Special-topic Lecture Bioinformatics: Modeling Cell Fate

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:

http://gepard.bioinformatik.uni-saarland.de/teaching/ss-2015/stl-bioinformatics-modcellfate-ss13

Biological topics to be covered:

This course will enter into details of three selected topics in current cell biology:

(1) Circadian rhythms

(2) Cell cycle

(3) Stem cell differentiation

(4) Cancerogenesis

Bioinformatics content

- microarray expression analysis
- DNA methylation analysis
- GO and pathway annotation
- interaction networks
- application of clustering techniques
- construction of gene-regulatory networks
- stochastic simulations
- time series analysis

Aim of this lecture, „Lernziele“

(1) The aim of this course is not to fully cover these four topics but to **enter deeply** into various **details** of these fields.

(2) This course should train you to **analyze** original biological **data** using modern bioinformatics tools.

(3) You shoud also become familiar with the biological processes (**pathways**) controlling cellular adaptation / cell fate.

(4) Also, you will get a routine in reading original research publications.

Tutorial

We will handout 6 **biweekly assignments**.

Groups of up to two students can hand in a solved assignment.

Send your **solutions** by e-mail to the responsible tutors :

Thorsten Will, Maryam Nazarieh, Daria (?)

until the time+date indicated on the assignment sheet.

The **tutorial** on Tuesdays 12.45 pm - 2 pm will provide help to understand the papers and the assignment solutions.

Schein condition 1

Only those students can get a „Schein“ who have obtained more than 50% of the points for all assignments.

Schein = pass 3 written tests

Schein condition 2

The successful participation in the lecture course („Schein“) will be certified upon fulfilling Schein condition 1 and upon successful completion of 3 written 45 minute tests.

Each test roughly covers the content of one of the three lecture topics.

Dates: probably at the beginning of lectures V5, V9, V13.

All students registered for the course may participate in the tests.

2 out of 3 tests have to be passed.

The final grade on the Schein is the average of your 2 best tests.

Rounding scheme: (1.0 + 1.3 -> 1.0 ; 1.3 + 2.0 -> 1.7)

written tests

The tests will cover the lecture material (slides on the lecture website)
and the theory behind the assignments for this topic.

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

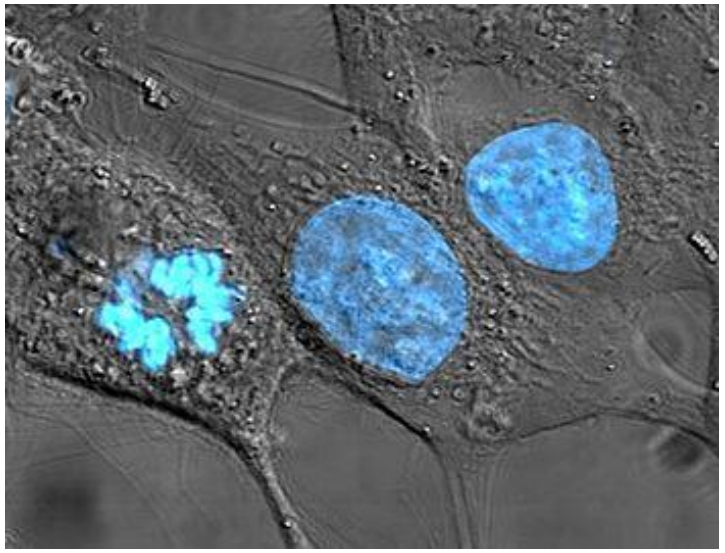
Those who miss or fail one test, will be given a second-chance oral exam.
If you fail or miss more than two tests, you cannot get a Schein.

Gene Transcription etc.

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

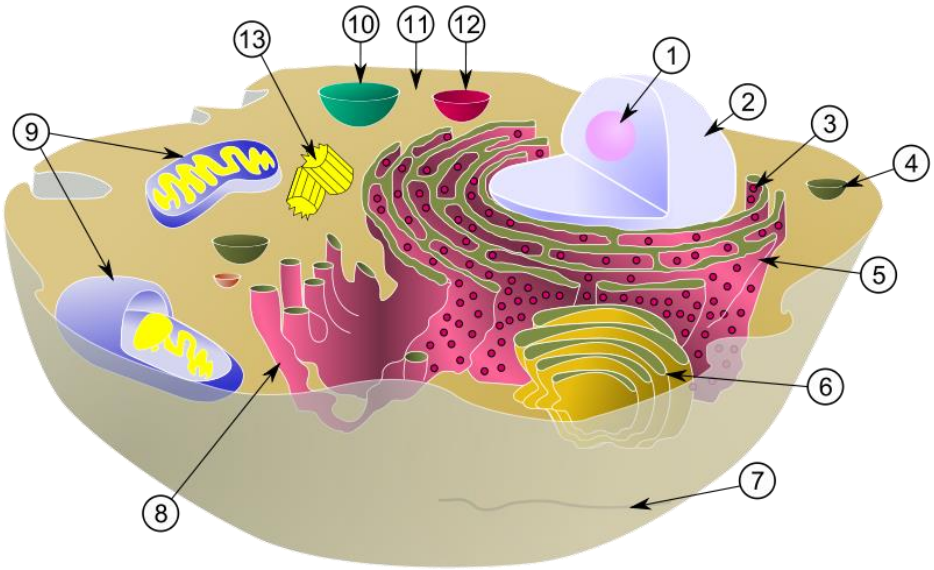
Genome
Genes
Introns, Exons
Nucleus
DNA-Polymerase
Transcription
mRNA
Splicing
Ribosome
tRNA
Translation

A 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

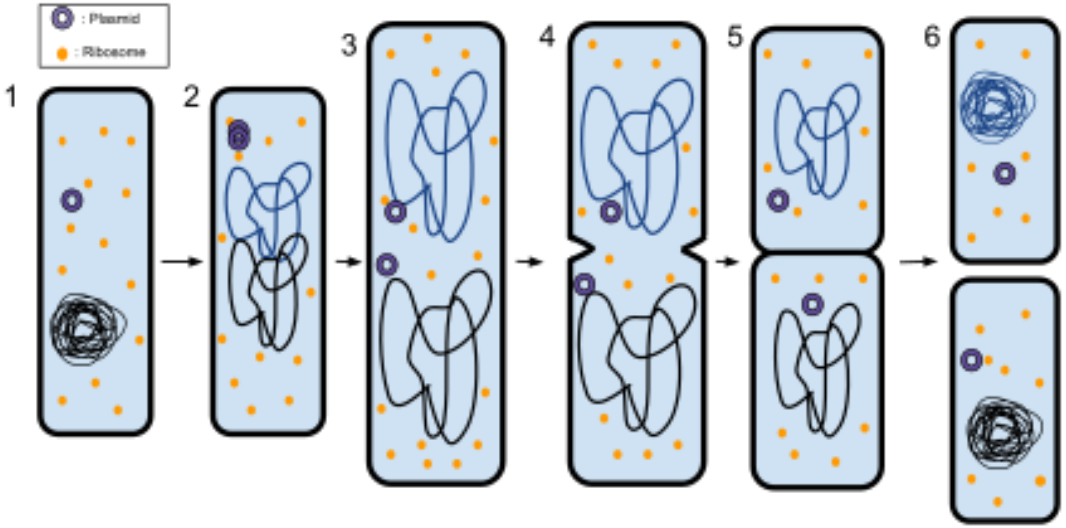


Schematic of typical animal cell, showing subcellular components. Organelles: (1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrioles

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".

Phases

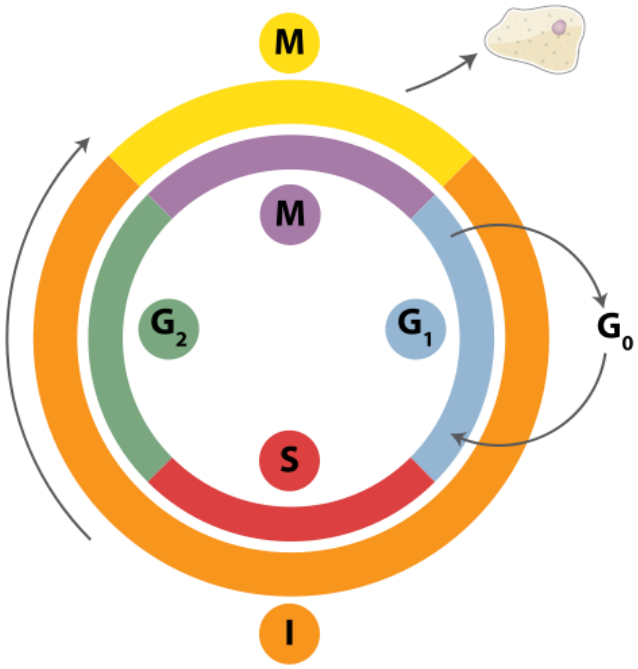
The cell cycle consists of **4 distinct phases**:

- 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**.

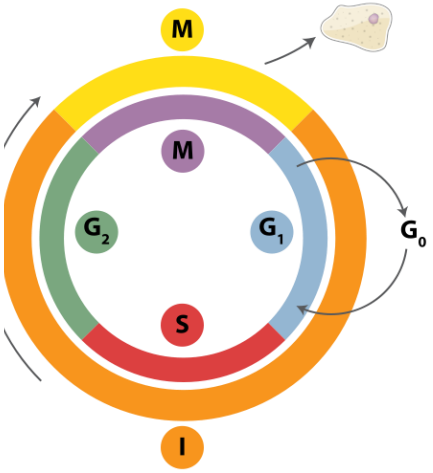


Schematic of the cell cycle.
Outer ring:
I = Interphase, M = Mitosis;
Inner ring:
M = Mitosis, G1 = Gap 1, G2 = Gap 2, S = Synthesis.

www.wikipedia.org

Activity during 4 phases

State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	G_0	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	G_1	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	S	DNA replication occurs during this phase.
	Gap 2	G_2	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	M	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.



M phase itself is composed of 2 tightly coupled processes:

- **mitosis**, in which the cell's chromosomes are divided between the two daughter cells, and
- **cytokinesis**, in which the cell's cytoplasm divides in half forming distinct cells.

www.wikipedia.org

Cellular differentiation

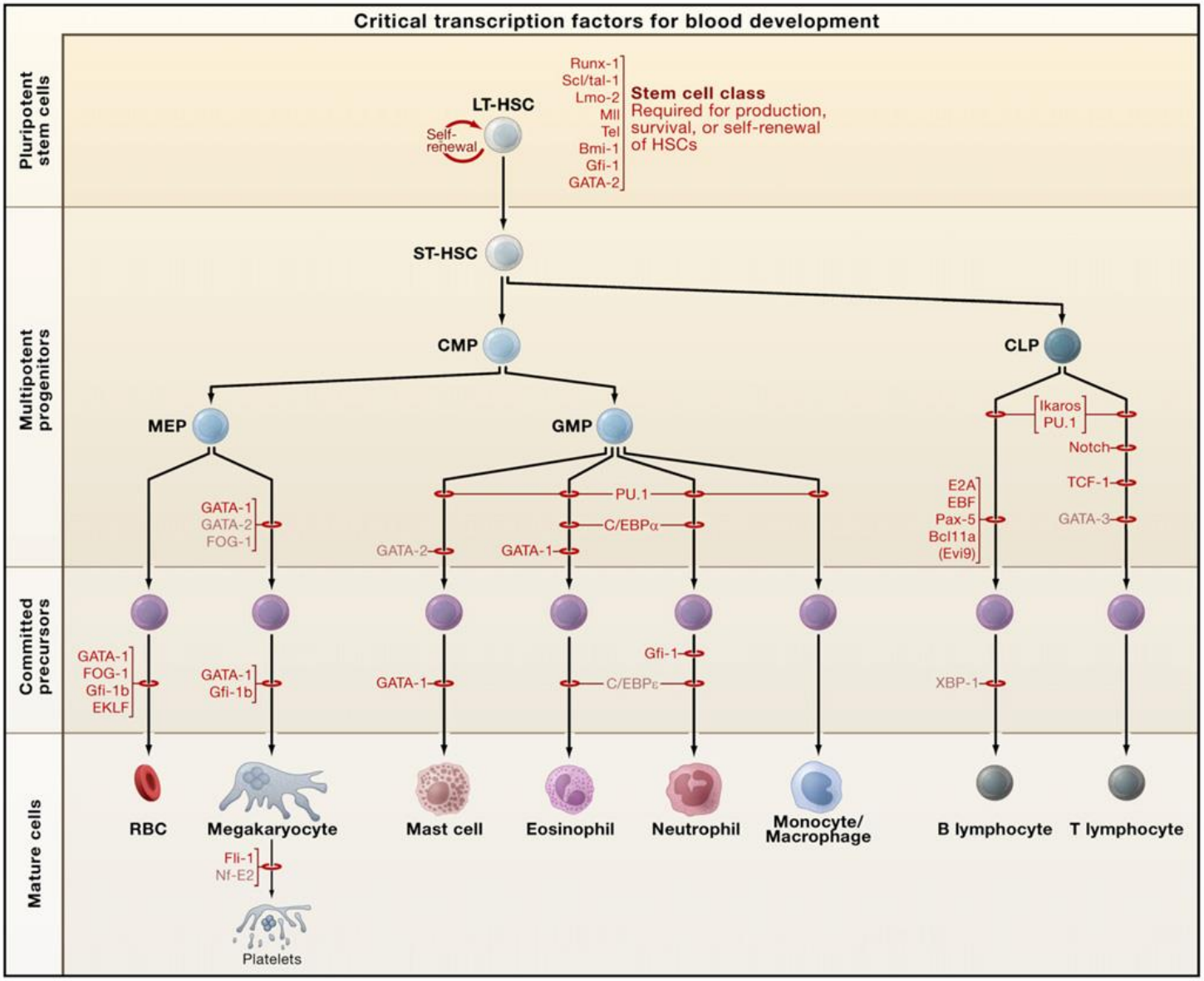
Differentiation is a key example of cell fate.

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

Hematopoiesis: development of blood cells



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

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

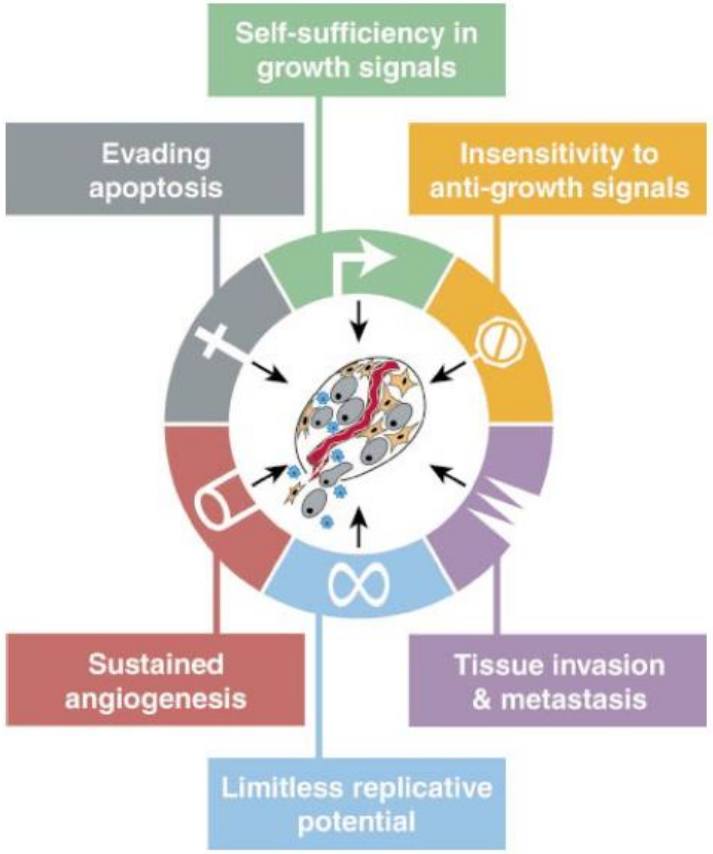


Figure 1. Acquired Capabilities of Cancer

The Hallmarks of Cancer

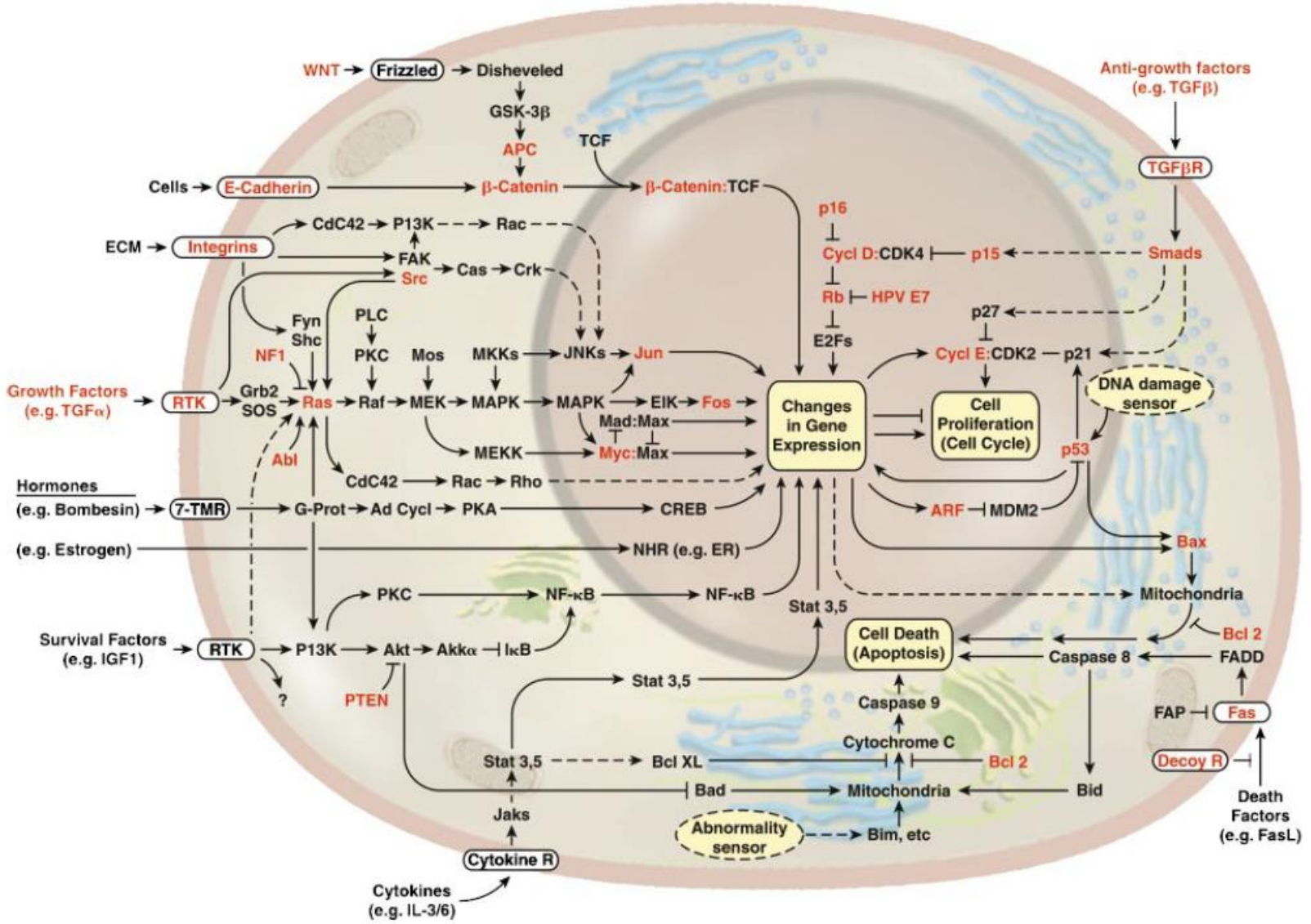


Figure 2. The Emergent Integrated Circuit of the Cell

The Hallmarks of Cancer

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

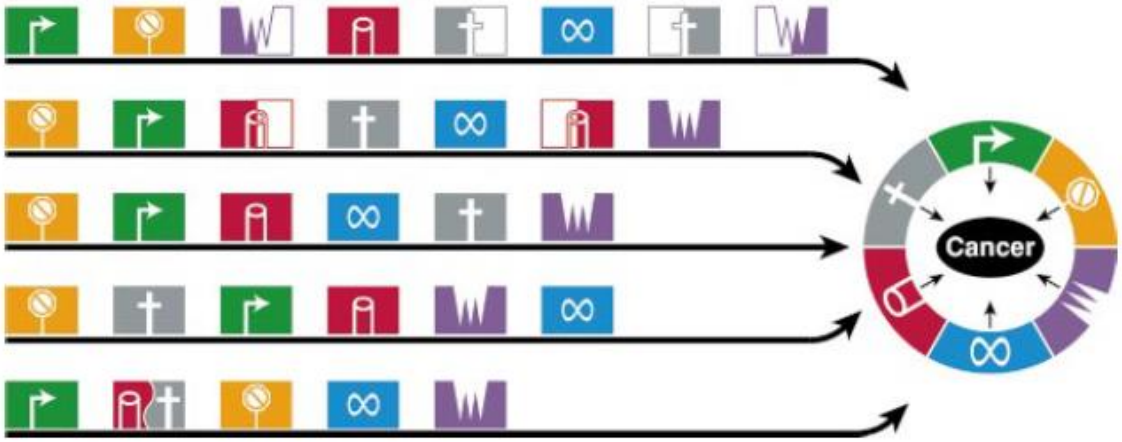
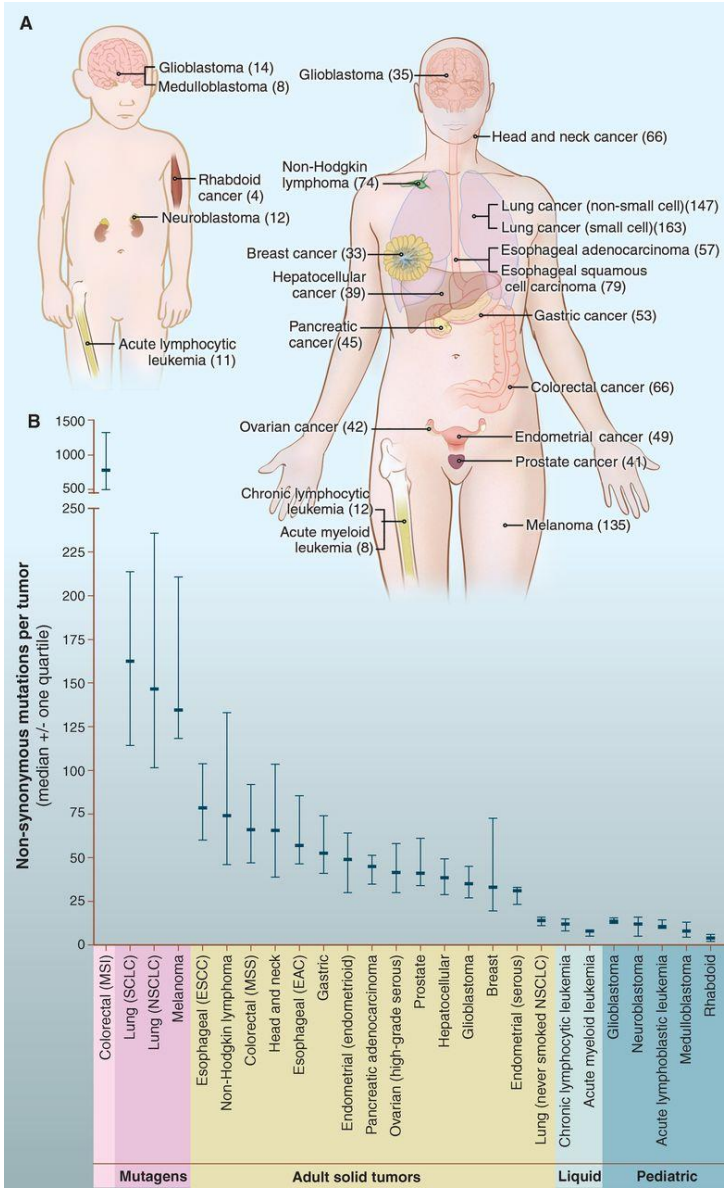


Figure 4. Parallel Pathways of Tumorigenesis

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.

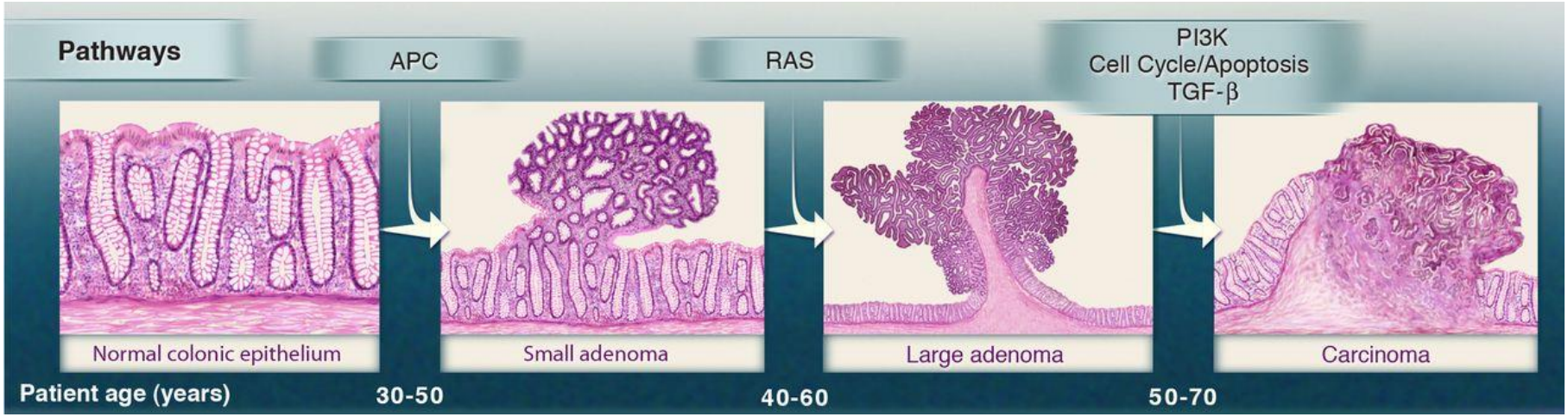
MSI, microsatellite instability;
SCLC, small cell lung cancers;
NSCLC, non–small cell lung cancers;
ESCC, esophageal squamous cell carcinomas;
MSS, microsatellite stable;
EAC, esophageal adenocarcinomas.

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

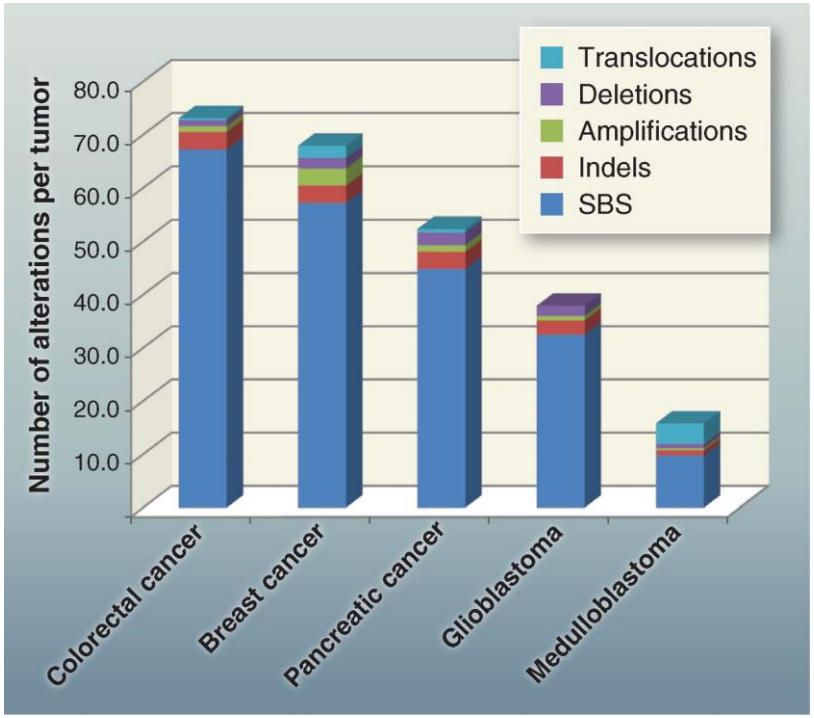


Patient age indicates the time intervals during which the driver genes are usually mutated.

TGF-β, transforming growth factor–β.

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

Alterations affecting protein-coding genes

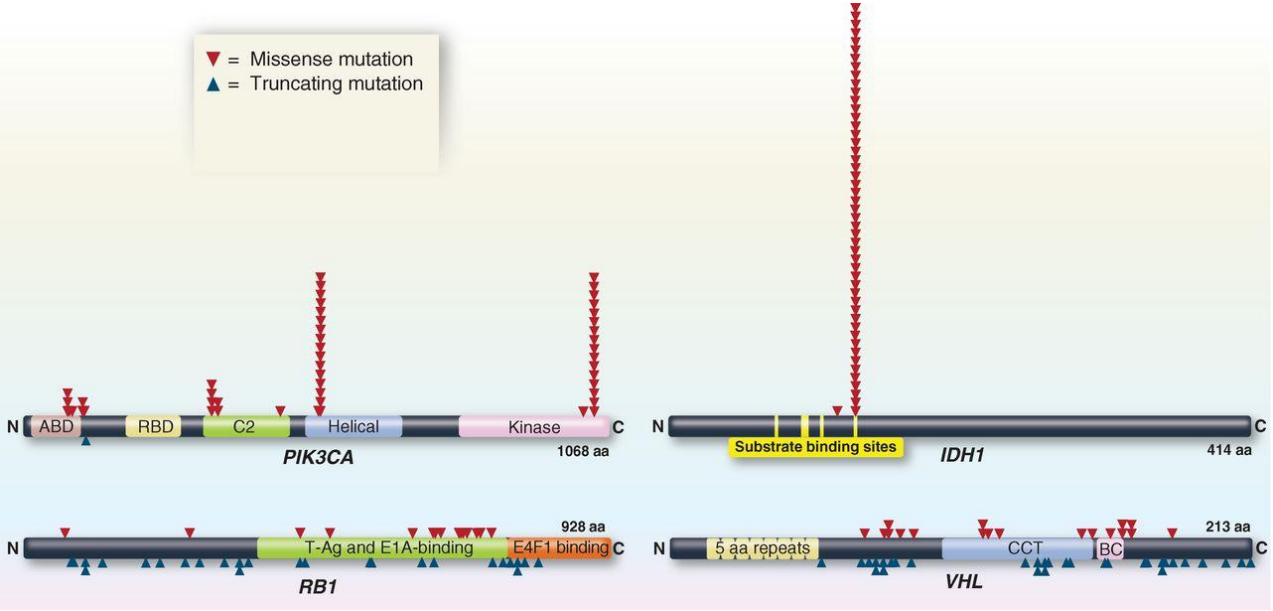


SBS: single-base substitutions (SBS),

Indels: small insertions and deletions,

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

Mutations in oncogenes and tumor suppressor genes



PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha)

IDH1: Isocitrate dehydrogenase 1

RB1: retinoblastoma protein

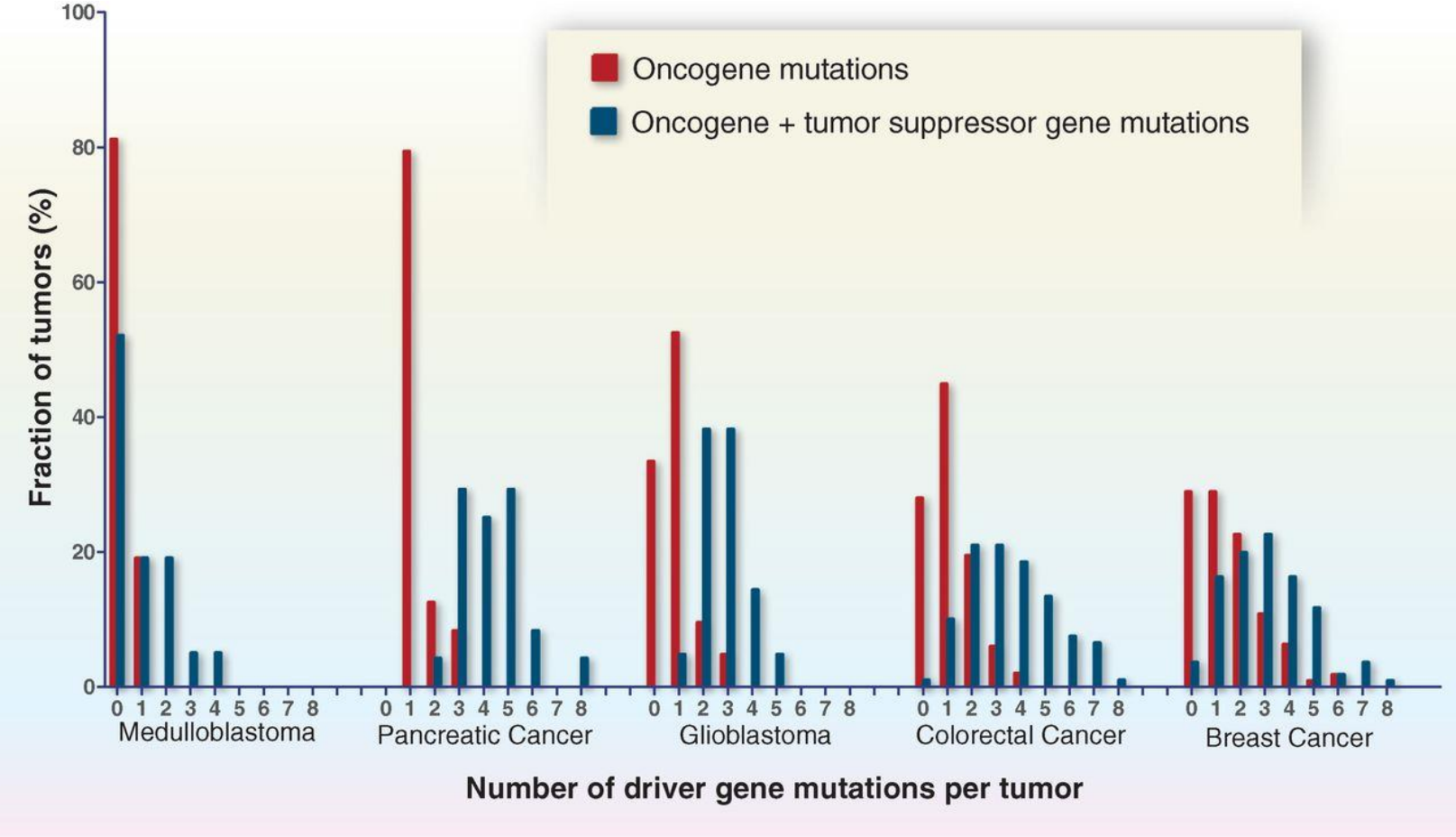
VHL: Von Hippel–Lindau tumor suppressor

Oncogenes PIK3CA and IDH1:
missense mutations accumulate at identical positions,
(almost) no truncation mutations

tumor suppressor genes RB1 and VHL:
truncating mutations and missense mutations
spread over the entire genes

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

Number of driver gene mutations per tumor



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

Genetic heterogeneity in tumors

Example: primary tumor in the pancreas and its metastatic lesions in the liver.

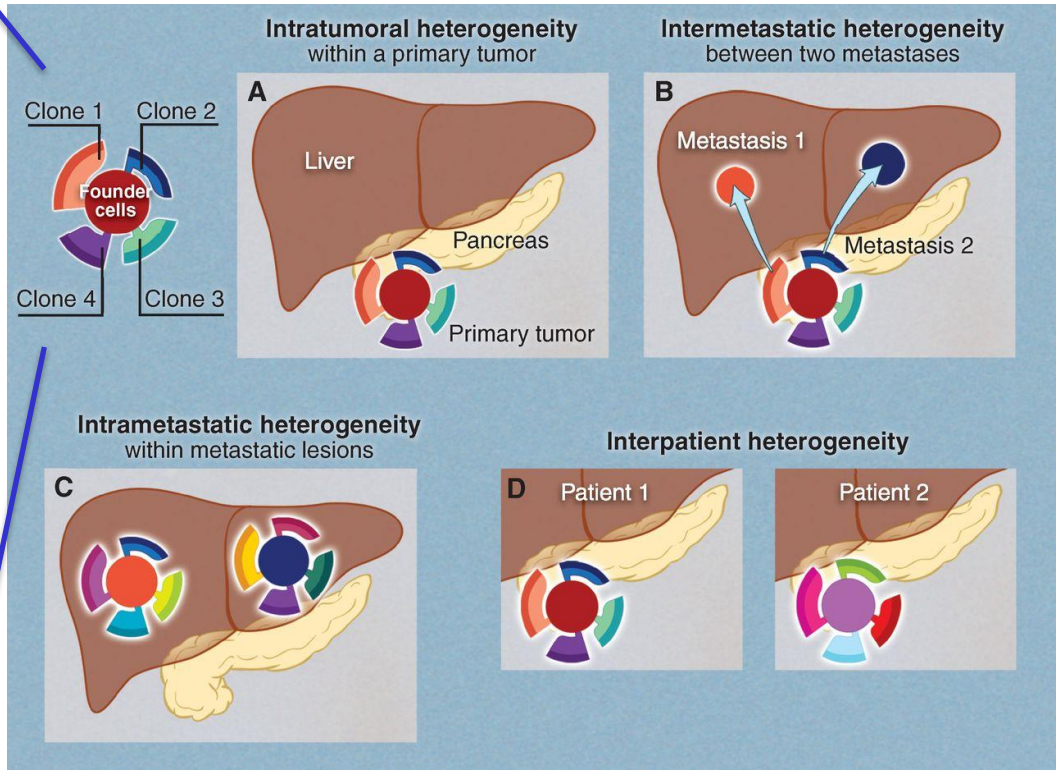
Mutations introduced during primary tumor cell growth result in clonal heterogeneity.

A typical tumor is represented by cells with a large fraction of the total mutations (founder cells) from which subclones are derived.

The differently colored regions in the subclones represent stages of evolution within a subclone.

heterogeneity among the cells of the primary tumor.

heterogeneity among different metastatic lesions in the same patient

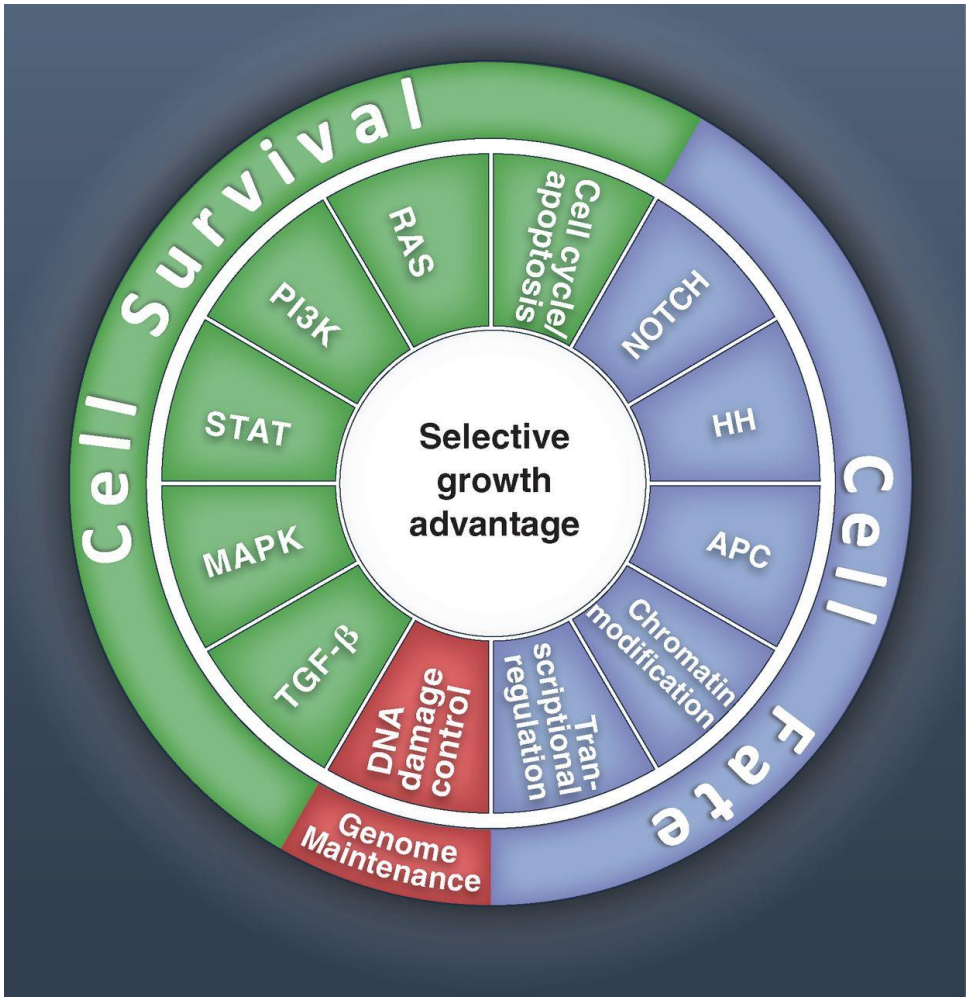


heterogeneity among the cells of each metastasis develops as the metastases grow

heterogeneity among the tumors of different patients. The mutations are almost completely distinct.

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

Cancer driver genes belong to 12 pathways



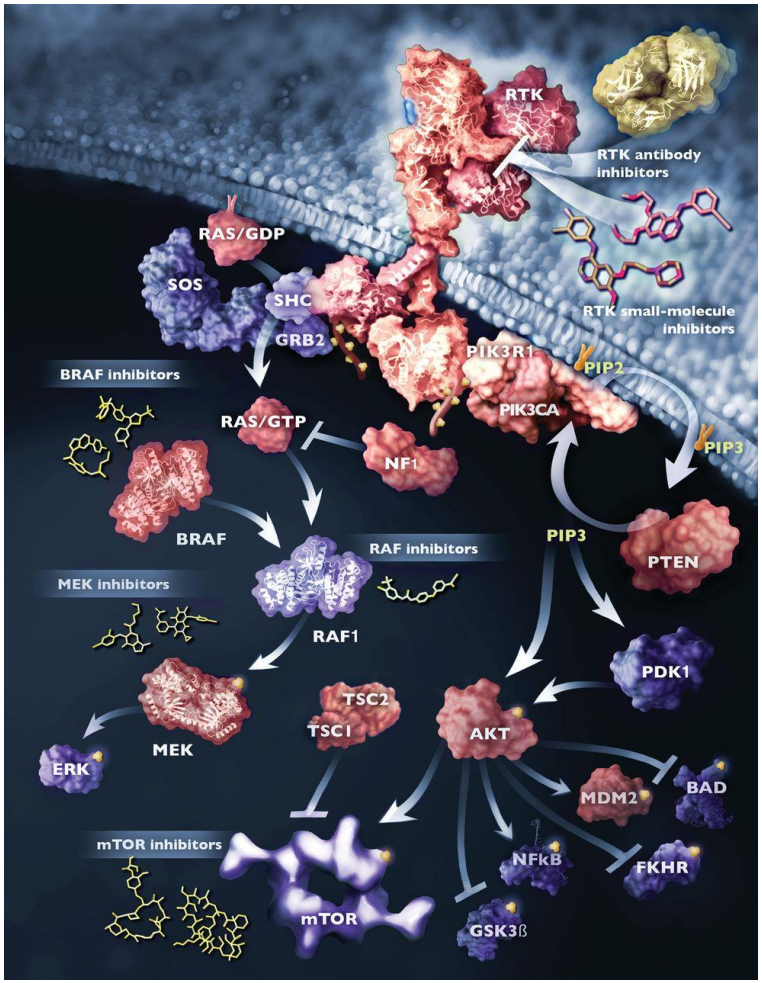
Cancer cell signaling pathways and the cellular processes they regulate.

All known driver genes can be classified into one or more of 12 pathways (middle ring).
All these pathways confer a selective growth advantage.

The pathways can themselves be further organized into 3 core cellular processes (outer ring).

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

Signal transduction pathways affected by mutations in human cancer



Two representative pathways (RAS and PI3K) are illustrated.
The signal transducers are color coded: red indicates protein components encoded by the driver genes;
yellow balls : sites of phosphorylation.
Stick models: therapeutic agents that target some of the signal transducers.
RTK, receptor tyrosine kinase;
GDP, guanosine diphosphate;
MEK, MAPK kinase;
ERK, extracellular signal–regulated kinase;
NFkB, nuclear factor κB;
mTOR, mammalian target of rapamycin.

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

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 behavioural 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.

What effect does temperature usually have on chemical reactions?

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

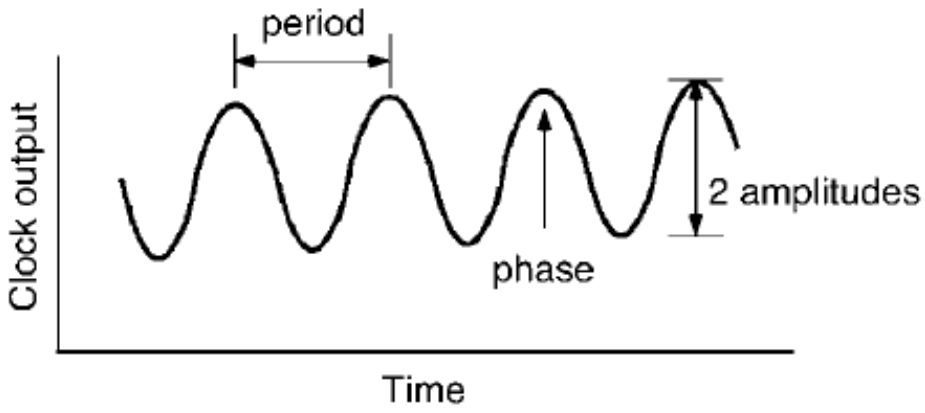
(3) a series of outputs tied to distinct phases of the oscillator
that regulate activity and physiology.

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)

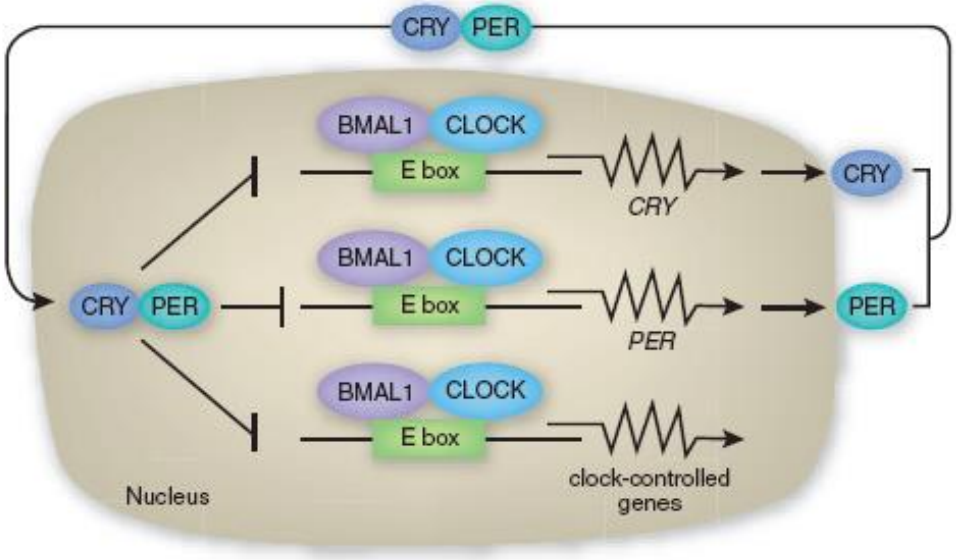
Autonomous oscillators everywhere

Remarkably, autonomous circadian oscillators are also present in all tissues of the body,
where they are synchronized by unidentified signals to regulate, in a tissue-specific manner, transcriptional activity throughout the day.

Paolo Sassone-Corsi,
UC Irvine

Eckel-Mahan & Sassone-Corsi,
Nat. Struct. Mol. Biol. 16, 462 (2009)

Basic molecular elements of the mammalian clock



This is the **minimal scheme** for the mammalian clock.

It requires several interconnecting transcriptional, translational and post-translational loops to achieve gene expression with circadian periodicity

(a) 2 TFs **CLOCK** and **BMAL1 heterodimerize**.

(b) BMA1:CLOCK binds to the **E-boxes** in the promoters of the *PER* and *CRY* genes, as well as in the clock-controlled genes, activating their transcription.

(c) The PER and CRY proteins dimerize, enter the nucleus and **inhibit** CLOCK-BMAL1–activated transcription.

Sancar,
Nat. Struct. Mol. Biol. 15, 23 (2008)

Full (?) circuit of circadian rhythms in mammals

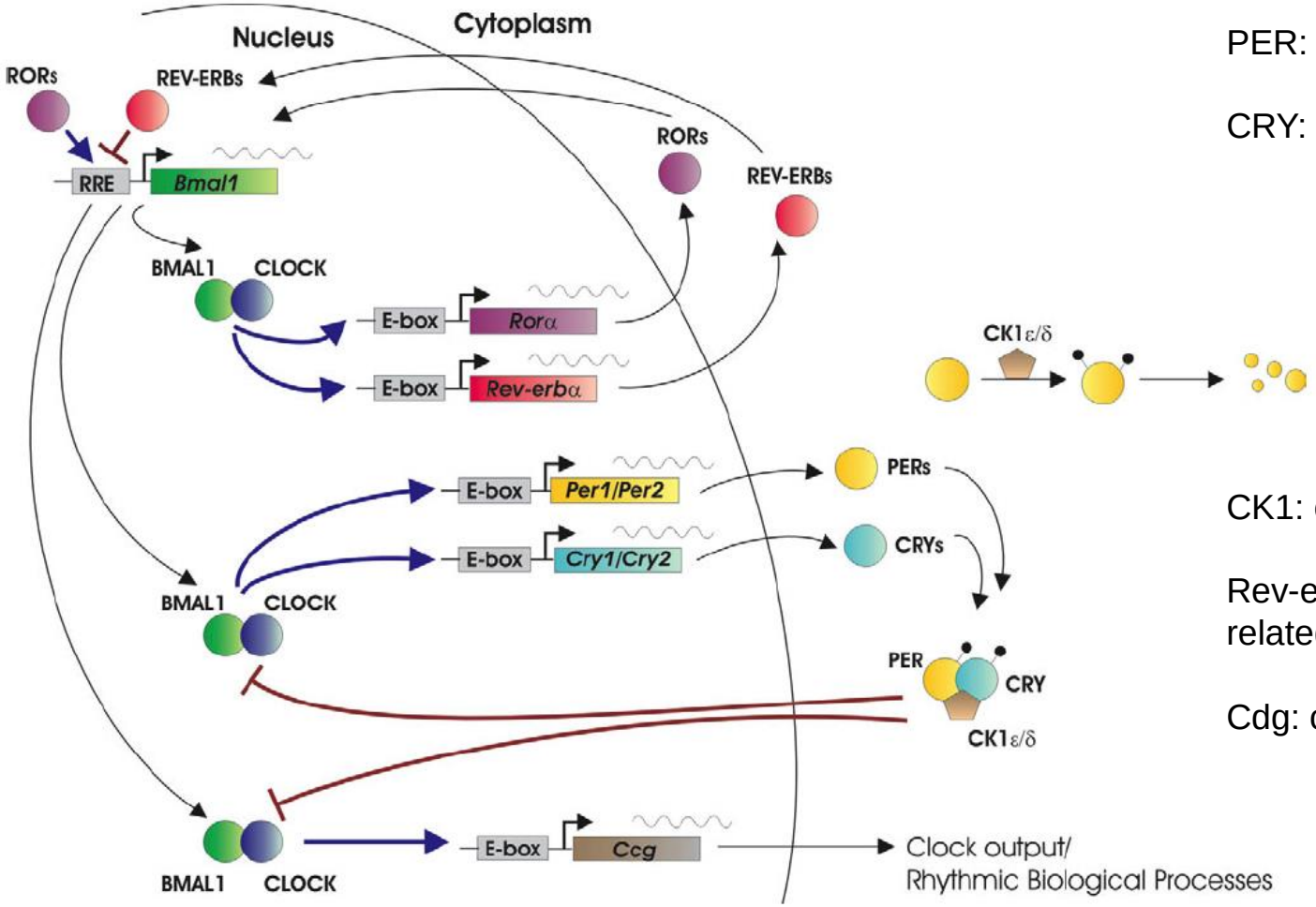


Figure 1. A network of transcriptional–translational feedback loops constitutes the mammalian circadian clock.

PER: period

CRY: cryptochrome

CK1: casein kinase

Rev-erb, ROR: retinoic acid-related orphan nuclear receptors

Cdg: clock-controlled gene(s)

Ko & Takahashi Hum Mol Genet 15, R271 (2006)

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.

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

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.*

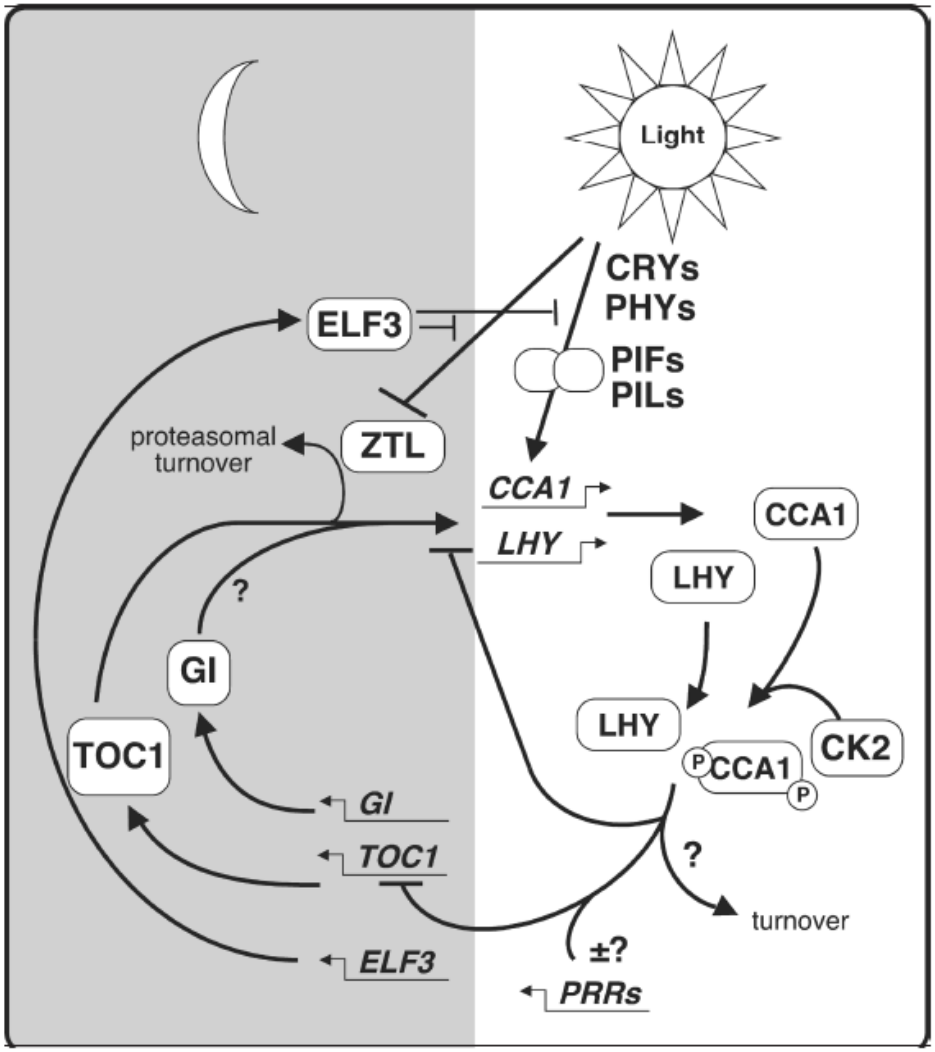
Salomé et al. J. Biol. Rhythms 19, 425 (2004)

Model of the *Arabidopsis thaliana* oscillator

Light perceived by the **PHYs** and **CRYs** 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.



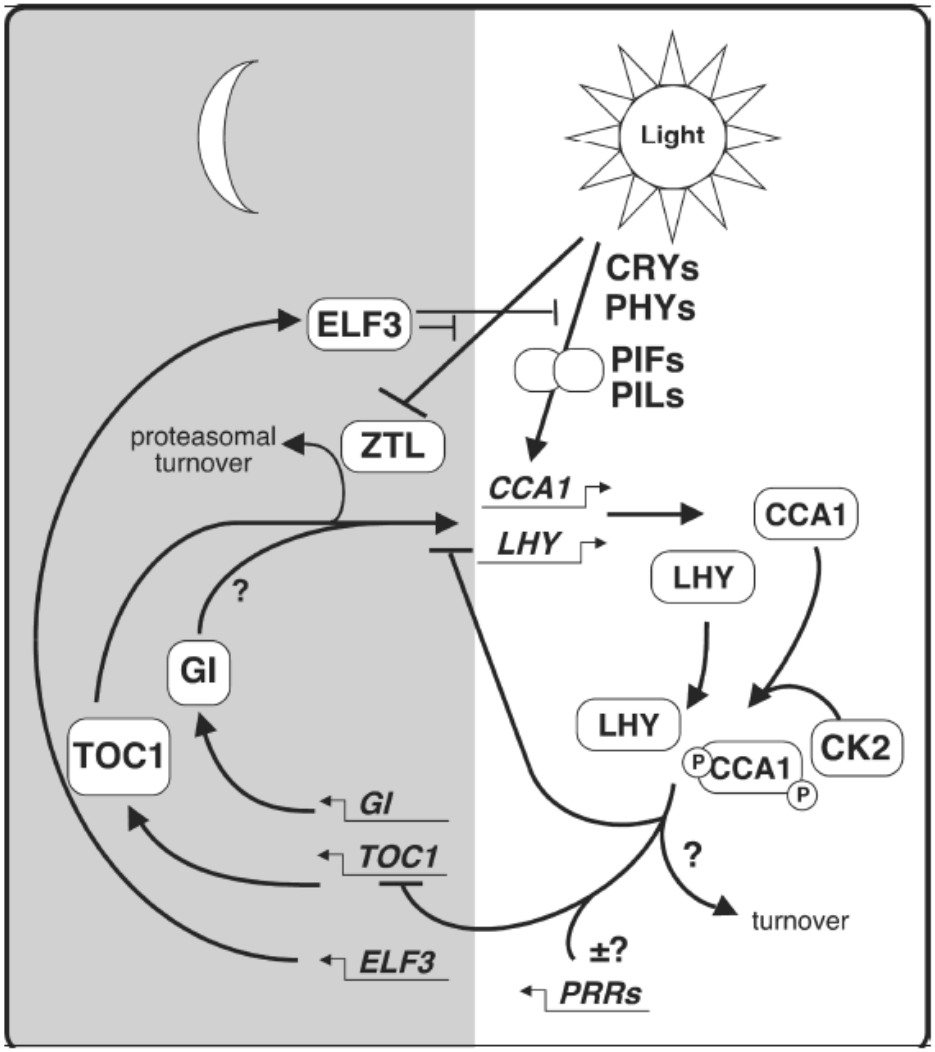
Salomé et al. J. Biol. Rhythms 19, 425 (2004)

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, following the turnover of CCA1 and LHY proteins.

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

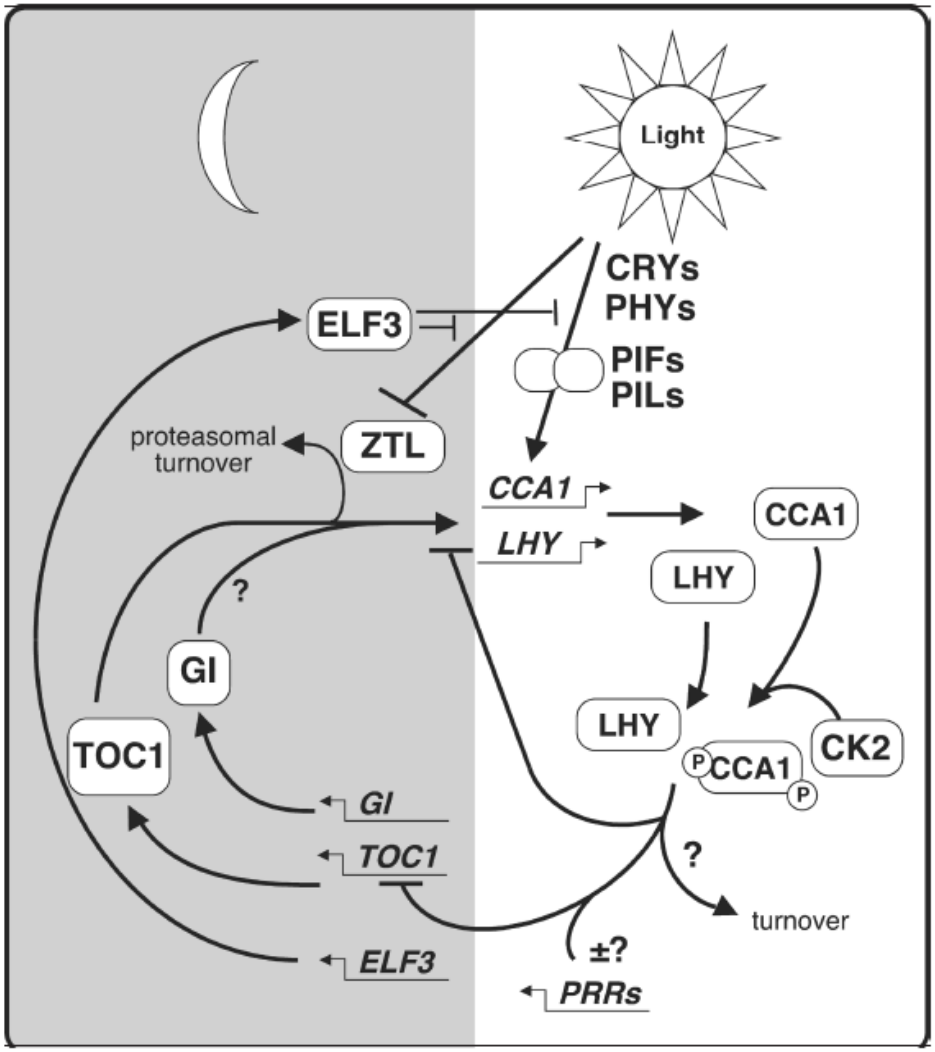


Salomé et al. J. Biol. Rhythms 19, 425 (2004)

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.

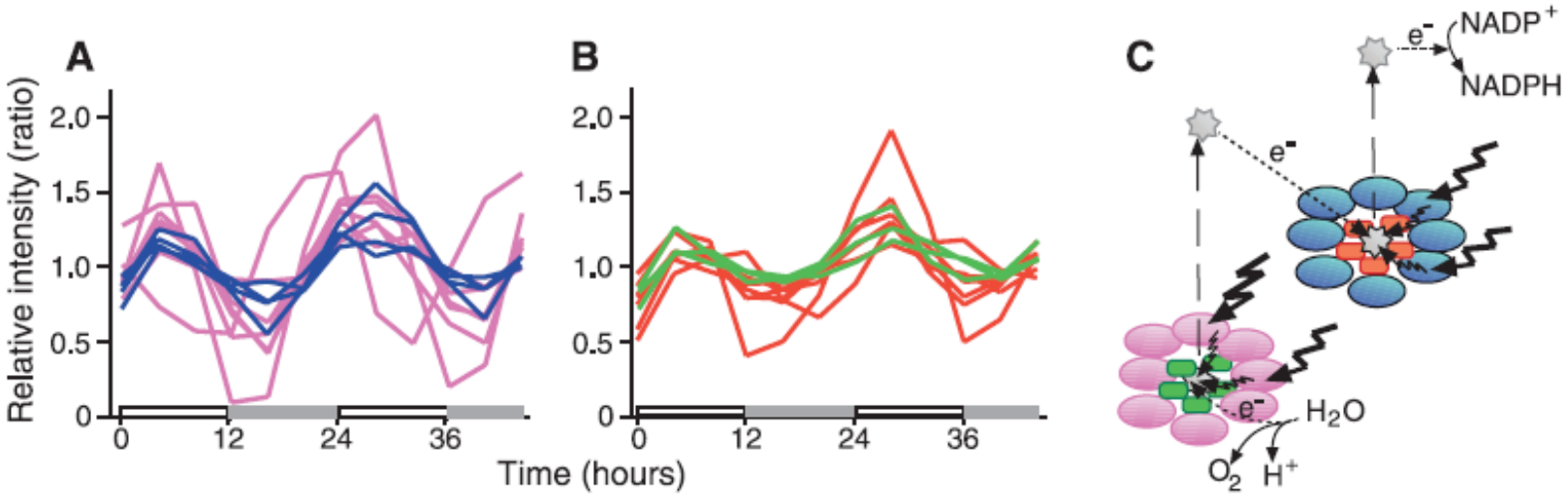
→ identify temporal patterns of gene expression in *Arabidopsis* plants under constant light conditions using GeneChip arrays representing about 8200 different genes.

Score all genes 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

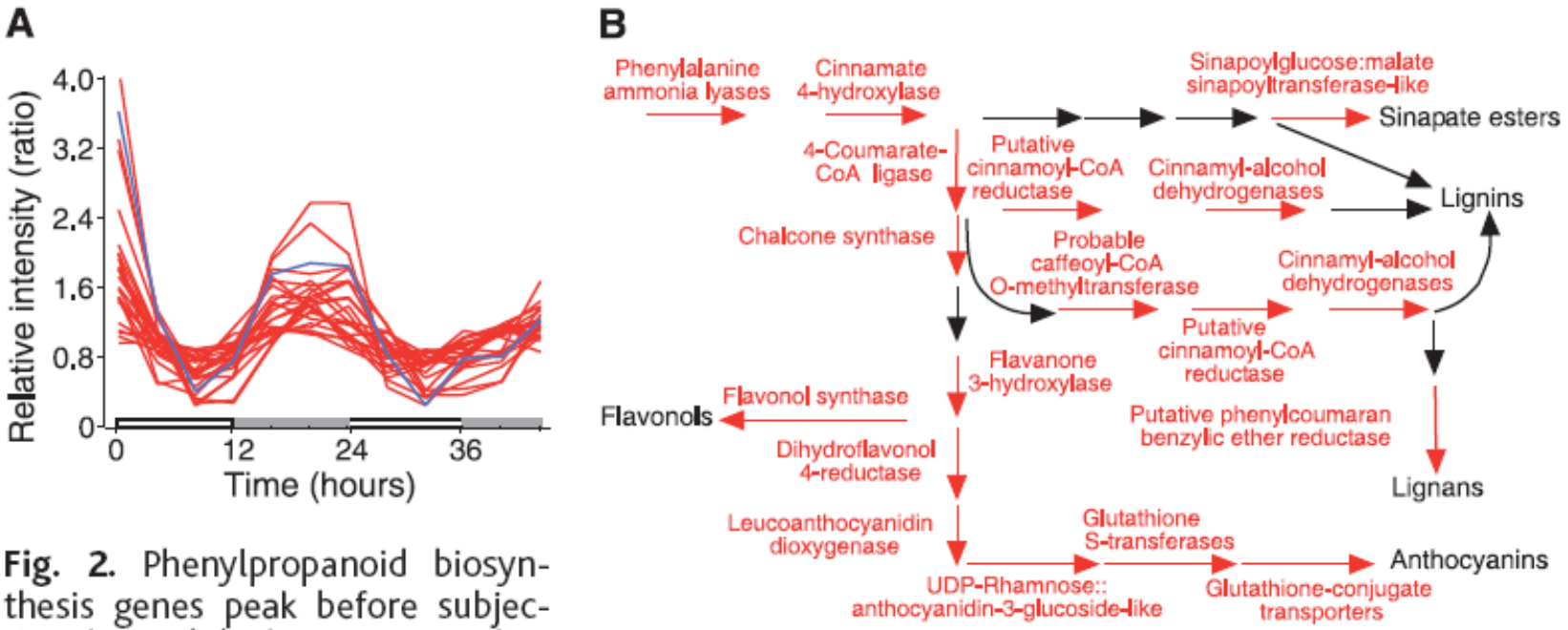


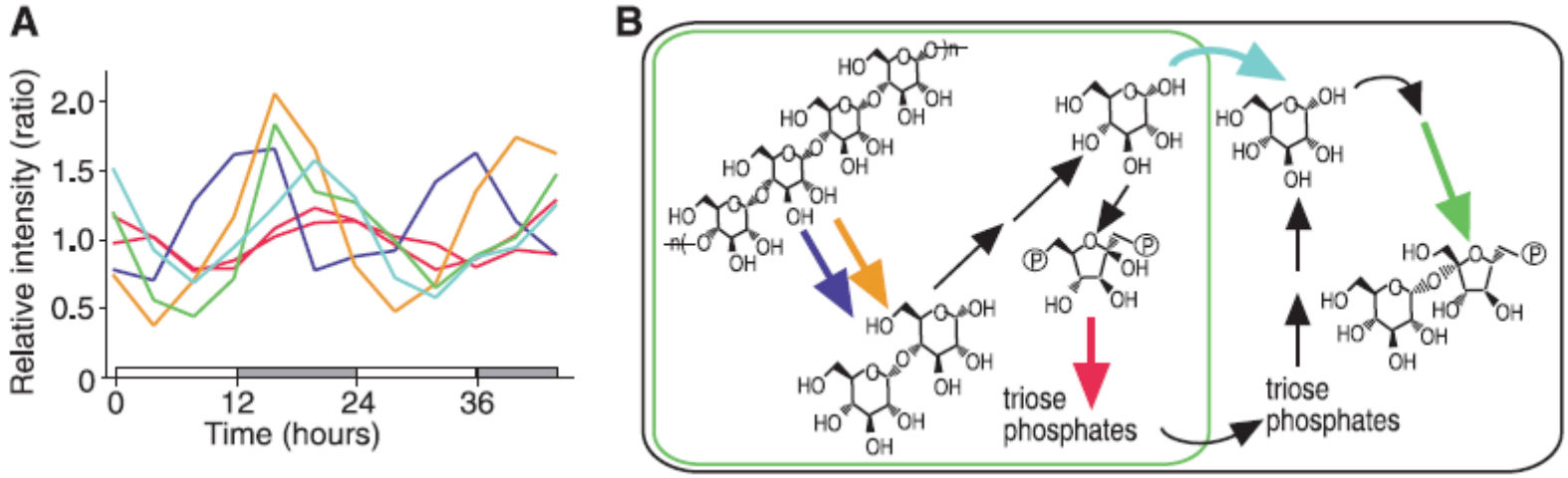
Fig. 2. Phenylpropanoid biosynthesis genes peak before subjective dawn. (**A**) The gene encoding the Myb transcription factor *PAP1* (accession number AAC83630) is in blue. The red traces represent phenylpropanoid biosynthesis genes. (**B**) Phenylpropanoid biosynthetic pathways. Genes encoding all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

„Phenolic sunscreen“

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

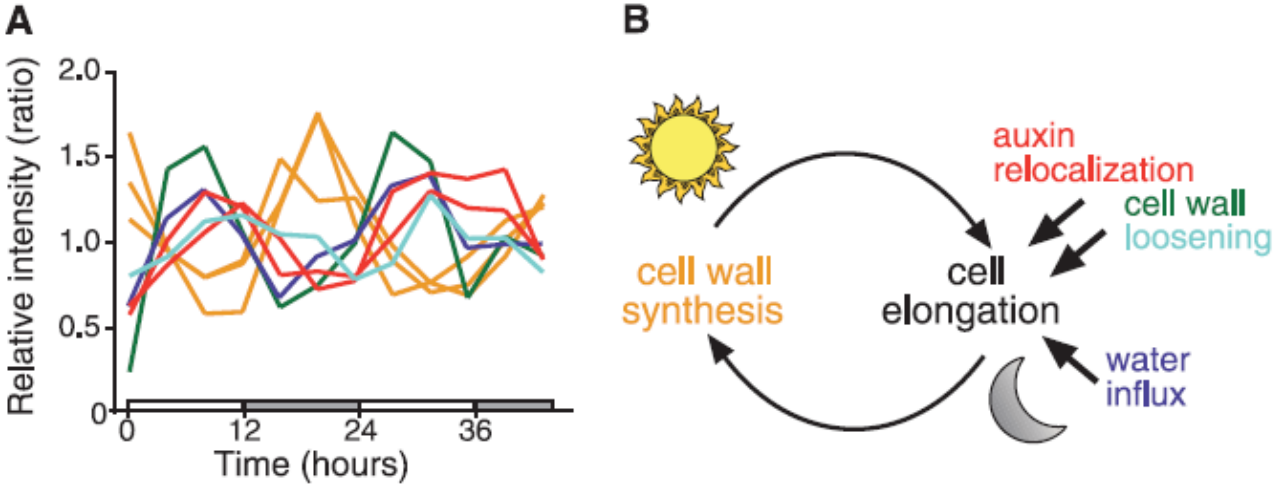
Chilling resistance is an important trait in plants.

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.

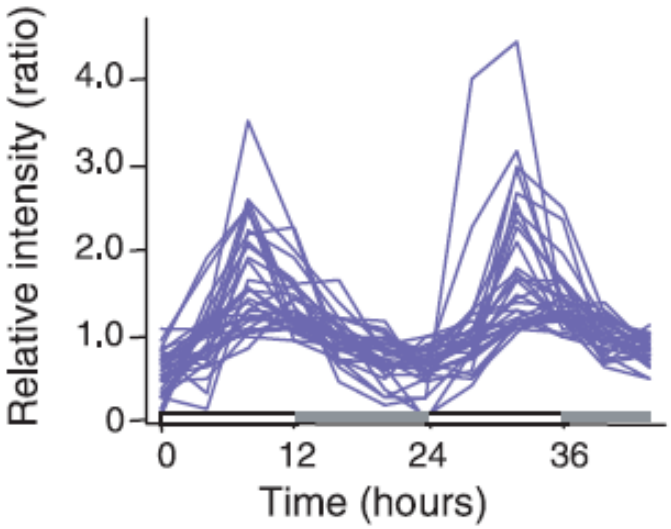
3 enzymes implicated in cell wall synthesis (all in gold) peak toward the end of the subjective night.

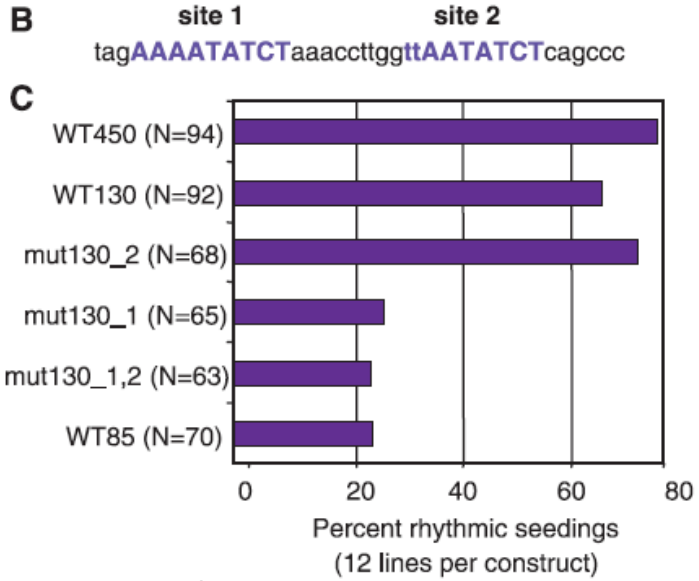
Harmer et al. Science 290, 2110 (2000)

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)

Summary

Cells need to tightly control their exact position in the cell cycle and in development.

Control during cell cycle: checkpoints + Cdk / cyclin system

Control during development: different chromatin states / epigenetics

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Cancerogenesis is determined by random apperance of driver mutations plus sofar poorly understood epigenetic changes.

Cellular differentiation and cancerogenesis involve similar players of the epigenetic machinery.

Next week: discuss paper
http://www.pnas.org/content/111/45/16219.abstract