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. Now this animation is similar to the one I showed you yesterday except now we have the positive transcription factor CYC and CLOCK, which actually bind to the per promoter at this e-box and drive transcription, turning on RNA synthesis and here is the production of the per protein by the ribosome, the unstable, pink proteins, which are rapidly degraded and then every other protein or so, molecule is stabilized and accumulates in the cytoplasm during the evening. And when sufficient protein has accumulated, then the protein migrates into the nucleus near the end of the night and per protein interacts with the CLOCK and CYCLE protein, probably directly, and that interaction extinguishes transcription and then in the morning the per protein is degraded, it disappears slowly over the course of the morning until all of the protein is gone and as the last protein molecule disappears the CLOCK and CYCLE transcription factors are activated and transcription, and the cycle begins anew. So here we have the period gene and the timeless gene and they are both drive, transcription is driven by CLOCK and CYCLE, which binds to both promoters. So these proteins accumulate and the RNA accumulates and is synthesized and you'll notice that the pink protein disappears, but when the pink protein interacts with the timeless protein then a stable heterodimer is formed. So the distinction between the pink per protein, which gets degraded and the red per protein, which is stabilized is an interaction with timeless. So the heterodimers now move into the nucleus so the form of per that moves into the nucleus is the heterodimer, and it's the heterodimers which interact with CLOCK and CYCLE and extinguish transcription. Now when the sun comes up, notice that that sunlight quickly causes the degradation of the timeless protein and then after timeless is degraded the period protein slowly disappears and that disappearance is then followed by the turn-on of transcription of both genes, which is then followed by the production of RNA and the beginning of the next cycle. And so I have, I think, next an animation, which now illustrates the contribution of both double time and cryptochrome to this cycle, which we're building in a more and more complex fashion. So here's the doubletime kinase, which puts phosphates on the period protein, and here's the cryptochrome, the light harvesting protein. And so now the cycle starts out as it did before but we see that the doubletime kinase, this casein kinase-1ε is actually the agent, which contributes to the degradation of the period protein, which kills the pink proteins, if you will. And if the period protein which is produced gets together with its partner, Timeless, and forms a heterodimer then the heterodimer is resistant to the effects of the doubletime kinase. So that's really the distinction between the protein which was degraded and the protein which is accumulated. Then the heterodimers get together and connect, and here it is the conversion of cryptochrome

from an inactive to an active form and it goes and kills timeless. And then the doubletime kinase goes and kills the per proteins, turns over the proteins and transcription becomes anew. So this has added two elements to the story. First, the doubletime kinase is actually the agent which turns over the period protein or assigns the protein as being a substrate for degradation in the manner that I referred to previously. And second, cryptochrome is actually the light harvesting protein, and cryptochrome, when it's converted from an inactive to an active form by light, cryptochrome actually then leads to that very rapid degradation of the timeless component, which then begins the day anew, leads to the turning over of the period protein and starts the cycle once again. This now gives you a flavor for how the doubletime mutant protein, this mutant kinase which doesn't work very well compares with its wildtype counterpart and the effect of that mutant on the cycle. So here's the doubletime kinase destroying the period protein and notice that it's having trouble functioning. It takes a couple of shots to kill the pink protein as compared to the normal one shot, which the wildtype protein is able to affect, yet the accumulation in the cytoplasm in flies occurs pretty normally. And so these heterodimers accumulate in fairly normal fashion. They migrate into the nucleus they make contact with the positive transcription factors extinguish transcription and now here's the conversion of cryptochrome to the active form. And now watch what happens, timeless disappears very quickly, and now the nuclear form of the kinase starts to degrade per and the mutant form has trouble keeping up. It's working more slowly, this takes longer to go away and as a consequence the turning on of transcription the next day occurs more slowly in the mutant strain, the strain with the mutant doubletime kinase as compared to the wild type strain. So that’s a fairly realistic depiction of how that mutant actually lengthens the protein.