

WS 2018/19 Grundvorlesung Modul 8 „Allgemeine und Molekulare Genetik“

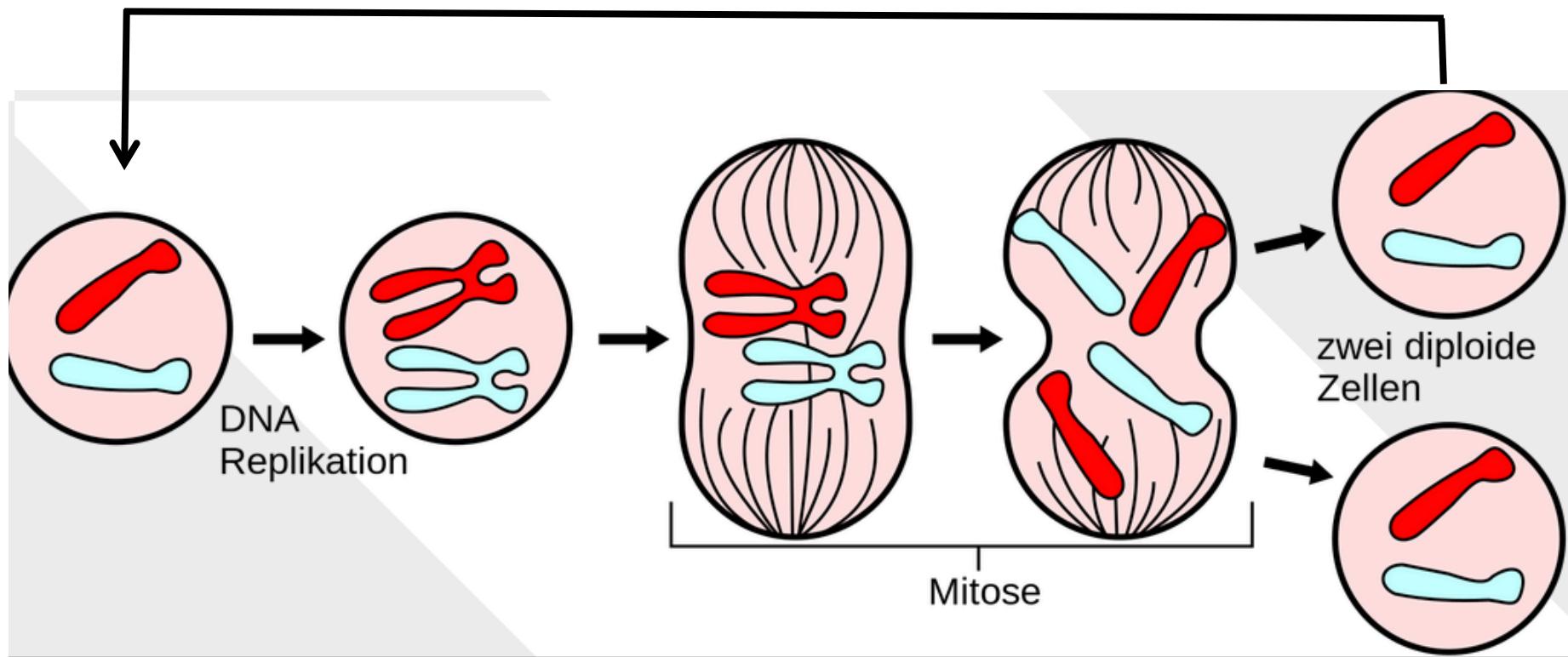
Replikation der DNA

Kap. 20



An animated primer on the basics of DNA, genes, and heredity.

Ohne DNA-Replikation gibt es nichts zu verteilen....



DNA replication at work



MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β -D-deoxyribofuranose residues with 3',5'-linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's² model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There

This figure is purely diagrammatical. The two phosphate-sugar chains, and the horizontal rods of bases, follow the same axes. The vertical line marks the fibre axis.

is a residue on each chain every 3.4 Å. in the α -direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical α -co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally^{3,4} that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{5,6} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material. Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

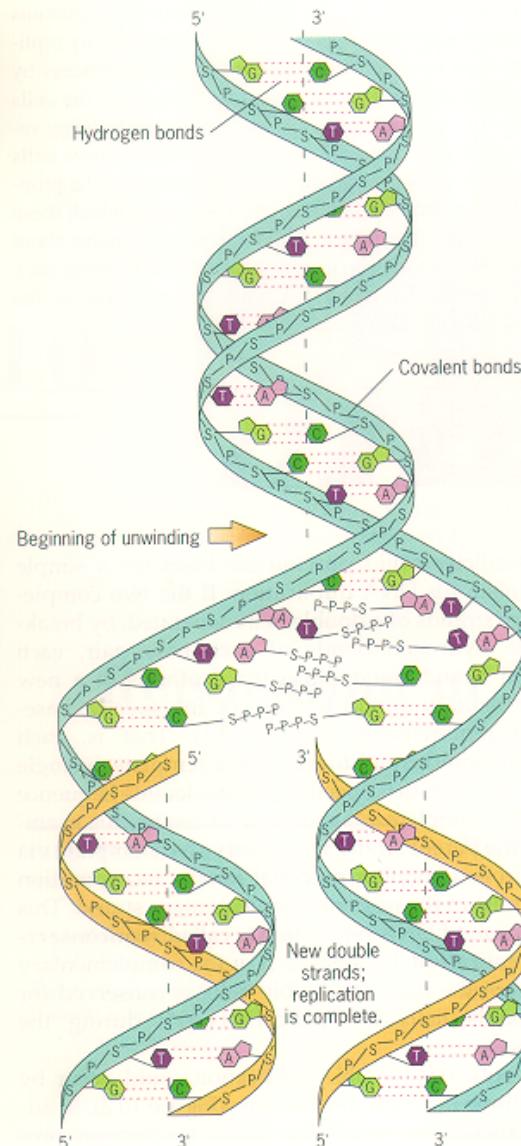
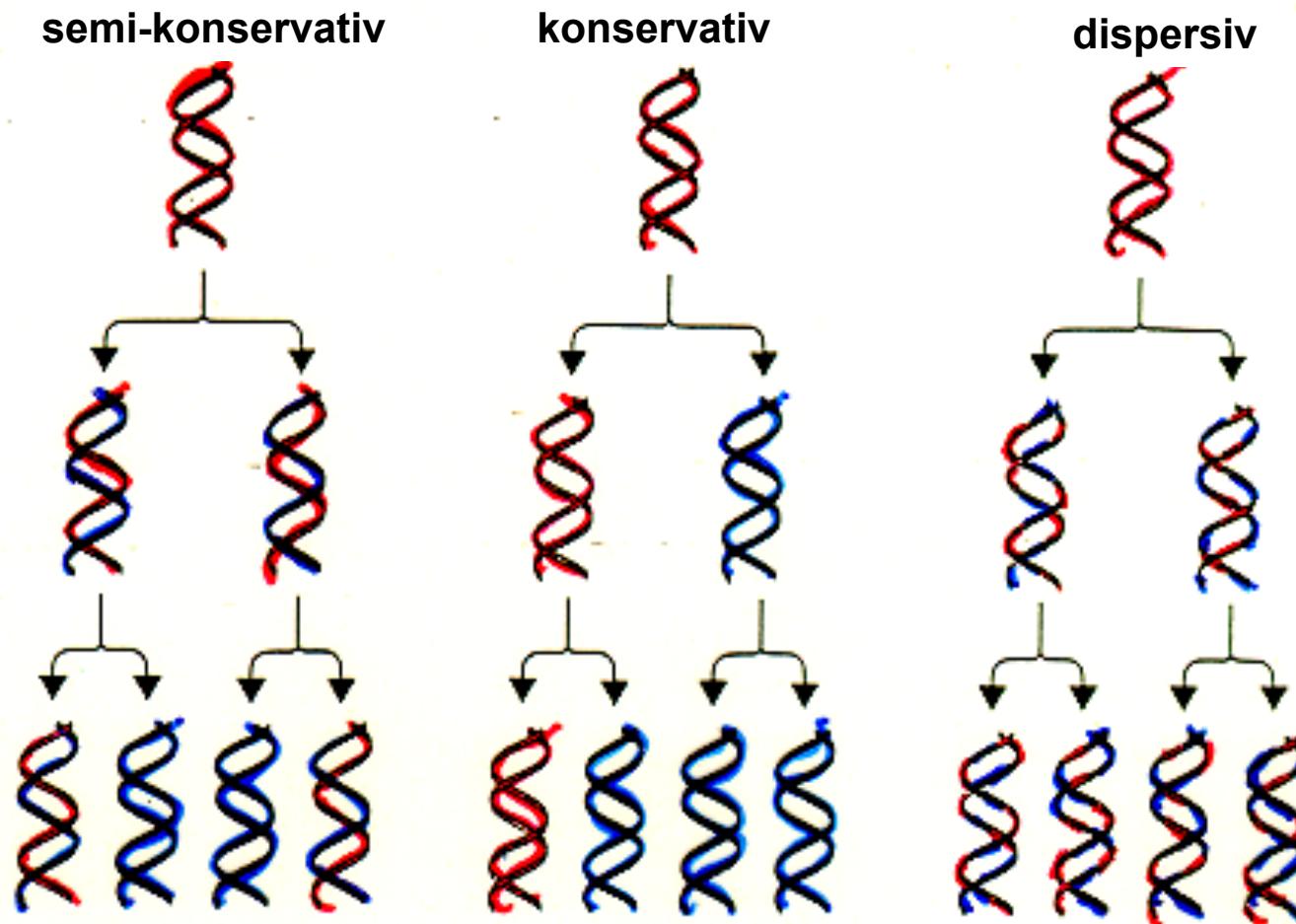


Figure 10.1 Semiconservative model of DNA replication based on complementary base pairing as proposed by Watson and Crick. Note that each of the parental strands is conserved and serves as a template for the synthesis of a new complementary strand. That is, the base sequence in each progeny strand is determined by the hydrogen-bonding potentials of the bases in the parental strand.

Modelle der DNA-Replikation



Das Meselson/Stahl-Experiment (1958)

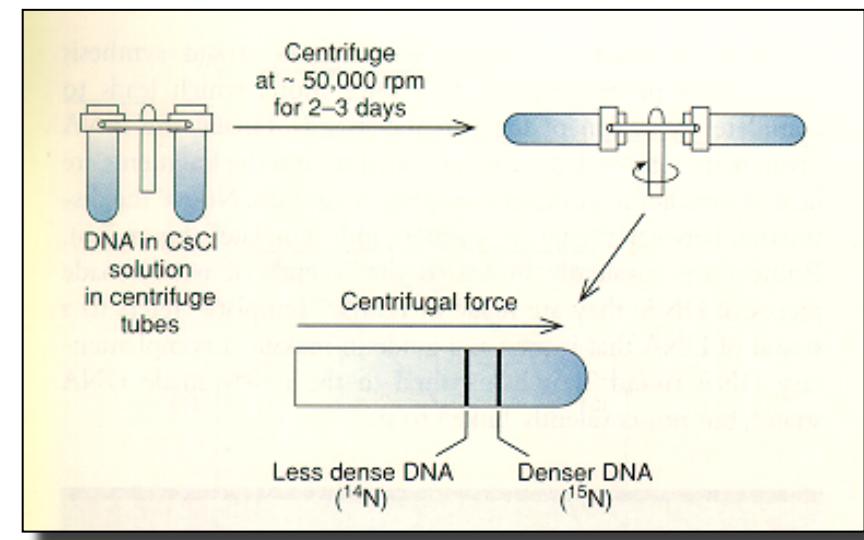


Mathew Meselson

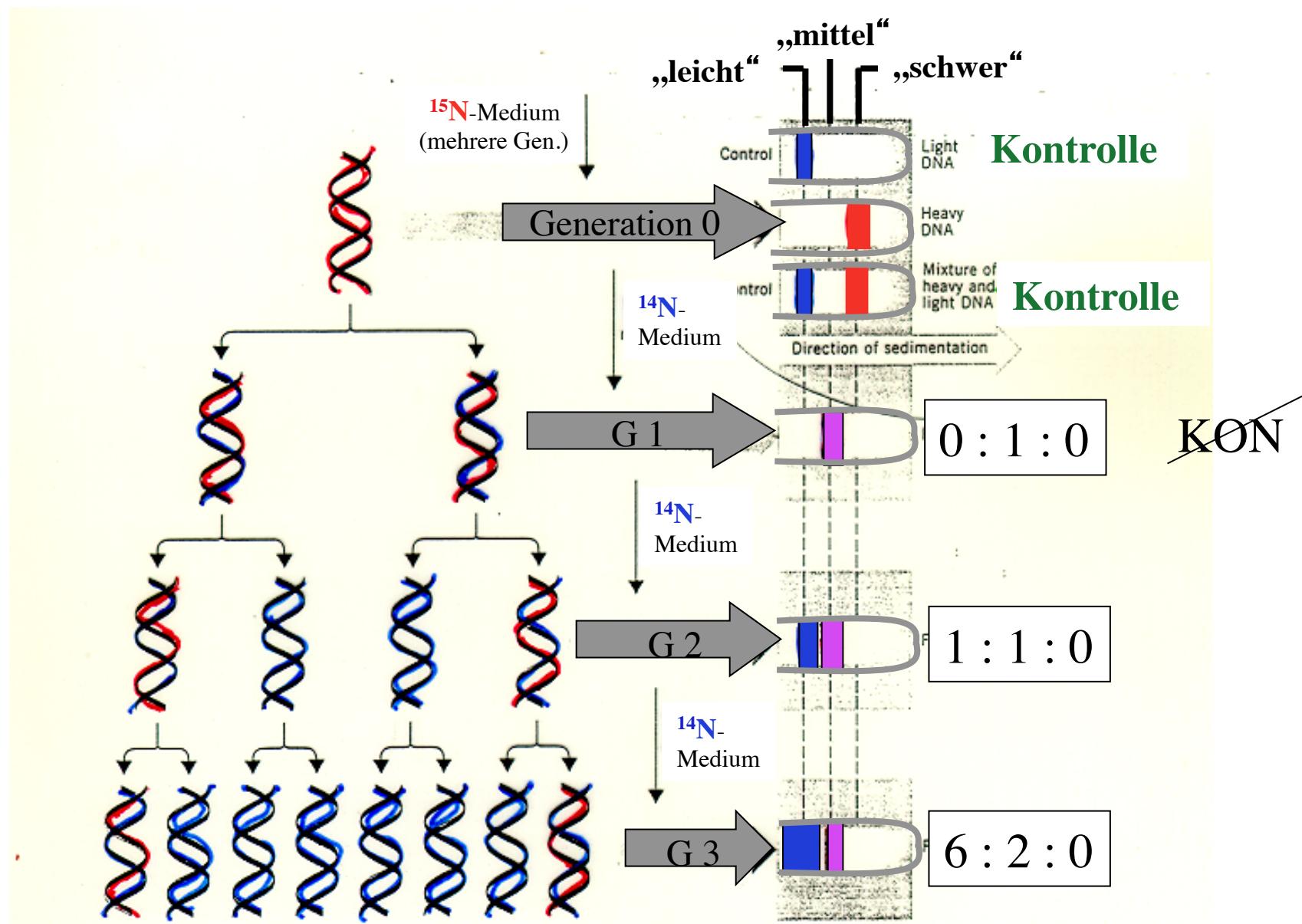


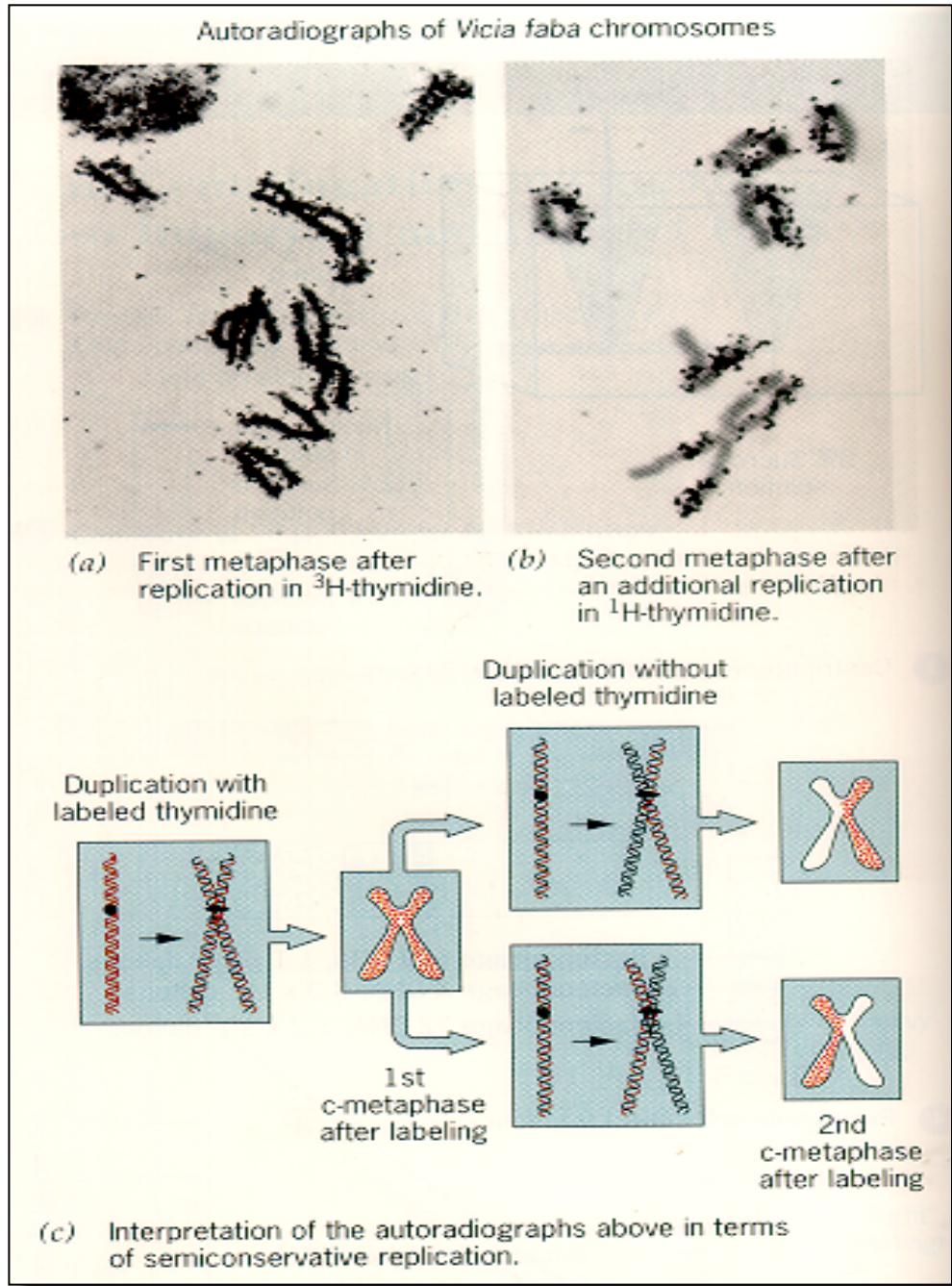
Franklin Stahl

- *E. coli* für 12 Std. in ^{15}N -Medium > DNA höherer Dichte
- Anschließendes Zurückhalten einer ^{15}N -Probe (Generation 0) und Überführen der restlichen Kultur in ^{14}N -Medium
- Weitere drei Probennahmen nach jeder Verdoppelung der Zellkultur (Generationen 1-3)
- Messung der DNA-Dichte durch CsCl-Gleichgewichts-Dichtezentrifugation



Das Meselson/Stahl-Experiment



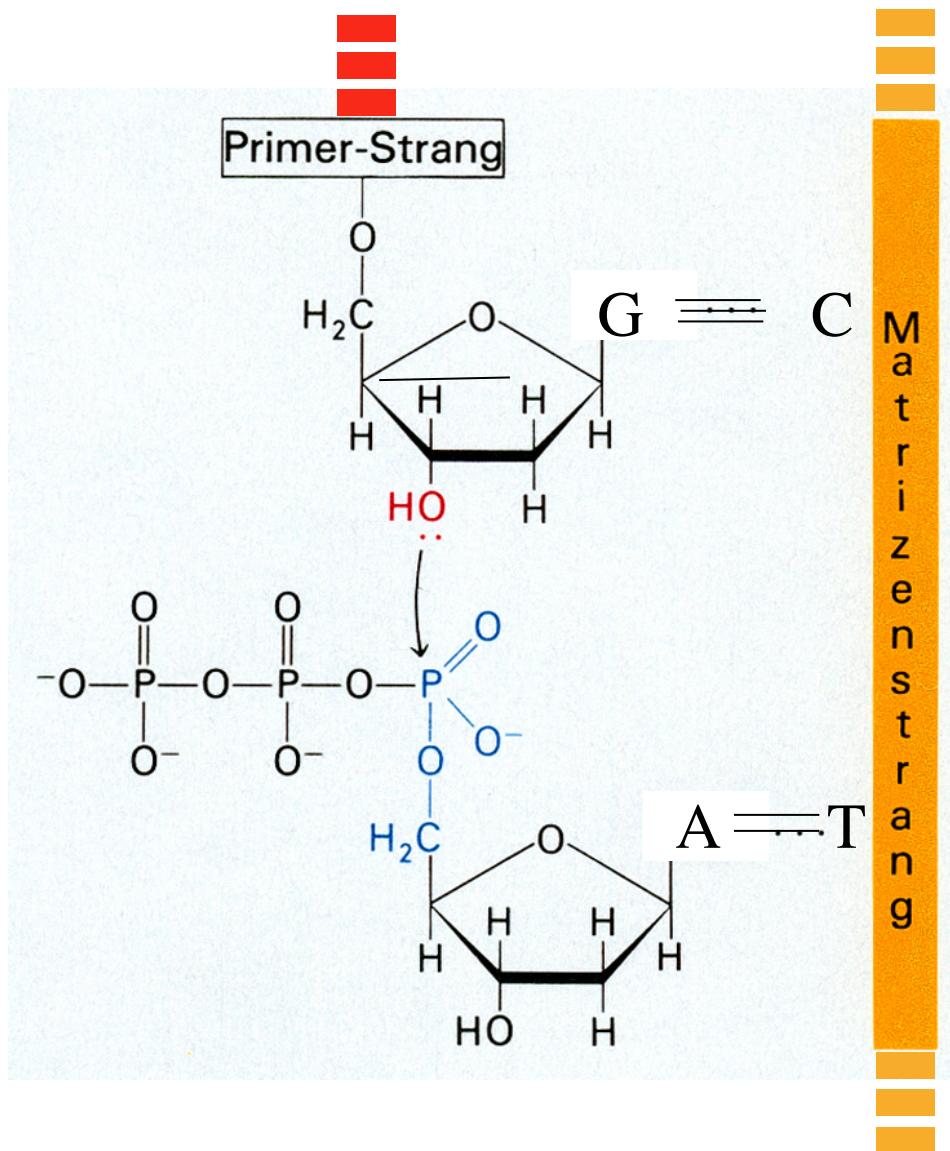


Das Taylor-Experiment (1957)

...zeigt semi-konservative Replikation bei eukaryot. Chromosomen

Lit: Klug/Cummings/Spencer - Genetik
S. 374

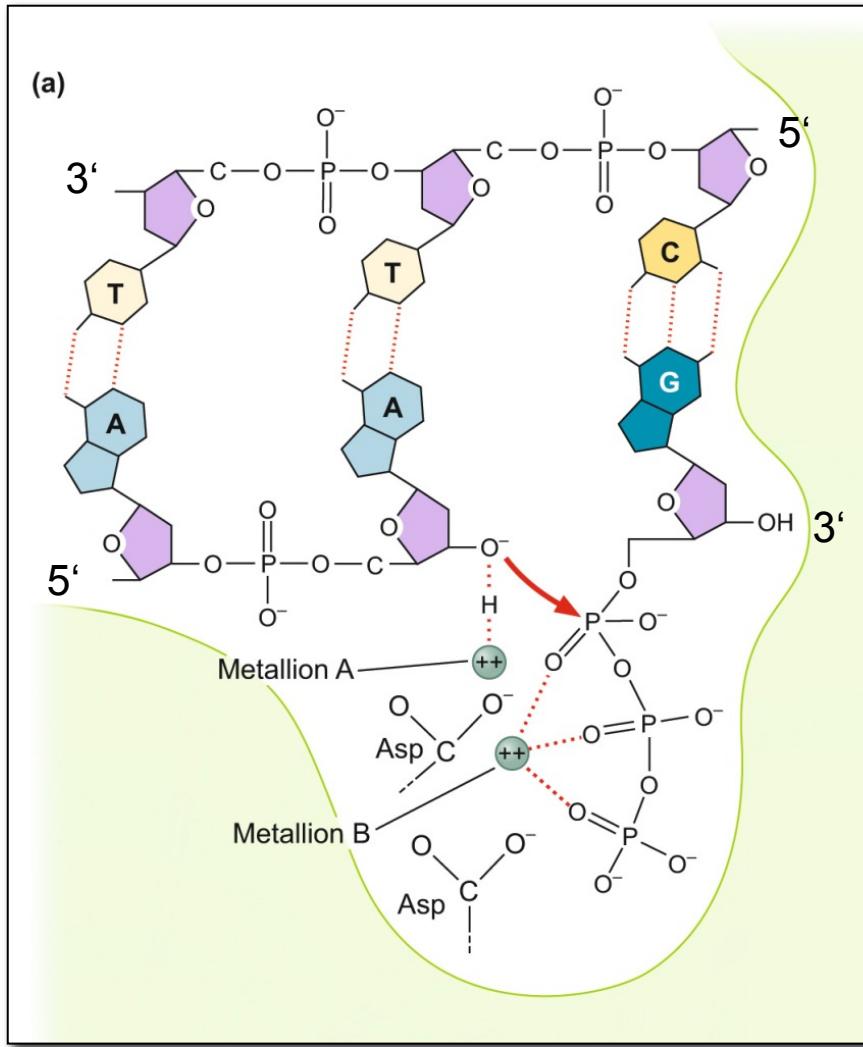
Die Polymerisation von DNA



Einbau neuer Nukleotide mit komplementären Basen anhand der Vorlage des Matrizen-Stranges.

Es ist ein **PRIMER** mit einem **freien 3'OH-Ende** notwendig!

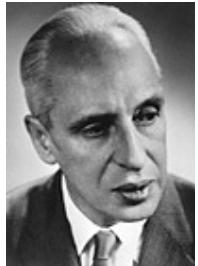
Aktives Zentrum der DNA-Pol



DNA Pol hat
Mg⁺⁺ Ionen im aktiven
Zentrum.

Ion A schwächt OH-Bindung
und erhöht nukleophile
Reaktivität des O-Atoms.

Ion B neutralisiert z. T.
die negative Ladung des
PPi.



DNA-Polymerasen



1958

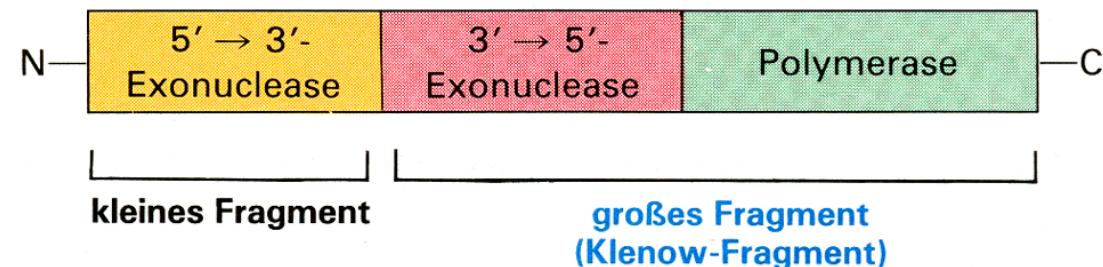
Severo Ochoa Arthur Kornberg

- alle bekannten DNA-Polymerase synthetisieren DNA von **5' nach 3'**
- alle DNA-Polymerasen benötigen zum Start ein **freies 3'OH-Ende**, das von einem „**Primer**“ geliefert werden muss.

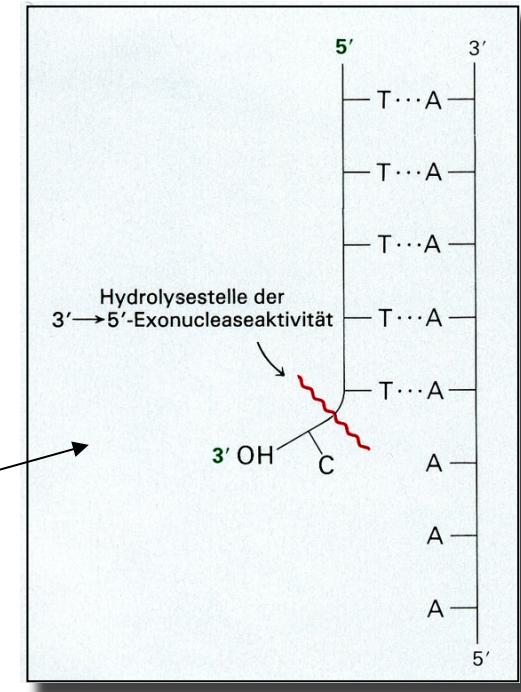
(nur RNA-Polymerasen kommen ohne Primer aus)

DNA-Polymerasen haben oft mehrere Funktionen

DNA-Polymerase I
aus E. coli



- 5' > 3' Polymerase-Funktion
- 5' > 3' Exonuklease-Funktion:
Primerentfernung, Reparatur
- 3' > 5' Exonuklease-Funktion:
„Korrektur-Lesen“





The Nobel Prize in Chemistry 2015
Tomas Lindahl, Paul Modrich, Aziz Sancar

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The Nobel Prize in Chemistry 2015



Photo: Cancer Research UK

Tomas Lindahl

Prize share: 1/3

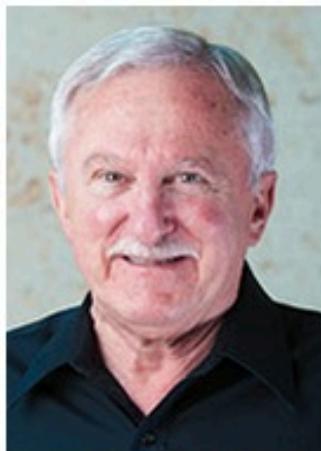


Photo: K. Wolf/AP Images for HHMI

Paul Modrich

Prize share: 1/3



Photo: M. Englund,
UNC-School of Medicine

Aziz Sancar

Prize share: 1/3

Vorlesung „Mutationen und Reparatur“, Jan 2019

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar *“for mechanistic studies of DNA repair”*.



Dennoch: bisweilen ist die DNA-Replikation fehlerhaft und erzeugt Mutationen!

Male-driven evolution of DNA sequences

Lawrence C. Shimmin, Benny Hung-Junn Chang & Wen-Hsiung Li*

Center for Demographic and Population Genetics, University of Texas, PO Box 20334, Houston, Texas 77225, USA

It is commonly believed^{1,2} that the mutation rate is much higher in the human male germ line than in the female germ line because the number of germ-cell divisions per generation is much larger in males than in females. But direct estimation of mutation rates is difficult, relying mainly on sex-linked genetic diseases³, so the ratio (α_m) of male to female mutation rates is not clear. It has been noted⁴ that if α_m is very large, then the rate of synonymous substitution in X-linked genes should be only 2/3 of that in autosomal genes, and comparison of human and rodent genes supported this prediction⁴. As the number of X-linked genes used in the study was small and the X-linked and autosomal sequences were non-homologous, and given that the synonymous rate varies among genes⁵, we sequenced the last intron (~1 kb) of the Y-linked and X-linked zinc-finger-protein genes (ZFY and ZFX) in humans, orang-utans, baboons and squirrel monkeys. The ratio Y/X of the substitution rate in the Y-linked intron to that in the X-linked intron is ~2.3, which is close to that estimated from synonymous rates in the ZFY and ZFX genes^{6–8} and implies $\alpha_m \approx 6$. This estimate of α_m supports the view that the evolution of DNA sequences in higher primates is male-driven. It is, however, much lower than the previous estimate⁴ and therefore raises a number of issues.

Figure 1 shows a condensed alignment of the sequences of the last intron of the human, orang-utan, baboon and squirrel

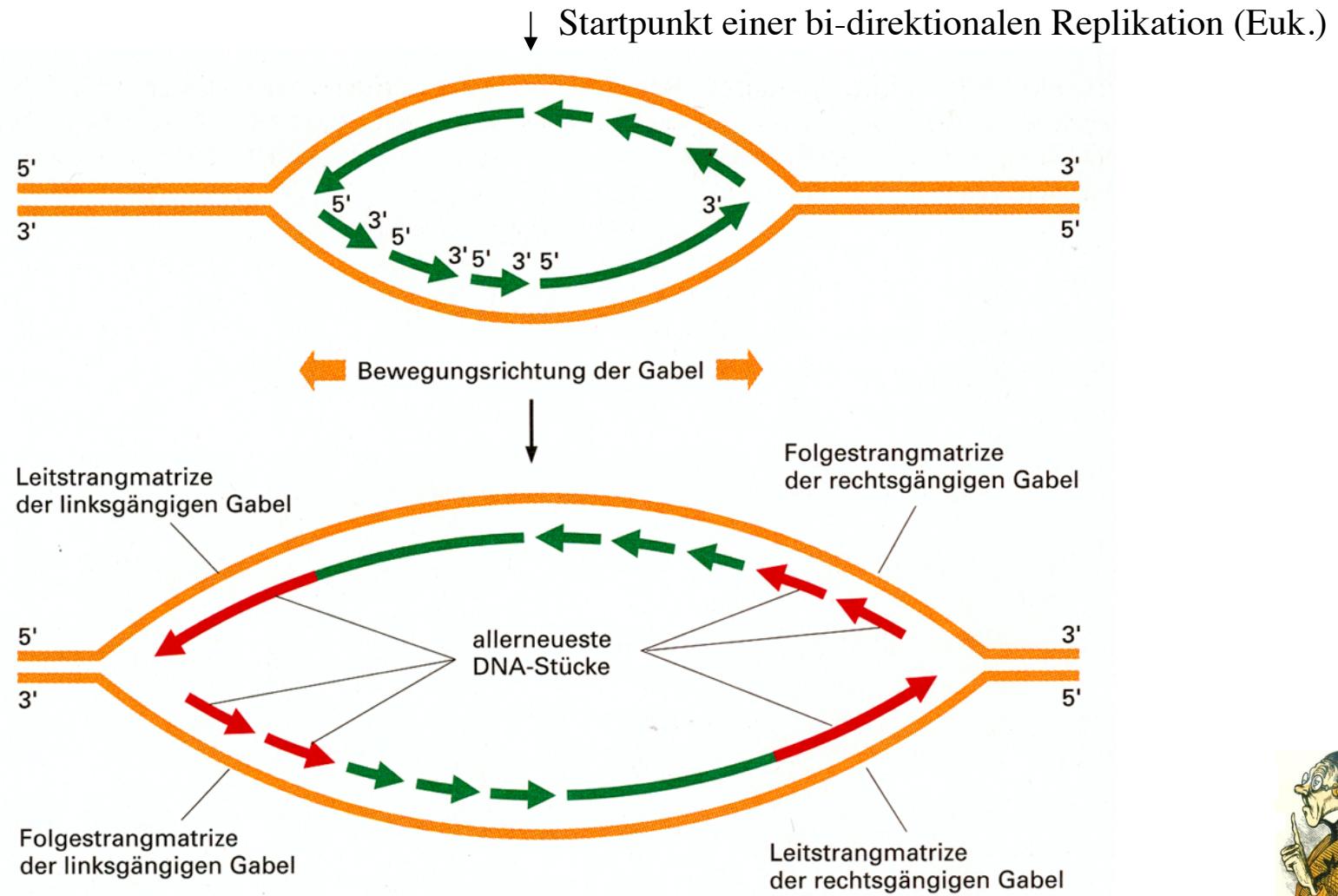
* To whom correspondence should be addressed.

Jonsson et al. 2017, Nature:

- ein Kind von 30jährigen Eltern erbt ca. 11 Neumutationen von Mutter und ca. 45 vom Vater!
- denn: Spermium eines 20jährigen hat 160 Replikationsrunden hinter sich, das eines 40jährigen 610!
- diese Neumutationen können Grund für seltene genetische Erkrankungen sein

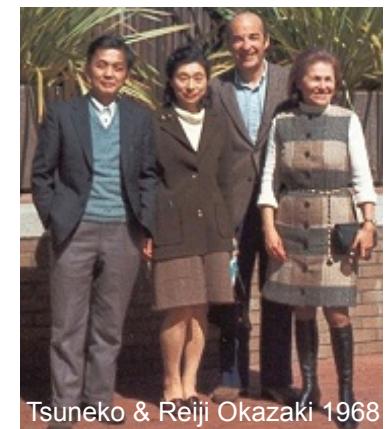
<https://www.theguardian.com/science/2017/sep/20/fathers-pass-on-four-times-as-many-new-genetic-mutations-as-mothers-study>

Die DNA-Replikation muss „semi-diskontinuierlich“ verlaufen



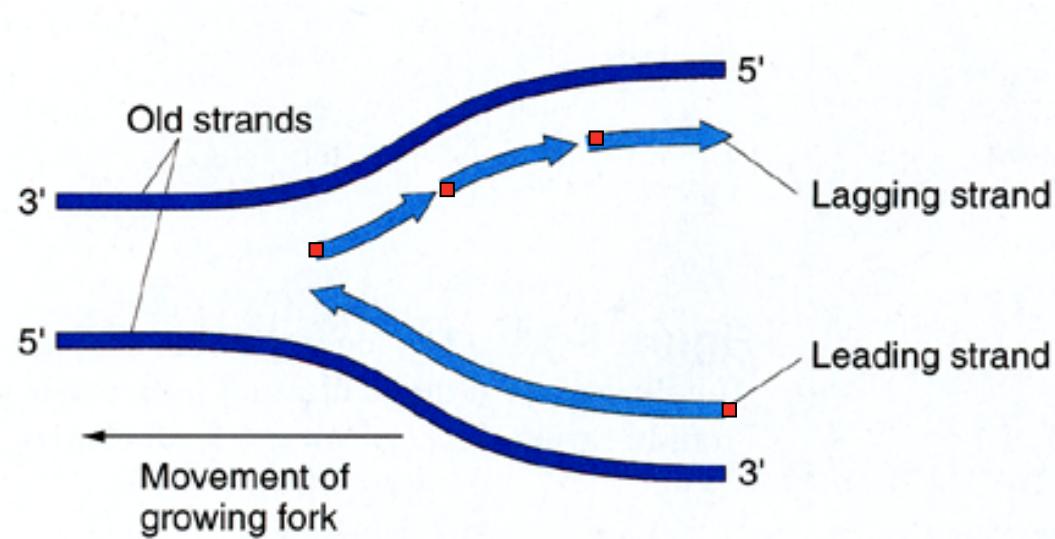
Wir halten fest...

- auf dem **Leit-Strang** („leading strand“) kann die DNA-Synthese **kontinuierlich** erfolgen
- auf dem **Folge-Strang** („lagging strand“) erfolgt die Replikation **diskontinuierlich** in kleineren Abschnitten (= Okazaki-Fragmente)



Tsuneko & Reiji Okazaki 1968

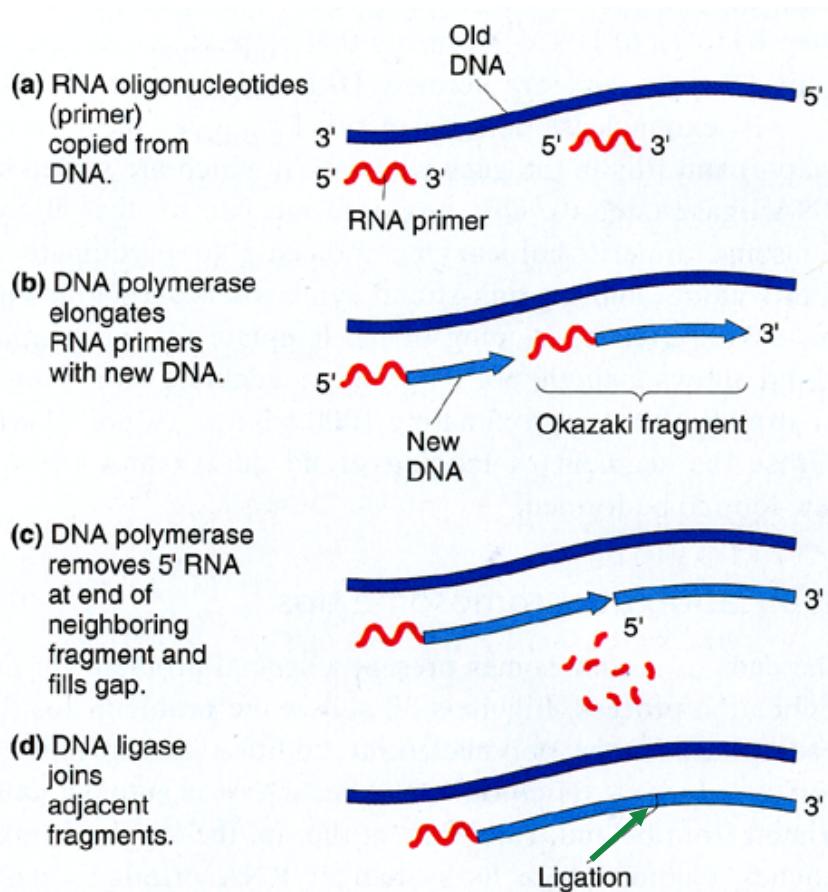
DNA-Synthese startet von RNA-Primern aus!



- beide Stränge werden ausgehend von **RNA-Primern** repliziert
- RNA-Primer werden von einer speziellen RNA-Polymerase (**„Primase“**) synthetisiert
- Grund: RNA-Polymerasen können ohne bereits existierendes 3 'OH-Ende eine Synthese beginnen

RNA-Primer müssen raus!

lagging strand



- Primer-Entfernung erfolgt durch 5 ' $>$ 3 ' Exonuklease-Aktivität der DNA-Polymerase (oder durch RNase H, s.u.)

- Okazaki-Fragmente werden durch **DNA-Ligasen** verknüpft (diese verbinden 5 'P-Ende mit 3 'OH-Ende)



Enzymatic Removal of Ribonucleotides from DNA Is Essential for Mammalian Genome Integrity and Development

Martin A.M. Reijns,^{1,3} Björn Rabe,^{1,3} Rachel E. Rigby,¹ Pleasantine Mill,¹ Katy R. Astell,¹ Laura A. Lettice,¹ Shelagh Boyle,¹ Andrea Leitch,¹ Margaret Keighren,¹ Fiona Kilanowski,¹ Paul S. Devenney,¹ David Sexton,¹ Graeme Grimes,¹ Ian J. Holt,² Robert E. Hill,¹ Martin S. Taylor,¹ Kirstie A. Lawson,¹ Julia R. Dorin,¹ and Andrew P. Jackson^{1,*}

¹Medical Research Council Human Genetics Unit, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK

The presence of ribonucleotides in genomic DNA is undesirable given their increased susceptibility to hydrolysis. Ribonuclease (RNase) H enzymes that recognize and process such embedded ribonucleotides are present in all domains of life. However, in unicellular organisms such as budding yeast, they are not required for viability or even efficient cellular proliferation, while in humans, RNase H2 hypomorphic mutations cause the neuroinflammatory disorder Aicardi-Goutières syndrome. Here, we report that RNase H2 is an essential enzyme in mice, required for embryonic growth from gastrulation onward. RNase H2 null embryos accumulate large numbers of single (or di-) ribonucleotides embedded in their genomic DNA (>1,000,000 per cell), resulting in genome instability and a p53-dependent DNA-damage response. Our findings establish RNase H2 as a key mammalian genome surveillance enzyme required for ribonucleotide removal and demonstrate that ribonucleotides are the most commonly occurring endogenous nucleotide base lesion in replicating cells.

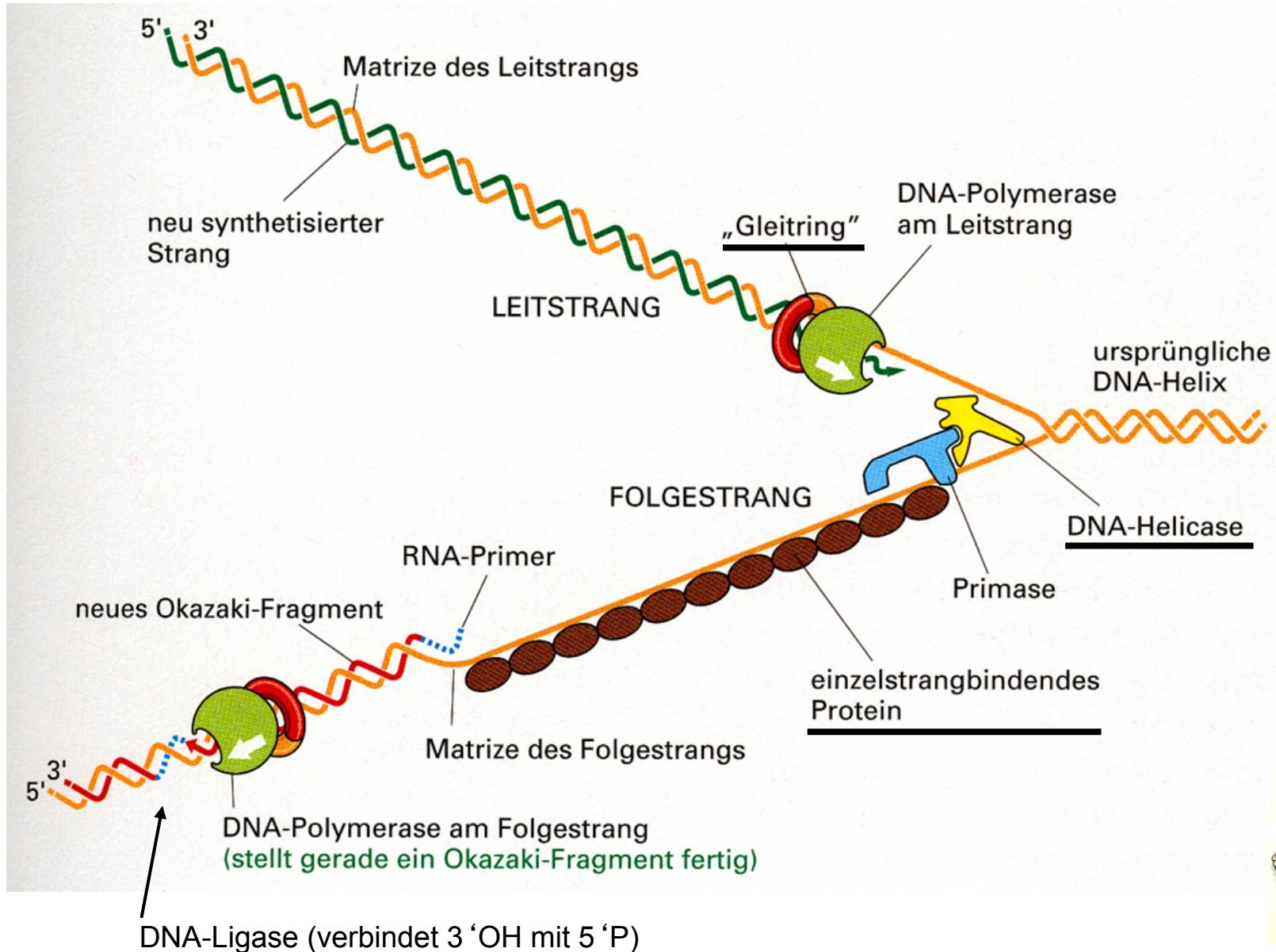
Lange Zeit übersehen:

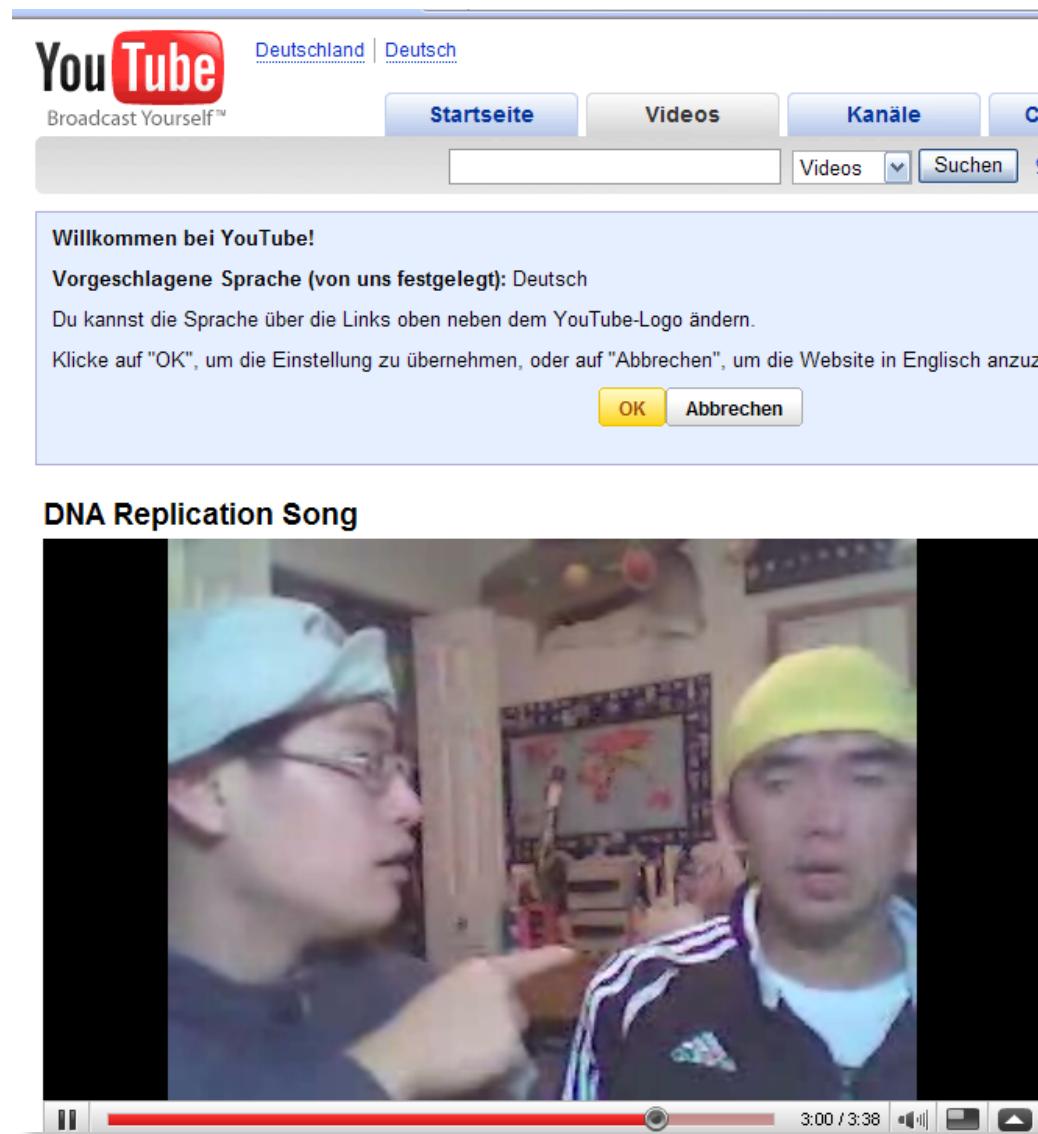
RNase H2 entfernt
NTPs aus der
replizierenden DNA

zurück zu den Basics...

Die Replikationsgabel

-Vereinfachte Version-

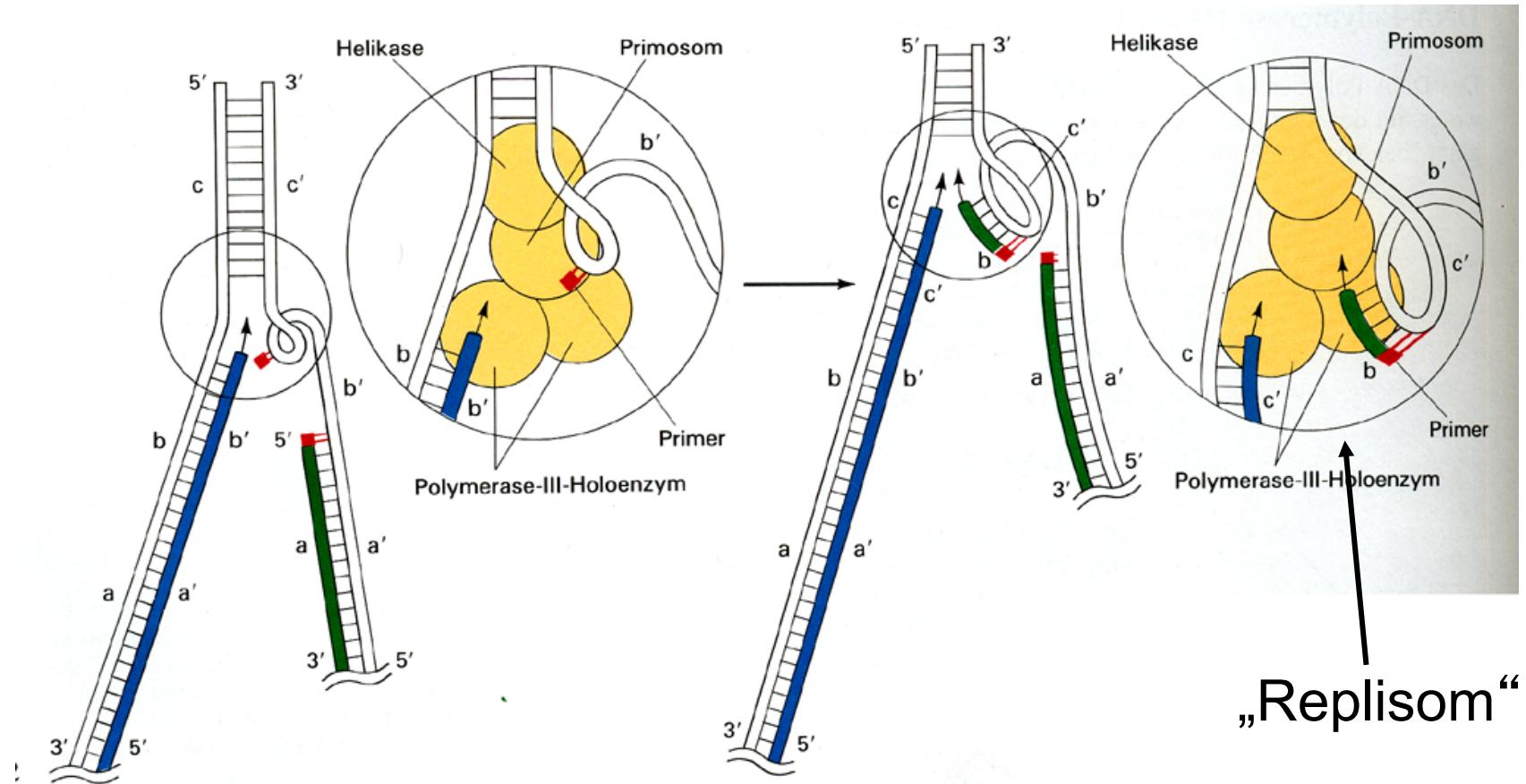




Kontrastprogramm aus den 1970ern? „protein synthesis dance“

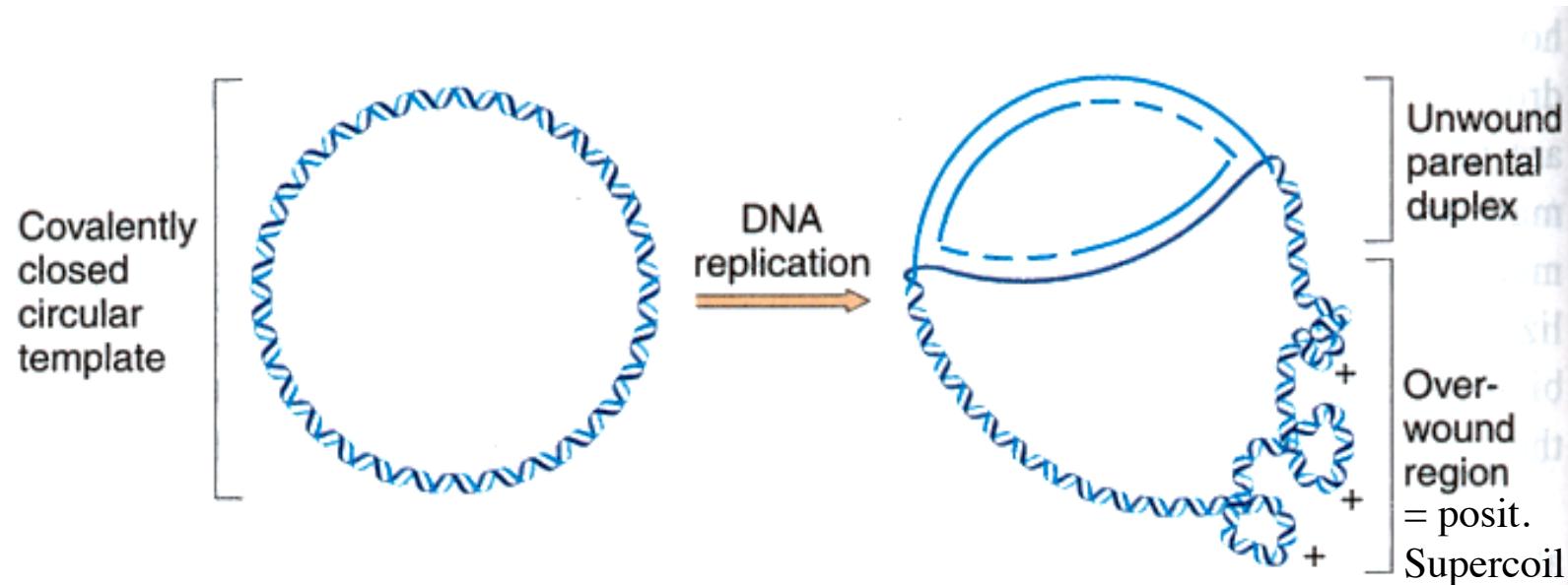
Die Replikationsgabel

-realistischer-



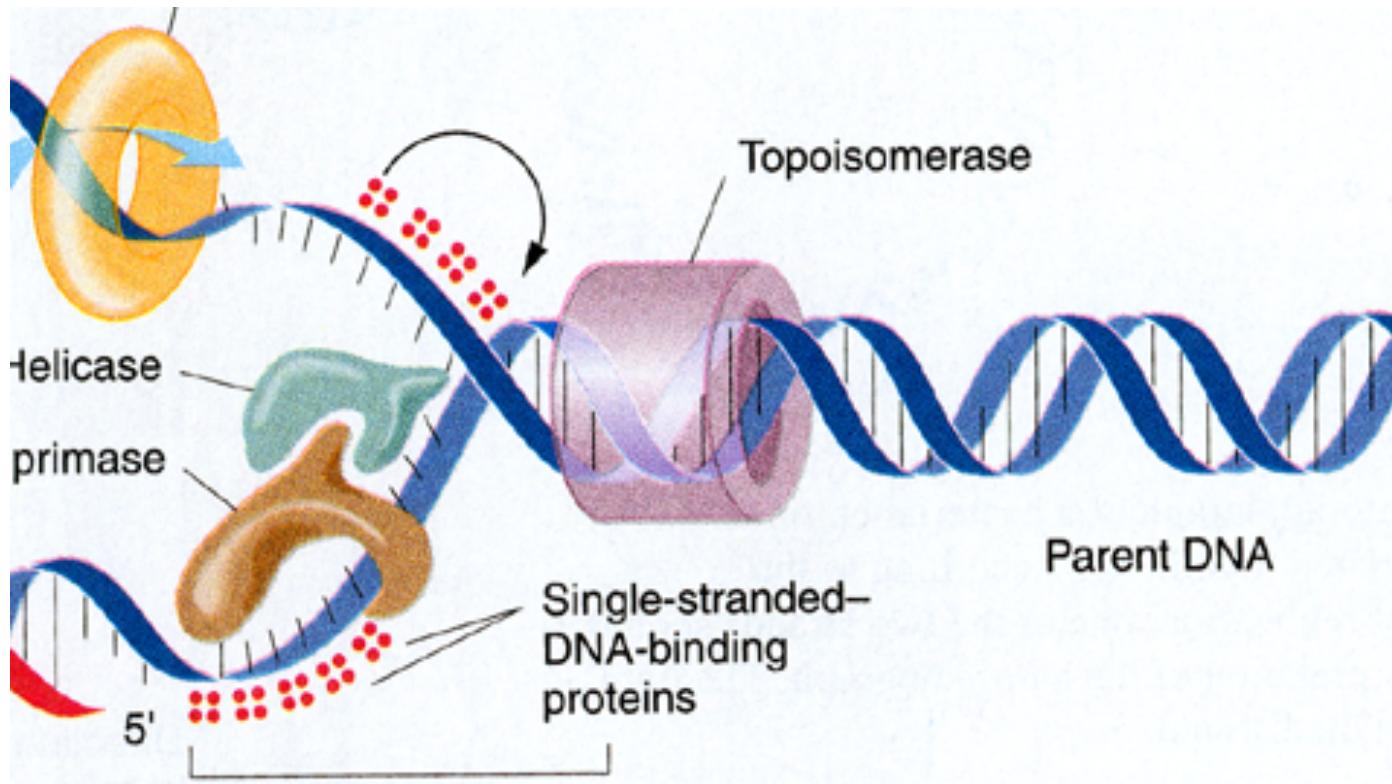
Schlaufenbildung des Folgestrangs ermöglicht es dem Polymerase-Komplex, auf beiden Strängen in die gleiche Richtung zu laufen

Stress an der Rep-Gabel!



Auch bei linearen, aber sehr langen und zudem an der Kernmembran verankerten Chromosomen entsteht **Windungsstress (Torsionen)**.

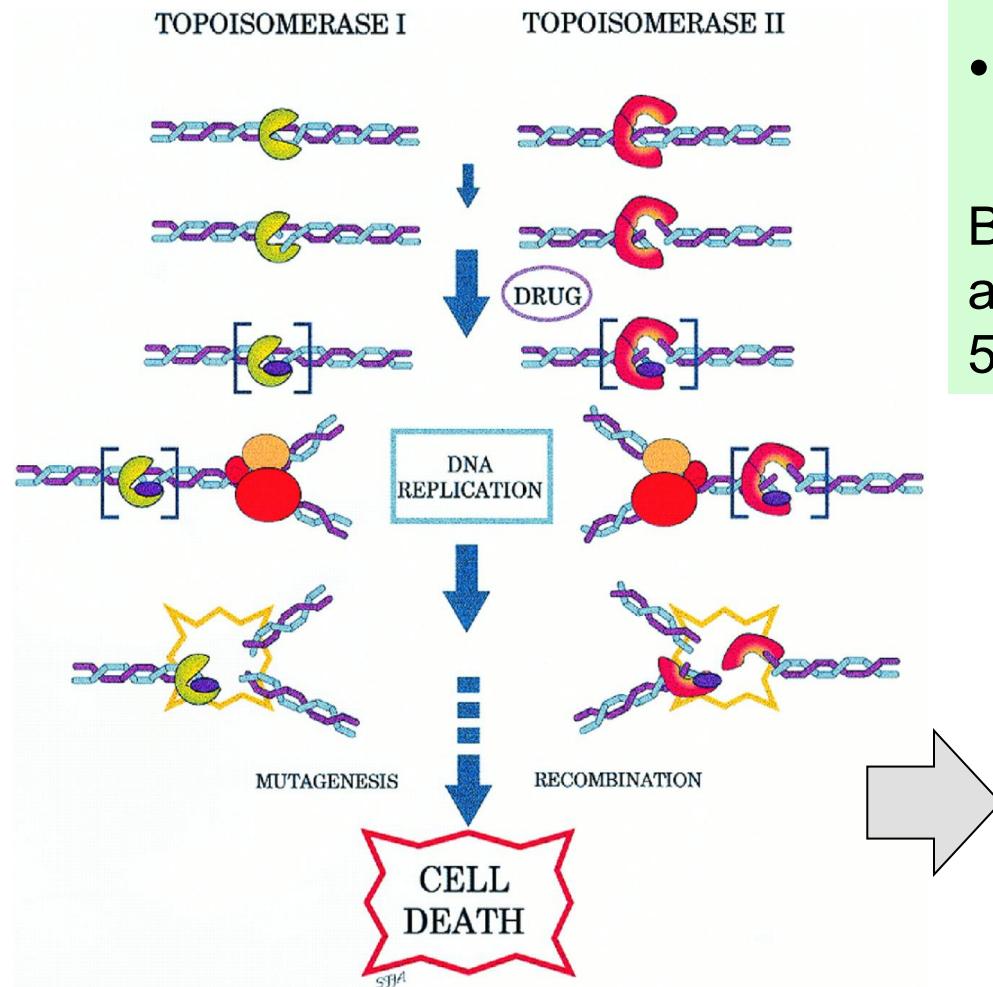
Funktion der Topoisomerasen



- oberhalb der Gabel entstehen **Torsionen**
- Topoisomerasen entspannen die Helix durch vorübergehende Durchtrennung und erneute Ligation („nicking-closing enzymes“)



DNA-Topoisomerasen



- Typ 1 > Einzelstrangbrüche
- Typ 2 > Doppelstrangbrüche
(z. B. Gyrase bei E. coli)

Bindungen der DNA werden zeitweise auf Protein übertragen, indem z.B. 5'P an OH-Gruppe von Tyrosin bindet

Topoisomerase-Hemmer
sind wirksame Zytostatika
in der **Krebstherapie!**

Key Players



DNA-Polymerasen:

- katalysieren Einbau von Trinukleotiden unter Abspaltung von Pyrophosphat
- brauchen (zumeist) Matrizenstrang und Primer
- können Zusatzfunktionen haben

DNA-Helikasen:

- lösen H-Brücken unter ATP-Verbrauch
- treiben die Gabel voran
- viele Helikasen bei Eukaryoten, z.T. an Rekombination u. Reparatur beteiligt

Einzelstrang-Bindeproteine:

- stabilisieren esDNA (E. coli ssb bedeckt 8-12 Nukleotide)

Polymerase-Klammer:

- verhindert vorzeitiges Ablösen der POL

Primase :

- synthetisiert Primer (ist eine RNA-Polymerase)

Topoisomerasen :

- beseitigen Torsionsstress durch Schneiden und Schließen der DNA

DNA-Ligase:

- schließt DNA-Rückgrat durch Verbindung von 5 'P und 3 'OH -Enden

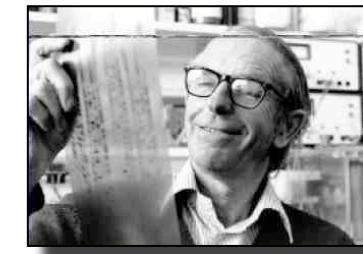
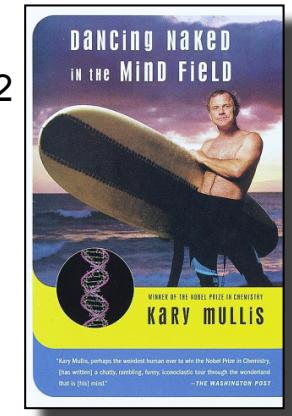
DNA-Replikation *in vitro*...



...ist eine weitere Basistechnik der Molekulargenetik und wird in zahlreichen Verfahren eingesetzt. Hier nur die zwei wichtigsten:

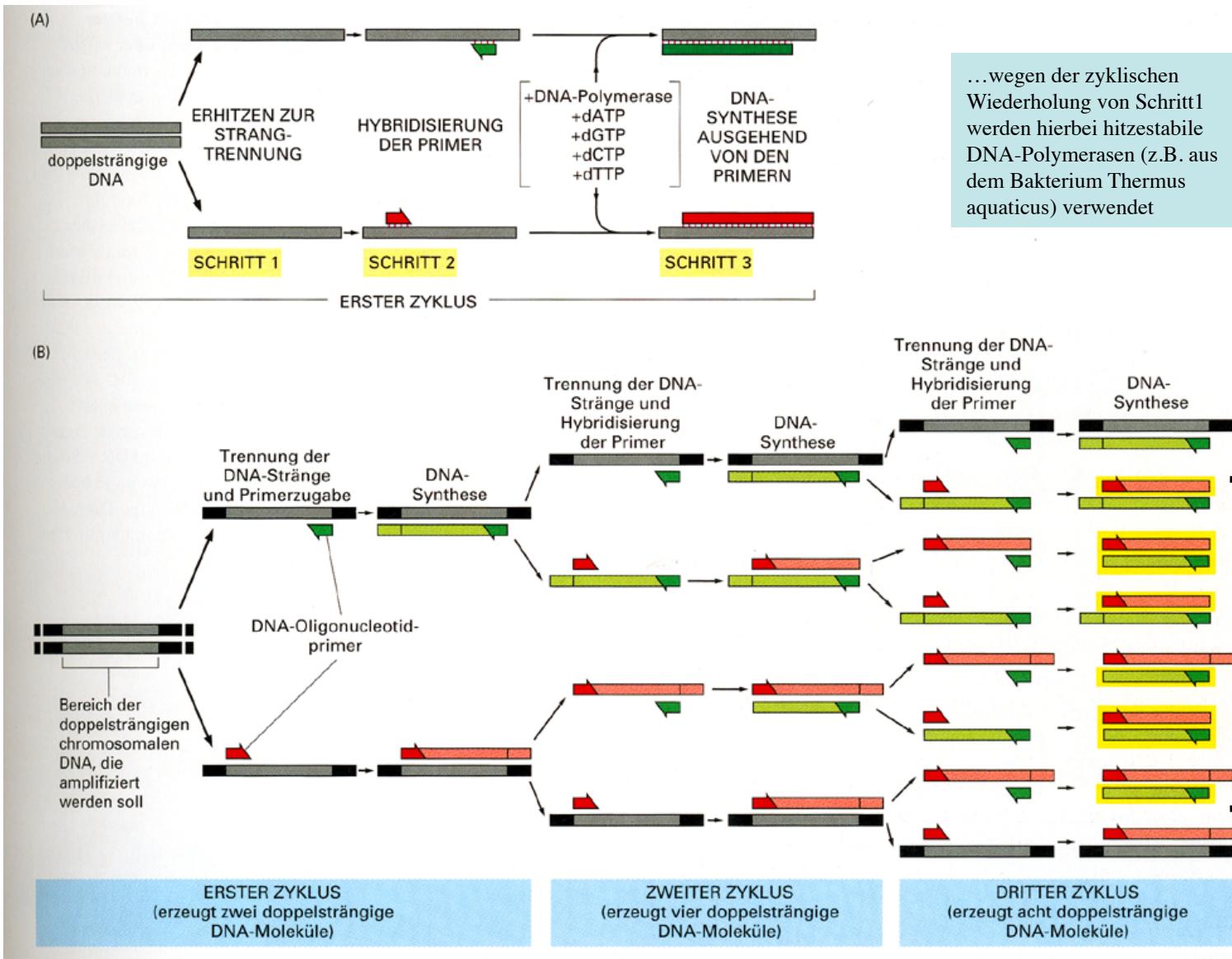
- Polymerase-Kettenreaktion (PCR)
 - > millionenfache Vervielfältigung eines definierten DNA-Abschnitts
 - > Grundlage für schnelle Gendiagnostik
- DNA-Sequenzierung (Sanger & neue Techniken)
 - > Entschlüsselung von Genomen

Kary B. Mullis
Nobelpreis 1992



Fred Sanger
Nobelpreis 1980

DNA-Replikation in vitro: PCR



Replikation bei Prokaryoten: weitere Details



Cambridge 1953.

Depressed by their lack of progress, Watson and Crick visit the local pub...

DNA-Polymerasen bei E. coli

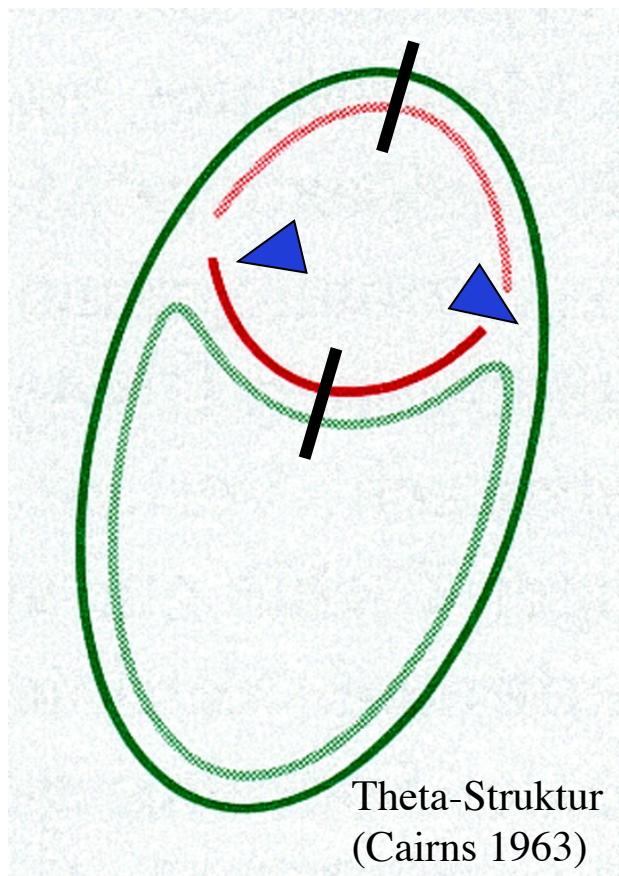
- DNA-POL III ist das Hauptenzym und repliziert leading + lagging strand (= „**Replikase**“)
(< 30/Zelle; schnell, prozessiv, hochakkurat, 3 ‘>5 ‘Exo)
- DNA POL I entfernt **RNA-Primer** und **repariert** Lücken
(400 Moleküle/Zelle, mit 20 nt/sec aber langsam)
- DNA Pol II ist **Reparaturenzym**
(kann im Gegensatz zu Pol III auch nach DNA-Lücken eine Synthese starten, ist aber „langsam“ und hat keine 5 ‘>3 ‘ Exonuclease; Sekunden nach Stress per SOS-Antwort induziert = „Ersthelfer“?)

- DNA Pol IV: Rettung blockierter R-Gabeln ?
(250/Zelle; zudem hohe Fehlerrate > gezielte Erzeugung von Mutationen?)
- DNA Pol V: Erzeugung von Mutationen nach Stress ?
(vor SOS-Induktion nur <15/Zelle)

Prokaryoten vs. Eukaryoten

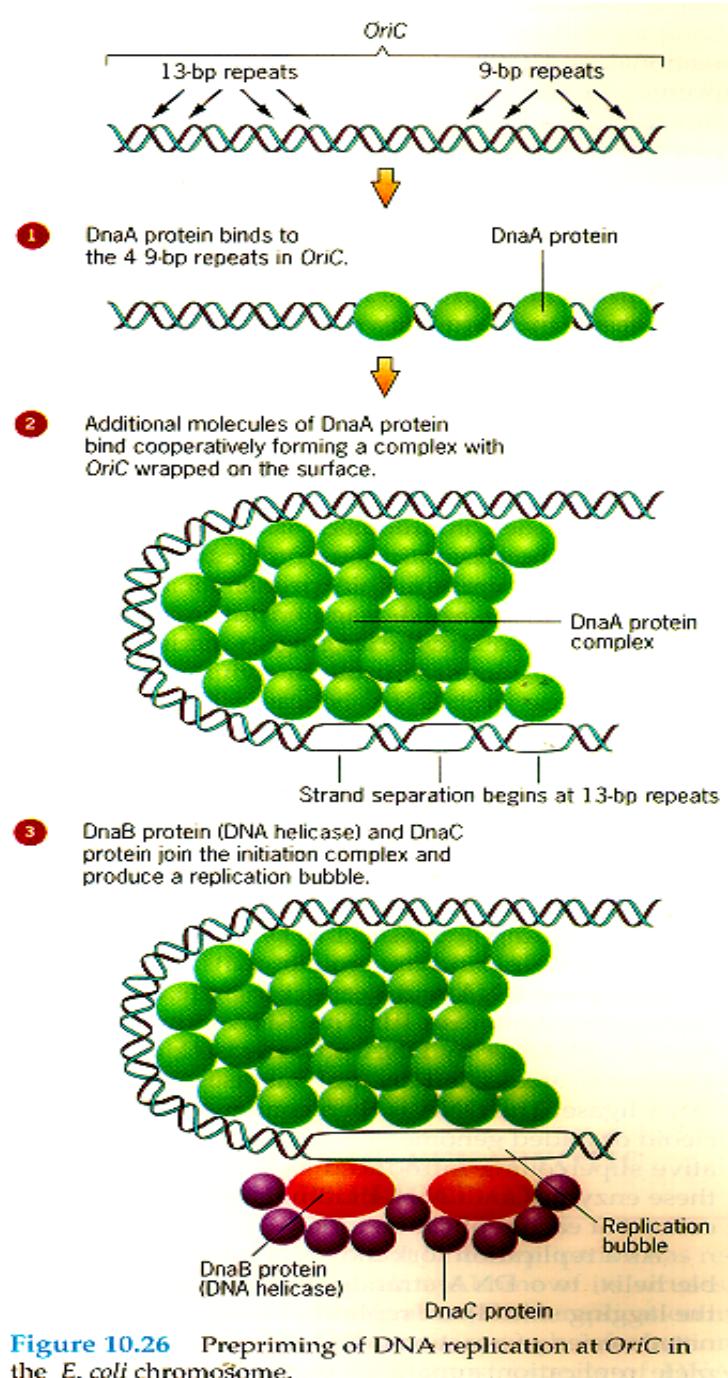
	Pro	Eu
Länge der Okazaki-F.	1000-2000 Nt.	100-200 Nt
Primerlänge	11-12 RNA-Nt	8-12 RNA-Nt von POL α um ca. 20 DNA-Nt verlängert
Rep.-Geschw.	50 000 Nt/min	ca. 2500 Nt/min
Anzahl d. Replikons	1	25 000 (Maus)
Länge von Replikons	4700 Kb (E. coli)	ca. 150 kb (Maus)

Die Replikation bei Prokaryoten verläuft bi-direktional



- meist ringförmige Genome
 - nur eine Replikationseinheit (syn. Replikon)
- / Startpunkt = „Origin“
- ▲ Rep-Gabel

Replikations-Initiation bei E. coli



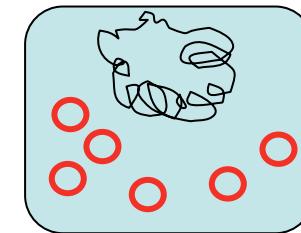
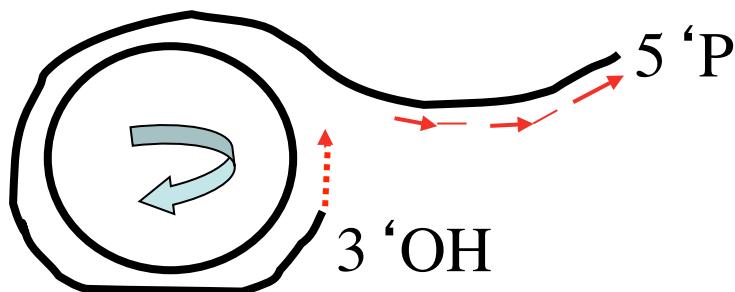
- *oriC* = Replikations-Startregion von *E. coli* (245 Bp)
- DnaA = Initiatorprotein
- autoreguliert und nur nach ATP-Bindung aktiv

Replikations-Termination bei E. coli

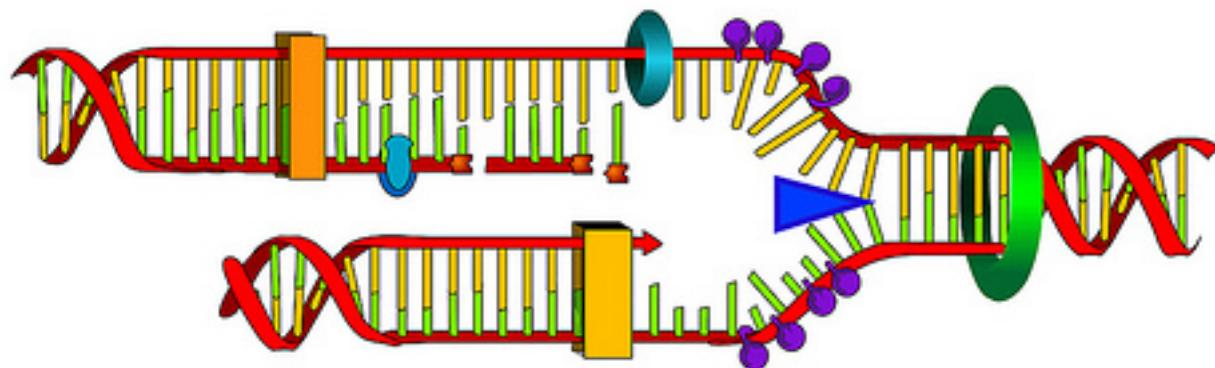
- ter1/ ter2 Elemente liegen gegenüber oriC
- binden 36 kD Terminationsprotein *tus*
- *tus* hindert Helikase an weiterer Entwindung
- nur **Notfall-Funktion** bei Asynchronie der beiden Gabeln!

Replikation von bakteriellen Plasmiden

- häufig nach dem „rolling circle“-Prinzip
- Plasmidmolekül = 1 Replikon
- Mehrfachkopie-Plasmide >> 200 Kopien pro Zelle
(wichtig für die Gentechnologie)

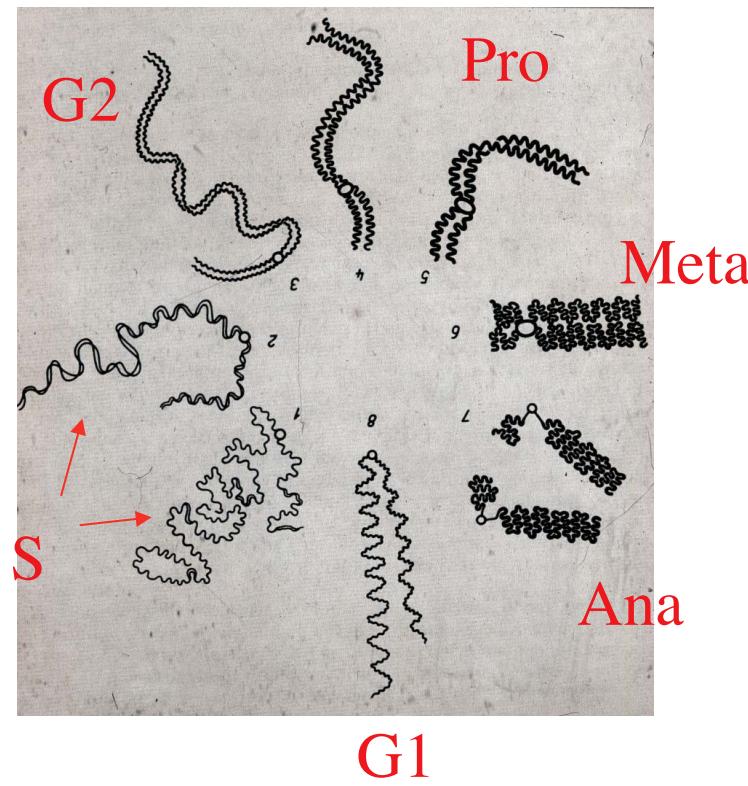
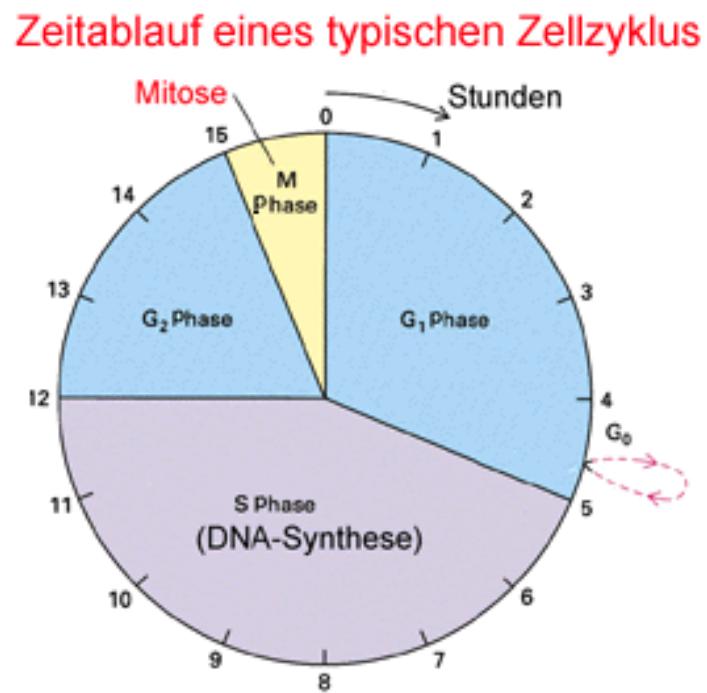


Replikation bei Eukaryoten

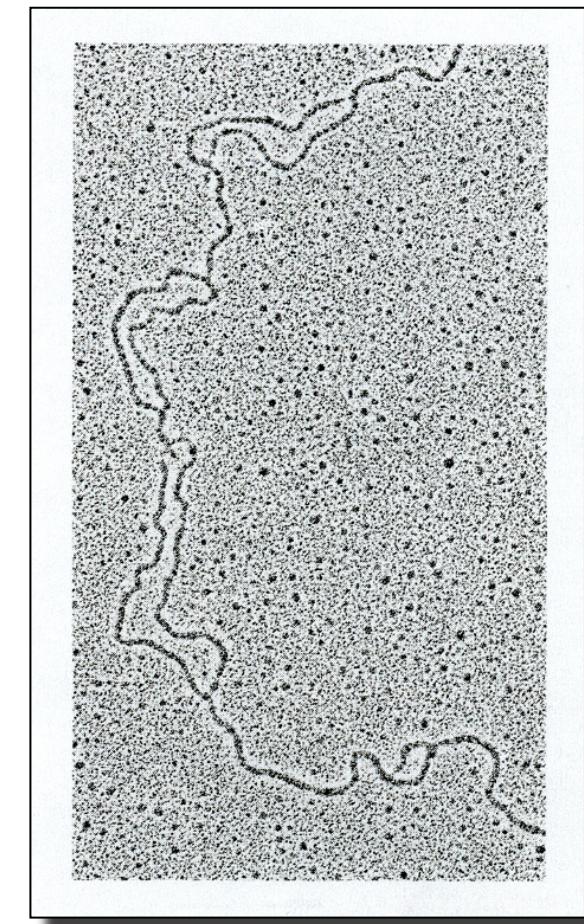
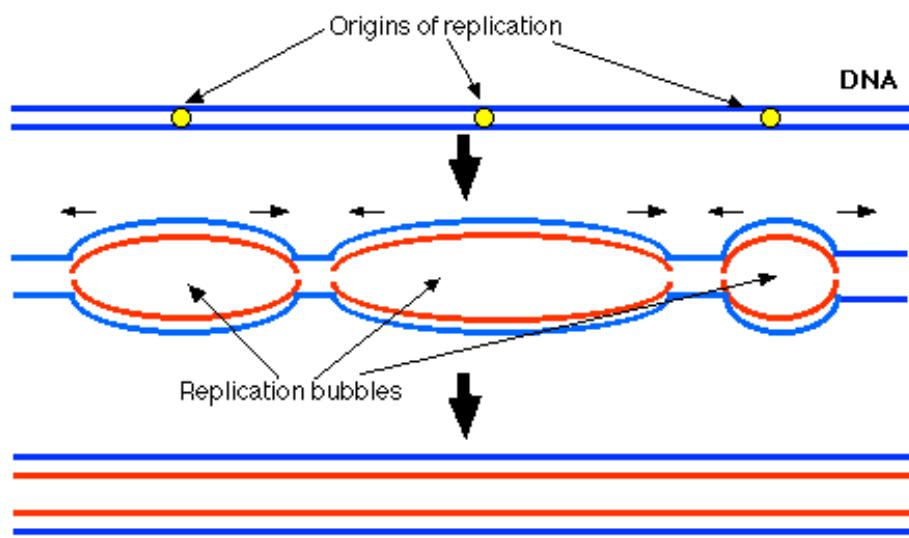


Review: Nature Reviews Genetics (2008) 9: 594ff

Die Replikation bei Eukaryoten findet in der S-Phase statt

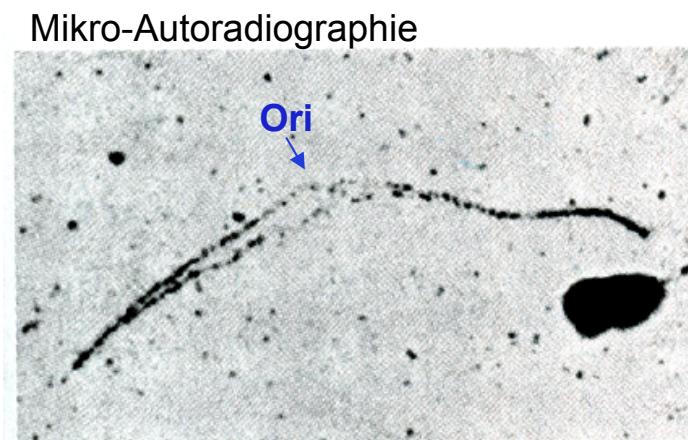


Eukaryotische Chromosomen haben eine Vielzahl von Replikons

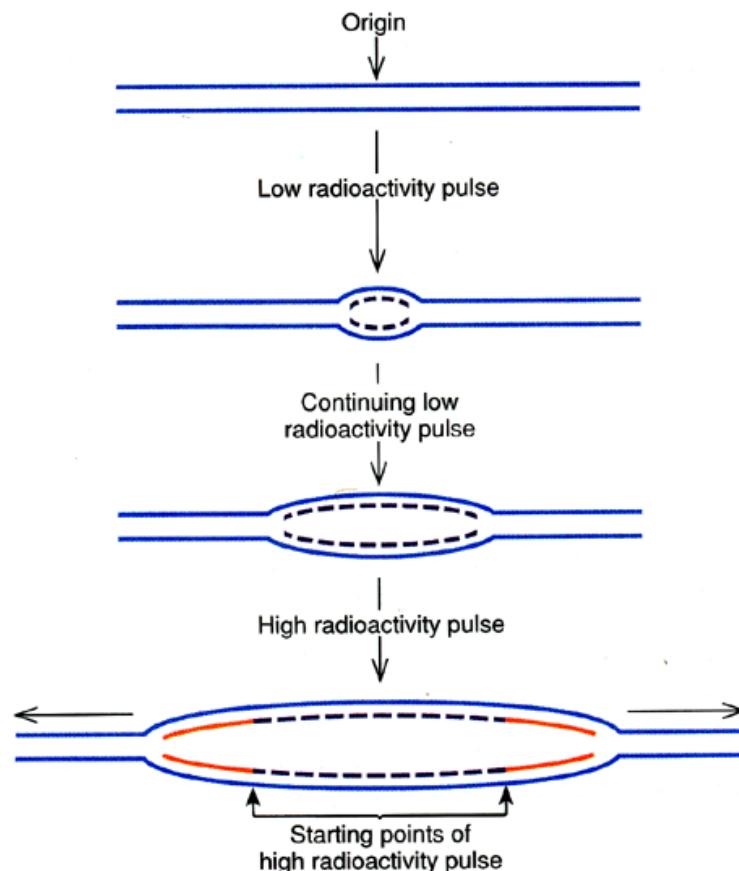


- Replikonlänge ca. 20-200 kb
- Anzahl aktiver Origins unterschiedlich nach Gewebe und Entwicklungsstand

Die Replikation eukaryotischer Chromosomen verläuft bidirektional



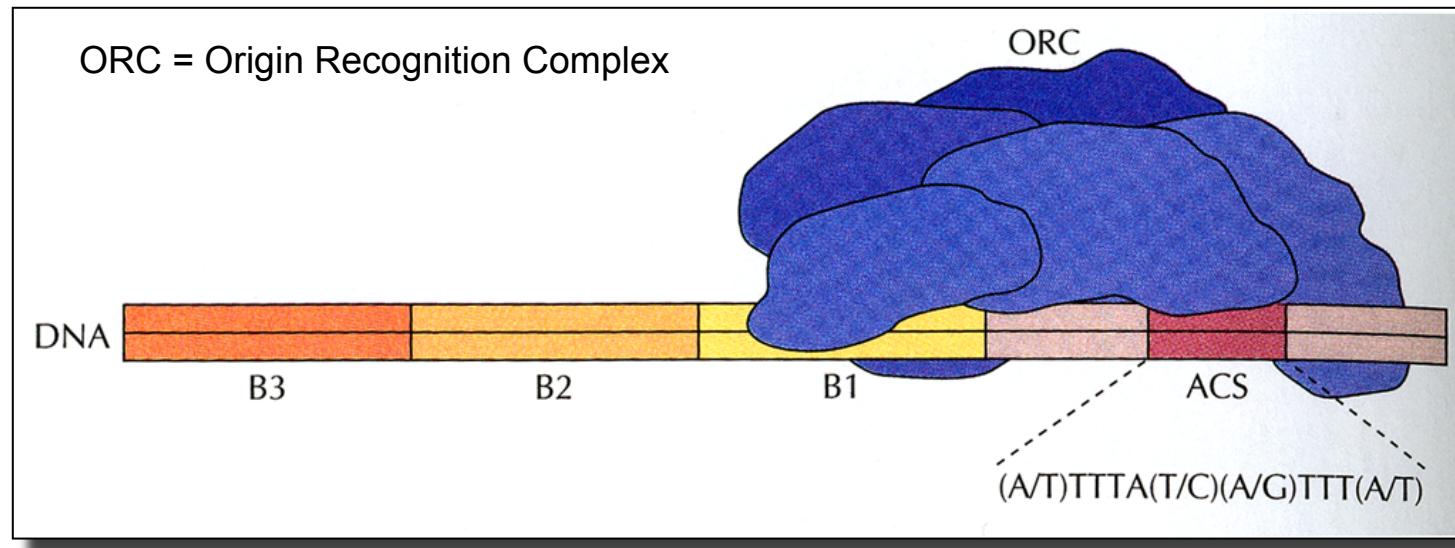
(a)



(b)

Eukaryotische Origins: nur in der Hefe einfach definiert!

ARS („autonomously replicating sequences“) in *S. cerevisiae*



- ca. 200 Bp lang
- 30-40 kb Abstand
- AT-reich

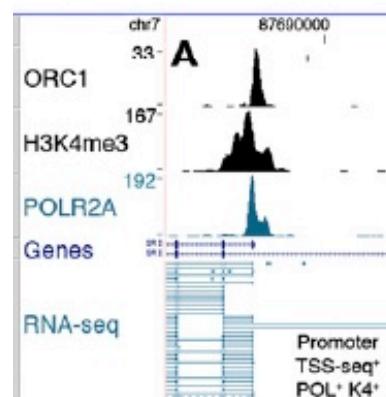


Genome-wide mapping of human DNA-replication origins: Levels of transcription at ORC1 sites regulate origin selection and replication timing

Gaetano Ivan Dellino,^{1,2,11} Davide Cittaro,^{3,6} Rossana Piccioni,¹ Lucilla Luzi,⁴ Stefania Banfi,^{1,7} Simona Segalla,^{1,8} Matteo Cesaroni,^{1,9} Ramiro Mendoza-Maldonado,^{5,10} Mauro Giacca,⁵ and Pier Giuseppe Pelicci^{1,2,11}

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We report the genome-wide mapping of ORC1 binding sites in mammals, by chromatin immunoprecipitation and parallel sequencing (ChIP-seq). ORC1 binding sites in HeLa cells were validated as active DNA replication origins (ORIs) using Repli-seq, a method that allows identification of ORI-containing regions by parallel sequencing of temporally ordered replicating DNA. ORC1 sites were universally associated with transcription start sites (TSSs) of coding or noncoding RNAs (ncRNAs). Transcription levels at the ORC1 sites directly correlated with replication timing, suggesting the existence of two classes of ORIs: those associated with moderate/ high transcription levels (≥ 1 RNA copy/cell), firing in early S and mapping to the TSSs of coding RNAs; and those associated with low transcription levels (< 1 RNA copy/cell), firing throughout the entire S and mapping to TSSs of ncRNAs. These findings are compatible with a scenario whereby TSS expression levels influence the efficiency of ORC1 recruitment at G₁ and the probability of firing during S.



- Anreicherung des Origin Recognition Complex ORC
- Histonmodifikation: offenes Chromatin
- RNA Pol 2 beginnt Transkription
- Genanfang
- passende mRNA

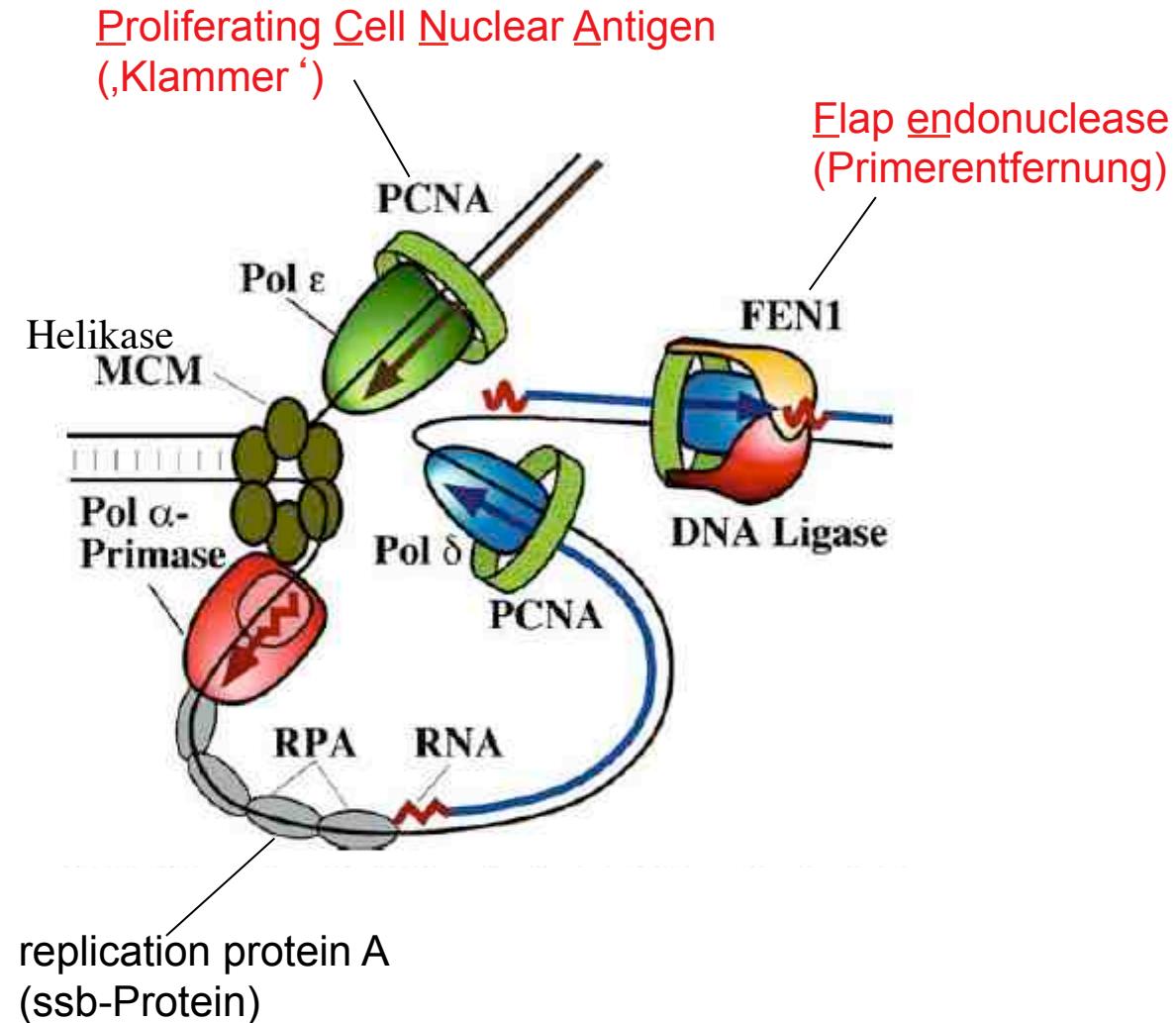
Replikationsstart
und Transkription
hängen zusammen...!

DNA Pol bei Eukaryoten



- haben nach Genomprojekt-Daten **15 !!** DNA-Polymerasen
- am besten erforscht sind POL α , β , γ , δ , ϵ
 - POL α hat **Primase**funktion u. DNA-Pol-Funktion
 - POL β ist Reparaturenzym (BER, base excision repair)
 - POL γ repliziert Organellen-DNA (mt, cp)
 - POL δ repliziert lagging strand u. entfernt Primer!
 - POL ϵ ist die leading strand-Polymerase
(aber δ kann sie ersetzen und ebenfalls leading strand replizieren)

Eukaryotische Replikationsgabel





Eukaryotische Replikation

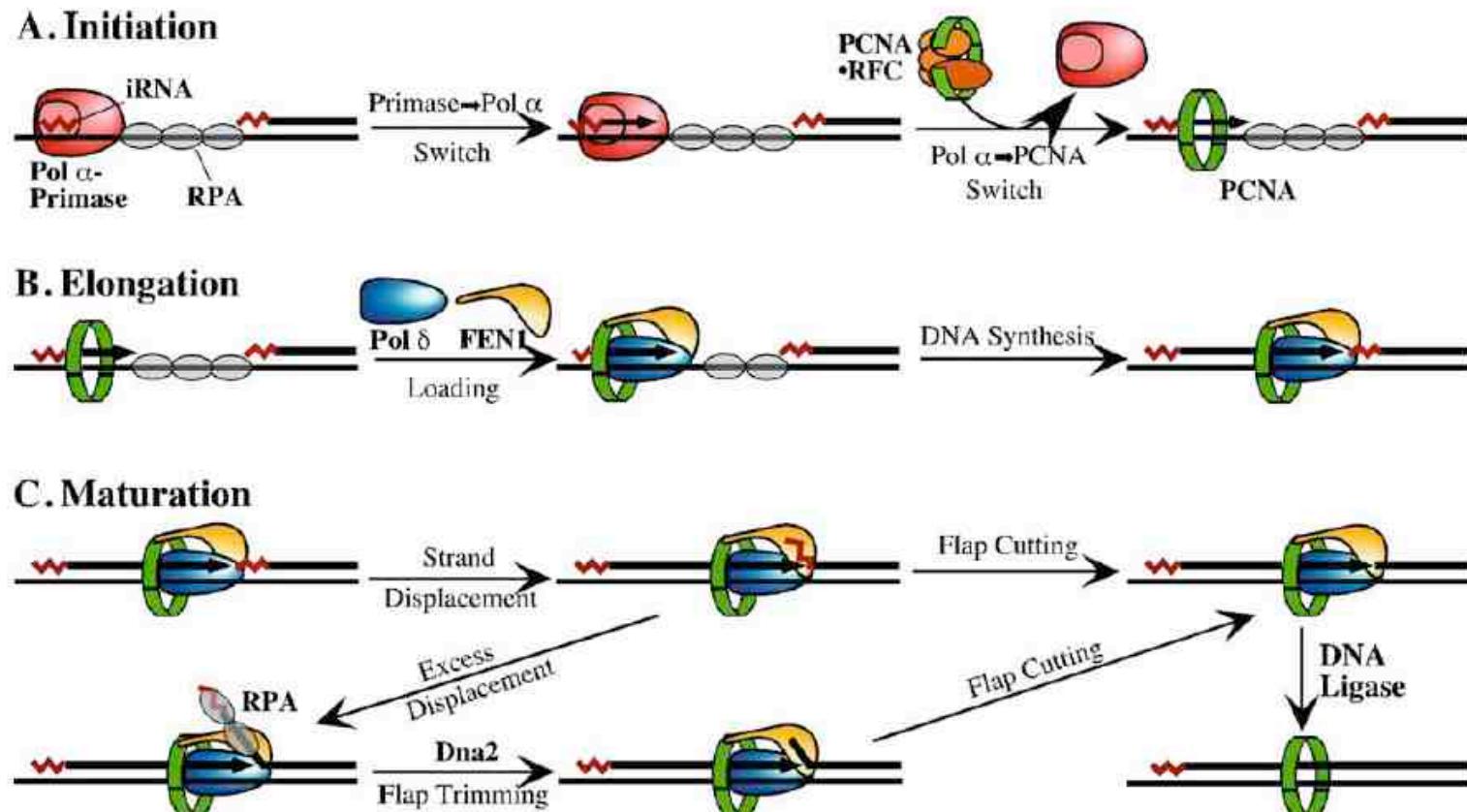


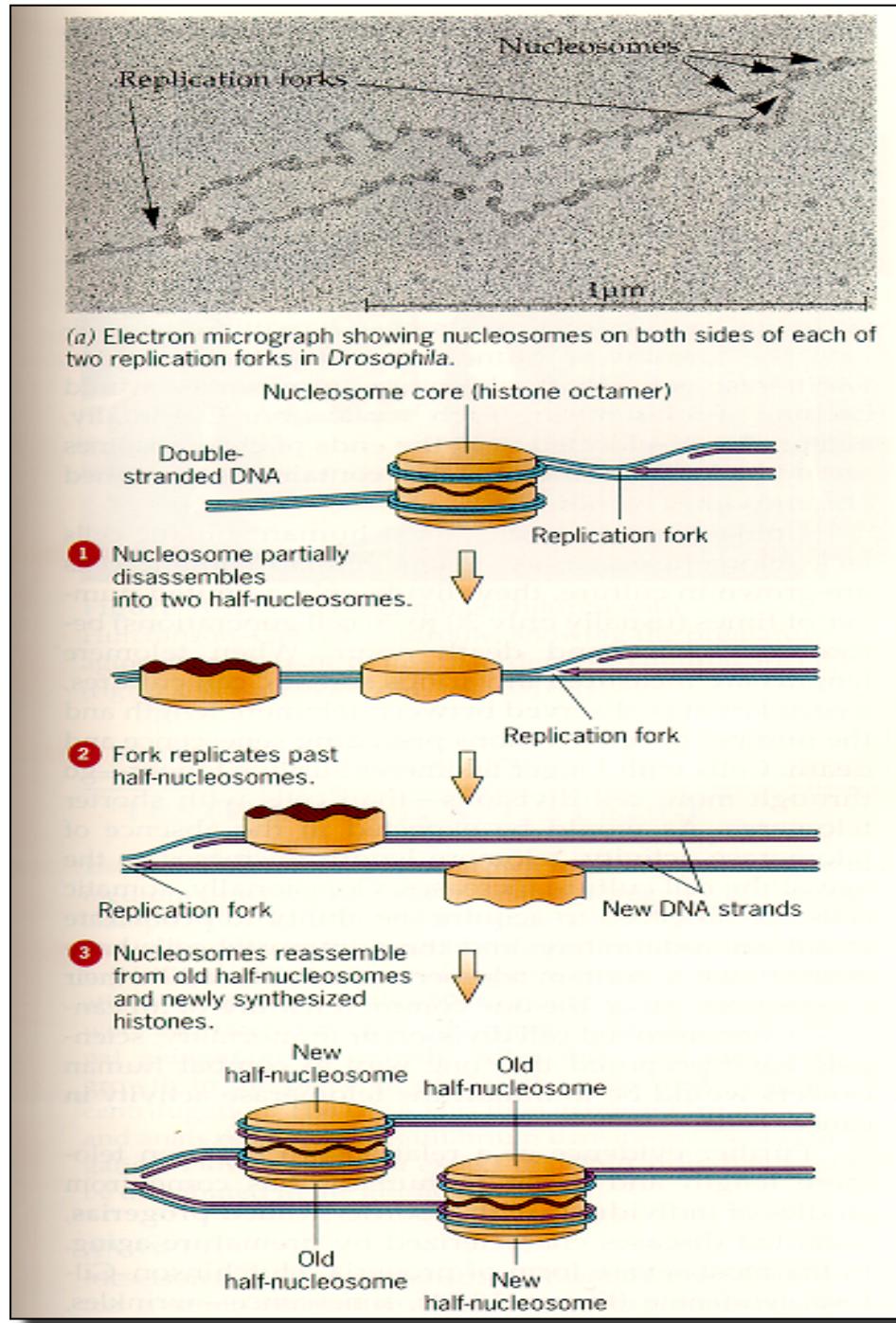
FIGURE 5 Replication stages of the lagging strand. The Pol α \rightarrow PCNA switch promotes loading of Pol ϵ on the leading strand (not shown), and Pol δ on the lagging strand. During elongation, FEN1 is proposed to be loaded together with Pol δ , but it is only activated upon encountering downstream DNA or RNA. In the model shown in Figure C, RPA binds to long flaps only, thus preventing cleavage by FEN1 and stimulating cleavage by Dna2. The trimmed flap then becomes a substrate for FEN1.

Termination bei Eukaryoten

Die Termination der Replikation in Eukaryoten erfolgt, sobald zwei Gabeln aneinanderstoßen.

Es sind **keine speziellen Signale** erforderlich.





Replikation und Chromatin

Die Histon-Oktamere werden hälftig auf die beiden Chromatiden verteilt

Defekte der Replikationsmaschinerie



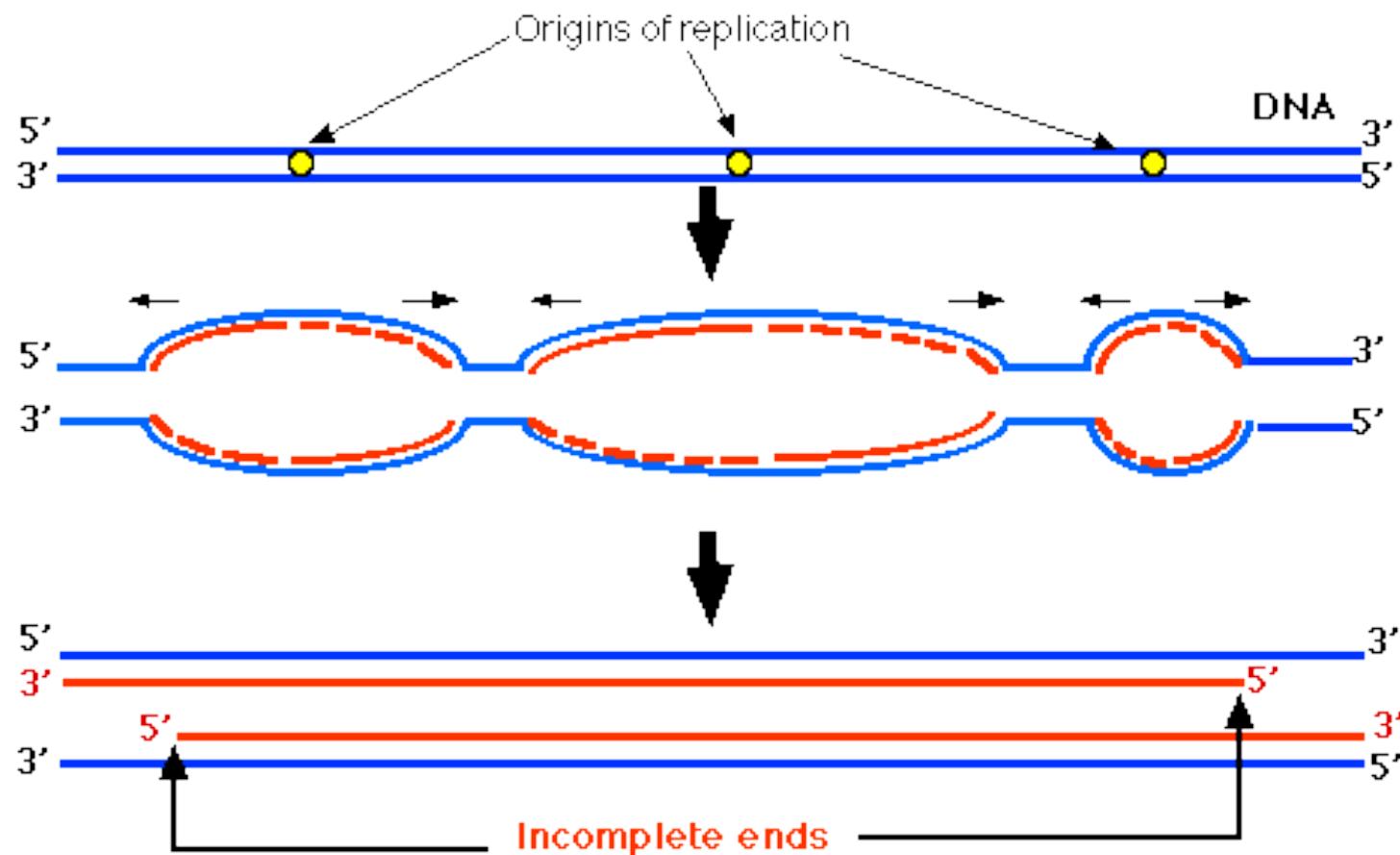
Progerie (Werner-Syndrom) > Helikase-Defekt



Ophtalmoplegia > DNA Pol γ -Defekt

DNA Pol δ -Mutationen und erhöhte Expression von DNA-Pol β sind mit Krebs assoziiert

Problem: Replikation der Enden linearer Chromosomen!!



Primerentfernung führt zu Verkürzung der Chromosomen mit jeder Replikation!!



The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"



Photo: Gerbil, Licensed by Attribution Share Alike 3.0

**Elizabeth H.
Blackburn**



Photo: Gerbil, Licensed by Attribution Share Alike 3.0

Carol W. Greider



Photo: Jussi Puikkonen

Jack W. Szostak



And, returning back from the University to a subway station
(and I was walking through the Moscow streets in the Fall,

https://www.youtube.com/watch?v=w_uS-kmHvwo

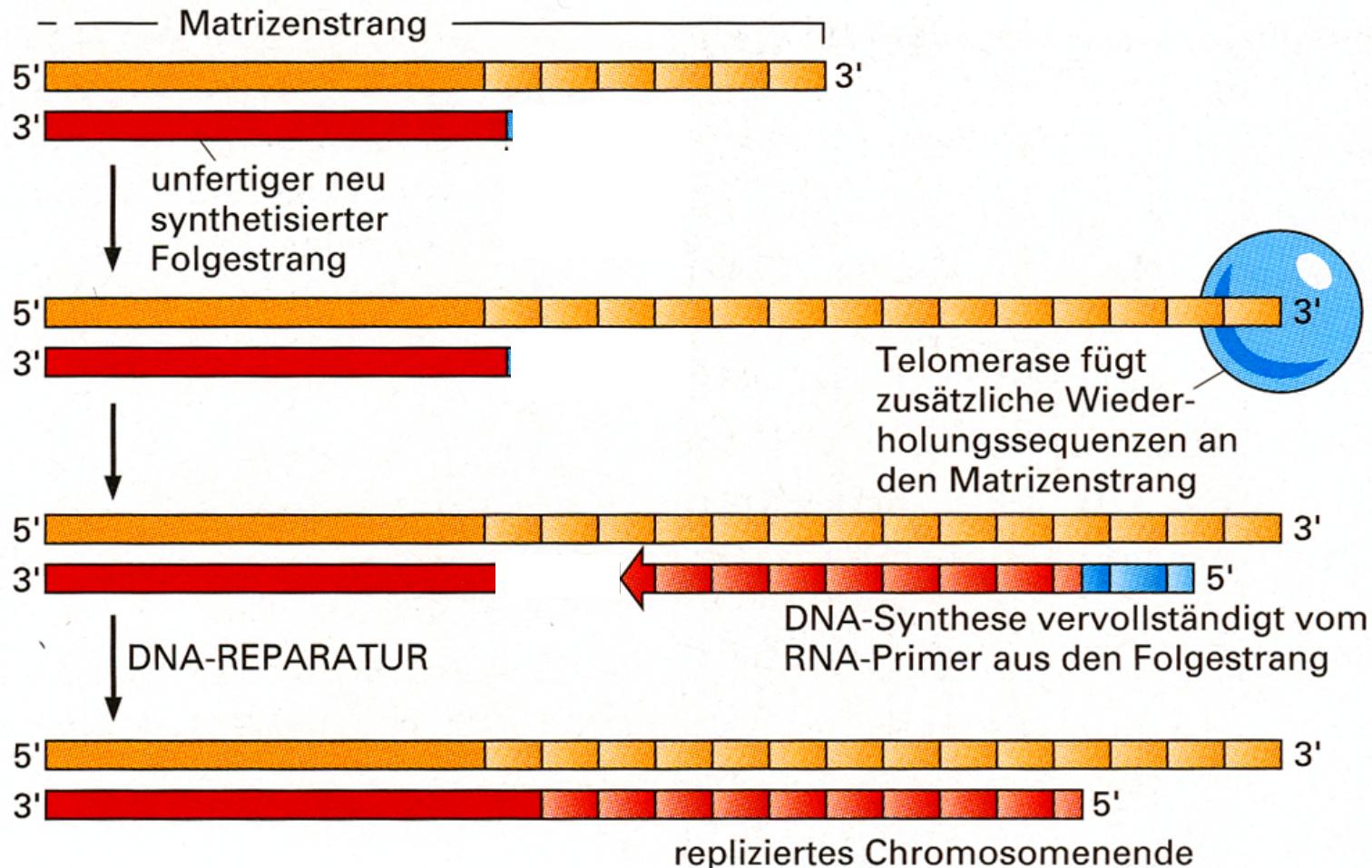
Lösung: Telomere & Telomerase

Speziell gebaute Chromosomen-Enden (**Telomere**) sowie Replikationsenzyme (**Telomerase**) kompensieren den Verlust von Nukleotiden!



- die Telomerregion der meisten Tiere und Pflanzen besteht aus simplen, tandem-repetitiven Sequenzen
 - z. B. $(TTAGGG)_{n= \text{ca. } 2000}$ beim Menschen
 - (Ausnahme: Dipteren wie Drosophila, sowie wenige Pflanzenarten)

Die Telomerase verlängert den überhängenden 3'-Strang



Telomerase

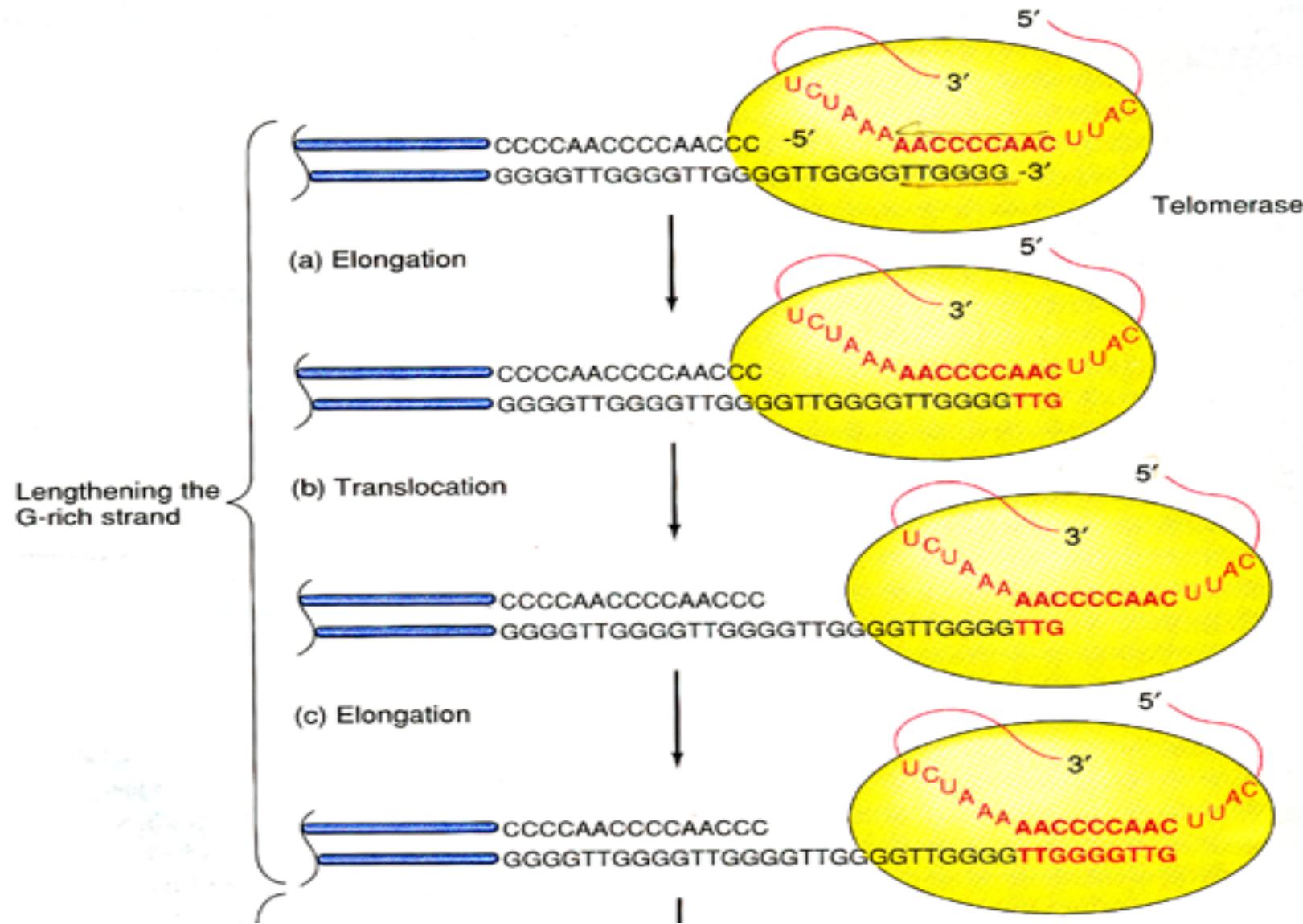


Die Telomerase ist ein Protein („TERT“) mit RNA-Anteil („TERC“), also ein Ribonukleoprotein.

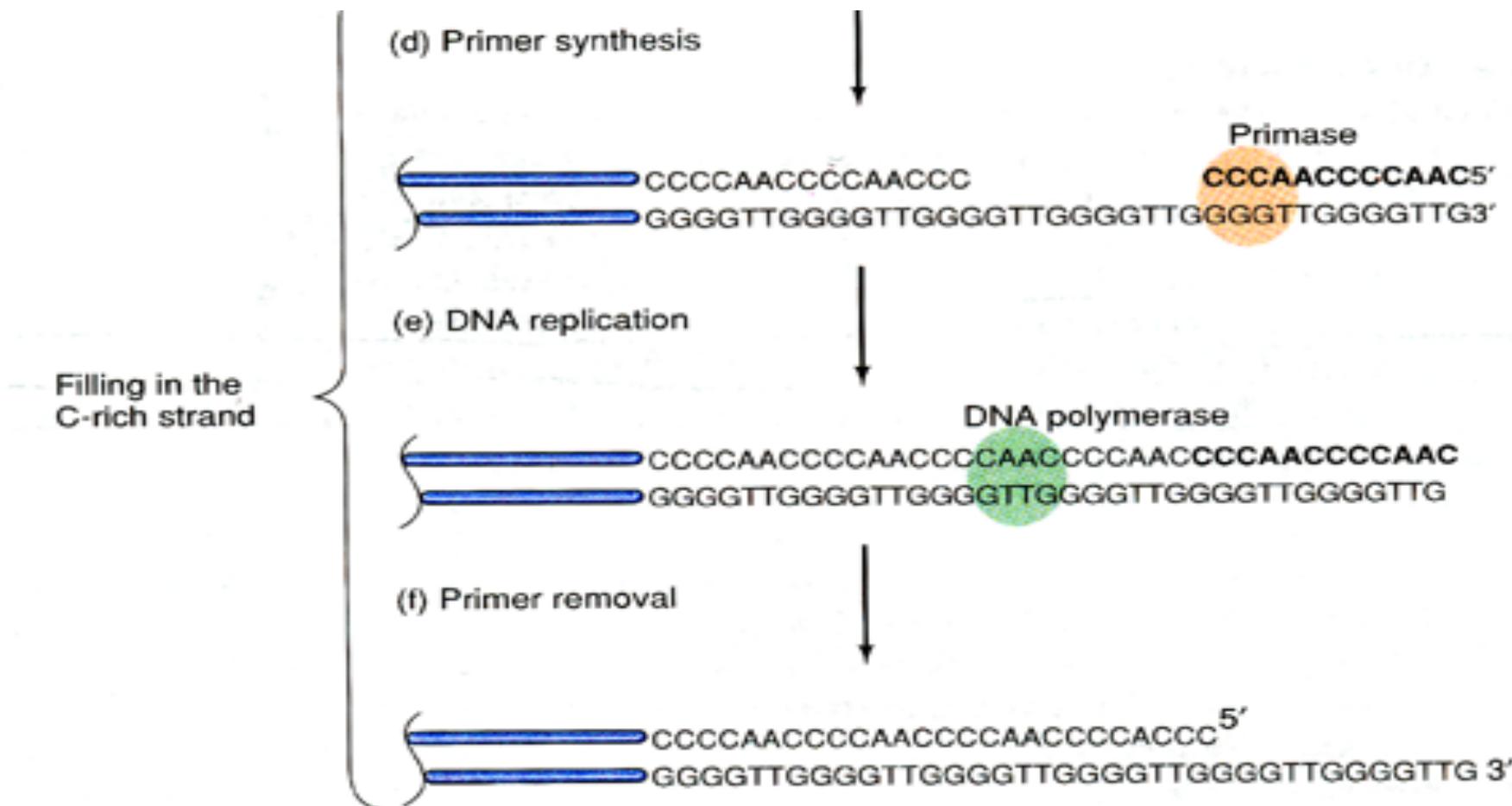
Der RNA-Anteil dient als Matrize für die Synthese von DNA-Telomersequenzen (z.B: TTAGGG_n).

Die Telomerase ist also eine „Reverse Transkriptase“.

Telomerase-Arbeitsmodell – etwas detaillierter-

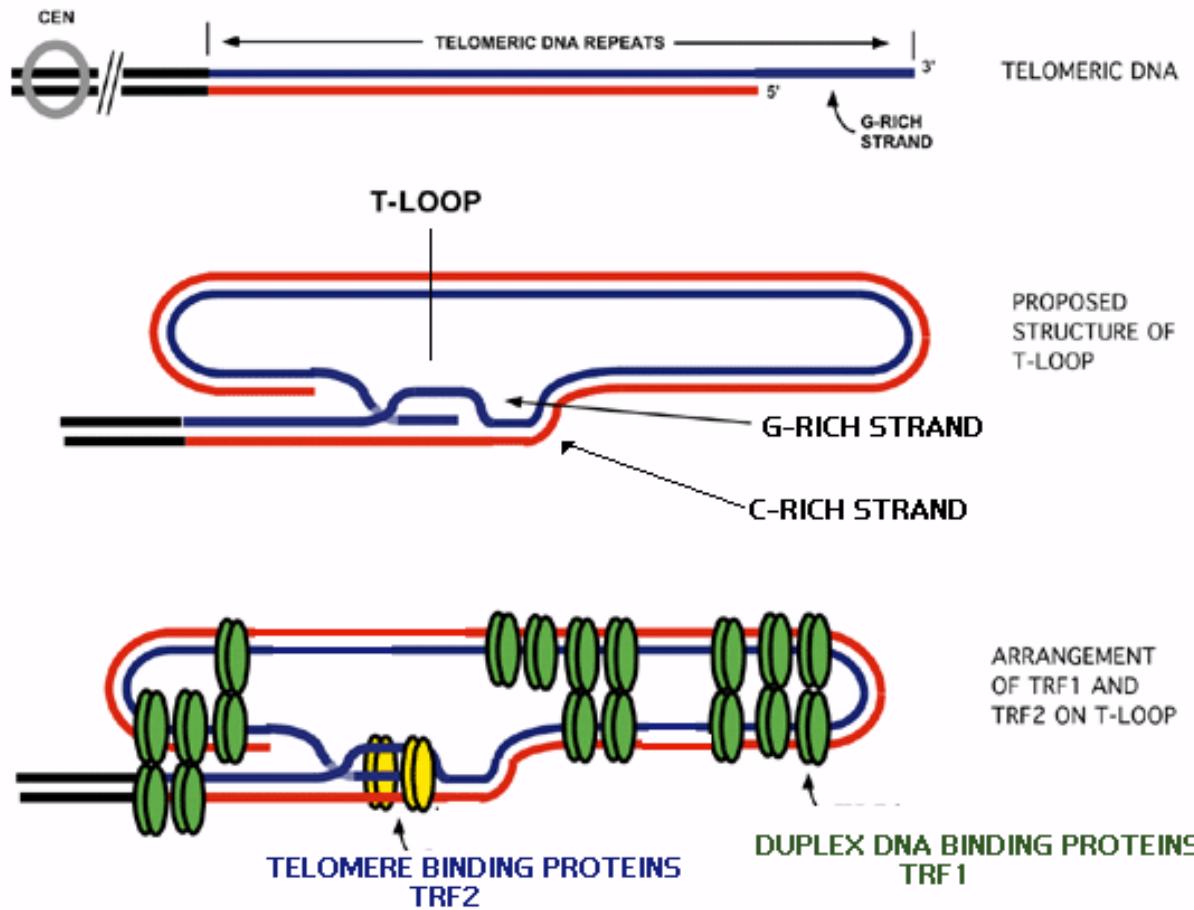


Telomerase-Arbeitsmodell (Fortsetzung)





Modell der Telomer-Struktur



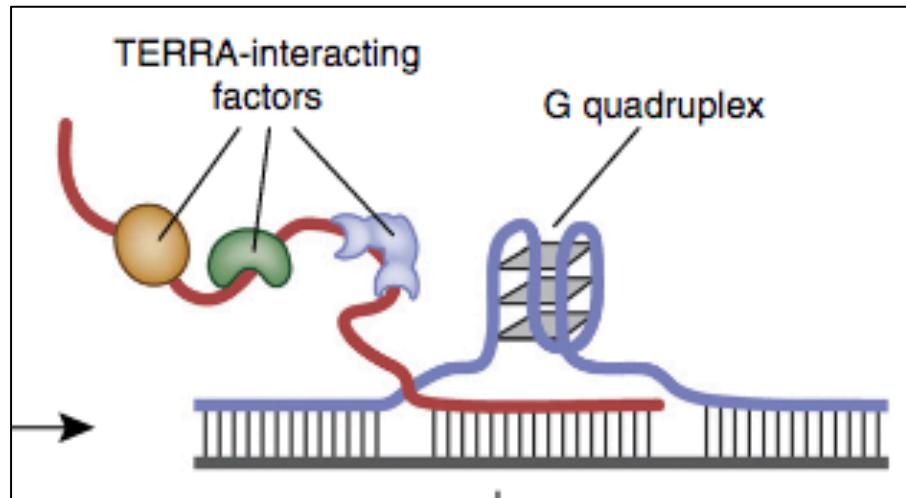
- T-Loop „versteckt“ das Ende und reguliert Aktivität der Telomerase
- SHELTERIN-Protein-Komplex versiegelt und schützt das Ende vor unbeabsichtigter DNA-Reparatur (die Instabilität erzeugen würde).

Gimme Shelterin !



Telomere produzieren eine long non-coding RNA (TERRA)

Article

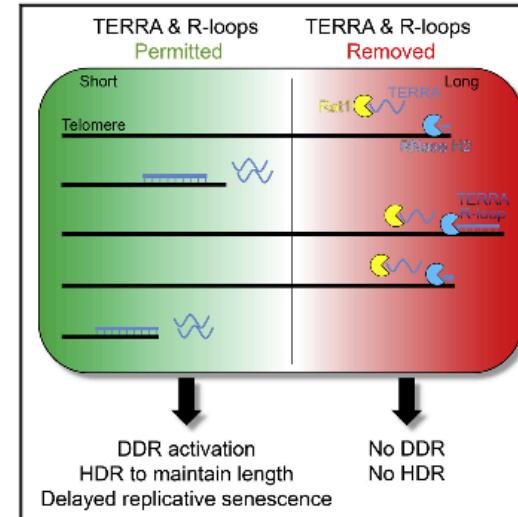


- an verkürzten Telomeren lockt TERRA die Telomerase an

Cell

Telomere Length Determines TERRA and R-Loop Regulation through the Cell Cycle

Graphical Abstract



Authors

Marco Graf, Diego Bonetti, Arianna Lockhart, ..., Pascale Jolivet, Maria Teresa Teixeira, Brian Luke

Correspondence

b.luke@imb-mainz.de

In Brief

TERRA balances repair mechanisms at short telomeres to preserve genome integrity.

Highlights

- Rif2 recruits RNase H2 and Rat1 specifically to long telomeres
- At long telomeres, TERRA and R-loops are degraded prior to telomere replication
- TERRA and R-loops accumulate as telomeres shorten and activate the DDR
- TERRA R-loops promote homology-directed repair to avoid premature senescence



Telomer-Verkürzung & Altern

...eine Bierdeckel-Kalkulation...

Da die Okazaki-Fragment-Synthese nicht am äußersten Ende des Matrizenstrangs beginnt, verliert die DNA ca. 100 Nt, d. h. circa 16 x TTAGGG, pro Replikation.

Nach nur 125 Mitosen ($16 \times 125 = 2000$) wären die ca. 2000 Telomer-Repeats des Menschen abgebaut!

Tatsächlich ist die Teilungszahl vieler Säuger-Zellen in Kultur auf etwa 100 Mitosen begrenzt („**Hayflick limit**“)!!

Ist die Telomer-Länge also eine innere Alters-Uhr?



Olovnikov 1971

Telomere & Altern

- Keimzellen: Telomerase-**positiv**
- normale Somazellen: Telomerase-**negativ!!!**

Wenn Telomerase-Aktivität fehlt:

- Verkürzung der Chromosomen
- zelluläre Seneszenz

Q: Warum schalten Soma-zellen die Telomerase ab?

Q: Jungbrunnen durch Telomerase-Aktivierung??

Lukas Cranach, *Der Jungbrunnen*



Telomere & Altern

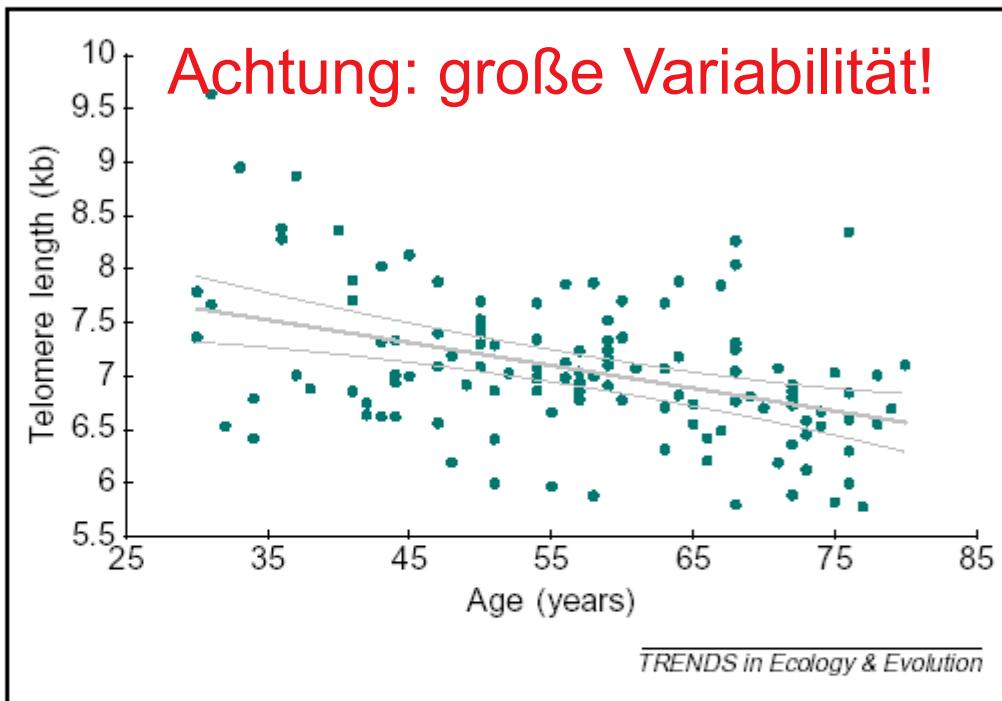


Figure 2. The relationship between age and telomere length in white blood cells in a sample of 125 randomly selected, healthy humans ($P<0.0001$, $r^2=0.18$) [52]. The grey lines indicate the regression line and confidence limits. Although there is a negative relationship between telomere length and age, there is wide variability; some individuals in their early 30s have shorter telomere lengths than do those in their late 70s.

- Abnahme der Telomerlänge mit dem Zell-Alter (aber große Variabilität)
- Primaten: Mensch hat kürzeste Telomere, aber längste Lebenszeit
- C. elegans (Nematode): Tiere mit gentechnisch verstärkter Telomerfunktion leben deutlich länger



Telomere & Stress

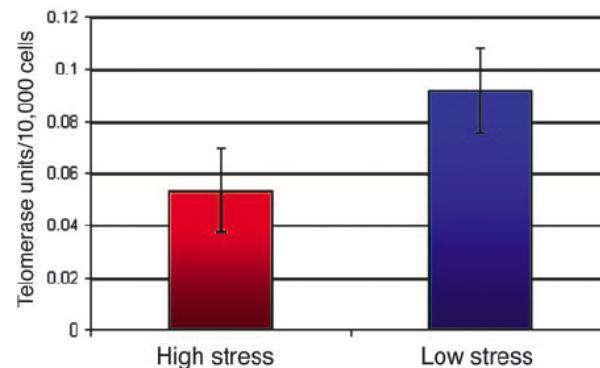
Accelerated telomere shortening in response to life stress

Elissa S. Epel*, Elizabeth H. Blackburn†, Jue Lin‡, Firdaus S. Dhabhar§, Nancy E. Adler*, Jason D. Morrow¶, and Richard M. Cawthon||

*Department of Psychiatry, University of California, 3333 California Street, Suite 465, San Francisco, CA 94143; †Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143; §Department of Oral Biology, College of Dentistry, and Department of Molecular Virology, Immunology, and Medical Genetics, College of Medicine, Ohio State University, Columbus, OH 43210; ¶Department of Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232; and ||Department of Human Genetics, University of Utah, 15 North 2030 E Street, Room 2100, Salt Lake City, UT 84112

Contributed by Elizabeth H. Blackburn, September 28, 2004

Numerous studies demonstrate links between chronic stress and indices of poor health, including risk factors for cardiovascular disease and poorer immune function. Nevertheless, the exact mechanisms of how stress gets “under the skin” remain elusive. We investigated the hypothesis that stress impacts health by modulating the rate of cellular aging. Here we provide evidence that psychological stress—both perceived stress and chronicity of stress—is significantly associated with higher oxidative stress, lower telomerase activity, and shorter telomere length, which are known determinants of cell senescence and longevity, in peripheral blood mononuclear cells from healthy premenopausal women. Women with the highest levels of perceived stress have telomeres shorter on average by the equivalent of at least one decade of additional aging compared to low stress women. These findings have implications for understanding how, at the cellular level, stress may promote earlier onset of age-related diseases.





Medical Daily

Vitality

Under the Hood

Innovation

The Hill

The Graph

VITALITY

Supercentenarians' Secret: Genes Of People Who Live Past 110th Birthday Have Nothing In Common

Nov 14, 2014 05:02 PM By Justin Caba  @jcaba33



Warum schalten Somazellen die Telomerase ab? Evolutionärer Vorteil?

- 85% aller Tumor-Formen: Telomerase + !!
> Telomerase-Aktivität gilt daher als Tumor-fördernd

Krebs bekämpfen durch Hemmung der Telomerase ?
Prinzipiell JA !

- Basenanaloga (z. B. AZT) bewirken initiale Telomer-Verkürzung!
Aber: Telomerase-unabhängiger 2. Mechanismus zur Telomererneuerung („ALT“)
lässt Telomere wieder wachsen. Die Hemmung der Replikationsfunktion der Telomerase funktioniert also nicht.
- Telomerase hat zweite zusätzliche Funktion, sie organisiert Chromatinkappe.
Diese Funktion kann durch „antisense-RNA“ gegen den Telomerase-RNA-Teil blockiert werden, was zum Zelltod führt!

<http://clincancerres.aacrjournals.org/content/12/10/3184.long>
<http://www.geron.com/imetelstat>



Jungbrunnen ohne Krebs?

Telomerase Reverse Transcriptase Delays Aging in Cancer-Resistant Mice

Antonia Tomás-Loba,^{1,5} Ignacio Flores,^{1,5} Pablo J. Fernández-Marcos,² María L. Cayuela,^{1,6} Antonio Maraver,² Agueda Tejera,¹ Consuelo Borrás,³ Ander Matheu,² Peter Klatt,^{1,2} Juana M. Flores,⁴ José Viña,³ Manuel Serrano,² and María A. Blasco^{1,*}

¹Telomeres and Telomerase Group

²Tumor Suppression Group

Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Madrid 28029, Spain

³Department of Physiology, University of Valencia, Valencia 46010, Spain

⁴Department of Animal Surgery and Medicine, Complutense University of Madrid, Madrid 28040, Spain

⁵These authors contributed equally to this work

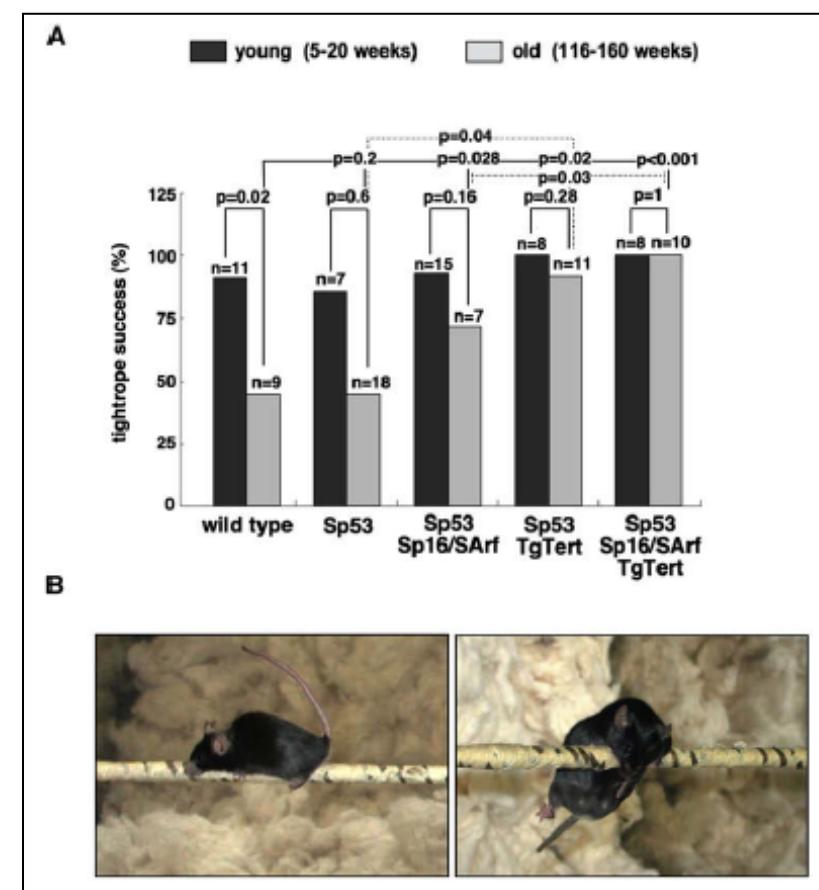
⁶Present address: Hospital Virgen de la Arrixaca, Murcia 30120, Spain

*Correspondence: mblasco@cnio.es

DOI 10.1016/j.cell.2008.09.034

SUMMARY

Telomerase confers limitless proliferative potential to most human cells through its ability to elongate telomeres, the natural ends of chromosomes, which otherwise would undergo progressive attrition and eventually compromise cell viability. However, the role of telomerase in organismal aging has remained unaddressed, in part because of the cancer-promoting activity of telomerase. To circumvent this problem, we have constitutively expressed telomerase reverse transcriptase (TERT), one of the components of telomerase, in mice engineered to be cancer resistant by means of enhanced expression of the tumor suppressors p53, p16, and p19ARF. In this context, TERT overexpression improves the fitness of epithelial barriers, particularly the skin and the intestine, and produces a systemic delay in aging accompanied by extension of the median life span. These results demonstrate that constitutive expression of *Tert* provides antiaging activity in the context of a mammalian organism.



zurück zum Kerngeschäft....

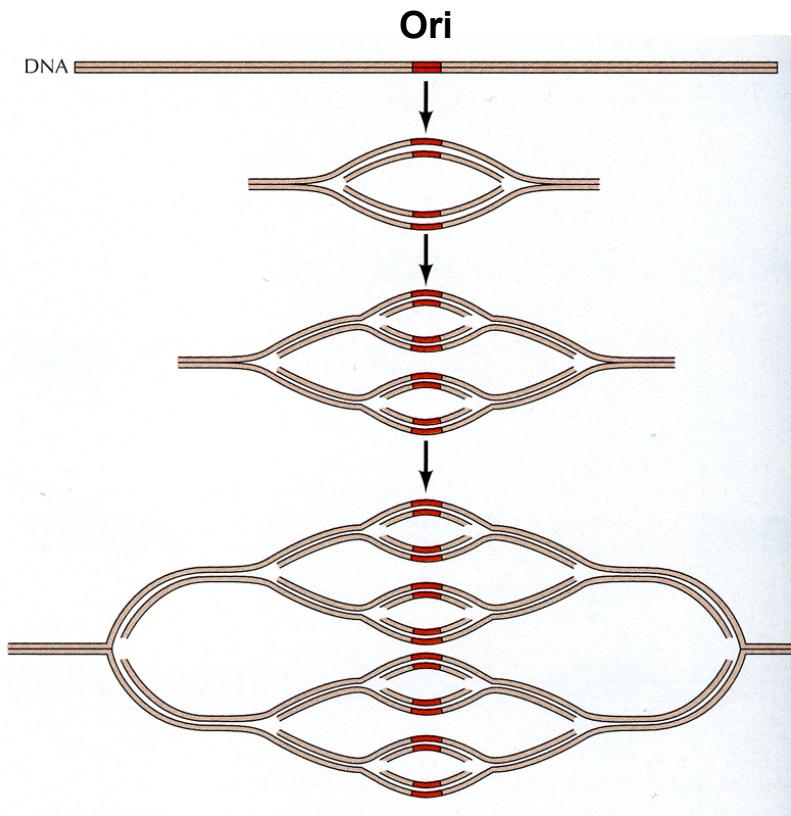
DNA-Amplifikation:

Eine selektive **Über-Replikation** definierter
Abschnitte im Genom von Eukaryoten

- in bestimmten Gewebetypen zur **gezielten Erhöhung der ‚Gendosis‘** und damit der Proteinproduktion
 - Chorion-Gene in Drosophila-Nährzellen
 - rRNA-Gene in Amphibien-Oocyten
- als **Mutationsereignis** in Krebszellen

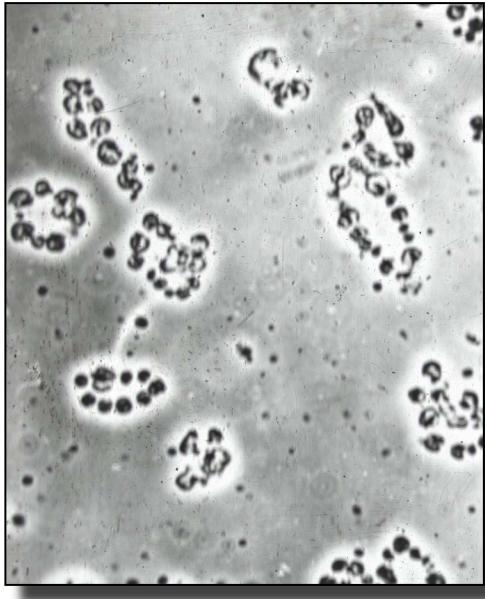


„Onion skin“-Modell der Amplifikation



- **Chorion-Gene** in Nährzellen während der Oogenese in Drosophila
- ein **Origin** mehrfach hintereinander aktiviert
- intrachromosomal Amplifikation (bis zu 64x)
- amplifizierter Bereich ca. 100 kb lang

Extrachromosomal Amplifikation per „rolling circle“

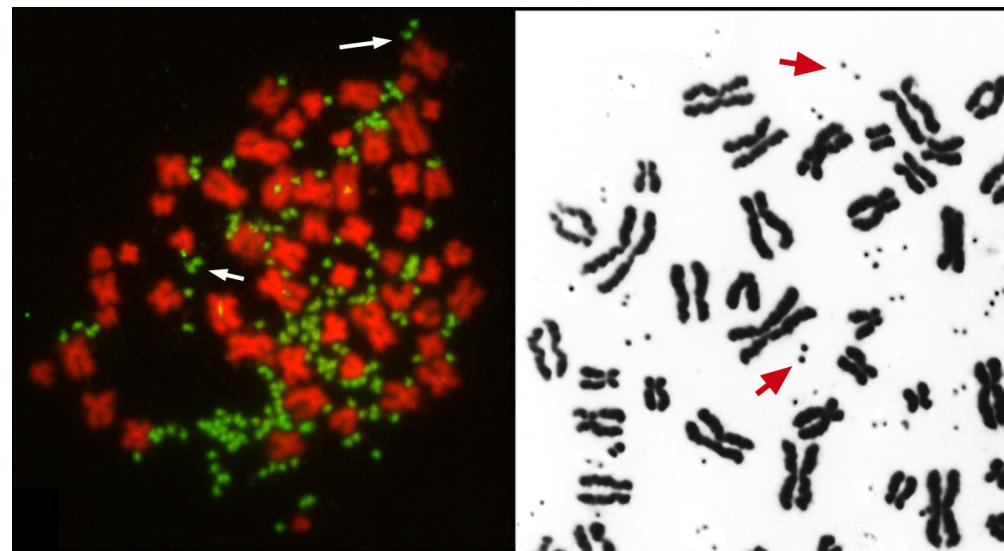
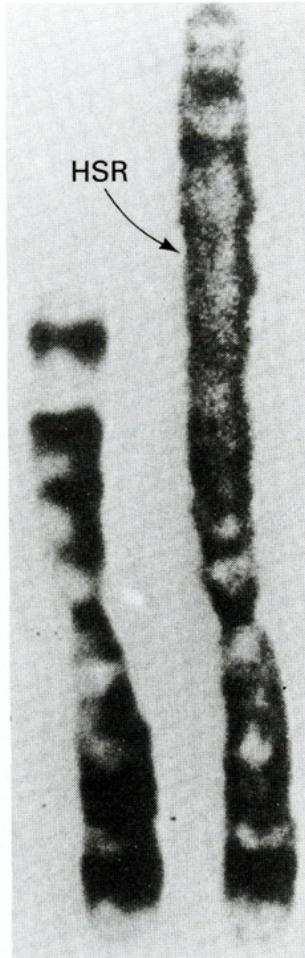


- Gene für die **rRNA** auf den DNA-Ringen
- in Oocyten von Amphibien sowie in Insekten (Käfern) besonders auffällig
- Ziel: überproportionale Vermehrung der rRNA-Gene für eine starke Transkription (und damit für eine Bildung vieler Ribosomen in einem sich schnell entwickelnden Embryo)

Amplifikation von Onkogenen in Krebszellen

HSR =
homogeneously
staining
region

= intrachromo-
somal DNA-
Amplifikation

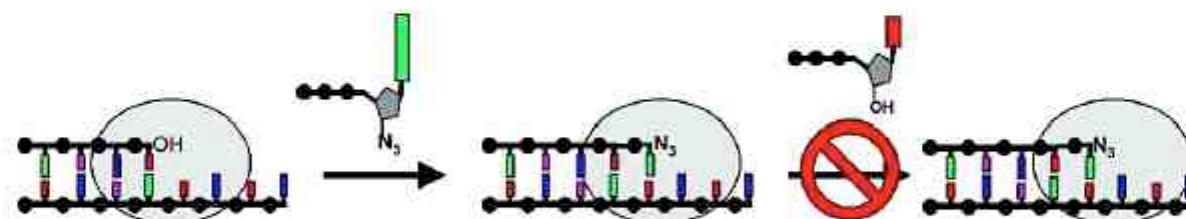


„Double Minute“-Minichromosomen
= extrachromosomal amplifizierte DNA

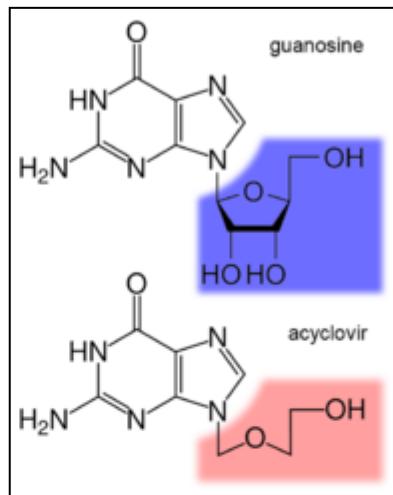
- Onkogene sind wachstumsfördernd
- erst „hyperaktive“ (amplifizierte)
Onkogene sind schädlich

Medikamente gegen unkontrollierte Replikation

...bei Krebs, Viren, Bakterien, Autoimmun-Erkrankungen



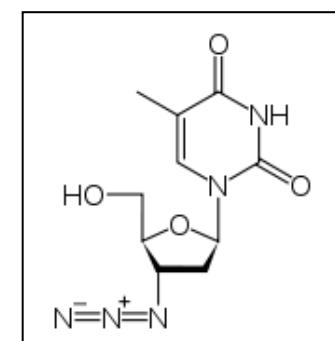
Strategie:
Kettenabbruch durch
Nukleotidanaloge



Azacyclovir (Herpes)

Selektiv durch virale Enzyme zum acyclo-GTP phosphoryliert

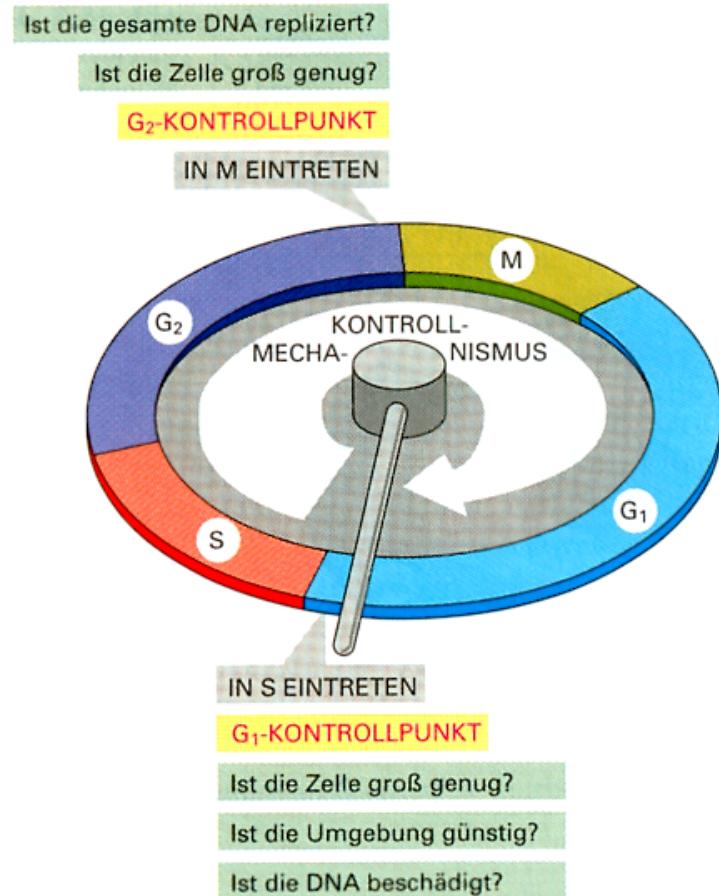
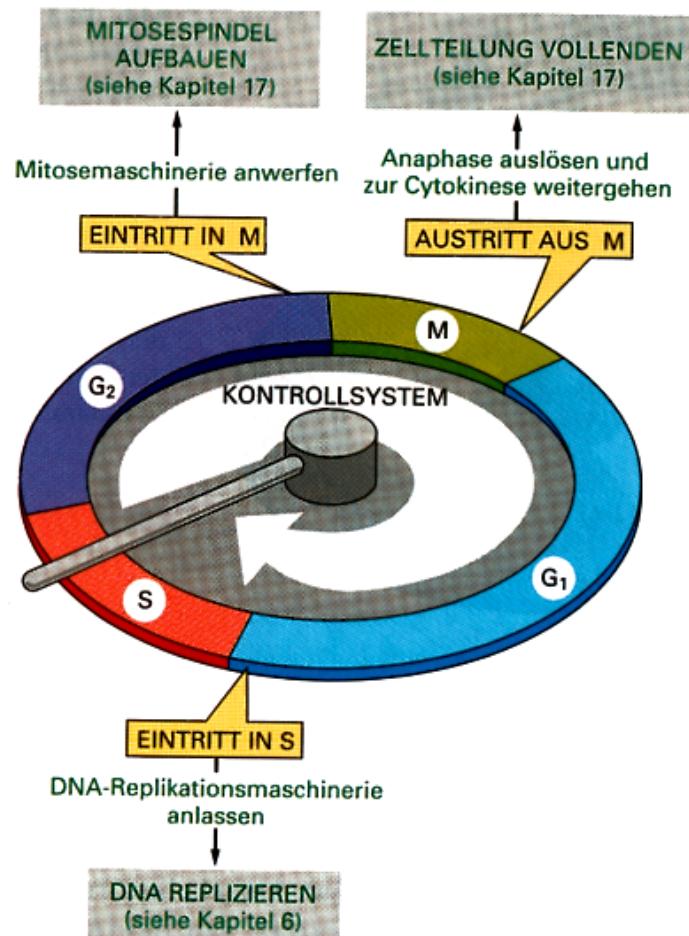
100x präferentiell durch virale DNA Pol eingebaut > Kettenabbruch



Azidothymidin/AZT (HIV)

AZT hemmt retrovirale Reverse Transkriptase 100x effizienter als zelluläre DNA-Polymerasen

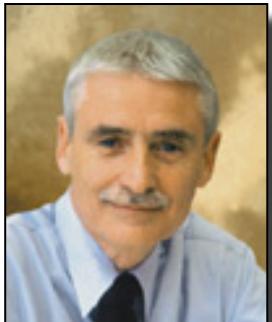
Wie werden S-Phase und Zellzyklus kontrolliert?



10.12.2001

Nobel-Preise

„key regulators
of the cell
cycle“



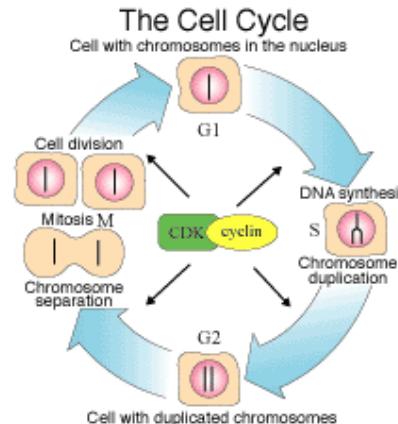
Leland Hartwell



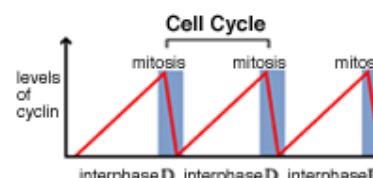
Tim Hunt



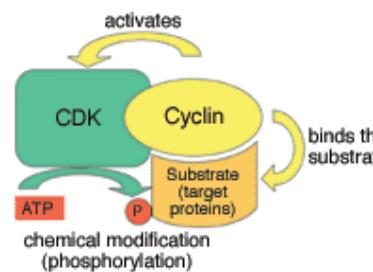
Paul Nurse



Hefe CDC-Gene
(cell division cycle)



Entdeckung der
Cycline



Isolierung der
CDKs

CDK-Cyclin-Komplexe kontrollieren den Zellzyklus

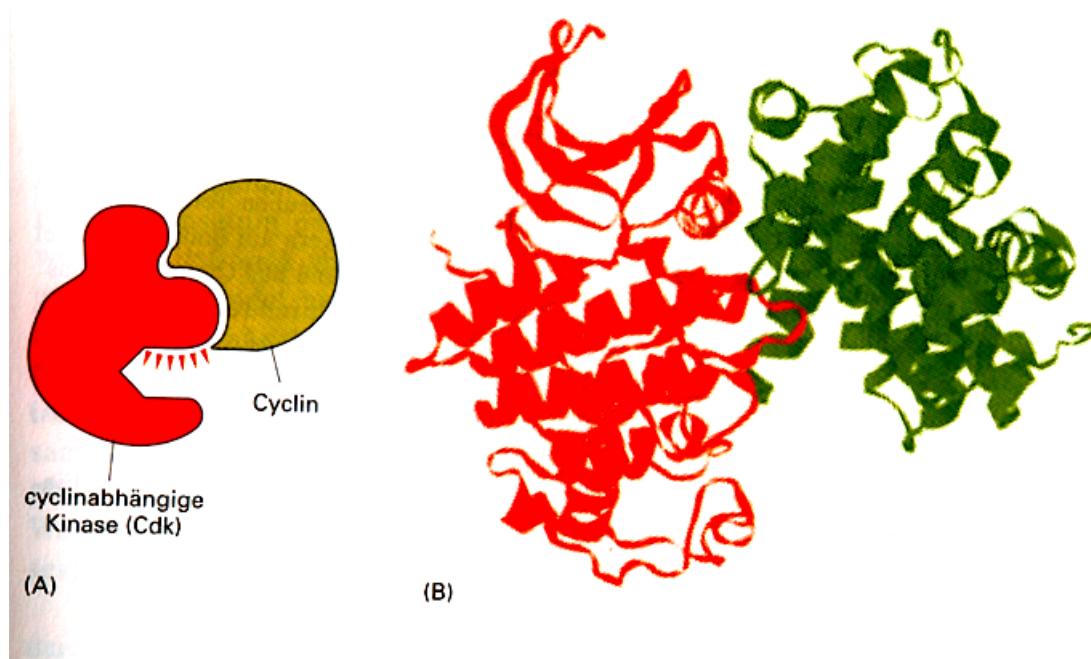
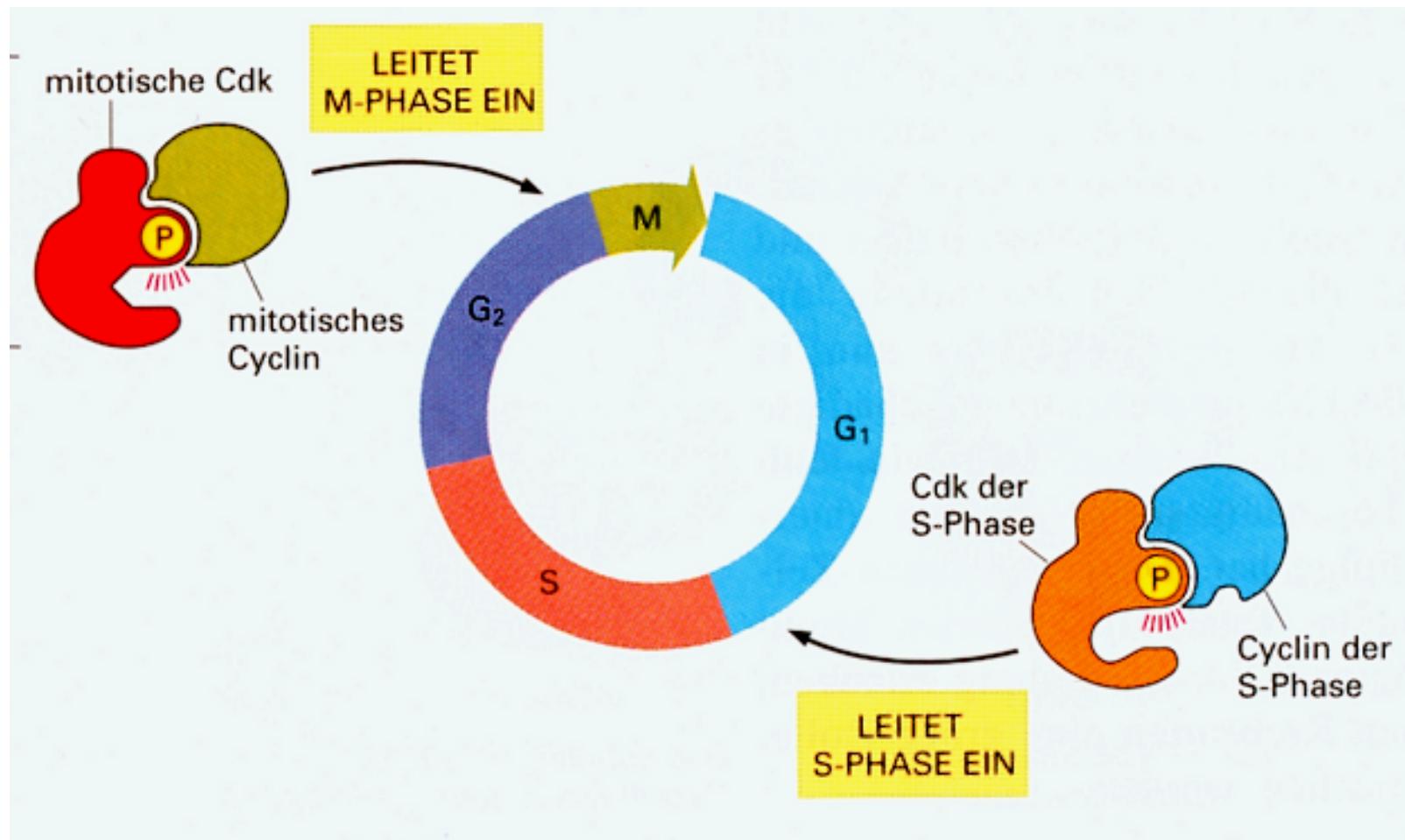


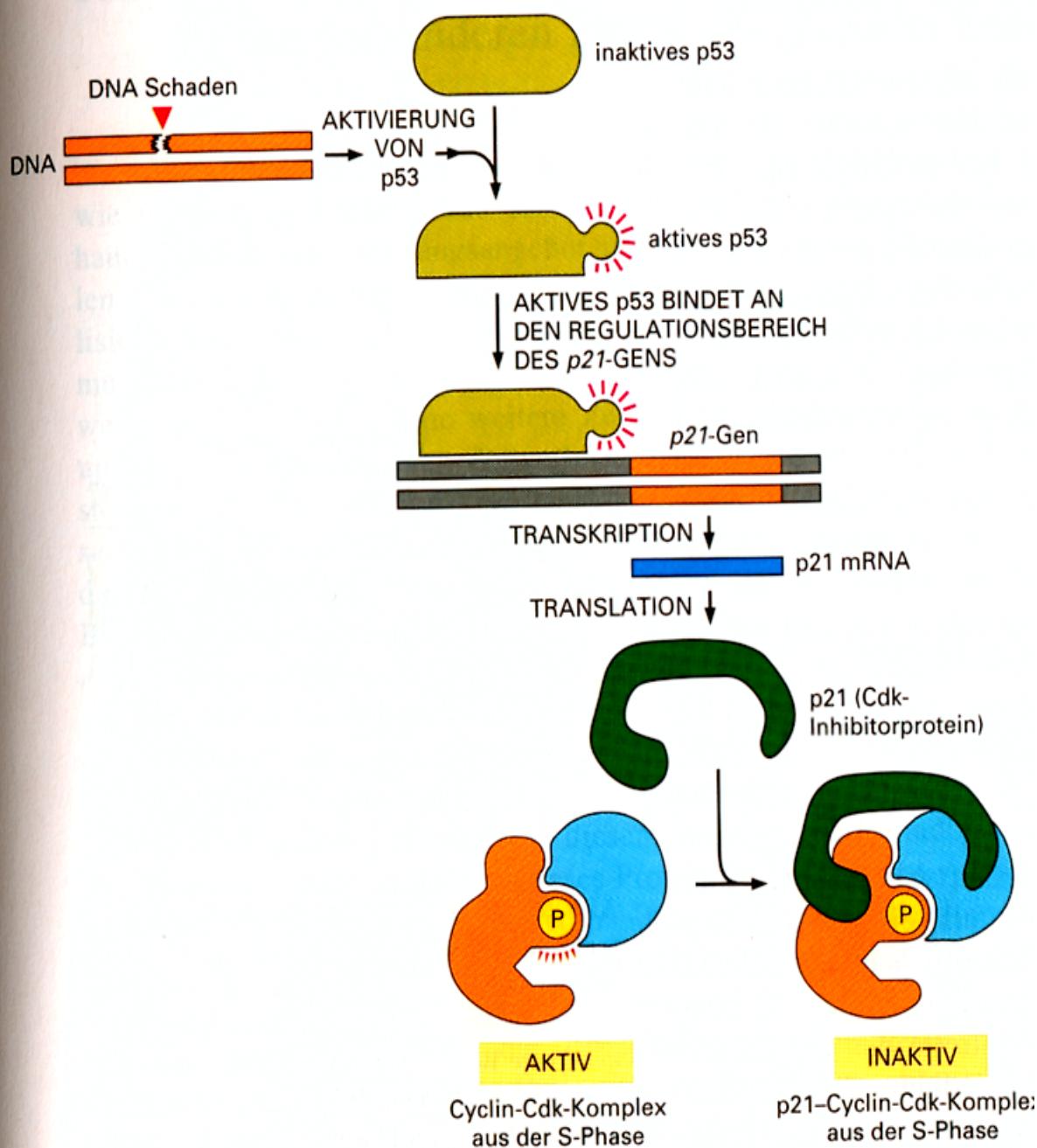
Abb. 18-4 Die zwei wesentlichen Proteinkomponenten eines Cyclin-Cdk-Komplexes. (A) Bei der cyclinabhängigen Kinase Cdk handelt es sich um ein Enzym, das die Phosphorylierung von Proteinen katalysiert, wohingegen Cyclin ein regulatorisches Protein ist, das für die enzymatische Aktivität der Cdk benötigt wird. Der aktive Komplex phosphoryliert Schlüsselproteine in der Zelle, die notwendig sind, um einen bestimmten Schritt innerhalb des Zellzyklus auszulösen. (B) Die dreidimensionale Struktur des Cyclin-Cdk-Komplexes (auch als MPF bezeichnet), der den Eintritt in die M-Phase kontrolliert. (B, nach P. D. Jeffrey et al. *Nature* 376:313–320, 1995.)

- CDK = cyclin-abhängige Kinase
 - Cyclin = regulator. Protein
- } aktiver Komplex phosphoryliert Zielproteine

Verschiedene CDK-Cyclin-Komplexe kontrollieren den Zellzyklus

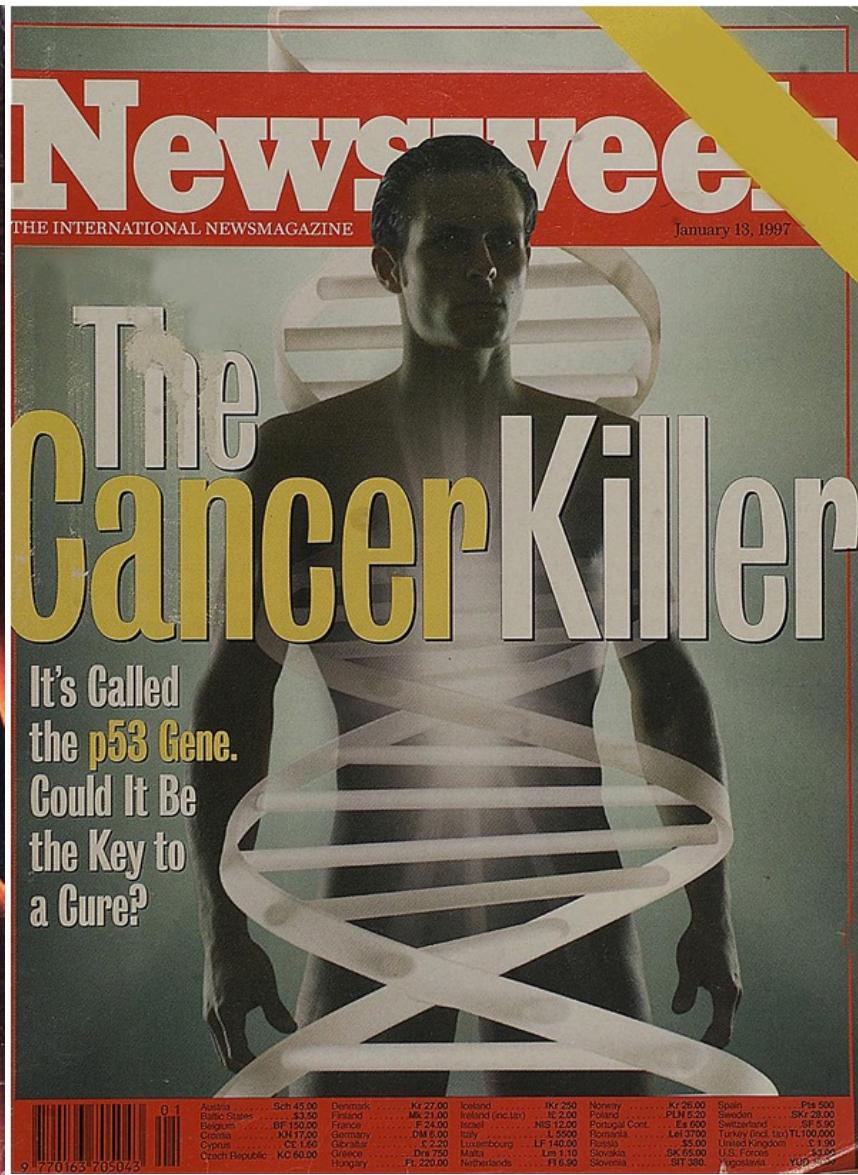


Der G1>S - Checkpoint wird von p53 überwacht!



Eintritt in S-Phase
(zunächst) verhindert

- p53 ist ein wichtiges „Anti-Tumor-Gen“!



P53, guardian of the genome

<http://www.nature.com/nature/journal/v358/n6381/pdf/358015a0.pdf>