Functional analysis of the clock genes in the bristletail, *Thermobia domestica*

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The clock gene homologues of *Clock* (*Td'Clk*), *cycle* (*Td'cyc*) and *timeless* (*Td'tim*) were cloned from an apterygote insect, *Thermobia domestica*. Structural analysis showed that *Td'*CLK includes bHLH, PAS-A, PAS-B domains but lacks a polyglutamine repeat in the C terminal region that is implicated for transcriptional activity in *Drosophila* CLK. *Td'*CYC contains a BCTR domain in its C terminal in addition to the common domains found in *Drosophila* CYC, i.e. bHLH, PAS-A, PAS-B domains. *Td'*TIM include four defined functional domains PER-1, PER-2, NLS and CLD like *Drosophila* TIM. Unlike in *Drosophila, Td'Clk* mRNA levels showed no significant daily fluctuation, while *Td'cyc* and *Td'tim* exhibited rhythmic expression. A single injection of double-stranded RNA (dsRNA) of *Td'Clk*, *Td'cyc*, or *Td'tim* into the abdomen of adult firebrats effectively knocked down respective mRNA levels and abolished the rhythmic expression of *Td'cyc* and *Td'tim*. Most those dsRNA-injected firebrats lost their circadian locomotor rhythm in constant darkness up to 30 days after injection, whereas those injected with *DsRed2* dsRNA as a negative control clearly maintained it. From these results, it is likely that *Td'Clk*, *Td'cyc* and *Td'tim* are involved in the circadian clock machinery in the firebrat.