Molecular mechanism of temperature sensing by the circadian clock of Neurospora crassa

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Supplementary Materials

Primers for mutagenseis of *frq* I-6.

D4 Opt: CTCGAAGGTGAGTAGACTAGTAGGCTTCTG;

A3 Opt: ACTTGAAACTAACCGGCTAGCGCGAGATACTAGCAGTAGGGTTACTTC and

D4 Mut: CTCGAACACTAGTAGACTAGTAGGCTTCTG;

A3 Mut: ACTTGAAAACCATCGGCTAGCGCGAGATACTTCCTCCCGTGTTACTTC

Construction of frqΔUTR. A *frq*-specific PCR fragment was amplified with the forward and reverse primers shown below and inserted into the MluI and SphI sites of the Cla I fragment carrying the *frq* gene.

Fwd: TGTGCATCTGTGGGAAAGTG

Rev: GGGCGCATGCCCTGGGATTTATCCCCACTATCCGCCATGTTCGGCGCGCCCAATGCCGTTTTTGCAATGG

The resulting 55 bp 5'UTR was then extended in a second step by inserting into the AscI and SphI site a short segment of the 5'UTR. In the resulting construct residues 50 to 1477 of the 5´-UTR are deleted. The following primers were used to amplify the 5´-UTR fragment:

Fwd2: GGCGCGCCGAGTAGCATCGCAGGCTTC

Rev2: GTGACCACGGCTGTCAAAGG

Mutagenesis of uORFs. The following oligonucleotides were used:

uORF1: CTCCATTGCAAAAACGGCCGTACGTGAAATTATTTCGATTACC

uORF6-mut: ACGCACACAGCCCGATCGTGAAAAGTCCCCACAA

uORF6-opt: ACGCACACAGCCCGACCATGGAAAGTCCCCACAA

RNA Analysis. To determine the I-6 spliced and unspliced portion of frq mRNA the following specific primers and probe were used:

I-6 F: 5'-TTCCATAGTCTCAGGCTTCGA-3'

I-6 splice junction specific R: 5'-GTGCGGAAGATGAAGTAACTGTT-3'

I-6 intron specific R: 5'-ACCCTATCAGATCTTCGTCAGAA-3'

I-6 Probe: FAM-AGCCGTATCATGAAAAGTCCCCACAA-TAMRA

Construction of *frq*-dmpi8.

frq-dmpi8 was amplified from *Drosophila* genomic DNA using the following primer:

Dmpi8 F: CCCCCCTTAAGACGAGTGAGCAATTGCCT

Dmpi8 R: CCCCCCTTAAGAGAACTTGAAGGGAATGGAA

The resulting PCR fragment was inserted into the AflII site of the *frq* gene (ClaI fragment).

Supplementary Figure Legends

Supplementary Figure 1. Conidiation of the strains I-6^{opt} and I-6^{mut}. Race tubes were inoculated in LL and incubated at 25°C in DD. After 24 h they were transferred to the indicated temperatures.

Supplementary Figure 2. The strains *frq*ΔUTR (A) and *frq*1/6mut (B) were grown at the indicated temperatures in LL. RNA was prepared and quantitfied by RT-PCR.

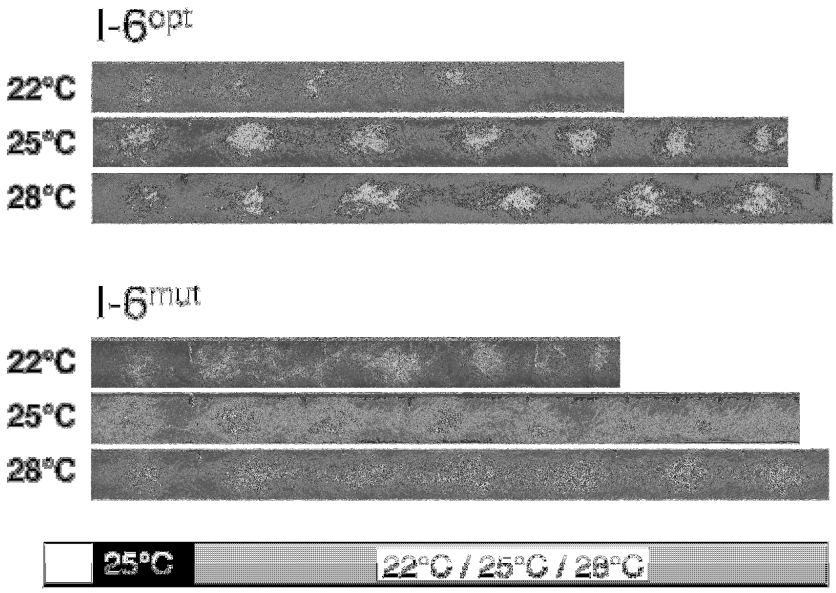
Supplementary Table 1. *Splice sites in frq*

Site	Pos.	Donor	Lariat	Dist. (nt)	Acceptor
					atAG-g
					ctAG-c
					ttAG-a
					gcAG-a
					gtAG-g
					cccgt
					caAG-t

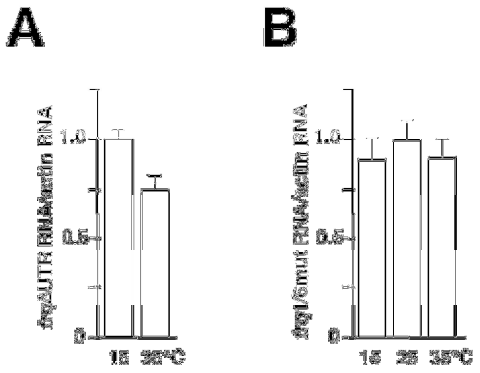
Supplementary Table 2. *Translation initiation sites in frq*

Site	Pos.	Sequence	Length of ORF(aa)
			19
			7
			27
			36
			27
			23
			11
			989
			64
			890

ion sites in the 5´-UTR
otides. The Neurospora
is indicated. 'T'= not T.



Diernfellner Supplementary Fig. 1



Diernfellner Supplementary Fig. 2