Current Biology

Supplemental Information

Metabolic Cycles in Yeast

Share Features Conserved among Circadian Rhythms

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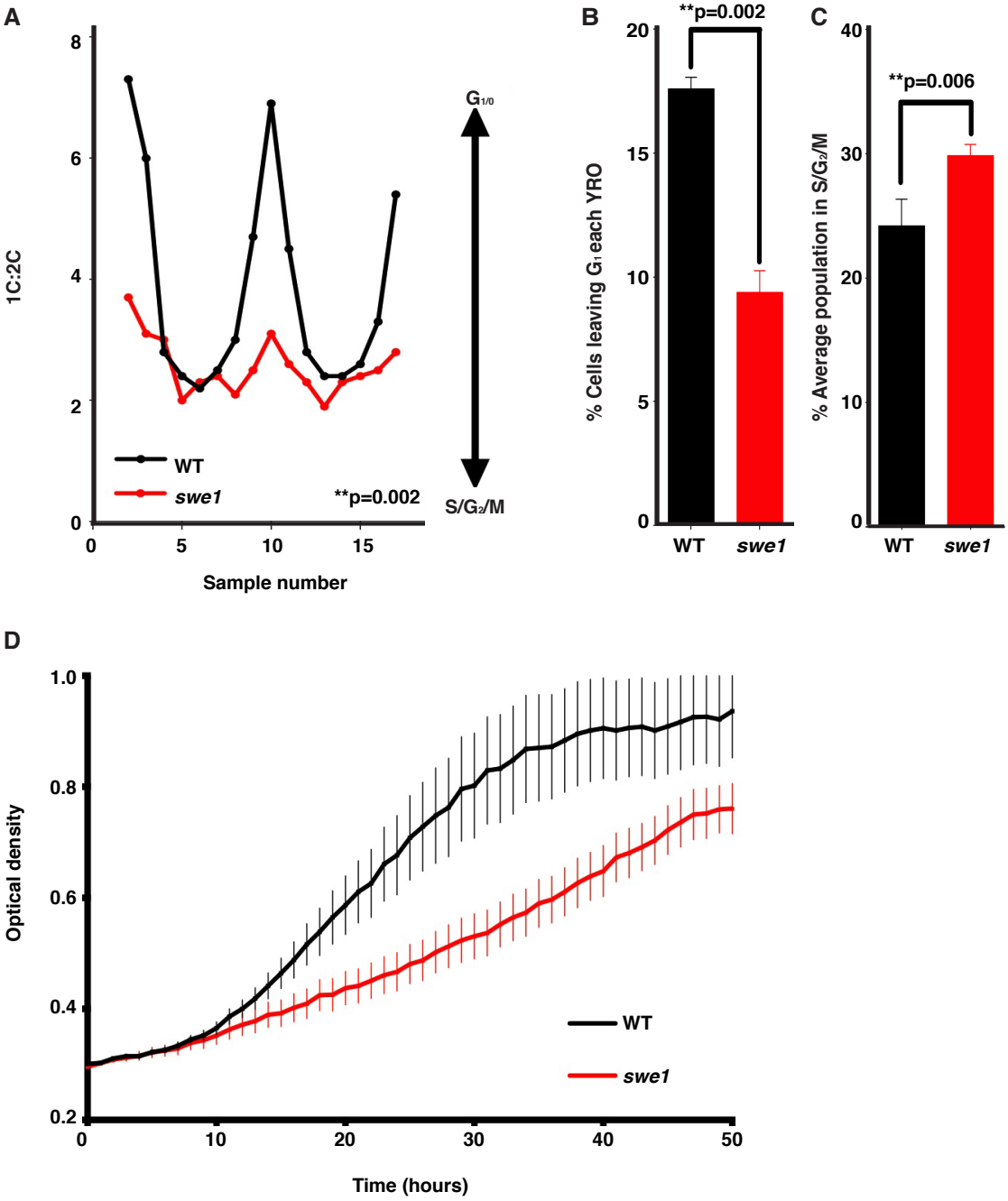


Figure S1, relating to Figure 1. Strains deleted for *SWE1* show less variation in DNA content across the YRO, leave G1 less frequently and grow slower than wild type under YRO/bioreactor and standard growth conditions (**A**) Samples harvested across two oscillations were analysed for DNA content by propidium iodide staining and FACs analysis. Paired t-test, p=0.002. (**B**) Bar graph showing fewer *swe1* cells leave G1 per respiratory oscillation when growing in the bioreactor. Paired t-test, p=0.002. (**C**) Bar graph showing that, on average, more cells are in the S/G2 or M phases of the cell cycle in *swe1* strains than in wild type under YRO/bioreactor conditions. Paired t-test, p=0.006. (**D**) Growth curves for wild type and *swe1* strains under standard growth conditions. Mean±SEM, n=4; 2-way ANOVA, p<0.0001 for time vs. genotype interaction.

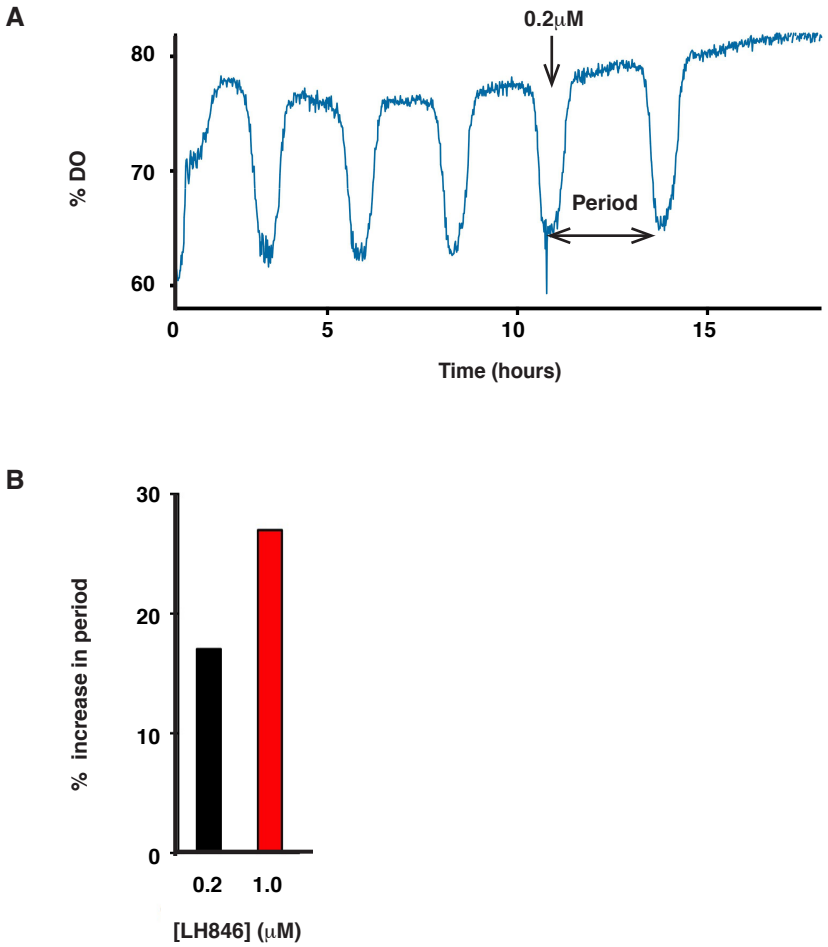
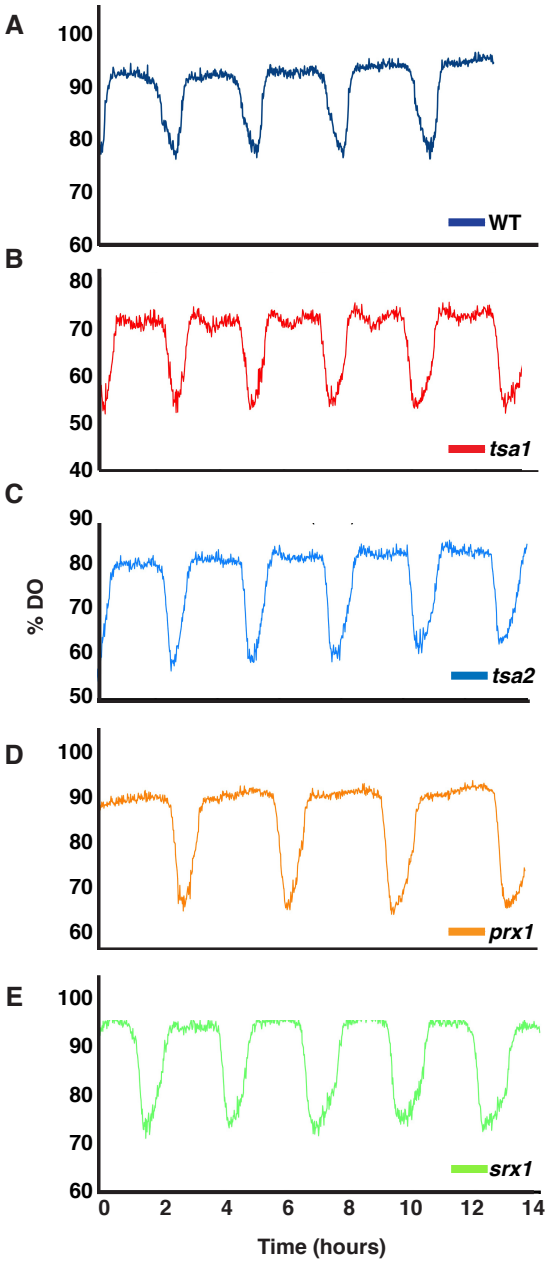
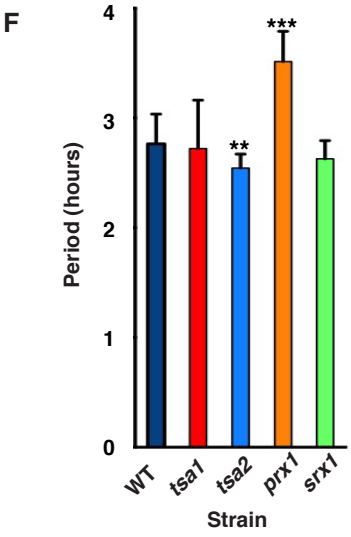


Figure S2, relating to Figure 2. Casein kinase inhibitor LH846 has a dose-dependent effect on the period of the YRO. (**A**) Representative dissolved oxygen trace indicating the time of addition of LH846. (**B**) Bar graph showing increase in period of oscillation in the presence of drug. Results from two independent experiments.





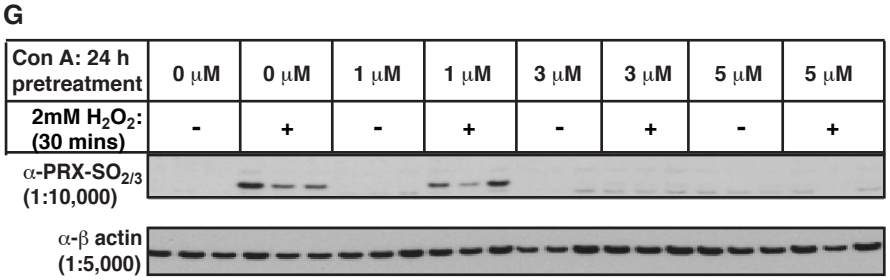


Figure S3, relating to Figure 4. Individual peroxiredoxins are not required for YROs and Conoidin A acts to block over-oxidation of cellular PRX in mouse fibroblasts treated with peroxide. (**A-E**) Representative dissolved oxygen trace for wild type and mutant strains. (**F**) Periods are wild type 2.77 h, SD 0.26, n=17; *tsa1* 2.72h, SD 0.44, n= 7, p=0.807; *tsa2* 2.53h, SD 0.13, n= 9, p=0.009; *prx1* 3.50h, SD 0.27, n= 5, p=0.001; *srx1* 2.62h, SD 0.17, n= 6, p=0.162. Mean ± SEM, p-values are t-test vs. wild type. (**G**) Western blot showing dose-dependent inhibition of PRX over-oxidation by 30 minutes 2 mM H_2O_2 treatment, following 24-hour pre-treatment with varying concentrations of conoidin A. SDS-PAGE was performed under reducing conditions, and performed as [S16] except that cells were pre-treated with 20 mM NEM in ice cold PBS for 15 minutes prior to lysis to alkylate any free thiol groups. Three biological replicates are shown per condition.

	Mammalian Cellular Circadian Rhythm	Yeast Respiratory Oscillation
Cell autonomous, persists under constant conditions	• Mouse fibroblasts and neurons [S1-S3].	• Period is consistent within a given strain/set of conditions [S4, S5-S10].
Temperature compensated	• In mouse fibroblasts [S11, S12].	• In *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* [S7, S13, S14].
Involves redox/metabolic cycles	• In human & mouse erythrocytes [S15, S16], mouse fibroblasts [S17, S18] and mouse myoblasts [S19]. • Mitochondrial metabolism [S19]. • Glucose utilization [S20].	• Redox balance NAD(P)H [S6, S8, S21, S22-S25]. Glutathione and GSH [S5, S21, S24] • Mitochondrial metabolism [S6, S23, S26, S27]. • Glucose utilization [S8, S9, S28].
Involves rhythmic transcription and chromatin modification	• In mouse fibroblasts [S29, S30-S32].	• Transcription [S6, S9, S33, S34]. • Histone acetylation [S35]. • Chromatin modification [S36].
Involves temporal regulation of haem and carbon monoxide	• Agonists of clock gene REV-ERB's haem binding site resets clock in mouse fibroblasts [S37]. • Haem synthesis is circadian regulated [S38]. • Other clock genes reported as functionally regulated by haem [S39, S40].	• The concentration of aminolevulinic acid, a rate-limiting metabolite in the synthesis of haem, oscillates [S24]. • Addition of CO can induce phase advancement, strains deleted for haem oxygenase oscillate with a longer period [S41].
Coupled with DNA replication and cell division	• In mouse fibroblasts [S2, S42-S44].	• DNA replication is gated to the reductive phase of the cycle [S6, S9, S33]. This may function to reduce the mutation rate [S7, S45]. • Oscillations are typically sychronised with the budding index [S25, S46, S47]. • Respiratory oscillations can occur without cell division cycling [S48]. • Swe1 is required for entry to the cell cycle after G_1 arrest [S49] and *SWE1* expression varies more than 10 fold across the YRO [S6].

Table S1.
Note that the mammalian references pertain specifically to observations made using cultured mammalian cells *in vitro* so as to be comparable with the yeast references.

		Casein kinase 1 (CK1)	Glycogen synthase kinase 3 (GSK3)
	Description	• Family of nucleocytoplasmic Ser/Thr kinases • Highly conserved in eukaryotes, ubiquitously expressed • Auto-phosphorylation inactivates • Usually requires a priming phosphate (pS/T-X-X-S) • Can act synergistically with other kinases, such as GSK3 and PKA, at multisite phosphorylation domains to regulate protein stability and nucleo-cytoplasmic shuttling [S50, S51].	• Family of nucleocytoplasmic Ser/Thr kinases • Highly conserved in eukaryotes, ubiquitously expressed • Akt/PKB phosphorylation inactivates • Usually requires a priming phosphate (S/T-X-X-X-pS) • Can act synergistically with other kinases, such as CK1 and CK2, at multisite phosphorylation domains to regulate protein stability and nucleo-cytoplasmic shuttling [S50, S52].
Cellular functions (non-circadian)	Mammals	• 5 isoforms (7 genes) in mammals (α, β, γ1-3, δ, ε) with multiple cellular substrates and partners. • Important roles in membrane trafficking, DNA replication/repair, cytokinesis, vesicular transport, ribosome biogenesis, and transcription [S53, S54]. • Several CK1 isoforms work co-ordinately regulate wnt signalling [S55]. • Targets proteins such as β-catenin and IκB for ubiquitin-mediated proteasomal degradation through recruitment of F-box proteins (e.g. β-TRCP) that recognise the phosphodegron (Dp-S-G-X-XpS) [S56].	• 2 isoforms in mammals (α/β) with multiple cellular substrates and partners [S52]. • Phosphorylates enzymes such as glycogen synthase and IRS1 to regulate glucose homeostasis [S57, S58]. • Essential role in development (part of canonical β-catenin/wnt signalling pathway) [S59]. • Regulates apoptosis, cell proliferation and migration [S60]. • Important signalling role in both the adaptive and innate immune responses [S61]. • Regulates cellular response to DNA damage e.g. through phosphorylation of p53 [S62].
	Flies	• 8 family members with diverse roles in cytoskeletal polarisation/morphogenesis [S63], glial cell migration [S64], olfactory learning [S65], sperm individualization [S66], regulator of Wnt and Hedgehog signaling [S67]. • Synergystic regulation of β-catenin/Armadillo with GSK3, similarly to mammalian cells [S68].	• Fly ortholog (*Shaggy, Sgg*) is a segment polarity gene best known as a repressor of Wingless (Wg) signalling [S69], but also required for normal growth of larval and imaginal tissues [S70]. • Also involved in attachment of the mitotic spindle at the cell cortex [S71], and repression of Hedgehog signaling where it acts synergistically with CK1 to regulate protein degradation [S72].
	Plants	• Plants encode dozens of CK1 homologs, mostly uncharacterised. Roles include subcellular targeting [S73], root development, plant hormone sensitivity [S74], microtubule organization [S75] and starch metabolism [S76].	• The large family of GSK3 homologs in plants are implicated in diverse roles such as regulation of vascular development [S77], abscisic acid signalling [S78], floral development [S79], cell growth/differentiation [S80]. • GSK3-like kinase BIN3 phosphorylates transcription factor BIN2, to stabilise it and thereby negatively regulate brassinosteroid signalling [S81].
	Fungi (*S. cerevisiae*)	• 4 CK1 isoforms in *S. cerevisae*: – Yck1/2 functions in ROS signalling, to directly integrate signals from oxygen and glucose and repress transcription [S82]. • CK1δ homolog, Hrr25 has multiple functions including repression of calcineurin signalling [S83].	• Yeast homolog, Rim11, part of a feed forward loop that switches diploid yeast from a proliferative cycle to the meiotic cycle in response to nutrients [S84]. • Acts with PKA to regulate the stability of the stress-responsive transcription factor Cin5 [S85].
Circadian clock function	Mammals	• The gain-of-function tau mutation in CK1ε shortens circadian period by ~3 hours in homozygotes [S86-S88]. • Human familial sleep disorders (early awakening) segregate with mutations in human CK1δ or hPER2 phosphorylation sites [S89, S90]. • CK1δ/ε determine PER1/2 protein degradation, complex formation and nuclear import/export in the absence of both enzymes, the canonical transcriptional oscillator stops completely [S91, S92]. CK1α also recently implicated [S93].	• GSK3 exhibits a cell-autonomous phosphorylation rhythm [S94, S95]. • Knockdown/pharmacological inhibition of GSK3β shortens circadian period [S96]. Constitutive activation lengthens circadian period [S94]. • Phosphorylates clock proteins BMAL1, CLOCK, CRY2, PER2 and REV-ERBα to regulate their stability [S97, S98, S99].
	Flies	• Early mutagenesis screens identified CK1δ/ε homolog (*doubletime, dbt*), which regulates period length through phosphorylation of dPER [S100]. • Phosphorylation determines dPER turnover kinetics, complex formation and nucleocytoplasmic shuttling [S100, S101].	• GSK3β homolog (*shaggy, sgg*) was originally implicated in timekeeping in Drosophila mutants that exhibit altered circadian period [S102]. • GSK3 phosphorylates components of the core clock machinery (*e.g.*, TIMELESS) to regulate clock complex stability, localisation and periodicity [S103].
	Plants/ algae	• CK1 over-expression or inhibition lengthens circadian period in *O. tauri* ([S104, S105]. • CK1 homologs phosphorylate Cryptochrome (blue light sensor) in *O. tauri* and *A. thaliana* [S105, S106].	• Pharmacological inhibitors of GSK3 shorten circadian period dose-dependently in *O. tauri* [S104]. • Clock-relevant targets are not known at present.
	Fungi (*N. Crassa*)	• A CK1δ/ε homolog phosphorylates FRQ and WC1/2 to regulate the kinetics of protein turnover, thereby determining circadian period length [S107, S108, S109].	• A GSK3 homolog phosphorylates WC-1/2 to regulate protein abundance, with knockdown leading to WC-1 accumulation and shortened circadian period [S110].

Table S2. A comparison between the cellular and circadian functions of CK1 and GSK3 in model eukaryotes.

All the strains are isogenic and were made in the prototrophic CEN.PK background [S111].

HCY1377	*Mata TSA1::KanMX*
HCY1381	*Mata TSA2::KanMX*
HCY1385	*Mata PRX1::KanMX*
HCY1414	*Mata SRX1::KanMX*
HCY1427	*Mata TSA1::NatMX TSA2::KanMX*
HCY1445	*Mata RIM11::KanMX*
HCY1465	*Mata SWE1::KanMX*

Table S3. Strains and genotypes

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