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Michael Brunner

Molecular Clocks

Goal

Circadian clocks are timekeeping devices that measure time on a molecular level and coordinate the temporal organization of global gene expression. The endogenous cell-autonomous pacemakers are synchronized via various signal transduction pathways with the exogenous geophysical 24 h day/night cycle. The molecular mechanisms underlying these phenomena are in the focus of our research.

Background

Circadian clocks are cell-autonomous oscillatory systems that modulate rhythmic expression of a large number of genes. In eukaryotes these clocks are based on networks of interconnected transcriptional, translational and posttranslational feedback loops. Circadian clocks are synchronized with the exogenous day by environmental cues such as light and temperature. In the absence of entraining cues clock-specific oscillations persist with an intriguingly precise period that corresponds to an endogenous robust self-sustained subjective day-night rhythm of approximately 24 h.

Research Highlights

Dawn- and dusk-phased circadian transcription rhythms in *Neurospora crassa* coordinate anabolic and catabolic functions

The transcription factor White Collar Complex (WCC) is at the core of the circadian clock of the filamentous fungus *Neurospora crassa*. WCC activates directly and indirectly activates transcription of clock-controlled genes (*ccgs*). Amongst the genes directly controlled by the WCC is the clock gene *frequency* (*frq*). FRQ is a circadian co-repressor that inhibits its own synthesis in a negative feedback loop by regulating the activity and abundance of the WCC in rhythmic fashion. Several hundred genes were found to be under the direct control of the WCC. Amongst these rhythmic genes are about 30 genes encoding transcription regulators (e.g. the transcriptional repressor CSP1), which themselves control the expression of subsets of genes. This way, the *Neurospora* circadian clock modulates transcription of ~10% of the genome, which in turn results in oscillations of physiology and metabolism.

The vast majority of these transcript rhythms are generated by dawn and dusk specific transcription. Analysis of the clock-controlled transcriptome of *Neurospora crassa* together with temporal profiles of elongating RNA polymerase II indicates that transcription contributes to the rhythmic

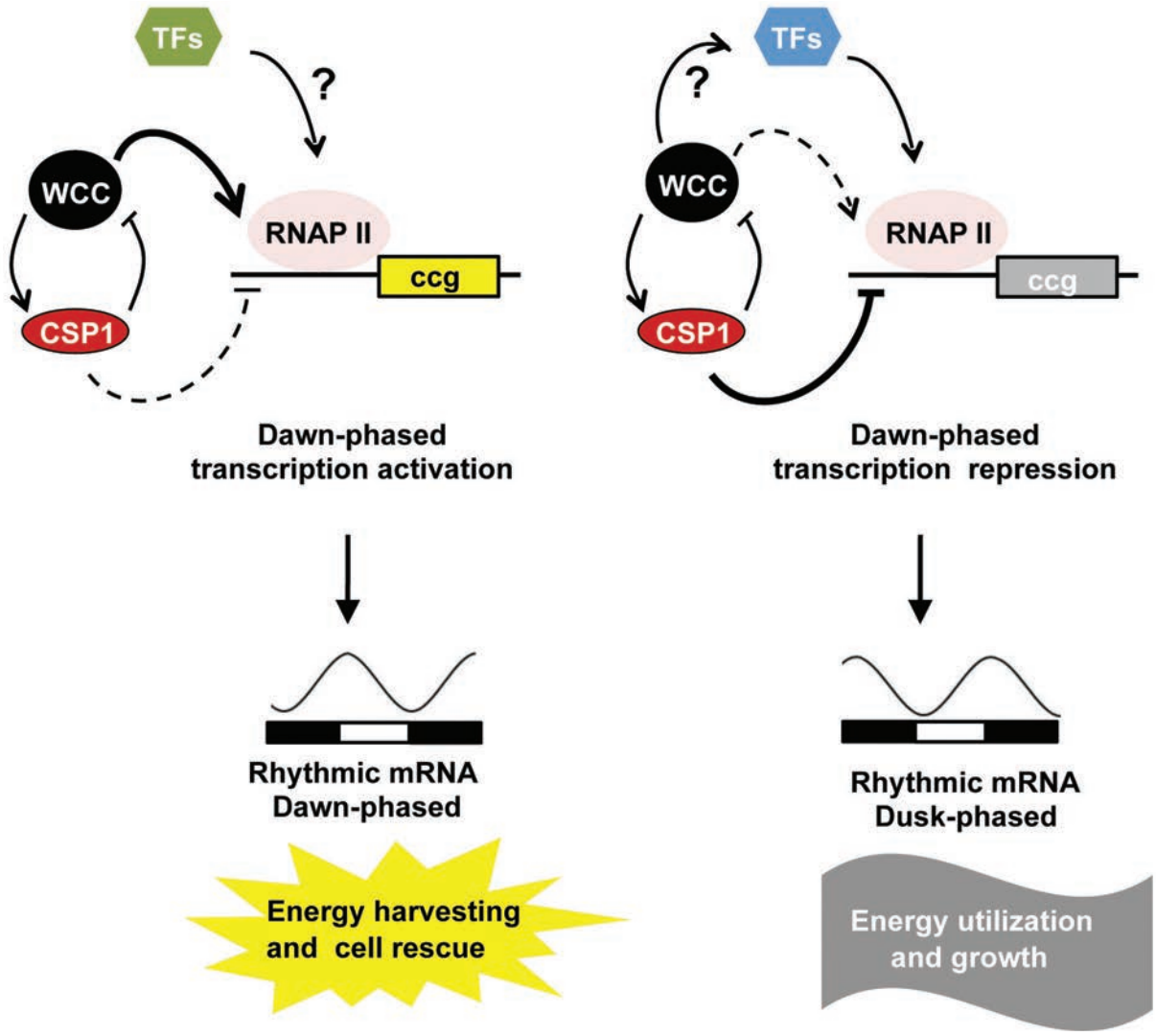

Fig. 1: Model of dawn- and dusk-phased transcription regulation. *The WCC, the core transcription factor of Genes activated by the WCC are transcribed predominantly during subjective late night to early morning where the activity of WCC is high. The activity of other transcription factors (TFs) including CSP1 appear to modulate phase and amplitude of dawn-phased genes. Dawn-specific genes are mainly involved in energy harvesting and cell rescue. The phases of genes repressed by CSP1 are clustered around dusk. Yet, unidentified TFs must be involved in rhythmic activation of these genes since phase and amplitude, but not the rhythmic expression of tested genes, were affected in Δcsp1. Our data suggest that expression of such TFs could be under the control of WCC. Dusk-phased genes are involved in energy utilization and growth, indicating a temporal distinction of cellular pathways and functions coordinated by the circadian clock of* Neurospora.

expression of the vast majority of ccgs. The ccgs accumulate in two main clusters with peak transcription and expression levels either at dawn or dusk. Dawn-phased genes are predominantly involved in catabolic and dusk-phased genes in anabolic processes, indicating a clock-controlled temporal separation of the physiology of *Neurospora*. Genes whose expression is strongly dependent on the core circadian activator WCC fall mainly into the dawn-phased cluster while rhythmic genes regulated by the glucose-dependent repressor CSP1 fall predominantly into the dusk-phased cluster. Surprisingly, the number of rhythmic transcripts increases about twofold in the absence of CSP1, indicating that rhythmic expression of many genes is attenuated by the activity of CSP1.

Transcription induced inactivation of promoters/transcriptional memory

mRNA transcripts are often present at only a few copies per cell and many genes are transcribed in bursts, with brief periods of high activity interspersed by long periods of inactivity. Burst size, i.e. the number of transcripts per burst, and burst frequency, i.e. the number of transcriptional bursts per time unit, are gene-specific and appear to depend on the promoter architecture.

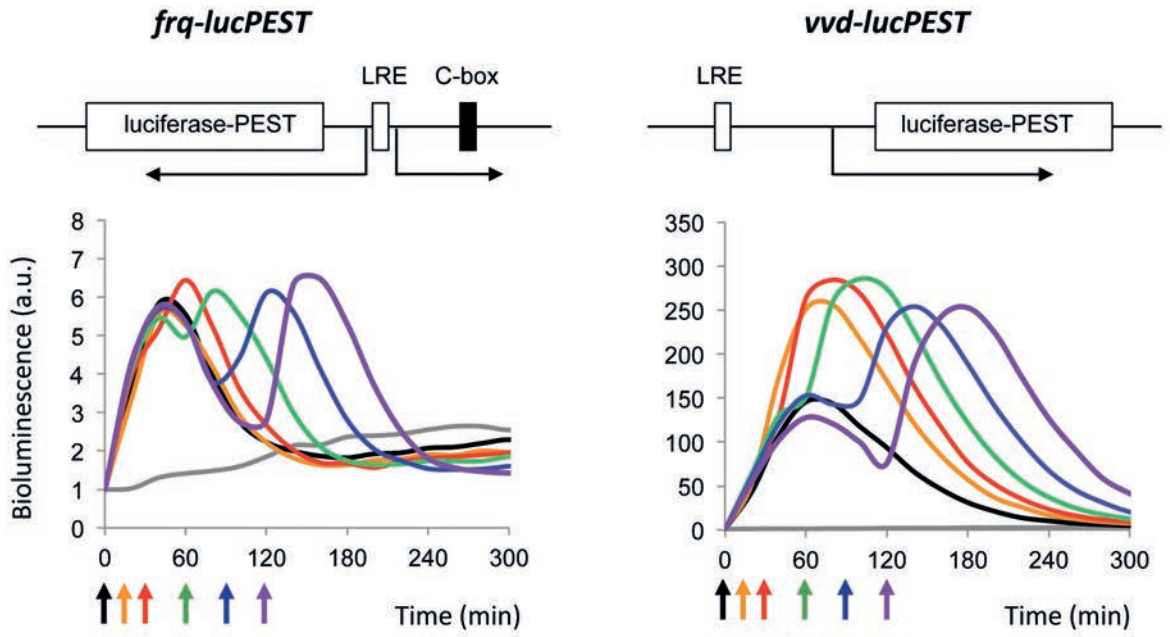

Fig. 2: The frq promoter is refractory towards restimulation. *Activation frq and vvd promoters by a single LP at t=0 min (black arrow, black curve) or restimulation by a 2nd challenging LP after 15, 30, 60, 90, and 120 min (colored arrows and curves). The data indicate that frq is refractory towards restimulation for ca. 45 min, while the vvd promoter is not under the same conditions.*

We use the natural light-inducible gene expression system based on the transcription activator and blue-light photoreceptor White Collar Complex (WCC) of *Neurospora*. The system allows repetitive stimulation of transcription within a short period of time. Activation of WCC by a single short light pulse (LP) triggers a synchronized wave of transcription at a large number of promoters. We have observed burst sizes between one and more than 50 transcripts per burst followed by periods of inactivity in the range of hours.

Challenging the *frequency* (*frq*) promoter with consecutive light pulses revealed that the promoter supports transcription of ~1 mRNA molecule and then becomes refractory towards further activation for about 45 min. This negative transcriptional memory is dependent on slow chromatin remodelling of the core promoter.

Coordination of the human circadian clock and the cell cycle

The circadian clock and the cell cycle are major cellular systems that organize global physiology in temporal fashion. The circadian

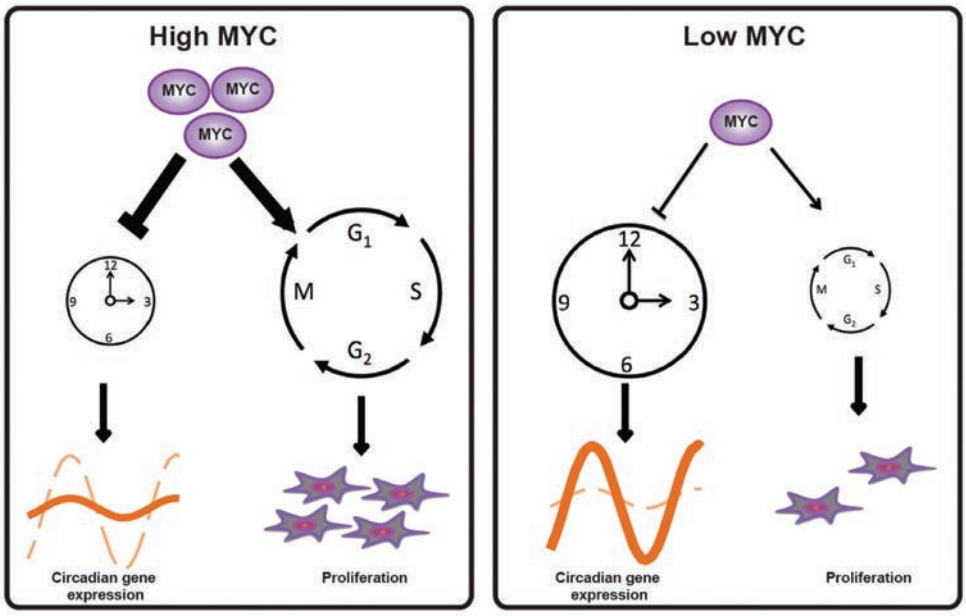

Fig. 3: Model of the co-ordinating function of MYC.
Left panel: high levels of MYC suppress the circadian clock by MIZ1-dependent down-regulation of BMAL1/CLOCK (see text), which results in low amplitude expression rhythms of clock-controlled genes. On the other hand, high MYC levels support cell growth and proliferation (for example, by inhibition of the cyclin dependent kinase inhibitor genes p15 *and* p21*). Right panel: at low levels of MYC, the circadian clock is not inhibited and supports high amplitude expression rhythms of clock-controlled genes. Low levels of MYC do not support cell growth and proliferation.*

clock of mammals is constituted by the core transcription factor BMAL1/CLOCK, which rhythmically activates expression of clock genes including *CRYs*, *PERs*, *REV-ERBs*, and *RORs*. CRYs and PERs are inhibitors of CLOCK/BMAL whereas REV-ERBs are repressors that control in coordination with ROR activators expression of *BMAL1* and *CLOCK*. The D-box-specific transcription factors E4BP, DBP, TEF and, HLF additionally contribute to the regulation of specific clock genes.

Disruption or misalignment of circadian rhythms in humans has been associated with numerous pathological conditions including cancer. *MYC* is an oncogene, which is severely deregulated in different cancers and, amplification of MYC often correlates with tumor aggression and poor prognosis. MYC is a transcription factor that supports cell growth and proliferation (cell cycle progression) by regulating transcription of up to 15% of the transcriptome.

We found that MYC is a key regulator that coordinates the circadian clock with cell growth. Overexpression of *MYC* attenuates the clock and conversely promotes cell proliferation while downregulation of *MYC* strengthens the clock and reduces proliferation. Inhibition of the circadian clock is crucially dependent on the formation of repressive complexes of MYC with MIZ1 and subsequent downregulation of the core clock genes *BMAL1* (*ARNTL*), *CLOCK* and *NPAS2*. In addition, MYC has the potential to activate expression of the circadian repressor REV-ERBα, which down-regulates *BMAL1*. *BMAL1* expression levels correlate inversely with *MYC* levels in 102 human lymphomas.

Selected Publications 2014 - 2016

Shostak A, Diernfellner A, Brunner M (2016) MYC inhibits the clock and supports proliferation. Cell Cycle. 2016 Sep 7:1-2.

Shostak A, Ruppert B, Ha N, Bruns P, Toprak UH; ICGC MMML-Seq Project, Eils R, Schlesner M, Diernfellner A, Brunner M (2016) MYC/MIZ1-dependent gene repression inversely coordinates the circadian clock with cell cycle and proliferation. Nat Commun. 2016 Jun 24;7:11807. doi: 10.1038/ncomms11807

Zhai Z, Kondo S, Ha N, Boquete JP, Brunner M, Ueda R, Lemaitre B (2015) Accumulation of differentiating intestinal stem cell progenies drives tumorigenesis. Nat Commun Dec 22;6:10219. doi: 10.1038/ncomms 10219.

Sancar C, Ha N, Yilmaz R, Tesorero R, Fisher T, Brunner M, Sancar G (2015) Combinatorial control of light induced chromatin remodeling and gene activation in Neurospora. PLoS Genet. 11(3):e1005105. doi: 10.1371/journal.pgen.1005105

Cesbron F, Oehler M, Ha N, Sancar G, Brunner M (2015) Transcriptional refractoriness is dependent on core promoter architecture. Nat Commun. 6:6753. doi: 10.1038/ncomms7753

Sancar C, Sancar G, Ha N, Cesbron F, Brunner M (2015) Dawn- and dusk-phased circadian transcription rhythms coordinate anabolic and catabolic functions in Neurospora. BMC Biol. Feb 24;13(1):17

Hoffmann J, Symul L, Shostak A, Fischer T, Naef F, Brunner M (2014) Non-circadian expression masking clock-driven weak transcription rhythms in U2OS cells. PLoS One 9: e102238

Lauinger L, Diernfellner A, Falk S, Brunner M (2014) The RNA helicase FRH is an ATP-dependent regulator of CK1a in the circadian clock of Neurospora crassa. Nat Commun 5: 3598

Lee CT, Malzahn E, Brunner M, Mayer MP (2014) Light-induced differences in conformational dynamics of the circadian clock regulator VIVID. J Mol Biol 426: 601-610

Ruger-Herreros C, Gil-Sanchez Mdel M, Sancar G, Brunner M, Corrochano LM (2014) Alteration of light-dependent gene regulation by the absence of the RCO-1/RCM-1 repressor complex in the fungus Neurospora crassa. PLoS One 9: e95069

Sancar G, Brunner M (2014) Circadian clocks and energy metabolism. Cell Mol Life Sci 71: 2667-2680

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