connection known at this time.

36. Q&A: What do fruit flies and humans have in common? (50:52)

So, the next question comes from Moscow. Go ahead, Moscow

My name is Ludmilla. I am a student of Lyceum. What is in common between a *Drosophila* and a human being besides the fact that we are affected by the same day and night cycles?

I'll answer that in two ways. I'll assume that that question really has two parts. The question is: what's in common as far as circadian rhythms are concerned, and secondly, what's in common more generally between fruit flies and humans, since that distinction wasn't completely clear to me. So, as far as circadian clocks go, you'll hear tomorrow from both of us about how the more elaborate machine, our more contemporary view of the oscillator at a molecular level, is really very similar between fruit flies and higher mammals. It's not identical, but there's a lot of similarity. So, we think the basic machine is very similar. Probably outputs, what the basic machine controls, is quite different because, physiologically, what we do is quite different. And, as for the more general question outside of circadian rhythms, I can only allude back to the points I made earlier. That is, that *Drosophila* has turned out to be a marvelous experimental organism for studies on learning and memory, studies even on cancer, studies on Alzheimer's disease. All these proteins, all these key proteins, which affect these processes and go awry in humans exist in *Drosophila*, and *Drosophila* is making monumental contributions to the study of these other processes well beyond circadian clocks.

37. Q&A: What kind of protein is PER and what does it do? (52:30)

So, the next question is from East Lyme High School. Go ahead, East Lyme.

Hi. My name is Elizabeth, and I'm a senior at East Lyme High School. My question is: what kind of protein is the per protein? What does it do?

So, that's a terrific question. I'm going to talk a little bit more about this next time, but in short, what we think that the period protein is, is itself a transcriptional repressor and that it actually directly interacts with the transcriptional machinery to turn off its own expression and to also turn off the expression of other genes. So that's the best guess in the field at the moment. That's its actual job as a biochemical entity.

38. Q&A: How does X inactivation affect the per gene on the fly’s X chromosome? (53:13)

So the next question is from the house.

The period gene in fruit flies is on the X chromosome. How does X inactivation factor into the expression of the gene?

So, I hate to be redundant. Another great question. So, indeed, females have two of these genes, and males have only 1 of these genes. And the basic phenomenon of dosage compensation in fruit flies takes care of that imbalance. So, unlike in mammals, which use X chromosome inactivation to re-create the imbalance that nature has given males and females, in fruit flies, the transcription gene expression from the male's single X is twice that from the female's two X so that the output from males and females is compensated by this general system of dosage compensation, which works on the period gene as it works on all other X-linked genes. So that system takes care of that problem.

39. Q&A: Does aging affect the circadian clock? (54:21)

The next question from the house, the last question, which has to be a quick one.

Has there been any evidence of variations in the clocks of fruit flies due to age?

So there's -- it appears at the moment that there is very little effect of aging on fruit flies, on fruit fly clocks, and my understanding is that that situation is actually similar in humans, that it's... it's been tough to define a specific thing that happens as far as aging. And quick question means it has to be quick from you, and the answer has to be quick from me. Thank you all very much.

40. Closing remarks by HHMI Vice President Dr. Peter Bruns (54:59)

Thanks very much for that very fascinating lecture, Michael. We will be back here tomorrow, same time, same place for two more exciting lectures on this. Michael is going to talk about not just the period gene, but now other genes as well, that work in a biological feedback network. And Joe is going to compare *Drosophila* and mammalian systems and give us some more information on recent discoveries in this whole system. So, thank you all very much for coming. And thank you, Joe and Mike, for these wonderful lectures.