Supplementary Text

Supplementary Figure Legends

Figure S1: Model parameters used in characterizing PER degradation in this study. (A) Onset of PER degradation, t_0, is defined as the time at which PER just starts to decrease after reaching maximum level. t_0 is monitored to see if, for example, onset is earliest for DBT^S expressing cells and delayed for DBT^L expressing cells (bottom panel). (B) PER half-life is defined as the time it takes for the protein level to drop to half of its peak value. The term "initial" value (at time t_0) is used interchangeably with "maximum" or peak value. PER half-life is measured in each cell to determine if it is altered by the presence of different DBT alleles (bottom panel). (C) The ratio of initial PER level (at time t_0) to post-degradation or final PER level is also calculated for each degradation profile

Figure S2: Total PER levels in cellular compartments. Total PER levels of the cell depicted in Figure 1A, B-D (blue line). In the cytoplasm, PER is much higher, >4 fold, than in the nucleus. In contrast to the total fluorescence values that are shown here, Figure 1 B-D show fluorescence levels per unit area computed by dividing the total fluorescence by the respective compartment areas.

Figure S3: Without exogenous DBT, PER is more stable. Compared to Figure 1, where PER levels drop several fold when coexpressed with DBT, when expressed alone in S2 cells PER remains relatively high for several hours. In this example, expressions of both PER and the control CFP were induced with the heat-shock promoter.

Figure S4: Nuclear entry of PER, TIM, DBT in S2 cells. (A) When expressed together, PER/TIM nuclear entry is mostly unaffected by the presence of overexpressed DBT with most of the cells showing nuclear translocation between 2 and 7 hours post induction. Nuclear entry of PER/TIM/DBT does not have a strong dependence on the respective protein levels. When expressing only PER and TIM and no exogenous DBT (as in Ref [2]), the proteins are seen entering the nucleus roughly within the same time window (data not shown). (B) Same data as in (A), but now timing of TIM and DBT nuclear entries are compared against PER nuclear entry. TIM and DBT sometimes enter the nucleus simultaneously with PER (data on the dashed line). However, most cells show that DBT enters before PER (red symbols below dashed line), presumably after completing phosphorylation of the subpopulation of PER unbound to TIM.

Figure S5: Onset of PER degradation is independent of PER abundance. The timing of PER turnover in cells expressing enzymatically-compromised forms of DBT (DBT^{ar} or DBT^{K38R}) or no exogenous DBT is independent of substrate abundance.

Figure S6: Mutations in DBT do not alter interactions with PER. (A, B) Co-immunoprecipitation of DBT variants with PER suggest equivalent binding strength between the two proteins. Differences in PER signal are roughly indicative of the activity of the corresponding kinase.

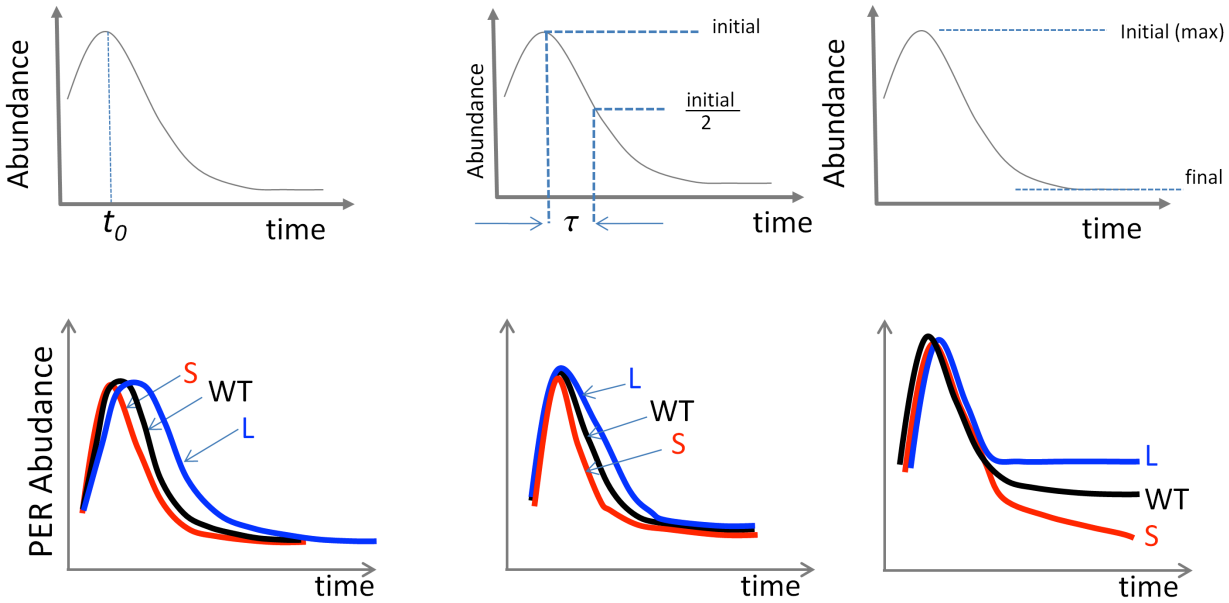
Table S1: Table summarizing the data used in constructing Figure 5. The cell results are from this study and the fly data are weighted-averages (see Experimental Procedures) from Refs. [1-3].

References

1. Rothenfluh A, Abodeely M, Young MW (2000) Short-period mutations of per affect a double-time-dependent step in the Drosophila circadian clock. Current Biology 10: 1399-402.

2. Suri V, Hall JC, Rosbash M (2000) Two novel doubletime mutants alter circadian properties and eliminate the delay between RNA and protein in Drosophila. Journal of Neuroscience 20: 7547-7555.

3. Muskus MJ, Preuss F, Fan J-Y, Bjes ES, Price JL (2007) Drosophila DBT lacking protein kinase activity produces long-period and arrhythmic circadian behavioral and molecular rhythms. Molecular and cellular biology 27: 8049-64.



Difference in Degradation Onsets

Difference in Degradation Rates

Difference in Amounts of Degradation

Figure S1

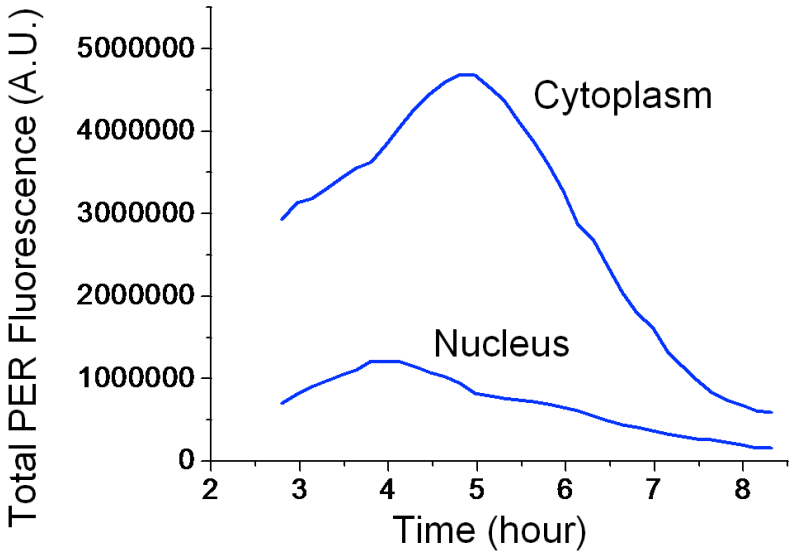

Total PER Fluorescence (A.U.)
5000000
4000000
3000000
2000000
1000000
0
2
3
4
5
6
7
8
Time (hour)
Cytoplasm
Nucleus

Figure S2

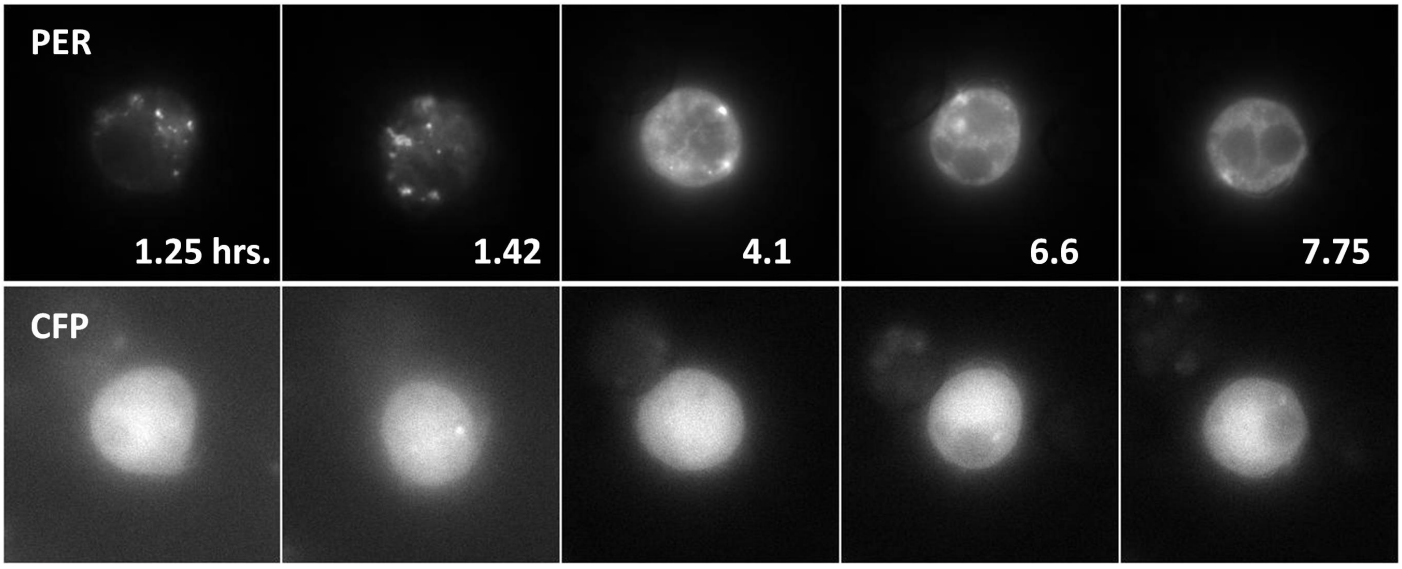


Figure S3

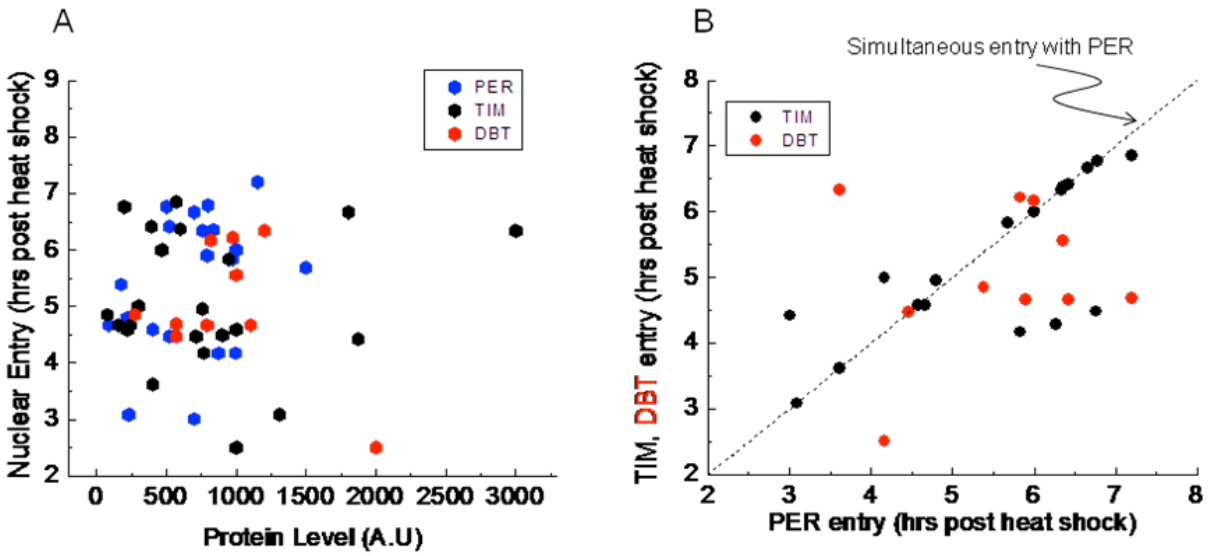

A
Nuclear Entry (hrs post heat shock)
Protein Level (A.U)
PER
TIM
DBT
B
Simultaneous entry with PER
TIM, DBT entry (hrs post heat shock)
PER entry (hrs post heat shock)
TIM
DBT

Figure S4

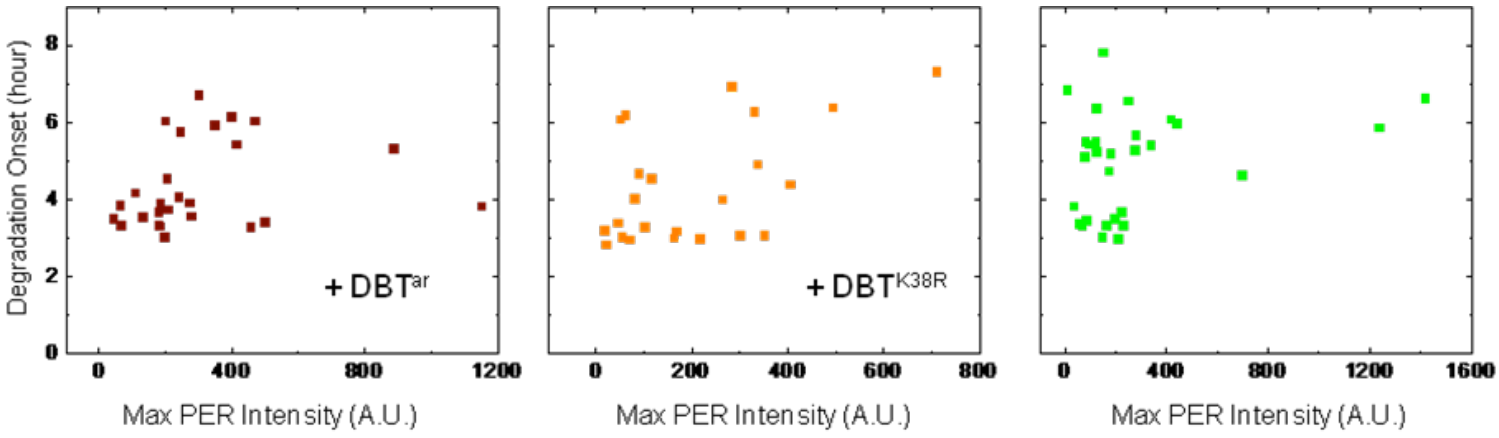

Degradation Onset (hour)
0
2
4
6
8
0
400
800
1200
Max PER Intensity (A.U.)
+ DBTar
0
200
400
600
800
Max PER Intensity (A.U.)
+ DBTK38R
0
400
800
1200
1600
Max PER Intensity (A.U.)

Figure S5

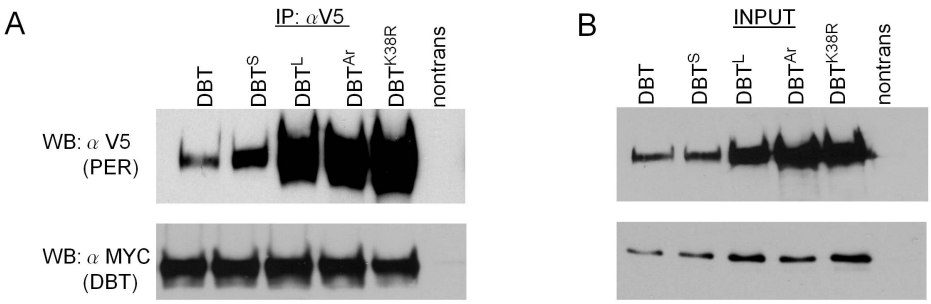

A
IP: αV5
DBT
DBT^S
DBT^L
DBT^Ar
DBT^K38R
nontrans
WB: α V5
(PER)
WB: α MYC
(DBT)
B
INPUT
DBT
DBT^S
DBT^L
DBT^Ar
DBT^K38R
nontrans

Figure S6

Table S1

REF	Genotype	Period (hr)	Standard Error (hr)	Rhythmic (x100%)	PER Half-life (hr)	Standard Error (hr)
18	*dbt*	24.2	0.2	0.36		
		24.1	0.1	0.94		
		24.7	0.2	0.77		
		24.8	0.5	0.31		
		24.9	0.2	0.38		
		24.9	0.2	0.49		
		24.3	0.2	0.94		
3	+/+	23.4	0.2	1		
4	+/+	24.1	0.08	0.93		
WT		**24.2**	**0.2**		**1.2**	**0.1**
18	dbt^{S}/+	18.4	0.1	0.74		
		18.4	0.09	0.47		
		18.2	0.07	0.5		
		18	0.1	0.65		
DBT-S		**18.2**	**0.09**		**1.24**	**0.1**
18	dbt^{L}/+	27.3	0.1	0.49		
		27	0.08	0.81		
		27.2	0.2	0.33		
		26.8	0.5	0.59		
		27.3	1.1	0.13		
		27.9	0.3	0.44		
DBT-L		**27.2**	**0.4**		**2.05**	**0.12**
3	dbt^{ar}/+	28.5	0.1	0.93		
		39.1	1.1	0.32		
		41.5		0.06		
DBT-Ar		**31.7**	**0.6**		**5.35**	**0.22**
18	dbt^{K38R}/+	28	0.2	0.69		
		33.2	0.8	0.38		
		33	1.9	0.16		
		34.9	1	0.09		
DBT-K38R		**33.4**	**1.2**		**6.5**	**0.7**