DNA Replication

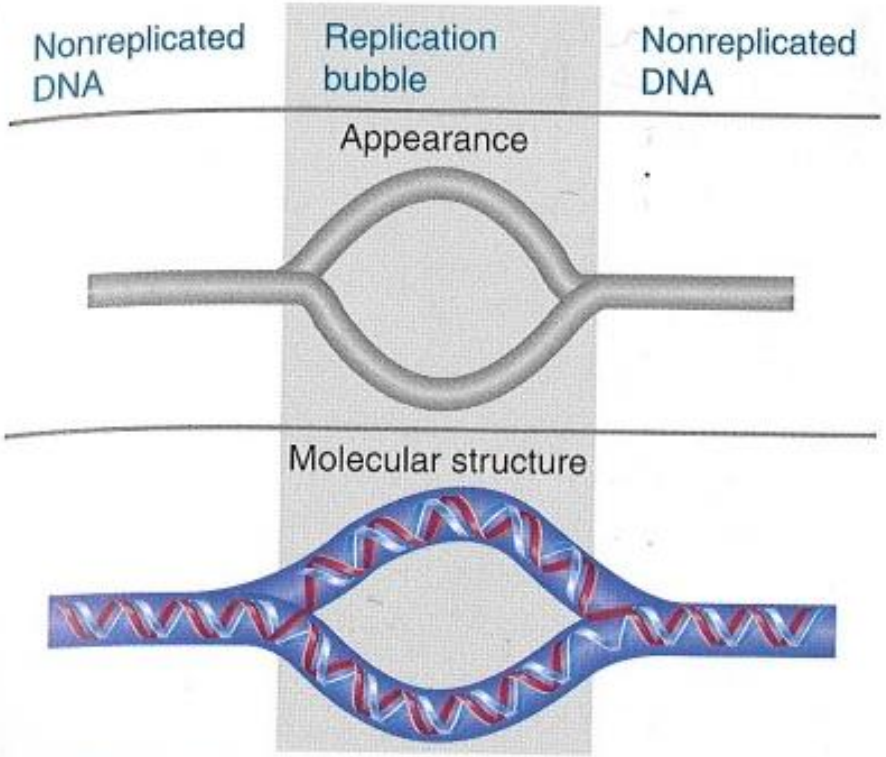


FIGURE 12.2 Replicated DNA is seen as a replication bubble flanked by nonreplicated DNA.

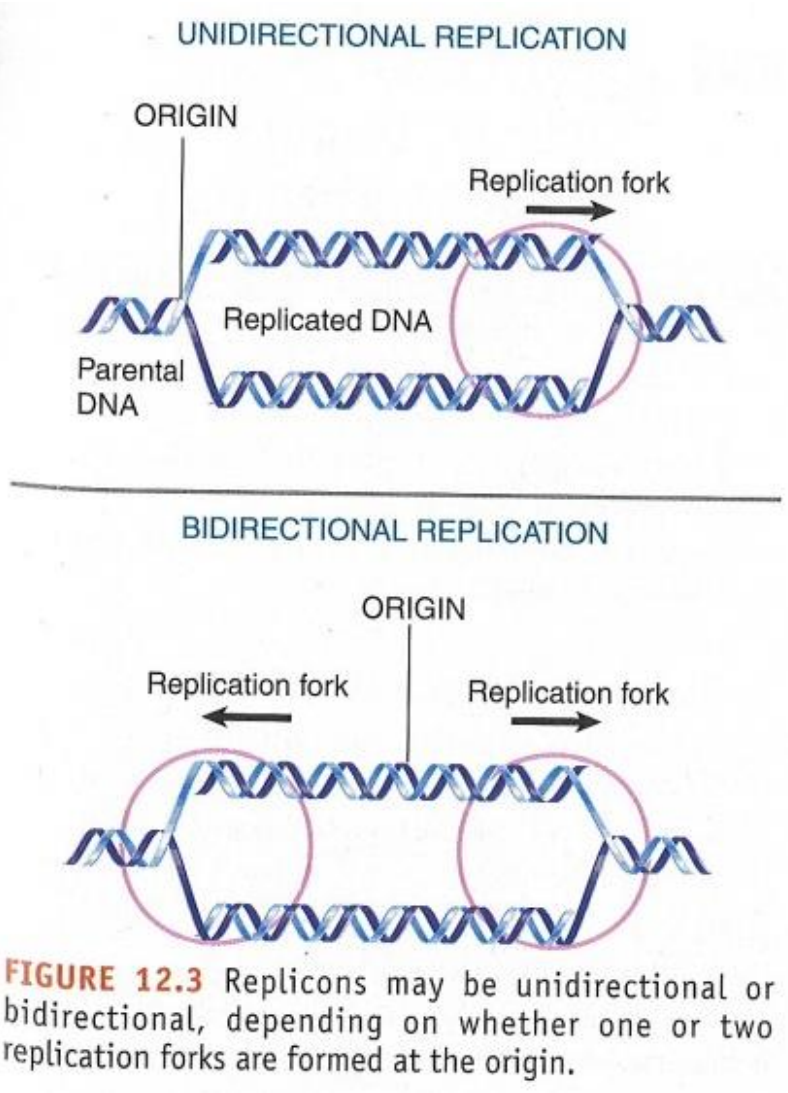


FIGURE 12.3 Replicons may be unidirectional or bidirectional, depending on whether one or two replication forks are formed at the origin.

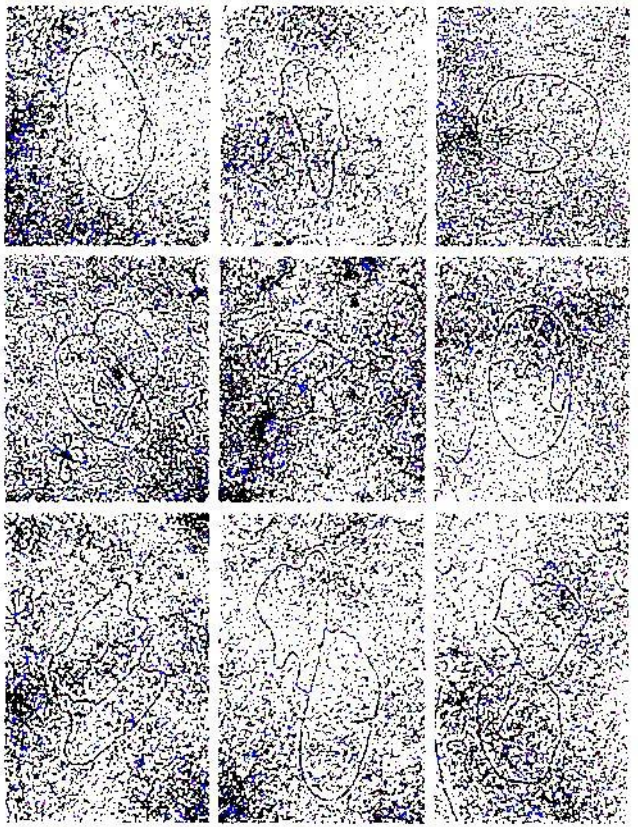
Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Replicating θ structure

Appearance of θ structure by electron microscopy

FIGURE 12.4 A replication bubble forms a θ structure in circular DNA.

Figure 12.4 The replication eye becomes larger as the replication forks proceed along the replicon. Note that the 'eye' becomes larger than the nonreplicated segment. The two sides of the eye can be defined because they are both the same length. Photograph kindly provided by Bernard Hirt.



Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

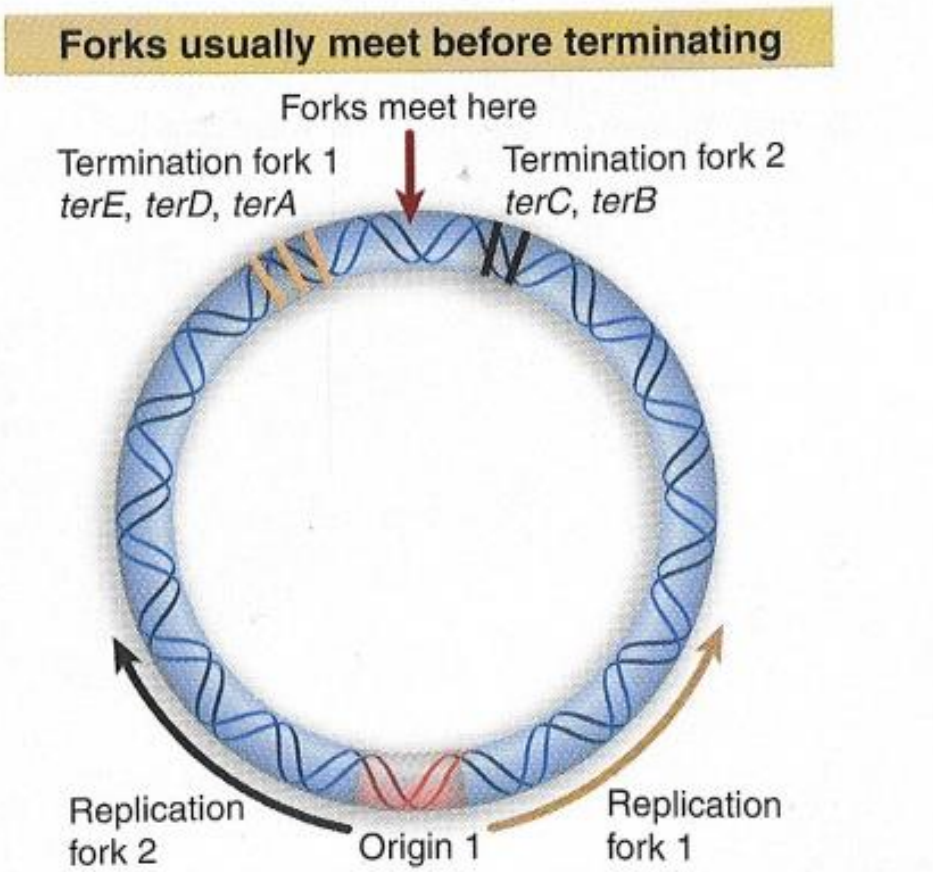


Figure 15.6 Replication termini in *E. coli* are located beyond the point at which the replication forks actually meet.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

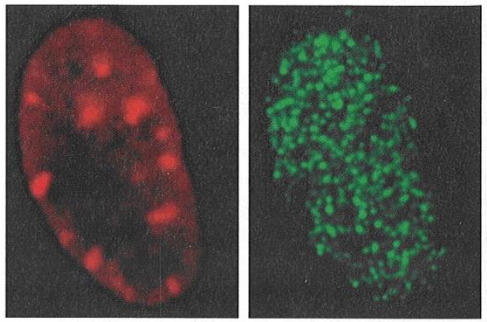


FIGURE 12.11 Replication forks are organized into foci in the nucleus. Cells were labeled with BrdU. The left panel was stained with propidium iodide to identify bulk DNA. The right panel was stained using an antibody to BrdU to identify replicating DNA. Photos of Anthony D. Mills and Ron Laskey, Hutchinson/MRC Research Center, University of Cambridge.

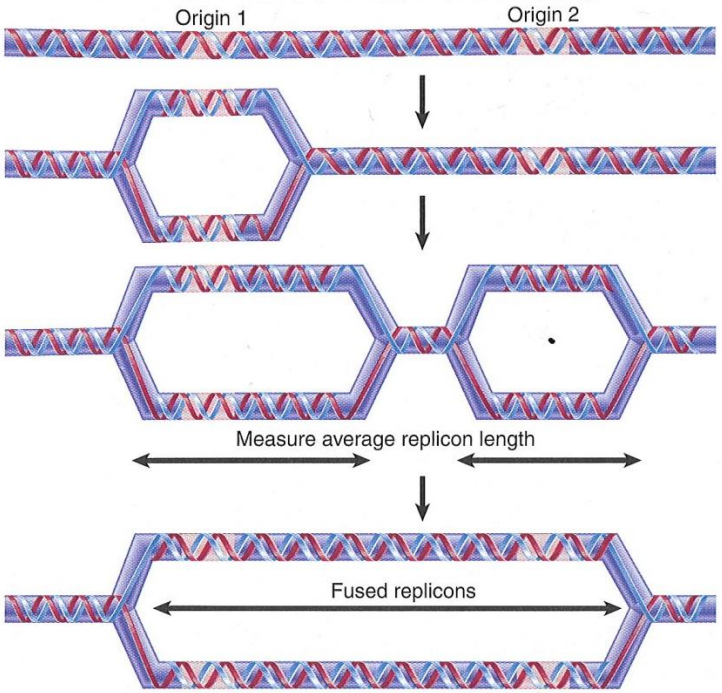


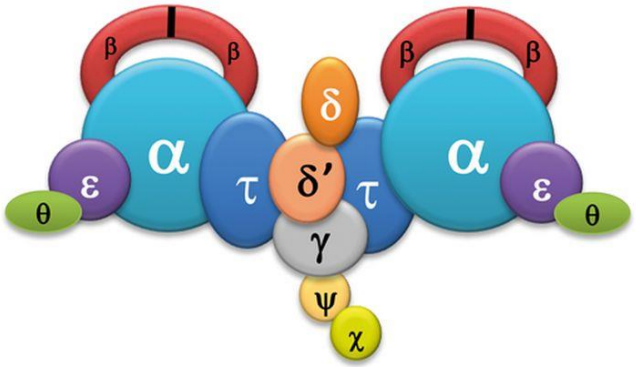
FIGURE 12.10 A eukaryotic chromosome contains multiple origins of replication that ultimately merge during replication.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

DNA Polymerases of *E.coli*

Type	Structure	Biochemical Function	Function in cell
DNA Polymerase I Pol I	1 Subunit 928 aa 103 kDa	DNA Polymerase 3‘-5‘ Exonuclease	Gap filling (Okazaki fragments) DNA Repair
DNA Polymerase II Pol II	88 kDa	DNA Polymerase	DNA Repair??
DNA Polymerase III Pol III	10 different subunits	DNA Polymerase 3‘-5‘ Exonuclease	The replication polymerase

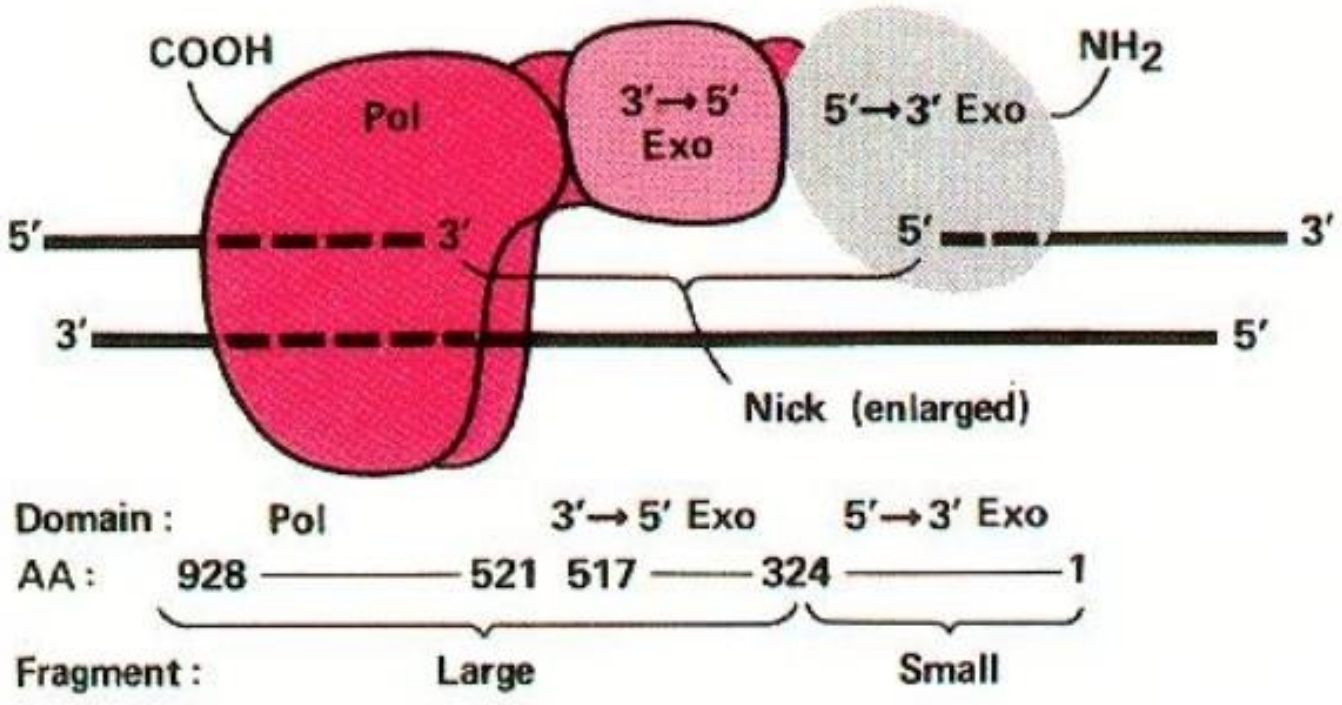
The five DNA polymerases of *Escherichia coli* and some of their relevant properties.

	Pol I	Pol II	Pol III	Pol IV	Pol V
DNA polymerase family	A	B	C	Y	Y
Activity	5'-3' polymerase 3'-5' exonuclease 5'-3' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase	5'-3' polymerase
	PolA	PolB		DinB	UmuC UmuD' UmuD'
Number of molecules/ cell					
– SOS	400	50 - 75	10 - 20	150 - 250	< 15
+ SOS	400	350 - 1000	10 - 20	1200 - 2500	200
Biological functions in the cell	DNA replication, Okazaki fragment maturation, DNA repair	DNA replication (backup DNA polymerase), DNA repair, TLS	DNA replication DNA repair	TLS	TLS

Iwona J. Fijalkowska et al. FEMS Microbiol Rev 2012;36:1105-1121

FEMS MICROBIOLOGY REVIEWS

DNA polymerase I (*E.coli*)



Klenow fragment

The three functional domains of DNA polymerase I: DNA-polymerase and 3´-5´-exonuclease at the 3´-OH end and 5´-3´-exonuclease at the 5´-NH_2-end.

http://crowngene.tistory.com/category/

DNA replication fork
- simplified model

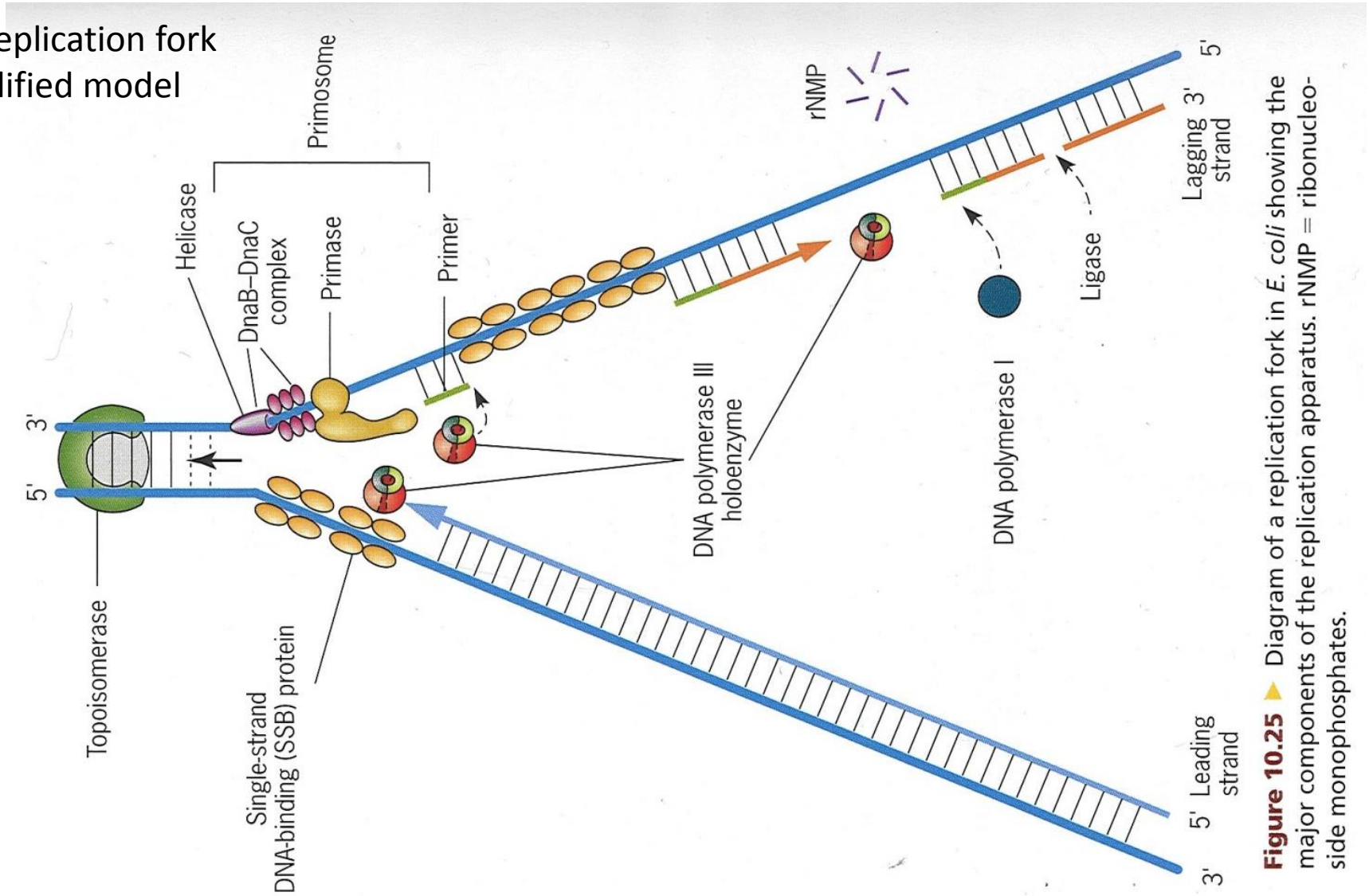
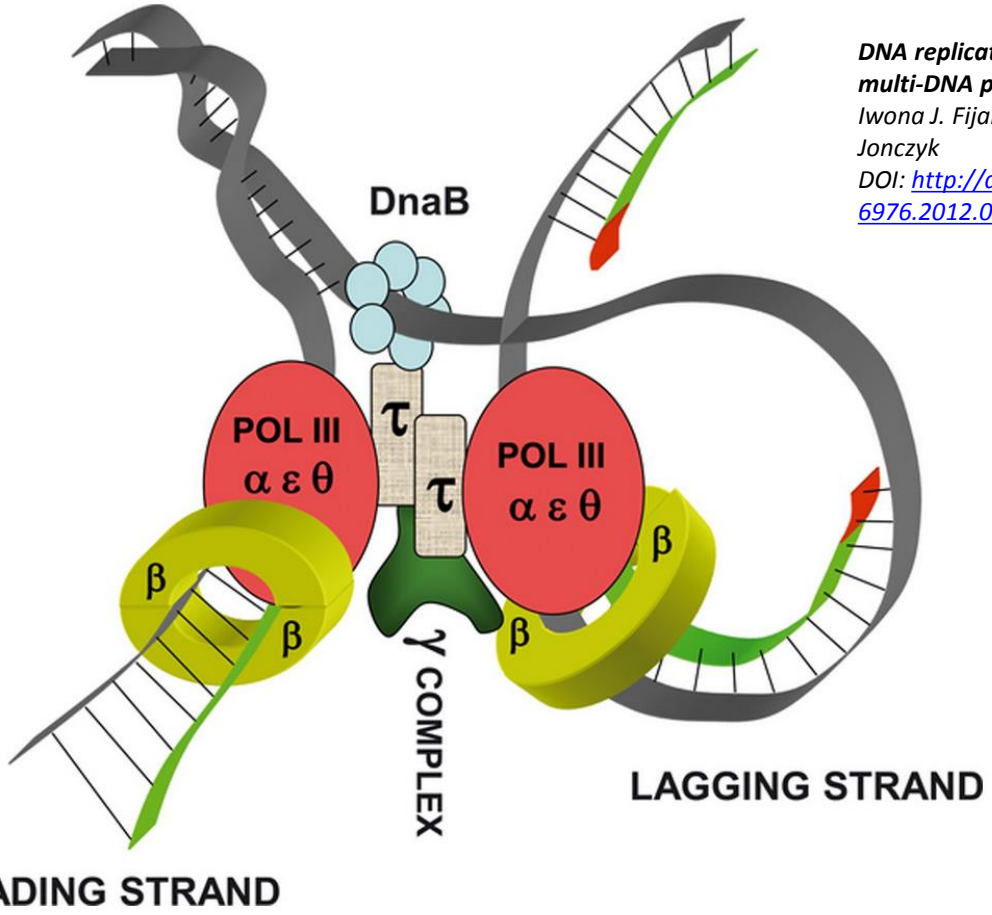


Figure 10.25 ▲ Diagram of a replication fork in *E. coli* showing the major components of the replication apparatus. rNMP = ribonucleoside monophosphates.

Taken from: D.P. Snustad, M.J. Simmons; Principles of Genetics, 5th edition; Wiley



DNA replication fidelity in Escherichia coli: a multi-DNA polymerase affair
Iwona J. Fijalkowska, Roel M. Schaaper, Piotr Jonczyk
DOI: http://dx.doi.org/10.1111/j.1574-6976.2012.00338.x 1105-1121

A model of the Escherichia coli DNA Pol III HE at the chromosomal replication fork, synthesizing, simultaneously, leading and lagging strands in, respectively, continuous (leading-strand) and discontinuous (lagging-strand) fashion. The αεθ complex represents the Pol III core, in which α is the polymerase, ε is the exonuclease (proofreading) subunit, and θ is a stabilizing subunit. See text for details. Not shown is the DnaG primase, which, in association with the DnaB helicase, produces lagging-strand RNA primers supporting the discontinuous synthesis in this strand. The γ complex (γδδ′χψ) conducts the cycling (loading and unloading) of the β2 processivity clamps, which is particularly important for the cycling of the polymerase in the lagging strand. Recent studies have suggested that the relevant form of the DnaX assembly (τ2γδδ′χψ as shown here) may be τ3δδ′χψ (noting that γ and τ are both products of the dnaX gene and differ only in their C-termini). As τ contains the extra C-terminal extension that mediates the τ–α interaction, the τ3δδ′χψ-containing HE is capable of binding a third Pol III core (McInerney et al., 2007; Reyes-Lamothe et al., 2010; Georgescu et al., 2012). This third Pol III core (not shown) may participate in polymerase switching and hence contribute to the chromosomal replication process (see text).

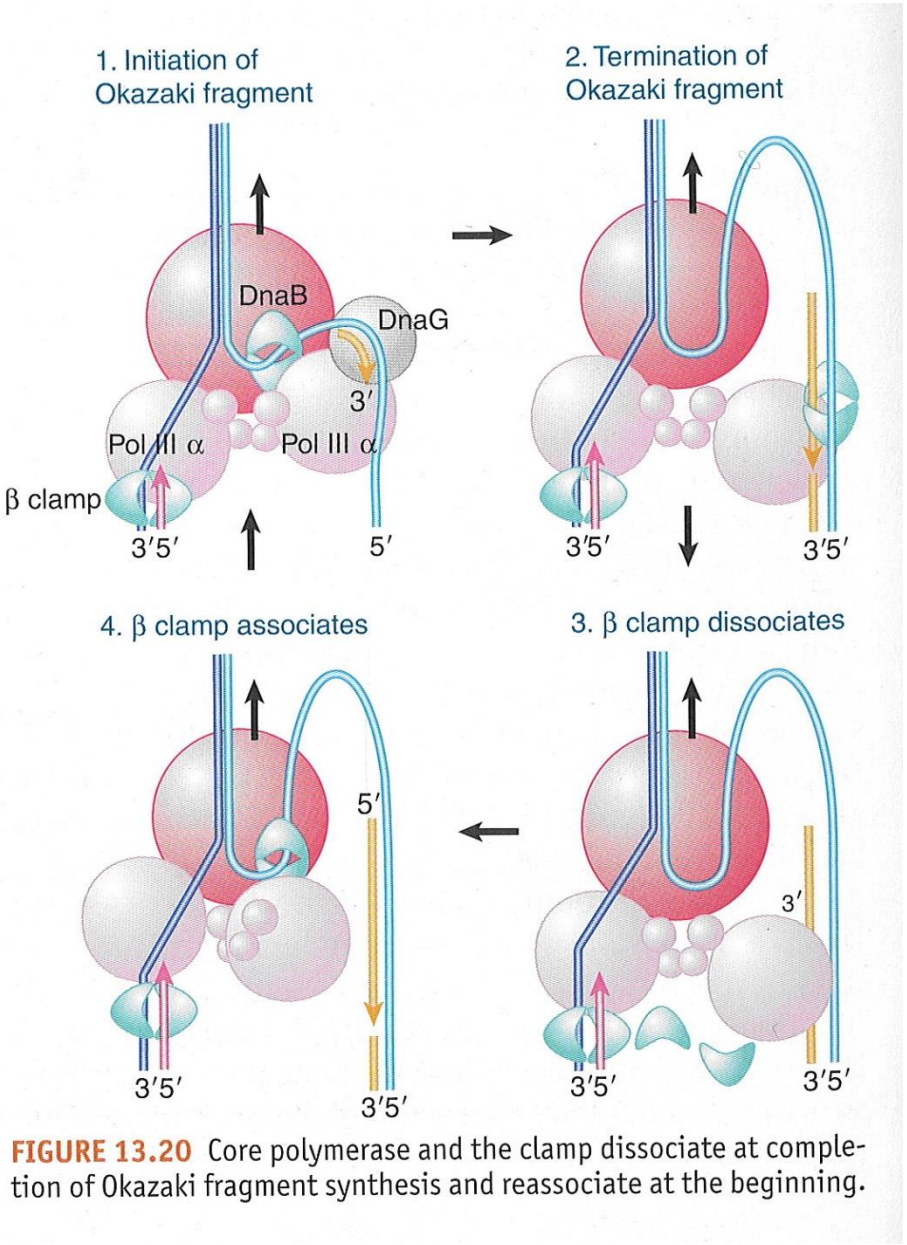


FIGURE 13.20 Core polymerase and the clamp dissociate at completion of Okazaki fragment synthesis and reassociate at the beginning.

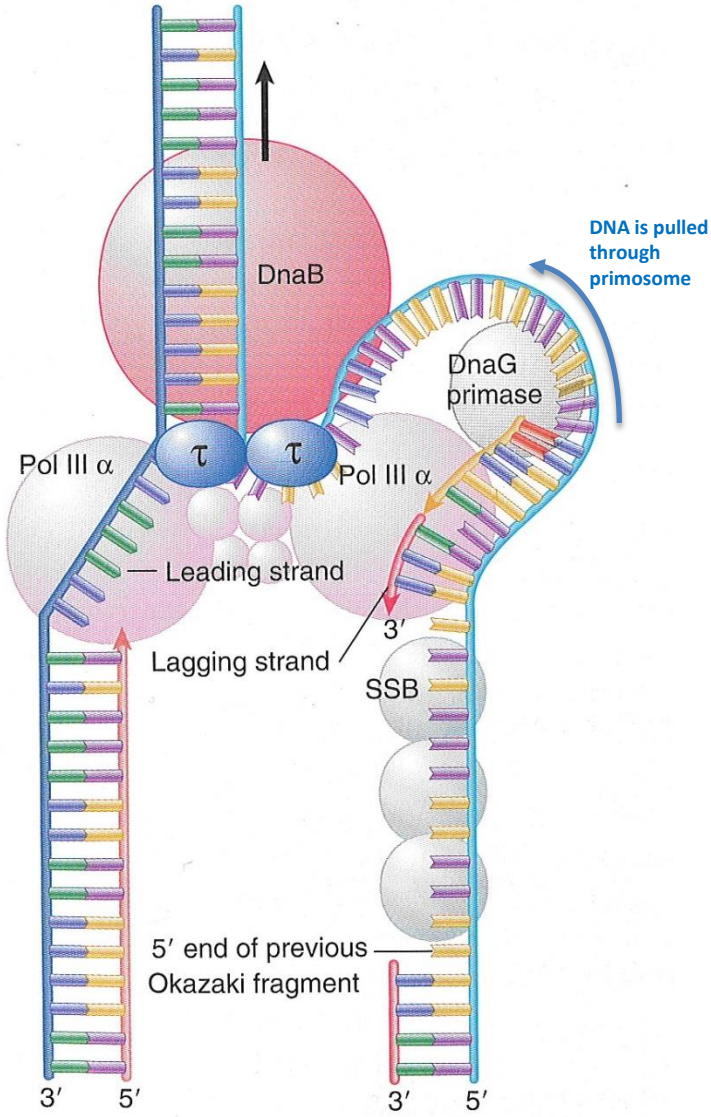


FIGURE 13.19 Each catalytic core of Pol III synthesizes a daughter strand. DnaB is responsible for forward movement at the replication fork.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

DNA Methylation Status Controls Replication Initiation

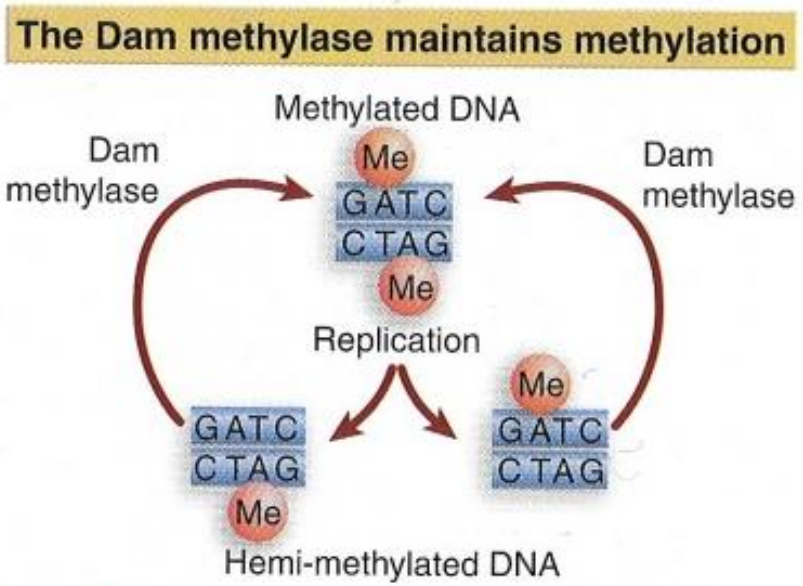


Figure 15.7 Replication of methylated DNA gives hemimethylated DNA, which maintains its state at GATC sites until the Dam methylase restores the fully methylated condition.

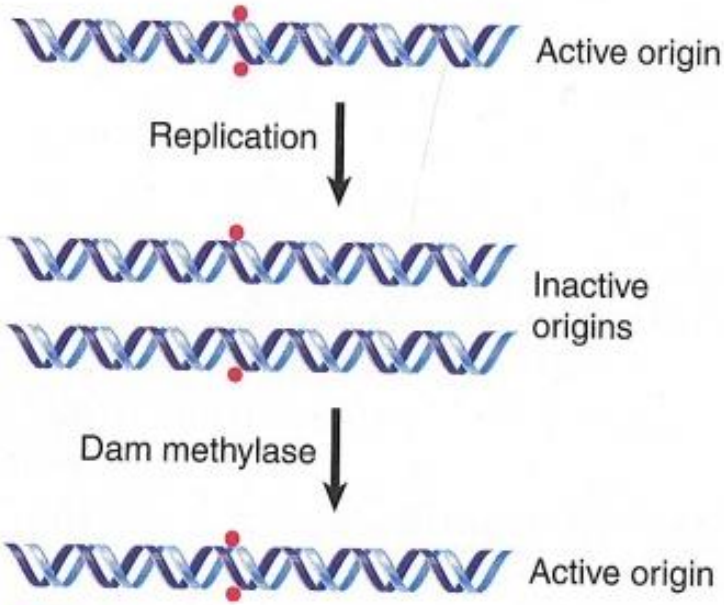
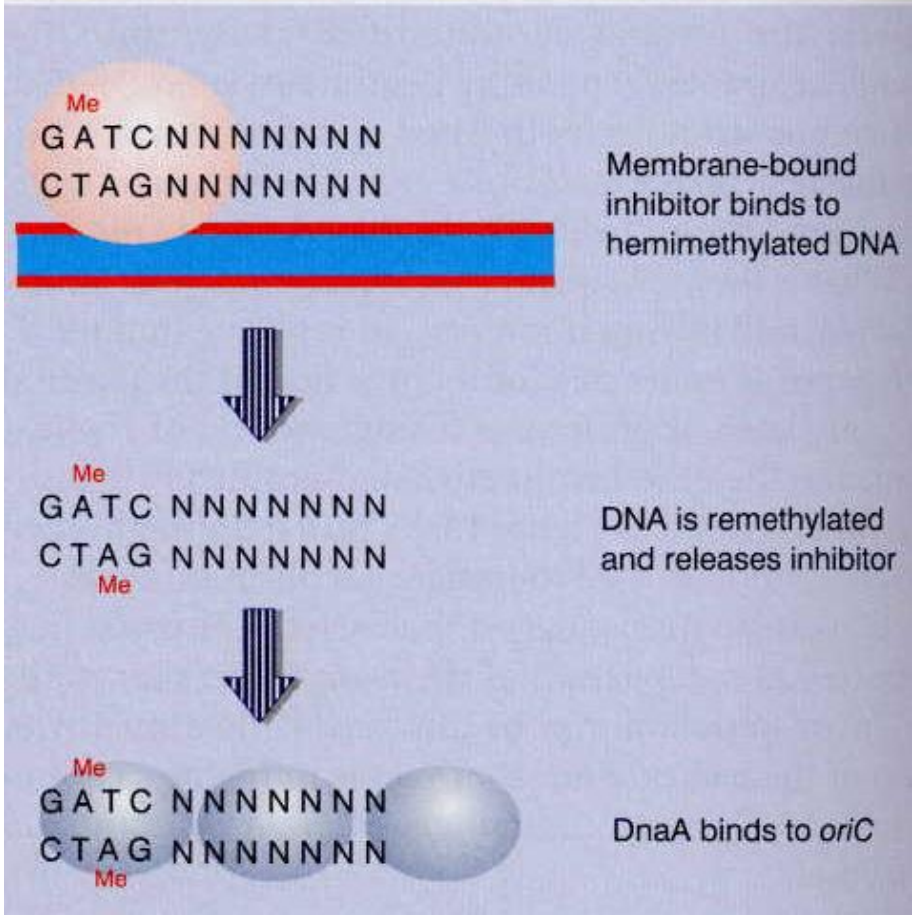


FIGURE 12.7 Only fully methylated origins can initiate replication; hemimethylated daughter origins cannot be used again until they have been restored to the fully methylated state.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Figure 13.26 A membrane-bound inhibitor binds to hemimethylated DNA at the origin, and may function by preventing the binding of DnaA. It is released when the DNA is remethylated.



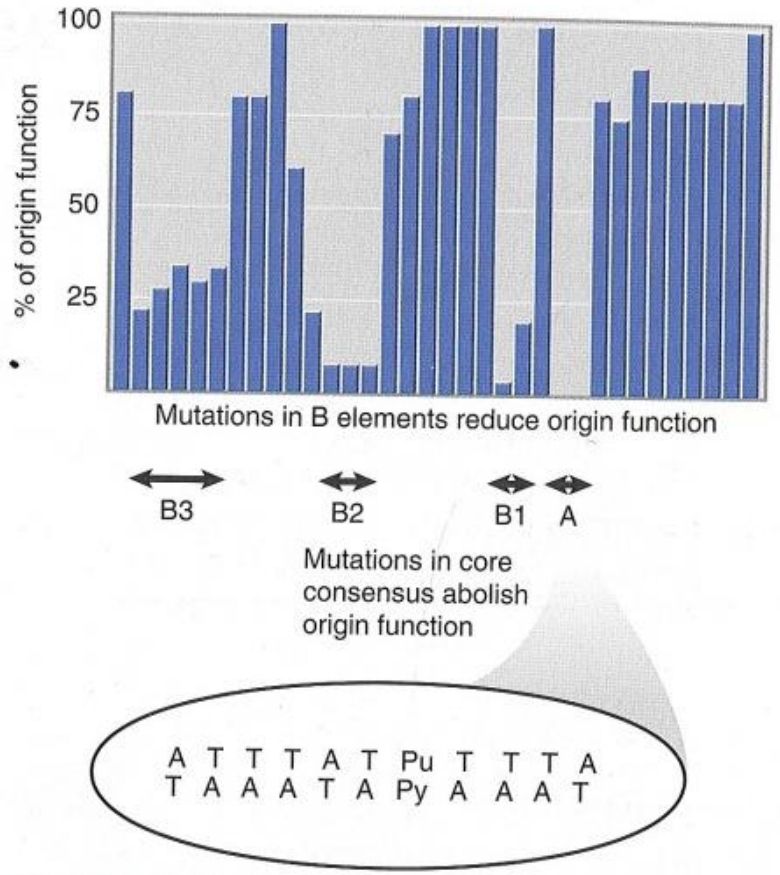


FIGURE 12.12 An *ARS* extends for ~50 bp and includes a consensus sequence (A) and additional elements (B1–B3).

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Alternate Strategies for Replication of Circular DNA

D-loop Displacement

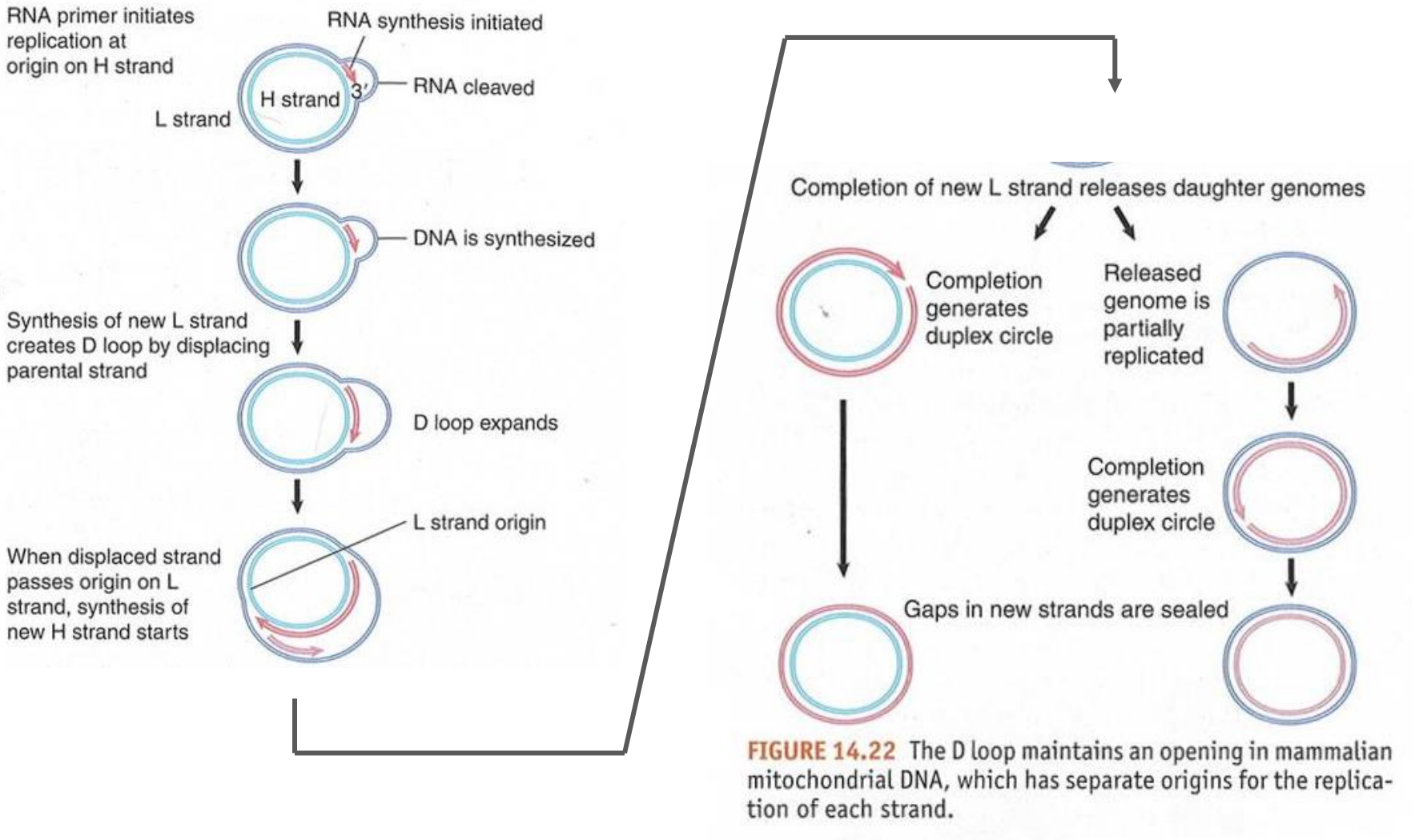


FIGURE 14.22 The D loop maintains an opening in mammalian mitochondrial DNA, which has separate origins for the replication of each strand.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

Alternate Strategies for Replication of Circular DNA

Rolling Circle Mechanism

→ no RNA Primer
→ 3‘ OH generated y nicking
→ different types of DNA generated
- ds circular DNA
- ss circular DNA
- concatemeric linear DNA

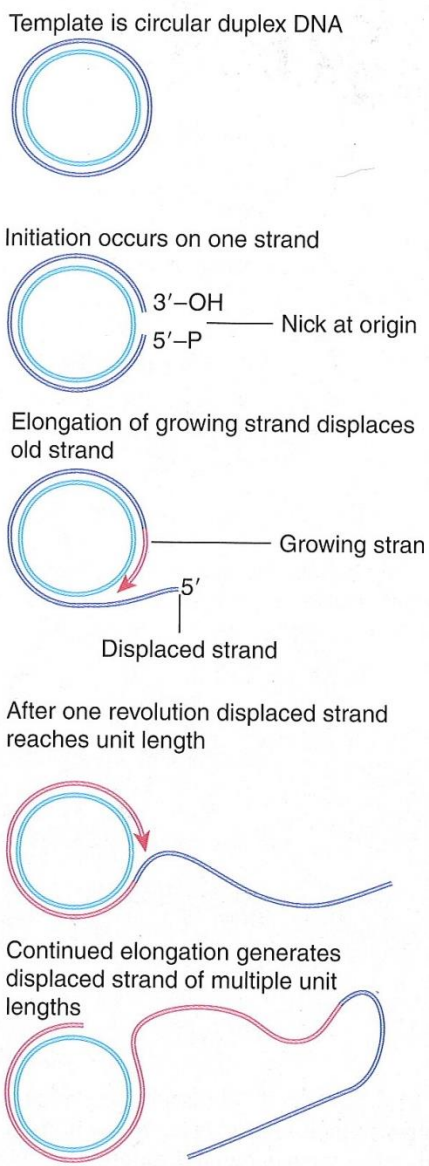


FIGURE 14.5 The rolling circle generates a multimeric single-stranded tail.

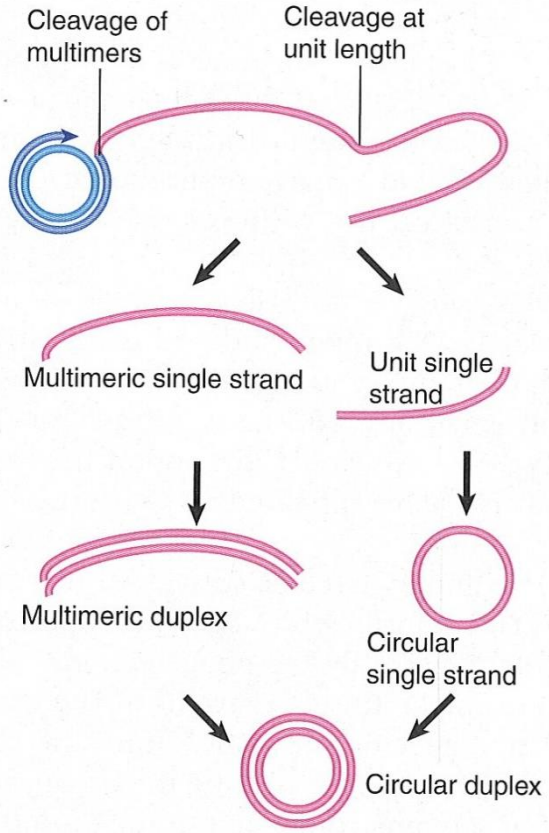


FIGURE 14.7 The fate of the displaced tail determines the types of products generated by rolling circles. Cleavage at unit length generates monomers, which can be converted to duplex and circular forms. Cleavage of multimers generates a series of tandemly repeated copies of the original unit. Note that the conversion to double-stranded form could occur earlier, before the tail is cleaved from the rolling circle.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI“; Jones&Bartlett Learning

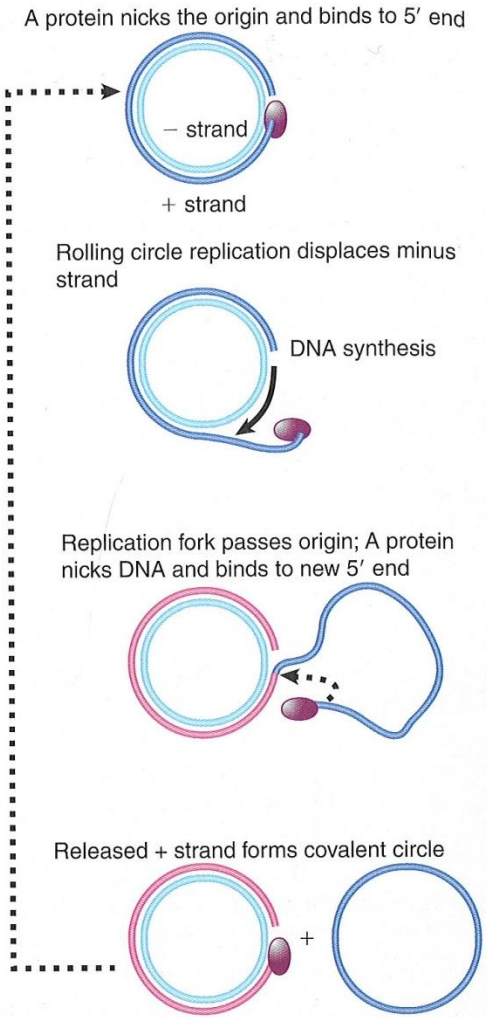


FIGURE 14.8 ΦX174 RF DNA is a template for synthesizing single-stranded viral circles. The A protein remains attached to the same genome through indefinite revolutions, each time nicking the origin on the viral (+) strand and transferring to the new 5′ end. At the same time, the released viral strand is circularized.

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

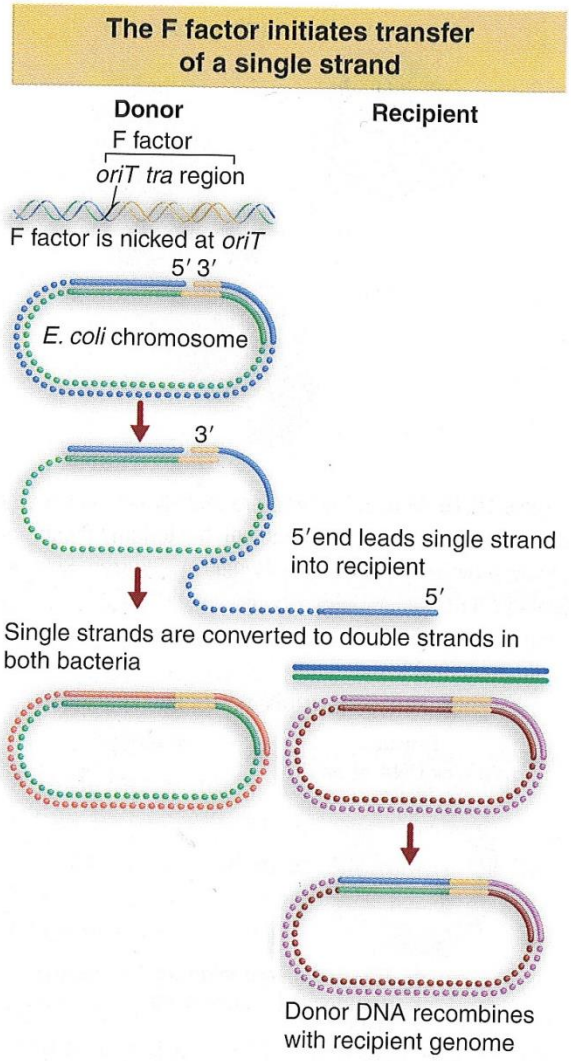


Figure 16.12 Transfer of chromosomal DNA occurs when an integrated F factor is nicked at *oriT*. Transfer of DNA starts with a short sequence of F DNA and continues until prevented by loss of contact between the bacteria.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

10.11.15

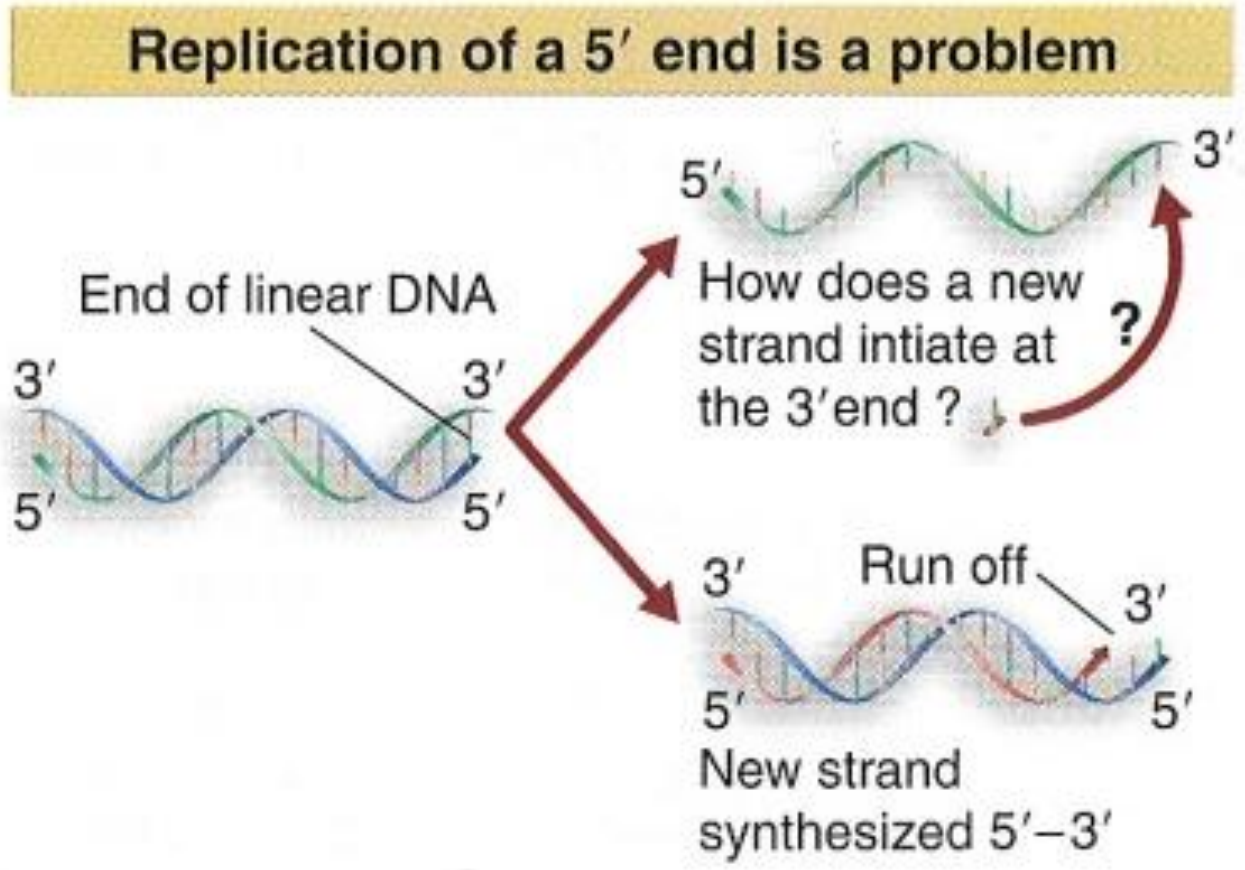


Figure 16.2 Replication could run off the 3′ end of a newly synthesized linear strand, but could it initiate at a 5′ end?

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

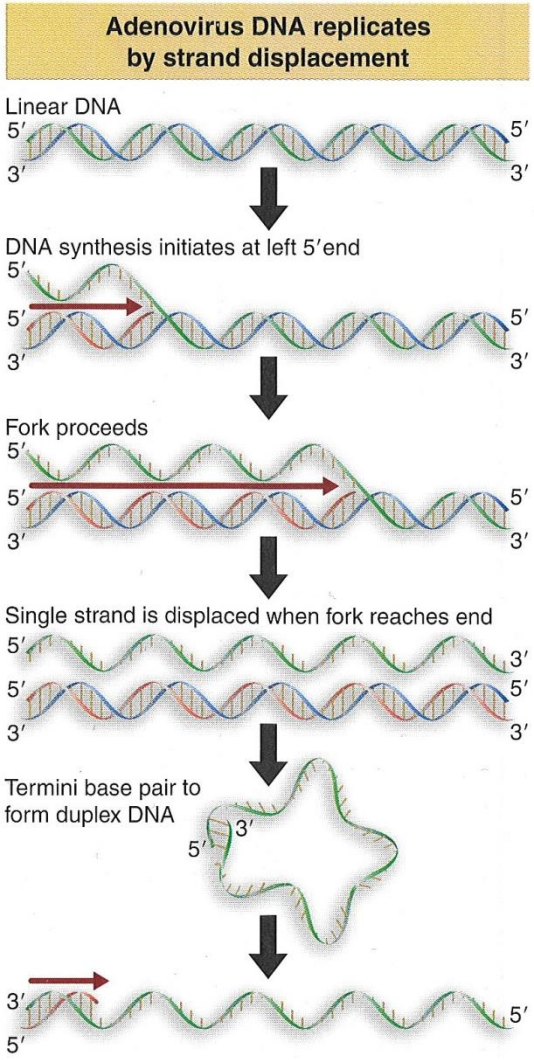


Figure 16.3 Adenovirus DNA replication initiates separately at the two ends of the molecule and proceeds by strand displacement.

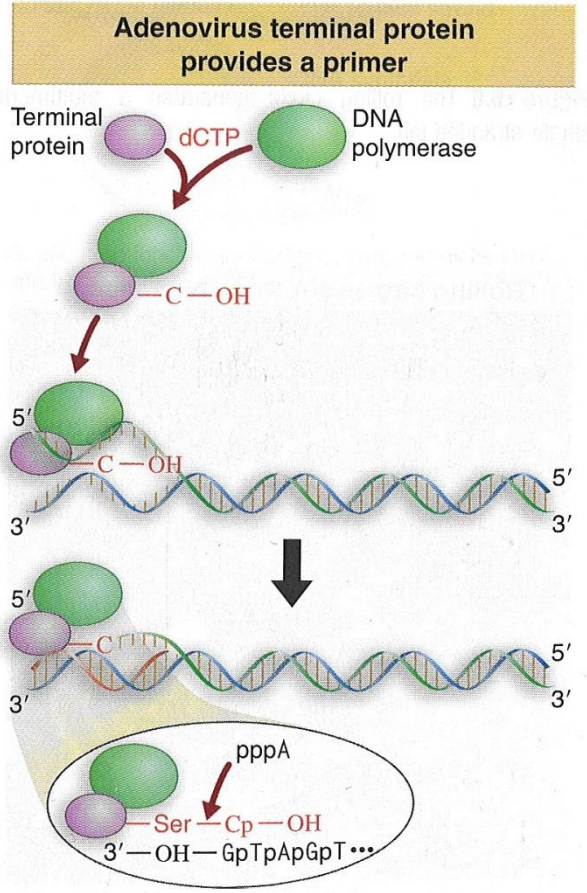


Figure 16.5 Adenovirus terminal protein binds to the 5′ end of DNA and provides a C–OH end to prime synthesis of a new DNA strand.

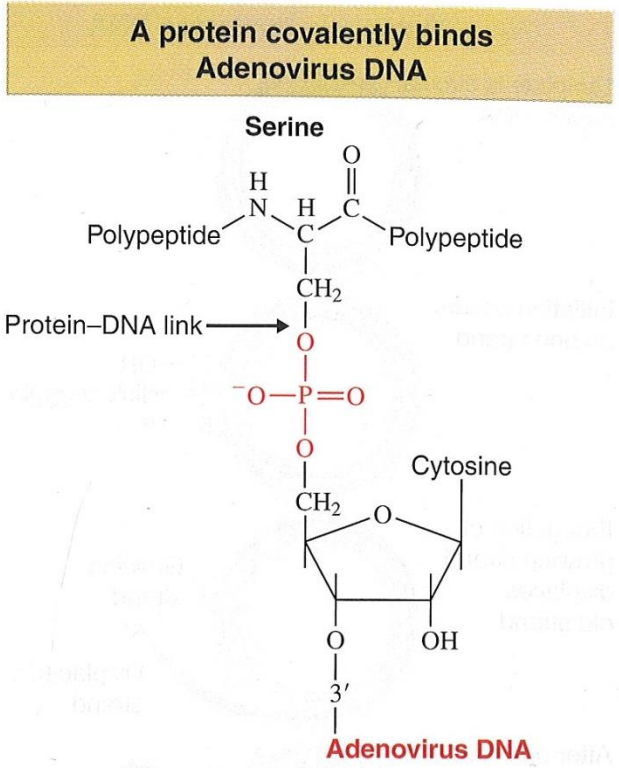


Figure 16.4 The 5′ terminal phosphate at each end of adenovirus DNA is covalently linked to serine in the 55 kD Ad-binding protein.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

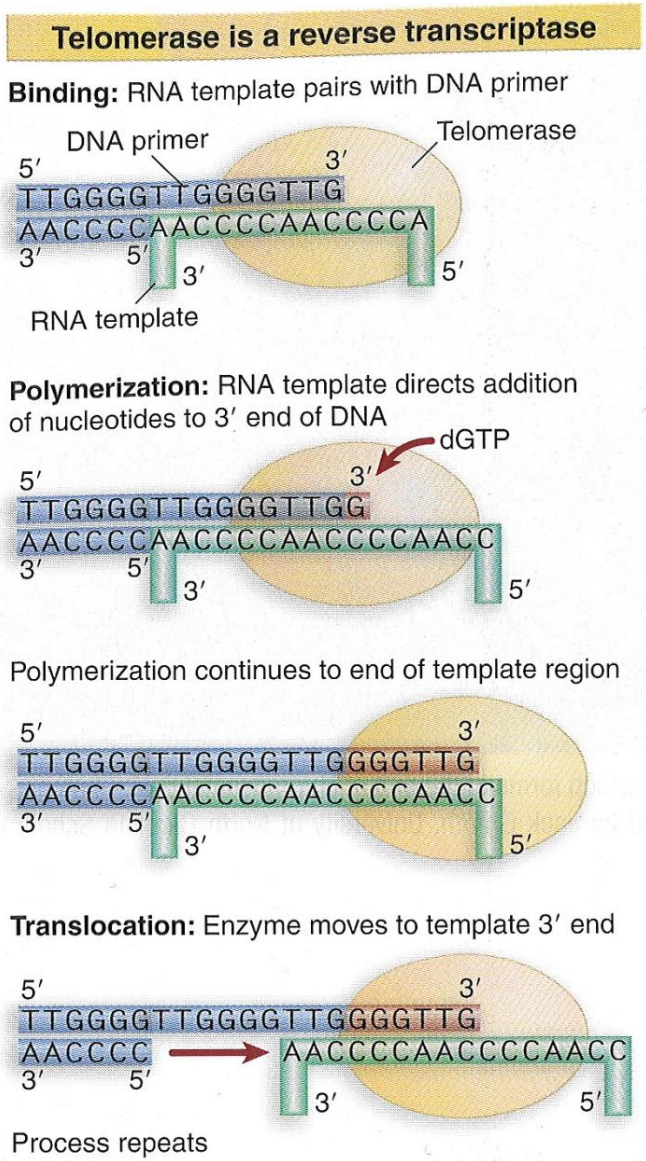
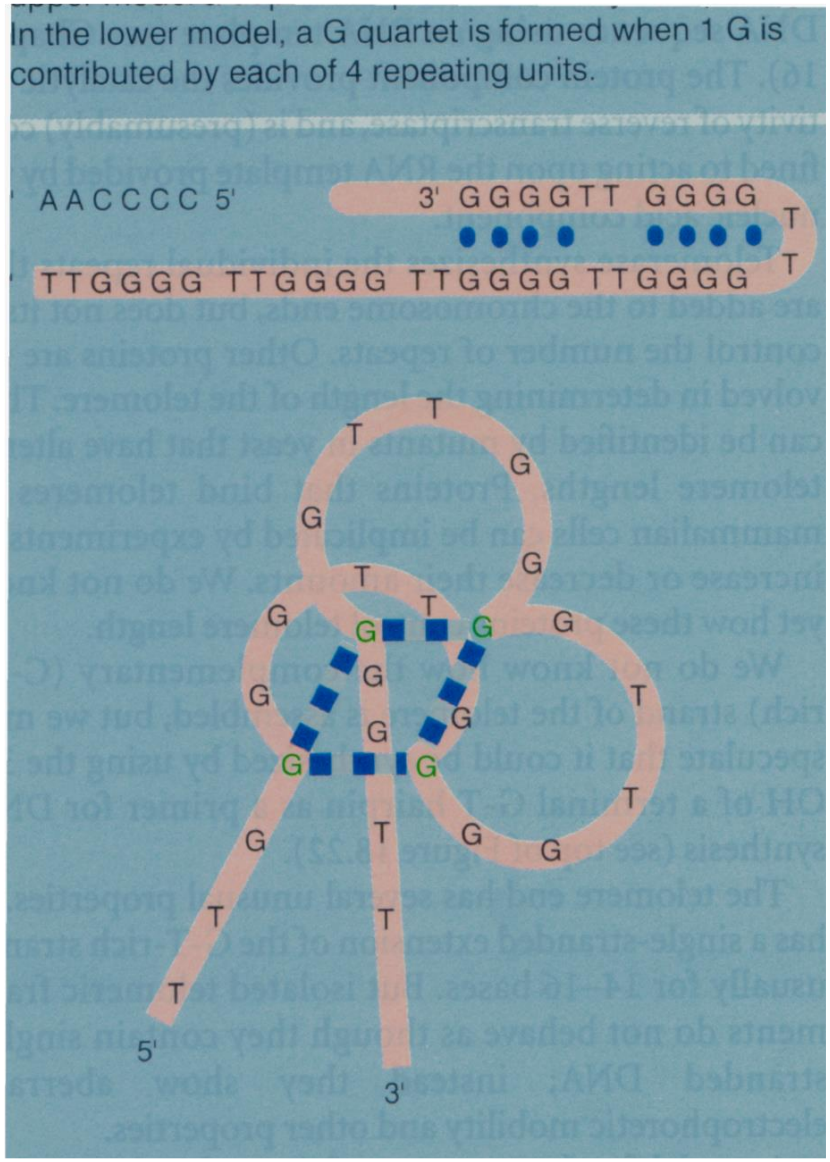


Figure 28.29 Telomerase positions itself by base pairing between the RNA template and the protruding single-stranded DNA primer. It adds G and T bases one at a time to the primer, as directed by the template. The cycle starts again when one repeating unit has been added.

Sequence repeats at the telomeres

Organism	Sequence
Tetrahymena (Ciliat)	TTGGG
Oxytricha (Ciliat)	TTTTGGGG
Trypanosoma	TTAGGG
Saccharomyces (Hefe)	$TG_{1-3}TG$
Homo sapiens	TTAGGG

Taken from: B. Lewin, Essential Genes, Pearson Ed. International



Telomeres have specific structures:

→ Looping by Hoogsten base pairing
→ No 3‘/5‘ free ends
→ Backfolding allows priming for synthesis of reverse strand

Segregation – Partitioning

Statistical Distribution

Active segregation mechanisms

True partitioning

Enhancement of maintenance

Multimer resolution systems → Plasmid monomerization
DNA-Configuration (e.g. pSC101)
Regulation of Cell Division
Killing of host cells

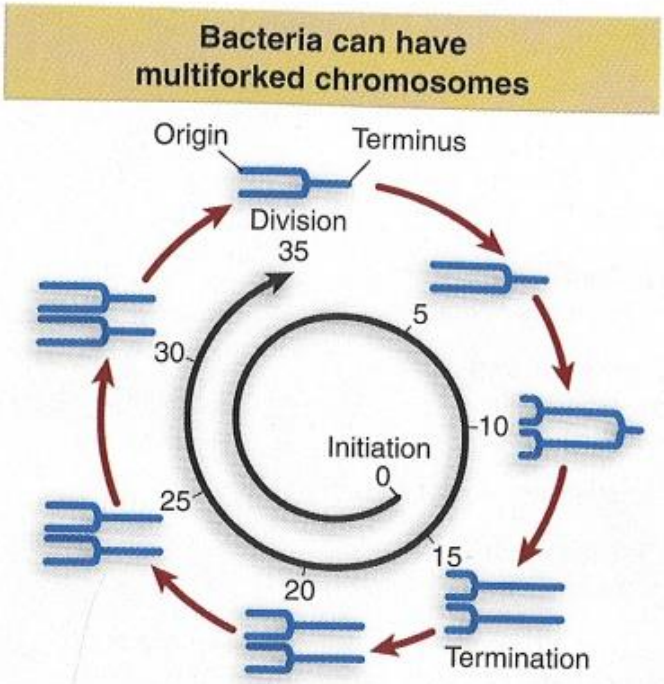
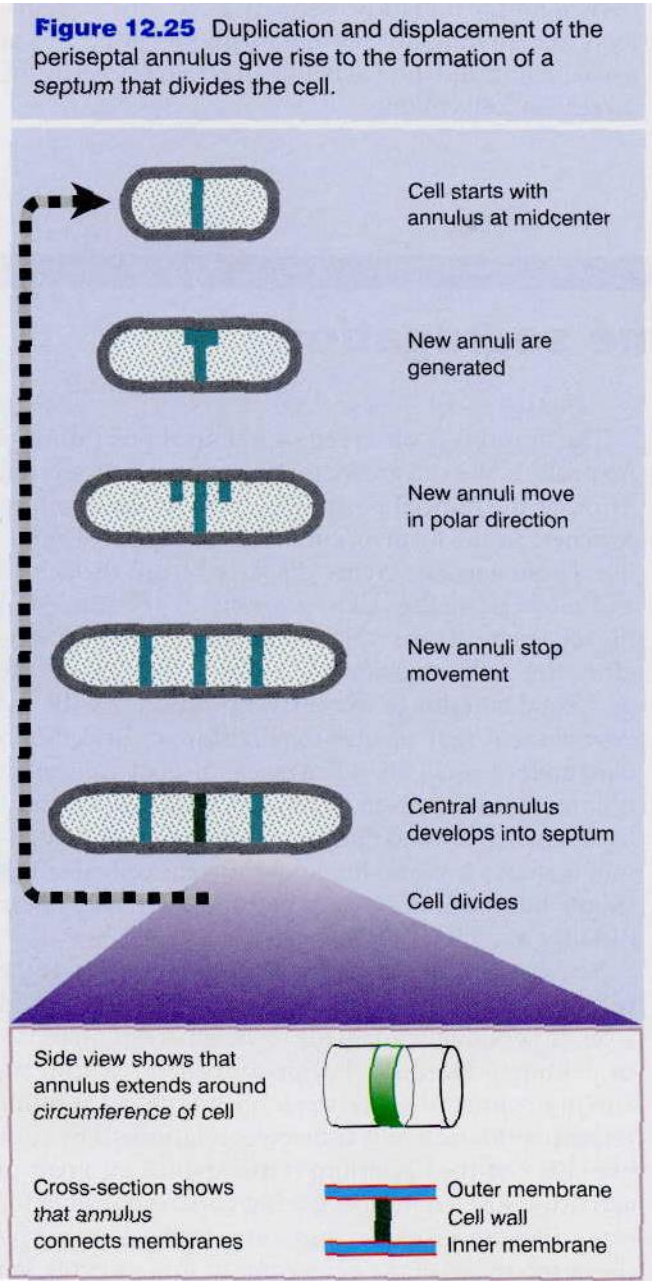


Figure 17.2 The fixed interval of 60 minutes between initiation of replication and cell division produces multiforked chromosomes in rapidly growing cells. Note that only the replication forks moving in one direction are shown; actually the chromosome is replicated symmetrically by two sets of forks moving in opposite directions on circular chromosomes.



Taken from: B. Lewin, Essential Genes, Pearson Ed. International

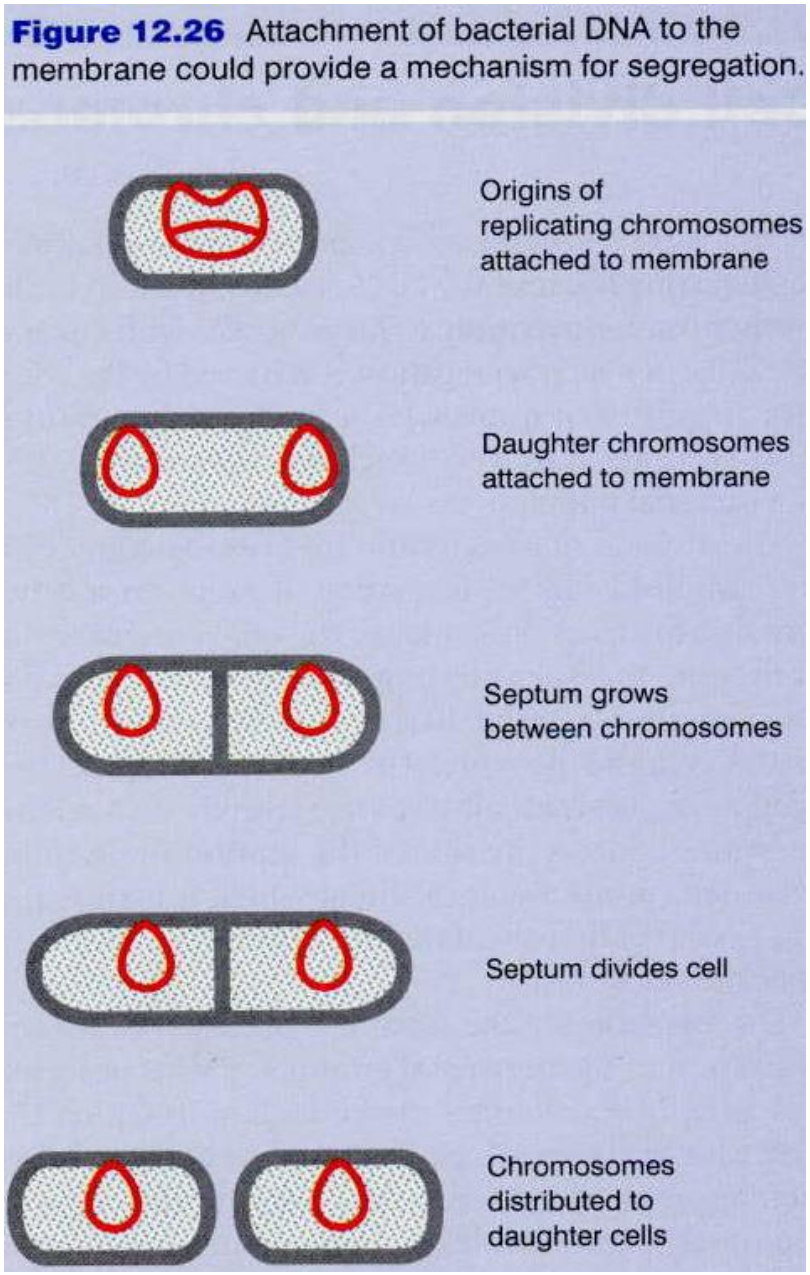


Figure 12.26 Attachment of bacterial DNA to the membrane could provide a mechanism for segregation.

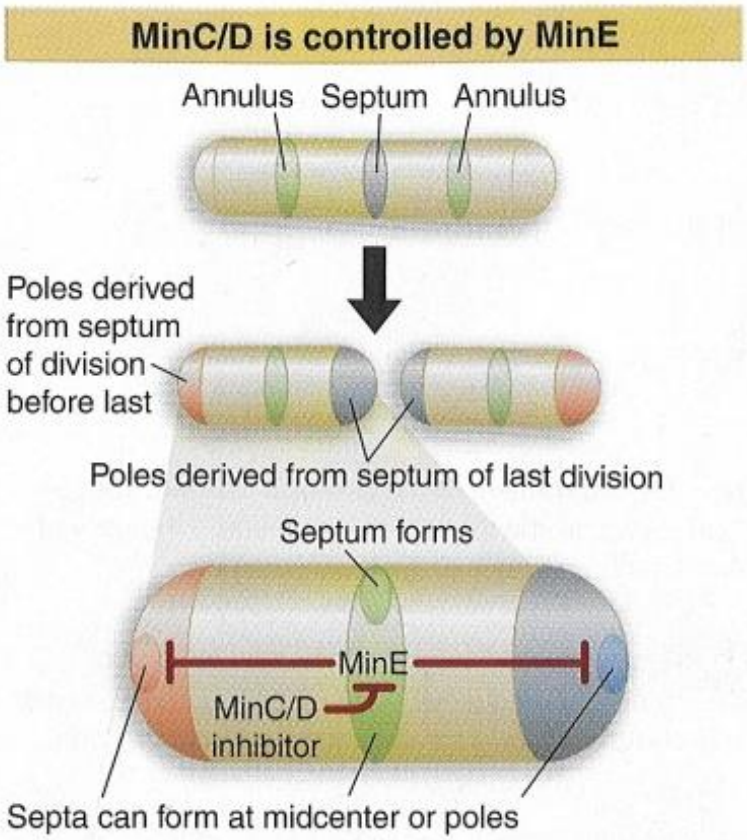


Figure 17.7 MinC/D is a division inhibitor, whose action is confined to the polar sites by MinE.

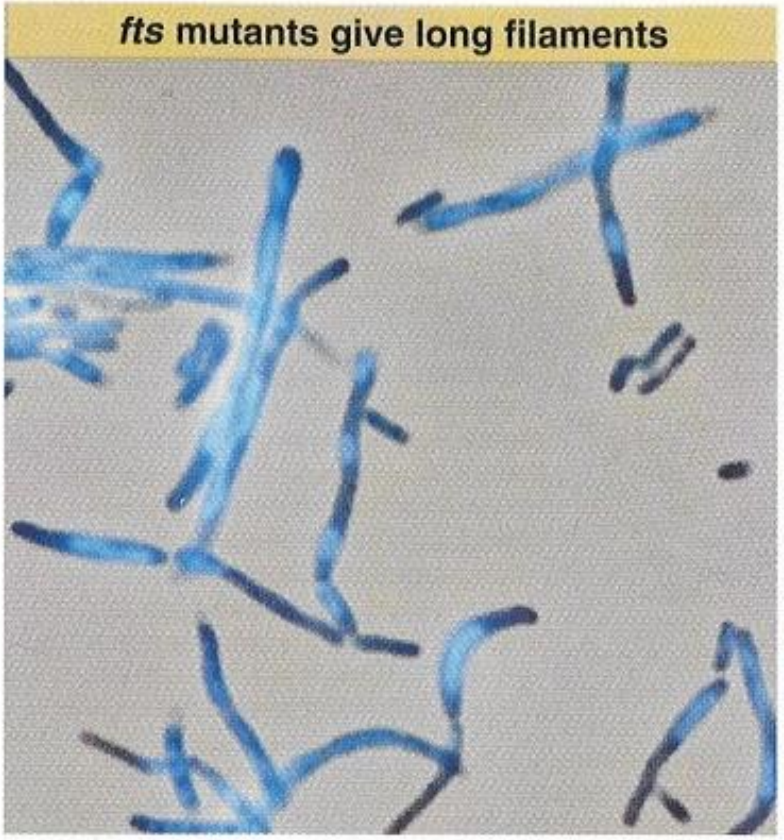


Figure 17.4 Failure of cell division generates multinucleated filaments. Photograph kindly provided by Sota Hiraga.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International

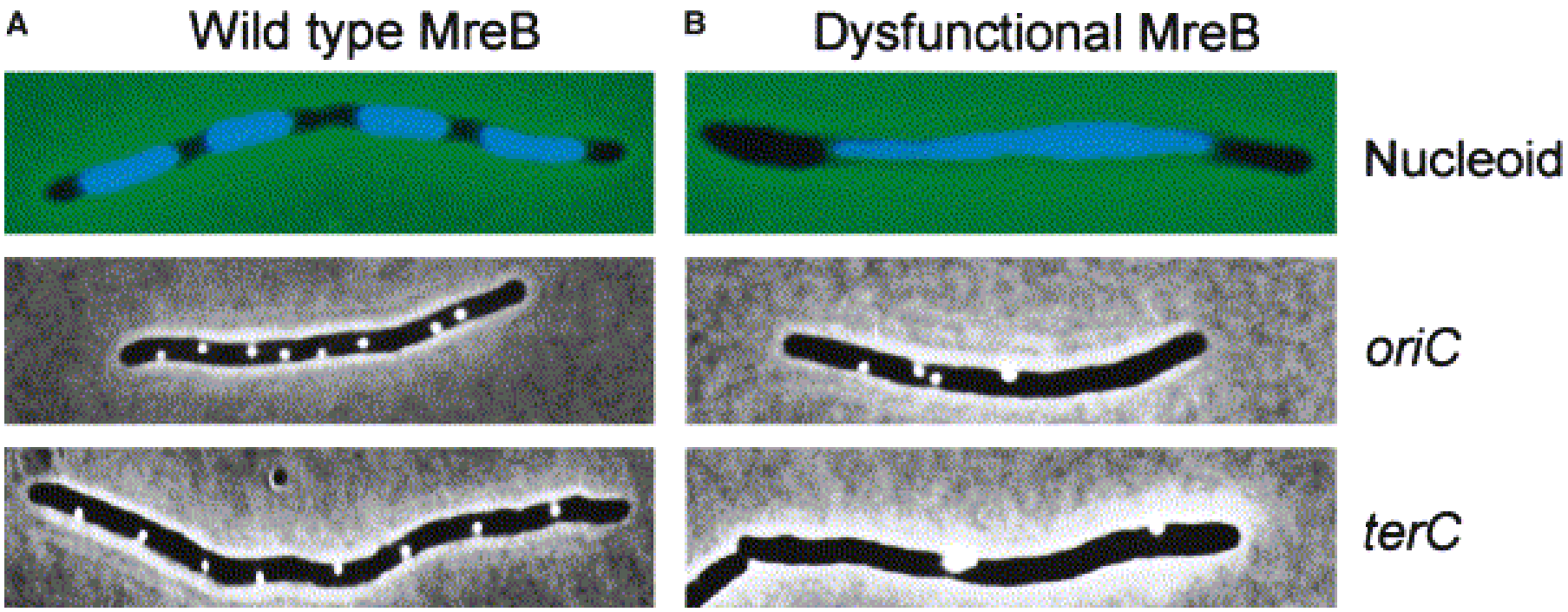


Figure 4. Dysfunctional MreB Inhibits Chromosome Segregation in *E. coli*
In (A), cells ectopically expressed wild-type MreB whereas in (B), the cells expressed an MreB derivative carrying a single aa change in the phosphate2 region (D165V). The top row shows DNA stained with DAPI, the second row cells expressing a GFP-ParB fusion protein that binds to *parS* inserted near *oriC*, and the bottom row cells expressing a GFP-ParB protein that binds to *parS* inserted near *terC* (modified from Kruse et al., 2003).

parA, parB: protein coding genes
parC : protein binding site on the DNA

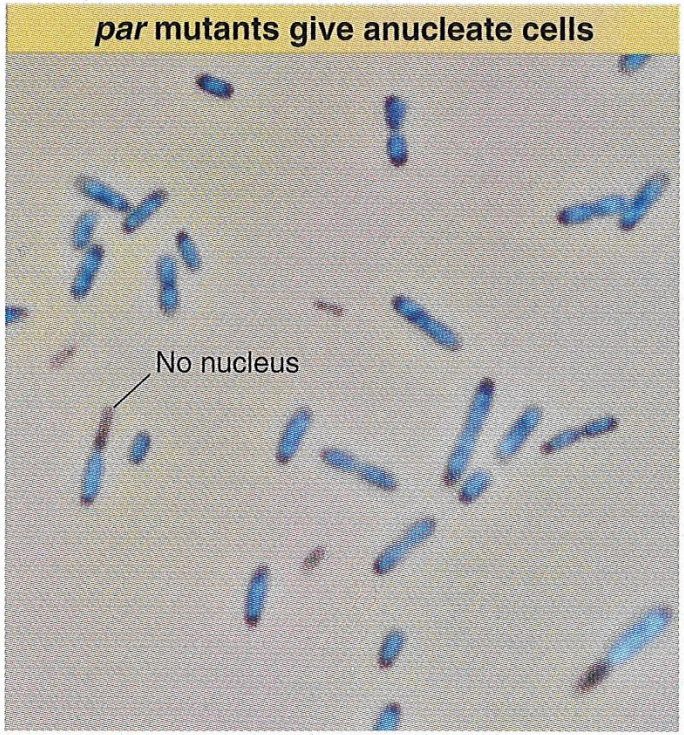


Figure 17.5 *E. coli* generate anucleate cells when chromosome segregation fails. Cells with chromosomes stain blue; daughter cells lacking chromosomes have no blue stain. This field shows cells of the *mukB* mutant; both normal and abnormal divisions can be seen. Photograph kindly provided by Sota Hiraga.

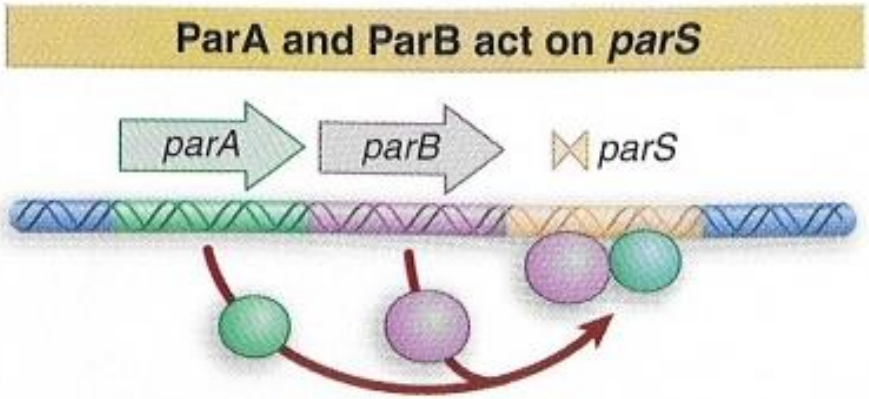
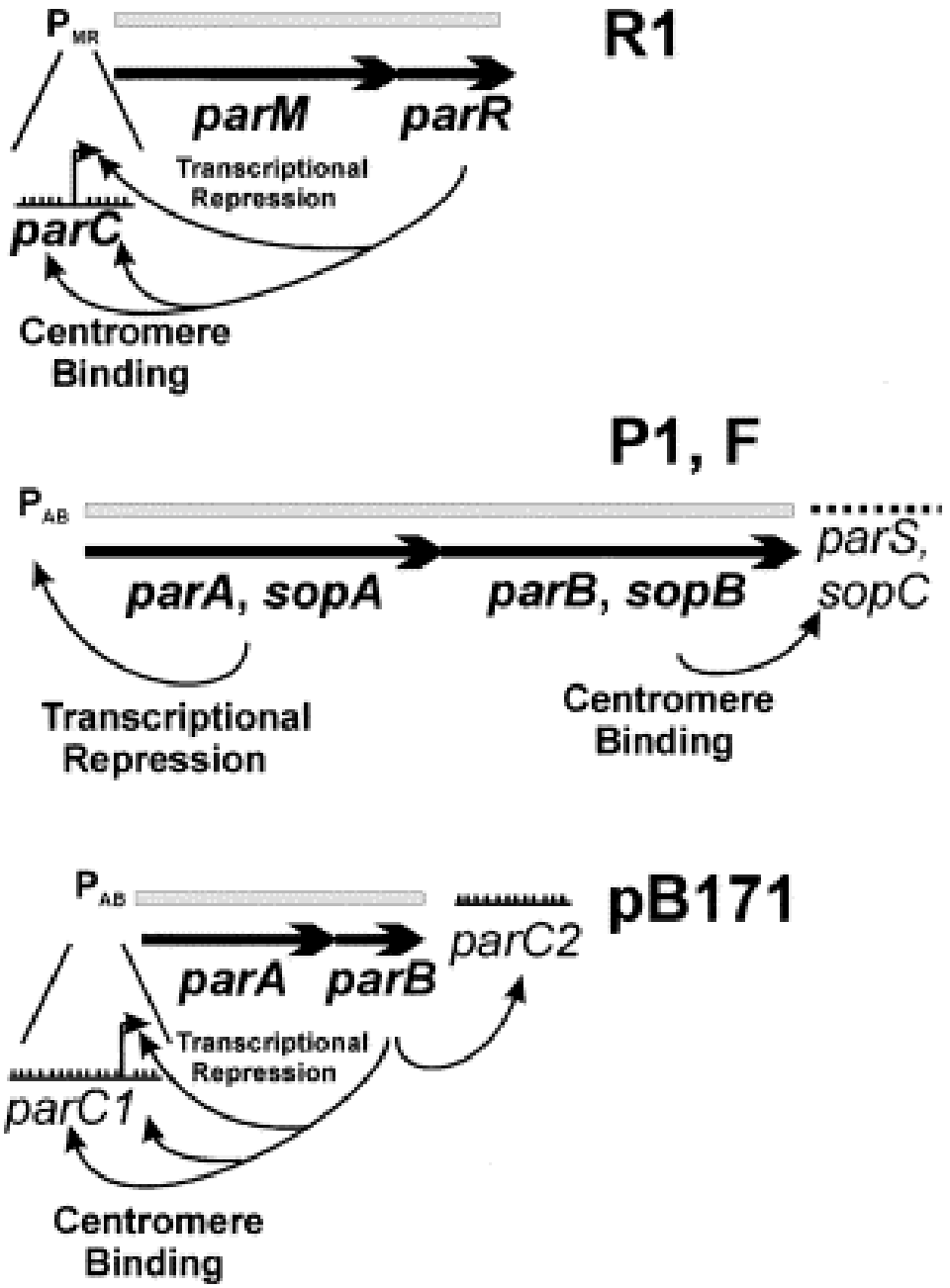


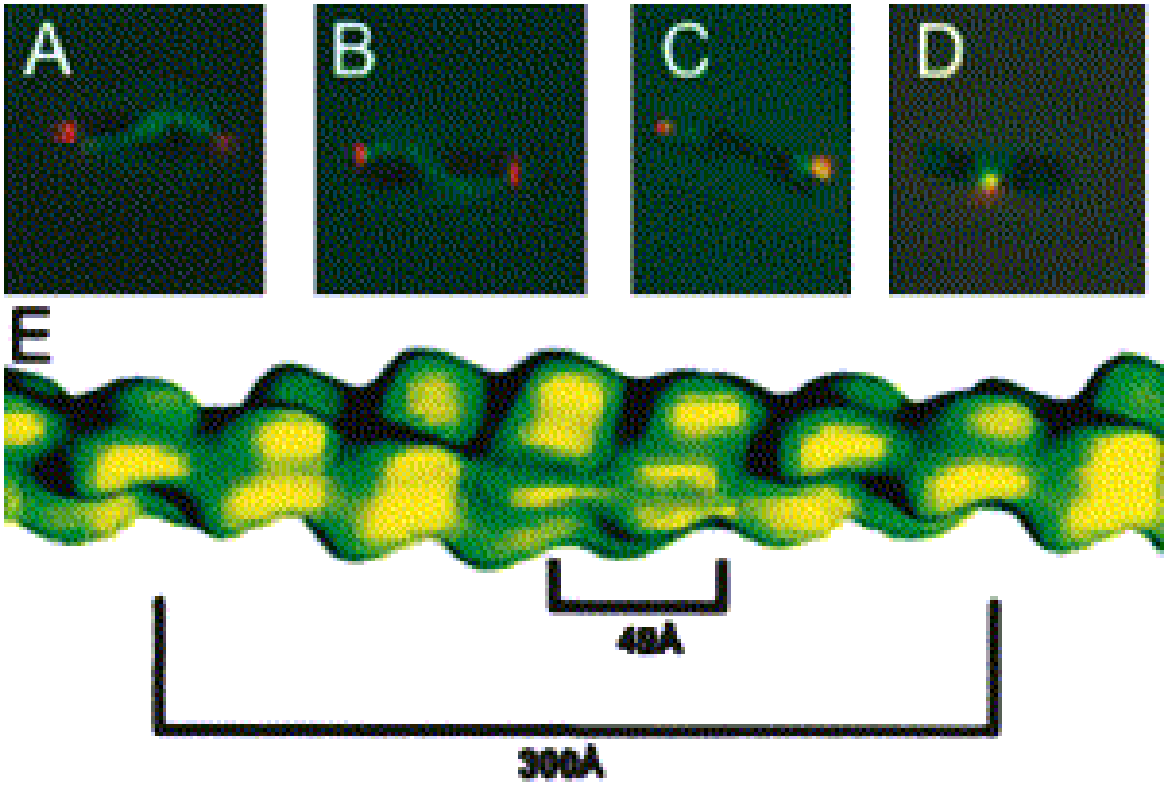
Figure 17.13 A common segregation system consists of genes *parA* and *parB* and the target site *parS*.

Taken from: B. Lewin, Essential Genes, Pearson Ed. International



Bacterial Mitotic Machineries.
Cell, Volume 116, Issue 3, Pages 359-366 K. Gerdes, J. Møller-Jensen, G. Ebersbach, T. Kruse, K. Nordström

Figure 1. Genetic Structure and Components of Type I (P1, F, and pB171) and Type II Partitioning Loci (R1)In *par* of R1, ParR binds to two times five direct repeats flanking the promoter region in the *parC* region and thereby autoregulates transcription of the *parMR* operon. The *parC* region acts as a centromere-like site and has partitioning activity when ParM and ParR are donated in *trans* (Dam and Gerdes, 1994). In *par*/*sop* of P1 and F, the A proteins bind to the *par*/*sop* promoter region and autoregulate transcription. The B proteins, when bound to the *parS*/*sopC* sites, enhance autoregulation by the A proteins (Hao and Yarmolinsky 2002 and Yates et al. 1999). The *par* region of pB171 has two *cis*-acting centromere-like sites to which ParB presumably binds (Ebersbach and Gerdes, 2001). Binding of ParB of pB171 to *parC1* autoregulates transcription of the *parAB* operon.



ParM: actin family ATPase

Figure 2. Actin-Like ParM Filaments In Vivo and In Vitro
In Vivo: (A) and (B) show cells with polar plasmids (red) located at the tip of ParM filaments (green) visualized by IFM. (C) shows decay of the filaments from mid-cell toward the cell poles. In (D), a single plasmid focus is located at mid-cell without a ParM filament (Møller-Jensen et al., 2003).
In Vitro: (E) shows a 3D reconstruction of a straightened ParM filament obtained by electron microscopy (modified from van den Ent et al., 2002).

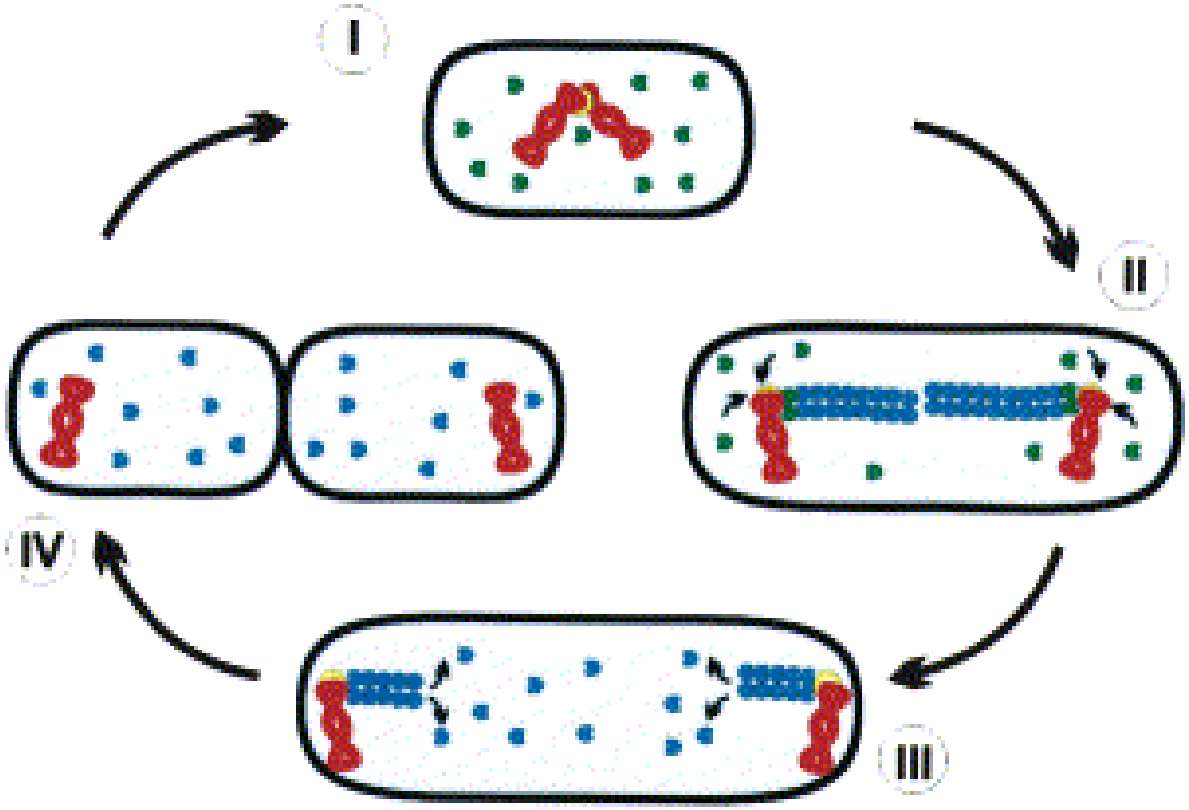
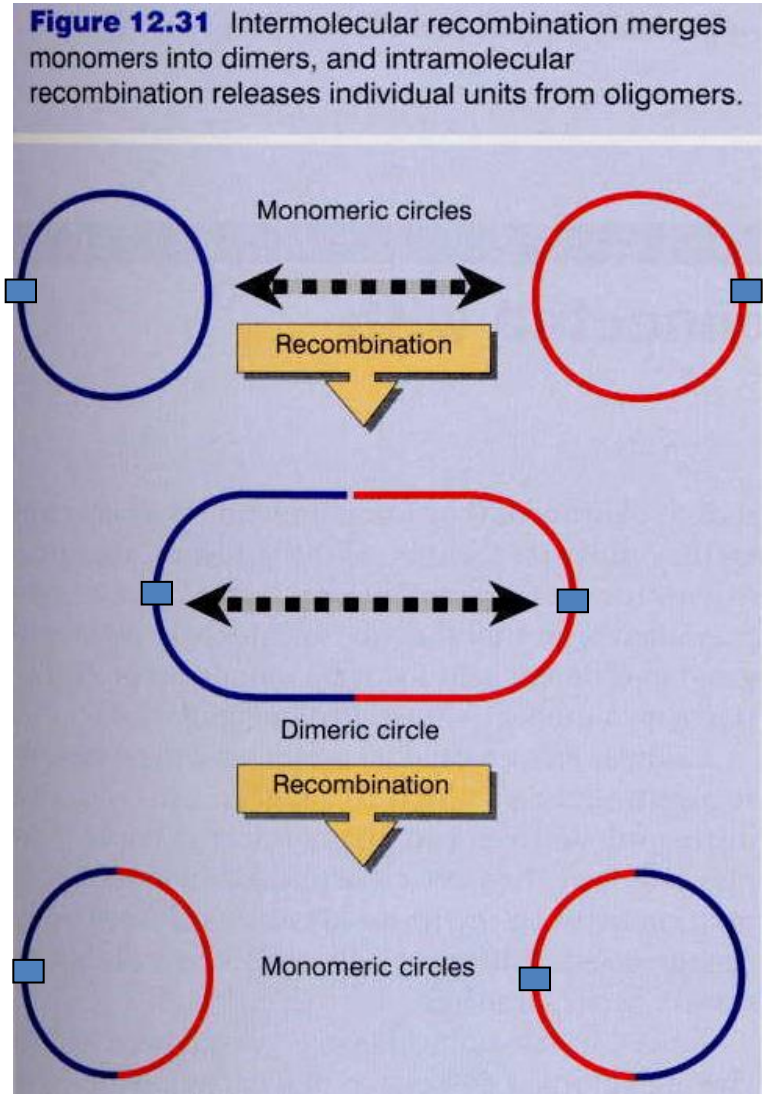


Figure 3. Model Explaining R1 *par*-Mediated Plasmid Partitioning during the Cell CyclePlasmids (red) are replicated by the host cell replication machinery, which is located at mid-cell. Replicated plasmids are paired by ParR bound to *parC* (yellow) thereby forming a partitioning complex (I). The partitioning complex forms a nucleation point for ParM filamentation. Continuous addition of ATP-ParM (green) to the filament poles provides the force for active movement of plasmids to opposite cell poles (II). Within the filaments, ATP is hydrolyzed, leading to destabilization of the ParM polymer (III). Nucleotide exchange is required to recharge the ADP-ParM (blue) molecules for a subsequent round of partitioning (IV). Modified from Møller-Jensen et al., 2003.

Systems facilitating plasmid maintenance in host cell



Site-specific recombination

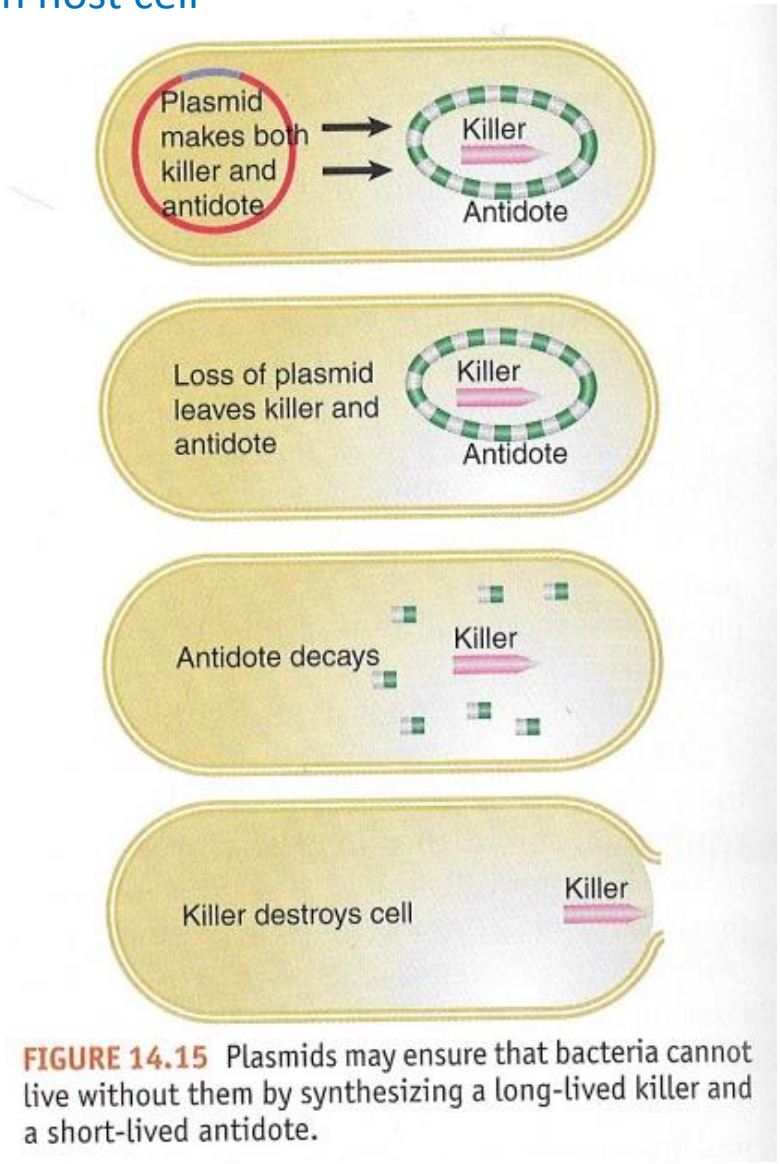


FIGURE 14.15 Plasmids may ensure that bacteria cannot live without them by synthesizing a long-lived killer and a short-lived antidote.

Host killing systems → Killer-Antidote

Taken from: J.E. Krebs, E.S. Goldstein, S.T. Kilpatrick; „Lewin´s Genes XI"; Jones&Bartlett Learning

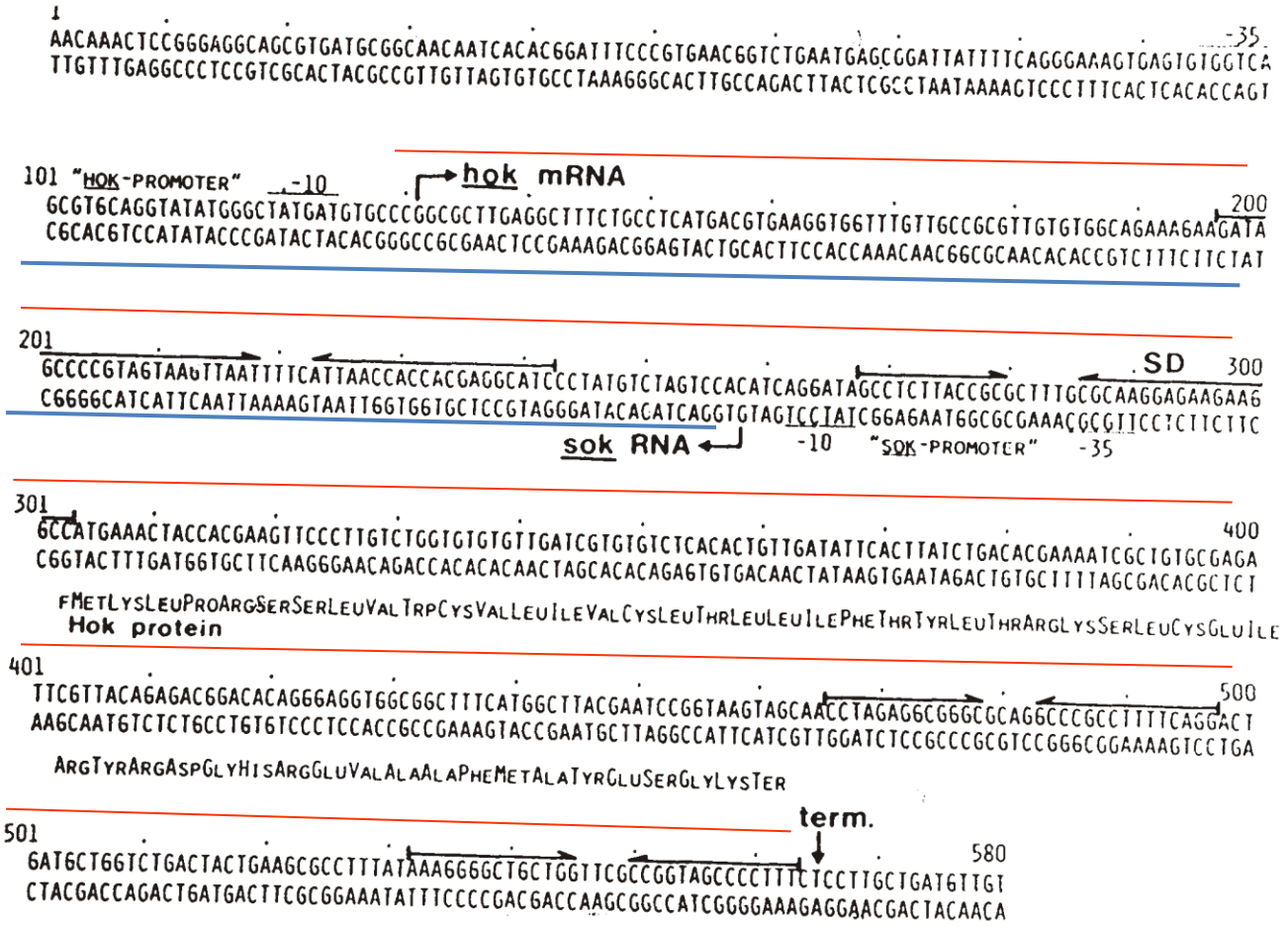
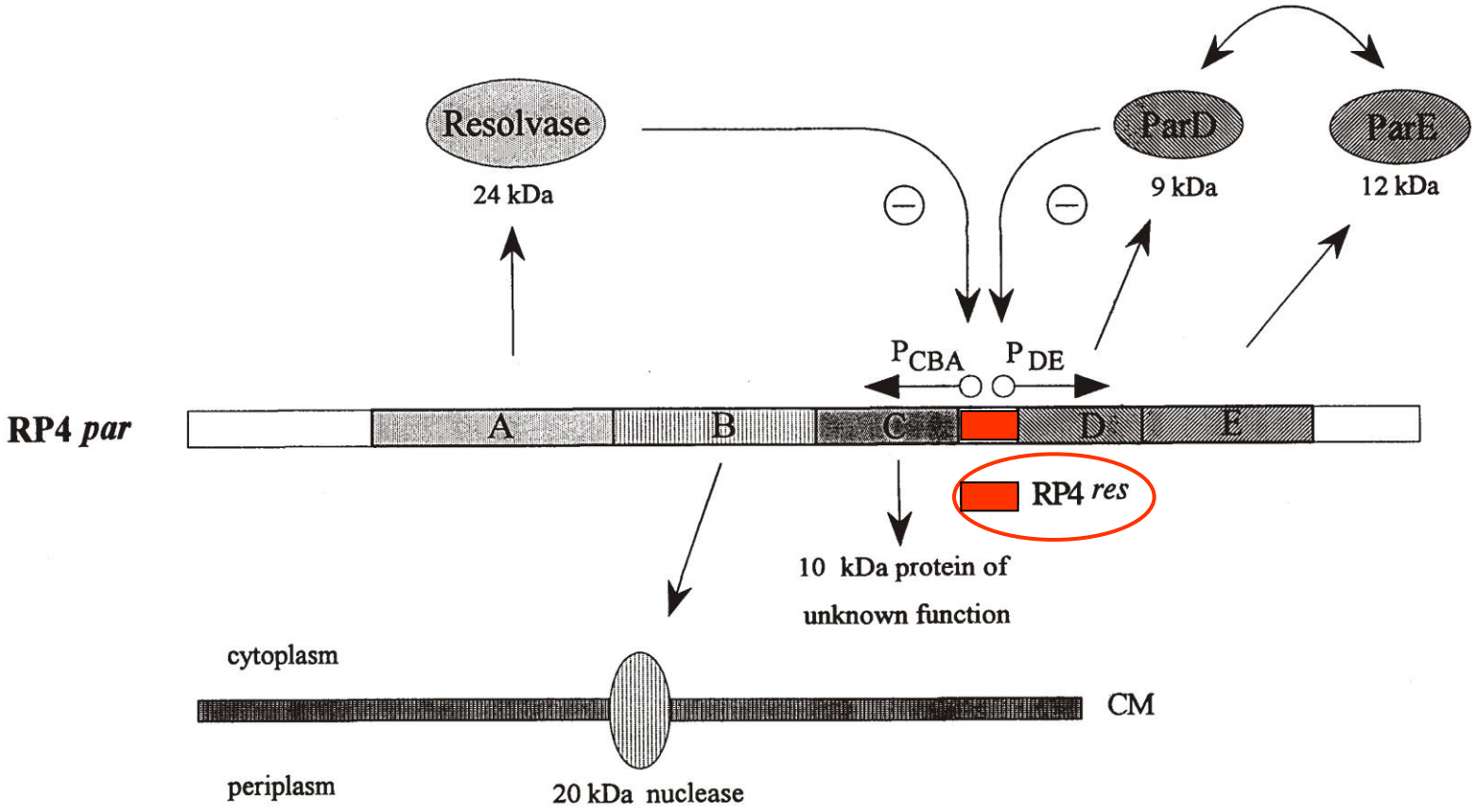


Fig. 5. Nucleotide sequence of the *parB* locus from plasmid R1. Shown are the location of the *hok* and *sok* genes encoding the toxin and antisense RNA, respectively. (Reproduced from Gerdes et al., 1986b, with permission of the publisher.)

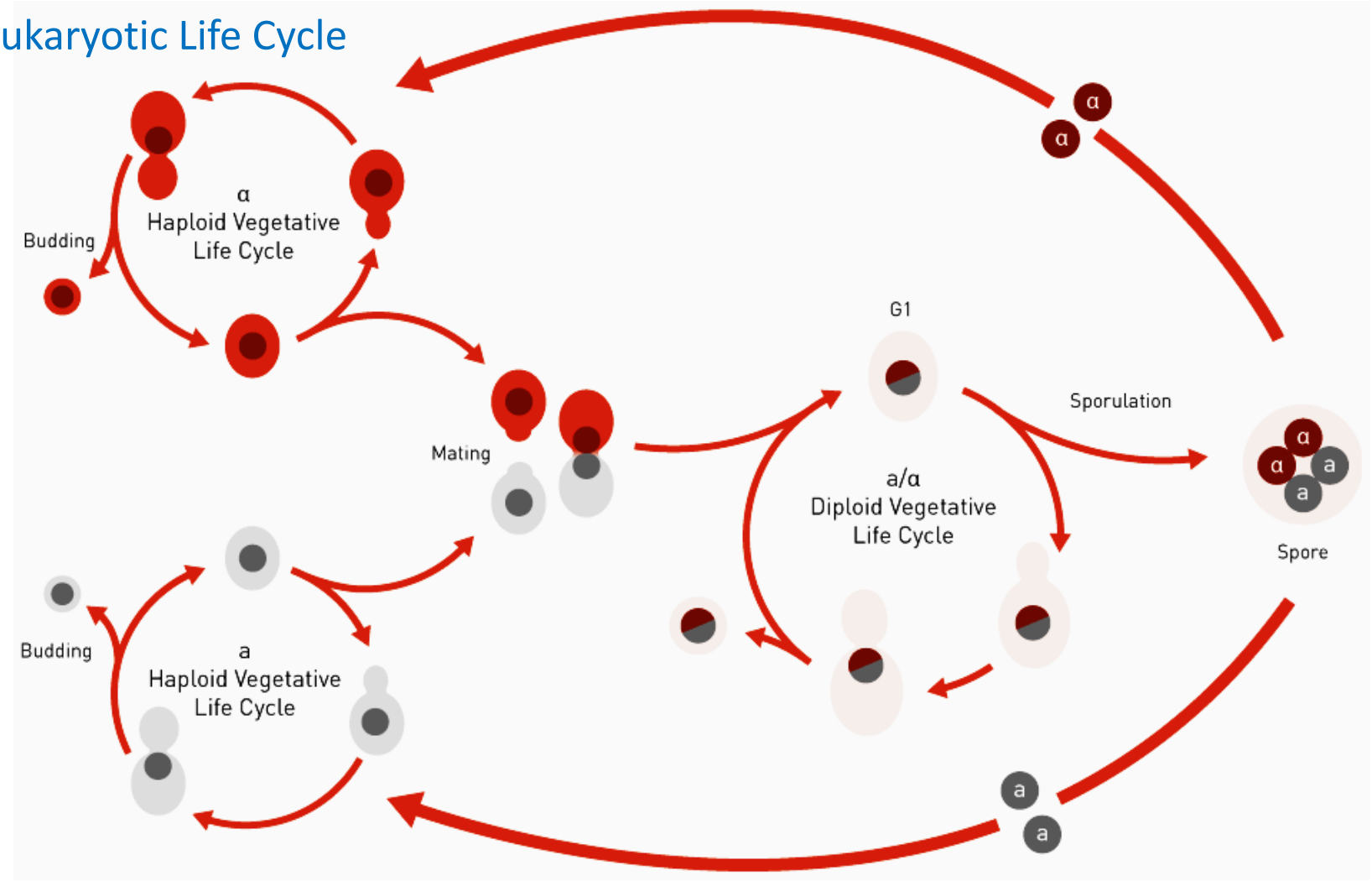
- Hok protein: toxic, kills cells
- Expression of Hok protein is triggered at the translational level by antisense RNA → *sok*
- *sok* RNA is less stable than *hok* RNA



Site specific resolution: *parA, (parB), res*

Killer – Antidote: *parE, parD*

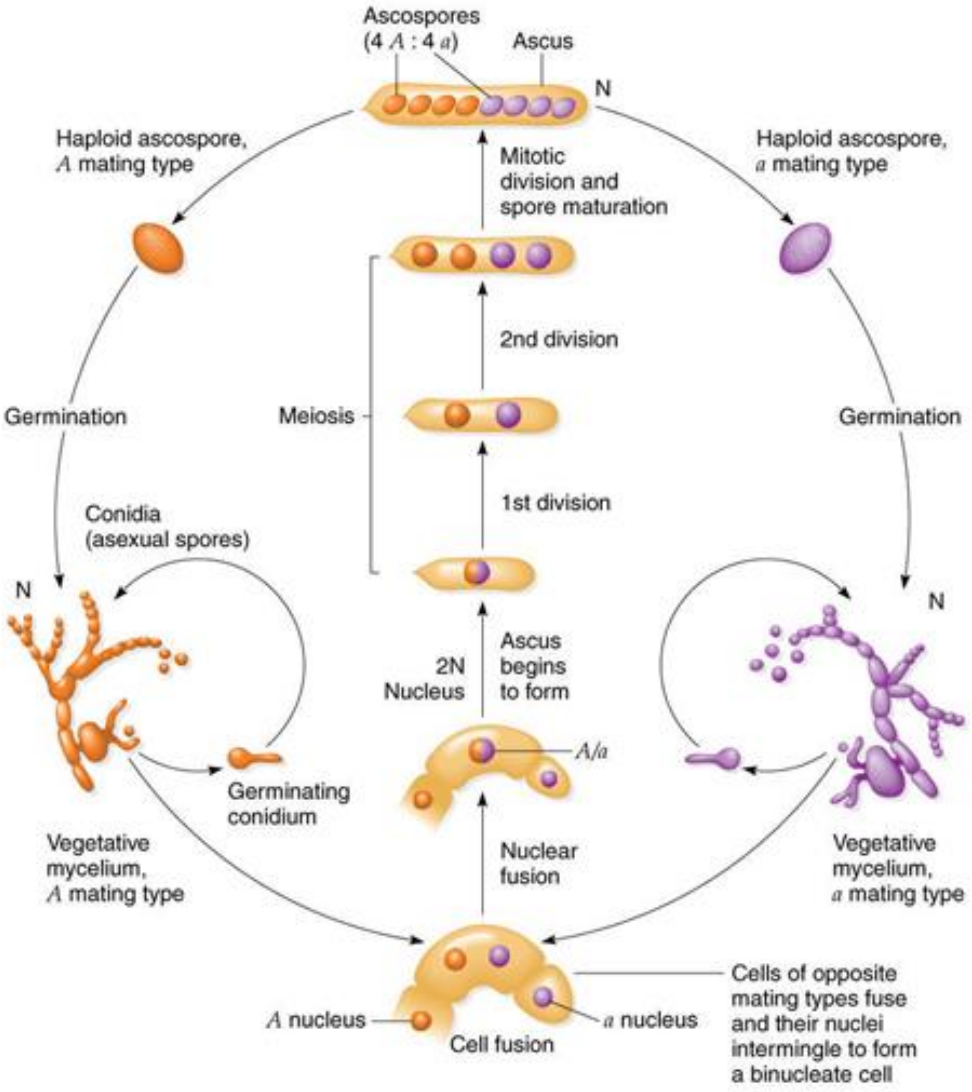
Eukaryotic Life Cycle



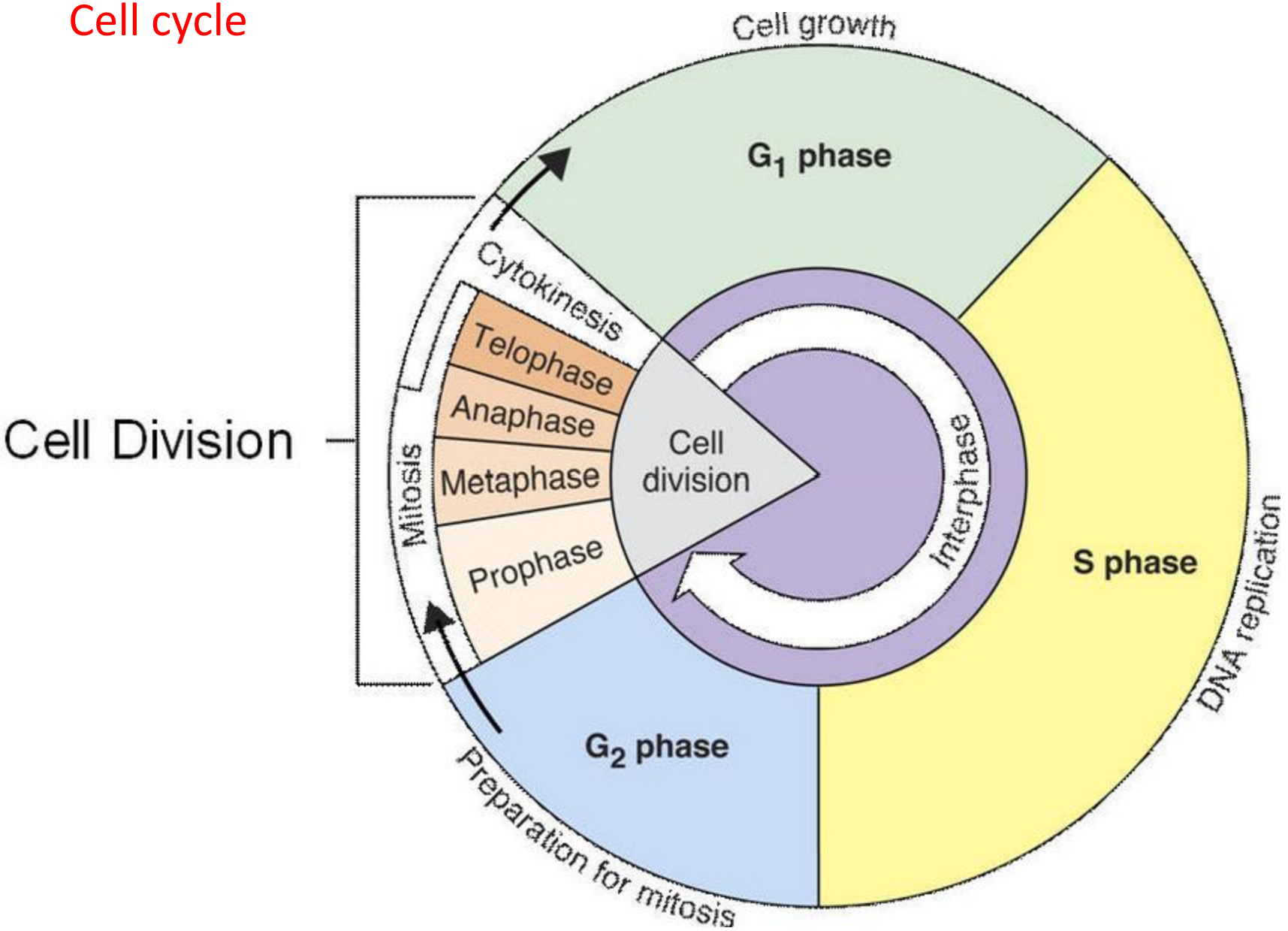
Yeasts have two opposite mating types (a and α for *S. cerevisiae*), and can exist in both haploid and diploid states. Depending on the environment, yeast undergo sexual or asexual reproductive life cycles to maintain or switch their ploidy. When nutrients are abundant, yeasts propagate using asexual reproduction. For *S. cerevisiae*, this is done via budding, where the daughter cell originates as a small bud on the mother cell and continues to grow until the daughter separates from the mother. This is why *S. cerevisiae* is commonly known as budding yeast. When nutrients are limiting or during other high-stress conditions, yeasts undergo meiosis to generate haploid spores, which are contained in an ascus (in the case of *S. cerevisiae*).

http://www.singerinstruments.com/resource/what-is-yeast/

Neurospora Life Cycle

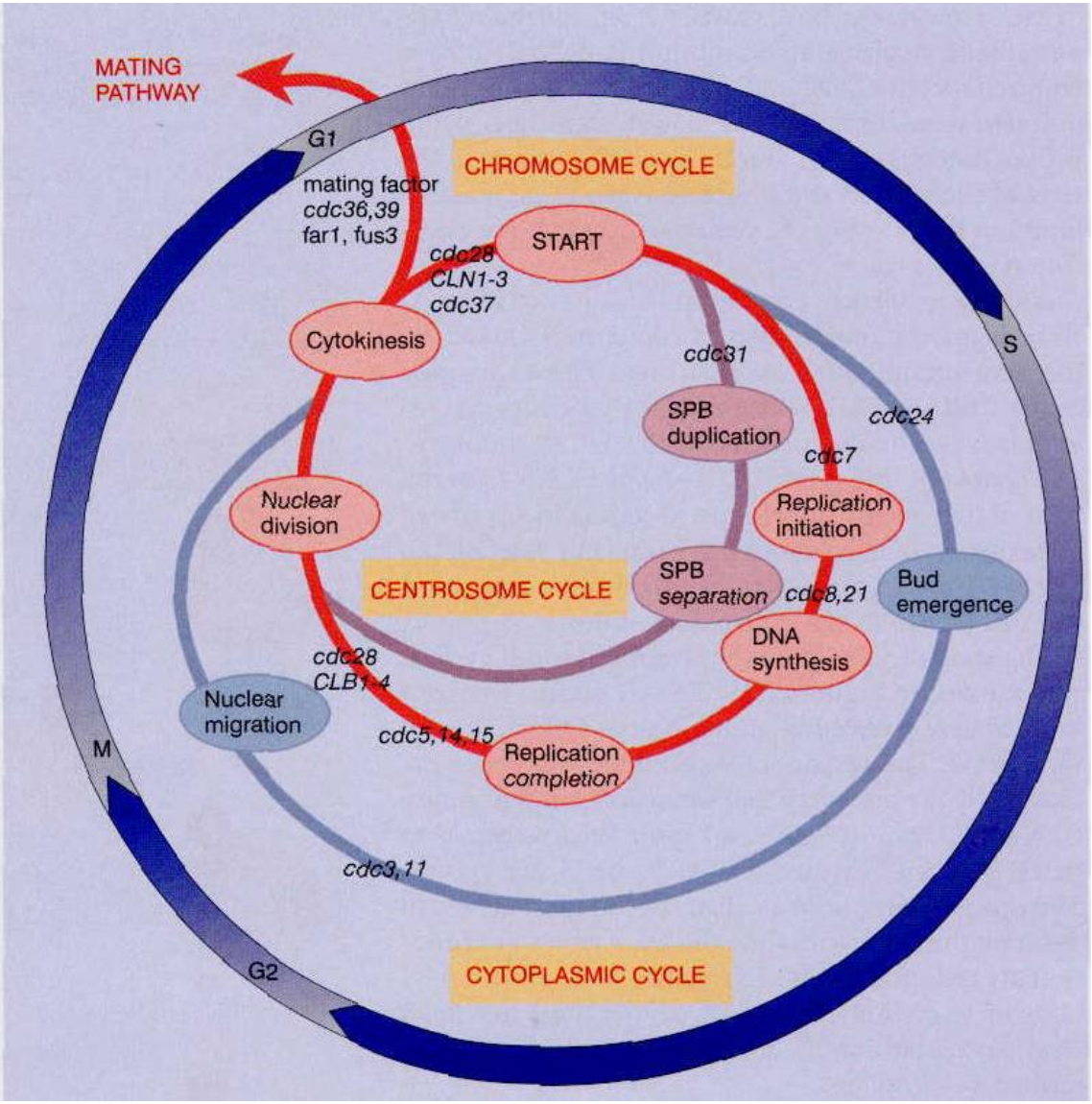


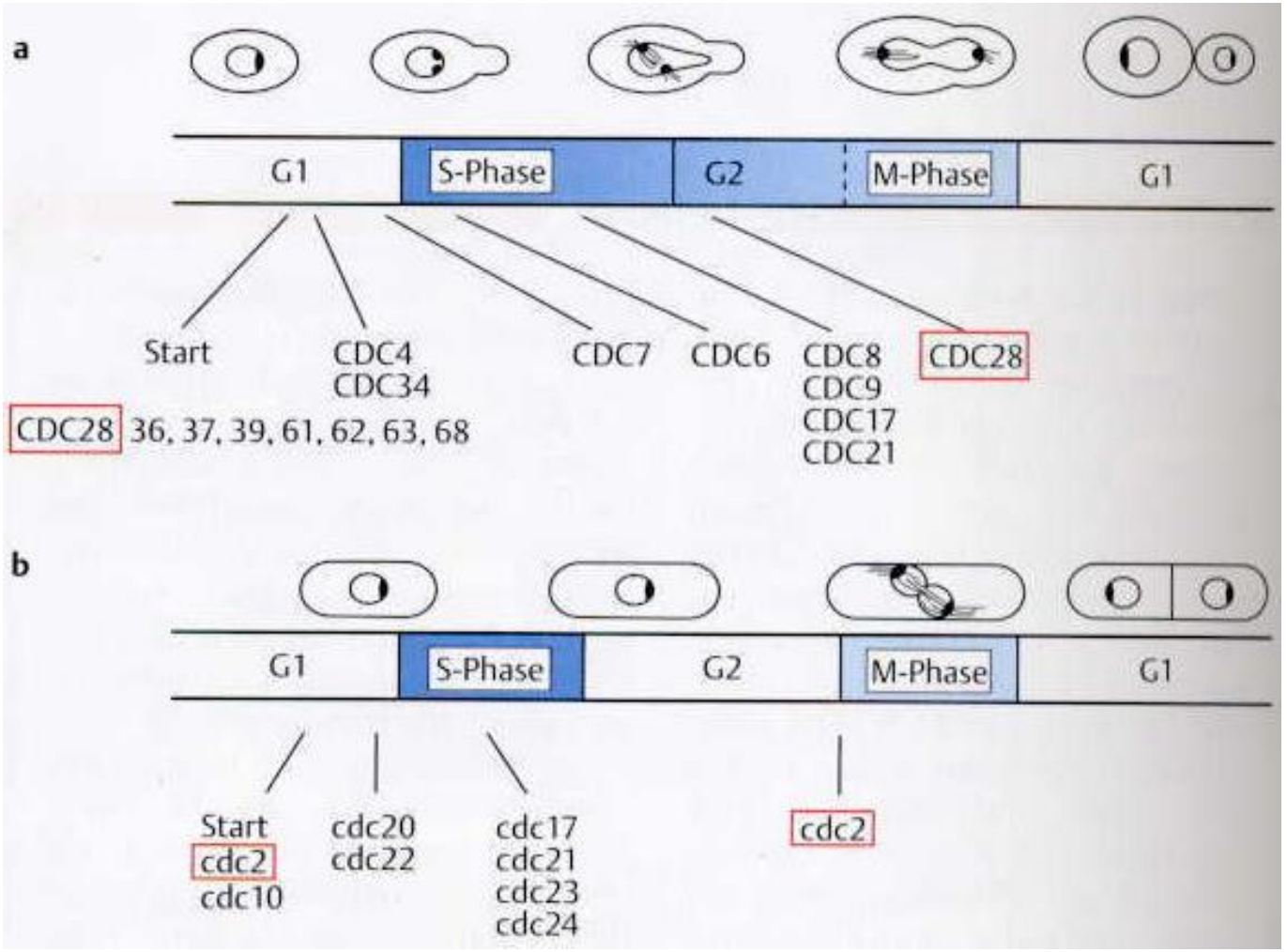
Cell cycle


Cell growth
G1 phase
Cytokinesis
Telophase
Anaphase
Metaphase
Prophase
Mitosis
Cell division
Interphase
S phase
DNA replication
G2 phase
Preparation for mitosis
Cell Division

Eukaryotic Life Cycle

Figure 27.18 The cell cycle in *S. cerevisiae* consists of three cycles that separate after START and join before cytokinesis. Cells may be diverted into the mating pathway early in G1.



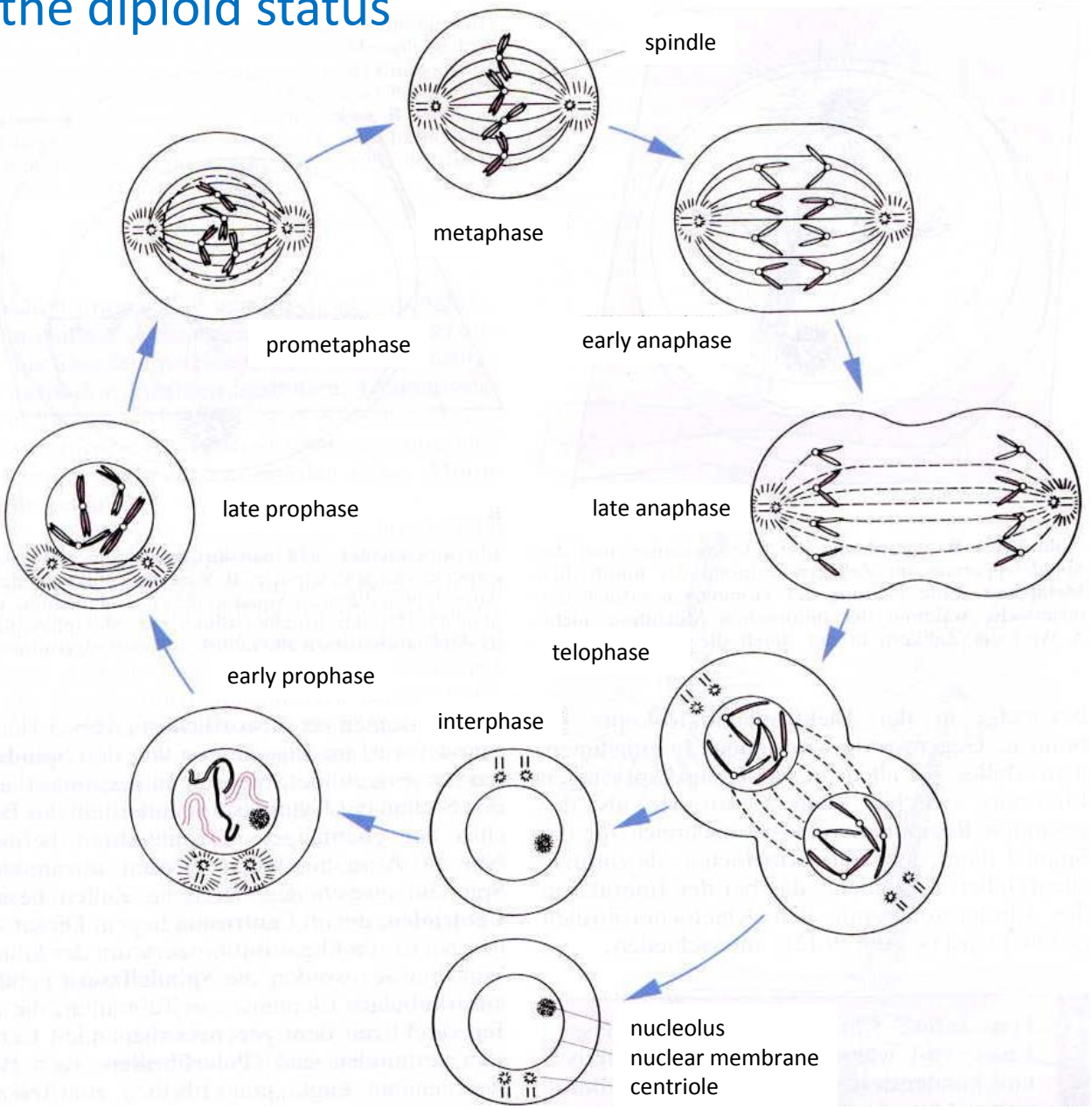


Cell cycle mutants. **a** *Saccharomyces cerevisiae* (baker´s yeast). The initiation of DNA replication, the duplication of the mitotic spindle and the formation of the bud occur approximately at the same time. Thus, S-phase, G2-phase and mitosis cannot be differentiated clearly from one another. The „daughter“ cell, arisen from the bud, is initially smaller than the „mother“-cell**. b** *Saccharomyces pombe* (fission yeast). Note that „START“ is overstepped as soon as the genes *CDC28* (*S. cerevisiae*) or *cdc2* (*S. pombe*) are active.

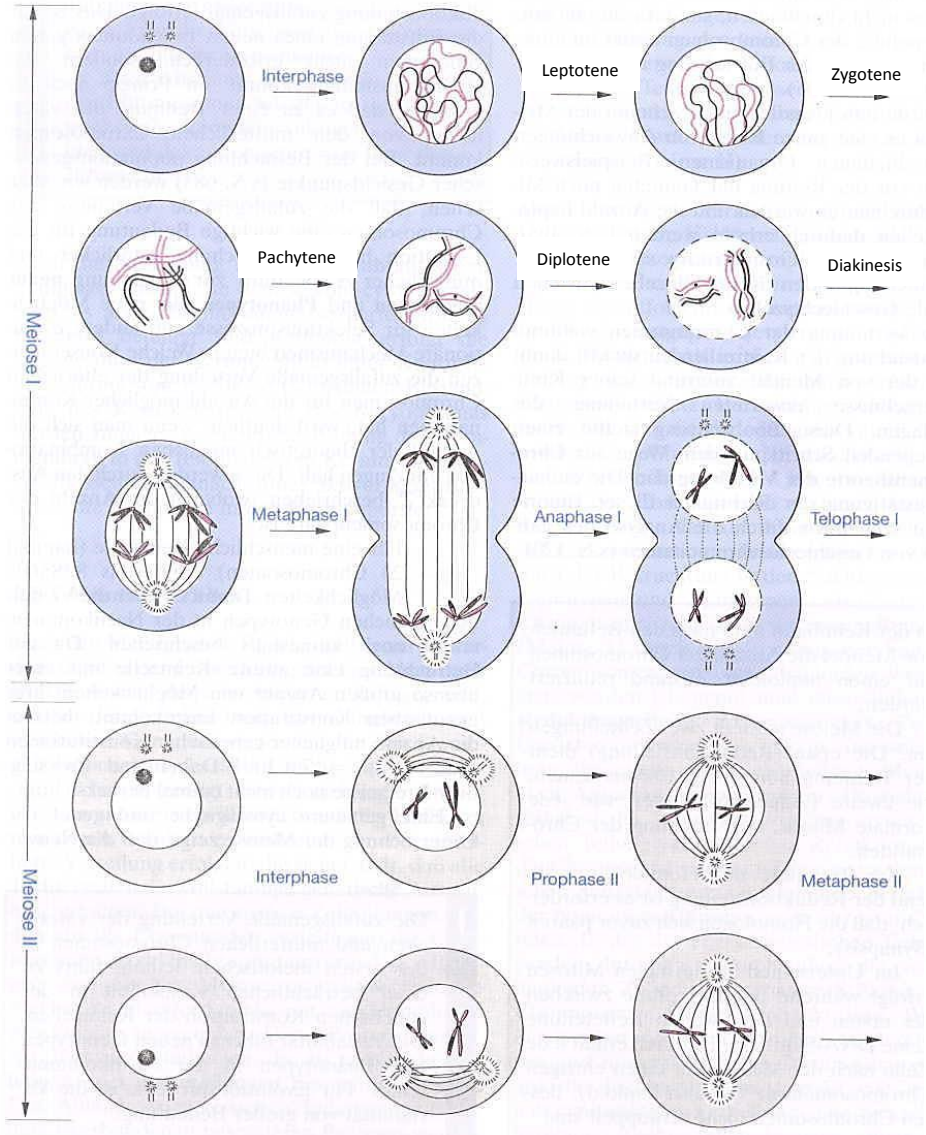
Taken from: R. Knippers, Molekulare Genetik, 9th Ed., Thieme

Mitosis: maintaining the diploid status

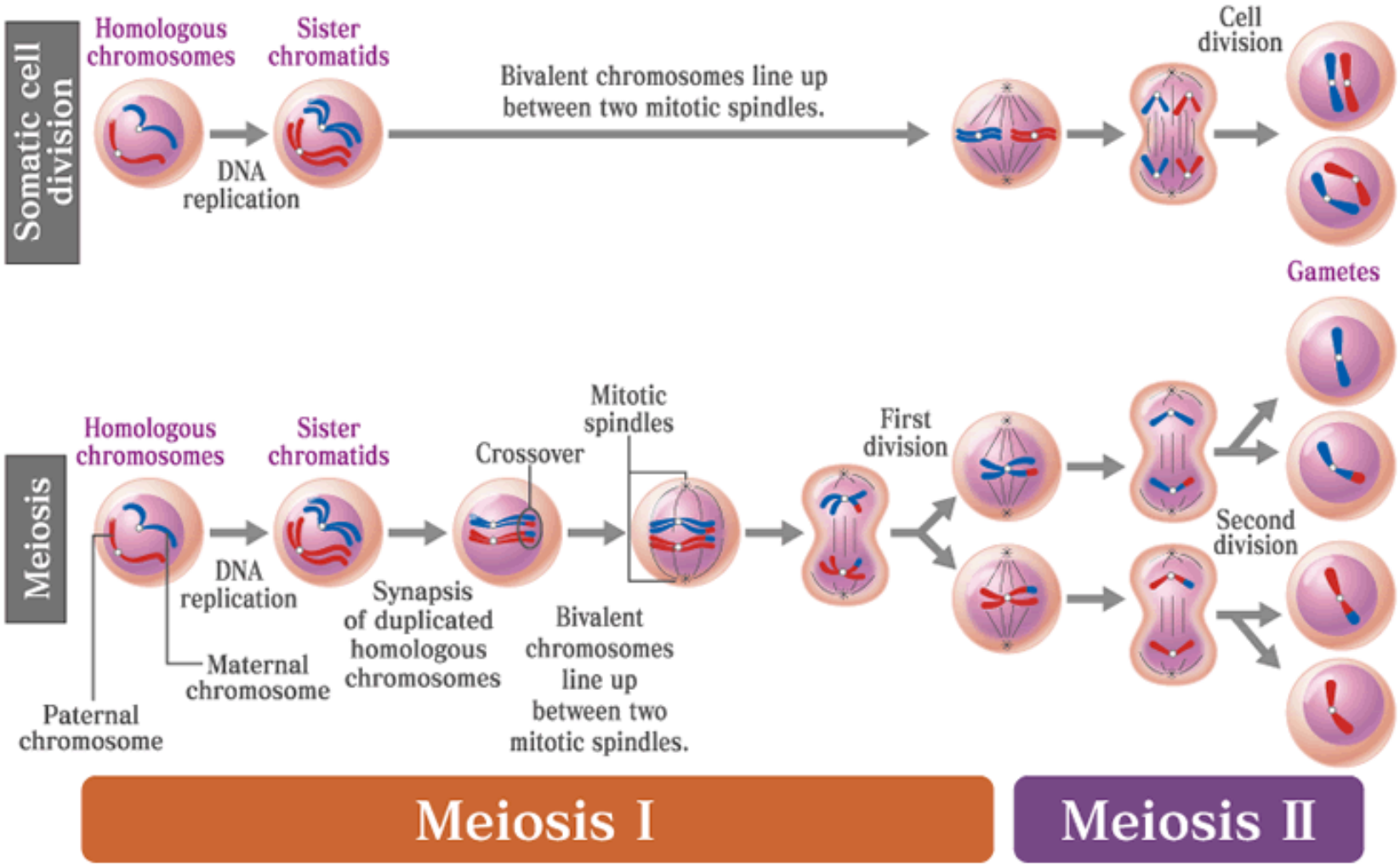
Mitosis. During the early prophase the centrioles move to opposite positions at the nuclear membrane and the chromatin starts to condense so that initially elongated chromosomes become visible. During the prophase, chromosomes contract more and more, the two chromatides become apparent and the nucleolus disintegrates. In the later prophase the nuclear envelope dissolves, the mitotic spindle forms and the chromosomes migrate to the equatorial plane of the former nucleus. In the metaphase, all chromosomes are located in the equatorial plane. Homologous chromosomes are in general distributed accidentally and unpaired. In the anaphase, the chromatides separate and migrate to opposite spindle poles. This ensures that each daughter cell gets a full set of chromosomes. In the late anaphase the chromatides are located close to the spindle poles and the constriction of the cell begins. In the telophase the new nuclear membrane is re-formed, centrioles duplicate and chromosome decondensation takes place. During the interphase, chromosomes decondensate and build a new chromatin matrix in the nucleus. The nucleolus was re-built. This scheme shows an animal cell.



Meiosis: reduction to haploid status



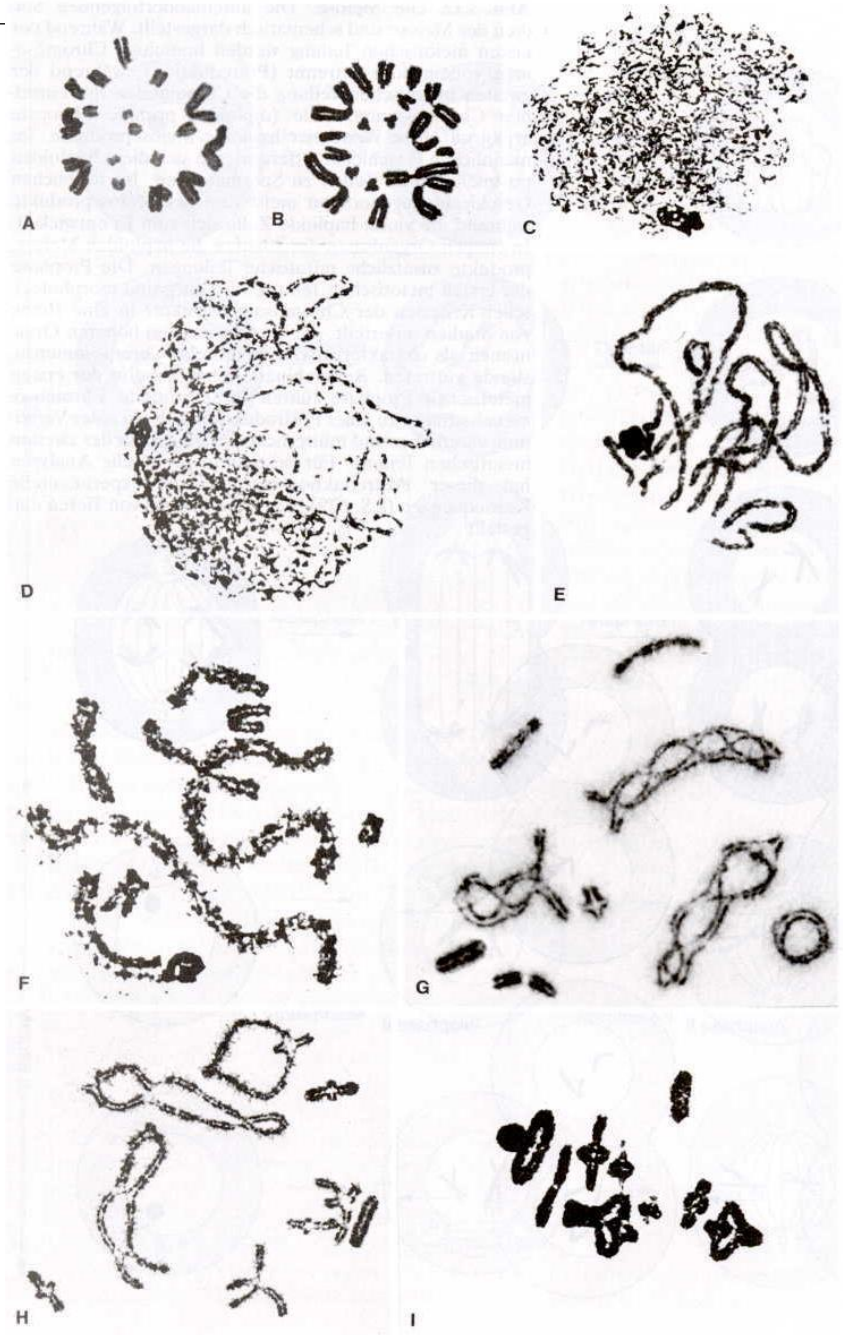
During the first meiotic division homologous chromosomes are separated (pre-reduction), during the second meiotic division chromatides of the single chromosomes are separated. Each diploid primary meiocyte results in four haploid meiosis products. In males, these four haploid post-meiotic cells develop into spermatozoa. In females, three of the haploid meiosis products degenerate whereas the fourth cell differentiates into an oocyte. In some organisms, the haploid post-meiotic cells undergo additional mitotic divisions. The prophase of the first meiotic division is morphologically divided into several stages which occur in most of the higher organisms as characteristic meiotic chromosome states. Recombination during the first meiotic prophase leads to a post-reduction of certain chromosome regions meaning to a distribution of paternal and maternal alleles not until the second meiotic division. For some genetic analyses, this post-reduction mechanism has experimental consequences. This scheme shows the meiosis of animals.



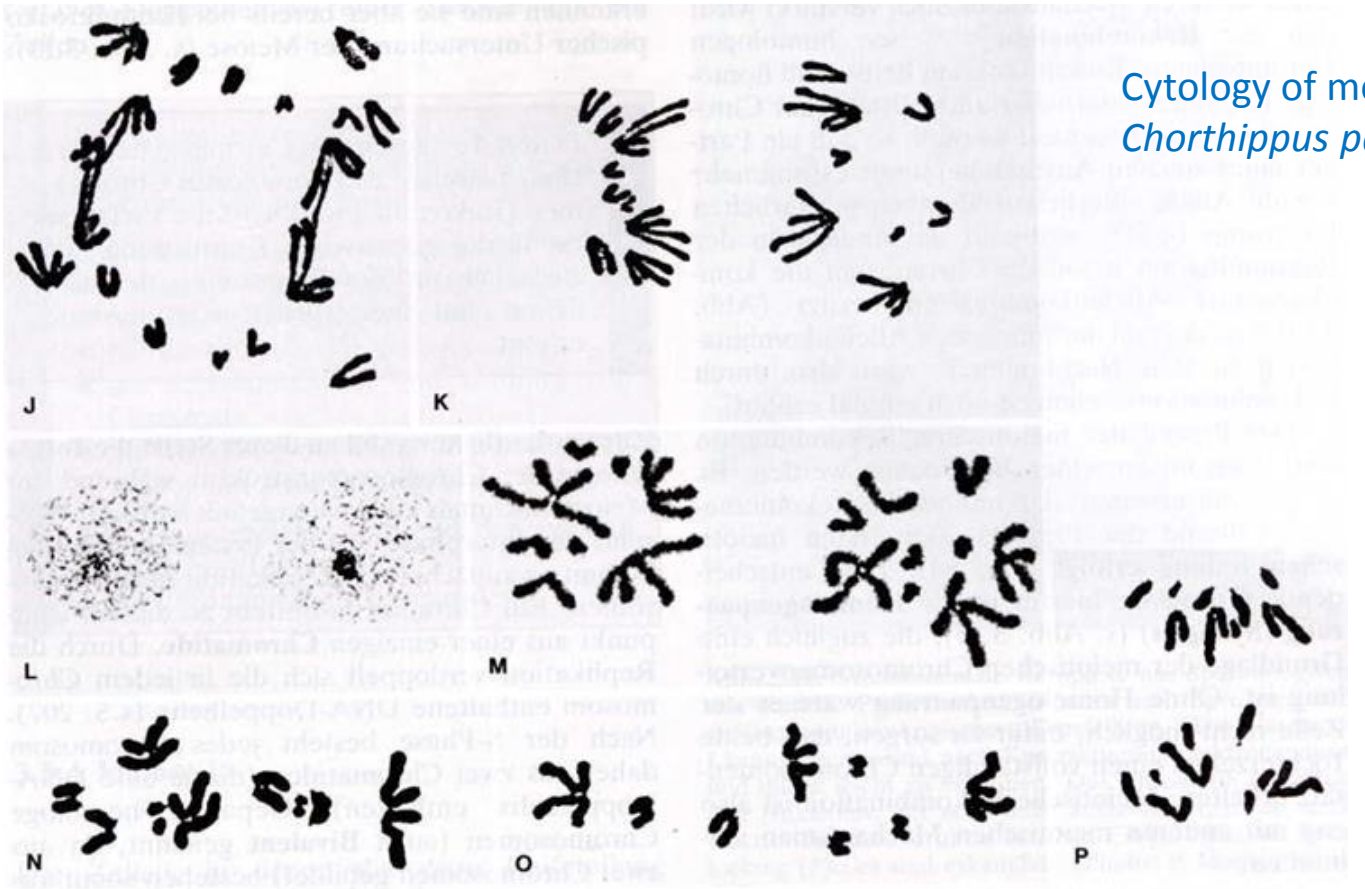
http://csls-text.c.u-tokyo.ac.jp/active/12_02.html

Cytology of meiosis of *Chorthippus parallelus*.

The male has 2n = 17 chromosomes (X/O), the female 2n = 18 chromosomes (X/X). **A** male and **B** female mitotic metaphase chromosomes. **C** Meiotic prophase I of the male. Leptotene. The X-chromosome is heterochromatic and condensed. **D** Meiotic prophase I, zygotene. The pairing of the chromosomes has begun, but is still incomplete. At higher magnification, the chromomeres are visible. **E** Meiotic prophase I. Zygotene. The pairing of the homologues is done, thus, eight bivalents are visible. **F** Meiotic prophase I. Pachytene. The chromosome pairs are significantly thickened and shortened. **G** Meiotic prophase I. Diplotene. Each bivalent is clearly four-stranded and chiasmata are visible. One chromosome pair is prematurely condensed (chromosome 6) as well as the X chromosome. **H** Meiotic prophase I. Diakinesis. Chromosomes contract by gradual condensation, chiasmata are partially terminated. **I** Meiotic metaphase I. Chromosomes are located in the equatorial plane of the spindle and centromeres of each bivalent are orientated above and below the equatorial plane towards the spindle poles. The X-chromosomes, which is unpaired, moves towards one spindle pole.



Cytology of meiosis of *Chorthippus parallelus*.



J Meiotic anaphase I. Most of the bivalents are separated and move towards the spindle poles. Solely the long arms of the biggest bivalents still touch the equatorial plane. **K** Meiotic late anaphase I. Chromosomes are located at the spindle poles. Both chromatids of each chromosome are visible. One of the forming secondary spermatocytes contains the X-chromosome, the other has no sex chromosome. **L** Meiotic interphase (interkinesis). Chromosomes are largely de-condensed. Only the X-chromosome in the right secondary spermatocyte nucleus is condensed. **M** Meiotic prophase II. Chromatids of each chromosome are completely separated and solely remain linked at the centromer. The X-chromosome is still more condensed than the other chromosomes. **N** and **O** Meiotic metaphase II. Chromosomes are located along the equatorial plane of the spindle. **P** Meiotic anaphase II. Chromatids spread to the spindle poles and form 2 haploid nuclei.