TIBBİ BİYOLOJİ ve GENETİK

Prof. Dr. KADİR TURAN

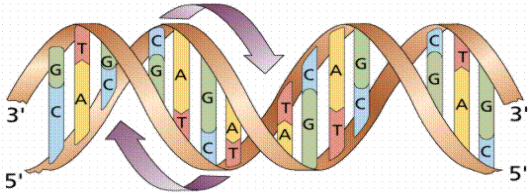
DNA (GEN) / PROTEİN ARASINDAKI İLİŞKİ

BİYOLOJİDE ANA KURAL (central dogma)

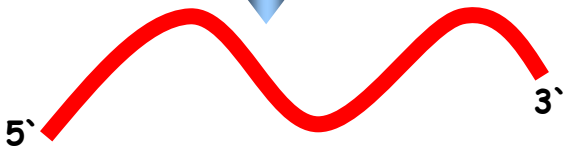
replikasyon

DNA

5`-CCTGAGCCAACTATTGAT-3`



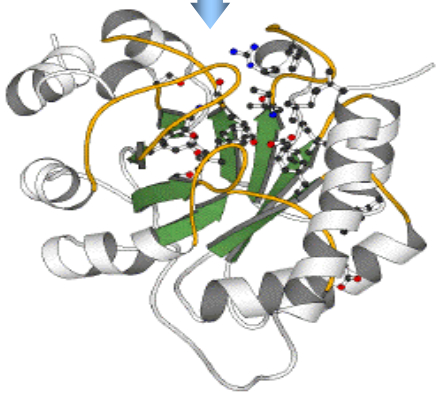
transkripsiyon



mRNA

5`-CCUGAGCCAACUAUUGAU-3`

translasyon



Protein

PEPTİT

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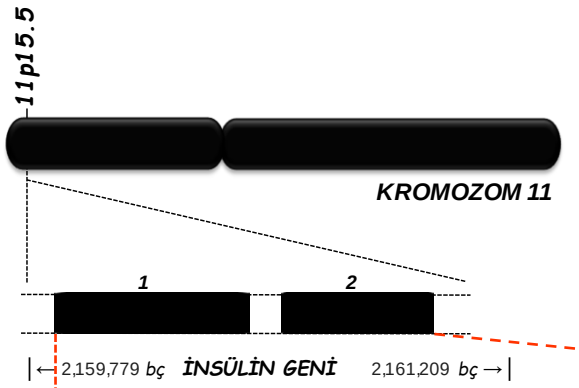
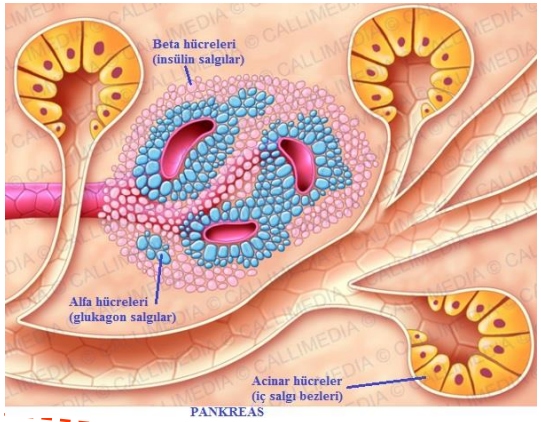
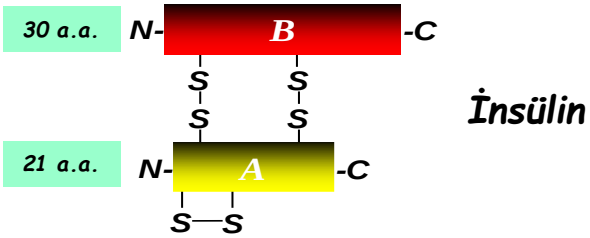
BİYOLOJİDE ANA KURAL (central dogma)





İnsülin DNA	ATG	GCC	CTG	TGG	ATG	CGC	---	CAG	CTG	GAG	AAC	TAC	TGC	AAC	TAG
İnsülin mRNA	AUG	GCC	CUG	UGG	AUG	CGC	---	CAG	CUG	GAG	AAC	UAC	UGC	AAC	UAG
İnsülin Protein	M	A	L	W	M	R	---	Q	L	E	N	Y	C	N	*
	1	2	3	4	5	6	---	104	105	106	107	108	109	110	



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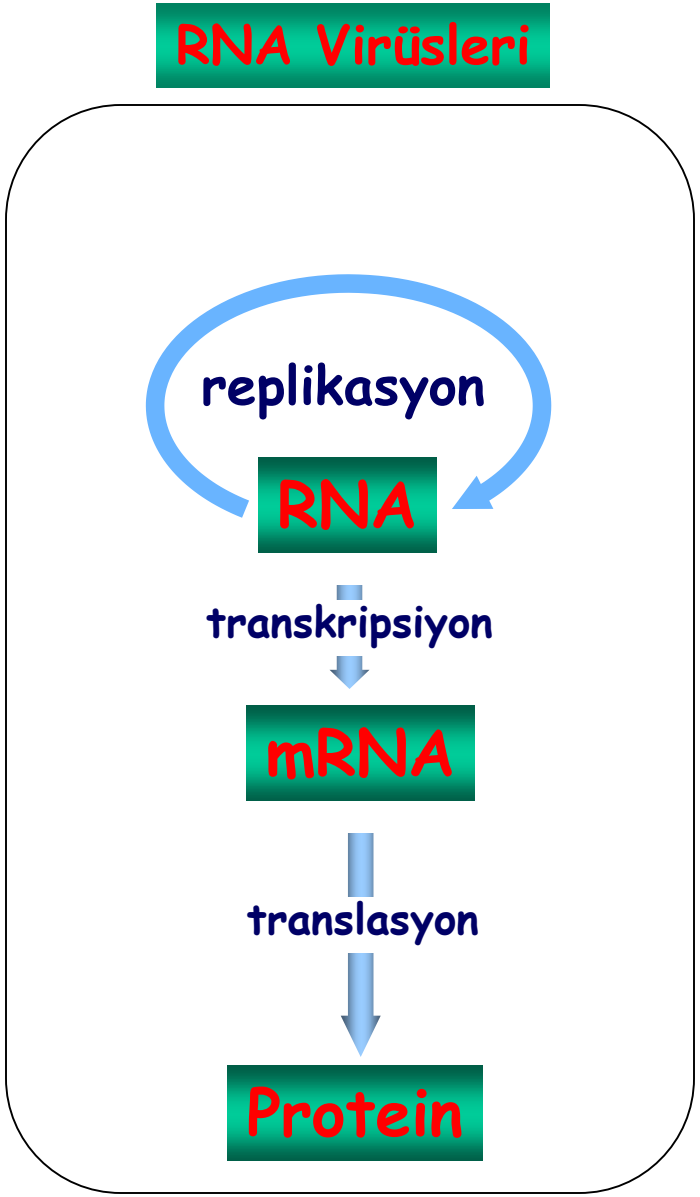
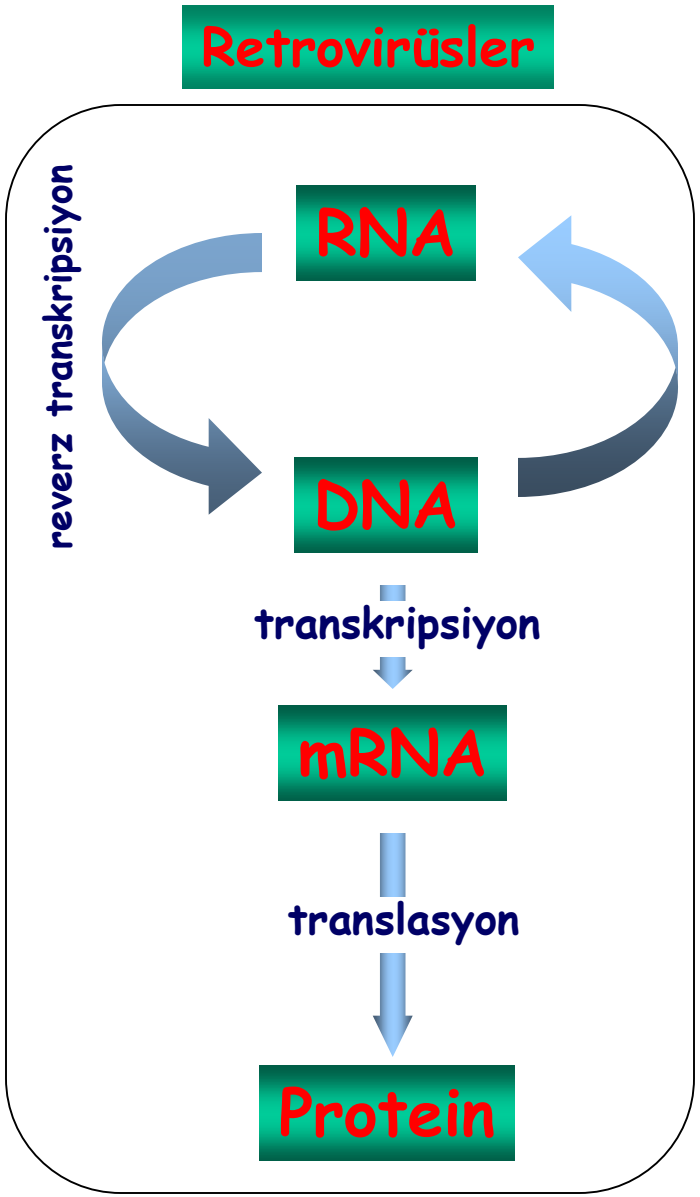
Ana Kural (Central Dogma): Doğada kalıtım materyalinin (DNA), replikasyon denilen bir olayla kendi benzerini sentez ettirmesi, kalıtım materyalinde nükleotid dizileri halinde şifrelenmiş olan genetik bilginin önce RNA halinde kopyalanması ve daha sonra bu şifreye uygun bir polipeptit sentez edilmesi Biyoloji'nin ana kuralı olarak tanımlanır.

Genotip: Bir organizmanın sahip olduğu genlerin tümüdür.

Fenotip: Bir organizmanın sahip olduğu iç ve dış karakterlerinin tümüdür.

Ana Kural'dan Sapmalar





PROTEİNLER

- ***Proteinler tüm biyolojik olaylar için temel olan moleküllerdir.***

- ***Canlılarda, nükleik asitler kalıtsal enformasyonu taşırken, proteinler tüm biyolojik olaylarda fonksiyonel olan moleküllerdir.***

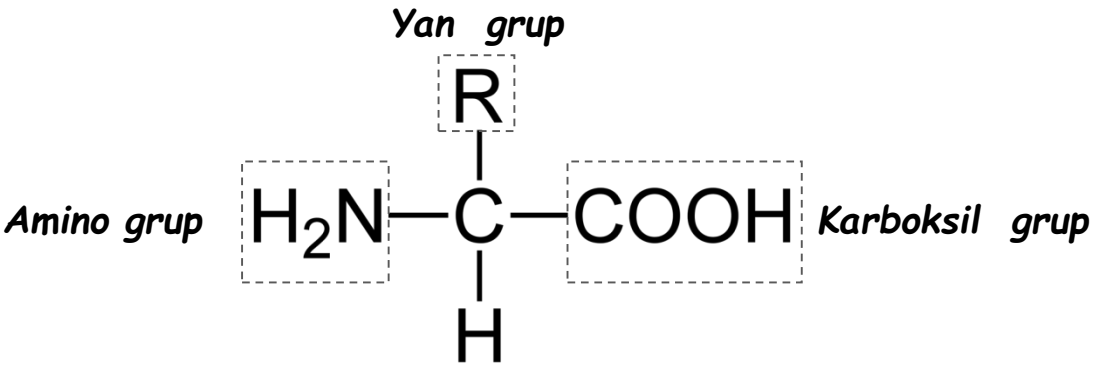
- ***Proteinler hem yapısal hem de fonksiyonel olarak büyük bir çeşitlilik gösterirler.***

- ***Proteinlerin temel yapı taşları L-amino asitlerdir***

Mirror

L-Alanine D-Alanine

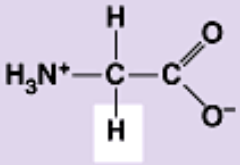
AMİNO ASİTLER



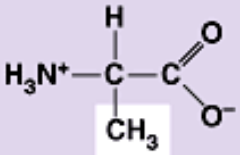
- *Asidik (- yüklü)*
- *Bazik (+ yüklü)*
- *Hidrofobik-Nonpolar (Sudan kaçan)*
- *Hidrofilik-Polar (Suyu seven)*

AMİNO ASİTLER

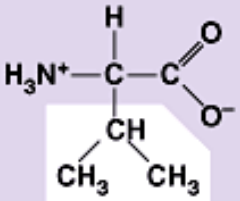
Nonpolar



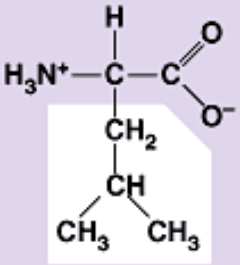
Glycine (Gly)



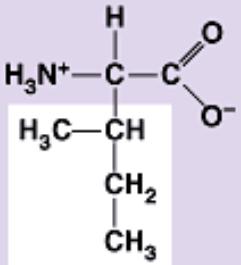
Alanine (Ala)



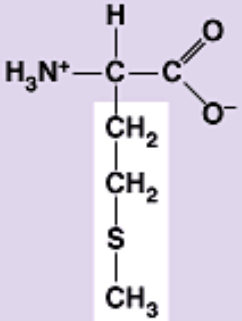
Valine (Val)



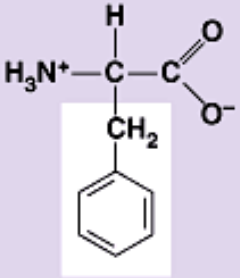
Leucine (Leu)



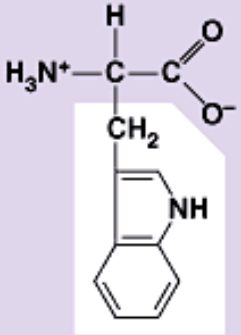
Isoleucine (Ile)



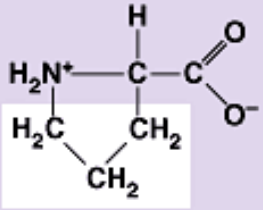
Methionine (Met)



Phenylalanine (Phe)



Tryptophan (Trp)



Proline (Pro)

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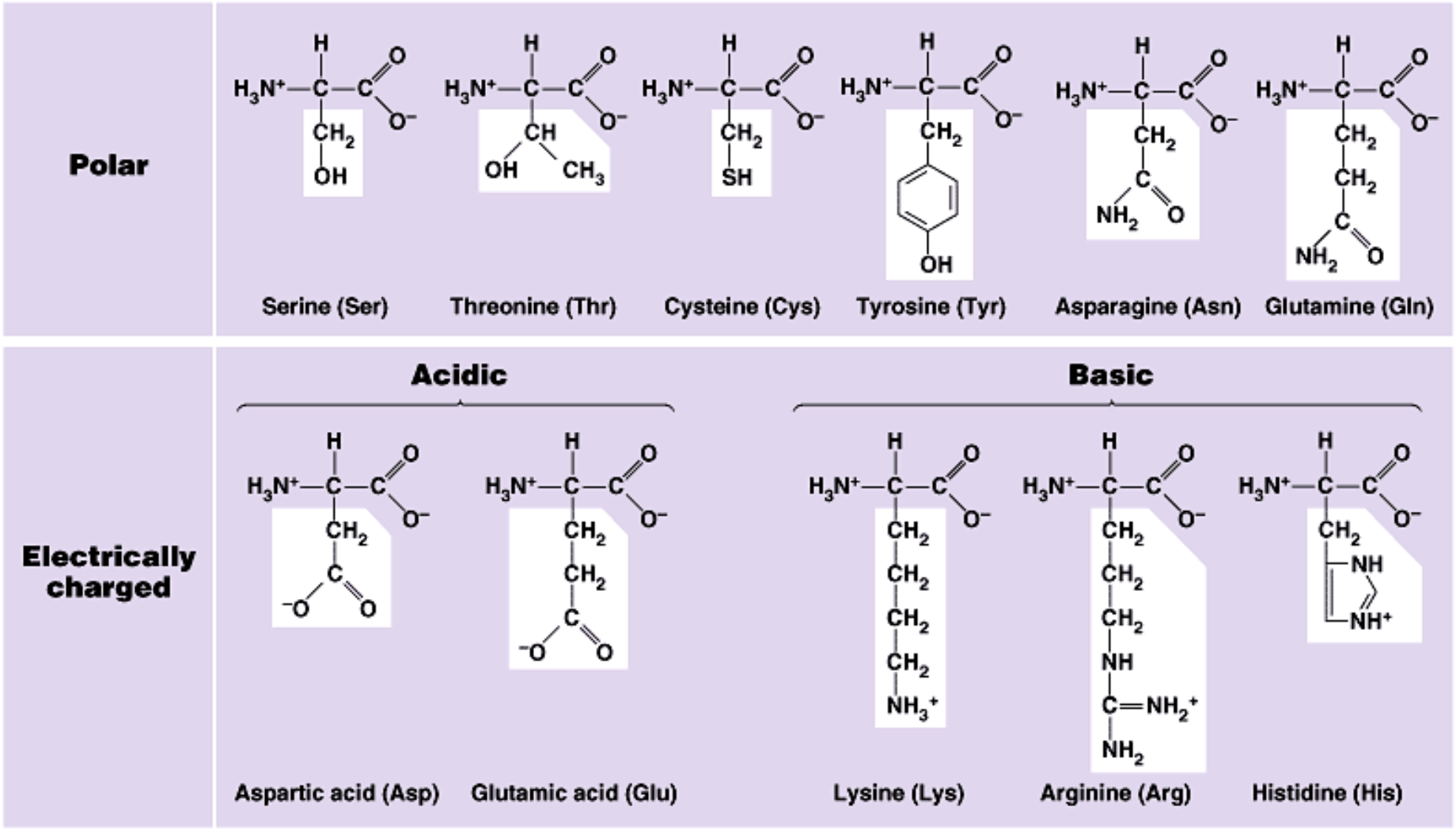
AMİNO ASİTLER



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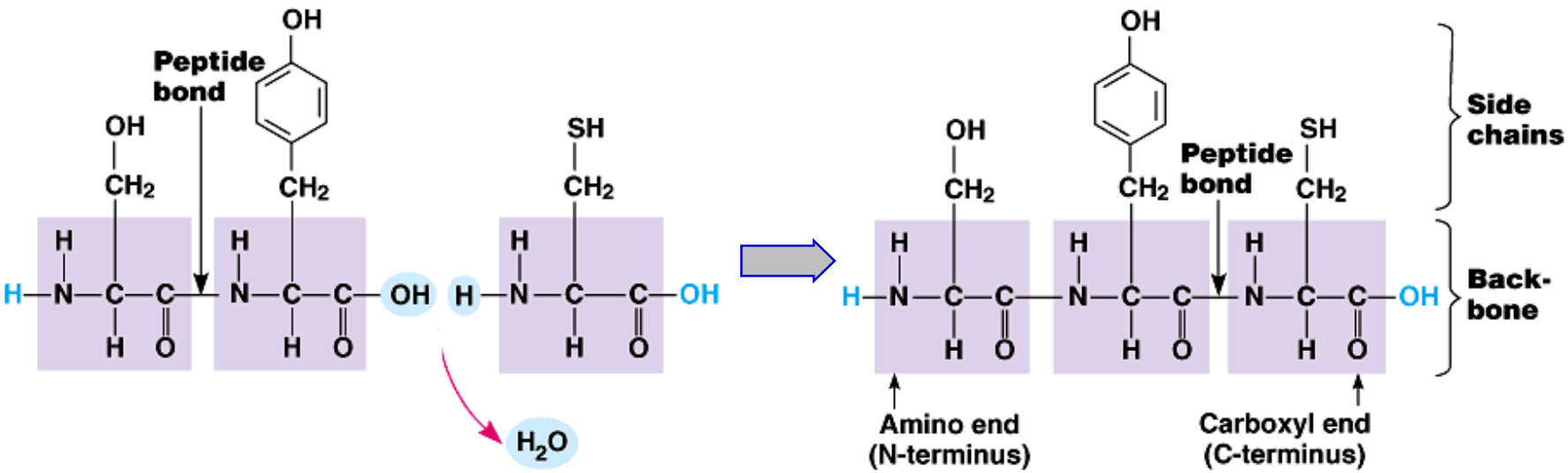
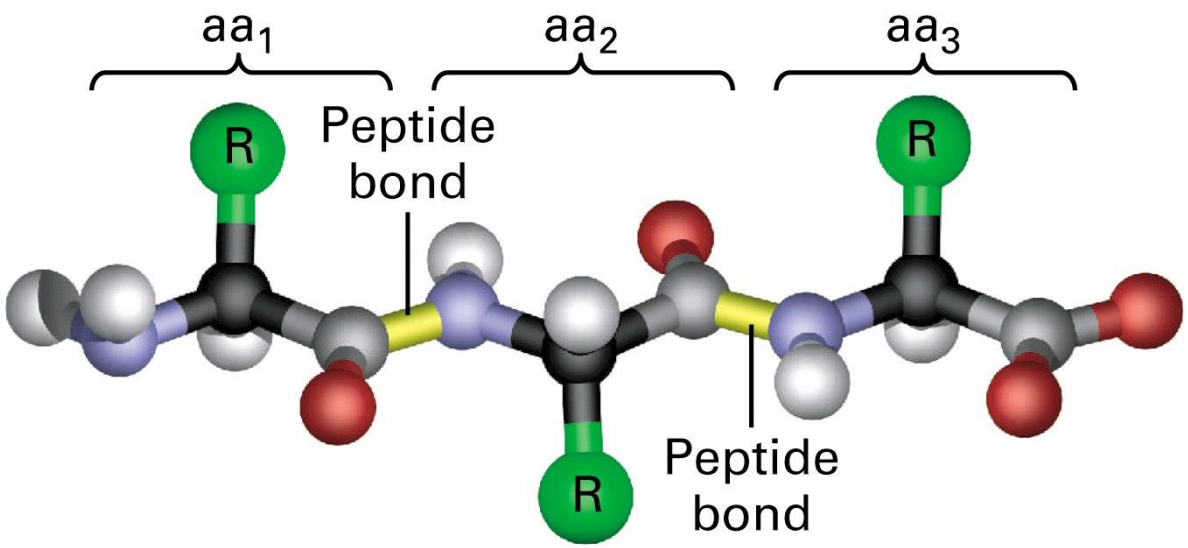
PEPTİD BAĞLARI





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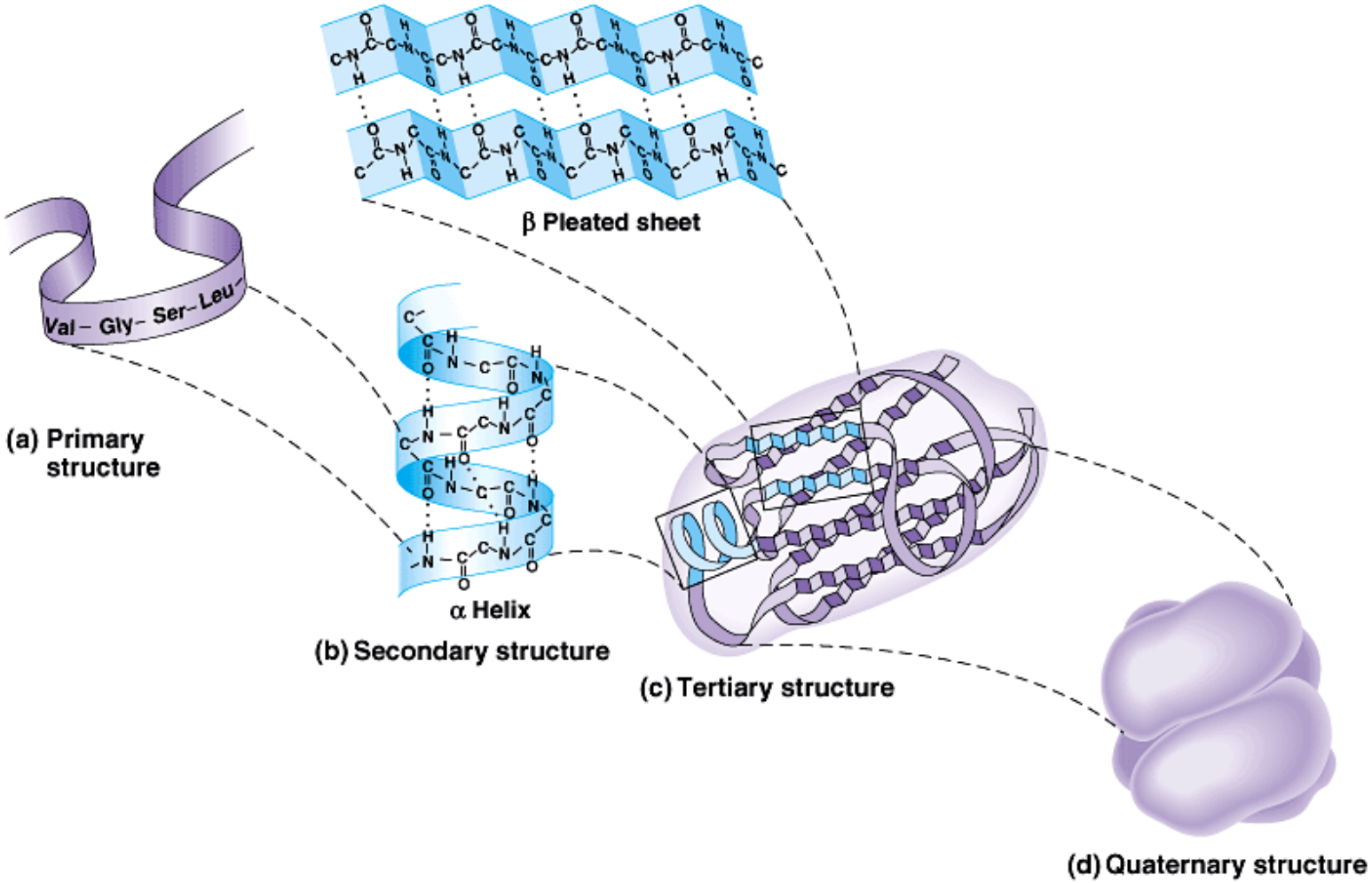
PROTEİNLERİN YAPISI

- *Primer Yapı*
- *Sekonder Yapı (a-helix, β-sheet)*
- *Tersiyer yapı*
- *Kuaterner Yapı*



PROTEİNLERİN YAPISINDA YER ALAN BAĞLAR

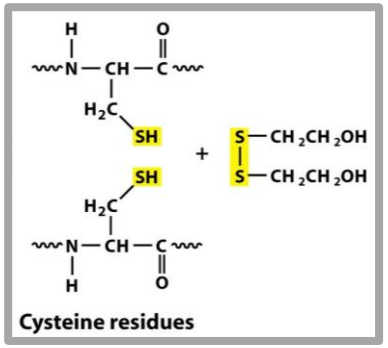
ionic bond

hydrogen bond

van der Waals attractions between atoms (*black*) in contact

Figure 3–5. Molecular Biology of the Cell, 4th Edition.

di-sülfit bağları



PROTEİNLER

Table 5.1 An Overview of Protein Functions

Type of Protein	Function	Examples
Structural proteins	Support	Insects and spiders use silk fibers to make their cocoons and webs, respectively. Collagen and elastin provide a fibrous framework in animal connective tissues. Keratin is the protein of hair, horns, feathers, and other skin appendages.
Storage proteins	Storage of amino acids	Ovalbumin is the protein of egg white, used as an amino acid source for the developing embryo. Casein, the protein of milk, is the major source of amino acids for baby mammals. Plants have storage proteins in their seeds.
Transport proteins	Transport of other substances	Hemoglobin, the iron-containing protein of vertebrate blood, transports oxygen from the lungs to other parts of the body. Other proteins transport molecules across cell membranes.
Hormonal proteins	Coordination of an organism's activities	Insulin, a hormone secreted by the pancreas, helps regulate the concentration of sugar in the blood of vertebrates.
Receptor proteins	Response of cell to chemical stimuli	Receptors built into the membrane of a nerve cell detect chemical signals released by other nerve cells.
Contractile proteins	Movement	Actin and myosin are responsible for the movement of muscles. Other proteins are responsible for the undulations of the organelles called cilia and flagella.
Defensive proteins	Protection against disease	Antibodies combat bacteria and viruses.
Enzymatic proteins	Selective acceleration of chemical reactions	Digestive enzymes catalyze the hydrolysis of the polymers in food.

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KALITIM MATERYALİNİN İŞLEVSEL ÖZELLİKLERİ VE YETENEKLERİNE GENEL BİR BAKIŞ

DNA'NIN İŞLEVSEL ÖZELLİKLERİ

REPLİKASYON

GEN ANLATIMI

KENDİNİ ONARMA

GENETİK ÇEŞİTLİLİK

Mutasyon

Rekombinasyon

Crossing-over
Transformasyon
Transdüksiyon
Konjugasyon
Transpozisyon

Crossing-over

Crossing-over (krossing over), ökaryotik hücrelerde mayoz bölünmenin profaz I evresinde, çiftler halde bulunan kromozomların (bivalent) kardeş olmayan kromatidleri arasında meydana gelen parça değişimleridir. Ökaryotik hücrelere özgü olan bu olay genetik rekombinasyona (gen alellerinin birbiriyle yer değiştirmesi) neden olur.

Transformasyon

Çıplak halde (hücreden izole edilmiş haliyle) DNA'moleküllerinin bir bakteri hücresine alınması ve bunun sonucu bakteride meydana gelen kalıtsal değişiklik olarak tanımlanabilir. Transformasyon olayı ilk defa 1927'de, İngiliz bakteriyolog, Frederick Griffith tarafından keşfedilmiştir. Bakterinin ortamdan yabancı DNA'yı içine alabilme özelliğine yetkinlik (competence) adı verilir. Doğal ortamda bakterilerin yetkinlikleri çok düşüktür, fakat Ca^{2+} gibi iyonlarla milyonlarca kat arttırılabilir. Bu şekilde etkinliği arttırılan E. coli bakterisi (kompetent bakteriler) gen klonlama çalışmalarında yaygın olarak kullanılır.

Transdüksiyon

Bir bakteri virüsü (bakteriyofaj) bir bakteriden diğerine genetik madde aktarılması olayıdır. Kısaca bakteri virüslerinin aracılık ettiği rekombinasyon olayıdır. Transdüksiyon olayı da gen klonlama çalışmalarında, ele alınan DNA moleküllerinin bakteriler aktarılmasında kullanılmaktadır.

Konjugasyon

Fiziksel temas halinde olan iki bakteride, birinden (F^+ ya da Hfr^+) diğerine (F^- ya da Hfr^-) gen aktarımıdır. F^+eşeme sahip bakteriden gen aktarımı sonucunda F^-olan bakteri de F^+özellik kazanır. Buna karşın Hfr^+bakteriden F^-bakteri gen transfer edildiğinde genellikle alıcı bakteri F^-özelliğini korur.

Transpozisyon

Transpozonlar (yer değiştiren elementler) ilk kez 1940 yılında Barbara McClintock tarafından mısır bitkisinde keşfedildi. Bu çalışmasından dolayı 1983 yılında Nobel ödülü aldı. ***Transpozonlar*** *bir hücre genomunda, farklı mekazimalarla yer değiştiren elementlerdir. Bu olay* ***transpozisyon*** *olarak adlandırılır. Transpozisyon olayı hem prokaryot hem de ökaryotlarda yaygın olarak gözlenen olaydır.*

REPLİKASYON

Replikasyon hangi özelliklere sahip olmalıdır?

TAMAMLANMAMIŞ KISA DNA MOLEKÜLLERİ OLUŞMAMALI

GENETİK BİLGİ BÜYÜK ORANDA DEĞİŞMEDEN KORUNMALI

HÜCRE BÖLÜNMESİ İLE DNA REPLİKASYONU BİRBİRİNİ İZLEYECEK ŞEKİLDE KONTROL ALTINDA TUTULMALI

Amoeba dubia
670,000,000,000

Amoeba proteus
290,000,000,000

Bufo bufo
6,900,000,000

REPLİKASYON

Kalıtım materyalinin değişmeden dölden döle aktarılmasını sağlayacak replikasyon modeli nasıl olmalı?

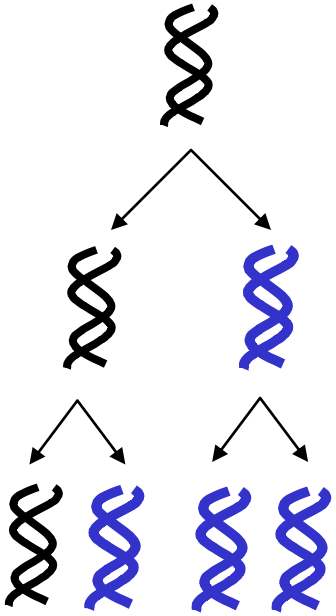
KORUYUCU (CONSERVATIVE) MODEL

YARI-KORUYUCU (SEMİ-CONSERVATIVE) MODEL

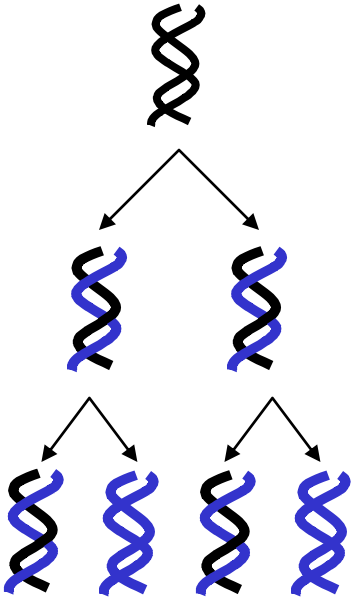
DAĞILIM (DISPERSIVE) MODEL

REPLİKASYON İÇİN İLERİ SÜRÜLEN MODELLER

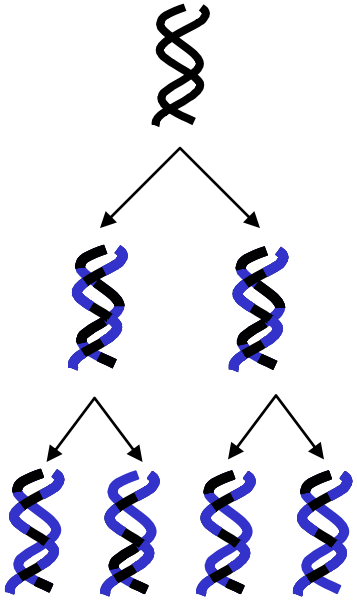
KORUYUCU (CONSERVATIVE) MODEL



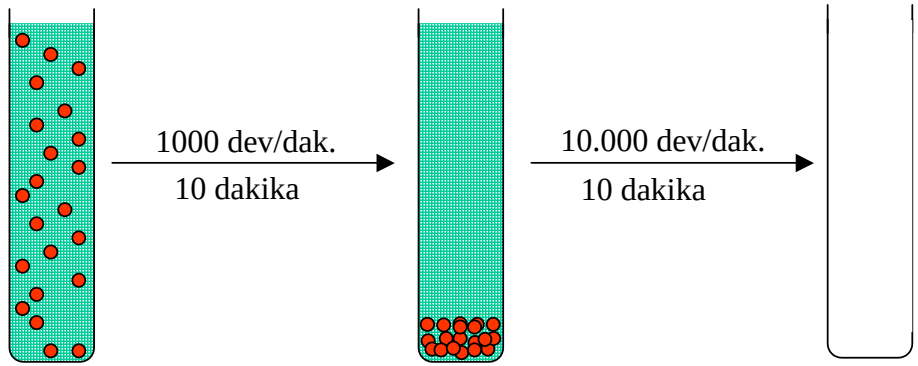
YARI-KORUYUCU (SEMİ-CONSERVATIVE) MODEL



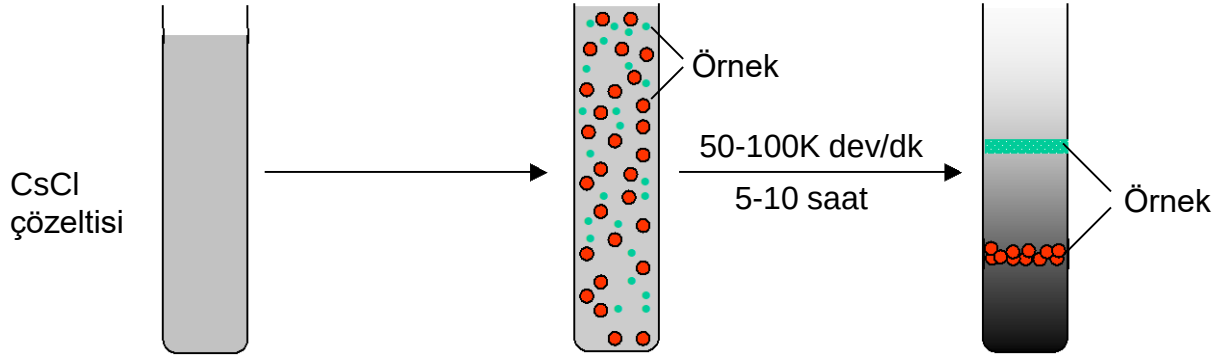
DAĞILIM (DISPERSIVE) MODEL



Acrobat Document

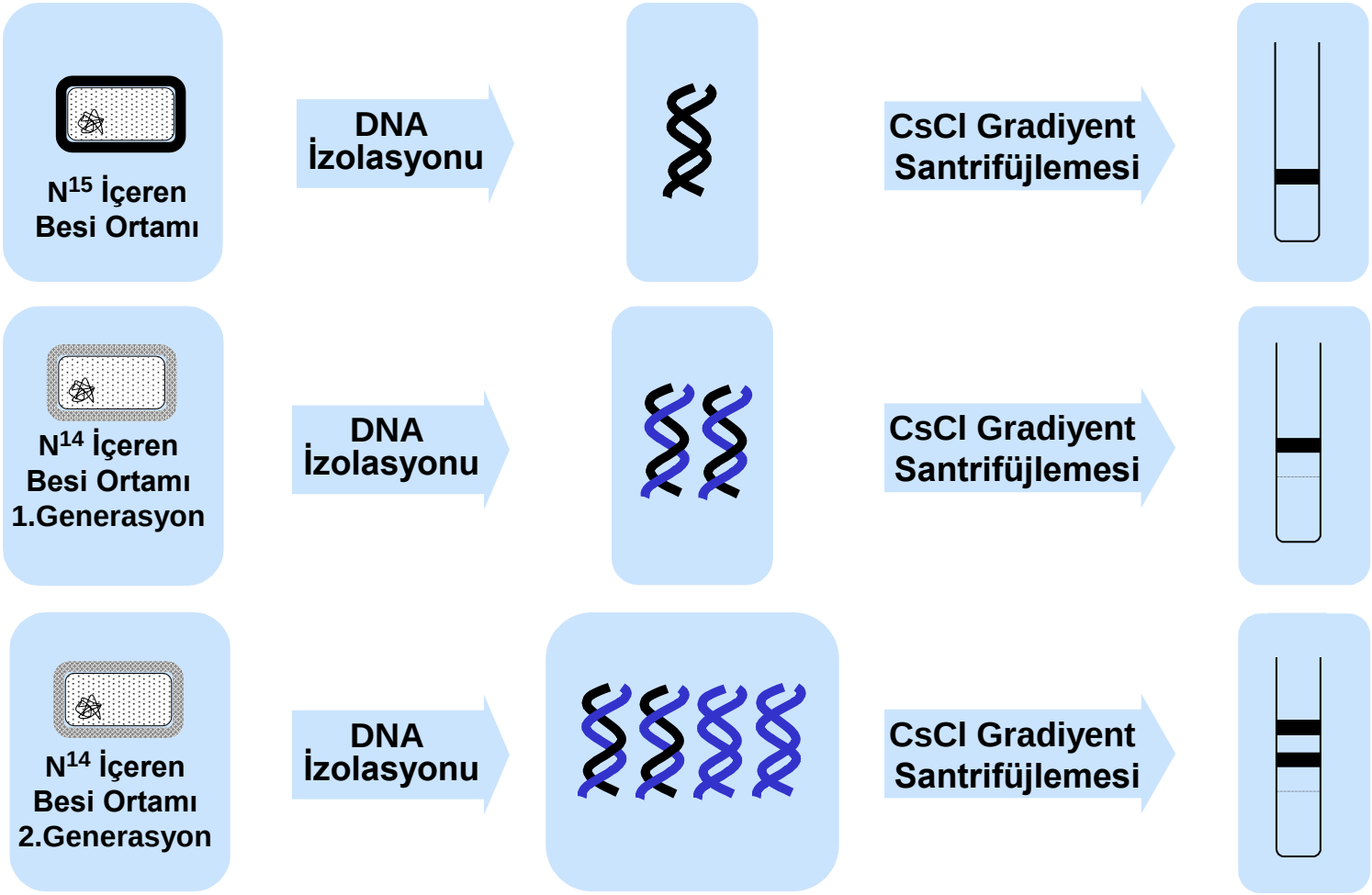


SANTRİFÜJLEME

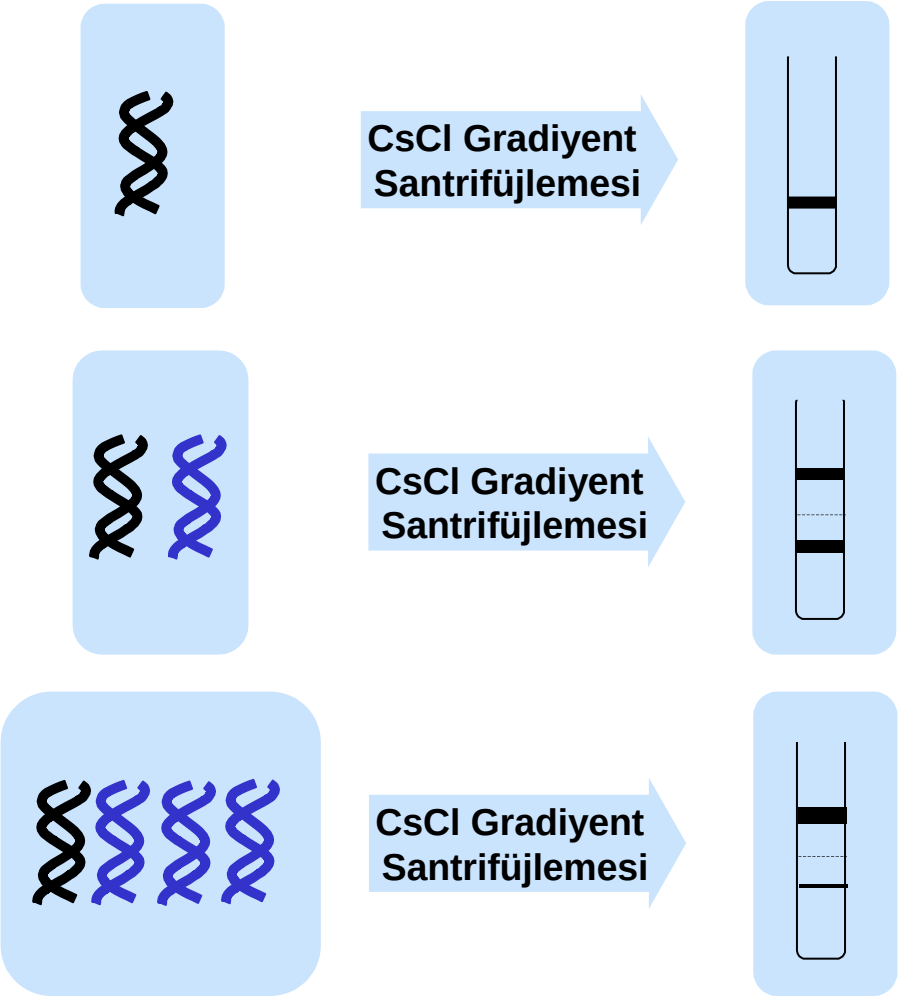


DENGE YOĞUNLUK GRADİYENTİ SANTRİFÜJLEMESİ
(CsCl Gradiyenti Santrifüjlemesi)

REPLİKASYON MODELİ
(Meselson & Stahl – 1958)



Meselson & Stahl'ın gerçekleştirdiği bu deneyler DNA replikasyonu'nun Yarı-koruyucu modele uygun olarak gerçekleştiğini ortaya koymuştur



Messelson&Stahl.swf

REPLİKASYONA KATILAN ENZİMLER

DNA POLİMERAZLAR

Bakteriler
- DNA Pol. I
- DNA Pol. II
- DNA Pol. III

Ökaryotik Organizmalar
- DNA Pol. α
- DNA Pol. β
- DNA Pol. γ
- DNA Pol. δ
- DNA Pol. ε

Virüsler
- T4 DNA Pol.
- T7 DNA Pol.
- Reverz transkriptaz vb.

PRİMAZ

LİGAZLAR

TOPOİZOMERAZLAR

PROTEİN FAKTÖRLER

DNA POLİMERAZLARIN GENEL ÖZELLİKLERİ

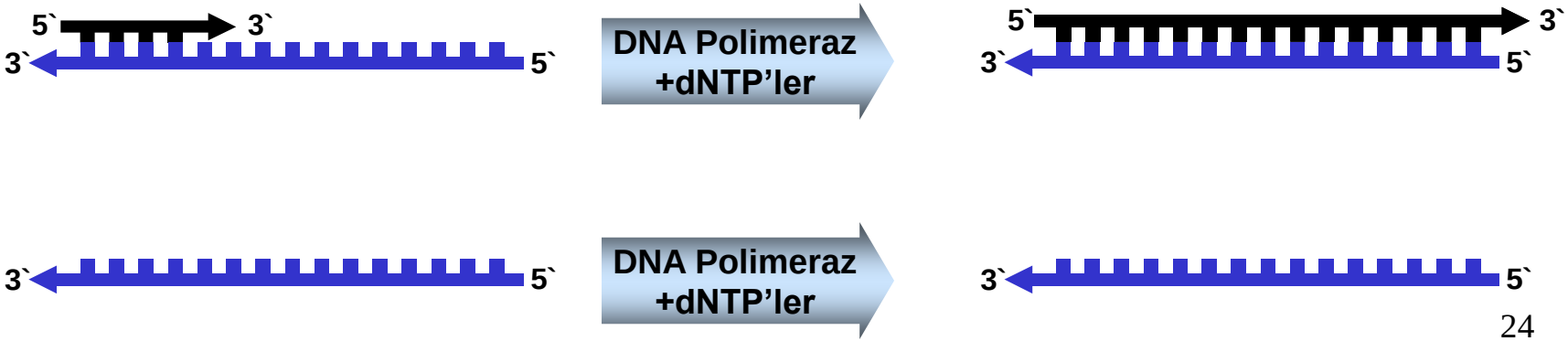
Bir DNA kalıba gereksinim duyarlar

Kendi başlarına polimerizasyon reaksiyonunu başlatamazlar, bir primere gereksinim duyarlar

Polimerizasyonu 5’-3’ yönünde kataliz edebilirler, yani serbest 3’-OH grubuna ihtiyaç duyarlar

Ortamda dört çeşit dNTP ve Mg^{+2} iyonlarının bulunması gerekir

DNA Polimerazların çoğu 3’-5’ ekzonükleaz aktivitesine sahiptir

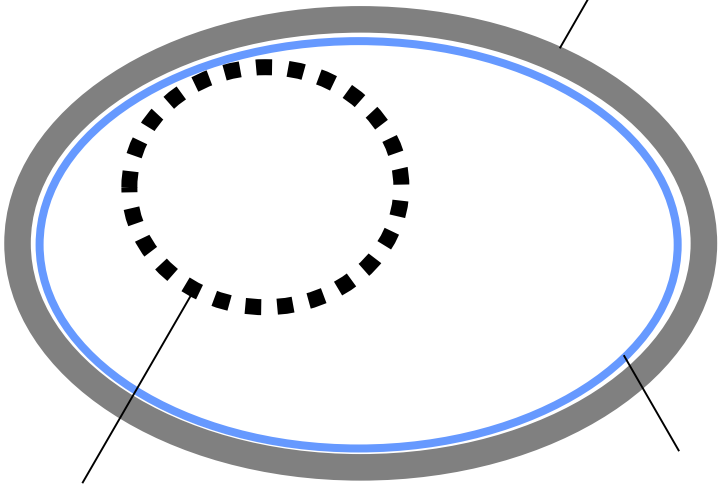


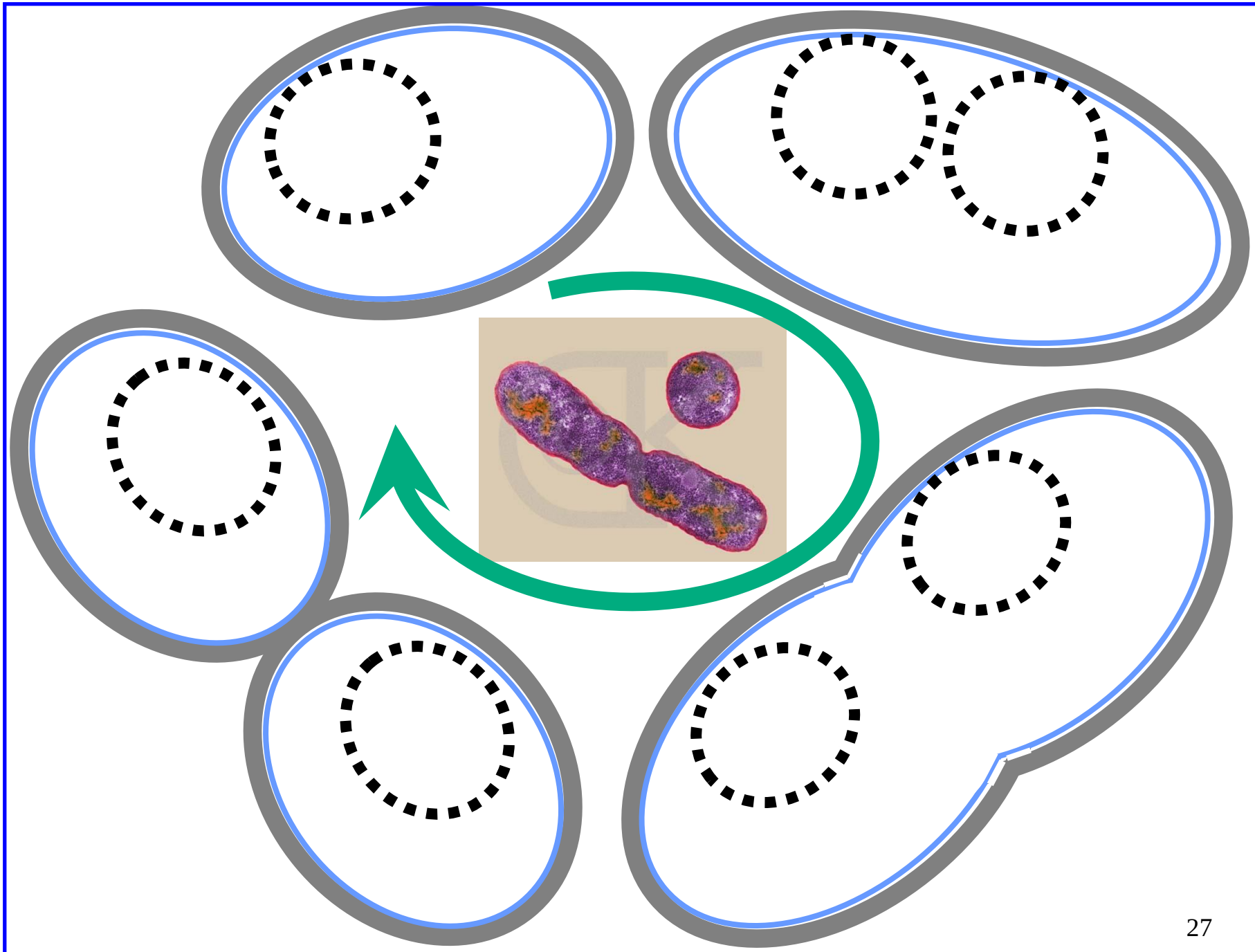
3'-5' ekzonükleaz aktivitesine sahip olan DNA polimerazların replikasyonda hata oluşma olasılığını önemli ölçüde düşürür

Bu nedenle DNA sentezi 10^{-8}-10^{-10} gibi yüksek bir doğrulukta gerçekleşir, bu da bakterilerde ortalama 1000 replikasyonda bir hata anlamına gelir (ortalama bakteri genomu 4200 kbç)

Enzim	Hata Oranları	
	- ekz.	+ ekz.
DNA pol I	10^{-5}	5×10^{-7}
DNA pol III	7×10^{-6}	5×10^{-9}
T4 DNA pol	5×10^{-5}	10^{-7}
Reverz transkriptaz	10^{-5}	

Bakterilerde Replikasyon ve Hücre Bölünmesi





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E. coli'de Replikasyonda İş Gören Bazı Protein ve Enzimler

DNA pol. I (*polA*) - DNA hasarlarının onarımı ve replikasyonda primerlerin yok edilmesi ve oluşan açıklıkların kapatılmasında rol alır (~400/hücre) (103 kDa).

DNA pol. II (*polB*): DNA onarımı (90 kDa).

DNA pol. III (*dnaE,dnaQ*): DNA replikasyonu; Çok sayıda altbirinden oluşur (10-20/hücre) (900 kDa).

Helikaz (DnaB) - DNA ikili sarmalının açılmasında rol oynar

Giraz - İkili sarmal yapının açılması ile oluşan stresin azaltılmasında rol oynar.

SSB (single-strand binding protein) – DNA sarmalı açıldıktan sonra DNA zincirlerine bağlanır.

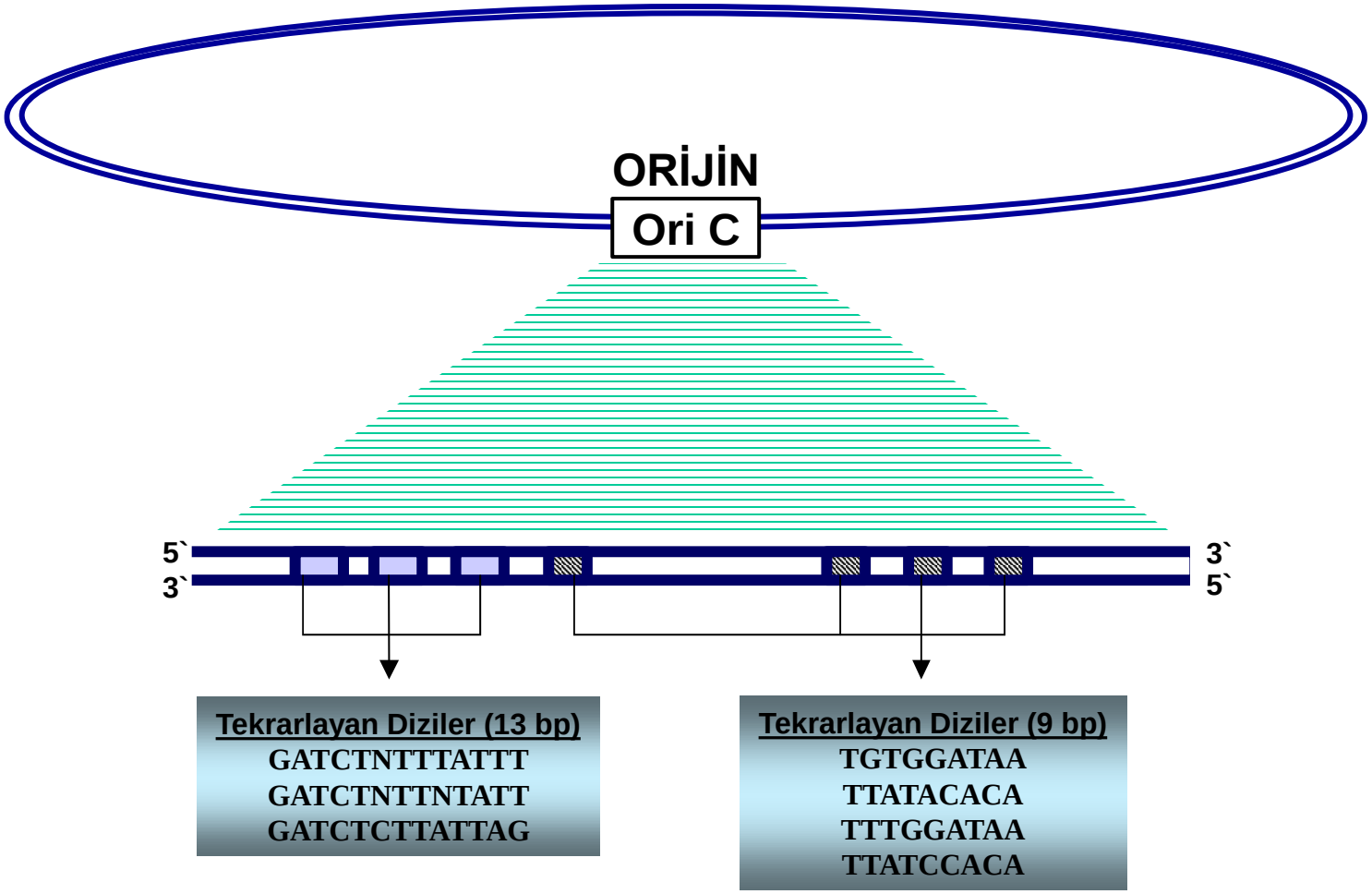
Primaz (dnaG) - RNA primerlerin sentezinde rol alır.

DNA ligaz -Kesikli DNA zincirlerleri arasında fosfodiester bağlarının kurulmasını sağlar.

E. coli'de DNA polimerazların enzimatik aktiviteleri

DNA plimeraz enzimi	5' → 3' sentez	3' → 5' ekzonükleaz	5' → 3' ekzonükleaz
DNA Pol I	+	+	+
DNA Pol II	+	+	-
DNA Pol III	+	+	-

BAKTERİLERDE REPLİKASYON



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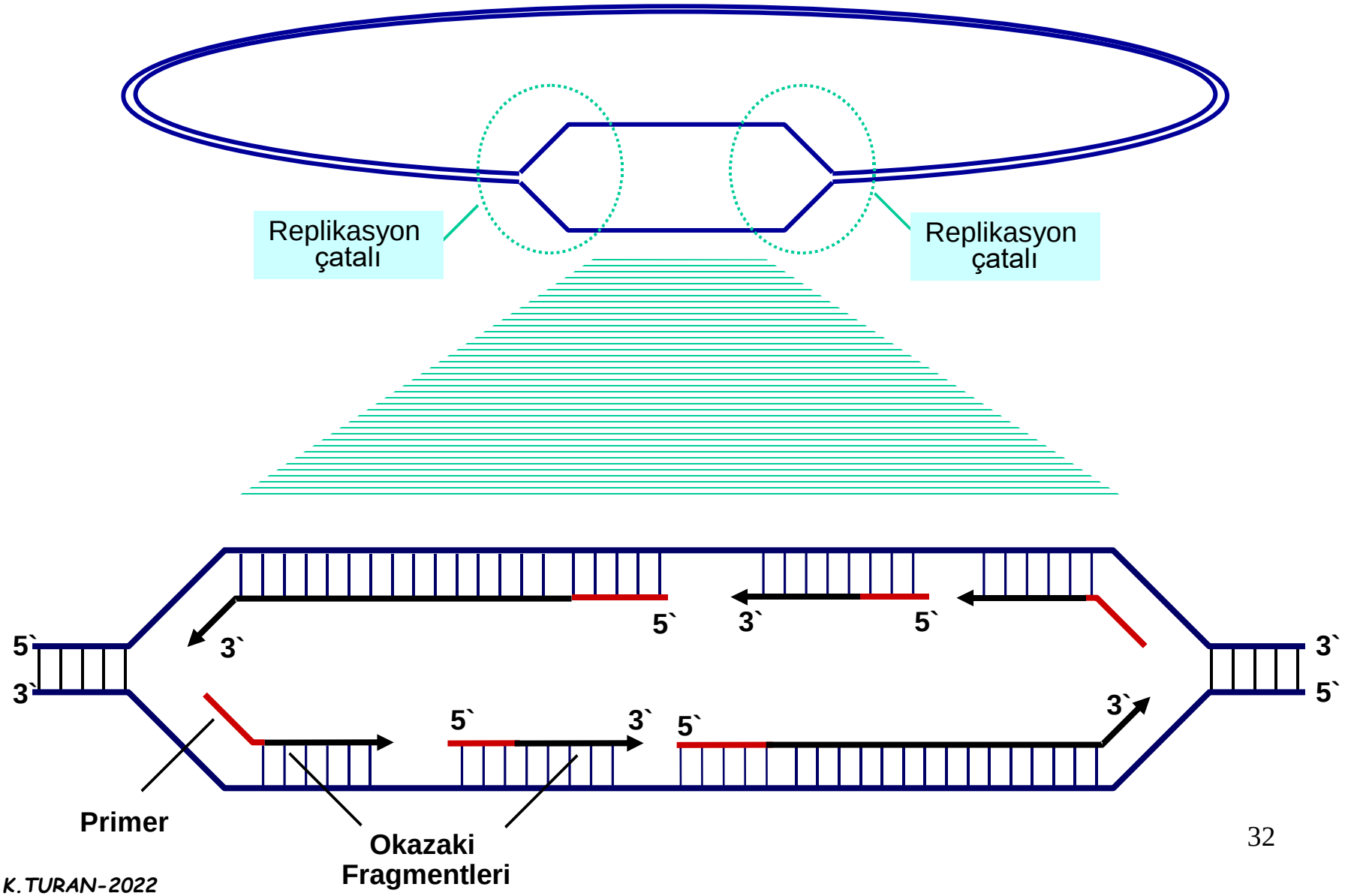
Bazı organizmalarda replikonların karşılaştırılması – (Lewin'denTable 14.1)

Organizma	Replikon sayısı	Replikon boyutu	Replikasyon hızı (bç/rep. çatalı)
E. coli	1	4,600 kb	50,000 bç/dk
S. cerevisiae	500	40 kb	3,600 bç/dk
D. melanogaster	3,500	40 kb	2,600 bç/dk
X. laevis	15,000	200 kb	500 bç/dk
M. musculus	25,000	150 kb	2,200 bç/dk

BAKTERİLERDE REPLİKASYON



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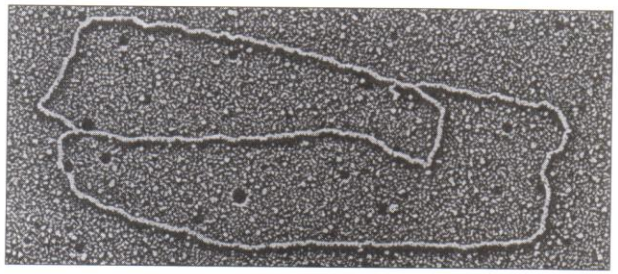
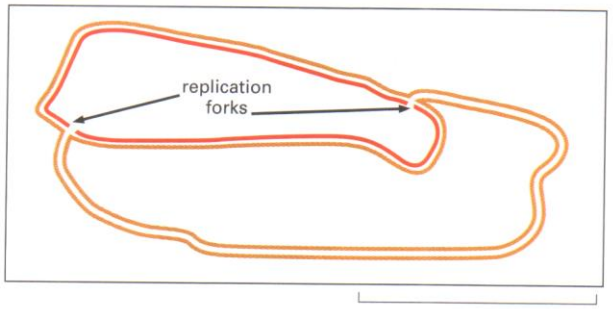
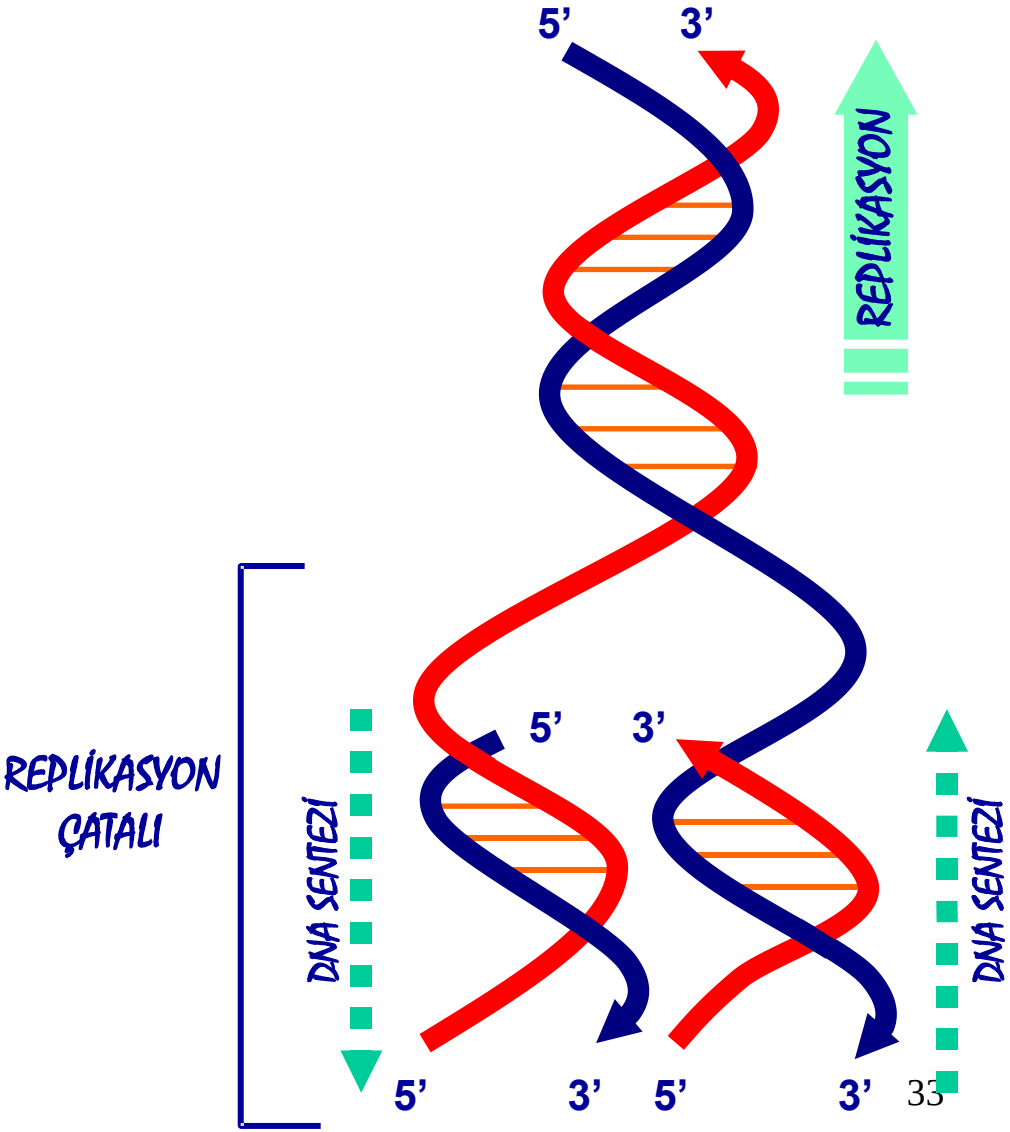
REPLİKASYON ÇATALI

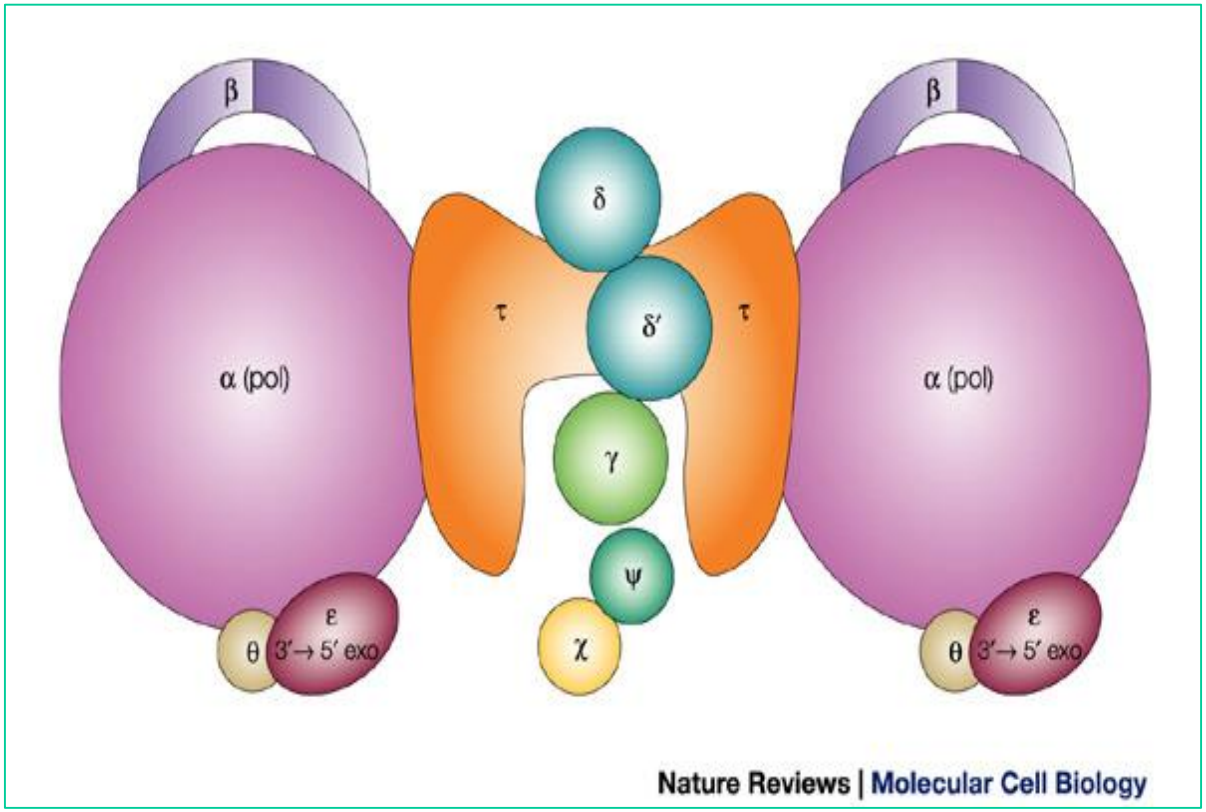




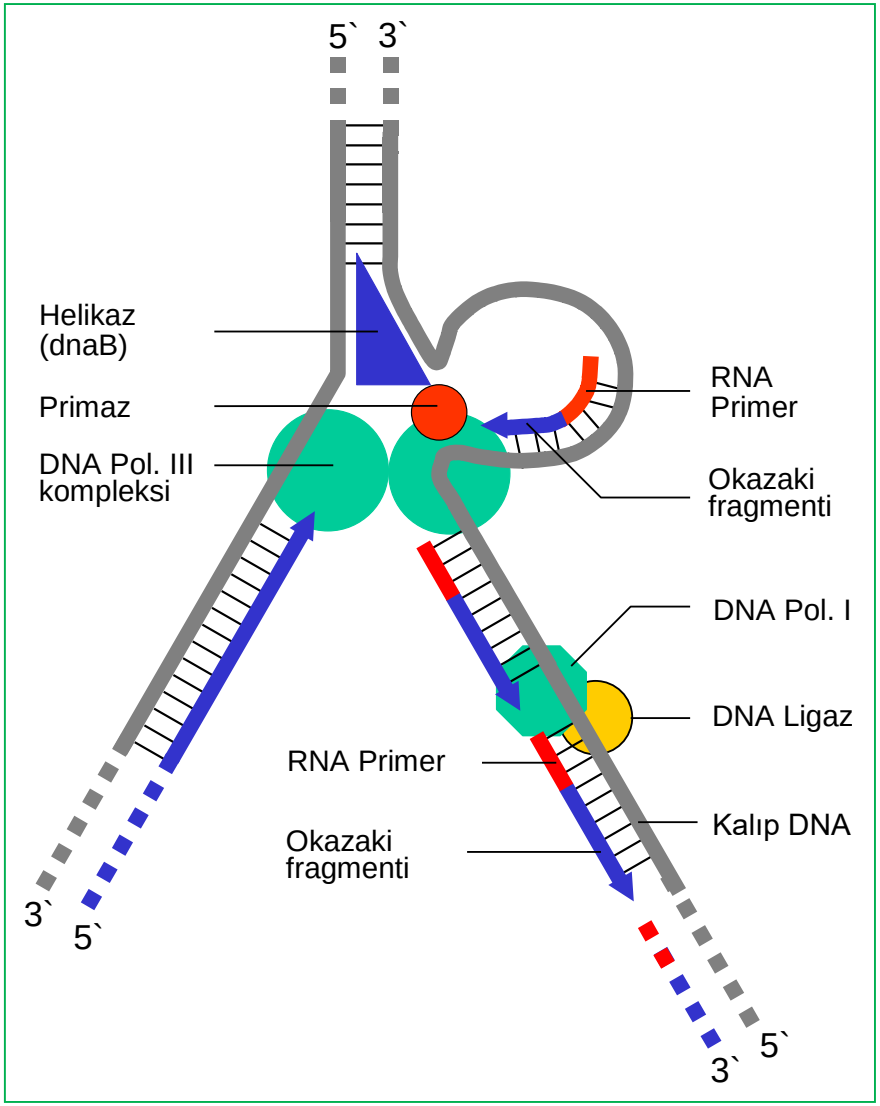
MBC-Fig.5-6

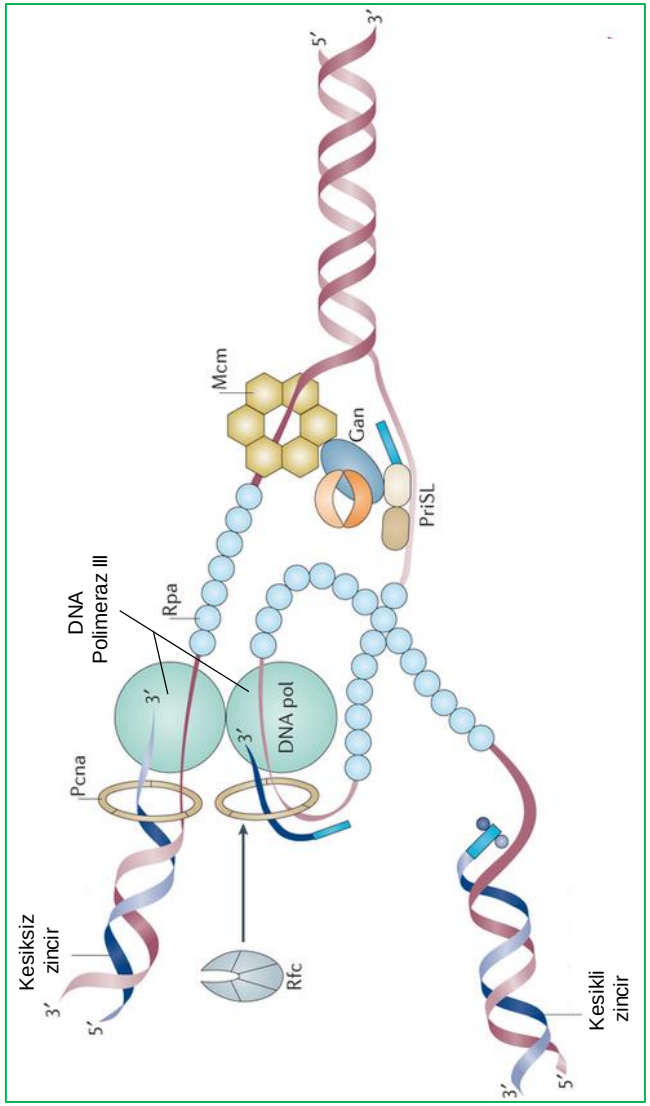


E. coli DNA polimeraz III kompleksi
(Shevelev and Hubscher, 2002)

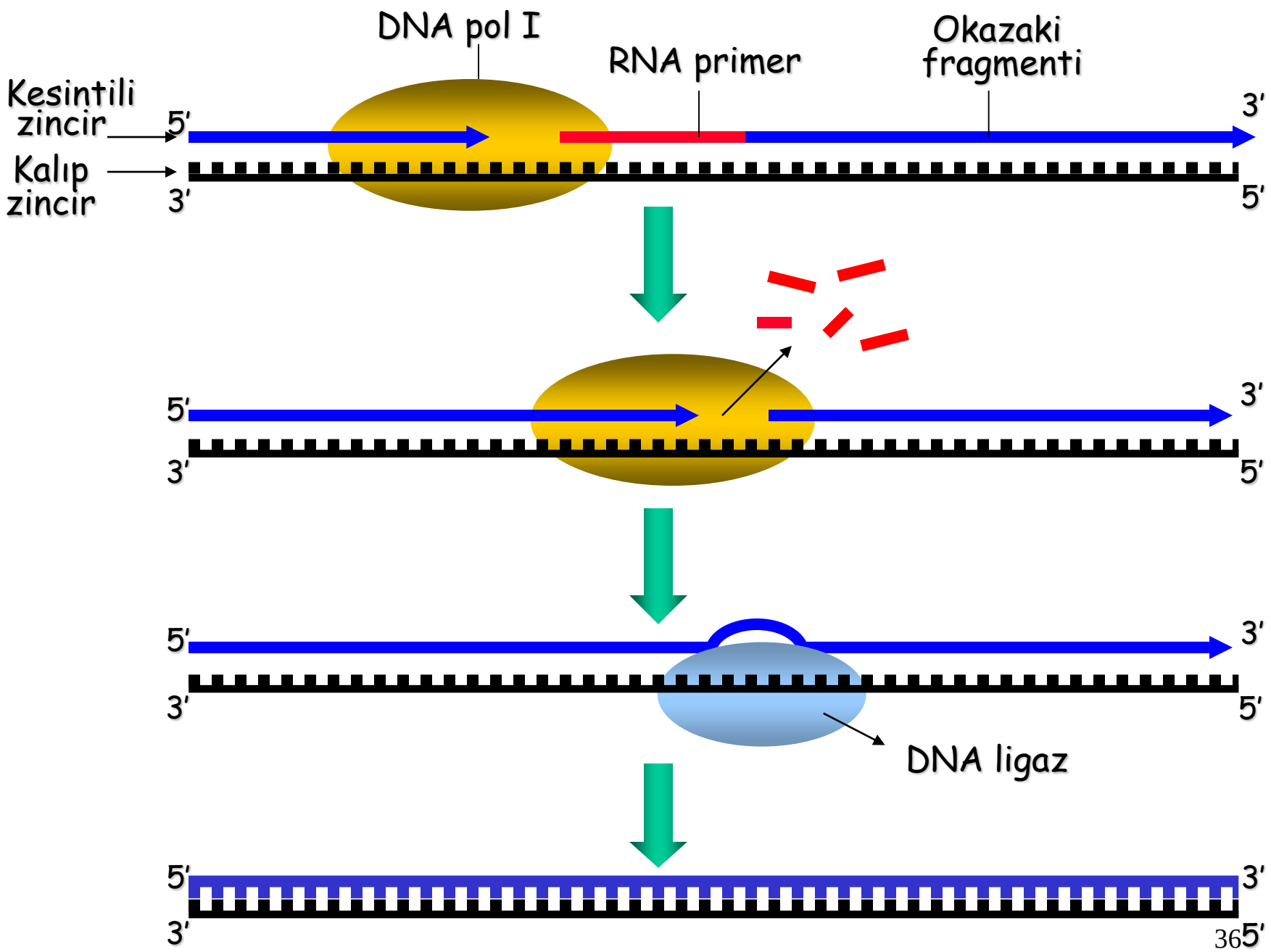


REPLİKASYON ÇATALI





Article *in Nature Reviews Microbiology*
11(9) · 627–638 July 2013


DNA pol I
RNA primer
Okazaki fragmenti
Kesintili zincir
Kalıp zincir
5′
3′
3′
5′
5′
3′
3′
5′
5′
3′
3′
5′
DNA ligaz
5′
3′
3′
5′

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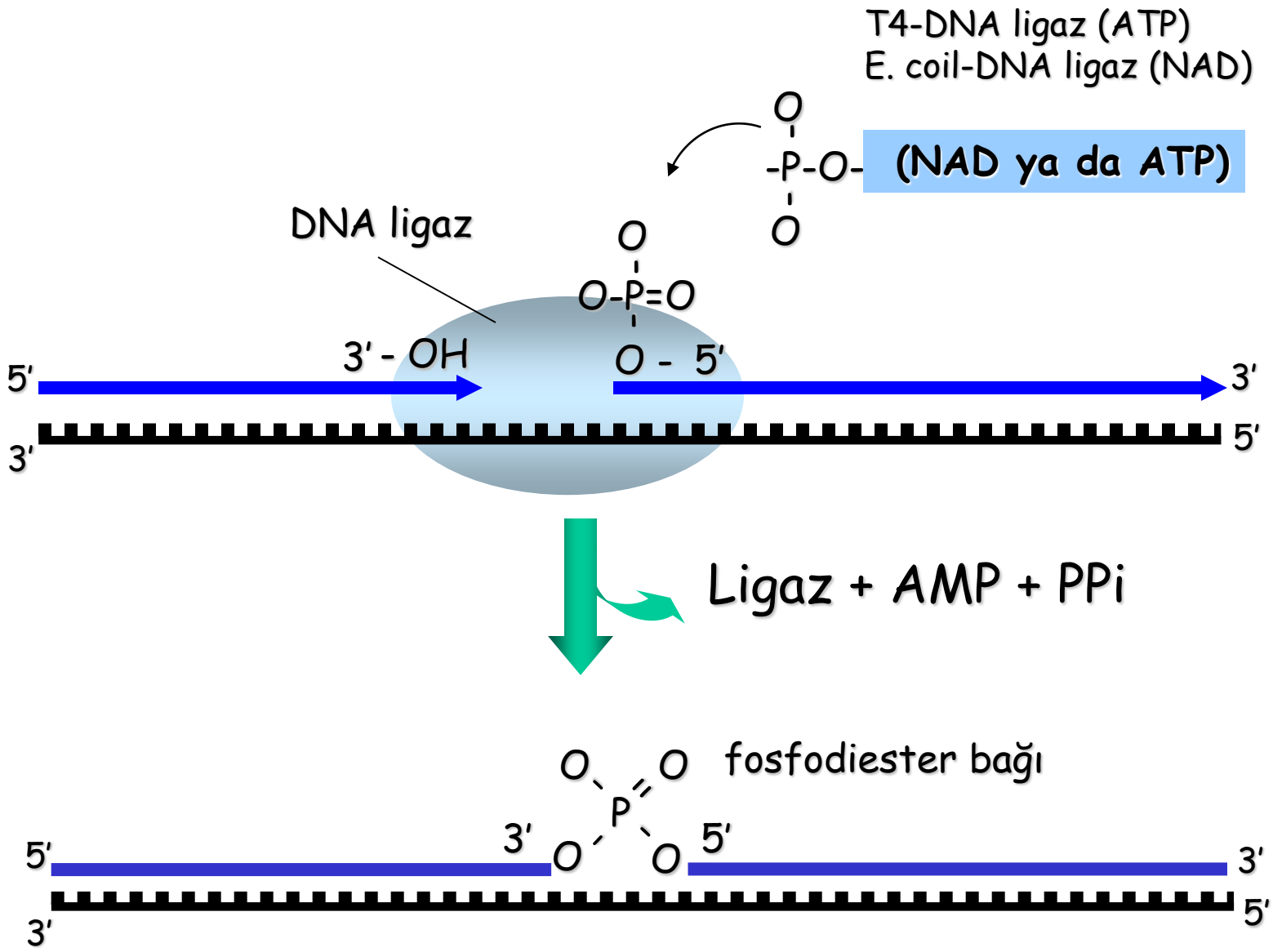
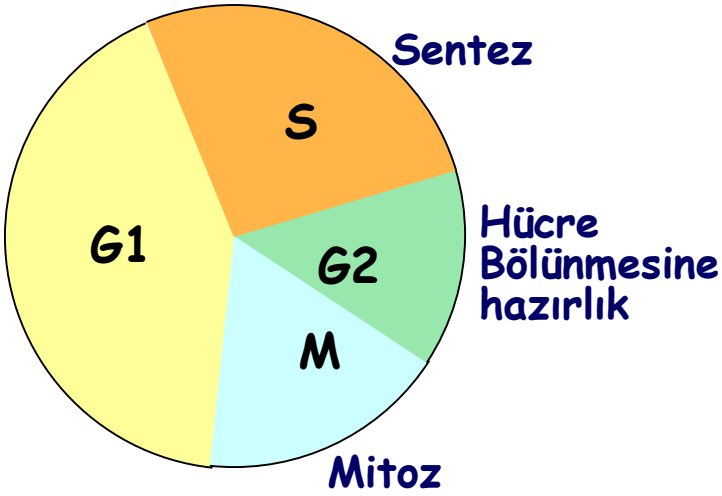
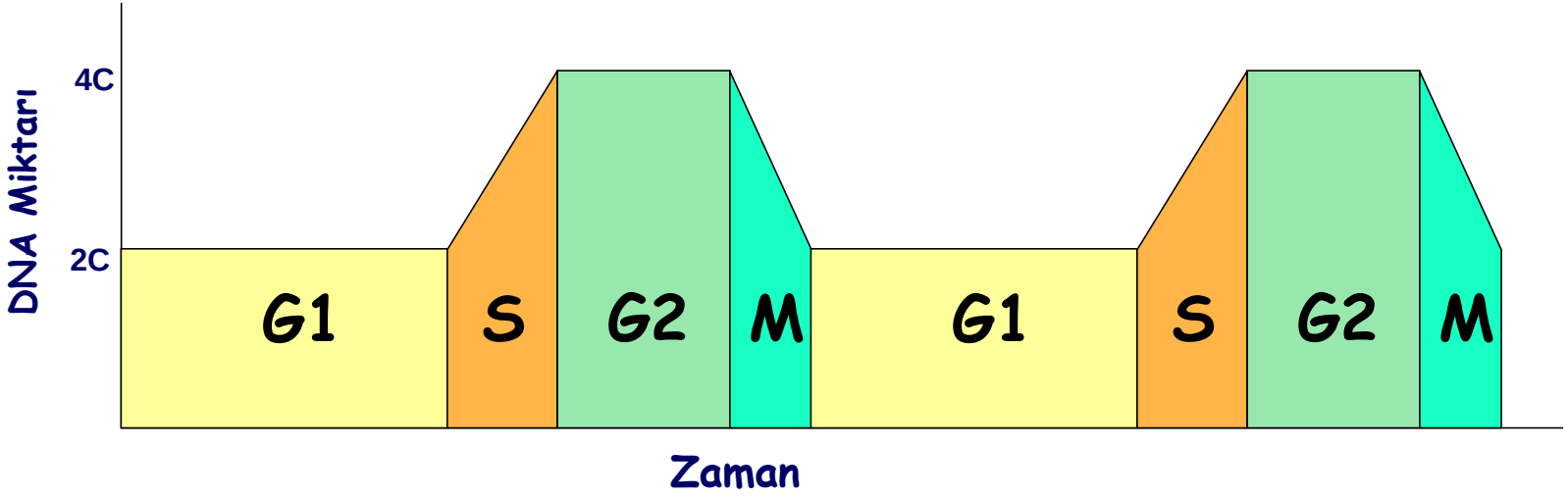
Ligasyon


T4-DNA ligaz (ATP)
E. coil-DNA ligaz (NAD)
O
-P-O-
O
(NAD ya da ATP)
DNA ligaz
O
O-P=O
3′ - OH
O - 5′
5′
3′
3′
5′
Ligaz + AMP + PPi
O
O
P
3′
O
O
5′
fosfodiester bağı
5′
3′
3′
5′

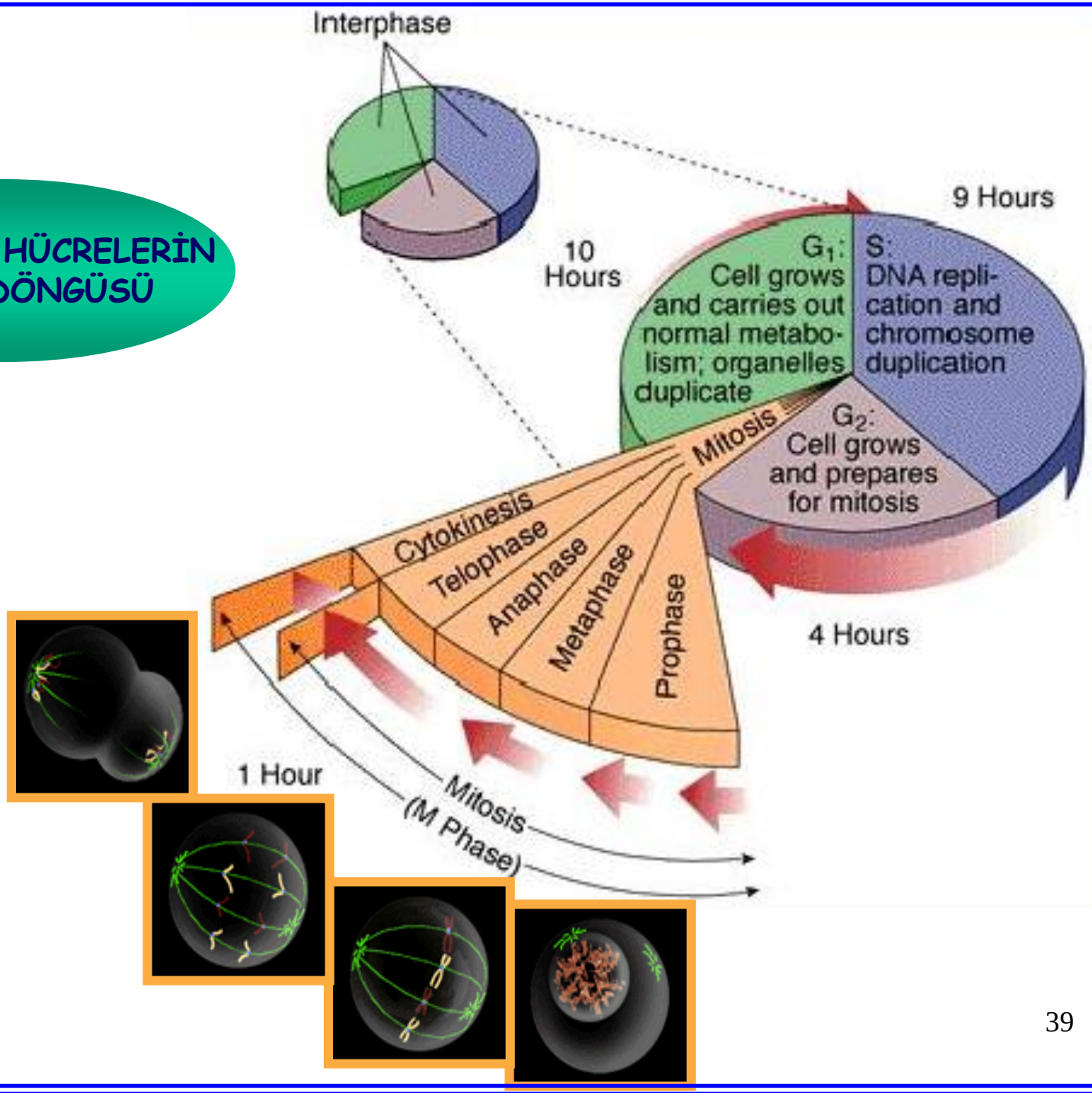
ÖKARYOTİK ORGANİZMALARDA REPLİKASYON





ÖKARYOTİK HÜCRELERİN YAŞAM DÖNGÜSÜ

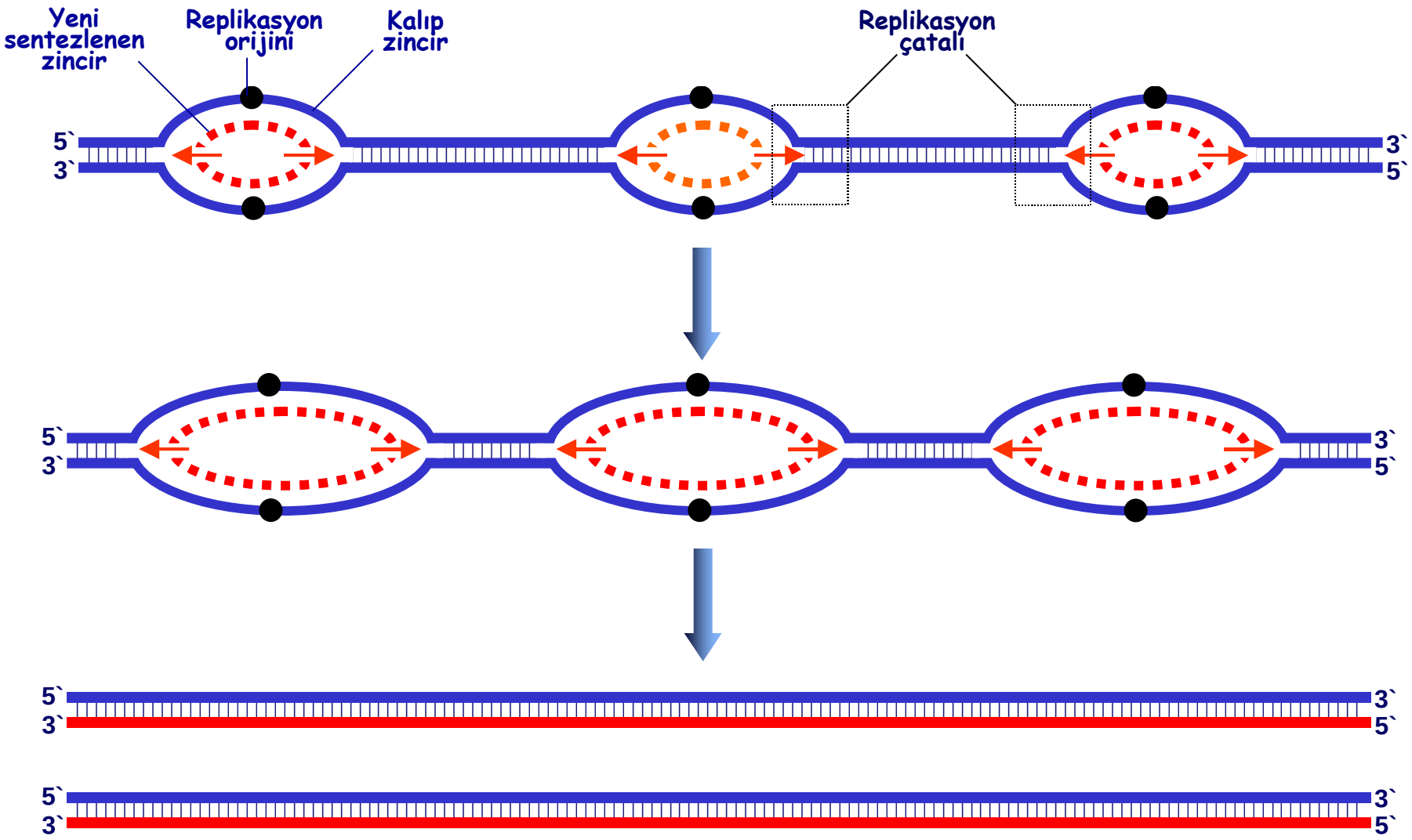
ÖKARYOTİK HÜCRELERİN YAŞAM DÖNGÜSÜ



Ökaryotik DNA polimerazlar

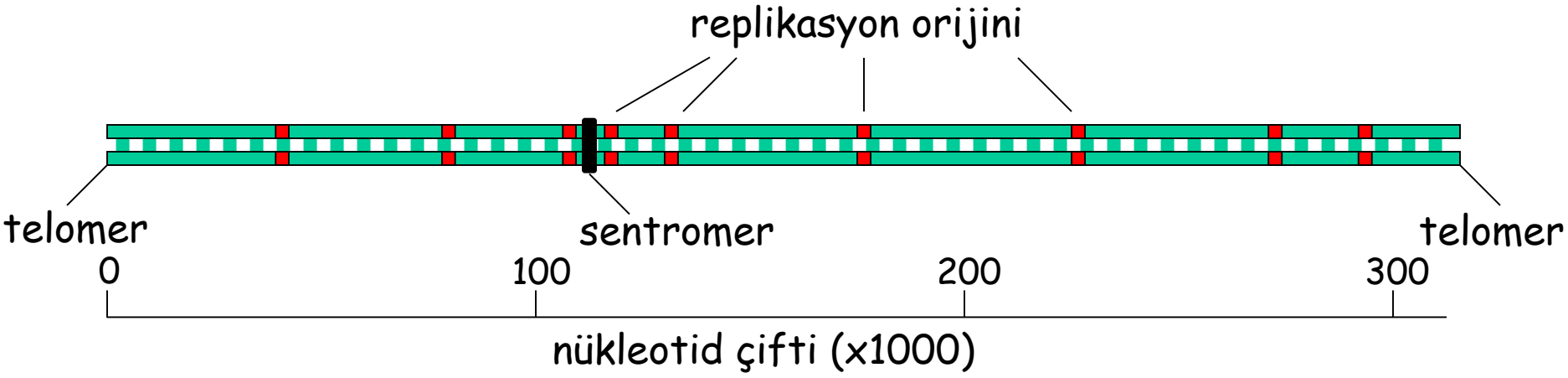
Enzim	α (I)	δ (III)	ε (II)	β	γ
Lokalizasyon	nukleus	nukleus	nukleus	nukleus	mitokondri
İşlev	Primer sentezi ve replikasyon	replikasyon	onarım	onarım	replikasyon
3′-5′ ekzo-nükleaz aktiv.	Yok	Var	Var	Yok	Var

ÖKARYOTİK ORGANİZMALARDA REPLİKASYON



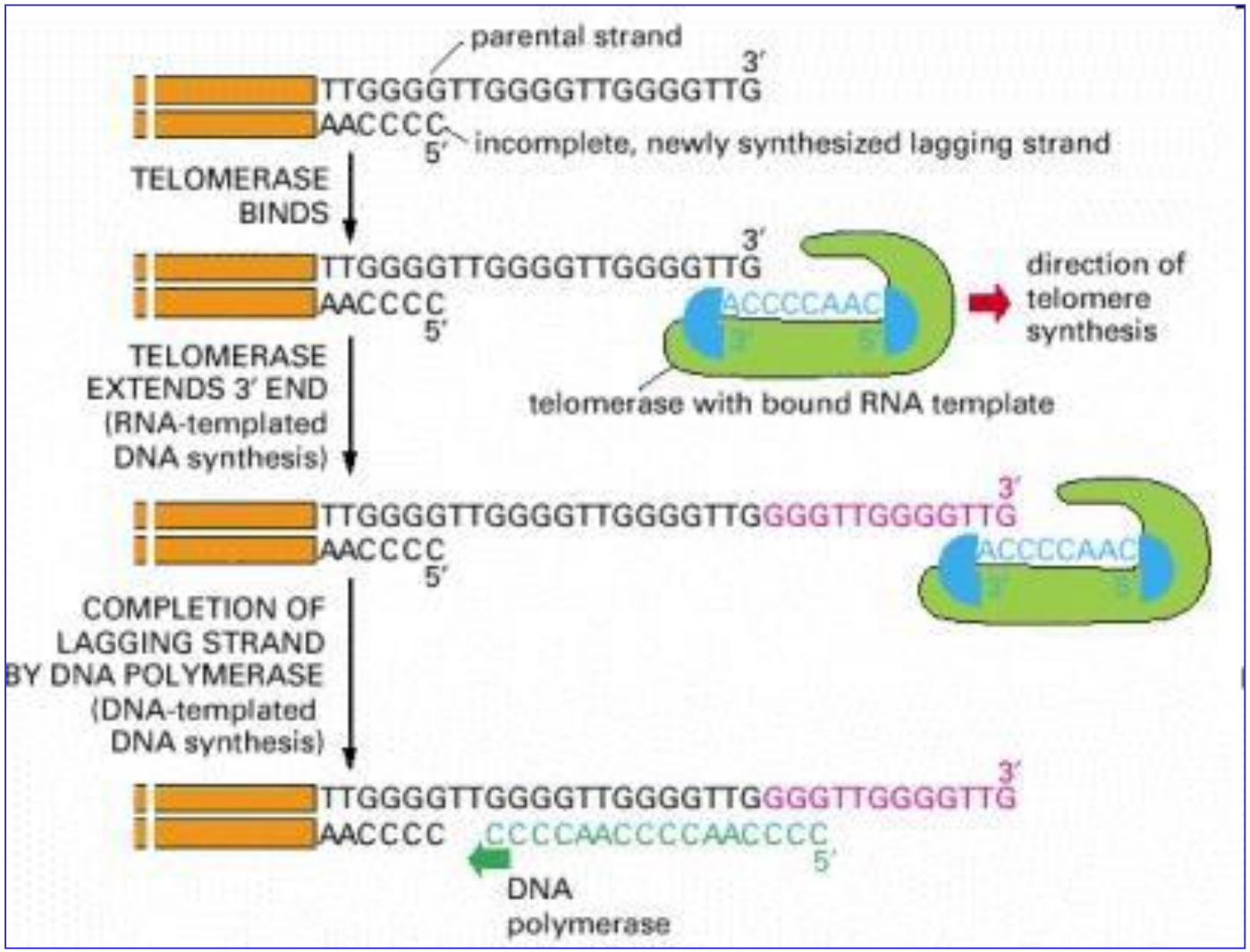
Yeni oluşan DNA molekülleri

3 nolu kromozom
(S. cerevisiae)



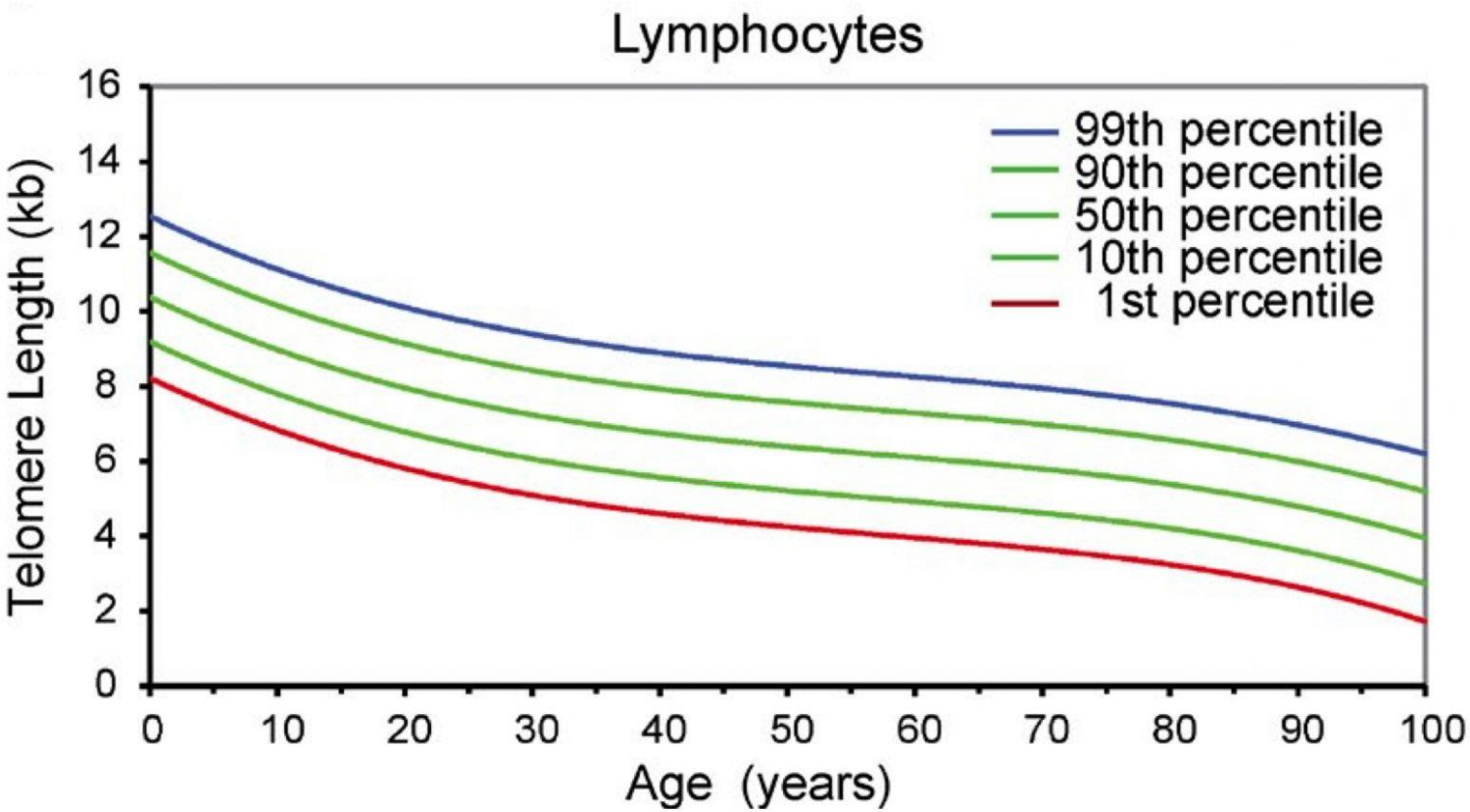
MBC, 4th edit. –Fig. 5-37

Telomer, Telomeraz,Telomer replikasyonu



İnsan Telomer Dizisi: TTAGGG

MBC, 4th edit. –Fig. 5-37



Average telomere length in relation to age.

Modified from J. Exp. Med. 2014 Vol. 211 No. 13.
PIK3R1 mutation augments PI3K signaling in new PID | Lucas et al.

Ökaryotik bir hücrenin interfaz (G1) nukleusunun şematik kesiti

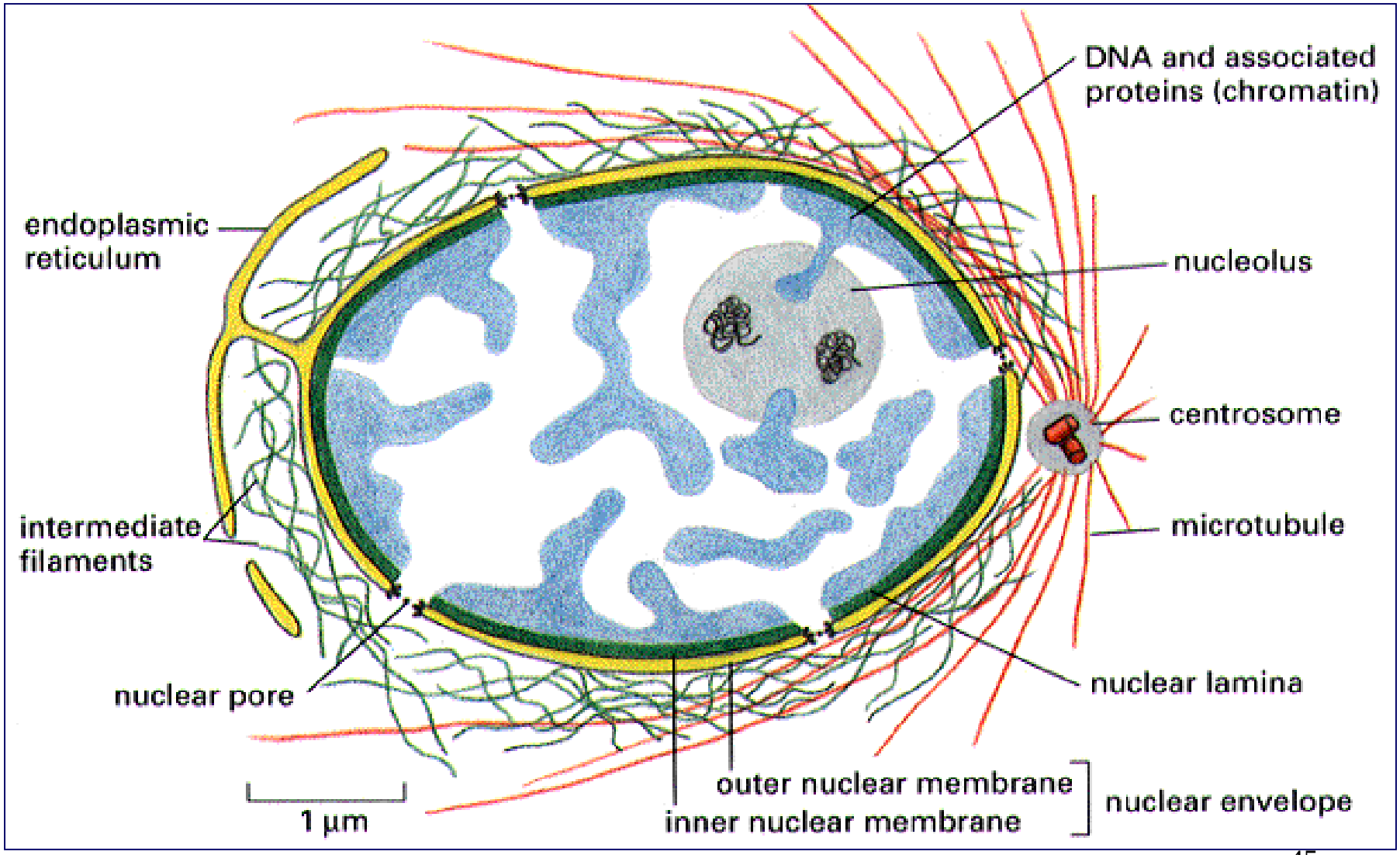
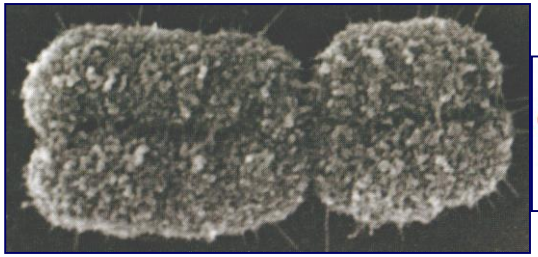
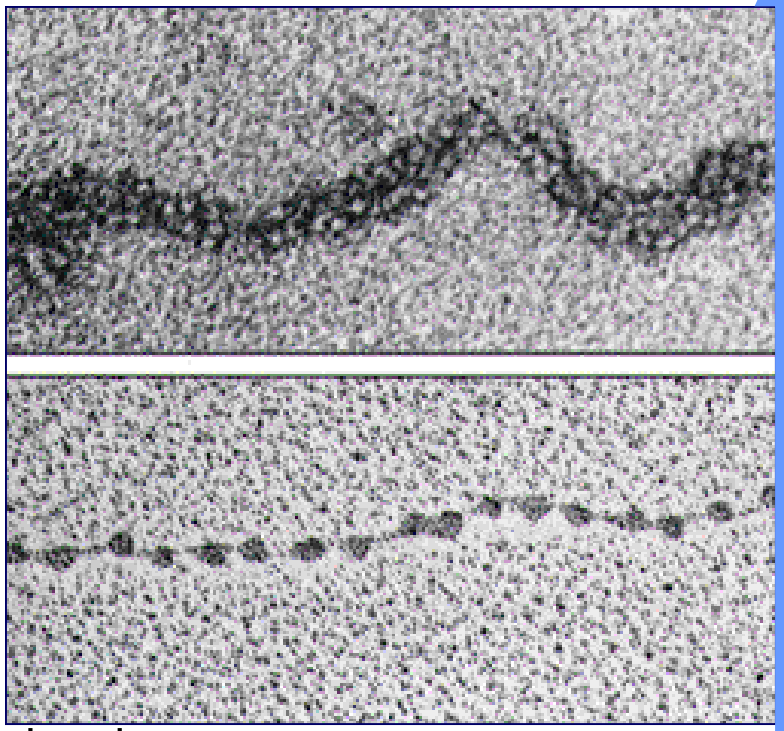


Figure 4-9A: MBC, IV.th edition

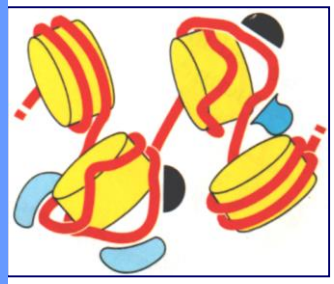
Kromatin/Kromozom Yapısı



700-1000 nm ***Kromozom***



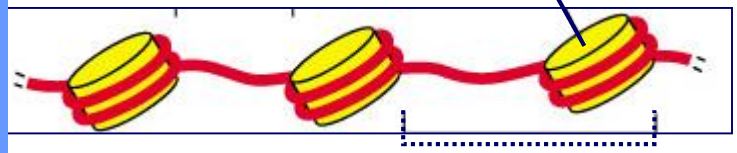
50 nm



30 nm

Kromatin

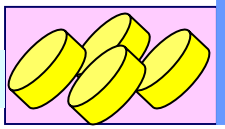
Nukleozom
DNA+2x(H2A+H2B+H3+H4)



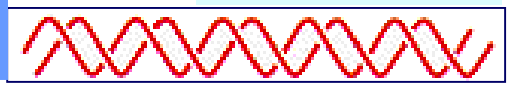
11 nm

~200 nç

HİSTONLAR



Çift Zincirli DNA



2 nm

Kromatin, Nukleozom ve Histonlar

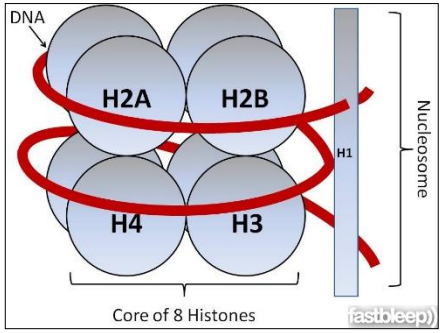


TABLE 2.1 THE HISTONES

HISTONE	TYPE	MOLECULAR WEIGHT (Da)	MOLAR RATIO
H1	Lysine-rich	23,000	1
H2A	Slightly lysine-rich	13,960	2
H2B	Slightly lysine-rich	13,744	2
H3	Arginine-rich	15,342	2
H4	Arginine-rich	11,282	2

Table 2.1 Gene Control (© Garland Science)

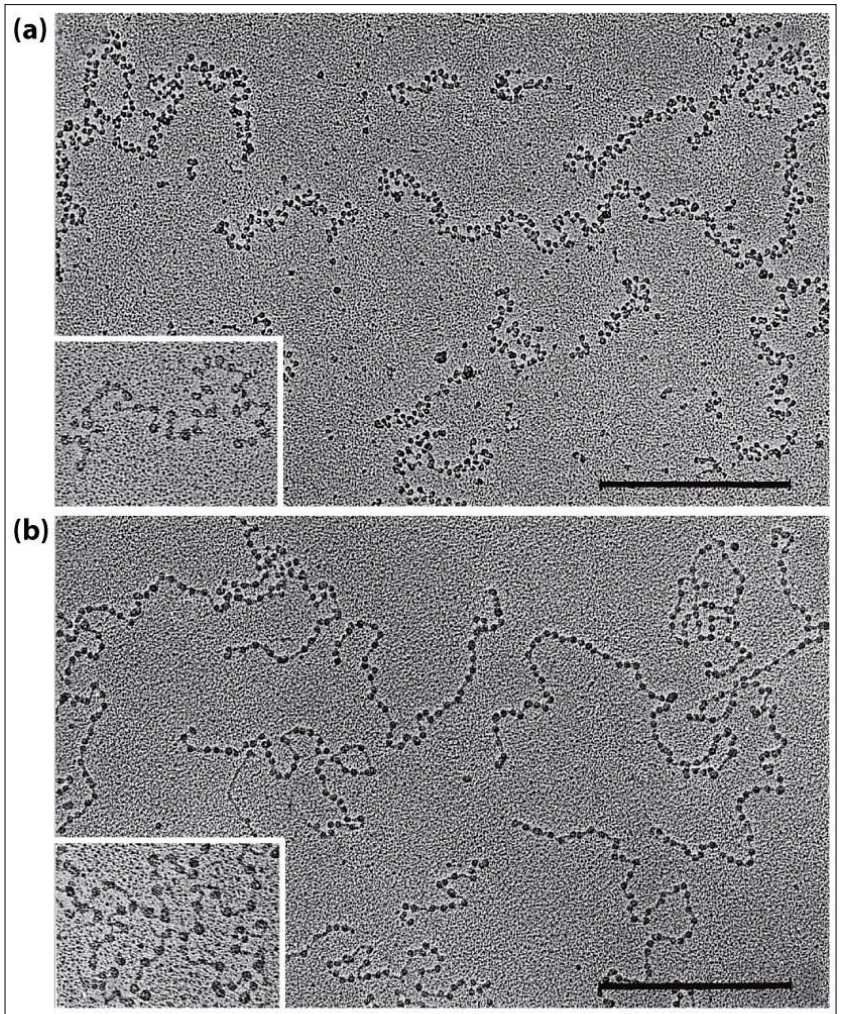


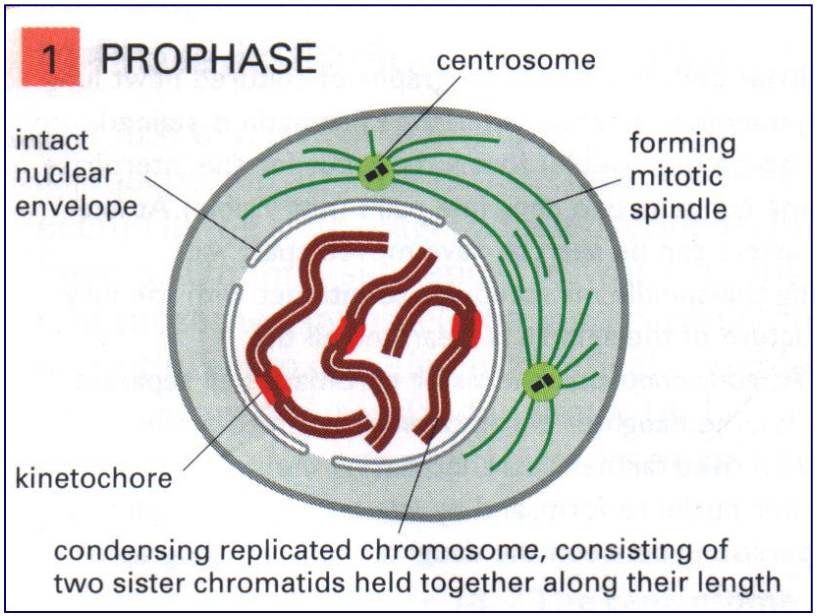
Figure 2.12 Gene Control (© Garland Science)

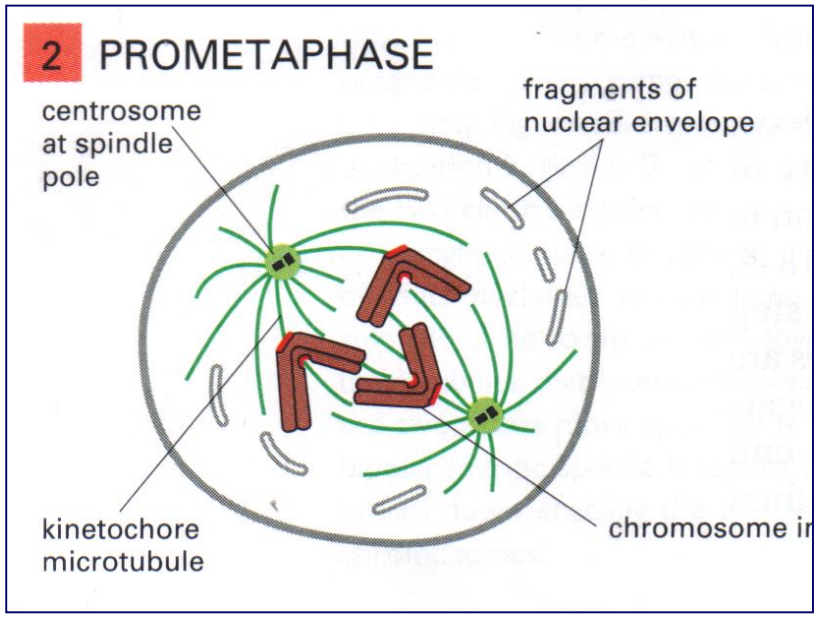
Kromatinin yapısında H1, H2A, H2B, H3 ve H4 olarak adlandırılan dört farklı histon proteini bulunun. Bu proteinlerin yapısı farlı organizma gruplarında büyük ölçüde korunmuştur. İkişer adet H2A, H2B, H3 ve H4 proteinin yaklaşık 150 bç'lik DNA bölgesi ile oluşturduğu komplek nükleozom olarak tanımlanır. Sekiz adet histon proteini histon korunu oluşturur.

A. H1 proteini varlığında
B. H1 proteini yokluğunda

Hücre Bölünmesi (MİTOZ)

Profaz

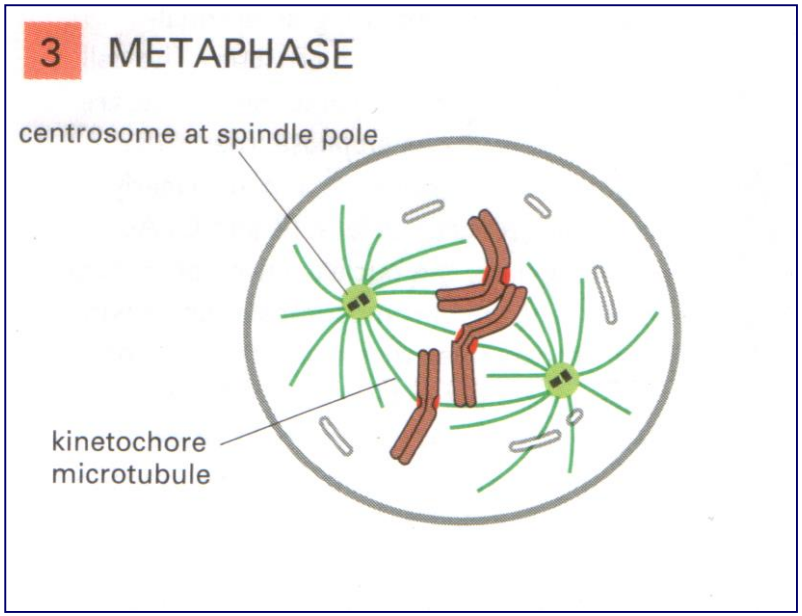




MBC, IV.th edition

- Nukleolus kaybolur, nukleus membranı bozulur
- Replike olan kromatinler kısalıp kalınlaşır, kısalma sırasında kardeş olan kromatinler bir arada tutulur
- Mitotik iğ iplikleri oluşur
- Kinetokor olgunlaşır

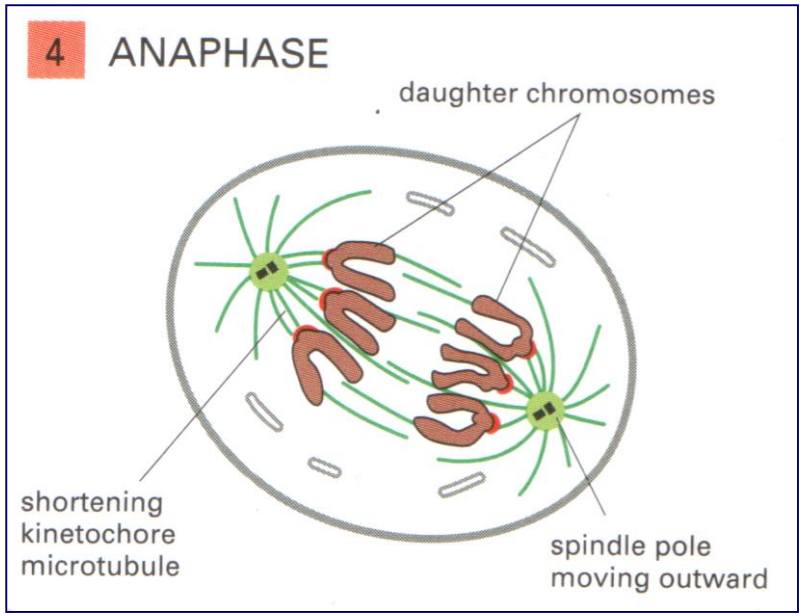
Metafaz



- ***İyice kısalmış ve kalınlaşmış olan kromozomlar hücrenin orta bölgesinde ekvator düzlemi ya da metafaz plağı adı verilen bölgede toplanır. Kromozomu oluşturan her bir kromatid sentromer bölgesi ile iğ ipliklerine bağlanır.***

MBC, IV.th edition

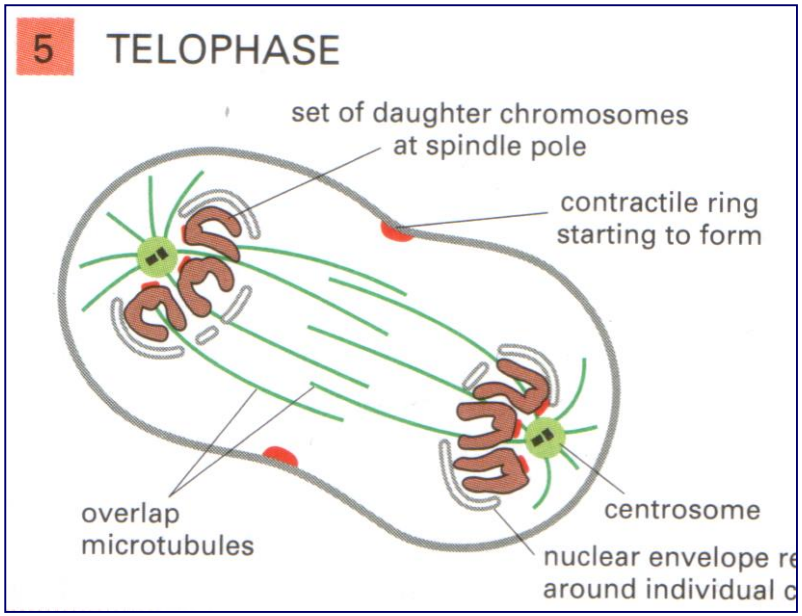
Anafaz



MBC, IV.th edition

- ***Kardeş kromatidlerin birbirlerinden ayrılıp iğ ipliklerinin yardımıyla kutuplara çekilmeye başladığı evredir. Bu evrede kromozomlar tek bir kromatidden oluşur ve yapılarında tek bir DNA molekülü taşırlar.***

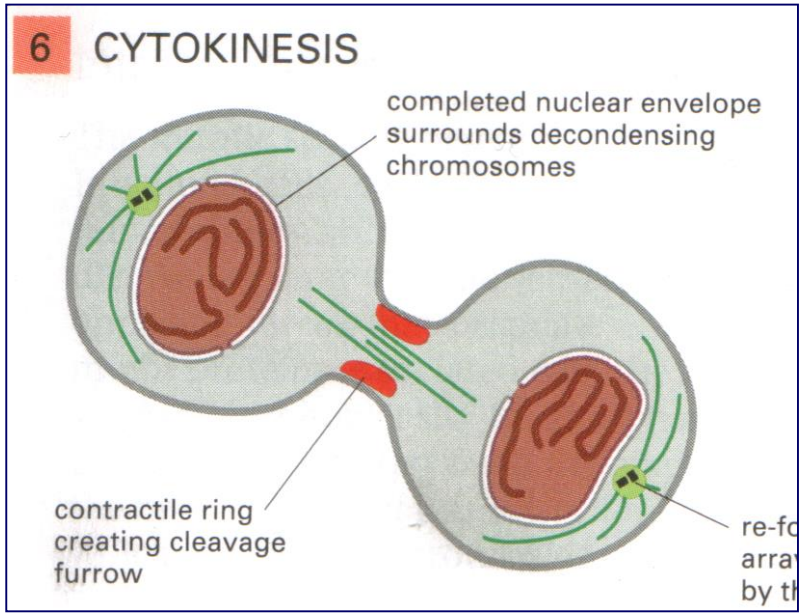
Telofaz



MBC, IV.th edition

- ***Birbirlerinden ayrılan kromozomların kutuplara erişmesi ile telofaz evresi başlar. Bu fazda profaz evresinin tersi olaylar gerçekleşir. Kromozomlar kromatin şeklini alır ve nukleus zarı yeniden oluşmaya başlar.***

Sitokinez



MBC, IV.th edition

- ***Mitoz bölünmede stoplazma bölünmesini ifade eder. Hayvan hücrelerinde boğumlanma şeklinde gerçekleşir.***

MAYOZ BÖLÜNME

•Mayoz bölünme eşey hücrelerini verecek olana ana hücrelerde meydana gelir.

•Bu hücreler ardı ardına iki bölünme geçirerek haploit genoma sahip gametleri oluştururlar

•Mayoz bölünmede hücreler, Mayoz I ve Mayoz II olarak tanımlanan iki bölünme geçirirler. Her bir bölünme Mitoz bölünmede olduğu gibi

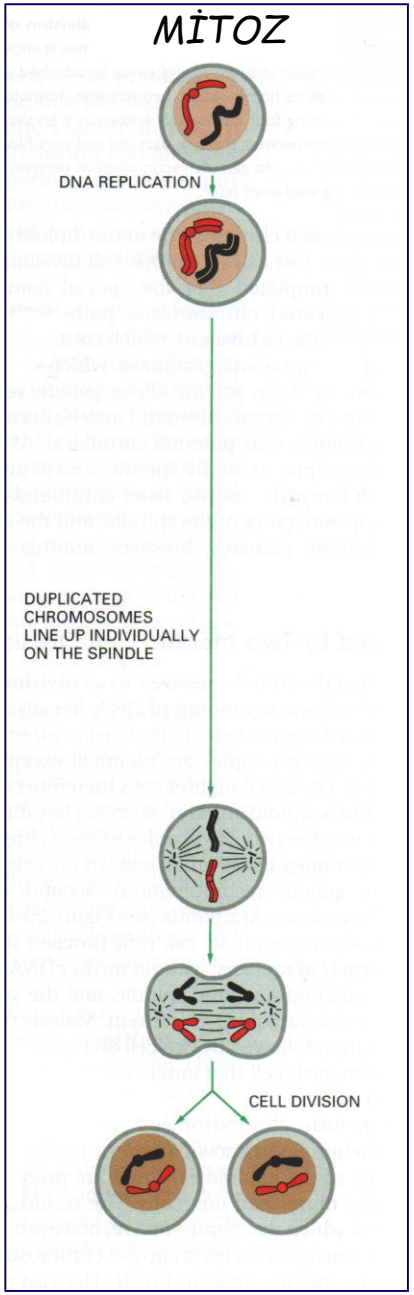
* Profaz,
* Metafaz
* Anafaz
* Telofaz

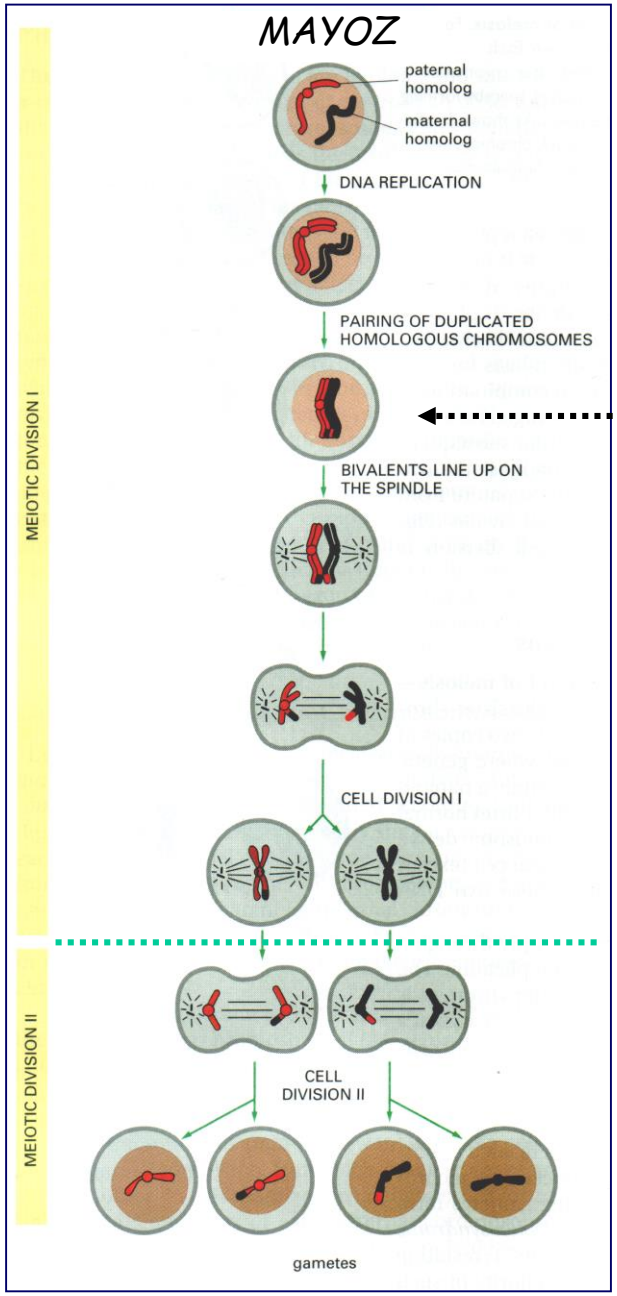
evrelerinden oluşur

I. Mayoz bölünmede profaz evresi (Profaz I)

*Leptoten – kromozom kondenzasyonu başlar
*Zigoten - homolog kromozomların eşleşmesi (bivalentlerin oluşması)
*Pakiten - krossing-over olayı gerçekleşir
*Diploten – Bivalentlerde homolog kromozomlar ayrılmaya başlar
*Diakinez - kromozomlarda maksimum kısalma ve kalınlaşma görülür

MİTOZ-MAYOZ





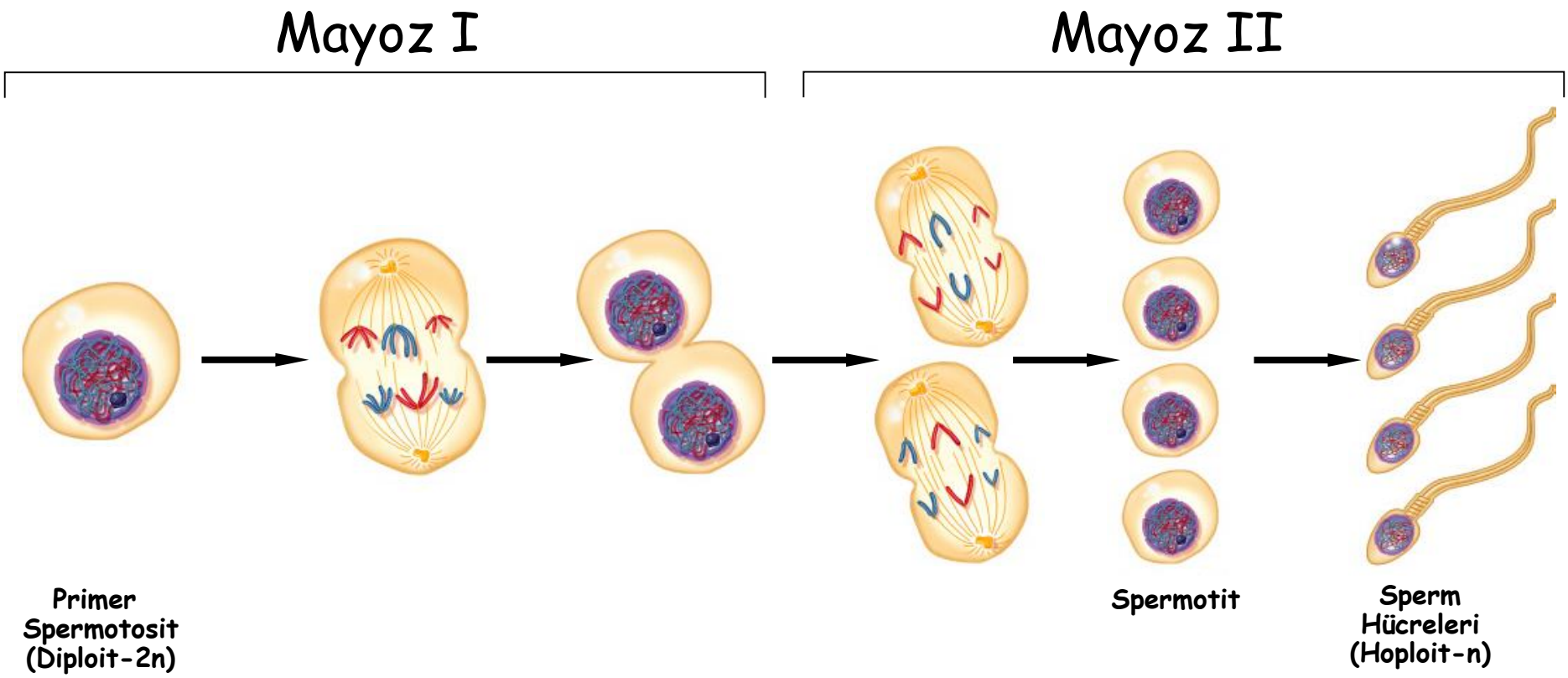
Krossingover

Mayoz aşamalar.swf

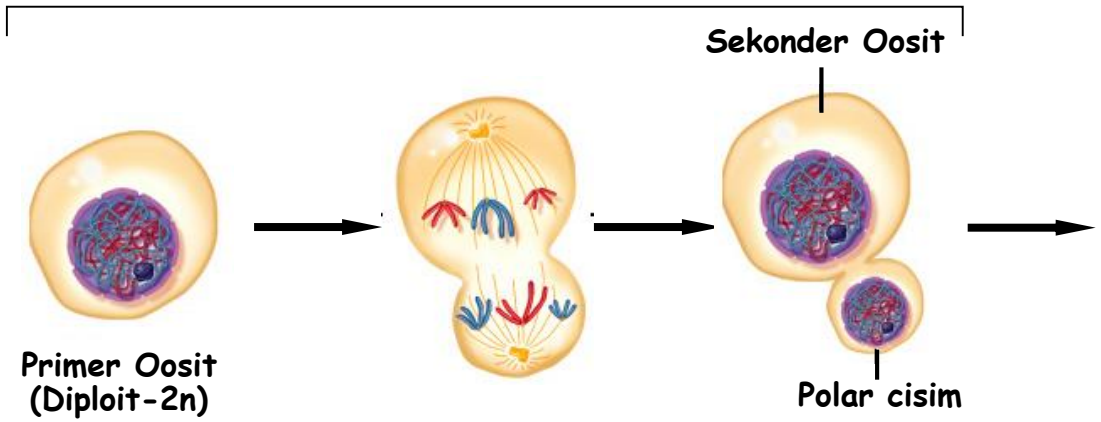
Mitoz-Mayoz.swf

Myz-Rnad.swf

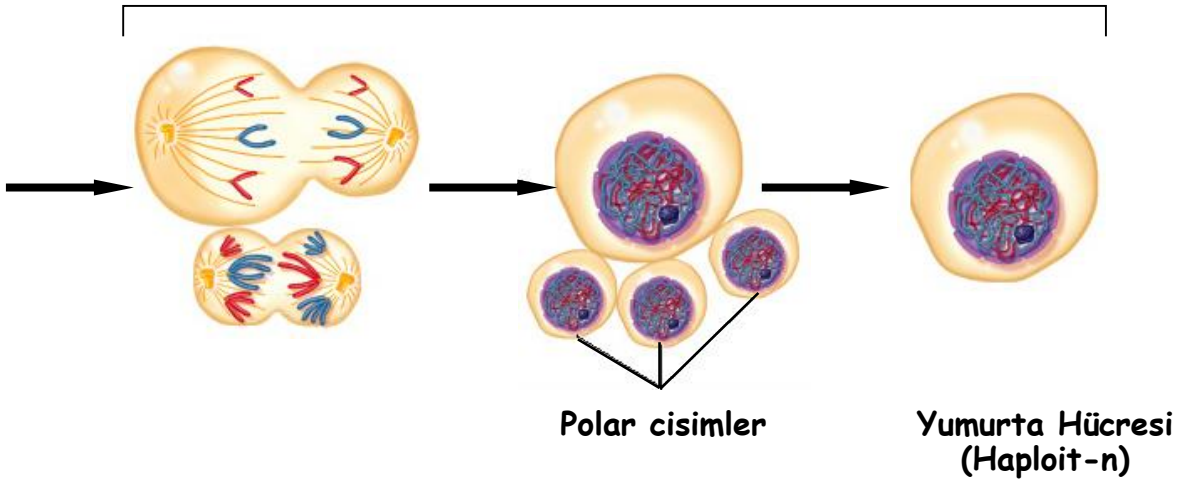
MBC, IV.th edition


Mayoz I
Mayoz II
Primer Spermotosit (Diploit-2n)
Spermotit
Sperm Hücreleri (Hoploit-n)

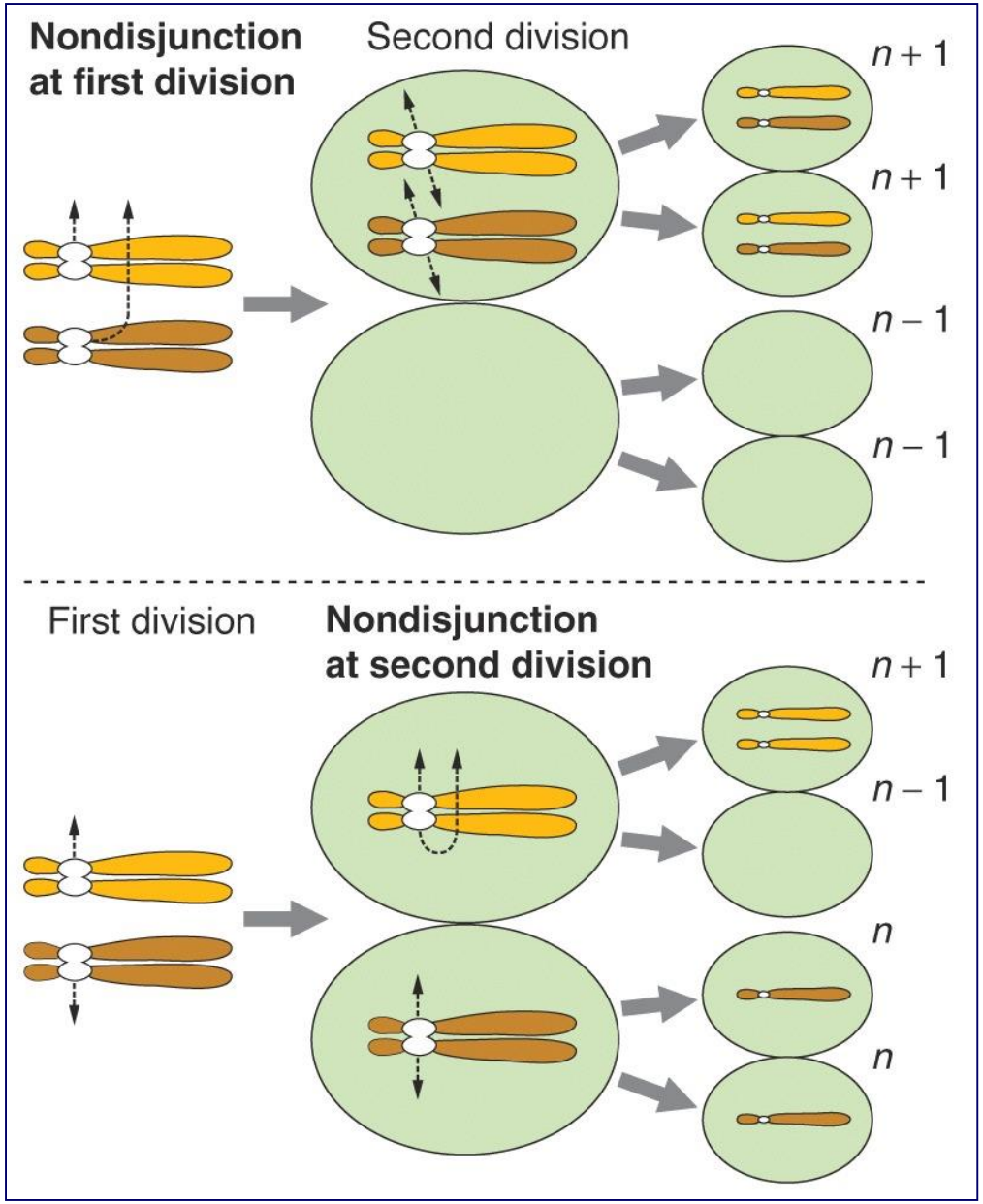
Mayoz I



Mayoz II



Non-disjunction (Homolog kromozomların ayrılmaması)



Anaphase-lag: Mayoz bölünme sırasında kromozomlardan birinin kaybolması

Bakterilerde;

Replikasyon tek bir noktadan başlamakta ve bu nedenle Bakteri genomu bir replikon birimi olarak tanımlanmaktadır.

DNA sentezi (replikasyon) hücrenin tüm yaşam döngüsü süresince devem etmektedir.

Replikasyon replikasyon orijininden her iki yöne doğru ve her iki iplikte birden gerçekleşmektedir

Replikasyon çatalında DNA sentezi ipliklerden birinde sürekli, diğerinde kesikli olarak gerçekleşmekte ve kesikli sentez sonucu oluşan fragmentler Okazaki fragmentleri olarak adlandırılmaktadır.

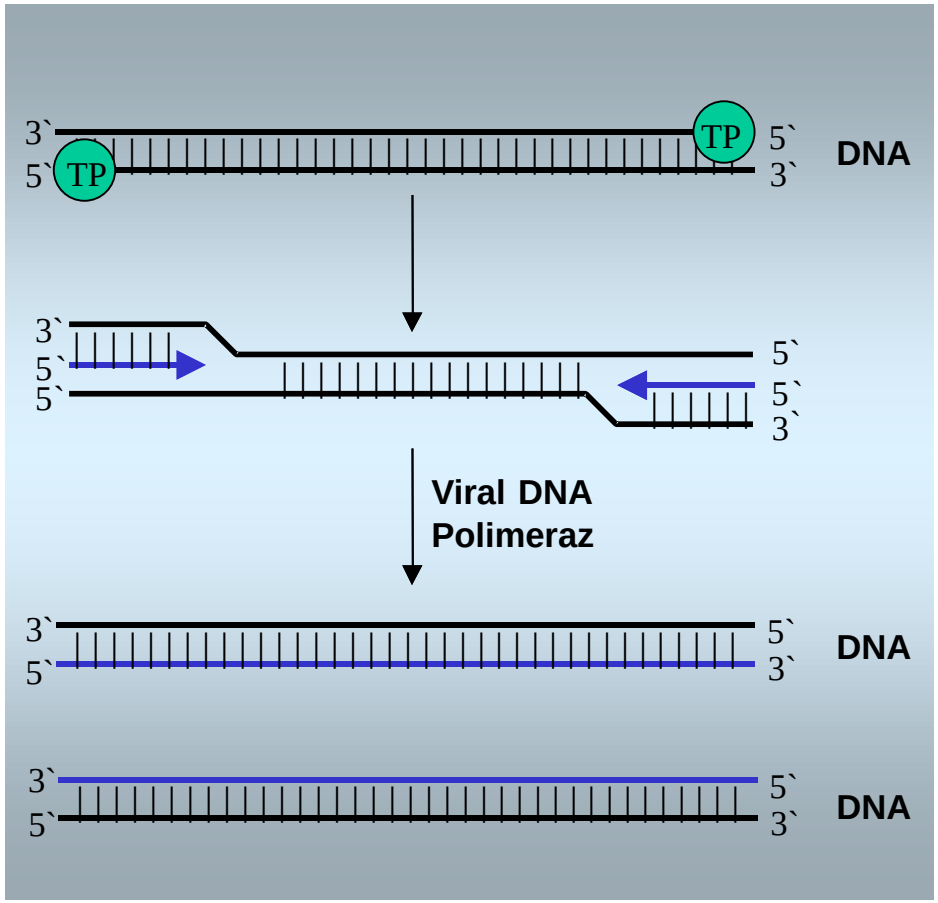
Ökaryotik Organizmalarda;

Replikasyon birden fazla noktadan başlamakta.

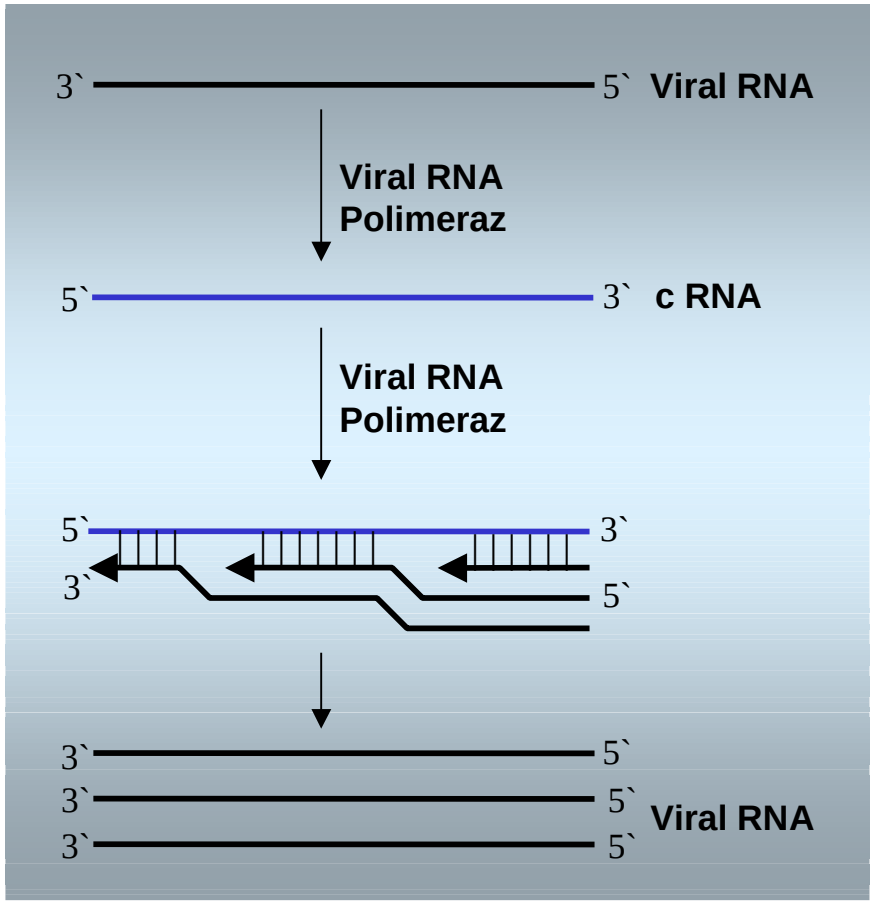
DNA sentezi (replikasyon) hücre yaşam döngüsünde sadece SENTEZ evresinde gerçekleşmektedir.

Replikasyona katılan enzim ve protein faktörler dışında replikasyon mekanizması bakterilere benzerdir.

ADENOVİRÜS`LERDE REPLİKASYON



İNFLUENZA VİRÜS`LERDE REPLİKASYON



PCR

(In Vitro DNA Replikasyonu)

POLYMERASE CHAİN REACTİON

POLİMERAZ ZİNCİR REAKSİYONU

PCR

- PCR, basit olarak nükleotid dizisi bilinen iki bölge arasındaki DNA fragmentinin in vitro koşullada enzimatik olarak çoğaltılmasıdır.

- Bir biri ardışık gerçekleştirilen reaksiyonlar sonucunda ele alınan DNA fragmentinin bol miktarda kopyası elde edilir.

DNA Sentezi İçin Temel Gereksinimler

- Kalıp DNA
- DNA polimeraz
- Mg^{++}
- Oligonükleotid Primerler
- dNTPs

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 244, No. 11, Issue of June 10, pp. 2996-3008, 1969
Printed in U.S.A.

Enzymatic Synthesis of Deoxyribonucleic Acid

XXVI. PHYSICAL AND CHEMICAL STUDIES OF A HOMOGENEOUS DEOXYRIBONUCLEIC ACID POLYMERASE*

(Received for publication, November 6, 1968)

THOMAS M. JOVIN,‡ PAUL T. ENGLUND,§ AND LEROY L. BERTSCH
From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

SUMMARY

A new purification procedure for *Escherichia coli* DNA polymerase yields about 10 mg of homogeneous enzyme per kg of cell paste, with exonuclease III as a by-product. DNA polymerase consists of a single polypeptide chain of molecular weight 1.09×10^5. This tentative conclusion is based on (*a*) an unchanged molecular weight after unfolding in solutions containing guanidine hydrochloride and mercaptoethanol, (*b*) the presence of approximately one residue of NH_2-terminal methionine, and (*c*) the presence of a single zone on polyacrylamide gel electrophoresis in a denaturing solvent at several pH values. The enzyme contains one disulfide bond and a single reactive sulfhydryl group, which can be modified by iodoacetic acid without change in either polymerase or its associated exonuclease activity. There is less than one phosphorus atom per molecule, thus minimizing the possibility of enzyme-associated nucleotide material in stoichiometric amounts.

tentative conclusion is that the enzyme consists of a single polypeptide chain. The following paper deals with the effects of chemical modifications of the polymerase molecule (2).

EXPERIMENTAL PROCEDURE

Materials

Nucleotides and Nucleic Acids—Unlabeled deoxyribonucleoside triphosphates were purchased from various suppliers and purified when necessary. α-^{32}P-dTTP was prepared as previously described (3), except that γ-^{32}P-dATP was used in the thymidine kinase reaction. ^{3}H-dTTP was purchased from Schwarz Bio-Research. Calf thymus DNA, isolated according to the procedure of Kay, Simmons, and Dounce (4), was "activated" according to the method of Aposhian and Kornberg (5). Poly d(A-T)[1] was prepared by the method of Schachman *et al.* (6), with either unlabeled substrates or ^{3}H-dTTP. Concentrations of nucleic acids are expressed as equivalents of nucleotide phos-

JOURNAL OF BACTERIOLOGY, Sept. 1976, p. 1550-1557
Vol. 127, No. 3
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Printed in U.S.A.

Deoxyribonucleic Acid Polymerase from the Extreme Thermophile *Thermus aquaticus*

ALICE CHIEN, DAVID B. EDGAR, AND JOHN M. TRELA*

Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221

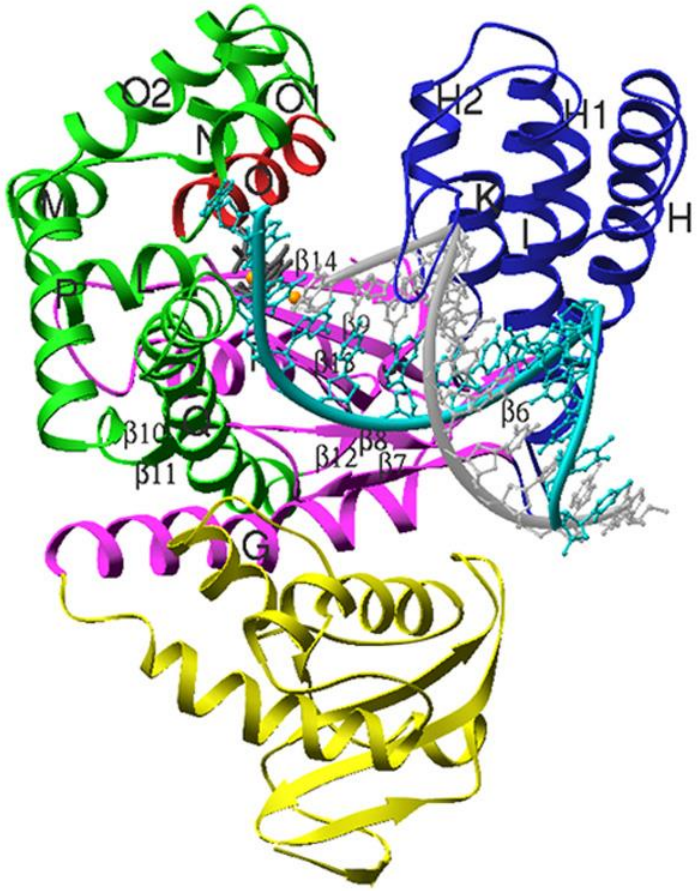
Received for publication 12 April 1976

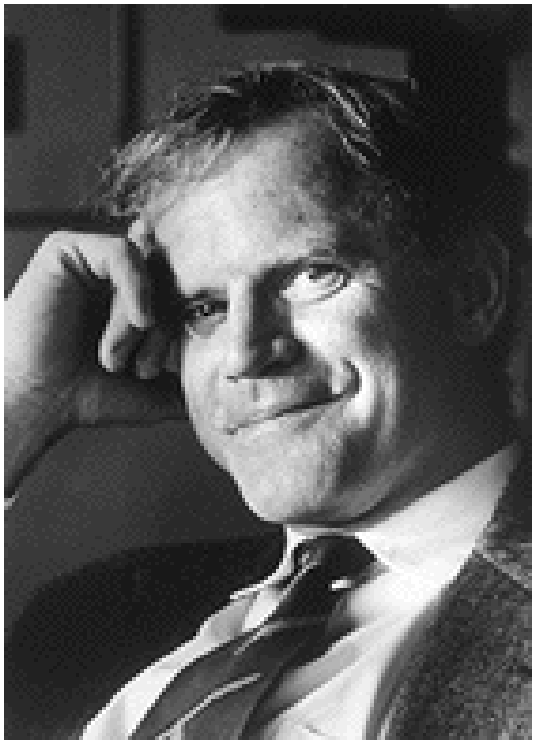
A stable deoxyribonucleic acid (DNA) polymerase (EC 2.7.7.7) with a temperature optimum of 80°C has been purified from the extreme thermophile *Thermus aquaticus*. The enzyme is free from phosphomonoesterase, phosphodiesterase, and single-stranded exonuclease activities. Maximal activity of the enzyme requires all four deoxyribonucleotides and activated calf thymus DNA. An absolute requirement for divalent cation cofactor was satisfied by Mg^{2+} or to a lesser extent by Mn^{2+}. Monovalent cations at concentrations as high as 0.1 M did not show a significant inhibitory effect. The pH optimum was 8.0 in tris(hydroxymethyl)aminomethane-hydrochloride buffer. The molecular weight of the enzyme was estimated by sucrose gradient centrifugation and gel filtrations on Sephadex G-100 to be approximately 63,000 to 68,000. The elevated temperature requirement, small size, and lack of nuclease activity distinguish this polymerase from the DNA polymerases of *Escherichia coli*.

PCR

Taq DNA polimeraz

Thermus aquaticus





- *1983-1985*

  Dr.Kary B.Mullis

- *1993 Nobel Kimya Ödülü*

: Science. 1988 Jan 29;239(4839):487-91. Related Articles, Links

Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA.

Cetus Corporation, Department of Human Genetics, Emeryville, CA 94608.

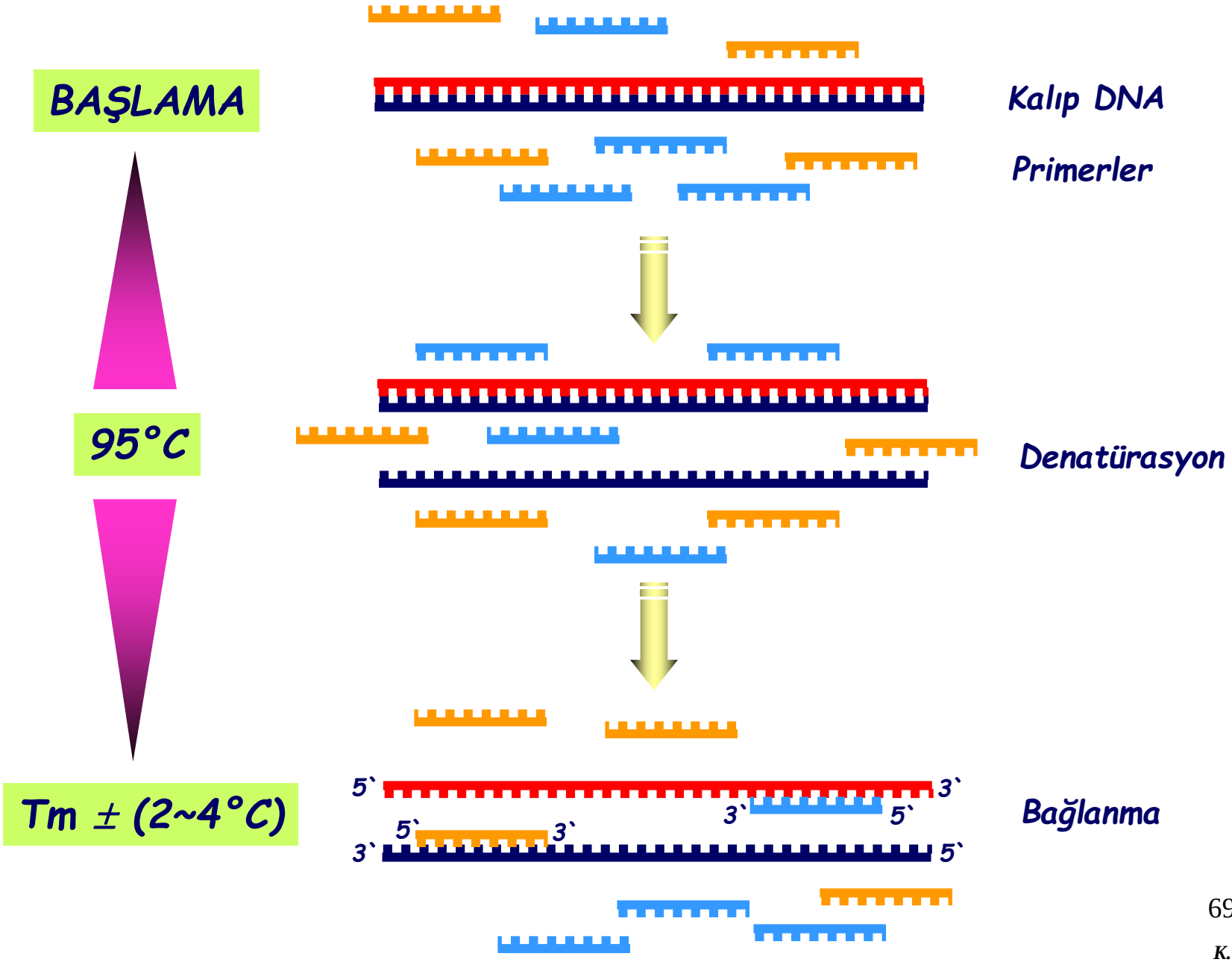
A thermostable DNA polymerase was used in an in vitro DNA amplification procedure, the polymerase chain reaction. The enzyme, isolated from Thermus aquaticus, greatly simplifies the procedure and, by enabling the amplification reaction to be performed at higher temperatures, significantly improves the specificity, yield, sensitivity, and length of products that can be amplified. Single-copy genomic sequences were amplified by a factor of more than 10 million with very high specificity, and DNA segments up to 2000 base pairs were readily amplified. In addition, the method was used to amplify and detect a target DNA molecule present only once in a sample of 10(5) cells.

PMID: 2448875 [PubMed - indexed for MEDLINE]

PCR

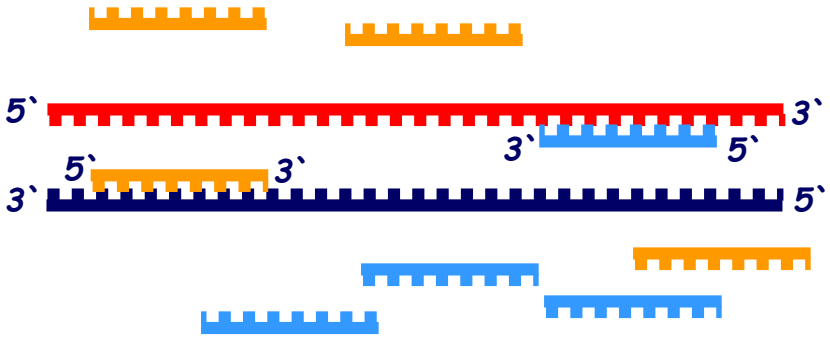
- DENATÜRASYON
- BAĞLANMA (ANNELING)
- SENTEZ

PCR


BAŞLAMA
Kalıp DNA
Primerler
95°C
Denatürasyon
Tm ± (2~4°C)
5`
3`
3`
5`
5`
3`
3`
5`
Bağlanma

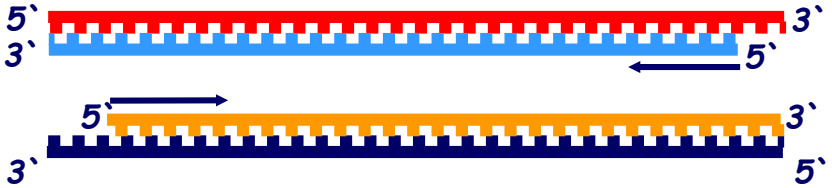
PCR

Tm ± (2~4°C)



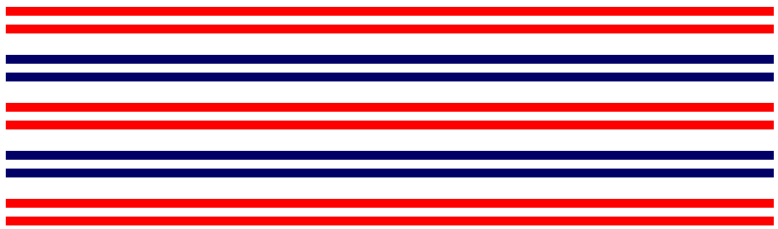
Bağlanma

72-74°C



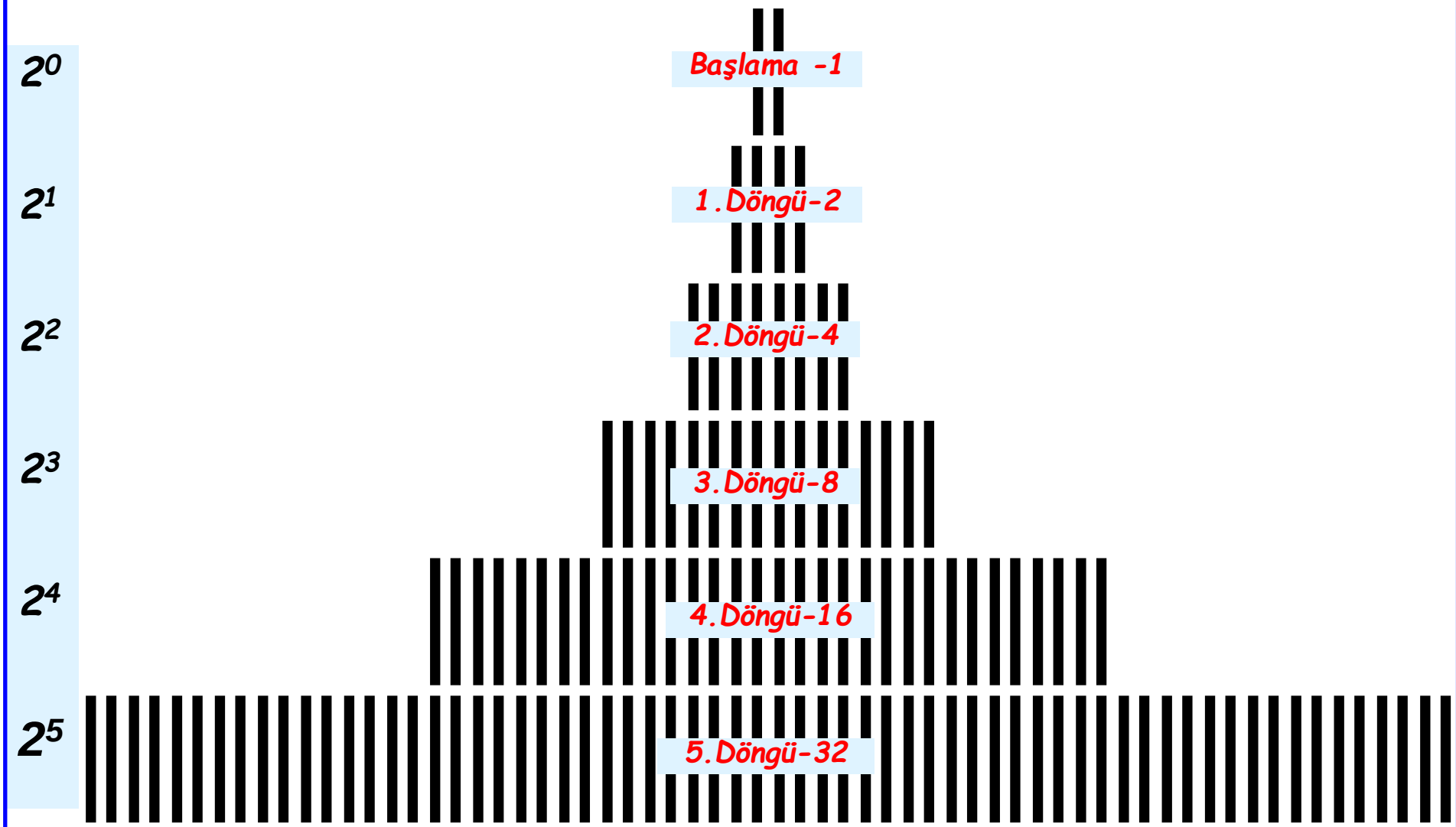
Sentez

25-35 Döngü



PCR Ürünleri

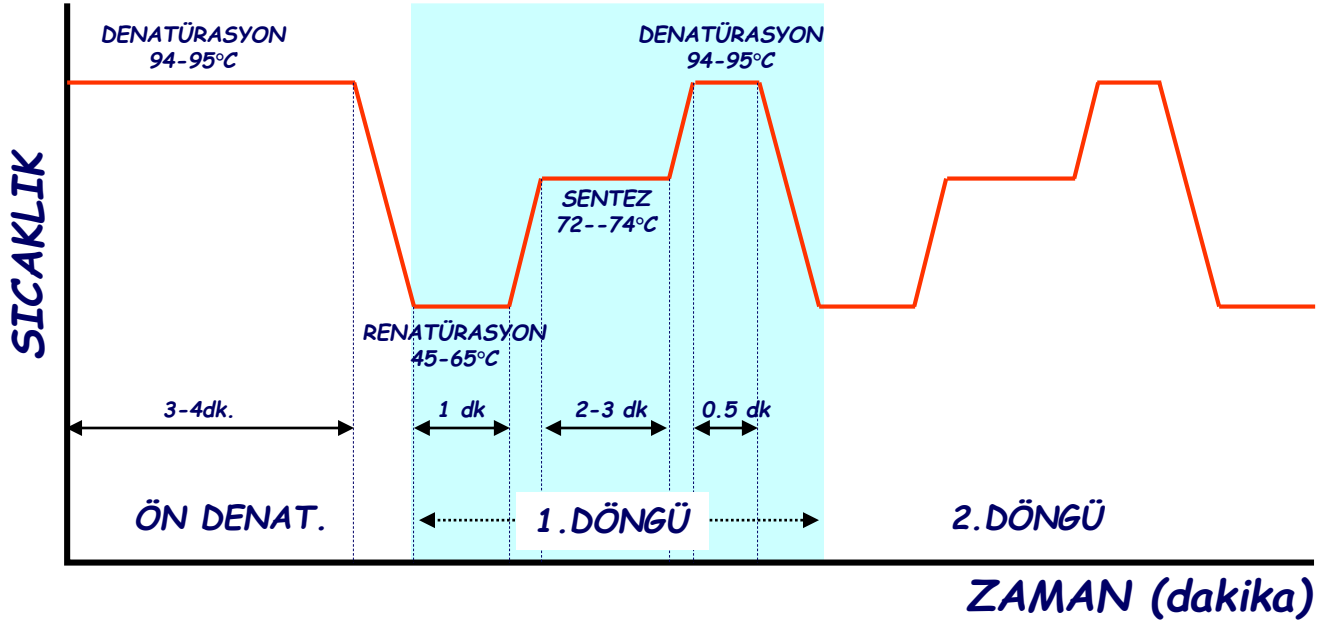
PCR



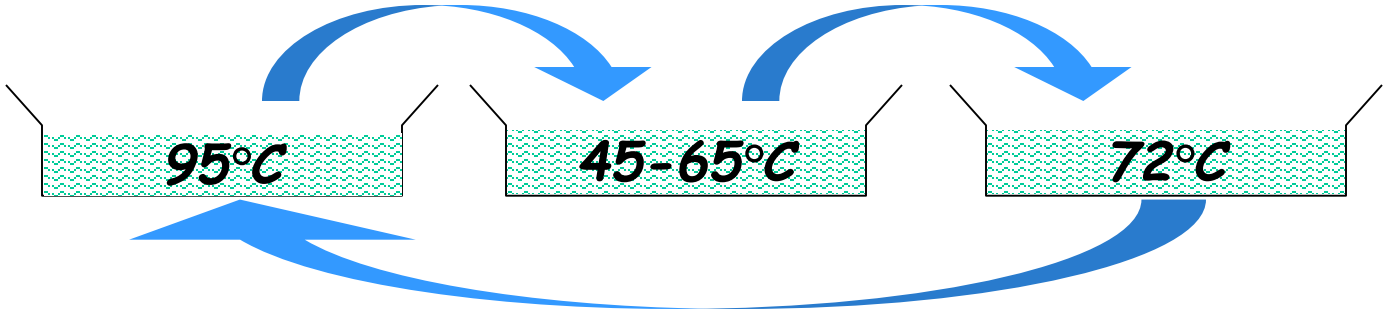
PCR sonrası elde edilecek DNA kopya sayısı [$(2^n-2n).N$] formülünden hesaplanabilir.
n - PCR döngü sayısı, **N** - orijinal kalıbın kopya sayısı

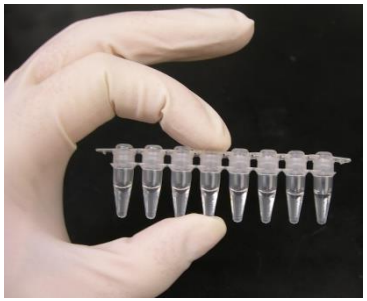
Pcr.swf

PCR-EVRELERİ



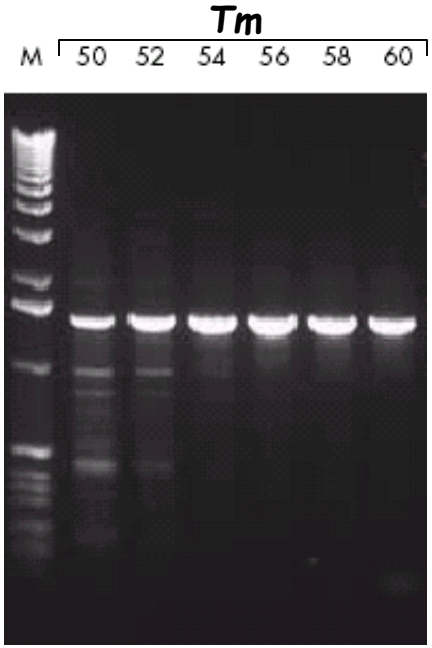
Thermal Cyclers

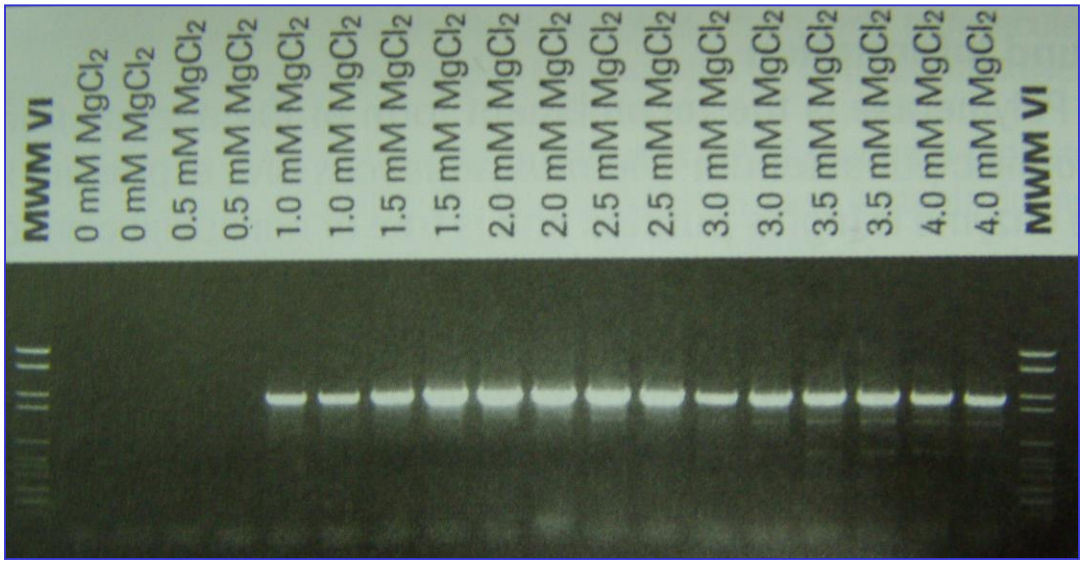




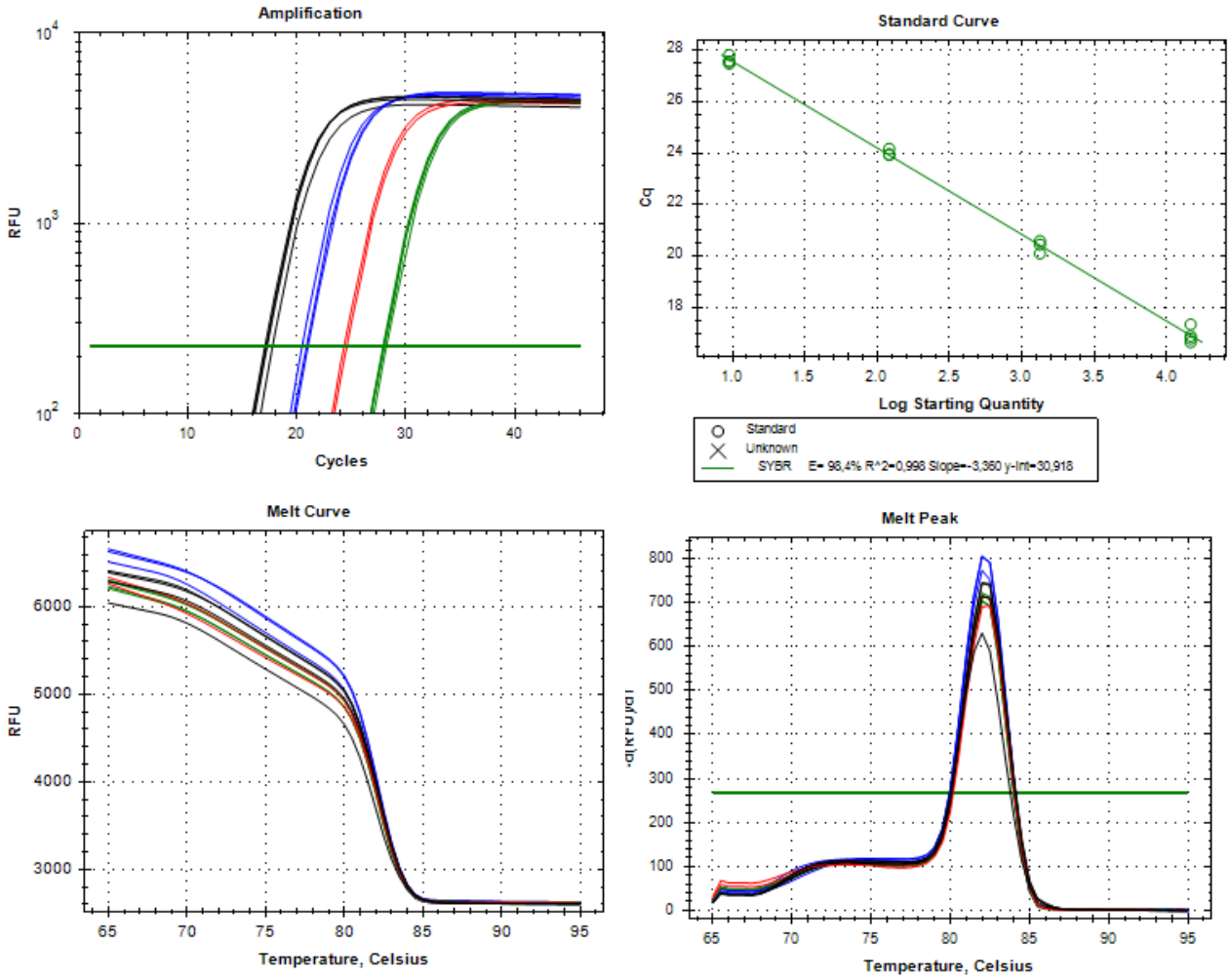
PCR

- *Primer Bağlanma Temperatürü (Annealing Temperature)*
- *Mg^{2+} Konsantrasyonu*





Gerçek Zamanlı (real time) PCR
Kantitatif PCR (qPCR)


Amplification
RFU
Cycles
Standard Curve
Cq
Log Starting Quantity
Standard
Unknown
SYBR E= 96,4% R^2=0,998 Slope=-3,360 y-Int=30,918
Melt Curve
RFU
Temperature, Celsius
Melt Peak
Temperature, Celsius

PCR

Uygulamalar

- Tanı (mutasyonlar, infektif ajanlar)
- HLA ya da kan grubu tiplendirmeleri
- Gen klonlama ve seleksyon çalışmaları
- Dizi analizleri
- In vitro mutagenez
- Doku ya da hücrelerde mRNA miktar tayinleri

Yaşam (canlı) nedir?

Doğada bilginin aktarım şeklidir.

K.Turan (2021)

Bu mekanizmanın temel araçları birbirleri ile etkileşim halinde çalışan nükleik asitler (DNA/RNA) ve proteinlerdir. (Replikasyon/Gen ekspresyonu)