HYPOTHESIS

On the genetic basis of temperature compensation of circadian clocks

VIJAY KUMAR SHARMA

Chronobiology Laboratory, Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, P.O. Box 6436, Jakkur, Bangalore 560 064, India

Circadian (about a day) rhythms recur at various levels of organisation, in organisms ranging from bacteria to humans, and researchers in the field believe that these rhythms are adaptive (Sharma 2003a). Although the adaptive significance of circadian rhythms is yet to be unequivocally demonstrated empirically, their functional importance is well recognized in a variety of organisms, and some circumstantial evidence suggests that circadian clocks enhance survival of organisms living in periodic environments (Sharma 2003b). Circadian rhythms were first demonstrated in 1729, but it was only after 1950 that they caught the attention of several renowned biologists, especially after it was demonstrated that these rhythms are temperature compensated, i.e. their periodicities do not change drastically with increase or decrease of temperature within the physiological range. After the discovery of the period (*per*) gene in *Drosophila melanogaster* (Konopka and Benzer 1971), and the frequency (*frq*) gene in *Neurospora crassa* (Feldman and Hoyle 1973), the molecular mechanisms regulating circadian rhythms began to become clear. The consensus view is that the molecular mechanism underlying circadian rhythms involves two interlocked feedback loops based on transcription-translation controls (Sharma 2003a).

Since the identification of various mutant alleles at the *per* and *frq* loci (short period mutants, per^S or frq^1, frq^2; and long period mutants per^L or frq^3, frq^7), several key components of the circadian molecular loops have been discovered (Dunlap *et al*. 2003). An interesting aspect of these mutants is that unlike the wild type organisms, which display robust temperature compensation, they show partial or complete loss of temperature compensation (Mattern *et al*. 1982; Loros and Feldman 1986; Huang *et al*. 1995). Further, both *per* and *frq* genes include within their coding regions an internally repetitive array of threonine-glycine codons, and in *D. melanogaster* this region is polymorphic in length, both in natural and laboratory populations (Costa *et al*. 1992). These repeat sequences show a direct relationship with the ability of flies to maintain a temperature compensated circadian rhythm at different temperatures (Sawyer *et al*. 1997), and deleting the threonine-glycine repeat region produces flies that have temperature sensitive circadian clocks (Costa *et al*. 1992), which suggests that the ability of temperature compensation involves the clock genes.

Given that most biochemical process are normally temperature dependent, i.e. their rates become faster or slower with increase or decrease of temperature, it is intriguing that circadian rhythms are temperature compensated. In this write-up I will try to put together ideas that are based on experiments on temperature compensation in clock mutants, and attempt to provide a framework for understanding temperature compensation of circadian rhythms, and designing future experiments to study this interesting and important phenomenon.

Unless the biochemical processes that constitute the molecular feedback loops of circadian clocks are themselves temperature compensated, which seems to be quite unlikely, temperature compensation of circadian rhythms can stem from either of the following two mechanisms:

1. Temperature compensation could depend upon the total concentration of proteins translated from two alternative transcripts of a clock gene, generated by different patterns of splicing, each capable of generating circadian rhythmicity independently. At low temperatures, protein coded by one transcript could be present at higher concentration, whereas at high temperatures the protein resulting from the alternative transcript could predominate. However, the absolute level of these alternative two protein products of the clock gene would remain relatively constant across

E-mail: vsharma@jncasr.ac.in; vksharmas@yahoo.com.

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temperatures, giving rise to temperature compensation of the overall mechanism. For example, temperature compensation in *Neurospora* is thought to depend upon the relative concentration of two FRQ proteins, produced by alternative splicing of the *frq* locus. Both these proteins, FRQ1 and FRQ2, can generate circadian rhythmicity independent of each other (Liu *et al*. 1997). At low temperatures, FRQ1 tends to be produced, whereas at high temperatures FRQ2 is produced, but the combined level of both these alternative products of the *frq* gene at any given phase of the circadian cycle remains similar across a range of temperatures. This view is supported by the observation that the temperature compensation ability of the *Neurospora* circadian clocks can be disrupted by introducing mutations that silence any one of the two alternative transcripts (Liu *et al*. 1997).

2. The temperature compensation of circadian clocks can also be thought to arise from the interactions of the constituent clock genes and their transcripts in the two interlocked feedback loops (figure 1). Normally, the mRNAs and the proteins of the clock genes in these feedback loops are in anti-phase. Therefore, it is possible that although the rates of biochemical processes forming the two feedback loops are separately temperature dependent, these effects in the two loops perhaps cancel out to give rise to an output relatively unchanged by temperature and, hence, temperature compensated. According to this model, the outputs from the circadian molecular clock depend upon the fine balance between the protein levels in the two interlocking feedback loops, and any disturbance in this balance, such as those created in the clock mutants, would result in either complete or partial loss of temperature compensation. For example, the mutants in *Drosophila* and the *frq-9* mutant in *Neurospora* lack the robustness of temperature compensation of their wild type counter parts (Loros and Fieldman 1986). This model can be better illustrated using the interlocked loops of *Drosophila* (Cyran *et al*. 2003), which consists of several transcripts of several clock genes and their protein products oscillating in tandem, either in phase or out of phase (figure 1). In *Drosophila*, the mRNA and protein levels of two of the clock genes *period* (*per*) and *timeless* (*tim*) show rhythmic abundance, with the mRNAs peaks during the early part of the night (ZT13-ZT16; ZT is an abbreviation of Zeitgeber Time; 'lights-on' of the light-dark cycles is taken as ZT0 and 'lights-off' as ZT12), whereas the proteins peak during the later part of the night (ZT18-ZT24). PER and TIM proteins enter the nucleus as a complex formed by protein-protein interaction. Although PER and TIM do not have DNA binding domains, they influence their own transcription by physically associating with a positive transcription factor, a heterodimer of the products of two other clock genes d*Clock* (d*Clk*) and *Cycle* (*Cyc*). The dCLK / CYC heterodimer binds to the E-box in the *tim* and *per* promoter region. The gene d*Clk* is rhythmically expressed 180° out of phase compared to *tim* and *per* expression, i.e. d*Clk* mRNA and protein levels peak late in the night (ZT23-ZT4), while *Drosophila cycle* does not cycle, and is constitutively expressed in the cytoplasm. It is also known that the dCLK / CYC heterodimer activates the transcription of the gene *vrille* (*vri*), whose protein inhibits transcription of d*Clk* and other output genes. Recently it has been shown that dCLK / CYC activates the transcription of the gene *Pdp1*, whose protein activates transcription of d*Clk* and other output genes. In this scheme of events, the molecular clock depends upon the dCLK / CYC heterodimer, which in turn depends upon the expression of four clock genes *per*, *tim*, *vri* and *Pdp1* (Cyran *et al*. 2003). It is perhaps the relative levels of the transcripts and

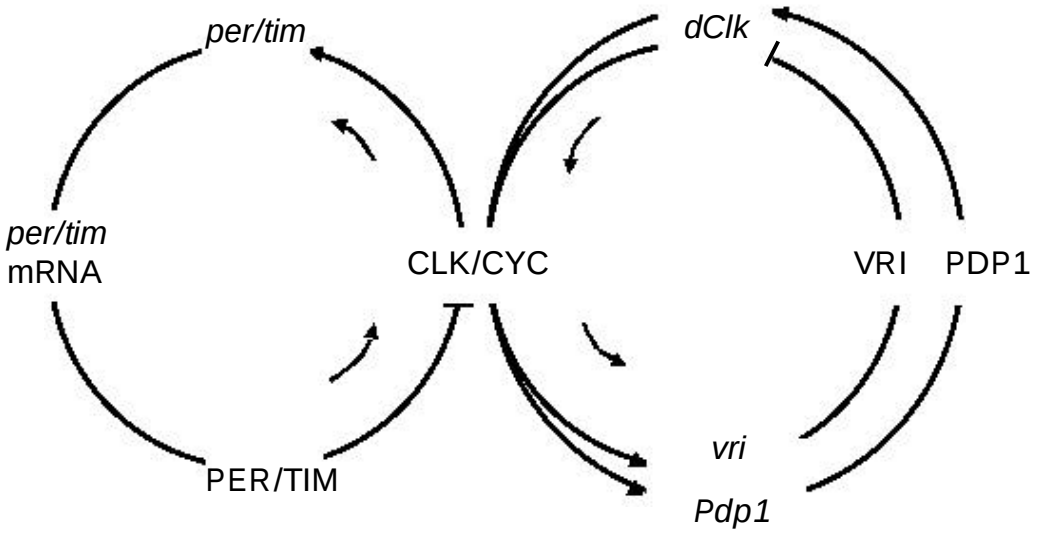

Figure 1. Backbone of the interlocked feedback loop of *Drosophila melanogaster* (modified after Cyran *et al*. 2003).

proteins of these genes that interact in a way so as to result in a temperature compensated output of dCLK/CYC heterodimer, although the expression of the genes themselves is temperature dependent.

Perhaps an extensive study of clock gene expression using mRNA and proteins from both the positive and the negative components of the interlocked feedback loops, carried out at different temperatures, might help us understand the regulation of temperature compensation of circadian timing mechanisms.

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