Special-topic lecture for Life Sciences: Cellular Programs

Leistungspunkte/Credit points: 5 (V2/Ü1)

This course is taught in English language.

The material (from books and original literature) are provided online at the course website:

https://www-cbi.cs.uni-saarland.de/teaching/ws-2020-21/special-topic-lecture-biosciences-cellular-programs-ws-20-21/

Biological topics to be covered:

This course will cover aspects of these four topics:

(1) Circadian rhythms

(2) Cell cycle

(3) Stem cell differentiation

(4) Cancerogenesis

Aim of this lecture, „Lernziele“

(1) The aim of this course is not to fully cover these 4 topics
but to **enter deeply** into various **details** of these fields
and to understand that they are deeply **interconnected**.

(2) This course should train you to **quickly read** and **understand**
the main messages in ca. 10 - 12 recent original, biological **research papers**.

Some of these papers apply modern **high-throughput techniques** that are
relevant to bioinformaticians.

(3) If needed, you should look up the experimental **methods** used in the papers.
This means that you should do background reading online, e.g. on wikipedia.

(4) Also, you (as a part of a small group) will **present** once a research paper
at the beginning of the lecture (first 25 minutes) and answer questions about it.

Conditions for certification

(1) There will be 5 biweekly **assignments** about 5 of the assigned papers.

Students need to answer written questions about the assigned research papers.

Every student submits his/her own solution. No team work.

There are three possible grades: excellent, pass, failed. Students need to get a "pass" grade on at least 4 assignments or 2 "pass" and one "excellent" grade.

(2) As mentioned, students need to **present** at least once during the lecture on the content of an assigned research paper (**team work**, ca. 25 min. powerpoint presentation and 10 min. discussion).

(3) If you have fulfilled the first 2 conditions for certification, you can participate at the **written final exam** (120 min) at the end of the winter term.

The exam will cover (parts of) the lecture and some of the assigned papers.

For those who do not live in Saarbrücken/Saarland (or if the Corona pandemic does not allow a written exam), an **oral online exam** will be offered.

Schein/Certification grade

Every student group will present their paper twice, once in the lecture on Tuesday morning and once in the lecture on Tuesday afternoon on the same day.

Both presentations will be graded by the audience. The better grade will count.

An averaged score will be computed from the mark of the final exam (counts 2/3) and the graded presentation (counts 1/3). This yields your grade of certification (“Schein”).

written exam

The exam will cover the lecture material (slides on the lecture website)
and the main principles addressed in the research papers and assignments.
No need to remember every experimental detail of each paper.

In the last lecture, I will provide a list what slides are relevant for the exam and which figures from the research papers.

In case of illness please send E-mail to:
kerstin.gronow-p@bioinformatik.uni-saarland.de and provide a medical certificate.

Those who failed or missed the final exam or the first oral exam due to illness

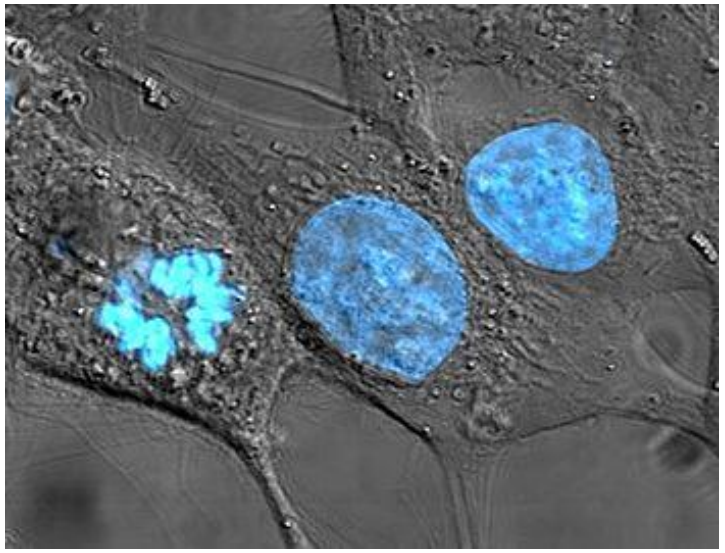
can take an **oral re-exam** at the beginning of SS 2021.

Gene Transcription etc.

Basic terms that you should remember from an introductory genetics lecture ...
or that you should read up about:

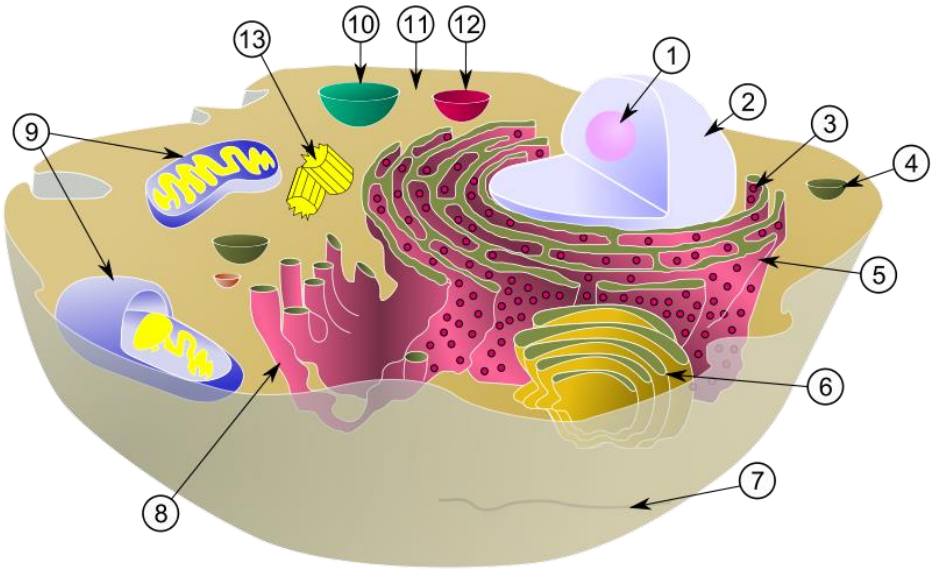
Genome
Genes
Introns, Exons
Nucleus
DNA-Polymerase
Transcription
mRNA
Splicing
Ribosome
tRNA
Translation

Components of a eukaryotic biological cell



HeLa cells stained for DNA with the blue Hoechst dye. The central and rightmost cell are in interphase, thus their entire nuclei are labeled. On the left a cell is going through mitosis and its DNA has condensed ready for division.

wikipedia.org

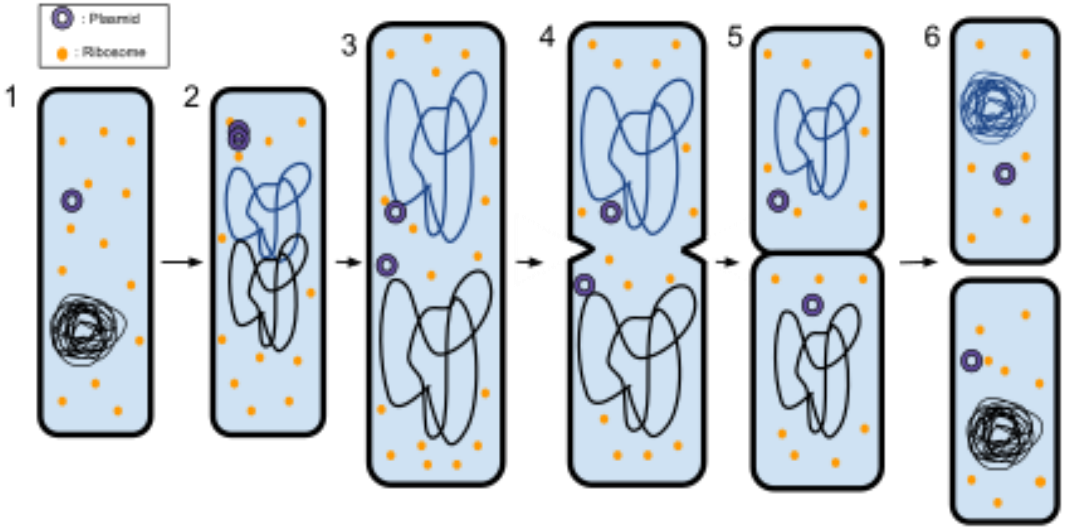


Schematic of typical animal cell, showing subcellular components. Organelles: (1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrioles

(Topic 2) cell cycle

The cell cycle, or cell-division cycle, is the series of events that takes place in a cell leading to its division and duplication (replication).

In cells without a nucleus (prokaryotes), the cell cycle occurs via a process termed **binary fission**.



www.wikipedia.org

In cells with a nucleus (eukaryotes), the cell cycle can be divided in 2 brief periods:

interphase—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and

the **mitosis** (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells".

Cell-cycle phases

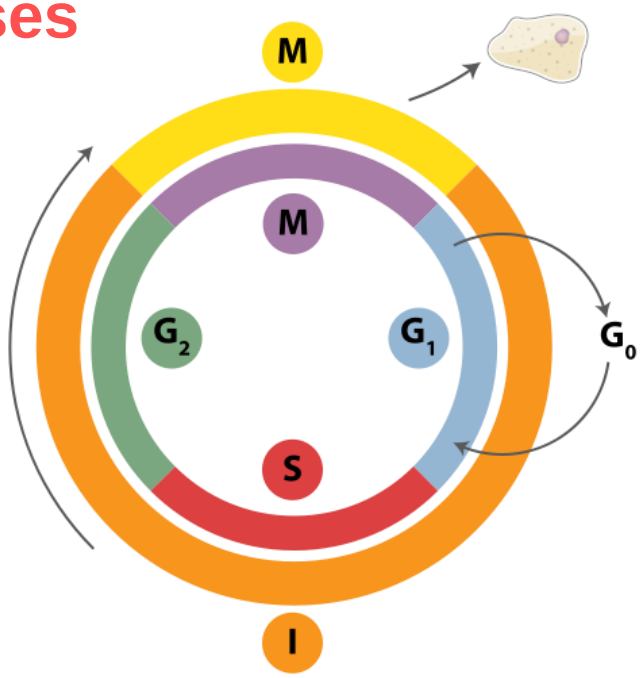
The cell cycle consists of **4 distinct phases**:

- G_1 phase,
- S phase (synthesis),
- G_2 phase
- and M phase (mitosis).

Interphase: combines G_1, S, and G_2

The activation of each phase is dependent on the proper progression and completion of the previous one.

Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called **G_0 phase**.



Schematic of the cell cycle.
Outer ring:
I = Interphase, M = Mitosis;
Inner ring:
M = Mitosis, G1 = Gap 1, G2 = Gap 2, S = Synthesis.

www.wikipedia.org

(Topic 3) Cellular differentiation

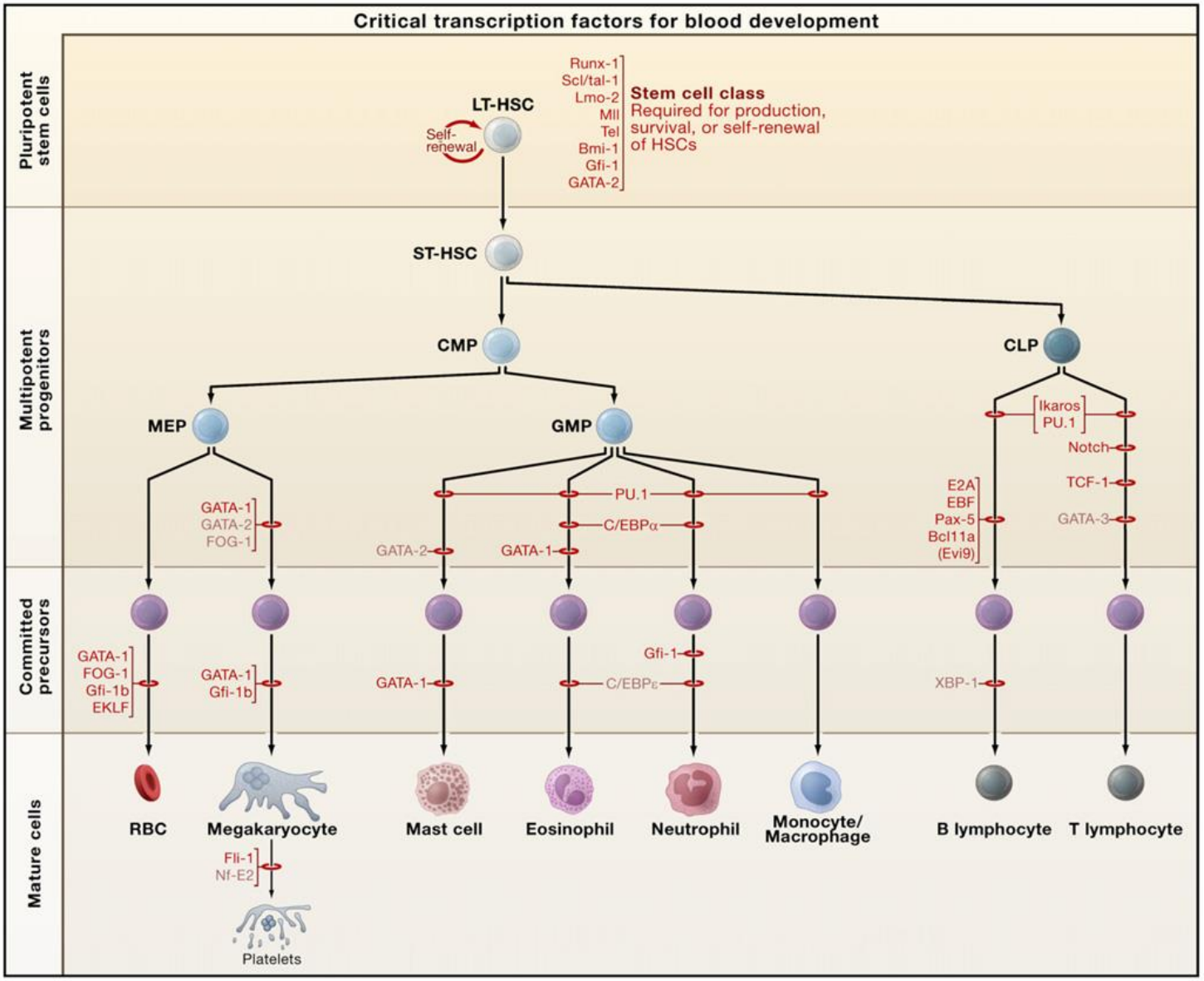
Differentiation is a key example of **cell fate**.

Differentiation does not depend on mutations.

So how does a cell know in which state it is?

-> This is controlled by **epigenetic modifications** of the genome and by activating different **transcriptional and post-transcriptional programs** in the cell (TFs and miRNAs).

Hematopoiesis: development of blood cells



Orkin & Zon, Cell (2008) 132: 631–644.

(Topic 4) The Hallmarks of Cancer

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

Douglas Hanahan* and Robert A. Weinberg†
*Department of Biochemistry and Biophysics and
Hormone Research Institute
University of California at San Francisco
San Francisco, California 94143
†Whitehead Institute for Biomedical Research and
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02142

Robert A. Weinberg

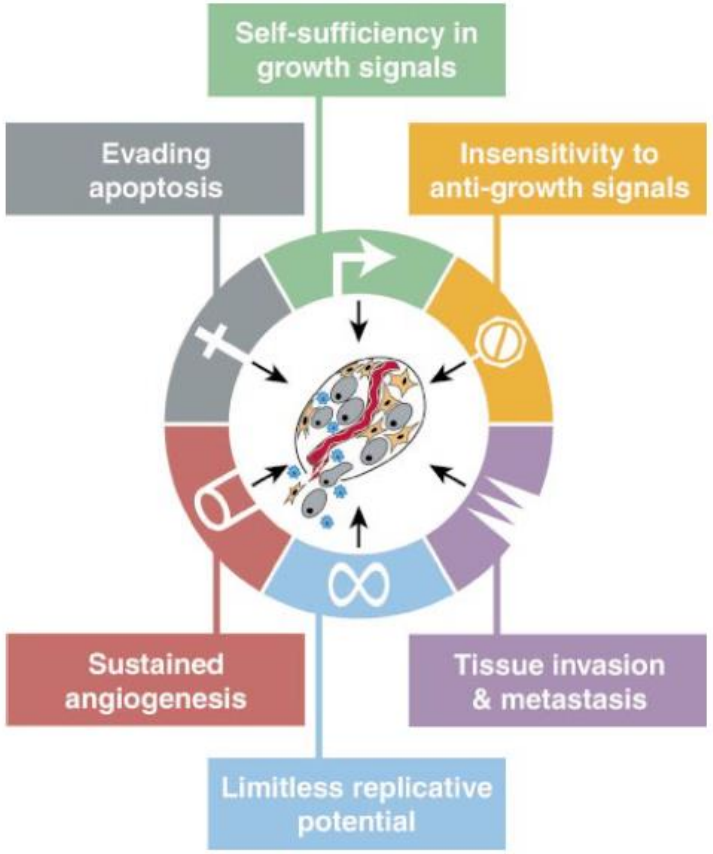


Figure 1. Acquired Capabilities of Cancer

Hallmark of Cancer Genes in the Cell Circuit

As for the genetic reprogramming of this integrated circuit in cancer cells, some of the genes known to be functionally altered are highlighted in red.

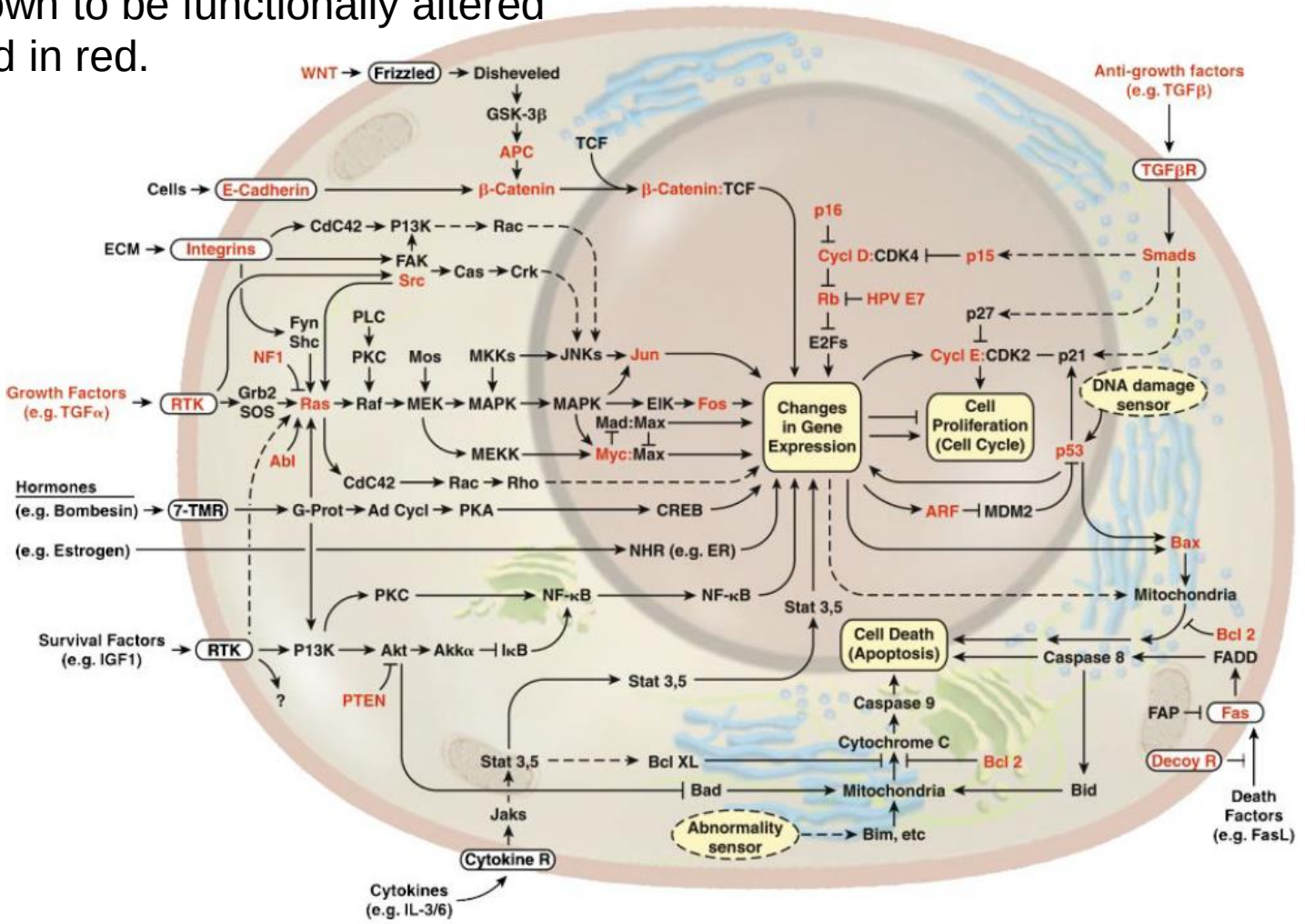


Figure 2. The Emergent Integrated Circuit of the Cell

Tumorigenesis

A

Component	Acquired Capability	Example of Mechanism
	Self-sufficiency in growth signals	Activate H-Ras oncogene
	Insensitivity to anti-growth signals	Lose retinoblastoma suppressor
	Evading apoptosis	Produce IGF survival factors
	Limitless replicative potential	Turn on telomerase
	Sustained angiogenesis	Produce VEGF inducer
	Tissue invasion & metastasis	Inactivate E-cadherin

B

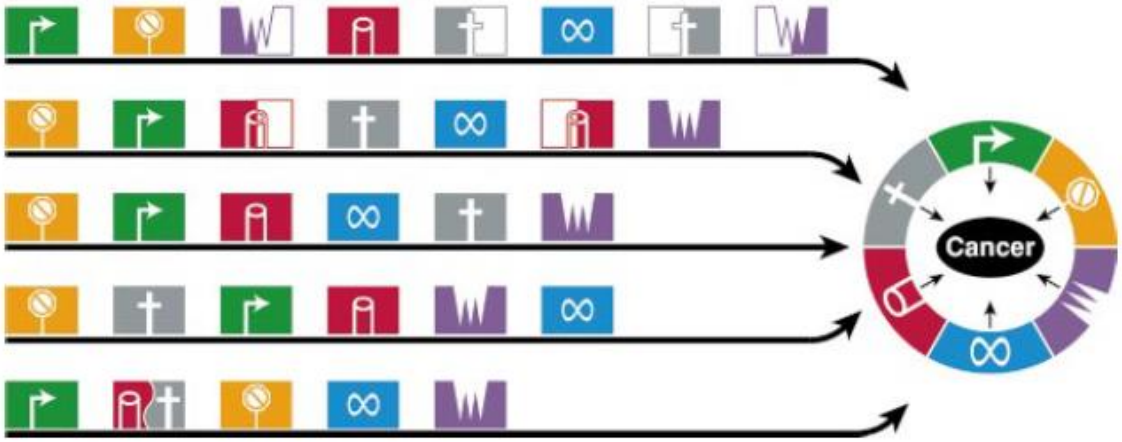
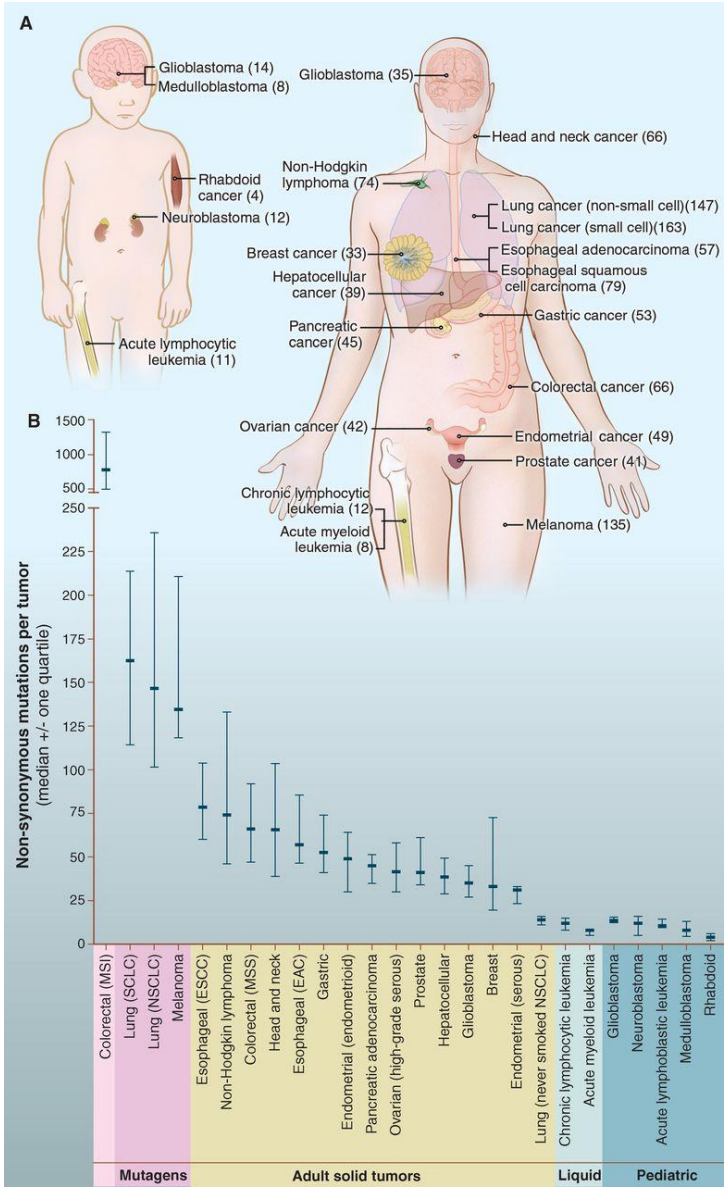


Figure 4. Parallel Pathways of Tumorigenesis

While we believe that virtually all cancers must acquire the same six hallmark capabilities (A), their means of doing so will vary significantly, both mechanistically (see text) and chronologically (B). Thus, the order in which these capabilities are acquired seems likely be quite variable across the spectrum of cancer types and subtypes. Moreover, in some tumors, a particular genetic lesion may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis. Thus, loss of function of the p53 tumor suppressor can facilitate both angiogenesis and resistance to apoptosis (e.g., in the five-step pathway shown), as well as enabling the characteristic of genomic instability. In other tumors, a capability may only be acquired through the collaboration of two or more distinct genetic changes, thereby increasing the total number necessary for completion of tumor progression. Thus, in the eight-step pathway shown, invasion/metastasis and resistance to apoptosis are each acquired in two steps.

Number of somatic mutations in human cancers



Top: children vs. adults

Numbers in parentheses : median number of nonsynonymous mutations per tumor.

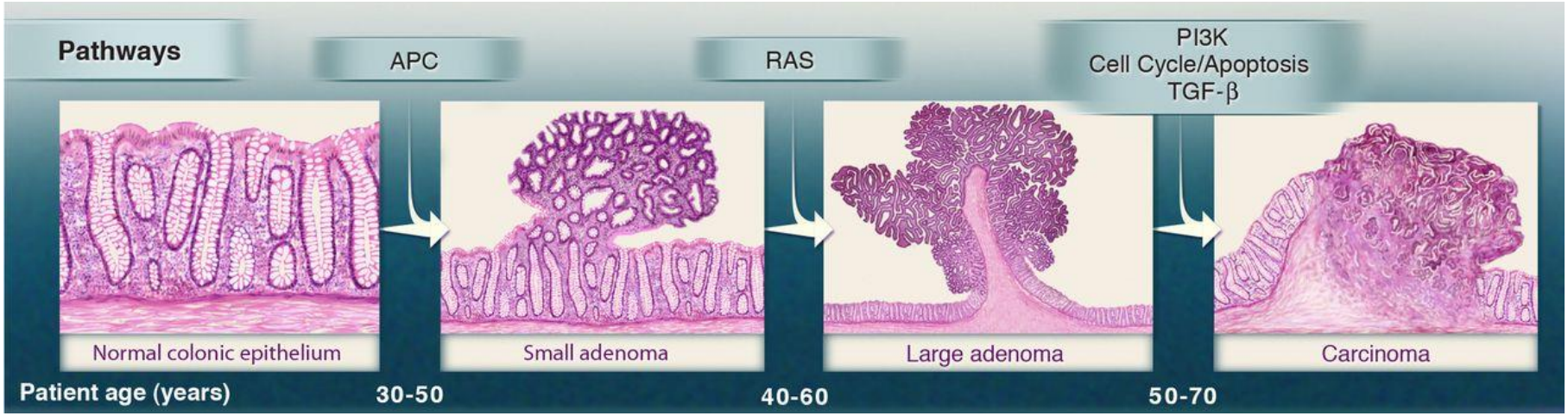
MSI, microsatellite instability;
SCLC, small cell lung cancers;
NSCLC, non–small cell lung cancers;
ESCC, esophageal squamous cell carcinomas;
MSS, microsatellite stable;
EAC, esophageal adenocarcinomas.

B Vogelstein et al. Science 2013; 339:1546-1558

Progression of colorectal cancer

The major signaling pathways that drive tumorigenesis are shown at the transitions between each tumor stage.

One of several driver genes that encode components of these pathways can be altered in any individual tumor



Patient age indicates the time intervals during which the driver genes are usually mutated.

TGF-β, transforming growth factor–β.

B Vogelstein et al. Science 2013; 339:1546-1558

(Topic 1) Circadian clocks in mammals and plants

Most organisms (animals, plants, fungi and cyanobacteria) enhance their fitness by coordinating their development with daily environmental changes through **molecular timekeepers (circadian clocks).**

Mammals display circadian rhythms in behavioral and physiological processes, such as

- sleep
- feeding
- blood pressure and
- metabolism

Roles in **plants** e.g.:

- opening of flowers in the morning and their closure at night

Circadian rhythms are guided by **external light–dark signals**
that are integrated through intrinsic central and peripheral molecular clocks

McClung Plant Cell 18, 792 (2006)

Circadian rhythms

(1) Circadian rhythms are the subset of biological rhythms with period of 24 h.
The term circadian combines the Latin words ‘‘circa’’ (about) and ‘‘dies’’ (day).

(2) Circadian rhythms are **endogenously generated** and **self-sustaining**.

They persist under constant environmental conditions, typically constant light (or dark) and constant temperature.
Under these controlled conditions, the free-running period of **24 h** is observed.

(3) For all circadian rhythms, the **period** remains relatively **constant** over a range of ambient temperatures.

This is thought to be one property of a general mechanism that buffers the clock against changes in cellular metabolism.

Chemical reactions are usually faster at higher temperatures.

McClung Plant Cell 18, 792 (2006)

Essential elements of biological clocks

Our biological clocks contain 3 essential elements:

(1) a **central oscillator** that keeps time;

(2) the ability to **sense time cues** in the environment and to **reset the clock** as the seasons change; and

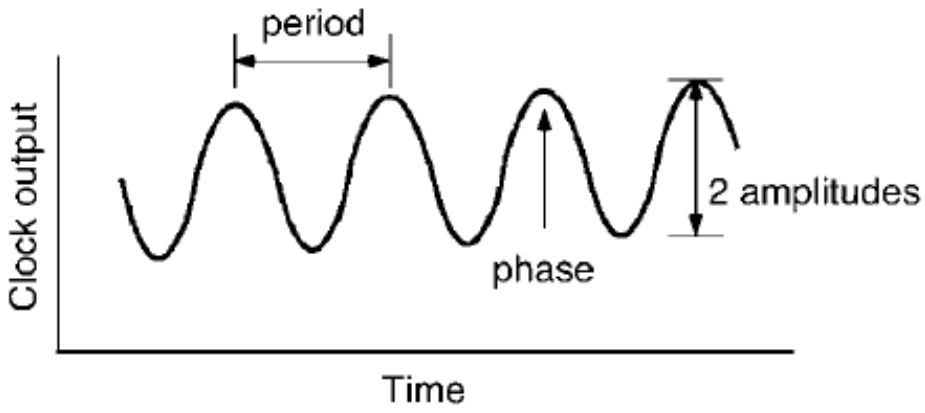
(3) a series of outputs tied to distinct phases of the oscillator
that regulate activity and physiology.

Gallego et al. Nat.Rev.Mol.Cell.Biol. 8, 140 (2007)

Parameters of Circadian clocks

Period : time to complete one cycle.

Amplitude of the rhythm :
one-half the peak-to-trough distance.



Phase : time of day for any given event.
E.g. if the peak in a rhythm occurred at dawn,
the phase of the peak would be defined as 0 h.

Phase is often defined in **zeitgeber time (ZT).**

Zeitgeber is German for „time giver“, and any stimulus
that imparts time information to the clock is a zeitgeber.
The onset of light is a powerful zeitgeber, and dawn is defined as ZT0.

McClung Plant Cell 18, 792 (2006)

Suprachiasmatic nucleus (SCN)

In mammals, the central clock resides in the suprachiasmatic nucleus (SCN), a small region of the brain that contains ca. 20,000 neurons.

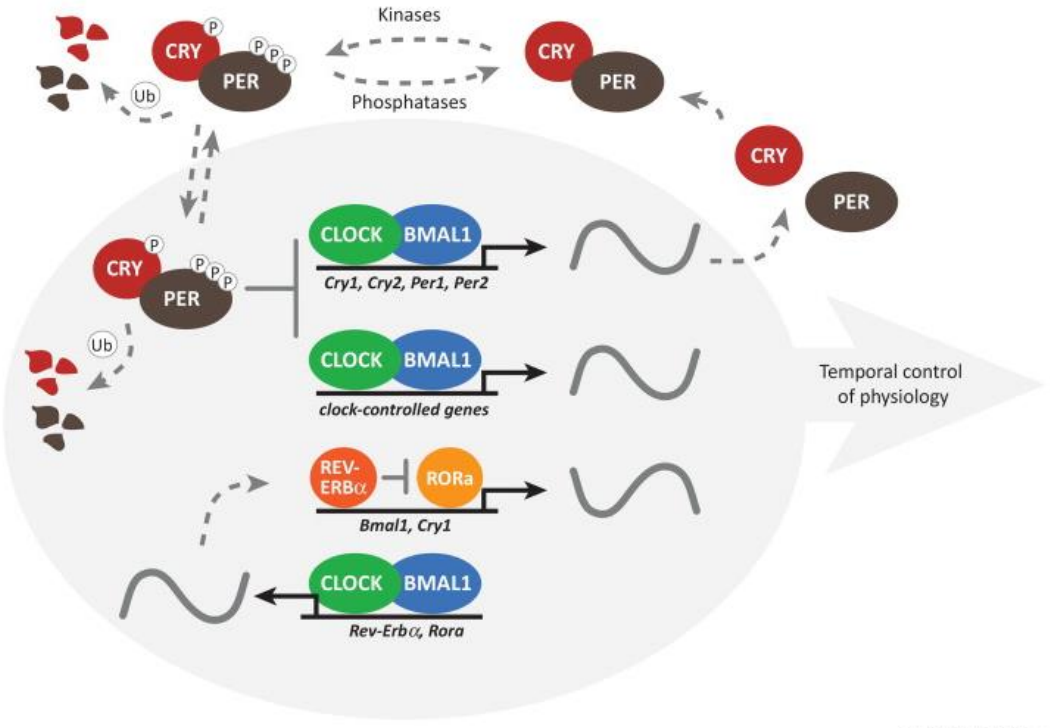
The SCN produces a **rhythmic output** that consists of a multitude of neural and hormonal signals that influence sleep and activity.

Most importantly, the SCN signals **set the peripheral clocks** present throughout the body.

The SCN clock is reset by external **light**,
which is **sensed** by the ganglion cells of the **retina**.

Gallego et al. Nat.Rev.Mol.Cell.Biol. 8, 140 (2007)

The molecular circadian clock in mammals



The cell-autonomous molecular clock in mammals is generated by 2 interlocking transcription/translation feedback loops (TTFL) that function together to produce robust 24 h rhythms of gene expression.

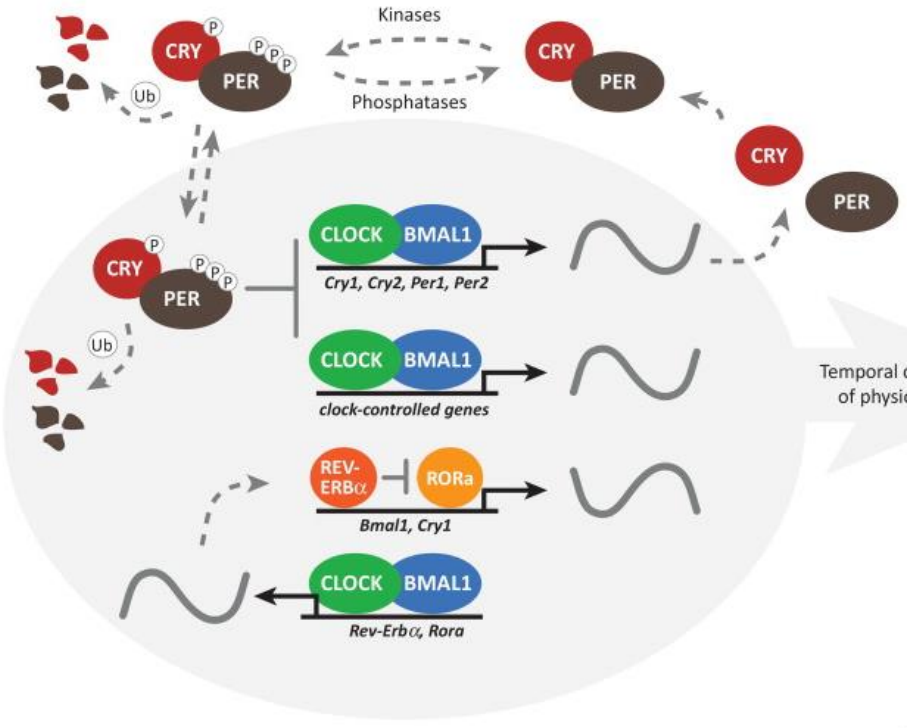
The core TTFL is driven by 4 integral clock proteins:

2 activators (CLOCK and BMAL1) and 2 repressors (PER and CRY), as well as by kinases and phosphatases that regulate the phosphorylation (P) and thereby localization and stability of these integral clock proteins.

BMAL1, brain and muscle ARNT-like 1
CLOCK, circadian locomotor output cycles kaput
CKI: casein kinases I CKIα, CKIδ, and CKIε;
CRY: cryptochrome
PER: period
PP: protein phosphatases PP1, PP5.

Partch et al. Trends Cell Biol 24, 90 (2014)

The molecular circadian clock in mammals



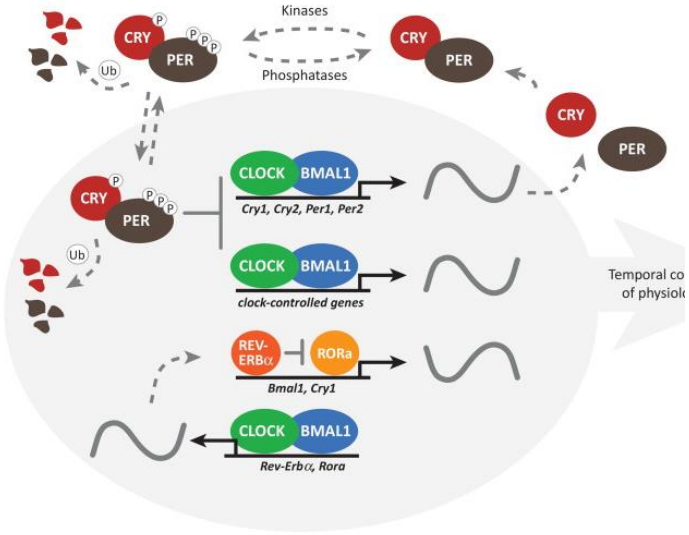
CLOCK and BMAL1 are subunits of the heterodimeric basic helix-loop-helix-PAS (PER-ARNT-SIM) transcription factor CLOCK:BMAL1, which activates transcription of the repressor *Per* and *Cry* genes, as well as other clock-controlled output genes.

PER and CRY proteins heterodimerize in the cytoplasm and translocate to the nucleus to interact with CLOCK:BMAL1, inhibiting further transcriptional activation.

As PER and CRY proteins are degraded through ubiquitin (Ub)-dependent pathways, repression on CLOCK:BMAL1 is relieved and the cycle begins again with ~24 h periodicity.

Partch et al. Trends Cell Biol 24, 90 (2014)

The molecular circadian clock in mammals



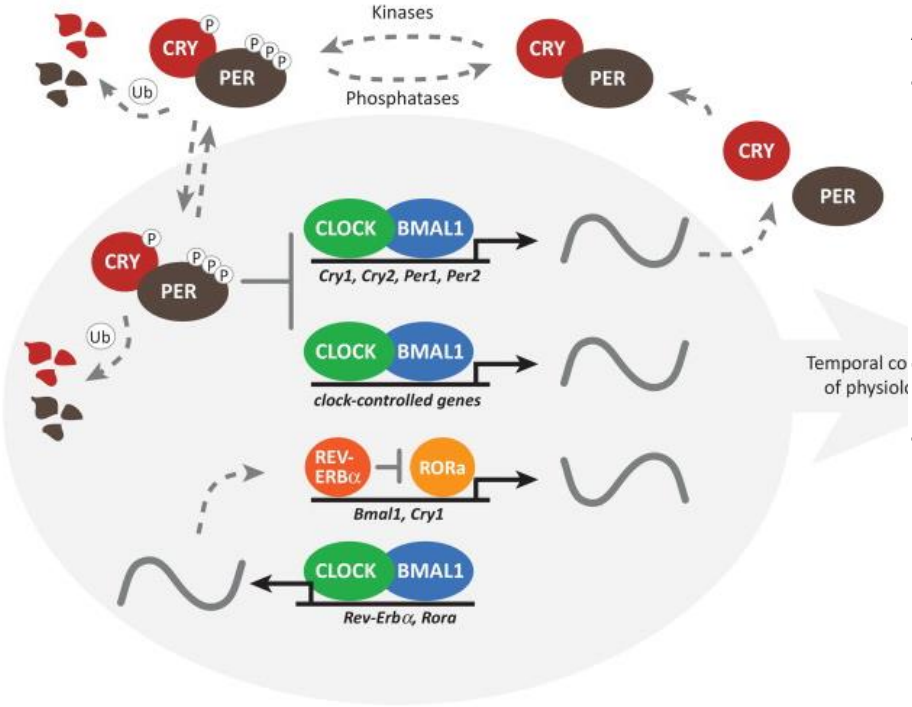
The casein kinases CKIδ and CKIε play an important role in determining the intrinsic period of the clock by controlling the rate at which the PER:CRY complexes are either degraded or enter the nucleus, and their activity is either counteracted or regulated by the phosphatases PP1 and PP5, respectively.

Notably, **familial mutations** resulting in the loss of a single phospho-acceptor site on PER2 (S662G) or a loss-of-function mutation in CKIδ (T44A) shorten the intrinsic period of the clock in mice and give rise to sleep phase disorders in humans.

A key role for the casein kinases in establishing period length has also been demonstrated pharmacologically via modulation of the kinases with **small-molecule inhibitors**, which dramatically lengthen the period by modulating PER localization and stability.

Partch et al. Trends Cell Biol 24, 90 (2014)

The molecular circadian clock in mammals



A second TTFL is generated through transcriptional activation by the retinoid-related orphan receptors (RORa, b, c) and repression by REV-ERBα/REV-ERBβ.

This TTFL drives rhythmic changes in *Bmal1* transcription and introduces a **delay** in *Cry1* mRNA expression that offsets it from genes regulated strictly by CLOCK:BMAL1 and is crucial for proper circadian timing

The presence of cooperative, interlocking feedback loops provides **robustness** against noise and environmental perturbations to help maintain accurate circadian timing, and also helps to generate **phase delays** in circadian transcriptional output that optimally time gene expression for local physiology.

Partch et al. Trends Cell Biol 24, 90 (2014)

Circadian clocks in Arabidopsis thaliana

Plants were the first organisms for which the observation of a circadian rhythm was published (de Mairan, 1729).

The molecular study of plant clocks began in 1985 with the observation that the mRNA abundance of the light-harvesting chlorophyll *a/b*-binding protein genes (***LHCB***) of peas oscillated with a circadian rhythm

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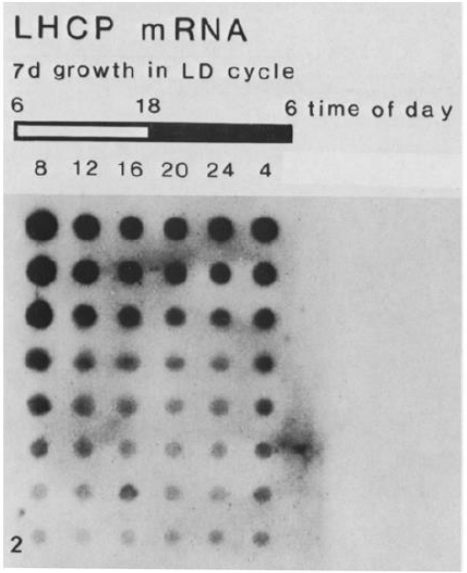


Salomé et al. J. Biol. Rhythms 19, 425 (2004)
Kloppstech, Planta 165, 502 (1985)

Key players in Arabidopsis thaliana

LHCB transcription is induced by light and shows a circadian pattern of expression with a peak in the middle of the subjective day.

The red-light photoreceptors, the **phytochromes (PHY)**,
mediate the light induction of *LHCB* through a motif in the *LHCB* promoter.
Comment: LHs absorb maximally at 850 nm (red light).

Minimal promoter fragments necessary and sufficient for light
and circadian regulation of *LHCB* were identified.

Tobin's group identified a protein with affinity to this promoter fragment.
This TF was named **CCA1** for CIRCADIAN CLOCK ASSOCIATED 1.

LATE ELONGATED HYPOCOTYL (***LHY***) is another gene
encoding a protein closely related to *CCA1.*

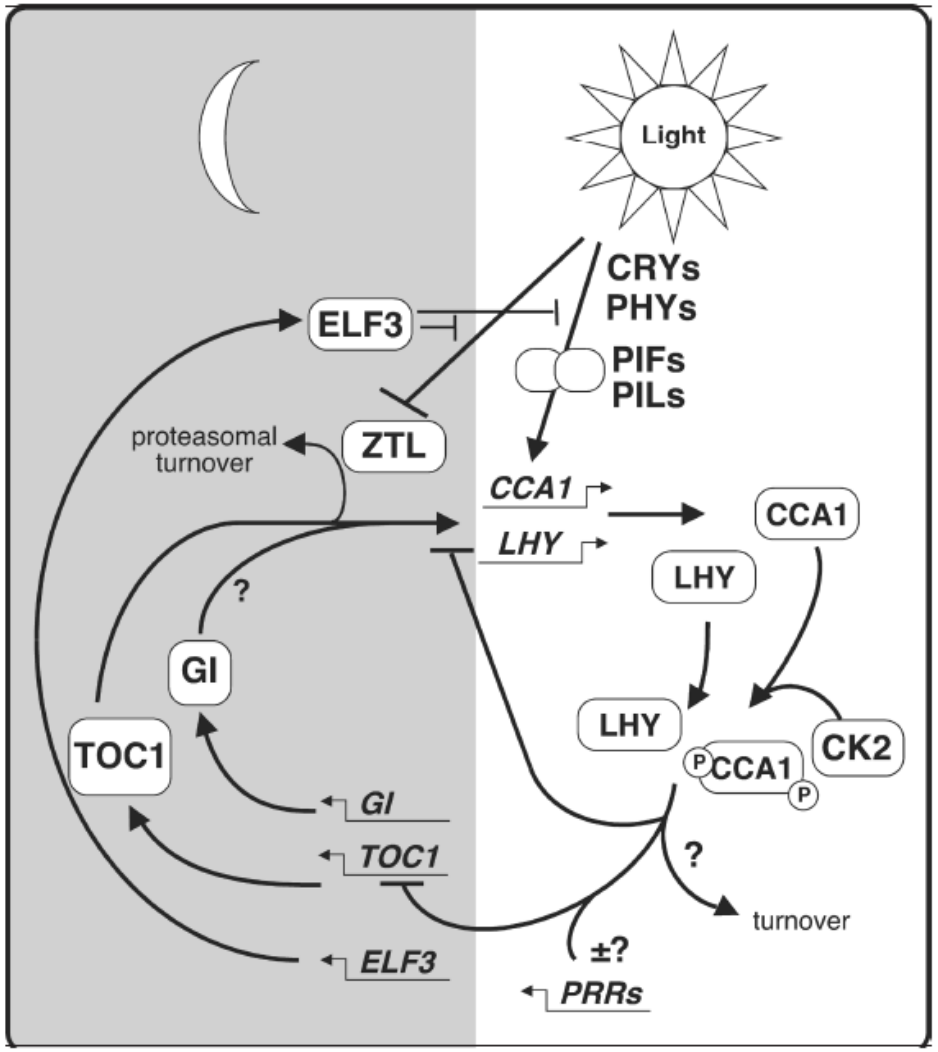
Salomé et al. J. Biol. Rhythms 19, 425 (2004)

Model of the *Arabidopsis thaliana* oscillator

Light perceived by the **PHYs** and **CRYs** (cryptochromes) induces the expression of 2 transcription factors, ***CCA1*** and ***LHY***.

CCA1 and *LHY* mRNA abundance peaks shortly after dawn (dt. *Morgendämmerung*).

CCA1 requires phosphorylation by **CK2** prior to binding to DNA.



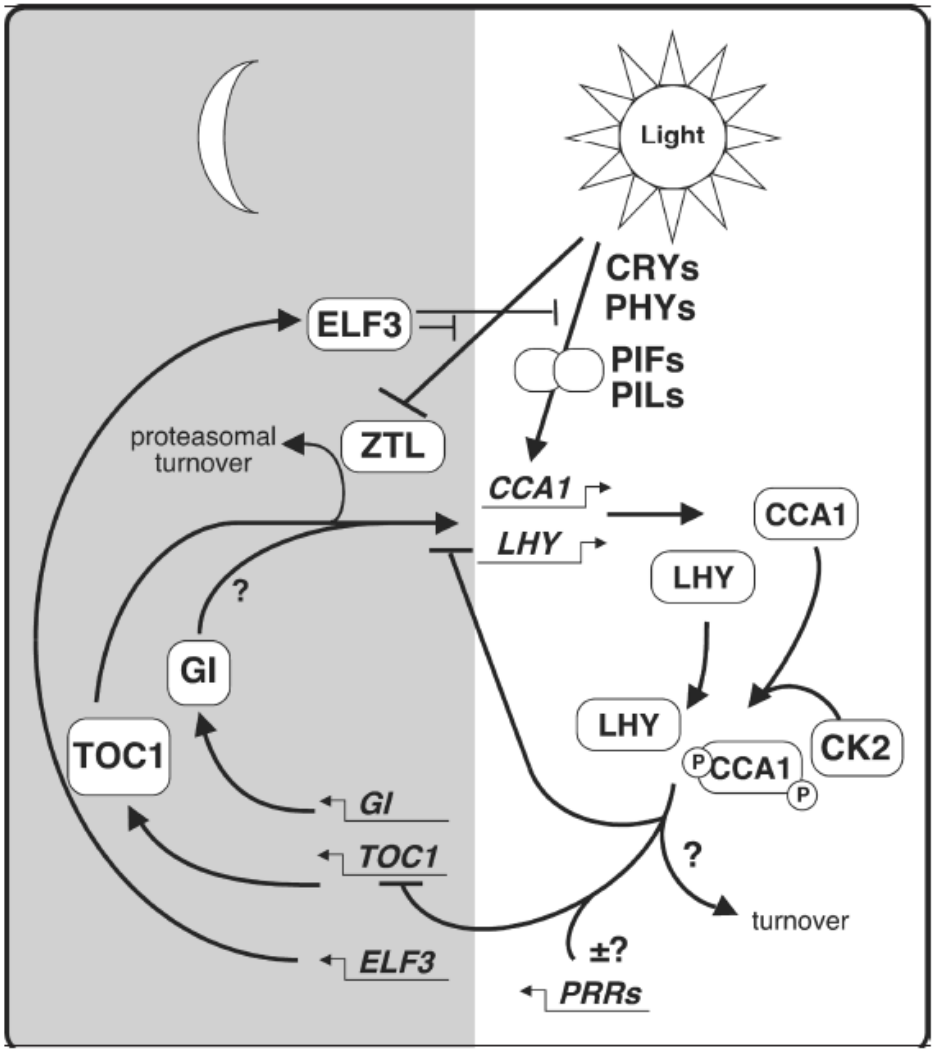
Salomé et al. J. Biol. Rhythms 19, 425 (2004)

Model of the *Arabidopsis thaliana* oscillator

One known target of the repressive activity of CCA1 and LHY is ***TOC1*** (Timing of Cab Expression 1).

Therefore, TOC1 mRNA abundance peaks around dusk (*dt. Abend-dämmerung*), following the turnover of CCA1 and LHY proteins.

TOC1 then feeds back onto *CCA1* and *LHY* and induces their expression for the next cycle.

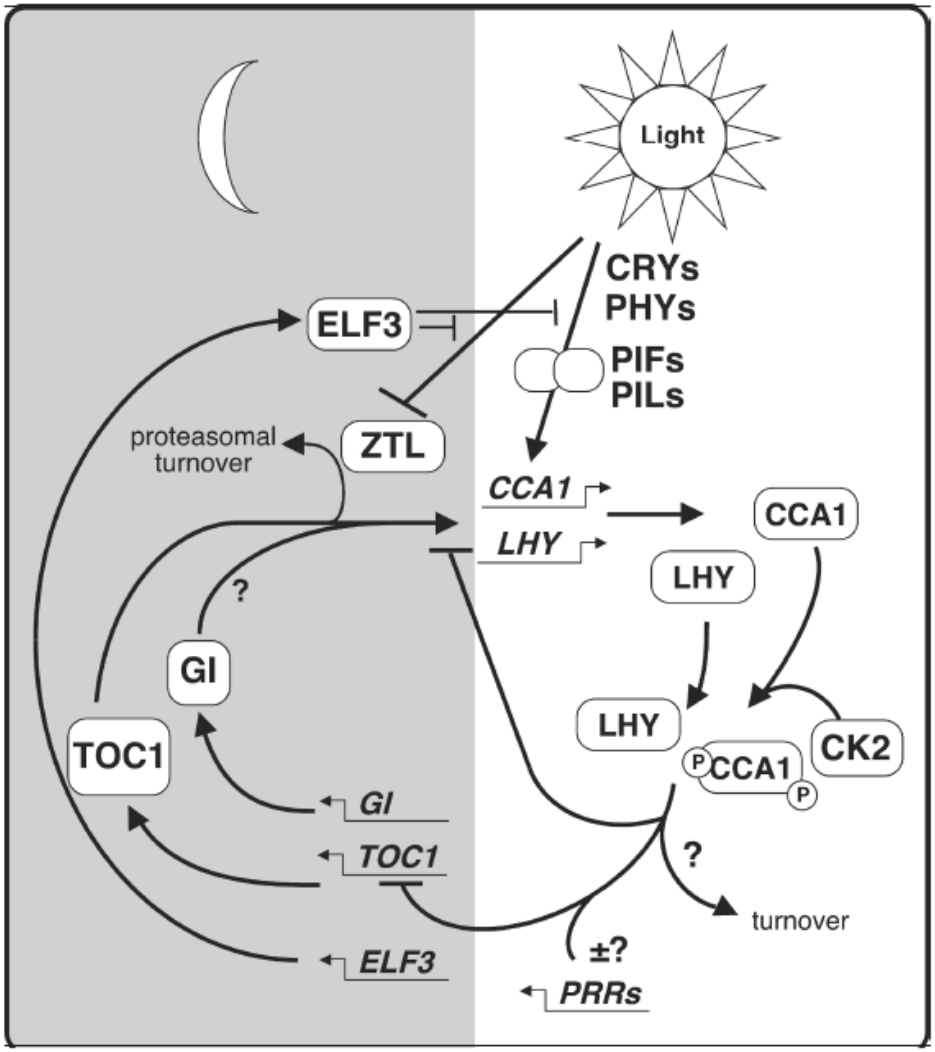


Salomé et al. J. Biol. Rhythms 19, 425 (2004)

Model of the *Arabidopsis thaliana* oscillator

TOC1 **degradation** is mediated by the F-box protein **ZTL** (Zeitlupe = *slow motion*), whose activity is negatively regulated by light.

CCA1 and LHY also negatively regulate their own promoters, possibly directly but possibly indirectly via TOC1.



Salomé et al. J. Biol. Rhythms 19, 425 (2004)

Detect unknown control mechanisms: Probe gene expression by microarrays

Harmer *et al.* used oligonucleotide-based arrays to determine steady-state mRNA levels in *Arabidopsis* at 4-hour intervals during the subjective day and night.

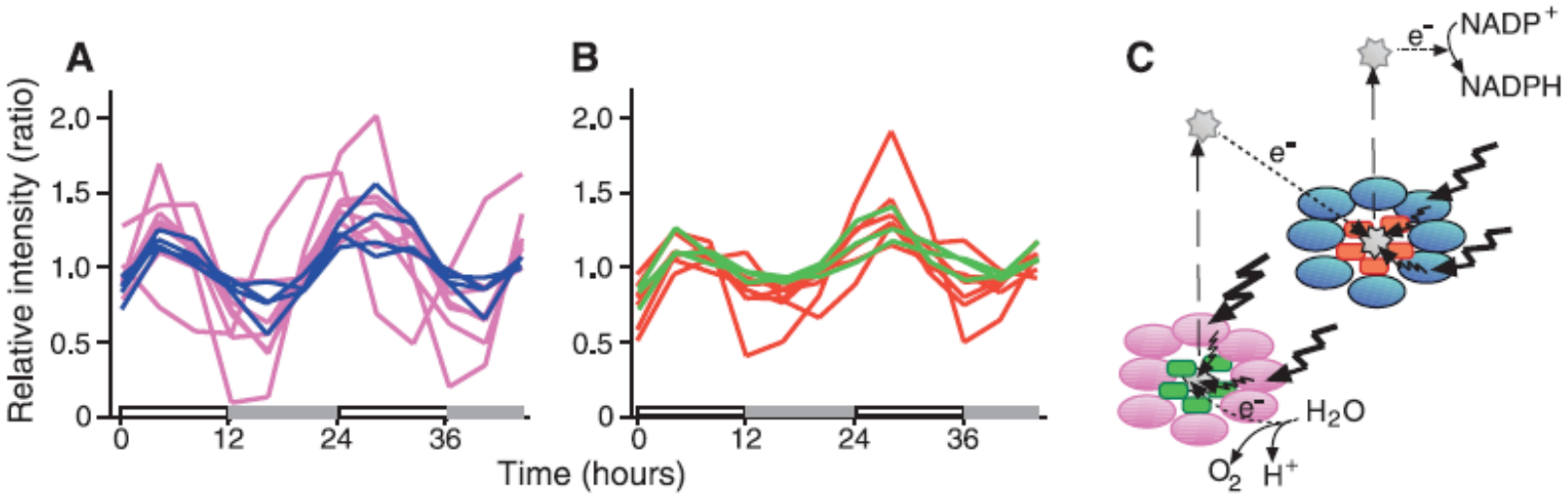
→ They identified temporal patterns of gene expression in *Arabidopsis* plants under constant light conditions using GeneChip arrays representing about 8200 different genes.

All genes were scored whether their expression is correlated with a **cosine** test wave with a period between 20 and 28 hours (probable correlation > 95%)
→ consider those genes as circadian-regulated.

→ 453 genes (6% of the genes on the chip) were classified as **cycling**.

Harmer et al. Science 290, 2110 (2000)

Photosynthesis genes peak near the middle of the day



Results after normalization of peak maximum.

(A) *LHCA* genes are in blue; *LHCB* genes are in pink.

(**B**) Photosystem I genes are in red;. Photosystem II genes are in green;.

(**C**) Model for function of photosynthesis gene products in photosystems II (left) and I (right). Colors of proteins match colors of corresponding gene traces.

Harmer et al. Science 290, 2110 (2000)

Synchronized production of photoprotective pigments

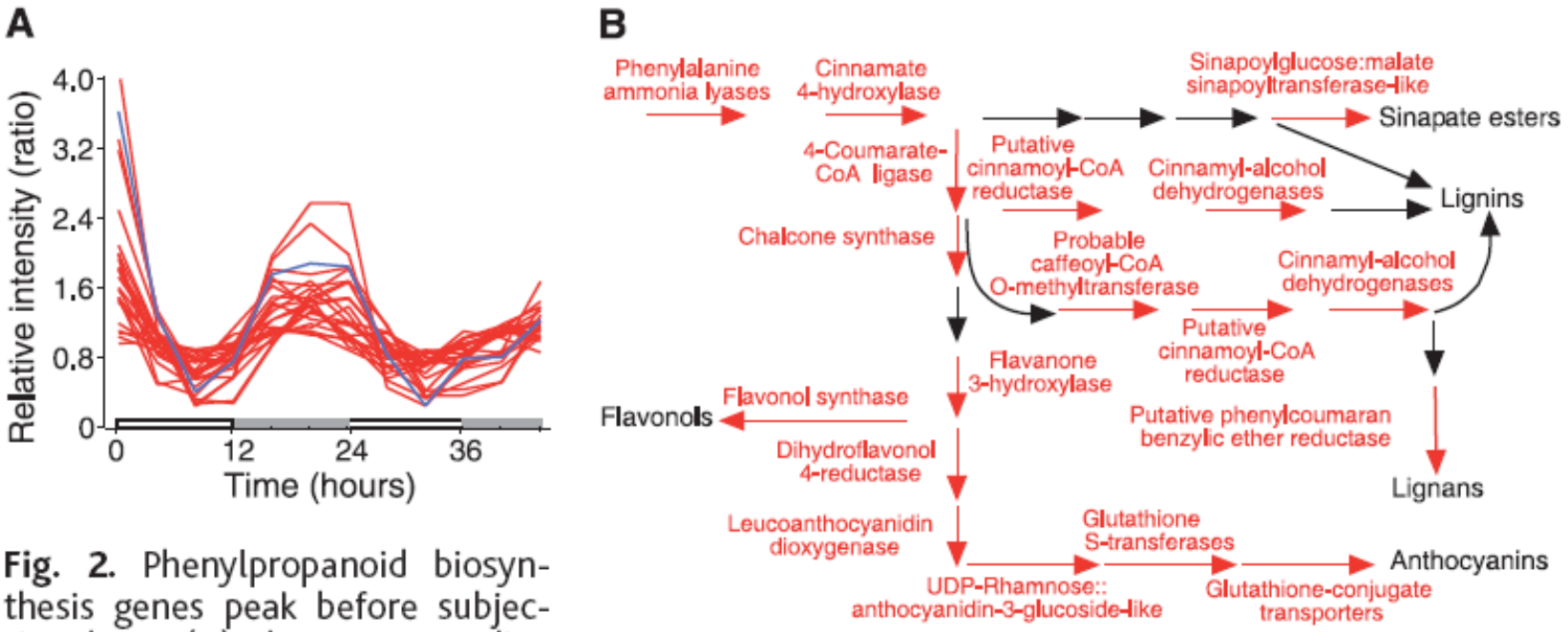


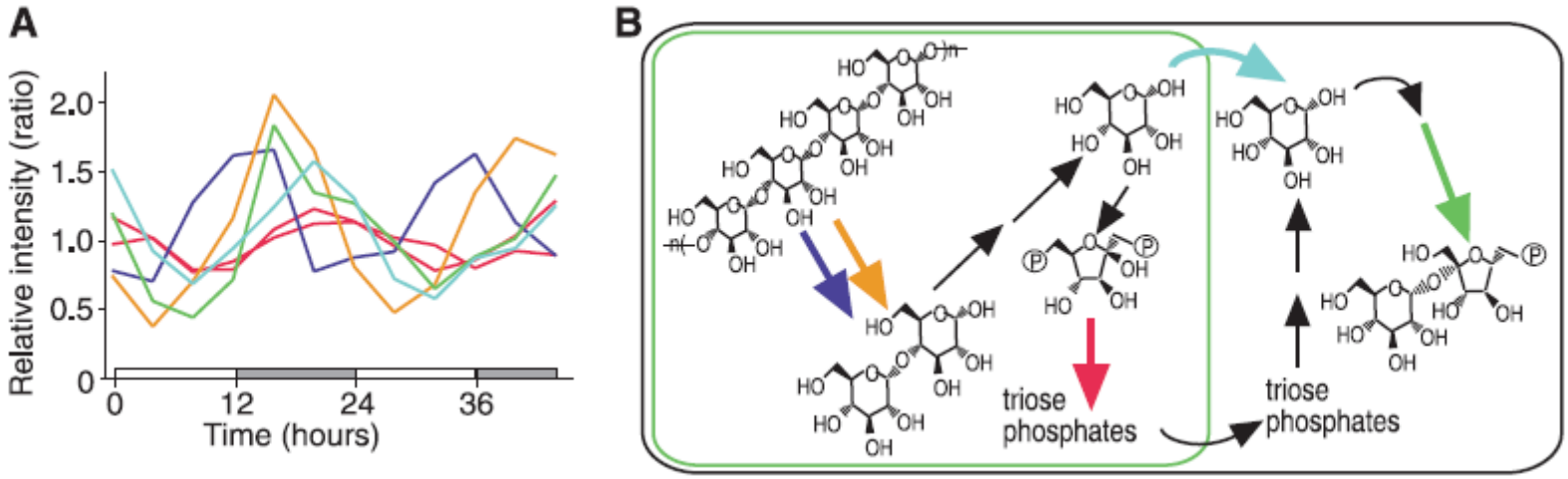
Fig. 2. Phenylpropanoid biosynthesis genes peak before subjective dawn. (**A**) The gene encoding the Myb transcription factor *PAP1* (accession number AAC83630) is in blue. The red traces represent phenylpropanoid biosynthesis genes. (**B**) Phenylpropanoid biosynthetic pathways. Genes encoding all enzymes indicated in red are clock-controlled. See Web table 2 (*8*) for gene names and accession numbers.

„Phenolic sunscreen“ is produced before sunrise.

Substances absorb light in the visible and UV range.

Harmer et al. Science 290, 2110 (2000)

Circadian regulation of sugar metabolism



Genes encoding starch-mobilizing enzymes peak during the subjective night because plants store starch in chloroplast for use during the night when the plant cannot do photosynthesis.

(**A**) Cycling genes encode a putative starch kinase that is related to potato R1 protein (dark blue); a β-amylase (gold); fructose-bisphosphate aldolase, (red); a putative sugar transporter (light blue); and a sucrose-phosphate synthase homolog (green).

(**B**) Model for the enzymatic functions of these gene products in the mobilization of starch. Colored arrows indicate the function of the corresponding gene indicated in (A).
The chloroplast is bounded by a green box and the cytoplasm by a black box.

Harmer et al. Science 290, 2110 (2000)

Chilling resistance

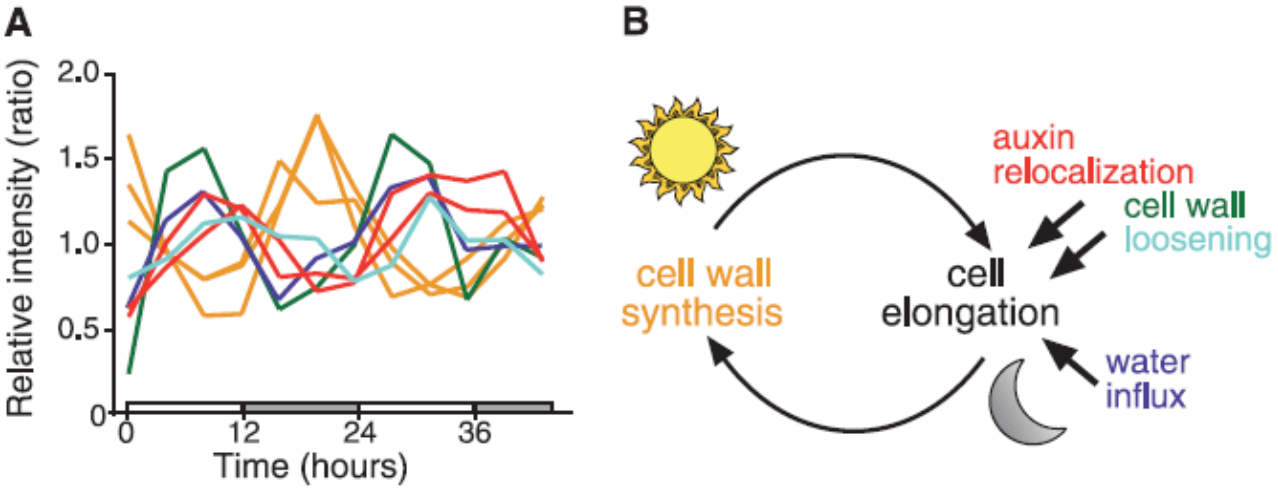
Chilling resistance is an important trait in plants.

A number of enzymes involved in **lipid modification**, including two desaturases, were found to be under clock regulation and peaked near subjective dusk.

This is consistent with previously observed rhythms in membrane lipid desaturation levels that correlate with increased resistance to cold treatments during the subjective night.

Gallego et al. Nat.Rev.Mol.Cell.Biol. 8, 140 (2007)

Genes implicated in cell elongation are circadian-regulated



(**B**) Proposed mode of action of the products of these clock-controlled genes in cell wall remodeling.

The rigid plant cell wall normally prevents cell expansion, but a simultaneous loosening of cell wall components, uptake of water, and synthesis of cell wall components seems allowed.

(**A**) Genes encoding the auxin efflux carriers *PIN3* and *PIN7* (red), a putative expansin (green), a putative polygalacturonase (light blue), and aquaporin d-TIP (dark blue) all peak toward the end of the subjective day.

Auxins are phytohormones – they regulate cell extension.

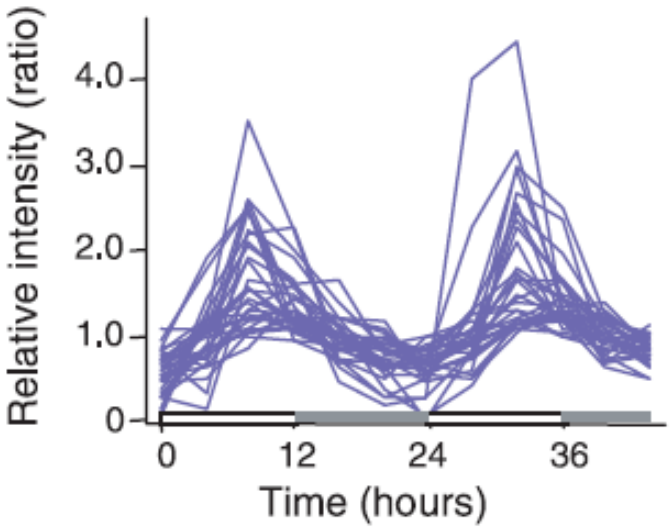
3 enzymes implicated in cell wall synthesis (all in gold) peak toward the end of the subjective night.

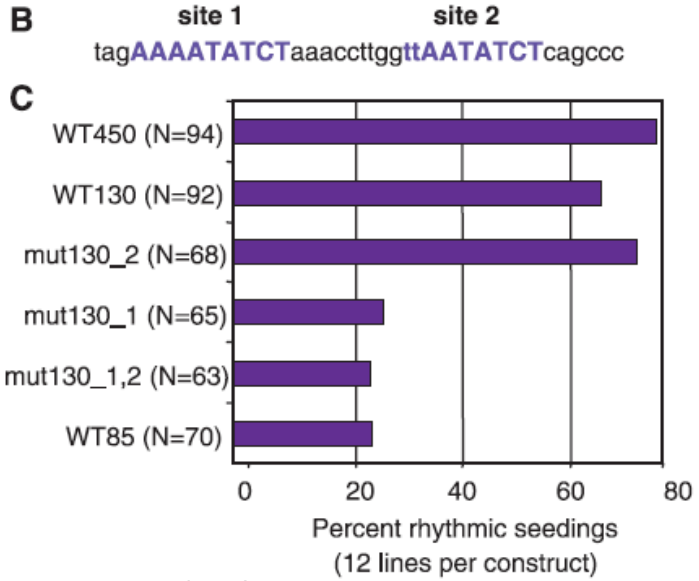
Harmer et al. Science 290, 2110 (2000)

Master regulator sequence of circadian-regulated genes?

Check genomic DNA regions upstream of cycling genes for overrepresented promoter elements

→ absolutely conserved motif, AAAATATCT "**evening element**," that occurs 46 times in the promoters of 31 cycling genes. All genes demonstrated impressive coregulation. All but one peak toward the end of the subjective day.





Mutation of the conserved AAAATATCT, but not a closely related motif, greatly reduced the ability of a promoter to confer circadian rhythmicity on a luciferase reporter gene in plants.

Harmer et al. Science 290, 2110 (2000)

Summary

Most organisms enhance fitness by coordinating their development with daily environmental changes through molecular timekeepers known as circadian clocks.

Clocks are generated by a transcription-translation negative feedback loop with a crucial delay between stimulus and response.

This system of multiple connected loops increases the clock's robustness and provides numerous points of input and output to the clock.

Many metabolic pathways are regulated by circadian clocks in plants and animals.

Kay & Schroeder Science 318, 1730 (2007)

Papers selected for this course

- Each paper should study and answer a particular research hypothesis related to the course content
- Some papers are game-changers, discover novel biology
- Papers are from different fields (cell biology, single-cell genomics, structural biology, clinical study …), using different technologies
- They should be well-written, not too long,
- They are typically published in a highly ranked journal. Often this means that they report „important research“. At least, text and figures are typically carefully polished.
- They should be either open access or accessible when logging in with UdS account.

Next week

Next week: we will discuss the freely accessible paper

https://www.sciencedirect.com/science/article/pii/S0092867419305070

Welz et al. Cell 177, 1436-1447 (2019) *„BMAL1-Driven Tissue Clocks Respond Independently to Light to Maintain Homeostasis “*

Presentations should address (in ca. 25 minutes → 20 - 25 slides):

- What is the main hypothesis of the paper?
  (maybe provide some essential background information to audience)
- What experiments were performed?
- Why did they perform these particular experiments?
- What are the main results (**not all, make a selection**)?
- What are the implications of these findings?
- Discuss possible limitations
- Your personal view at this paper

All members of the group should participate in the oral presentation.

Now we need to find the **first group of volunteers**.