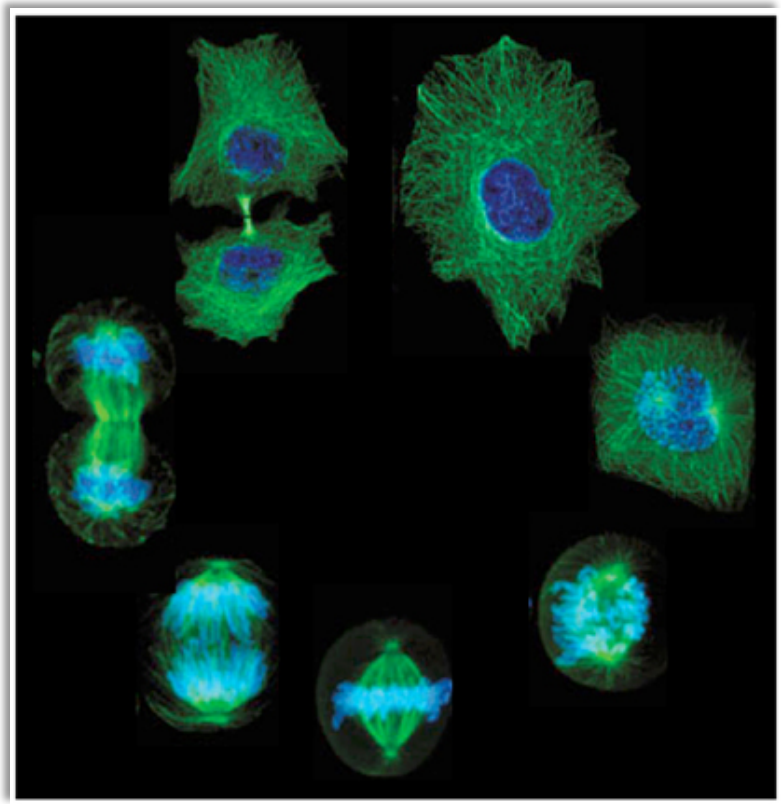
Cell Biology III

(The Cell Cycle)



http://25.media.tumblr.com/tumblr_lcn6tfAey11qezvqko1_500.jpg

Oct 29, Nov.5, Nov.1, Dec.3

Shin SUGIYAMA

Office: E207 Ext. 5039

ssugiya@bio.nagoya-u.ac.jp

図の出典

ECB

Essential 細胞生物学　原書第3版（Bruce Alberts他著、中村桂子・松原謙一監訳、南江堂、2011年）

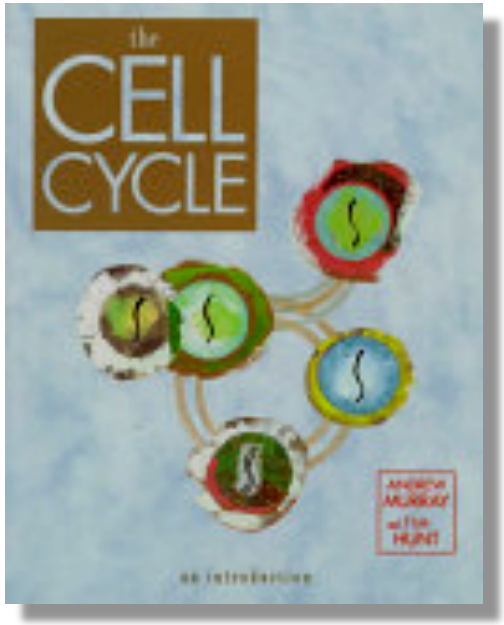
細胞の分子生物学　第5版（Bruce Alberts他著、中村桂子・松原謙一監訳、Newton Press、2010年）

The Cell Cycle an Introduction. Andrew Murray and Tim Hunt. Oxford Univ. Press 1993

Wikipedia

教科書

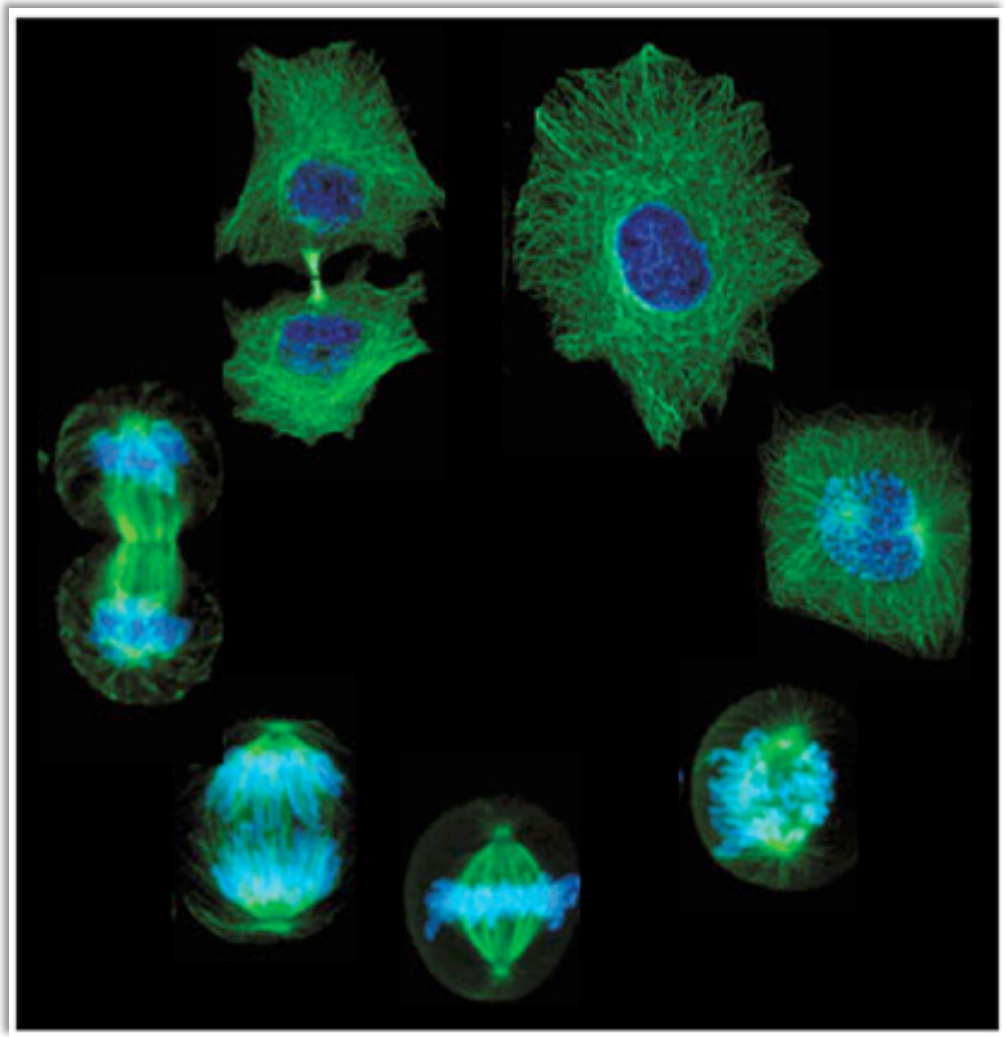
WIKIPEDIA



MBoC

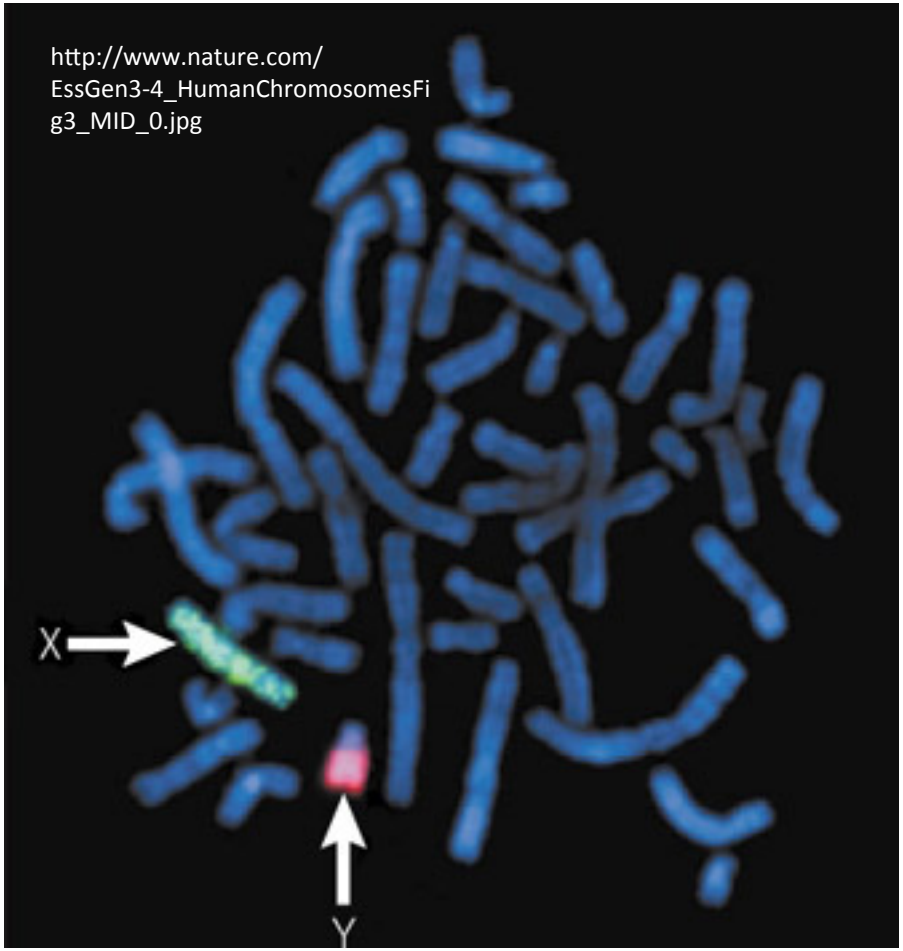
高価だけど、レフェランス本としては必要。

What is the cell cycle？



http://25.media.tumblr.com/tumblr_lcn6tfAey11qezvqko1_500.jpg

During the cell cycle the chromosomes are replicated and then equally divided to sister cells



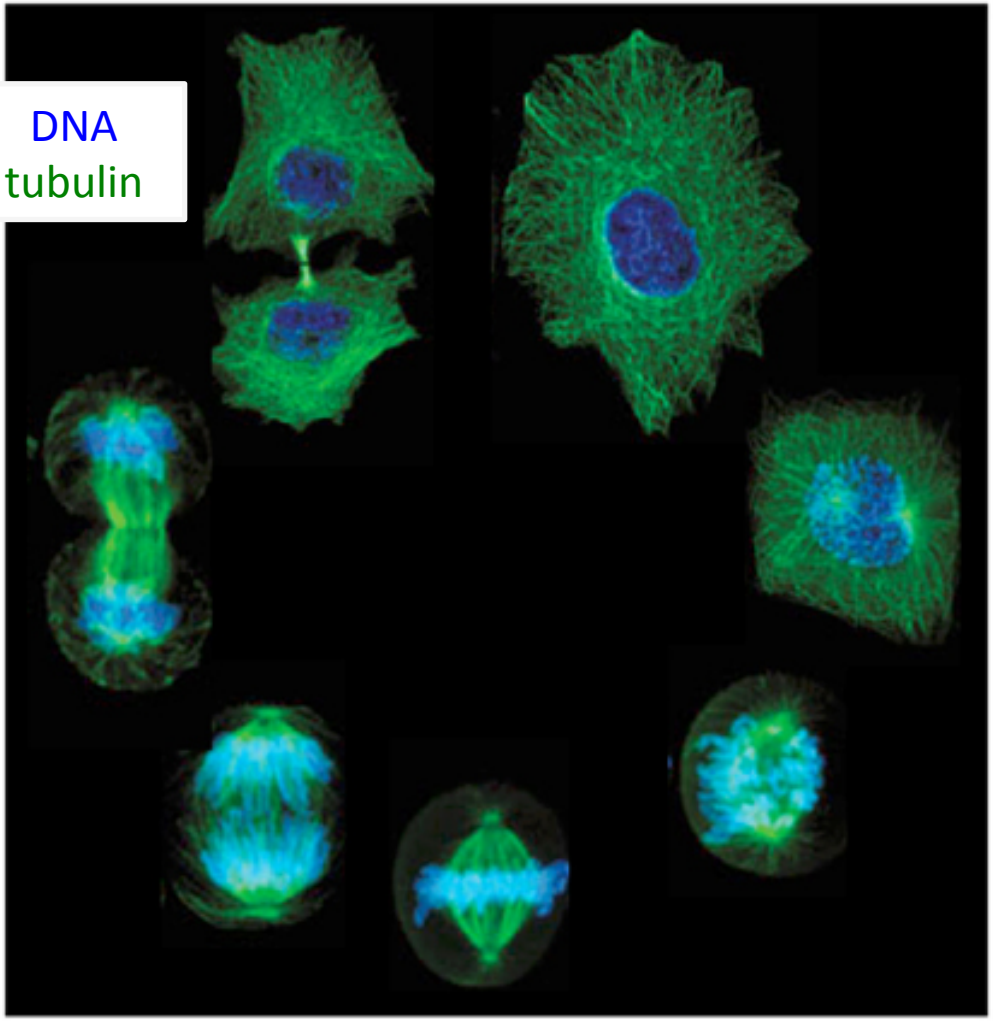
The cell cycle can be considered as being the replication and precise distribution of genetic material

Today's Concept I

Mitosis: Walther Flemming of Germany first descibed it in 1882

https://upload.wikimedia.org/wikipedia/commons/f/fe/Walther_flemming.gif

DNA
tubulin

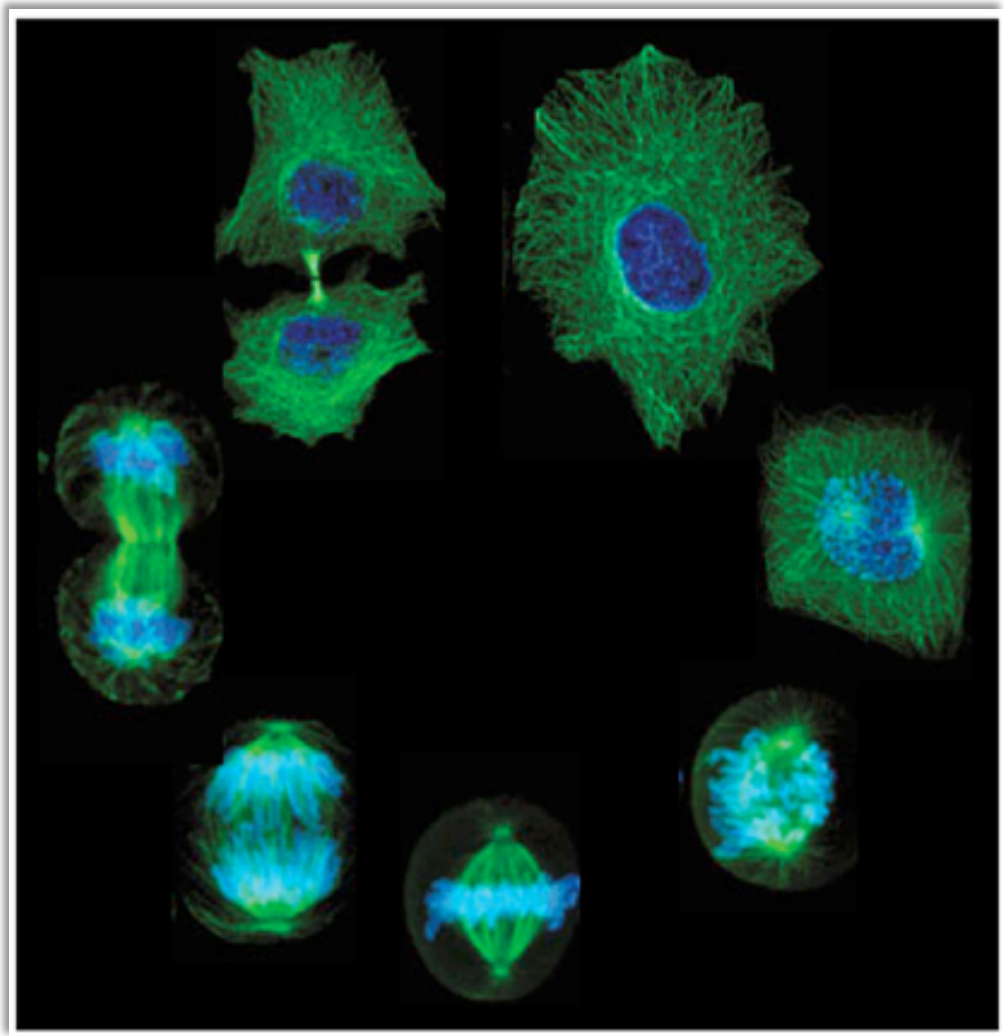


http://25.media.tumblr.com/tumblr_lcn6tfAey11qezvqko1_500.jpg

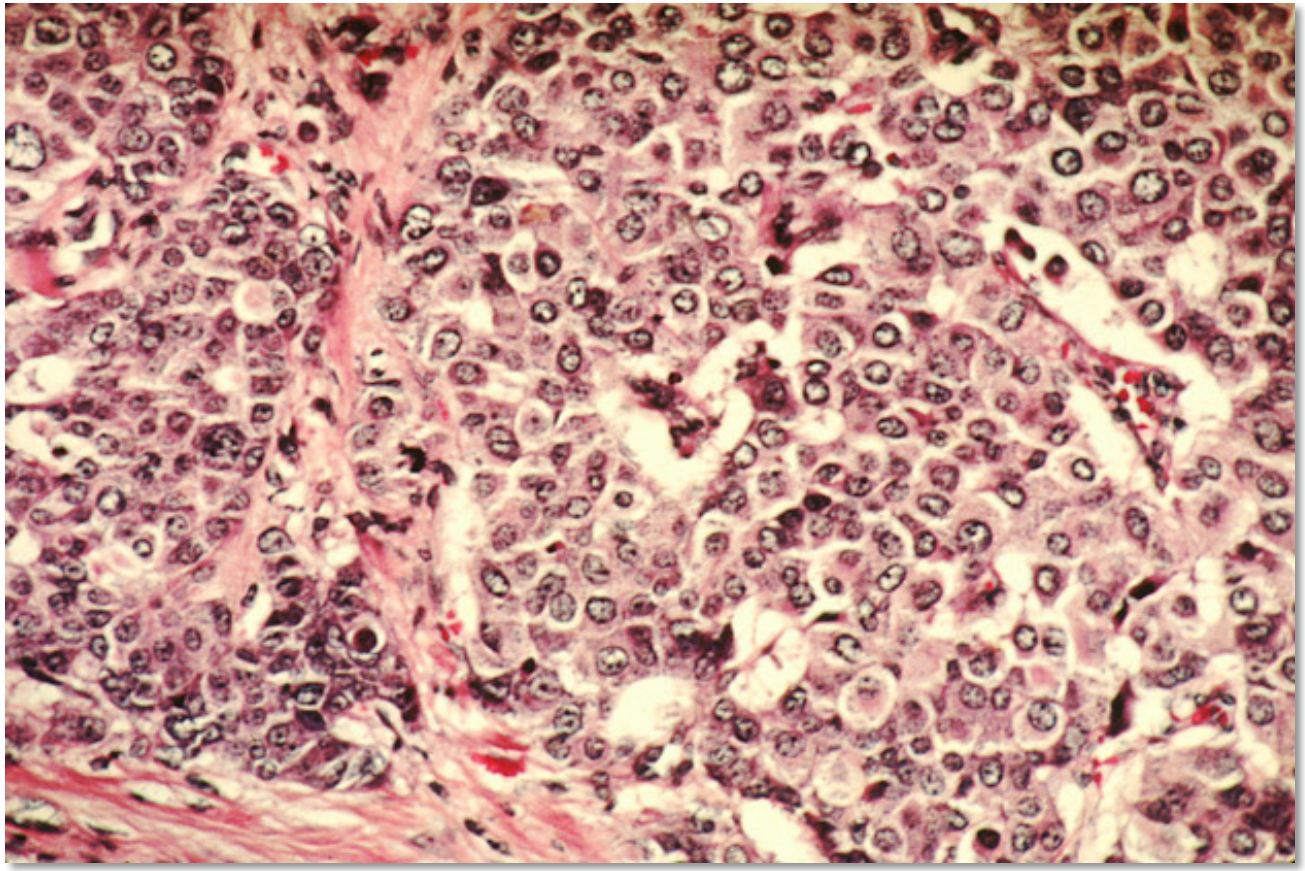
https://upload.wikimedia.org/wikipedia/commons/6/6d/Zellsubstanz-Kern-Kerntheilung.jpg

Mendel's theories were not widely known so he didin't appreciate the behavior of the chromosomes 。

Why is an understanding of the cell cycle important?



What happens when the cell cycle goes out of control?



http://www.nature.com/principles/ebooks/principles-of-biology-104015/9523064

example: **Breast Cancer**

Today's Concept II

Differentiation and the Cell Cycle

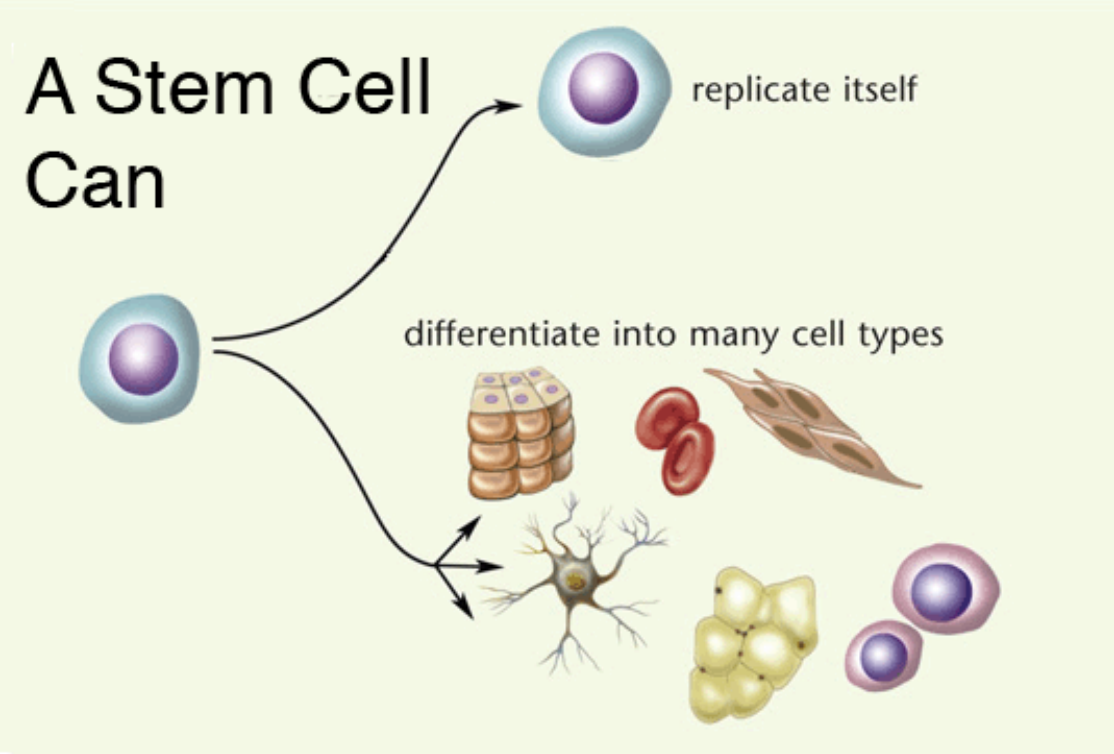


Image prepared by Catherine Twomey for the National Academies, *Understanding Stem Cells: An Overview of the Science and Issues* from the National Academies, www.nationalacademies.org/stemcells.

Undifferentiated : Proliferation ⟷ Differentiation : Less (No) Cell Division

Today's Concept III

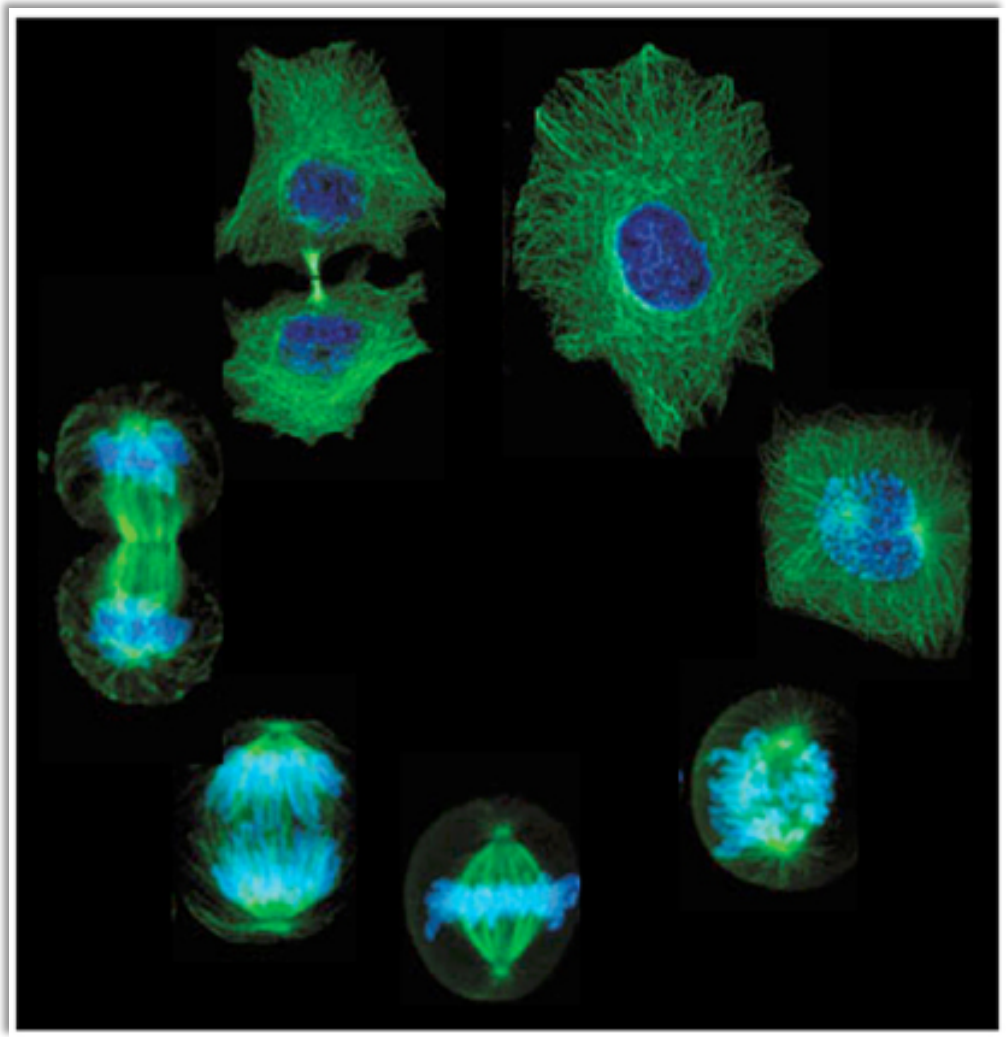
What will be taught

- Control of the cell cycle
- Mechanisms of mitosis
- Exceptions to the rule

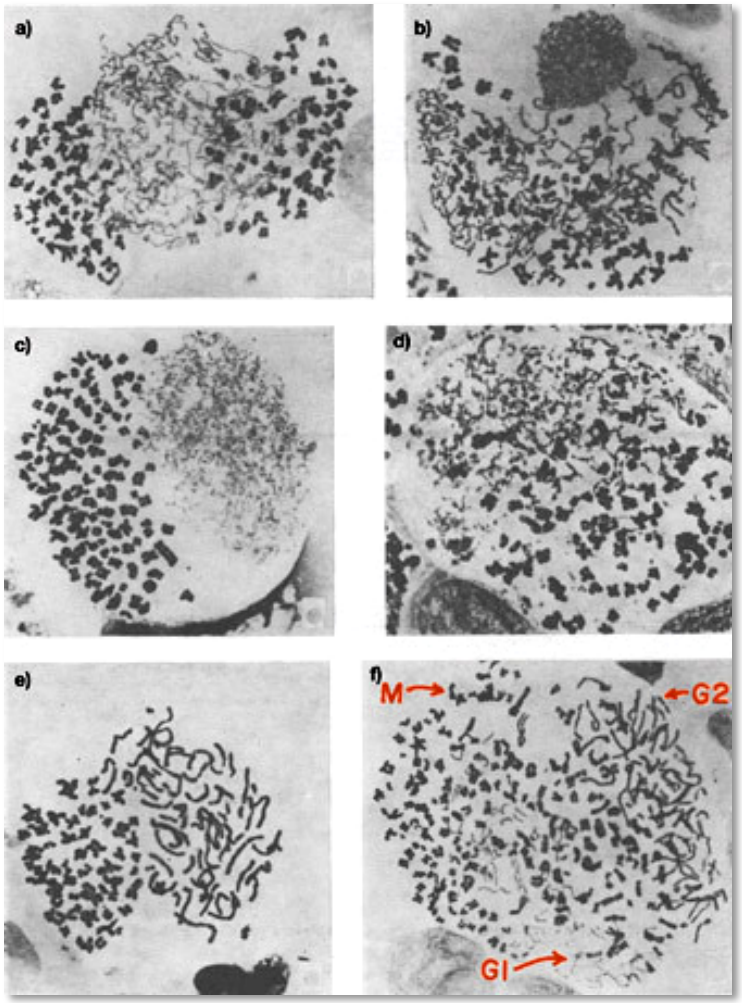
What won't be taught

- The prokaryotic cell cycle
- Meiosis
- DNA replication

How is the cell cycle controlled？



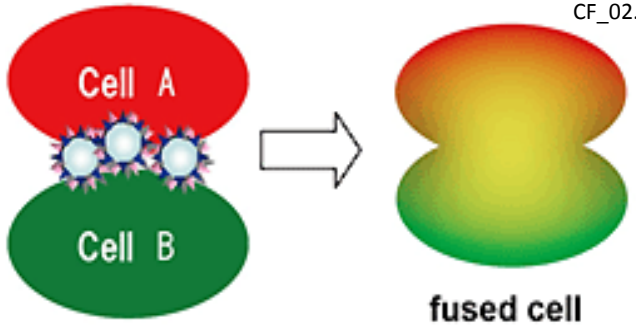
The experiment that got things started



Cells at different phases of the cell cycle were fused together and sometmes the cell phases were shifted or delayed.

Cell fusion

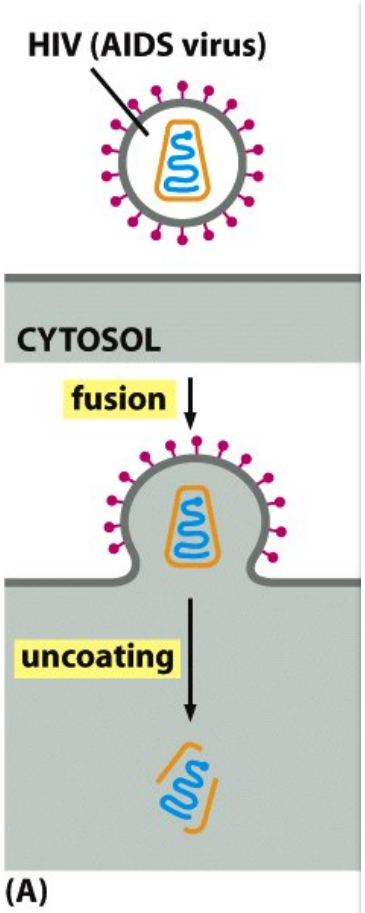
http://www.cosmobio.co.jp/export_e/products/cells/products_ISK_20070518/CF_02.gif



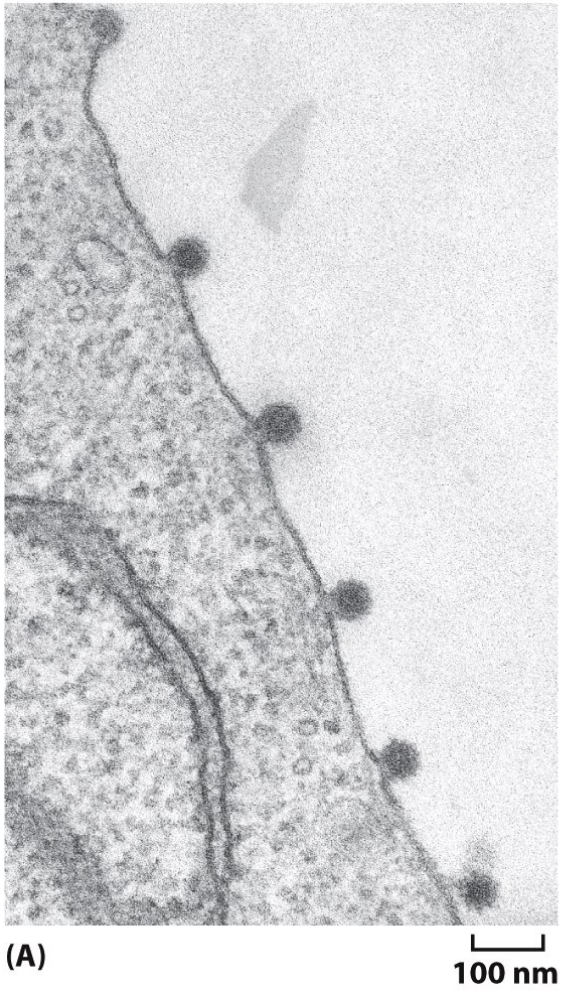
Viruses can be used to make cells fuse.

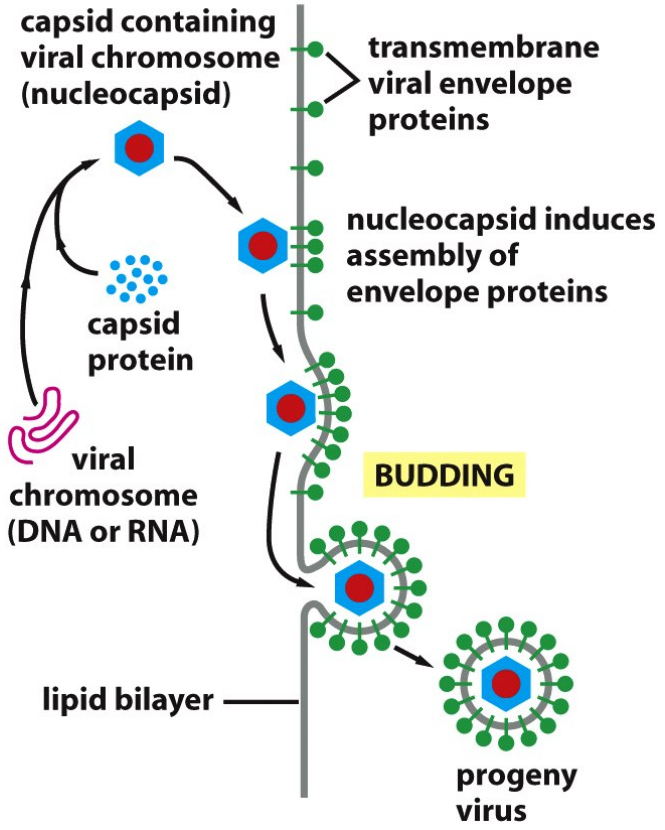
Johnson, R.T. & Rao, P.N., Mammalian Cell Fusion: Induction of Premature Chromosome Condensation in Interphase Nuclei.
Nature 226, 717–722 (1970)

How do viruses induce cell fusion?



MBoC p.1506





MBoC p.1497

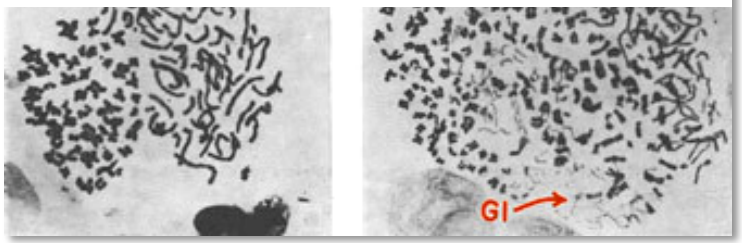
Figure 24-24 *Molecular Biology of the Cell* (© Garland Science 2008)

The experiment that got things started

1. When S phase cells and G1phase cells were fused, the G1 cells replicated DNA prematurely.

2. However, when S phase cells and G2phase cells were fused, G2 cells would NOT replicate DNA.

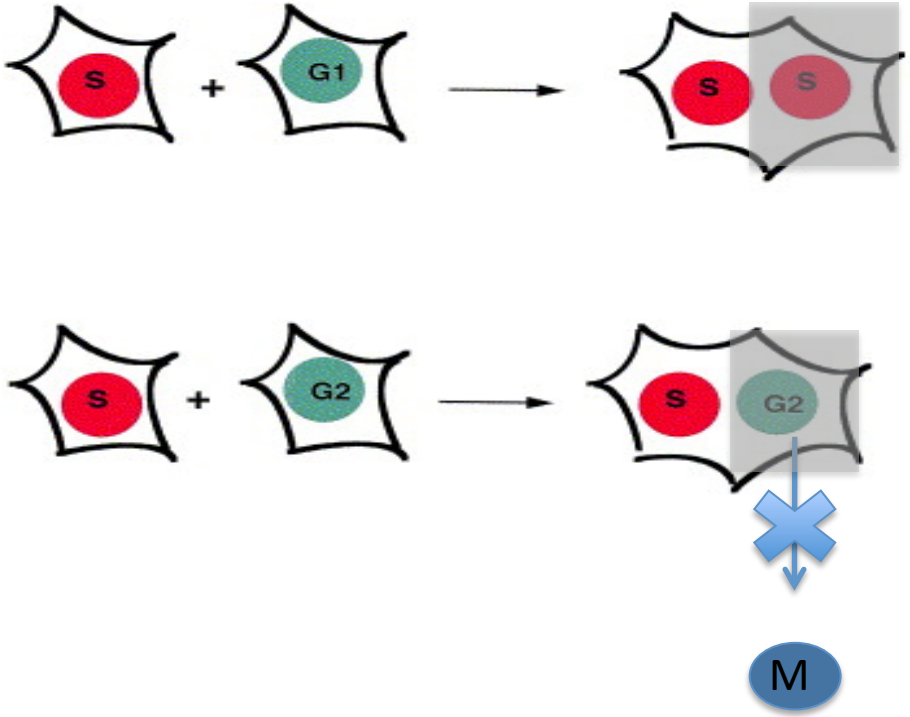
3. When S phase cells and G2phase cells were fused, G2 cells did not proceed to M phase.



Usually: G1 → S → G2 → M → G1

What happened…

http://ars.sciencedirect.com/content/image/1-s2.0-S0304419X97000334-gr1.jpg



Johnson, R.T. & Rao, P.N., Mammalian Cell Fusion: Induction of Premature Chromosome Condensation in Interphase Nuclei.
Nature 226, 717–722 (1970)

The experiment that got things started

(i) Existance of factor in S phase cells that induces DNA replication.

Licensing Factor

(ii) G1 phase cells can replicate DNA but G2 phase cells can not.

Re-replication block

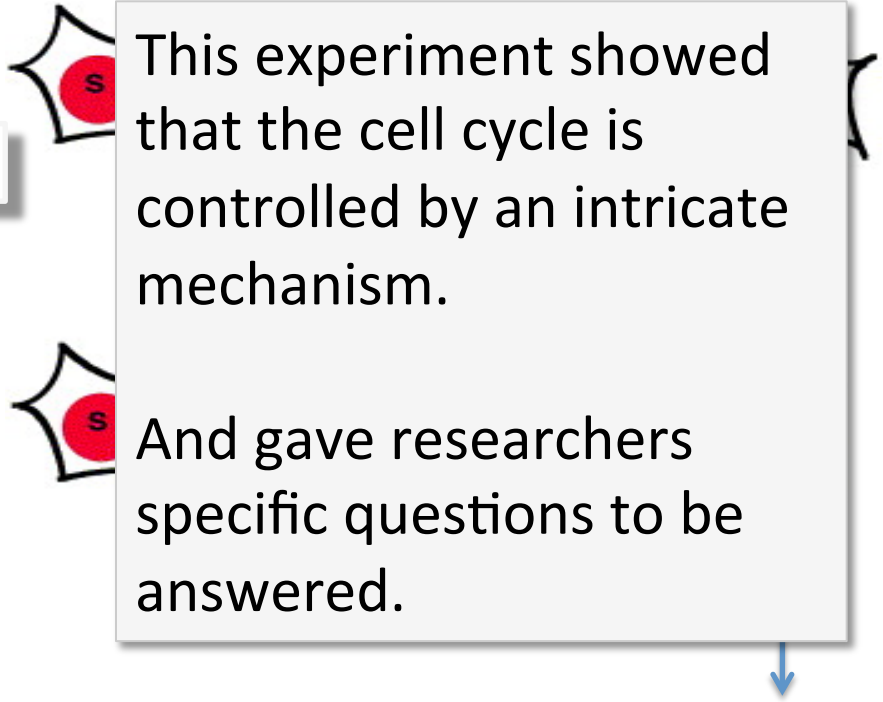
(iii) Existance of factor in S phase cells that blocks progression to M phase.

Check Point

(iv) Once cells have gone through M phase they can replicate DNA again.

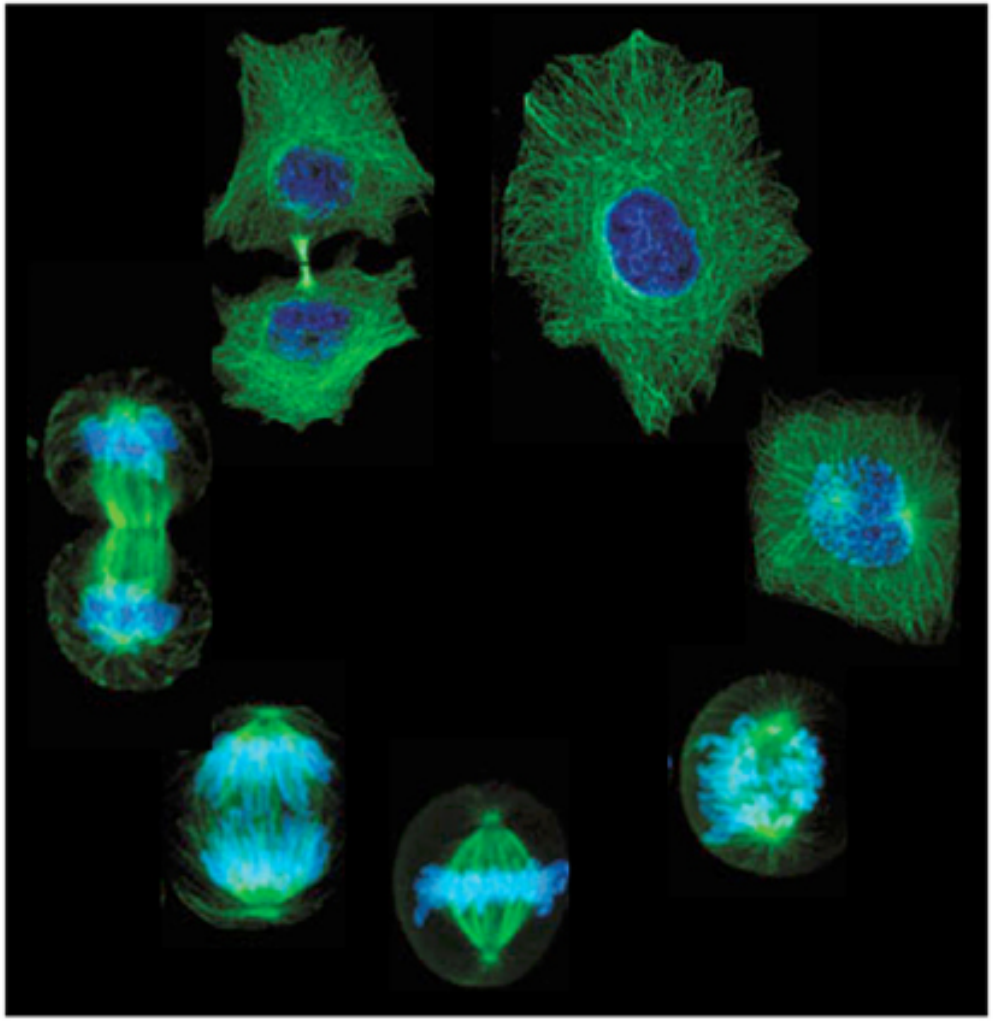
Re-licensing

What does all this suggest?



Johnson, R.T. & Rao, P.N., Mammalian Cell Fusion: Induction of Premature Chromosome Condensation in Interphase Nuclei.
Nature 226, 717–722 (1970)

MPF, Cyclin, Cdk



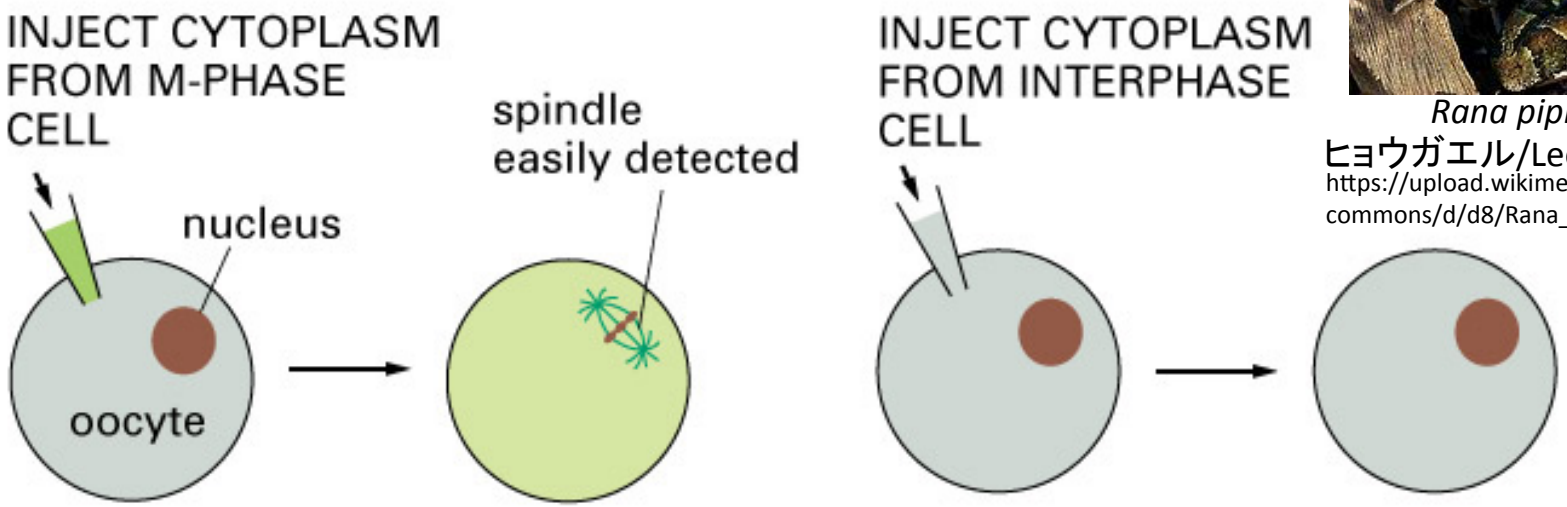
Discovery of MPF activity

(maturation/mitosis promoting factor)

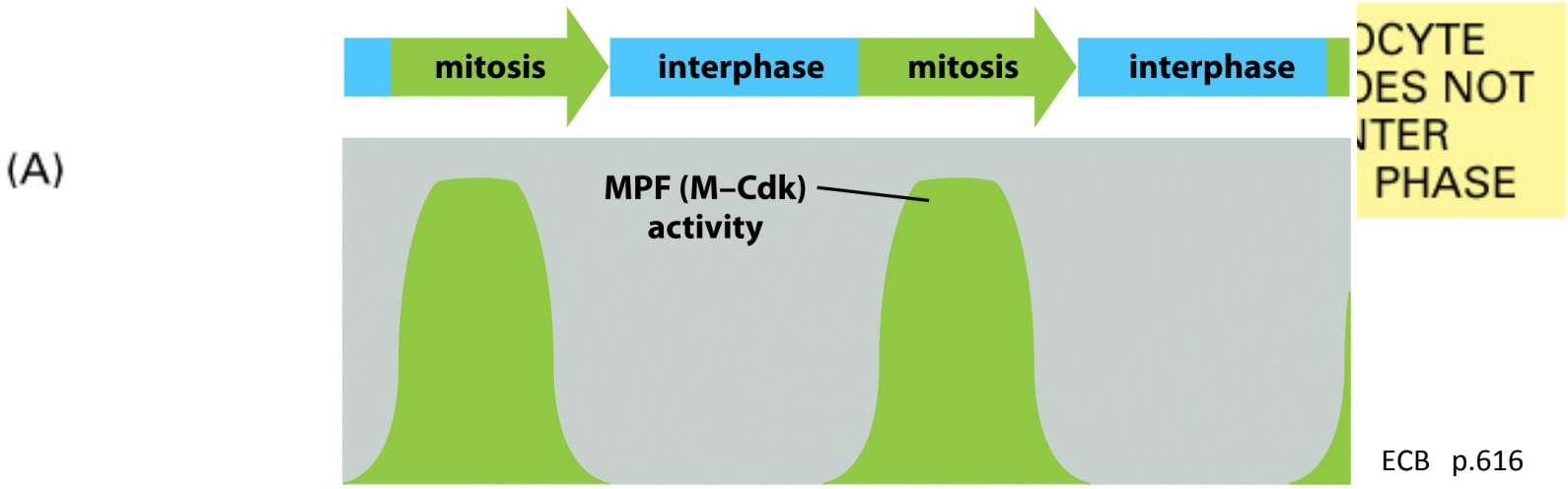
Rana pipiens

ヒョウガエル/Leopard Frog

https://upload.wikimedia.org/wikipedia/commons/d/d8/Rana_sphenocephala.jpg



ECB p.616



ECB p.616

Figure 18-9 Essential Cell Biology, Figure 18-8 Essential Cell Biology 3/e (© Garland Science 2010)

Cytoplasmic Control of Nuclear Behavior during Meiotic Maturation of Frog Oocytes [1]

YOSHIO MASUI [2] AND CLEMENT L. MARKERT
Department of Biology, Yale University, New Haven, Connecticut 06520

ABSTRACT Fully grown oocytes of the frog (*Rana pipiens*) undergo cytoplasmic and nuclear maturation when treated with progesterone after the follicular envelopes have been removed. The mechanism of this maturation was investigated by injection of cytoplasm from progesterone-treated oocytes at various stages of maturation into fully grown but immature oocytes. The injected cytoplasm becomes effective in inducing maturation by 12 hours after progesterone administration, reaches a maximum effectiveness around 20 hours, and then declines after the donor oocytes complete maturation. However, even cytoplasm from early embryos retains some capacity to induce oocyte maturation. The frequency with which maturation is induced is proportional to the volume of the injected cytoplasm. Progesterone itself is not directly responsible for the maturation-producing effect of injected cytoplasm since injected progesterone does not promote maturation. However, externally applied progesterone does induce the completion of the first meiotic division, presumably by releasing a cytoplasmic "maturation promoting factor." The production of this cytoplasmic factor was not affected by removal of the nucleus.

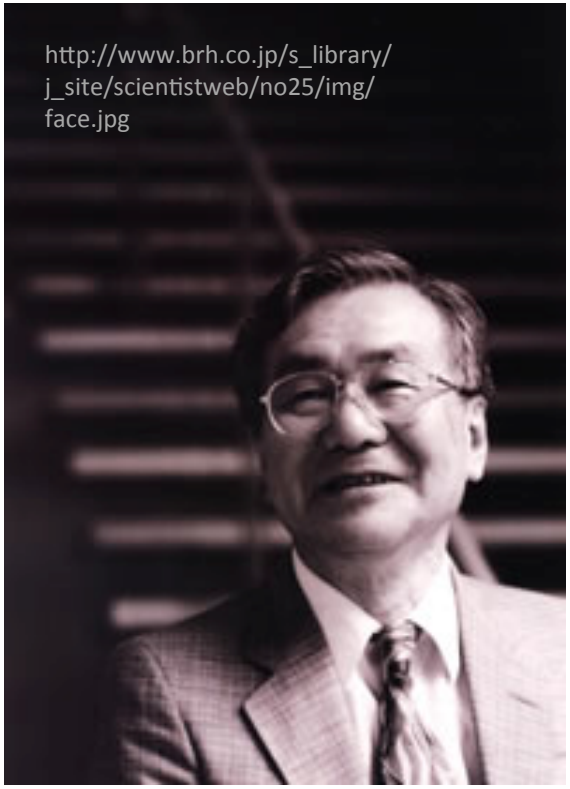
After completion of the first meiotic division, oocytes cease further development at the metaphase of the second meiotic division, where they remain until fertilized or activated to develop. Cytoplasm from such secondary oocytes when injected into one of the blastomeres at the two-cell stage of development suppresses mitosis as well as cleavage. Mitosis is usually arrested at metaphase. No such inhibition was brought about by injection of cytoplasm from cleaving blastomeres. Thus, the arrest of mitosis and cleavage can be attributed to a specific "cytostatic factor" in the cytoplasm of the secondary oocyte. Activation of donor secondary oocytes by insemination or pricking with a glass needle soon destroys the cytostatic factor. Likewise, addition of cortical cytoplasm to endoplasm from the secondary oocyte rapidly destroys the cytostatic capacity. This result implies that cortical material is involved in the process of removing the cytostatic factor at the time of normal activation or fertilization. Enucleation of oocytes demonstrated that production and removal of the cytostatic factor is independent of the nucleus.

Cytoplasmic control of nuclear activities during the mitotic cell cycle has been investigated by nuclear transplantation in amphibian eggs (Graham, '66; Graham et al., '66; Gurdon and Woodland, '68) and in protozoans (DeTerra, '60, '67; Goldstein and Prescott, '67) and by cell fusion in avian and mammalian cells (Harris, '67). The activity of a nucleus transferred into a cell at different stages of mitotic activity tends to conform to the state of the host cell. This nuclear behavior suggests a predominant role for the cytoplasm in regulating the mitotic activity of the nucleus.

A few observations also indicate that the cytoplasm may control the meiotic as well as the mitotic behavior of the nucleus. For example, the nuclei of spermatocytes and spermatids are synchronized in development, perhaps by means of the syncytial cytoplasmic bridges that link these cells (Fawcett, '61). Another example is provided by the behavior of sperm nuclei introduced into immature oocytes of sea urchins (Brachet, '22) and amphibians by precocious insemination

[1] This research was supported by NSF grant GB-5440X.

[2] Present Address: Department of Zoology, University of Toronto, Toronto 5, Ontario, Canada.



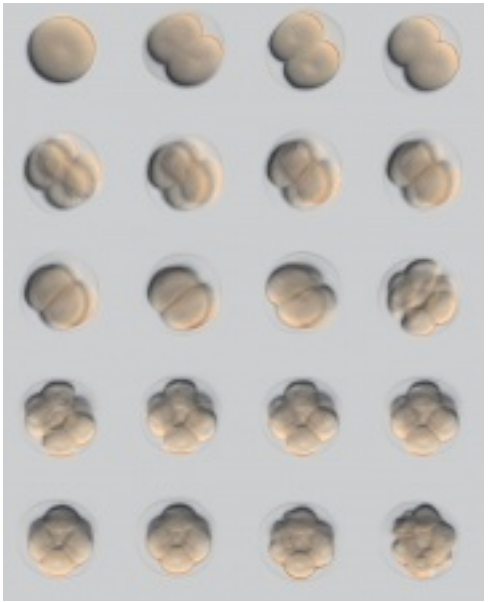
So what kind of molecule was MPF?

KCl
treatment

http://www2s.biglobe.ne.jp/~nkazu/jugyou/image/uni_jusei/Resized800/s-DSCN0920.jpg

https://upload.wikimedia.org/wikipedia/commons/2/2d/Echinometra_mathaei_MHNT_Philippines.jpg

Synchronized cell division



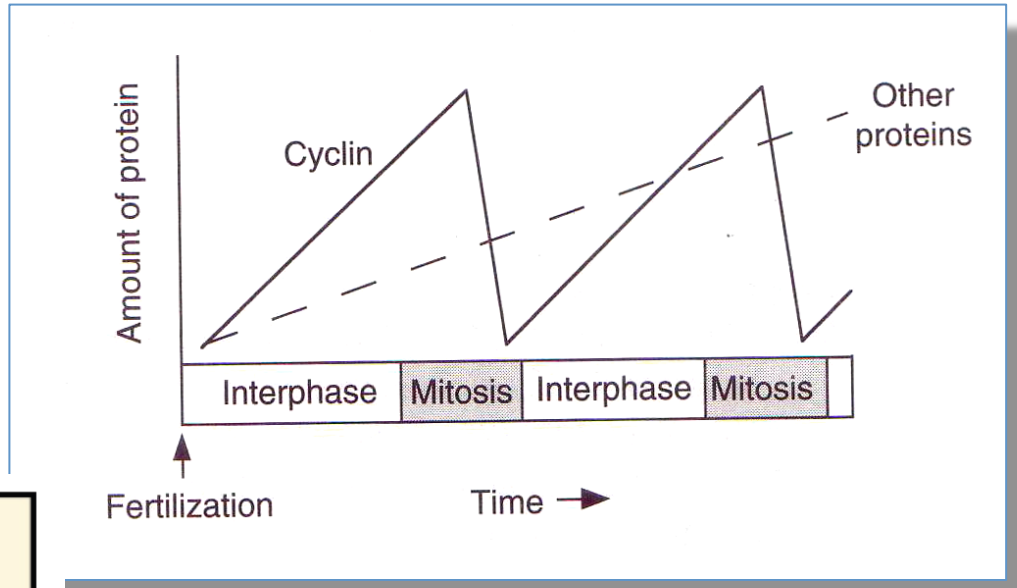
https://embryology.med.unsw.edu.au/embryology/images/b/b6/Sea_Urchin-_early_embryo_cleavage_pattern.jpg

https://upload.wikimedia.org/wikipedia/commons/4/48/Sea_urchin_eggs.jpg

https://upload.wikimedia.org/wikipedia/commons/a/a3/Tim_Hunt_at_UCSF_05_2009_%284%29.jpg

Wood's Hole
海洋学研究所

https://upload.wikimedia.org/wikipedia/commons/c/c6/Marine_Biological_Laboratory%2C_Woods_Hole_by_Pam_Wilmot.jpg



The Cell Cycle an Introduction p.25

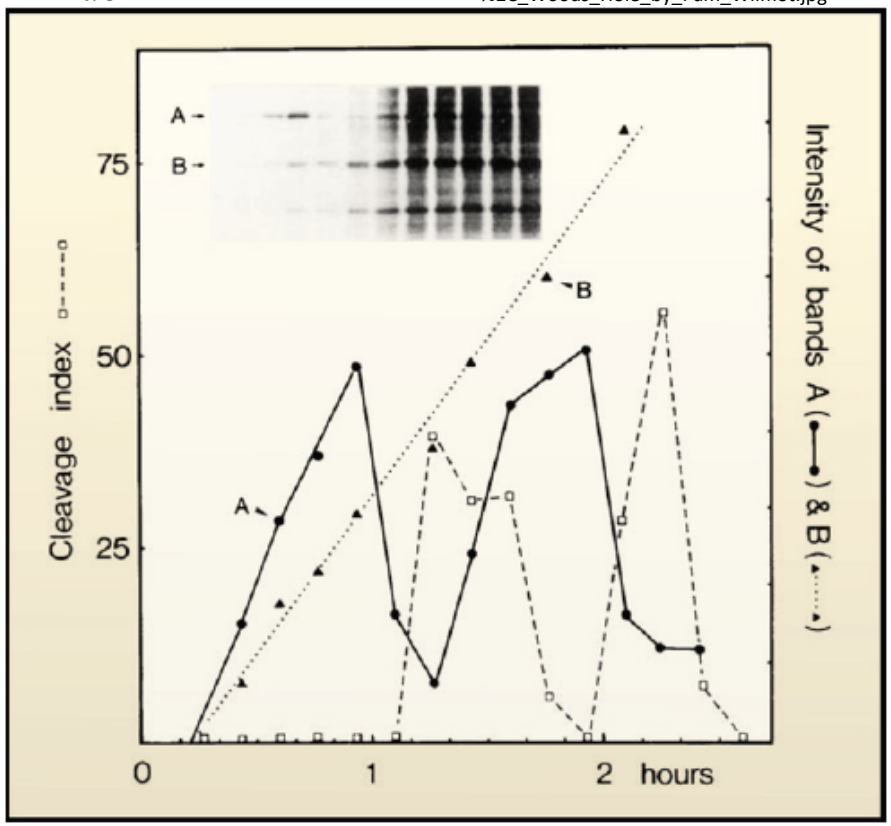


Figure 1. A Simple Experiment

http://www.cell.com/cell/abstract/S0092-8674(08)00888-X

Cell, Vol. 33, 389–396, June 1983, Copyright © 1983 by MIT

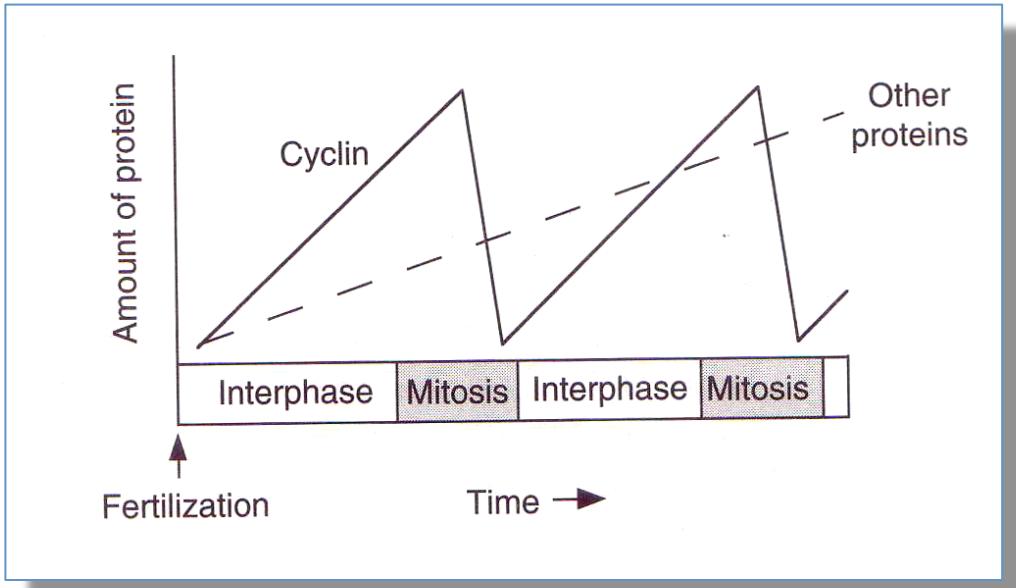
Cyclin: A Protein Specified by Maternal mRNA in Sea Urchin Eggs That Is Destroyed at Each Cleavage Division

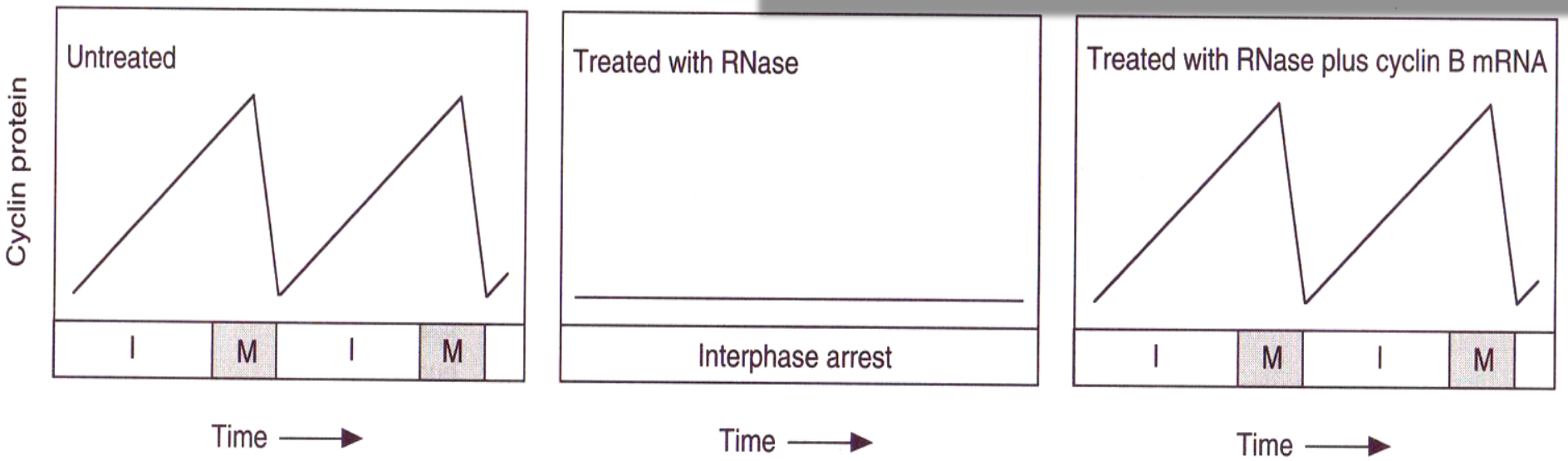
Tom Evans,* Eric T. Rosenthal,† Jim Youngblom,‡ Dan Distel,§ and Tim Hunt‖
Marine Biological Laboratory
Woods Hole, Massachusetts 02543

https://upload.wikimedia.org/wikipedia/en/e/ed/Nobel_Prize.png

Experiment focusing on proteins newly synthesized in fertilized sea urchin eggs.

Proof of the function of cyclin





- left: control
- center: *de novo* protein synthesis is inhibited by RNase
- right: same, with subsequent blocking of RNase and addition of cyclin mRNA

The Cell Cycle an Introduction p.54

Why is it called “Cyclin”？

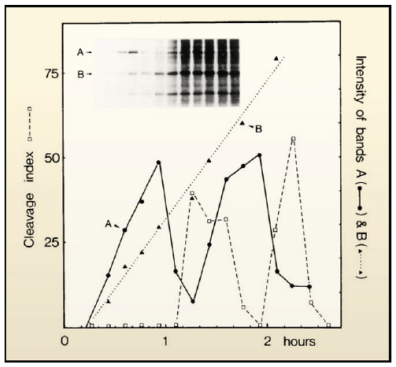
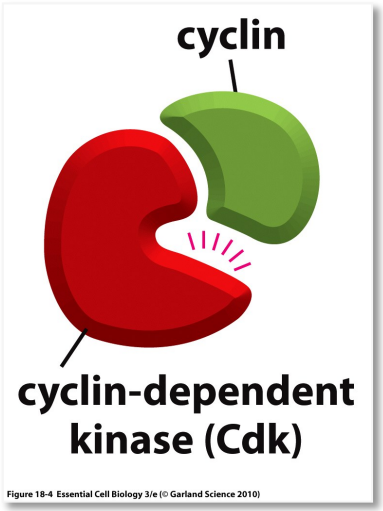


Figure 1. A Simple Experiment

"By the way the name cyclin, which I coined, was really a joke, it's because I like cycling so much at the time but they did come and go in the cell..."



ECB 614頁

Cyclin binds to Cdk which was discovered in yeast

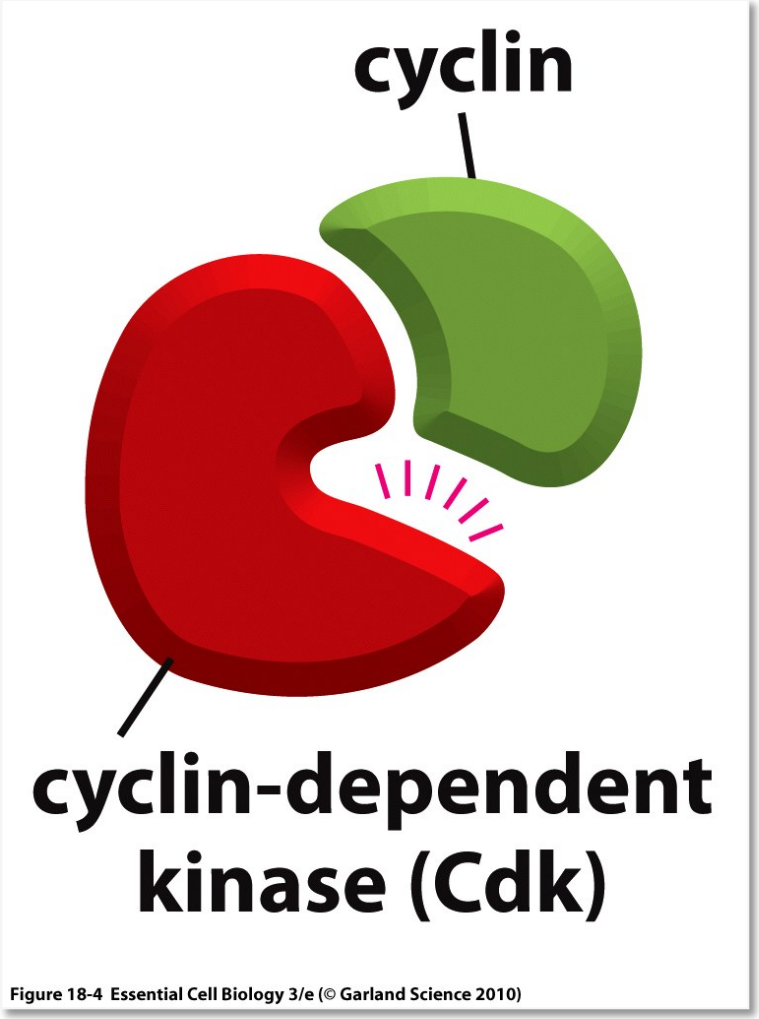
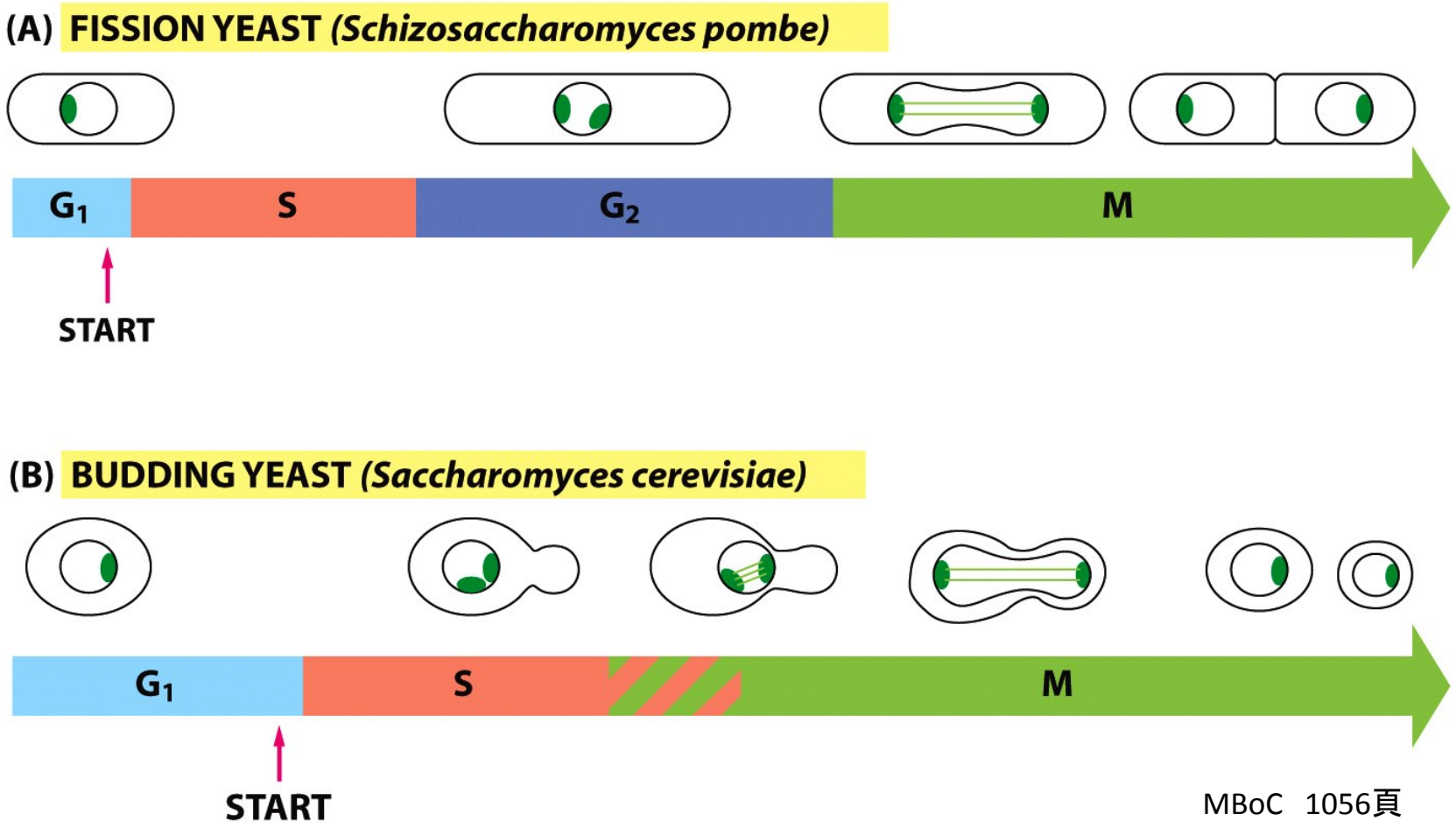


Figure 18-4 Essential Cell Biology 3/e (© Garland Science 2010)

ECB 614頁

So how was Cdk discovered?



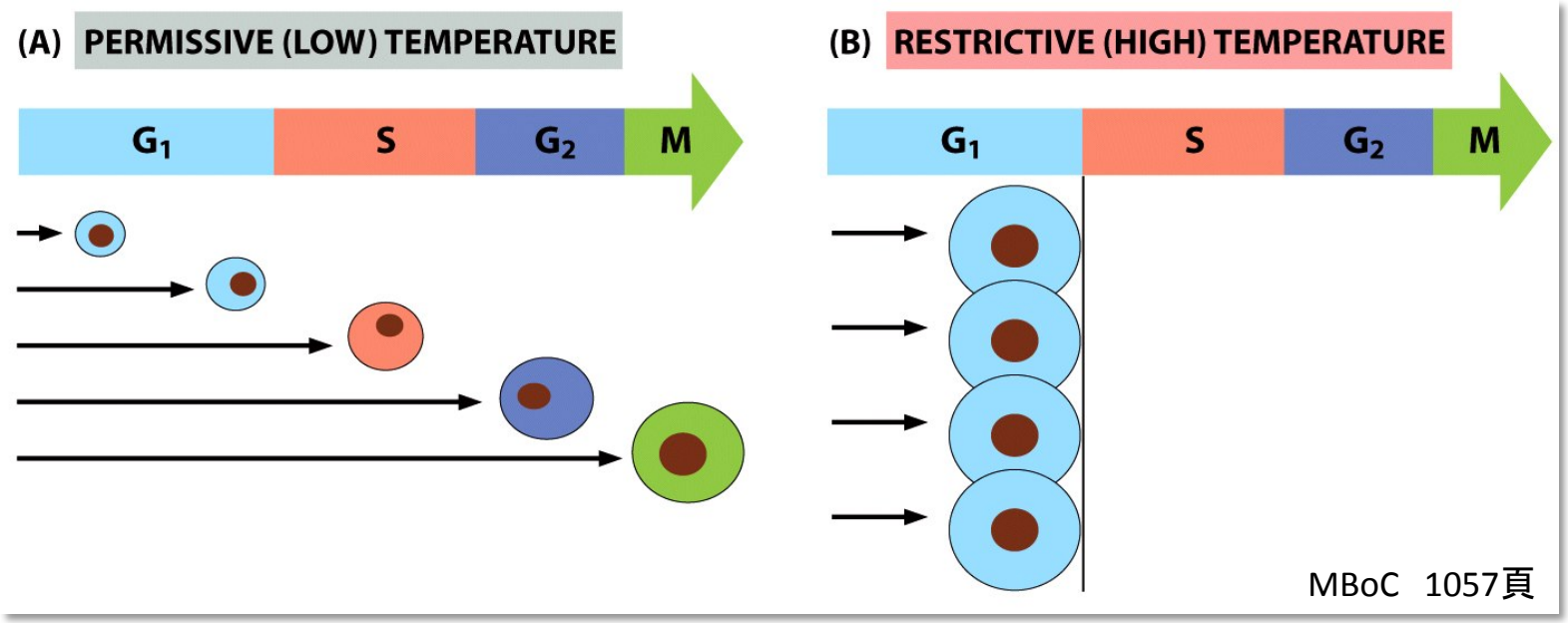
MBoC 1056頁

The size and shape of yeast indicate the phase of cell cycle

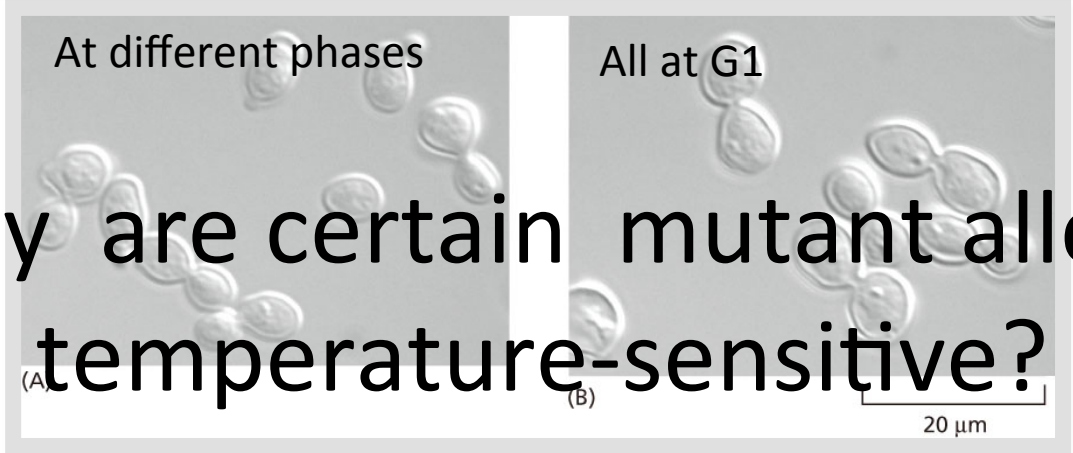
(possible with live cells)

Figure 17-5 *Molecular Biology of the Cell* (© Garland Science 2008)

A very useful type of mutation



MBoC 1057頁



Why are certain mutant alleles temperature-sensitive?

When cells are returned to permissive temperature they all resume cell cycle progression.

The cell cycle can be synchronized with temperature shifts.

JOURNAL OF BACTERIOLOGY, Dec. 2011, p. 6639–6650 Vol. 193, No. 23
0021-9193/11/$12.00 doi:10.1128/JB.05868-11

Temperature-Sensitive Mutants of RNase E in *Salmonella enterica*▽

Disa L. Hammarlöf,[1] Lars Liljas,[1] and Diarmaid Hughes[2]*

Department of Cell and Molecular Biology, Box 596,[1] and Department of Medical Biochemistry and Microbiology, Box 582,[2] Uppsala University, Uppsala, Sweden

- In this case amino acid changes of Ile207 caused temperature- sensitivity.
- TS mutants usually affect higher structure and not catalytic domains.

SHOWN HERE AS AN EXAMPLE

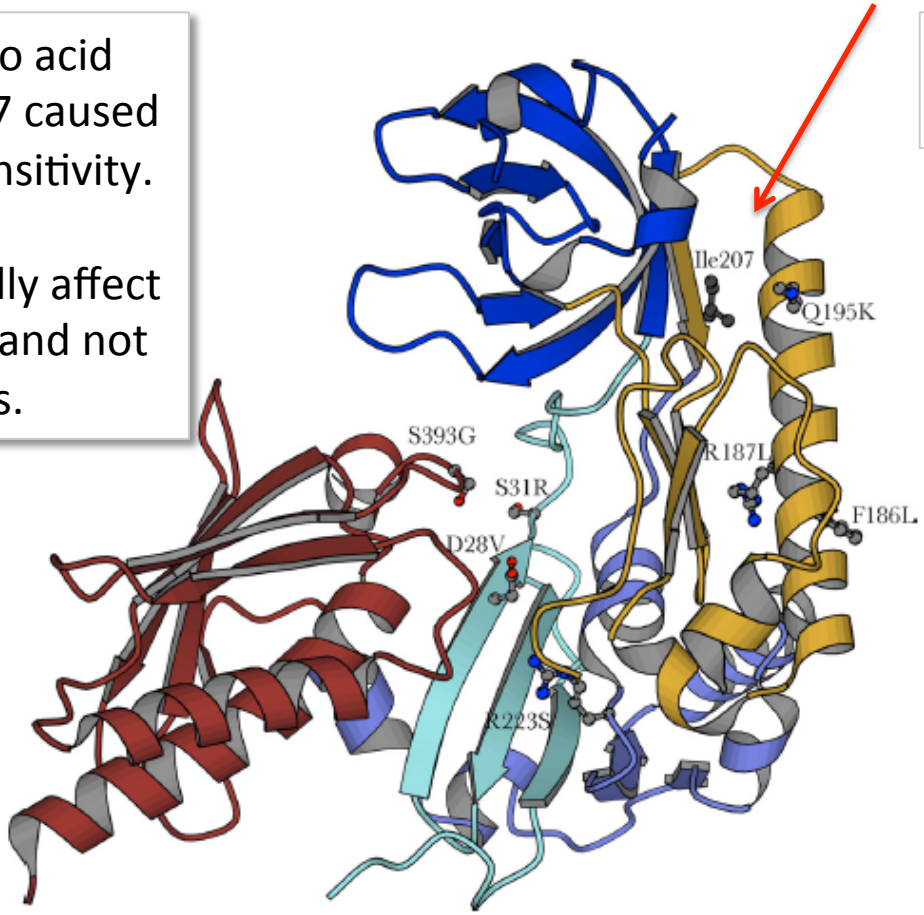
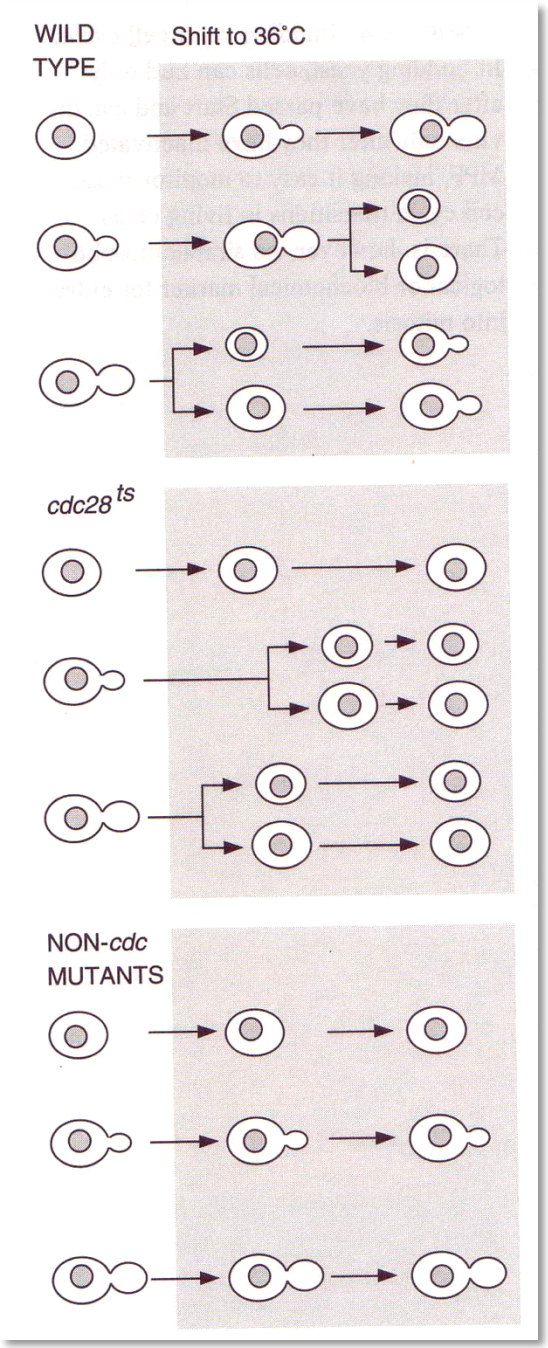


FIG. 4. Suppressor mutations for the *me-6* (Ile207Ser) and *me-9* (Ile207Asn) temperature-sensitive mutations. The coloring is the same as in Fig. 1. The suppressor mutations are found both in the 5′ sensor subdomain and in the RNase H and DNase I subdomains. The drawing is based on PDB entry 2bx2 (4).

Many of the cell cycle genes were identified as Cdc ts-mutants

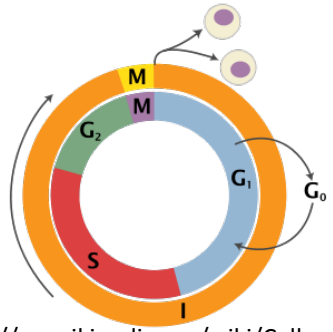


The Cell Cycle an Introduction 32頁

Wildtype continues to grow when shifted from 30℃ to 36℃.

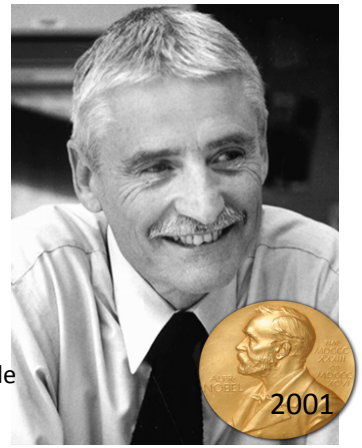
In this example, all cells of the cdc28 temperature-sensitive mutant stop at G1 phase.

Non-cdc temperature-sensitive mutants stop growth at various phases.



https://en.wikipedia.org/wiki/Cell_cycle

http://images.the-scientist.com/content/figures/images/yr2003/apr21/hartwell.jpg

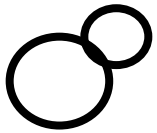


Lee Hartwell

Searched for temperature-sensitive cell cycle mutants

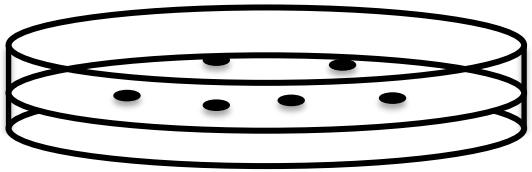
They are known as Cdc (Cell division cycle) mutants and 32 loci were identified.

Screening of Cdc (Cell division cycle) Mutants



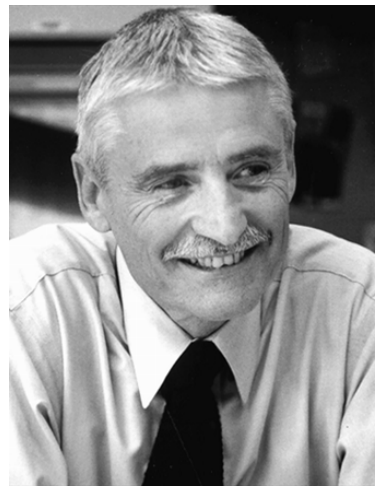
Budding yeast
（Normally cultured at 30℃）

Mutagenesis
（EMS or UV)



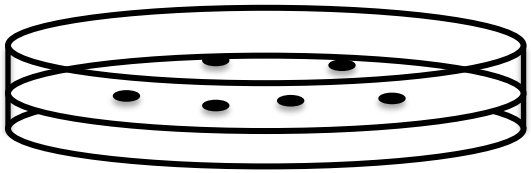
Grown from single cells at 25℃

A) Grow ◎
B)Don't grow X



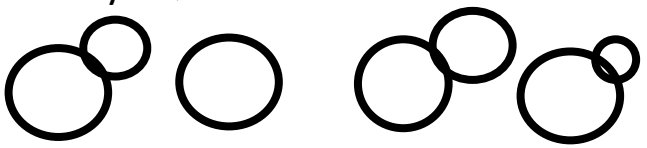
Lee Hartwell (USA)

"Replica" made of colonies



Replica grown at 36℃

A-B-A) non-cdc mutant（stop at any phase in the cell cycle）X

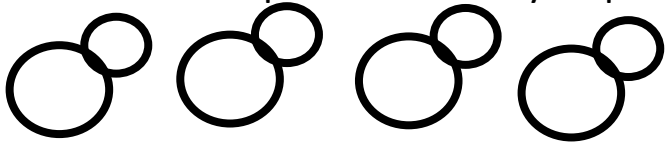


A-A) Grow X

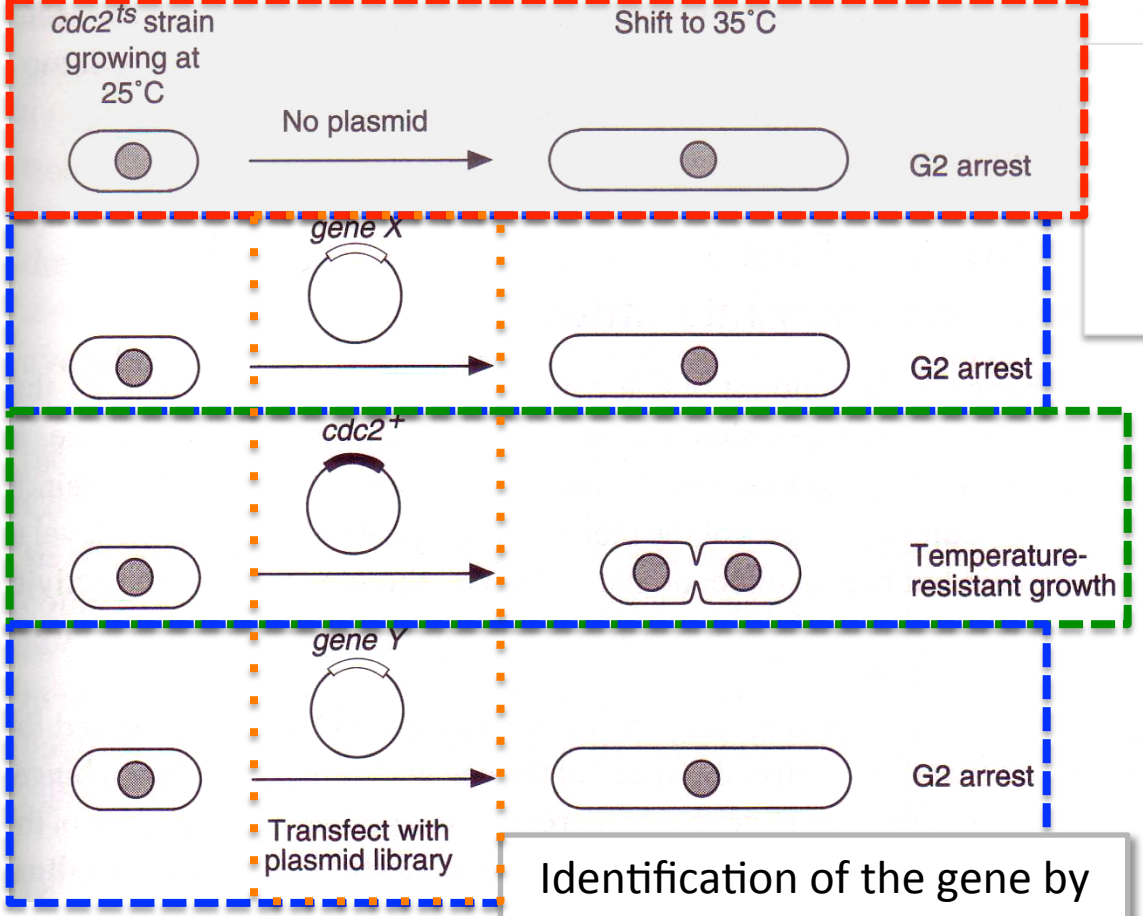
A-B) Don't grow this time ◎
（temperature sensitive）

Study cell cycle phase at 36℃

A-B-B)（cdc mutant）◎
Growth blocked at a particular cell cycle phase



Identified Cdk1 (cdc2) in Fission Yeast



Identification of the gene by mutant rescue

The Cell Cycle an Introduction 49頁

2001 Paul Nurse

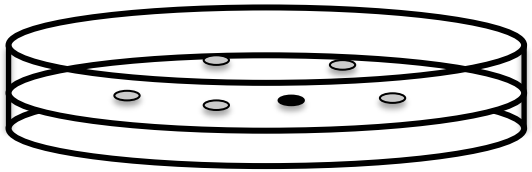
https://upload.wikimedia.org/wikipedia/commons/a/ac/Paul_Nurse_portrait.jpg

- When the cdc2^{ts} mutant is shifted to 35℃ it is arrested at G2 phase. Because cell growth continues it becomes longer.
- The mutant is randomly transformed with a plasmid library of genomic DNA.
- Most plasmids will not change (rescue) the mutant phenotype.
- However if the plasmid contains the full-length cdc2 gene it will complement (rescue) the mutant. The plasmid is then, recovered and sequenced.
- Fission yeast was later found to be homologous to budding yeast Cdc28 and human Cdk1.

Identified Cdk1 (cdc2) in Fission Yeast

Fission yeast Cdc2 temperature sensitive mutant
（permissive temperature　25℃）

Transformed with plasmids carrying random fragments of genomic DNA



Grown at 35℃

A) Non-transformed yeast do not grow X
B) Yeast transformed with genes other than cdc2 do not grow X
C) Yeast transformed with partial fragments of cdc2 do not grow X
D) Yeast transformed with the full length cdc2 gene are “rescued” ◎

Plasmid isolated from yeast that grow

Sequence of plasmid that “rescued” the mutant is determined

Sequence of same gene in the original mutant strain is also sequenced
（protein coded bycdc2 gene is identified）

Paul Nurse (UK)

The gene was first identified as the mutant (cdc2) and the DNA sequence was not known

“Mutant Rescue”
（”Plasmid Rescue” method）

Purification of maturation-promoting factor, an intracellular regulator of early mitotic events

(cell cycle/mitosis/protein phosphorylation)

MANFRED J. LOHKA*, MARIANNE K. HAYES†, AND JAMES L. MALLER

Department of Pharmacology, University of Colorado School of Medicine, Denver, CO 80262

Communicated by Raymond L. Erikson, December 22, 1987 (received for review October 10, 1987)

ABSTRACT **Maturation-promoting factor causes germinal vesicle breakdown when injected into *Xenopus* oocytes and can induce metaphase in a cell-free system. The cell-free assay was used to monitor maturation-promoting factor during its purification from unfertilized *Xenopus* eggs. Ammonium sulfate precipitation and six chromatographic procedures resulted in a preparation purified >3000-fold that could induce germinal vesicle breakdown within 2 hr when injected into cycloheximide-treated oocytes. Proteins of 45 kDa and 32 kDa were correlated with fractions of highest activity in both assays. These fractions contained a protein kinase activity able to phosphorylate the endogenous 45-kDa protein, as well as histone H1, phosphatase inhibitor 1, and casein. The highly purified preparations described here should help to identify the mechanism of action of maturation-promoting factor and to elucidate the role of protein kinases in the induction of metaphase.**

Summary (for part1)

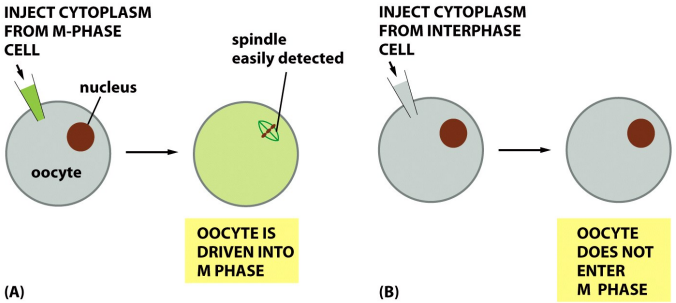
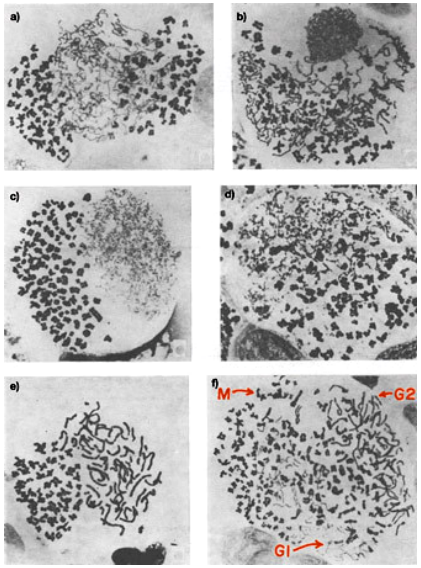
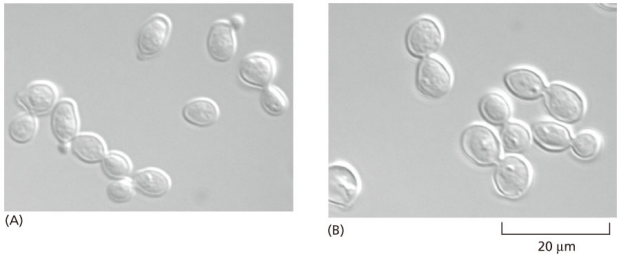


Figure 18-7 Essential Cell Biology 3/e (© Garland Science 2010)

Frog Egg
MPF
(Experimental Biology)



Cell fusion experiments indicated existance of a control mechanism



Yeast
Cdk
(genetics)

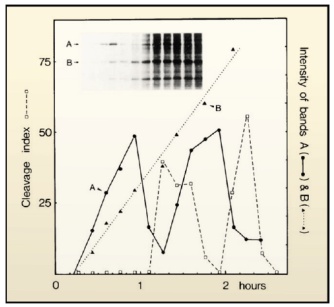
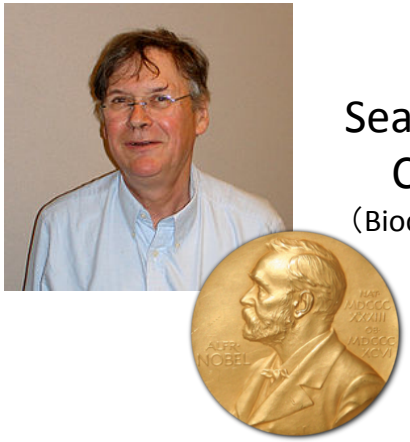


Figure 1. A Simple Experiment



Sea Urchin
Cyclin
（Biochemistry）

Activation of M-Cdk

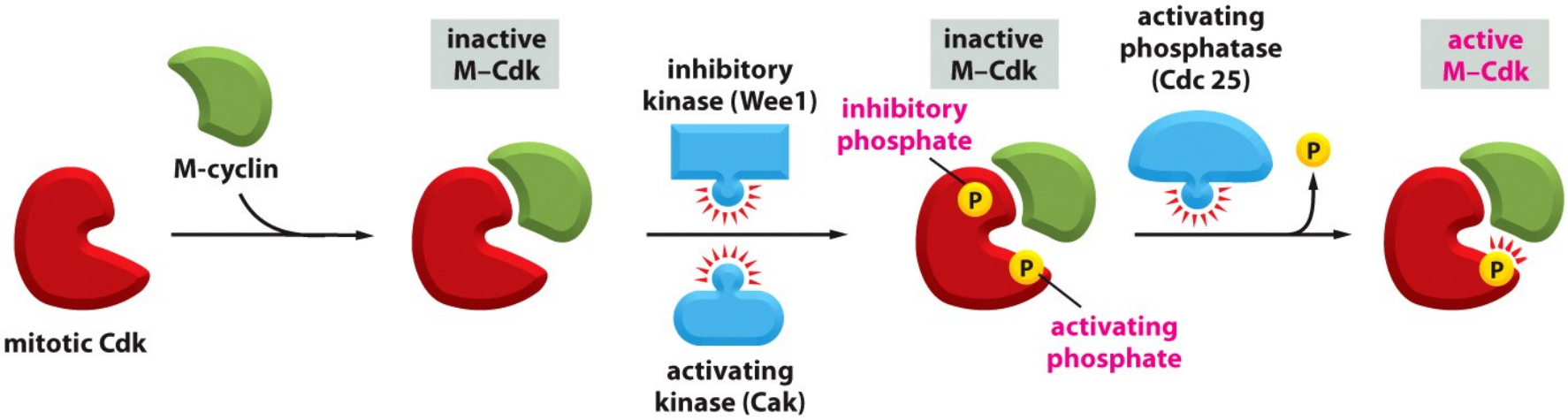


Figure 18-17 Essential Cell Biology 3/e (© Garland Science 2010)

You learned this last week

Regulation of Cdk-Cyclin by Wee1 & Cdc25

Both were identified as mutants in fission yeast (S. pombe)

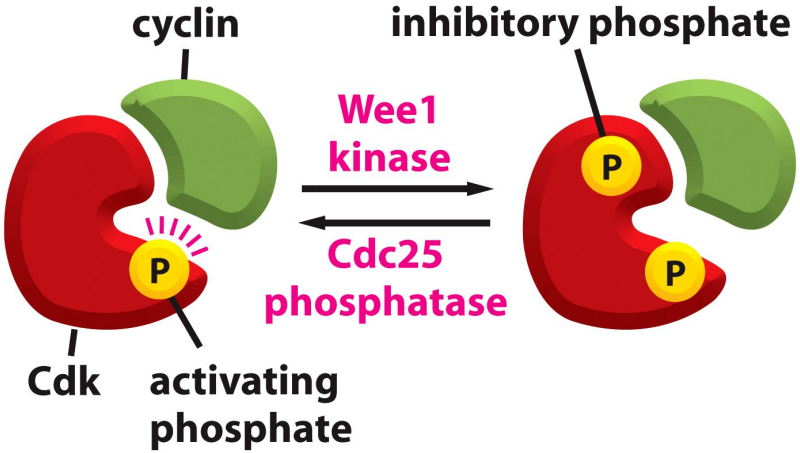
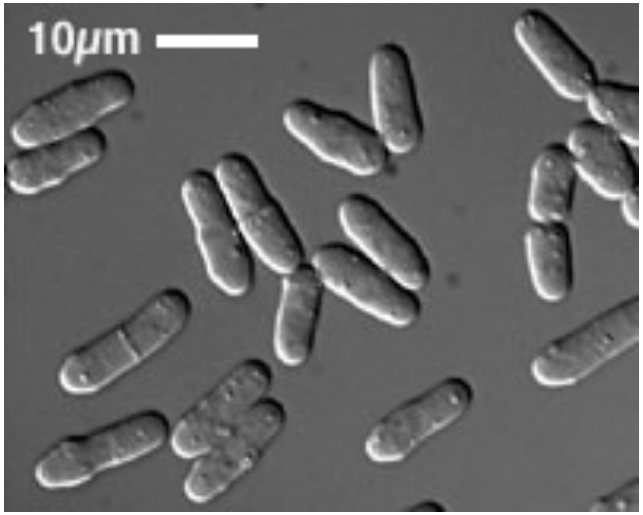


Figure17-18 Molecular Biology of the Cell S/e (©Garland science 2008)

ACTIVE **INACTIVE**



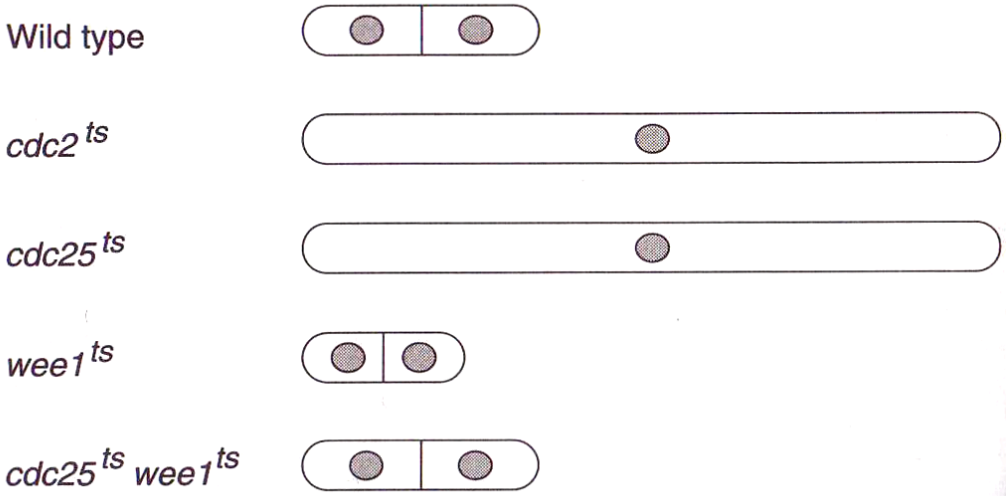
http://eishinoguchi.com/NoguchiLab.html

cdc2: Cdk (Cyclin-dependent kinase)

wee1: Cdk inhibitory kinase

cdc25: Cdk activating phosphatase

Paul Nurse



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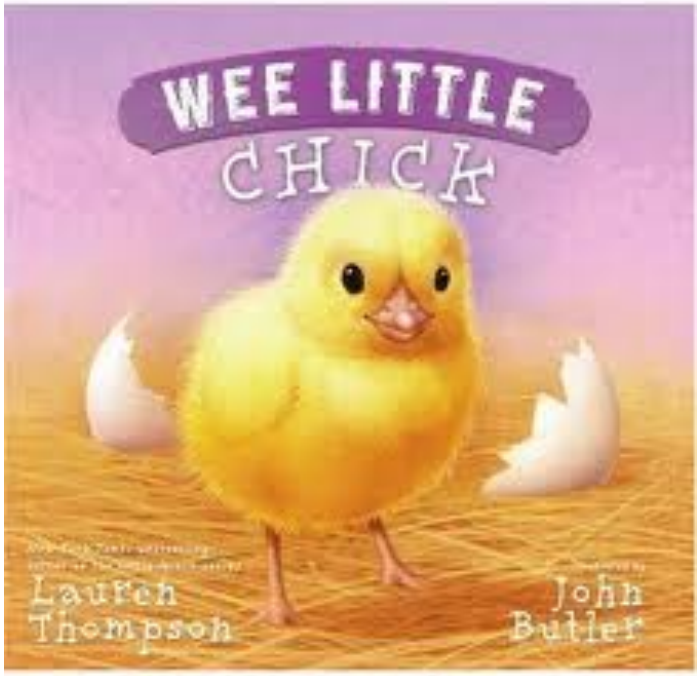
Paul Nurse

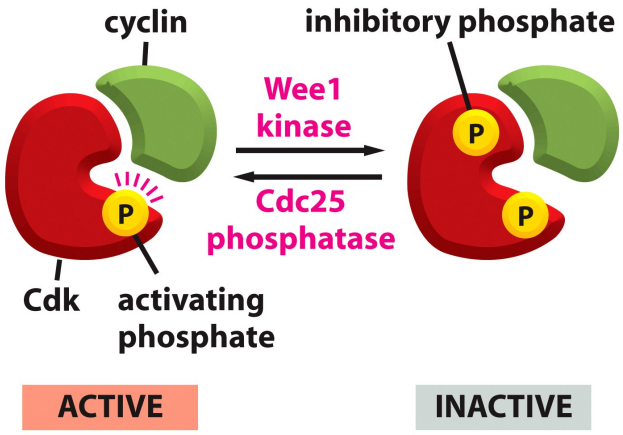
Wee1

(his first mutant)

wee (adj.) definition;

1. **Very small; tiny.**
2. **Very early: *the wee hours of the morning.***



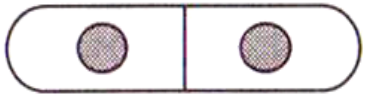


cdc2: Cdk (Cyclin-dependent kinase)

wee1: Cdk inhibitory kinase

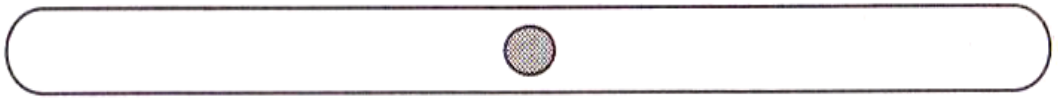
cdc25: Cdk activating phosphatase

Wild type



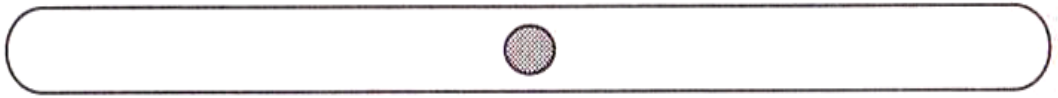
Normal mitosis
(fission yeast)

cdc2 ^{ts}



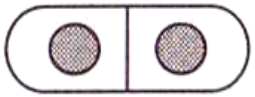
Mitosis is inhibited but cell growth continues

cdc25 ^{ts}



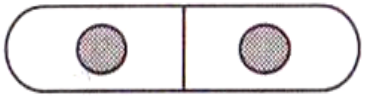
Mitosis is inhibited but cell growth continues

wee1 ^{ts}

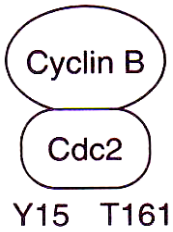


Mitois induced before G2
growth is finished.

cdc25 ^{ts} *wee1* ^{ts}



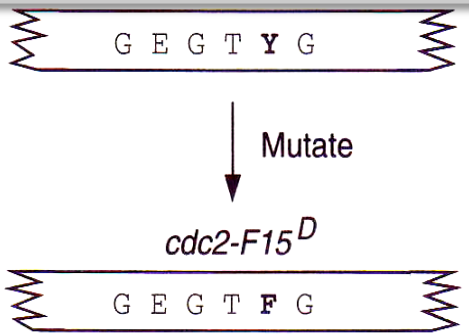
Cancel each other



Analysis of the phosphorylation sites

Inactive

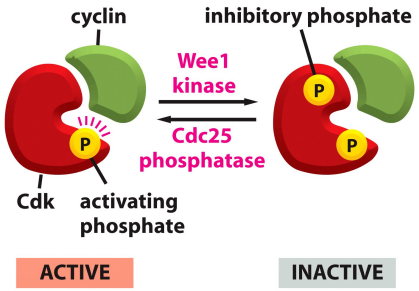
Tyrosine15 of Cdc2 exchanged with Phenylalanine

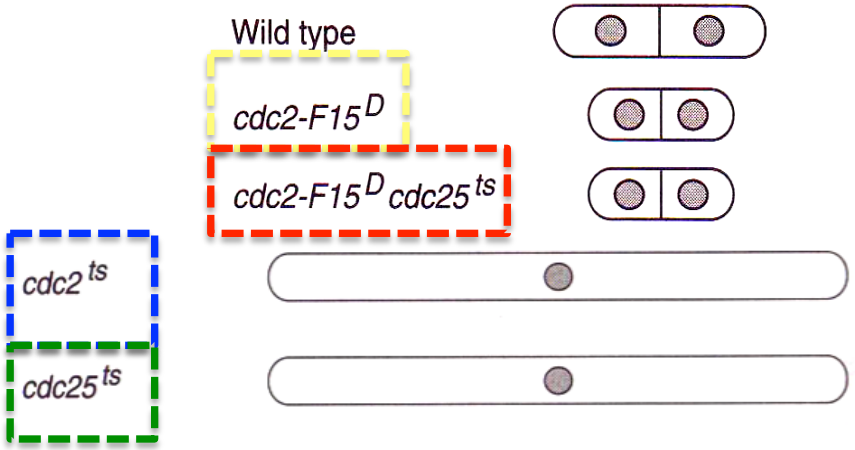


human Cdk(Cdc2) phosphorylation

Tyrosine15: inhibition

Threonine161: activation





- Cdc2 regular ts mutation: inhibition of mitosis
- Cdc25 regular ts mutation: inhibition of mitosis
- Cdc2 Y15→F mutant : induction of mitosis
- $Cdc2^{Y15F}$ and Cdc25 mutants do not cancel each other out, and the $Cdc2^{Y15F}$ phenotype is dominant.
- Indicates Cdc25 functions through Cdk Tyrosine 15

The Cell Cycle an Introduction
p.58

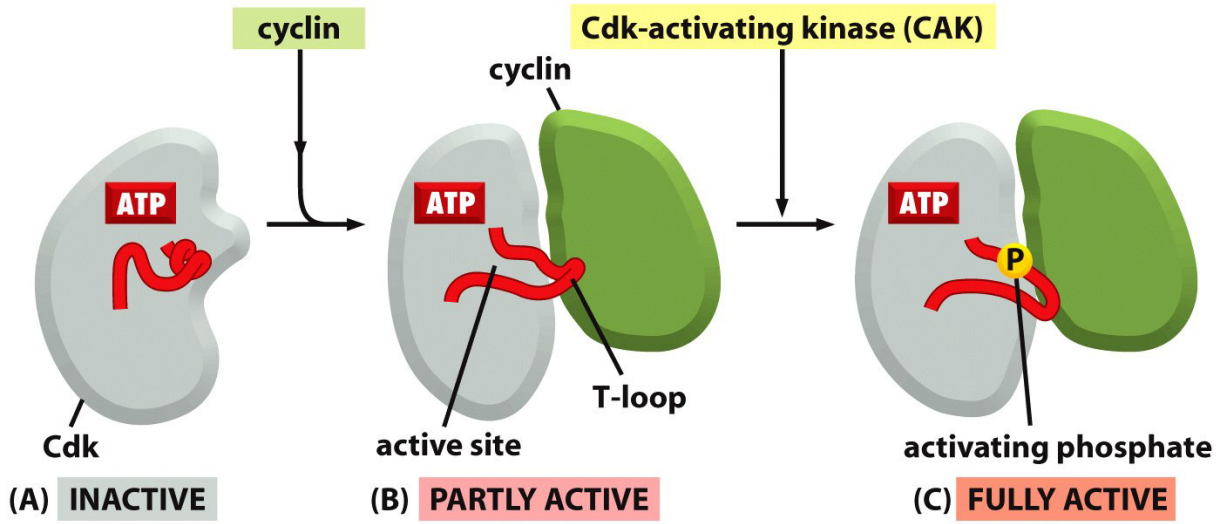


Figure 17-17 Molecular Biology of the Cell 5/e (© Garland Science 2008)

1. Binding of Cyclin
2. Phosphorylation of activating site
3. Dephosphorylation of inhibitory site
4. Release from Cdk inhibitor protein

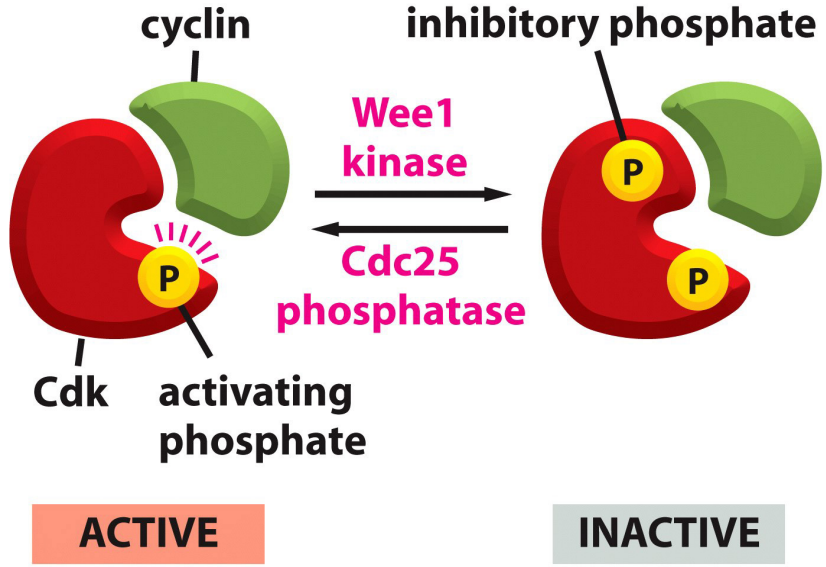


Figure 17-18 Molecular Biology of the Cell 5/e (© Garland Science 2008)

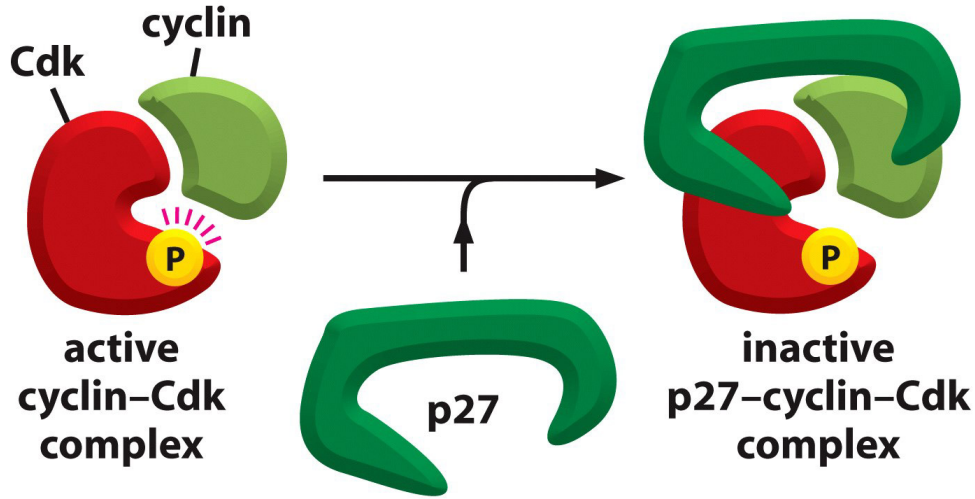
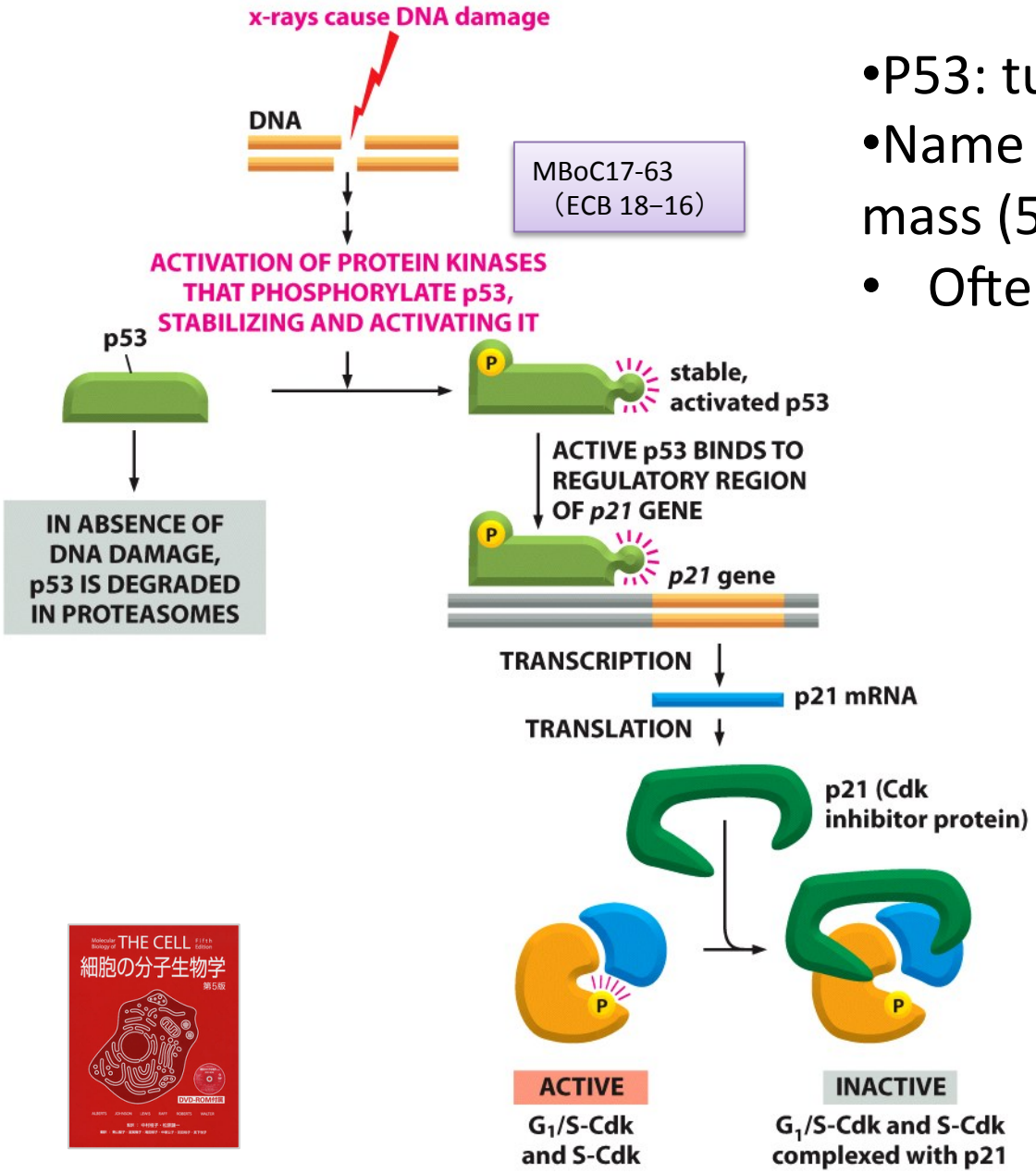
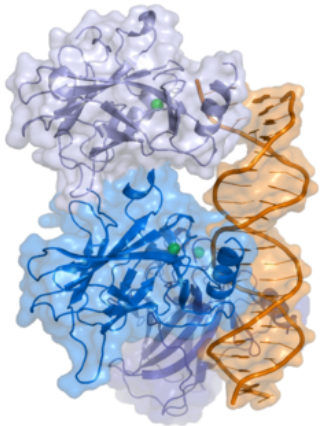


Figure 17-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

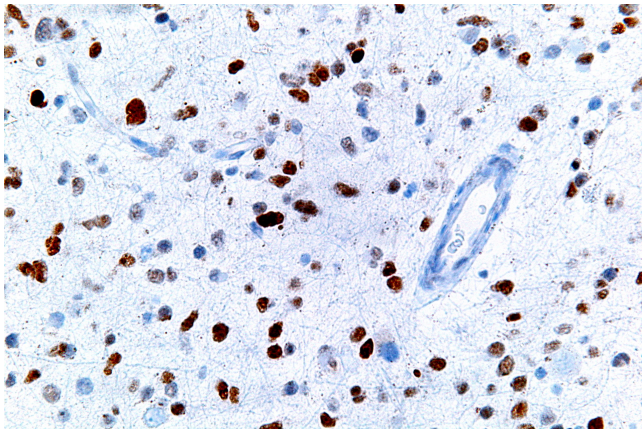
Cdk inhibitor proteins (CKIs)



- P53: tumor suppressor protein
- Name from apparent molecular mass (53kD)
- Often mutated in tumors



https://en.wikipedia.org/wiki/P53



https://en.wikipedia.org/wiki/P53

先週！

International New York Times

SCIENCE

Elephants: Large, Long-Living and Less Prone to Cancer

Carl Zimmer

OCT. 8, 2015

African elephants at Utah's Hogle Zoo. Researchers say elephants are exceptional cancer fighters.
University of Utah Health Sciences

Br. J. Cancer (1975) **32**, 411

CANCER AND AGEING IN MICE AND MEN

R. PETO,[1] F. J. C. ROE,[2] P. N. LEE,[3] L. LEVY[2] AND J. CLACK[2]

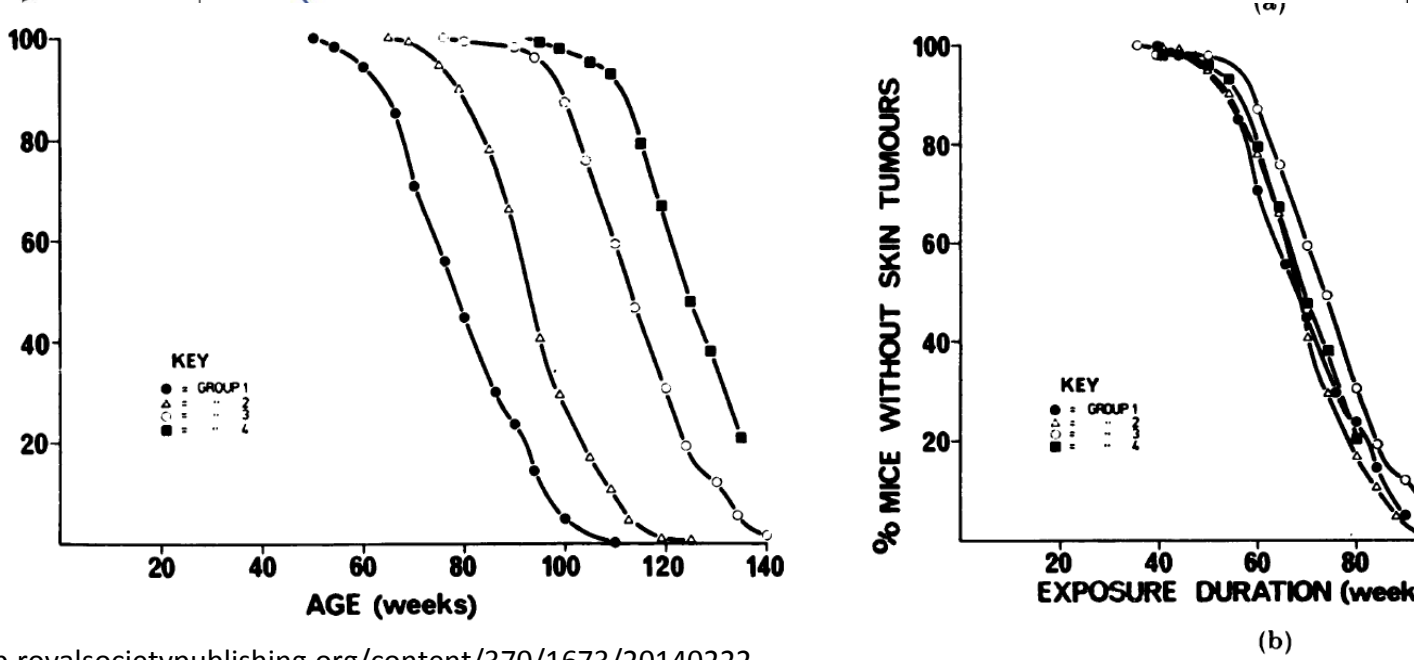
From the [1]Radcliffe Infirmary, University of Oxford, [2]Chester Beatty Research Institute (Pollard's Wood Research Station), Institute of Cancer Research, London, and the [3]Tobacco Research Council, Glen House, Stag Place, London SW1E 5AG

Received 2 June 1975. Accepted 10 July 1975

Peto's Paradox

推定変異率

estimated mutation rate versus no. stem cells in colon

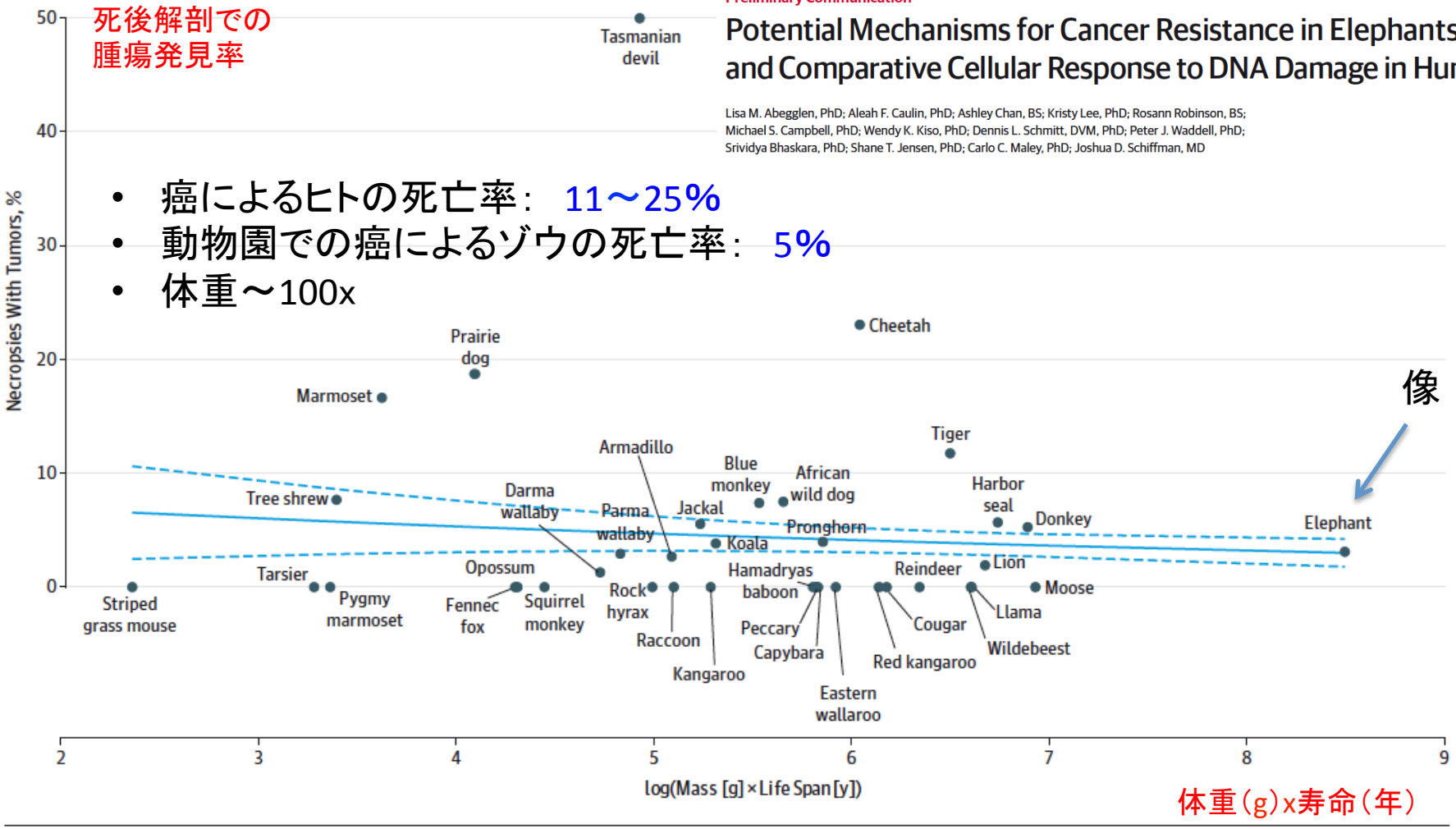


- 変異率は年齢で変わらない？（マウス）

http://rstb.royalsocietypublishing.org/content/370/1673/20140222

JAMA Published online October 8, 2015

Figure 1. Cancer Incidence Across Species by Body Size and Life Span



Preliminary Communication

Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans

Lisa M. Abegglen, PhD; Aleah F. Caulin, PhD; Ashley Chan, BS; Kristy Lee, PhD; Rosann Robinson, BS; Michael S. Campbell, PhD; Wendy K. Kiso, PhD; Dennis L. Schmitt, DVM, PhD; Peter J. Waddell, PhD; Srividya Bhaskara, PhD; Shane T. Jensen, PhD; Carlo C. Maley, PhD; Joshua D. Schiffman, MD

- 癌によるヒトの死亡率： 11～25%
- 動物園での癌によるゾウの死亡率： 5%
- 体重～100x

The mammalian species studied span the striped grass mouse to the elephant. Cancer incidence is not associated with mass and life span, as shown by the logistic regression (model fit shown as blue line; 95% CIs shown as dashed lines). Each data point in the graph is supported by a minimum of 10 necropsies for the included mammals (San Diego Zoo) and 644 annotated deaths for elephants (Elephant Encyclopedia database). The risk of cancer depends on both the number of cells in the body and the number of years over which those cells can accumulate mutations; therefore, cancer incidence is plotted as a function of mass × life span. All data with 95% CIs are presented in eTable 1 in the Supplement.

eLIFE

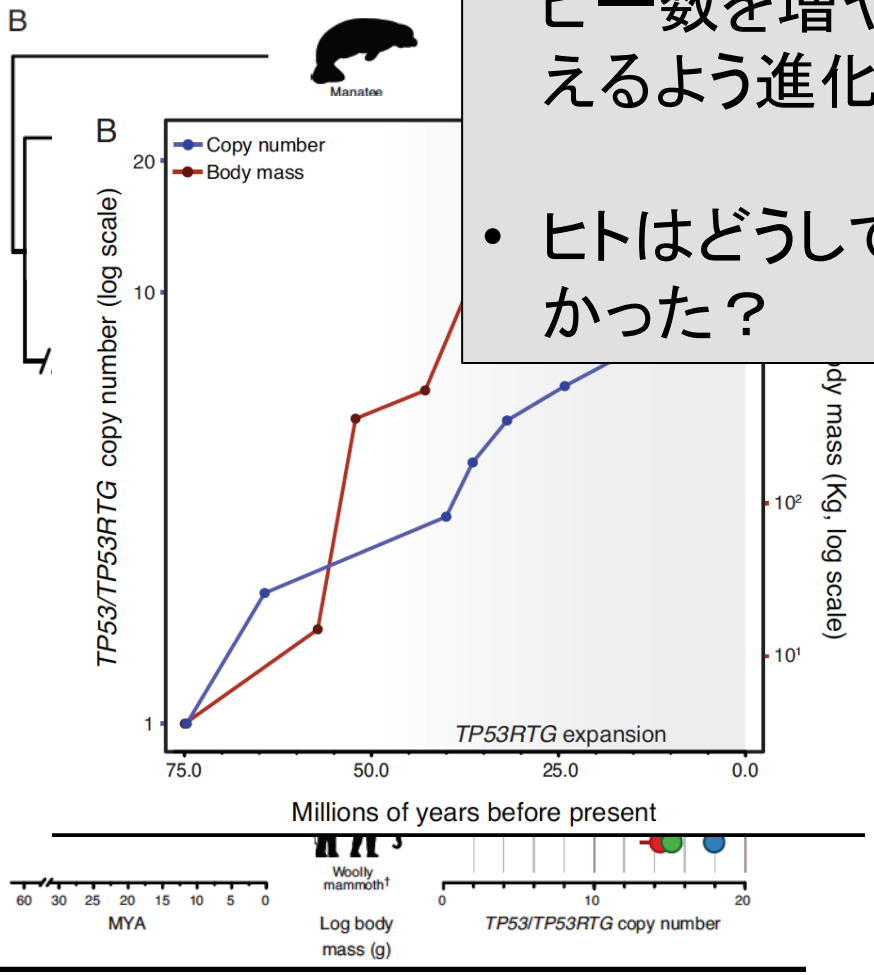
TP53 copy number expansion correlates with the evolution of increased body size and an enhanced DNA damage response in elephants

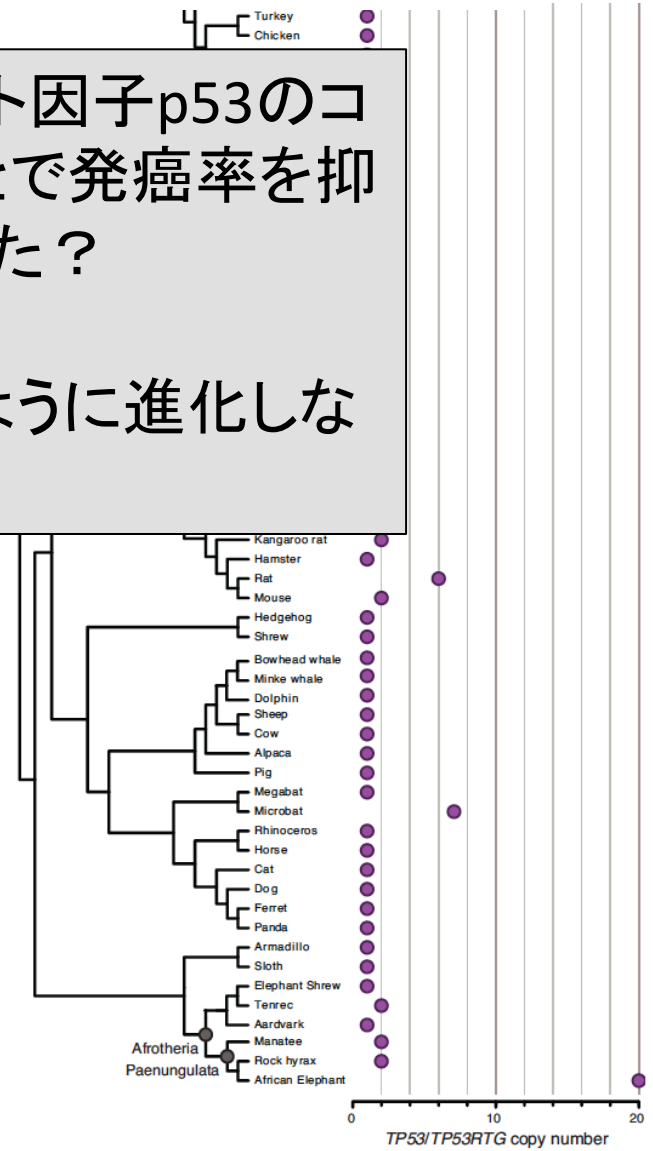
Michael Sulak, Lindsey Fong, Katelyn Mika, Sravanthi Chigurupati, Lisa Yon, Nigel P. Mongan, Richard D. Emes, Vincent J. Lynch

doi: http://dx.doi.org/10.1101/028522

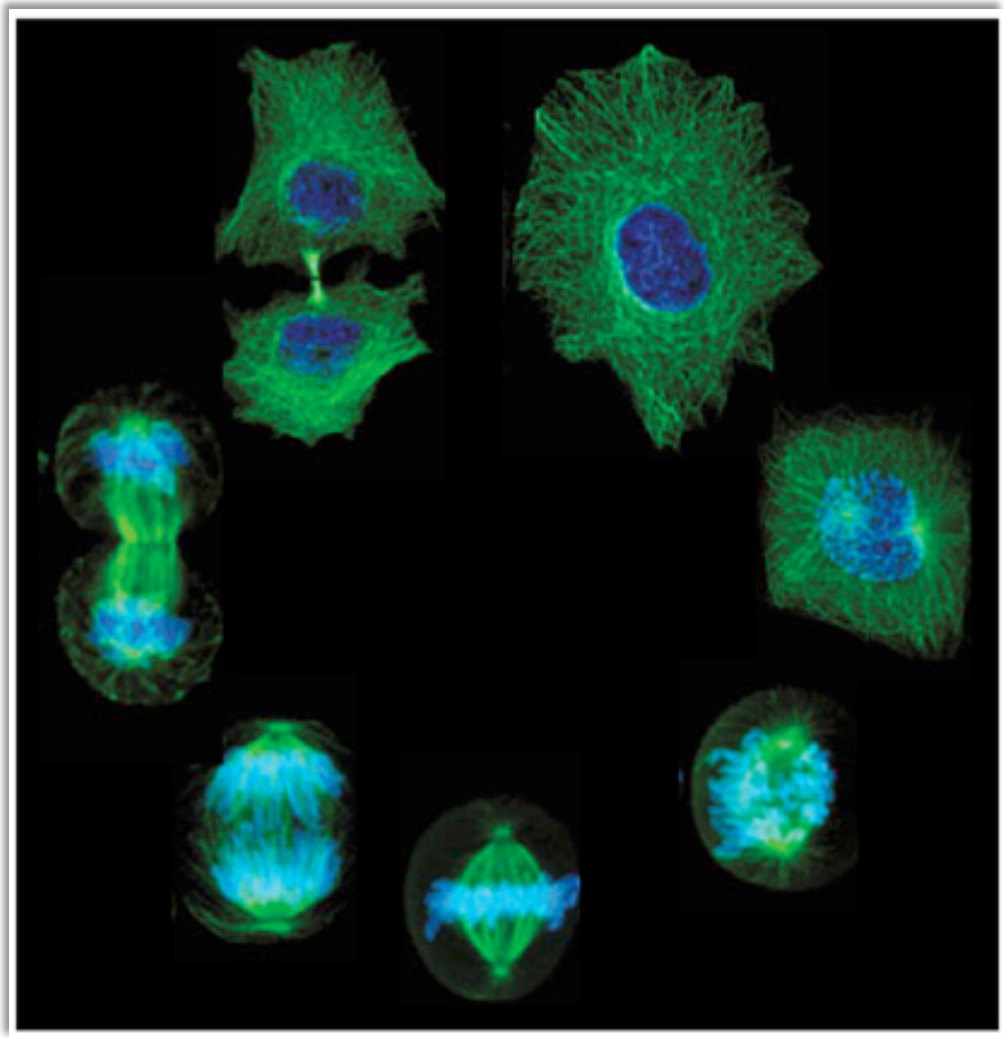
p53

- ゾウはチェックポント因子p53のコピー数を増やすことで発癌率を抑えるよう進化してきた？
- ヒトはどうして同じように進化しなかった？



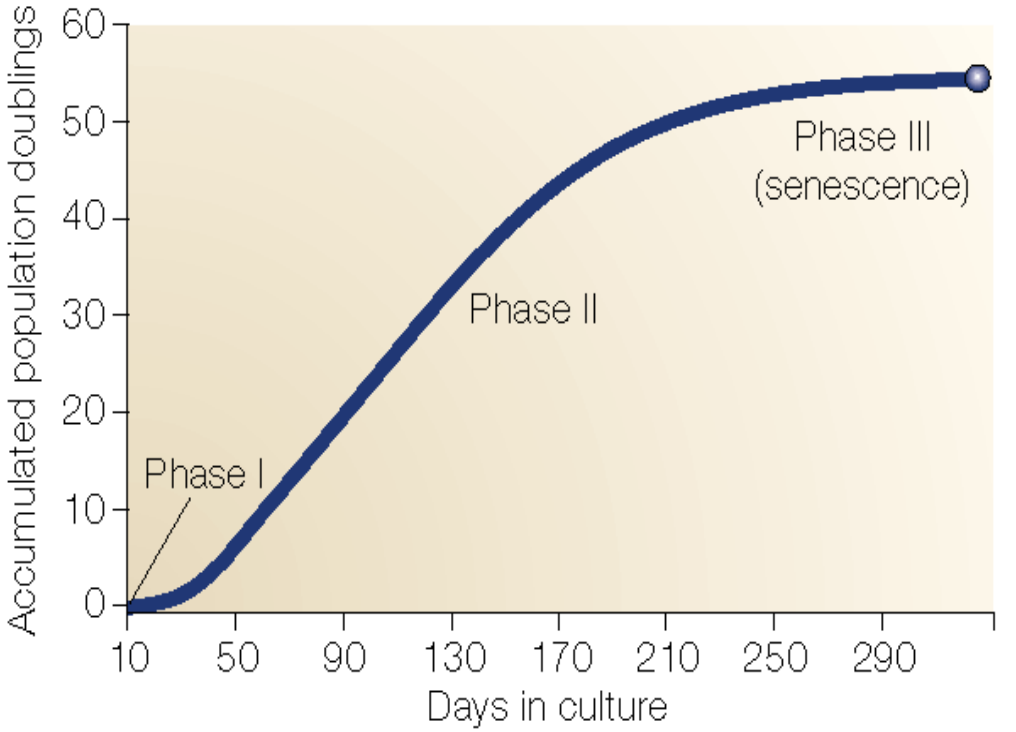


How many times can a cell divide ?



Hayflick limit (1965)

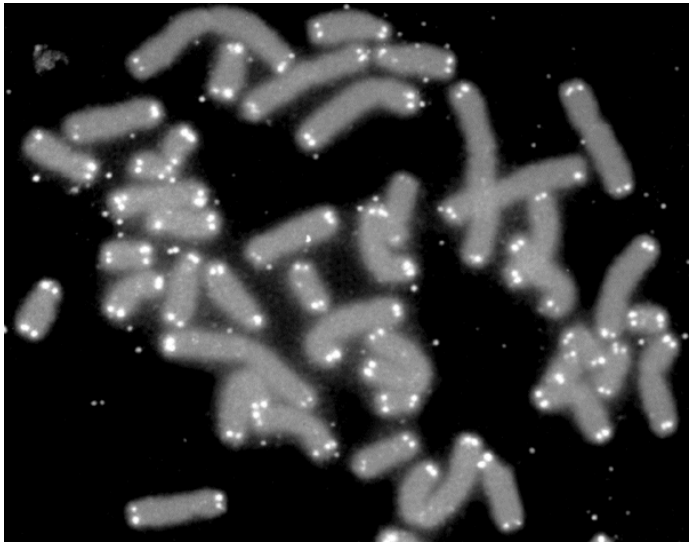
http://images.the-scientist.com/content/figures/images/yr1997/may/may_art/hayflick.jpg



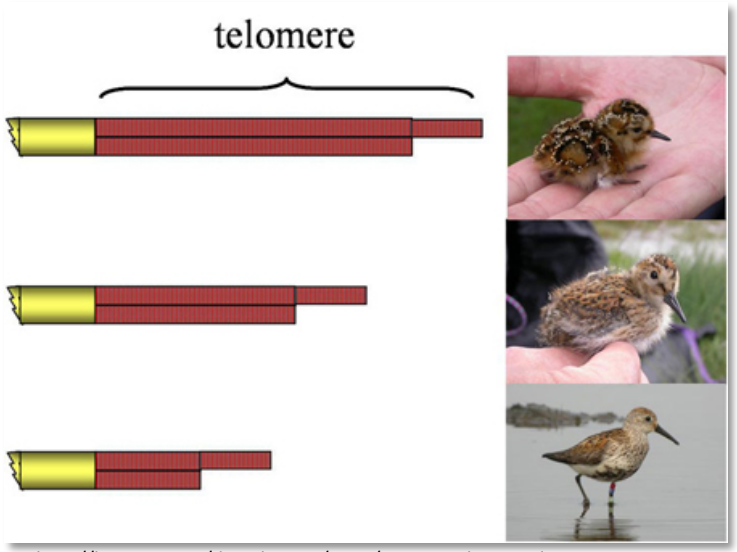
Nature Reviews | Molecular Cell Biology

Human culture cells cease growth after about 50 divisions.
Individual cells have a life span too!
aging= stop in proliferation ?
Why do they stop after 50 divisions?

The telomere theory



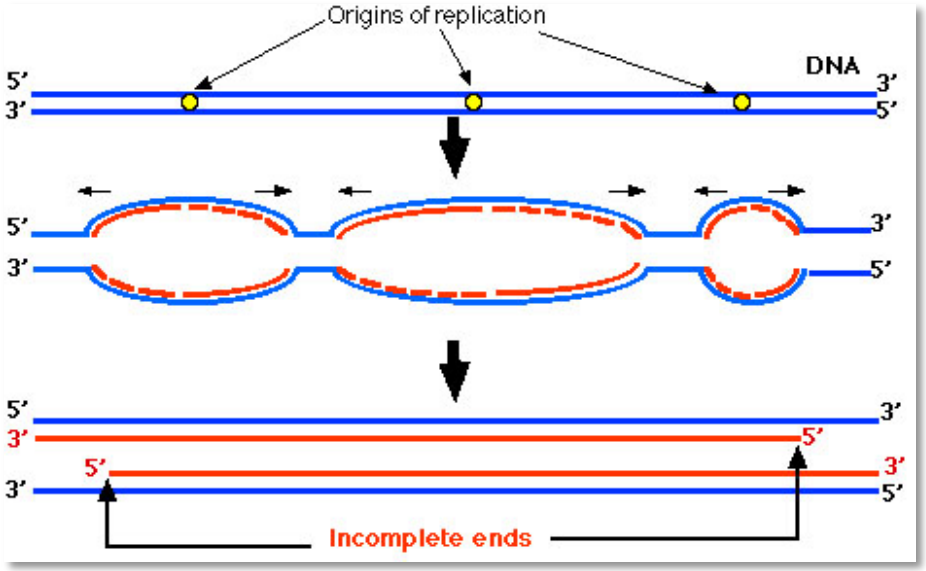
https://upload.wikimedia.org/wikipedia/commons/4/4a/Telomere_caps.gif



http://bioenv.gu.se/digitalAssets/1311/1311740_bpop_telomeres_picture3.jpg

What are telomeres?
Specialized structures at the end of chromosomes

Chromosomes become shorter each time DNA is replicated.



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/telomere3.gif

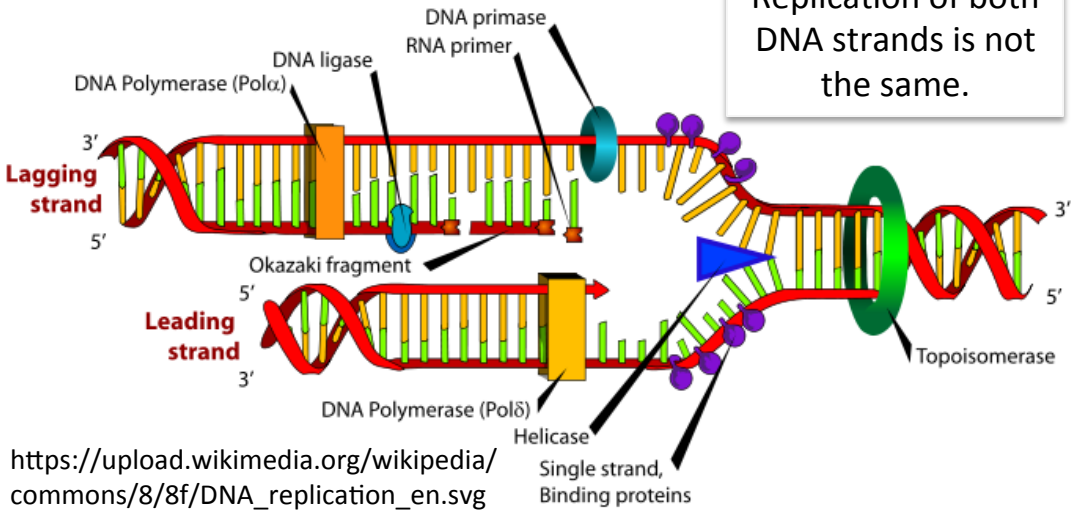
Telomeres

(: end structures of chromosomes)

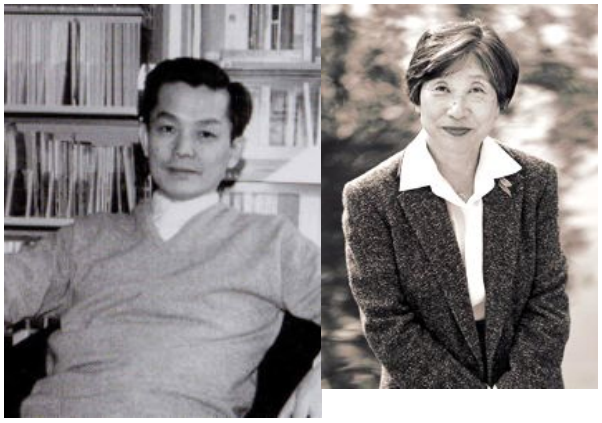
and DNA replication

DNA replication at the telomeres

Replication of both DNA strands is not the same.



https://upload.wikimedia.org/wikipedia/commons/8/8f/DNA_replication_en.svg



Reiji and Tsuneko Okazaki

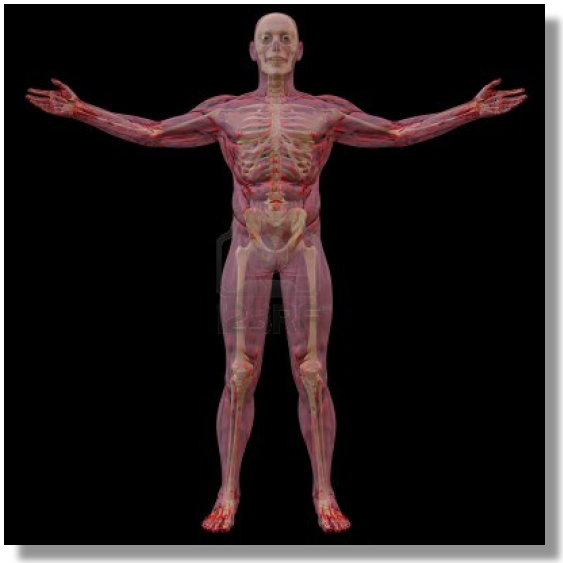
Chromosomes in most cells get shorter with each division

How many cells in the human body？

37,200,000,000,000
(=about 2^{46})

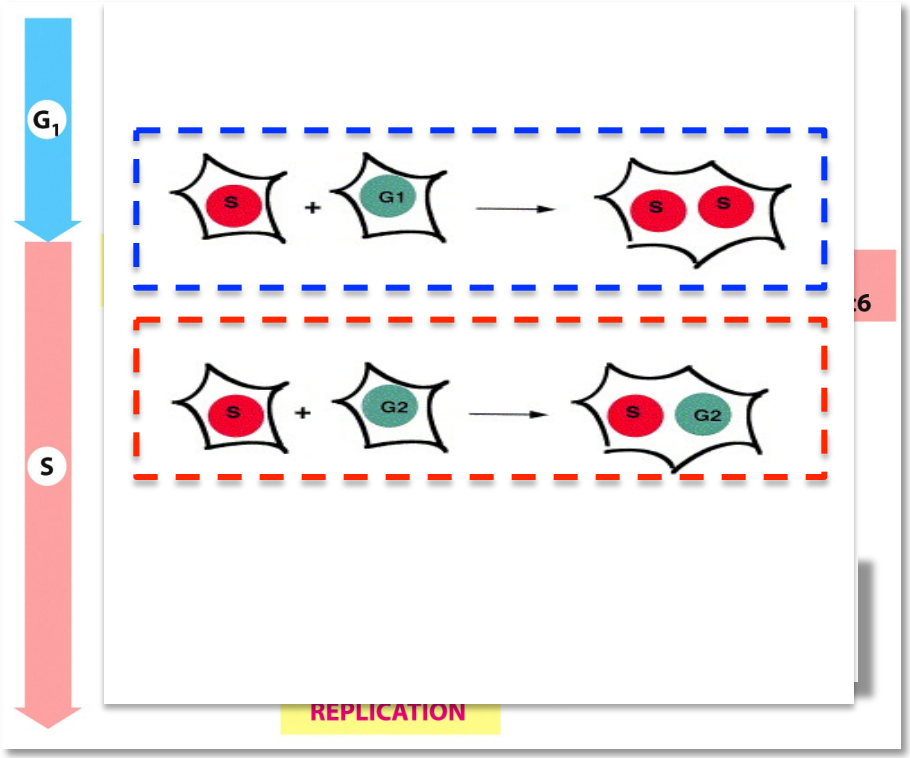
Average number of divisions:　47

If you believe Hayflick, how many divisions do you have left？



Ann Hum Biol. 2013 **An estimation of the number of cells in the human body.Bianconi E, Piovesan, Facchin F, Beraudi A, Casadei R, Frabetti F, Vitale L, Pelleri MC, Tassani S, Piva F, Perez-Amodio S, Strippoli P, Canaider S.**

So how do they actually function?

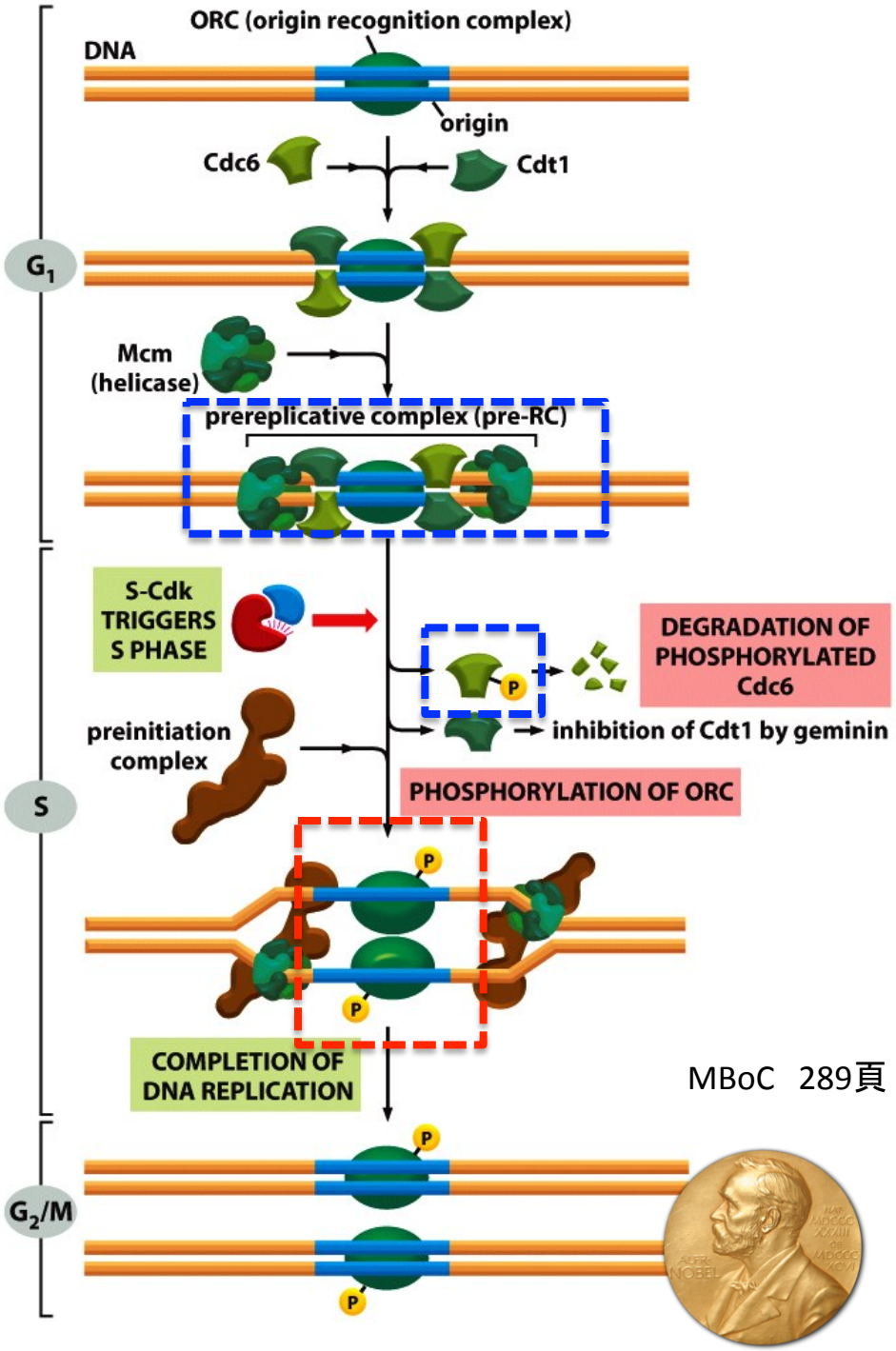


INTERPRETATION

The assembly of the pre-RC is the "licensing".

Phosphorylation of Cdc6 by Cdk induces its destruction and the start of DNA replication

Phosphorylation of ORC by S-Cdk is the "re-replication block".



MBoC 289頁

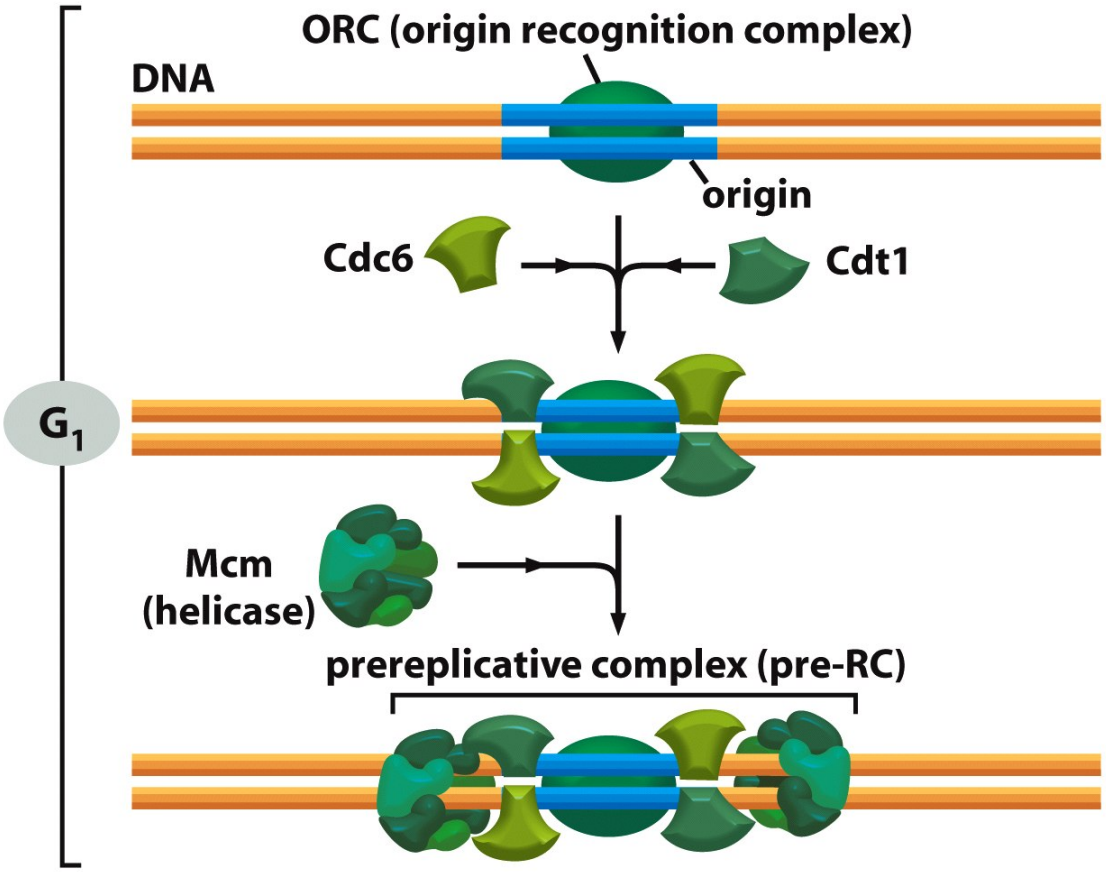


Figure 17-23 (part 1 of 3) *Molecular Biology of the Cell* (© Garland Science 2008)

MCM
designer "genes"

Bik-Kwoon Tye

http://bmcb.cornell.edu/faculty/tye.html

- Origin Recognition Complex:
  Orc1-Orc6 subunits

- Cdc6 : Identified as one of the yeast cdc mutants. Upregulated in many tumors.

- Cdt1 : cdc10-dependent transcript 1

- Mcm : minichromosome maintenance (6 subunits) Isolated as genes required to maintain artificial chromosomes in yeast.

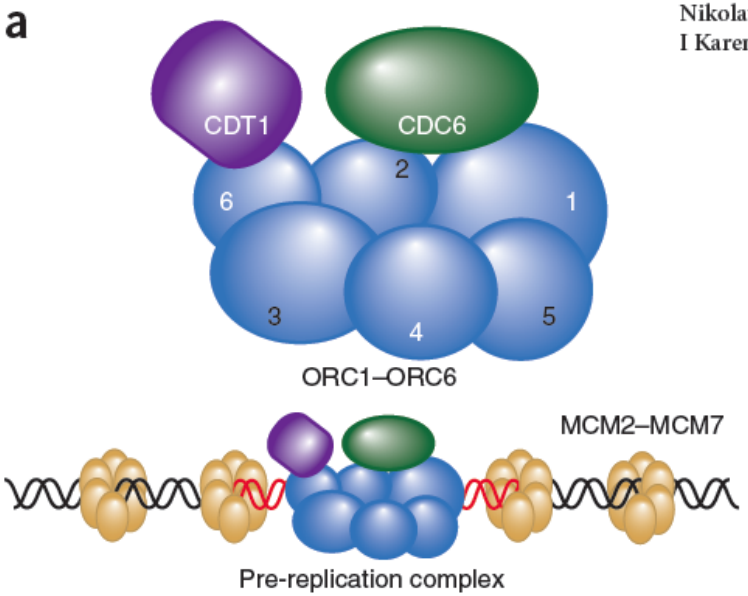
nature genetics

VOLUME 43 | NUMBER 4 | APRIL 2011 NATURE GENETICS p.356-359

Mutations in the pre-replication complex cause Meier-Gorlin syndrome

Louise S Bicknell[1,20], Ernie M H F Bongers[2,20], Andrea Leitch[1], Stephen Brown[1], Jeroen Schoots[2], Margaret E Harley[1], Salim Aftimos[3], Jumana Y Al-Aama[4,5], Michael Bober[6], Paul A J Brown[7], Hans van Bokhoven[8], John Dean[9], Alaa Y Edrees[5], Murray Feingold[10], Alan Fryer[11], Lies H Hoefsloot[2], Nikolaus Kau[12], Nine V A M Knoers[13], James MacKenzie[7], John M Opitz[14], Pierre Sarda[15], Alison Ross[9], I Karen Temple[16], Annick Toutain[17], Carol A Wise[18], Michael Wright[19] & Andrew P Jackson[1]

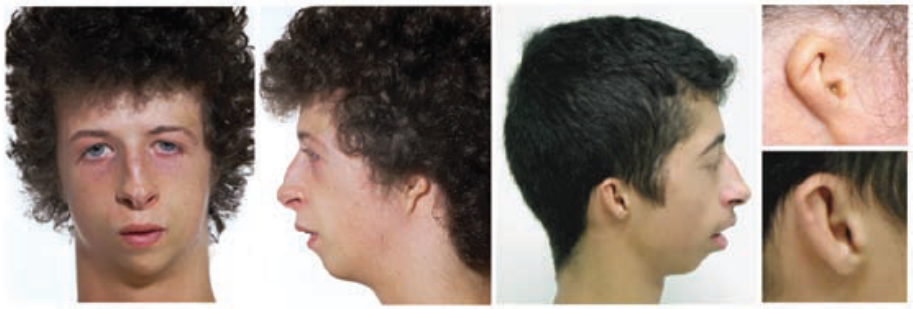
a



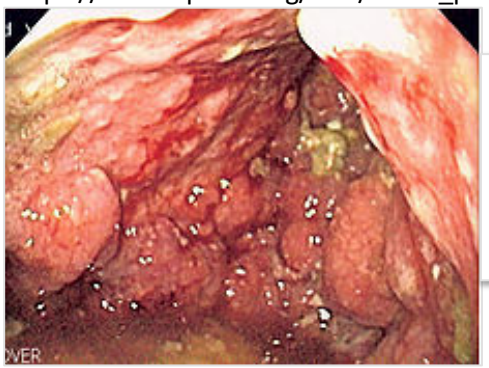
Meier-Gorlin syndrome: mutants of subunits in white

Growth of specific tissues is inhibited

c



https://en.wikipedia.org/wiki/Linitis_plastica



Scirrhous gastric carcinomas are caused by cdc6 upregulation

Cdc6 RNAi treatment

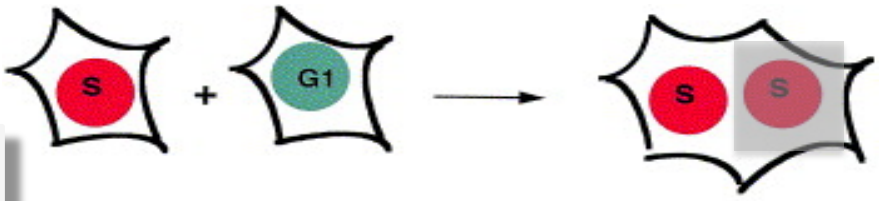
The experiment that got things started

(i) Existance of factor in S phase cells that induces DNA replication.

Licensing Factor

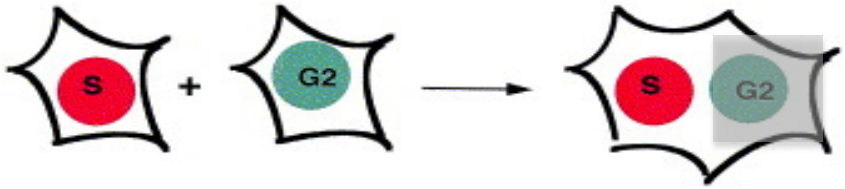
(ii) G1 phase cells can replicate DNA but G2 phase cells can not.

Re-replication block



(iii) Existance of factor in S phase cells that blocks progression to M phase.

Check Point



(iv) Once cells have gone through M phase they can replicate DNA again.

Re-licensing

Johnson, R.T. & Rao, P.N., Mammalian Cell Fusion: Induction of Premature Chromosome Condensation in Interphase Nuclei.
Nature 226, 717–722 (1970)

Trigger Mechanism for Mitosis

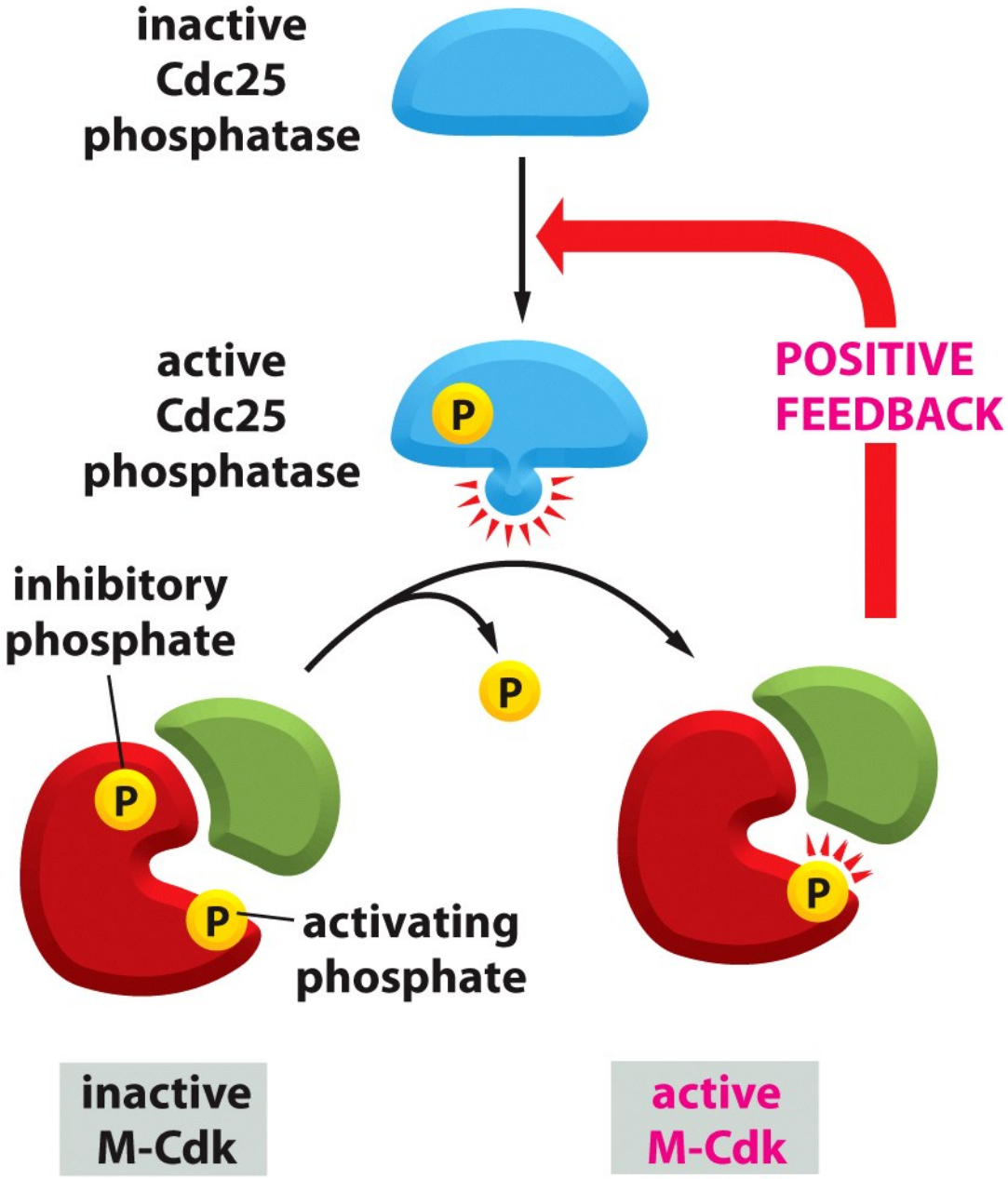
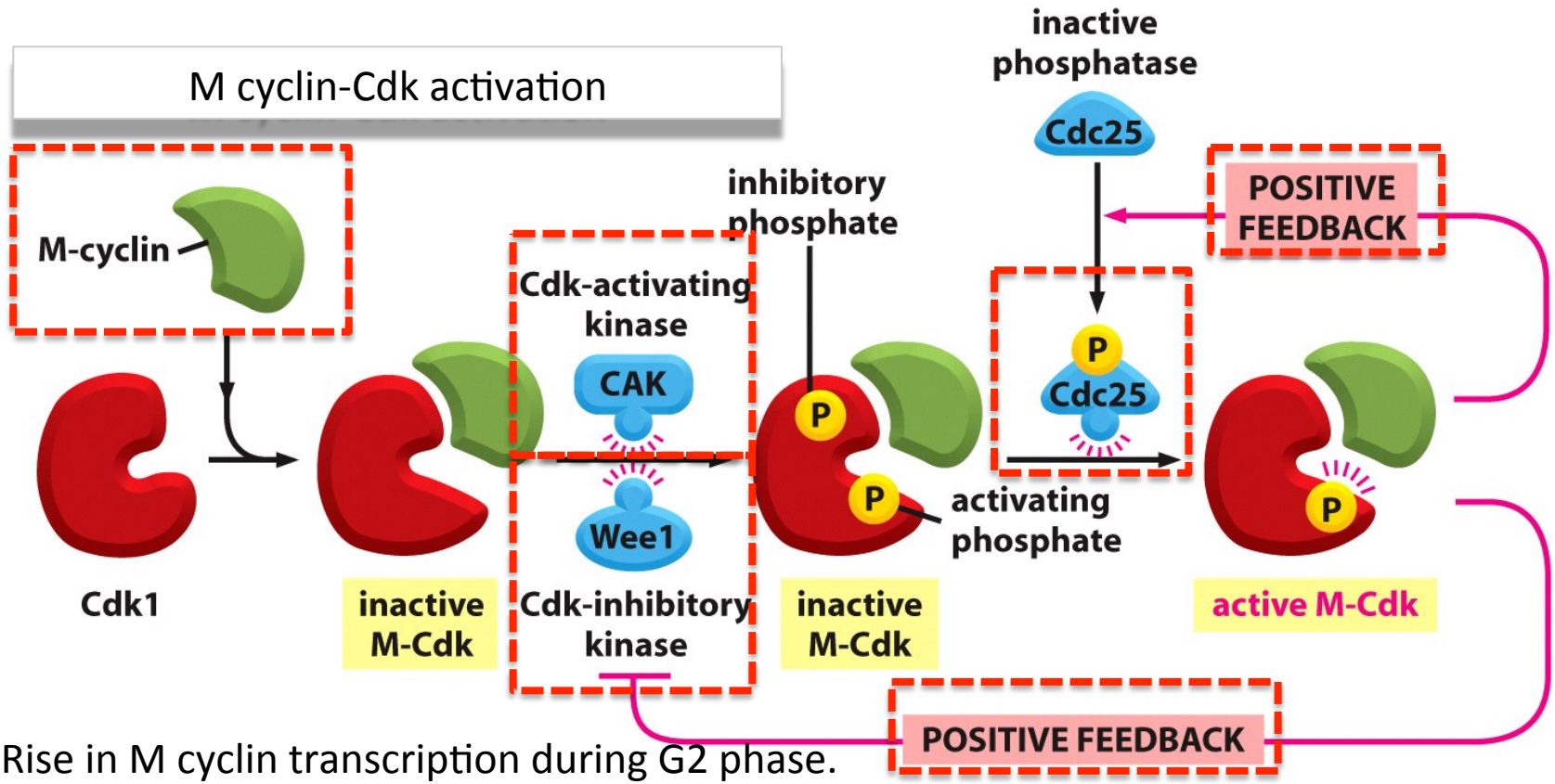
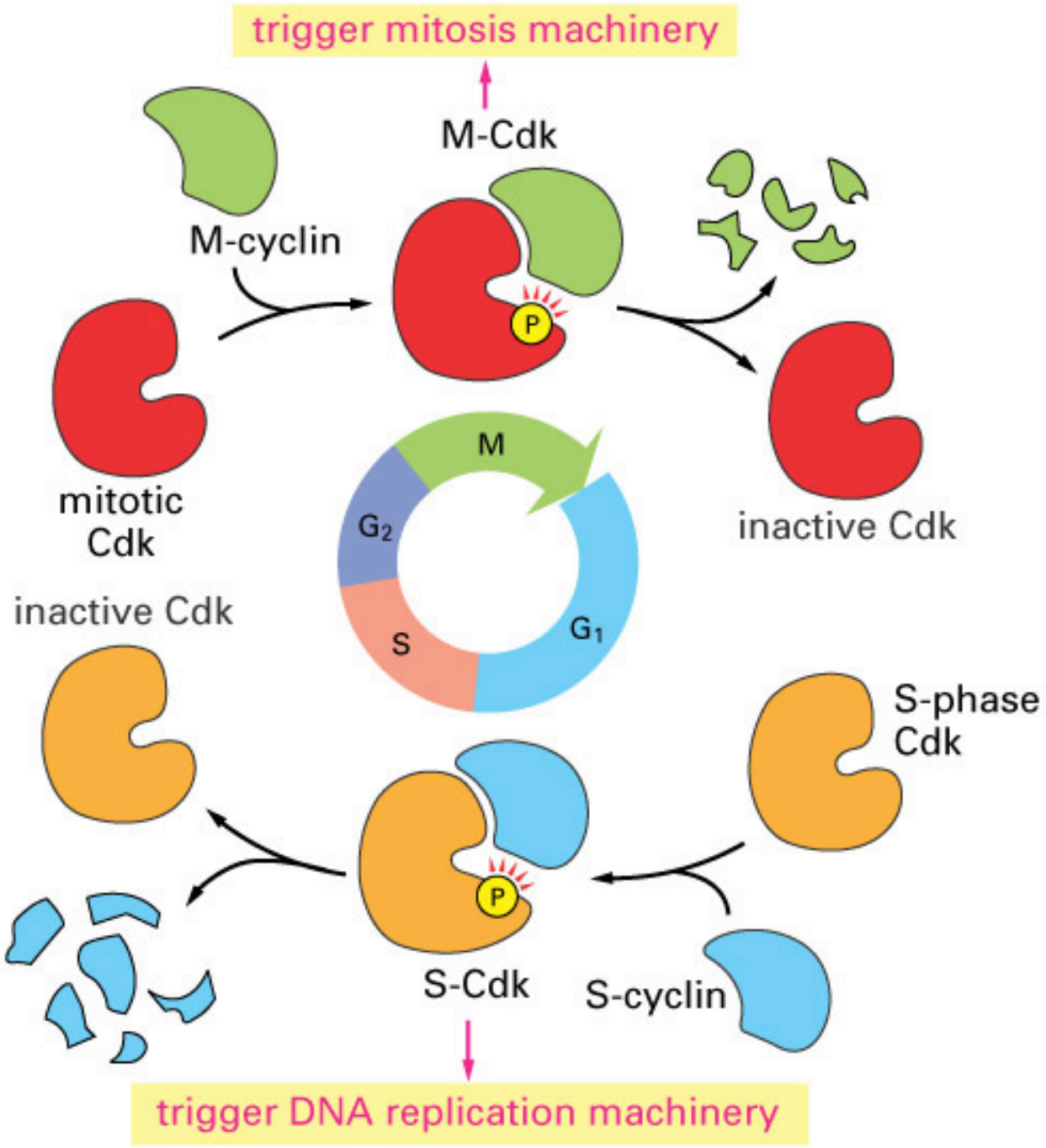


Figure 18-18 *Essential Cell Biology* (© Garland Science 2010)



1. Rise in M cyclin transcription during G2 phase.
2. Cdk-Activating Kinase (CAK) phosphorylates Cyclin-Cdk on its activation site.
3. At the same time Wee1 phosphorylates Cyclin-Cdk on its inhibitory site.
4. Dephosphorylation of the inhibitory site by Cdc25 tips the balance towards activation. The mechanism that initially induces Cdc25 activation is unknown.
5. Activated Cdk-Cyclin induces inhibition of Wee1 (double-negative feedback).
6. Activated Cdk-Cyclin induces activating phosphorylation of Cdc25 forming a positive feedback loop.

MBoC17-25
（ECB18–17）


trigger mitosis machinery
M-Cdk
M-cyclin
P
mitotic Cdk
inactive Cdk
M
G2
G1
S
inactive Cdk
S-phase Cdk
P
S-Cdk
S-cyclin
trigger DNA replication machinery

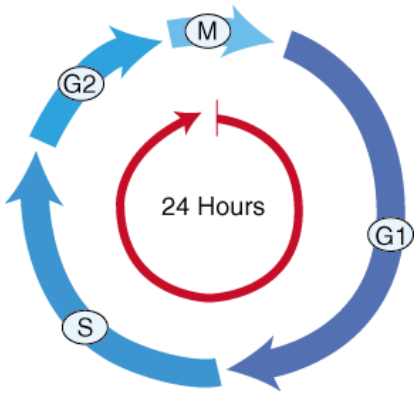
Cell-cycle times

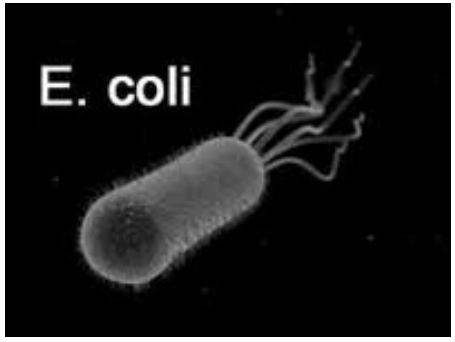
TABLE 18–1 SOME EUCARYOTIC CELL-CYCLE TIMES

CELL TYPE	CELL-CYCLE TIMES
Early frog embryo cells	30 minutes
Yeast cells	1.5–3 hours
Mammalian intestinal epithelial cells	~12 hours
Mammalian fibroblasts in culture	~20 hours
Human liver cells	~1 year

Table 18-1 Essential Cell Biology 3/e (© Garland Science 2010)

typically





Only 20 minutes!

https://endtimessigns.files.wordpress.com/2011/08/ecoli-bacteria.jpg

The paradox of E. coli cell division

•E. coli can divide once every 20 minutes.

•Their genome is 4,600,000 base pairs

•Their DNA replication rate is 1000bases／sec.

• 4.6 million/1000/2=2300sec.=38min

（Replication

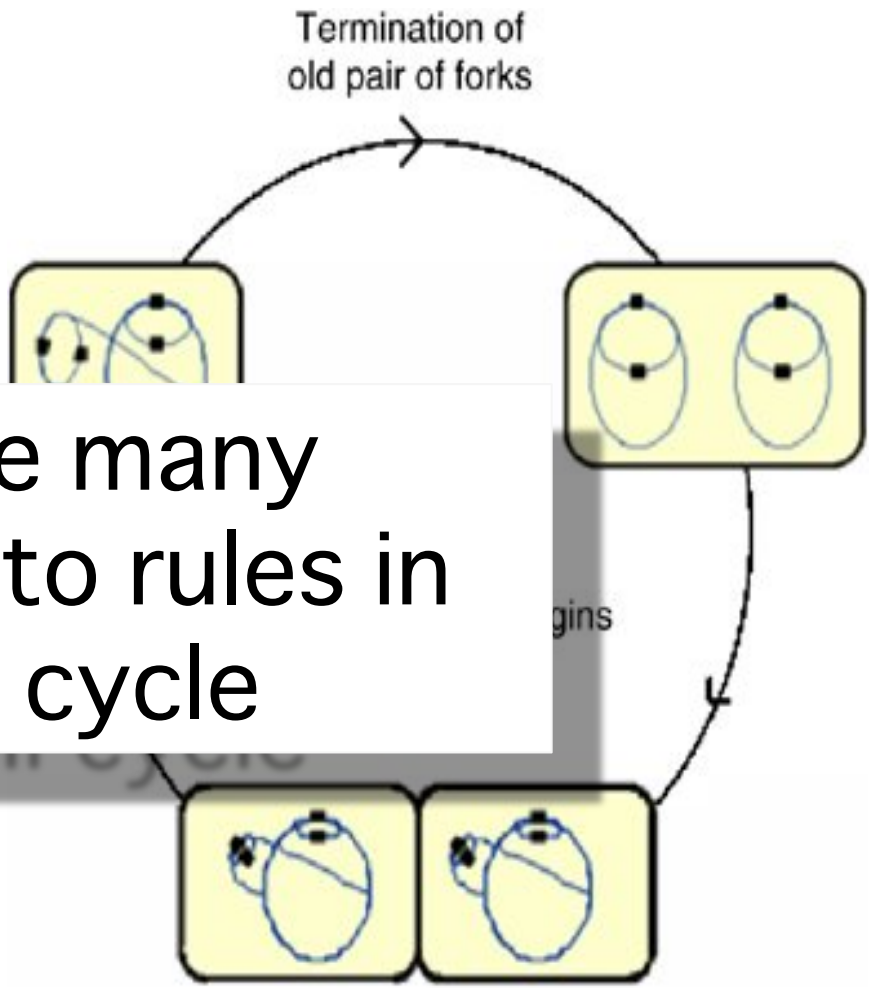
•Not

•Single repl

•Replication starts before the previous cycle is finished and division occurs even during replication

•They cheat！

There are many exceptions to rules in the cell cycle



http://sandwalk.blogspot.jp/2008/05/dna-replication-in-e-coli-solution.html

Summary

- A cell fusion experiment provided insight on the mechanisms regulating the cell cycle.
- Cyclin was discovered by biochemistry, and Cdk was discovered by genetics.
- Many cell cycle factors were identified as temperature-sensitive Cdc (Cell division cycle) mutants.
- Partial destruction of the pre-replicative complex (Orc, MCM proteins etc.) triggered by Cdk enables initiation of S phase (Replication).
- Phosphorylation of ORC by Cdk blocks re-replication within the same cell cycle.
- Activation of Cdk-Cyclin by Cdc25 tips the balance of activation-inhibition toward activation and in reinforced by positive feedback.

Next week

- The Mitotic Spindle
- The Cell Cycle Checkpoint
- Anaphase Progression