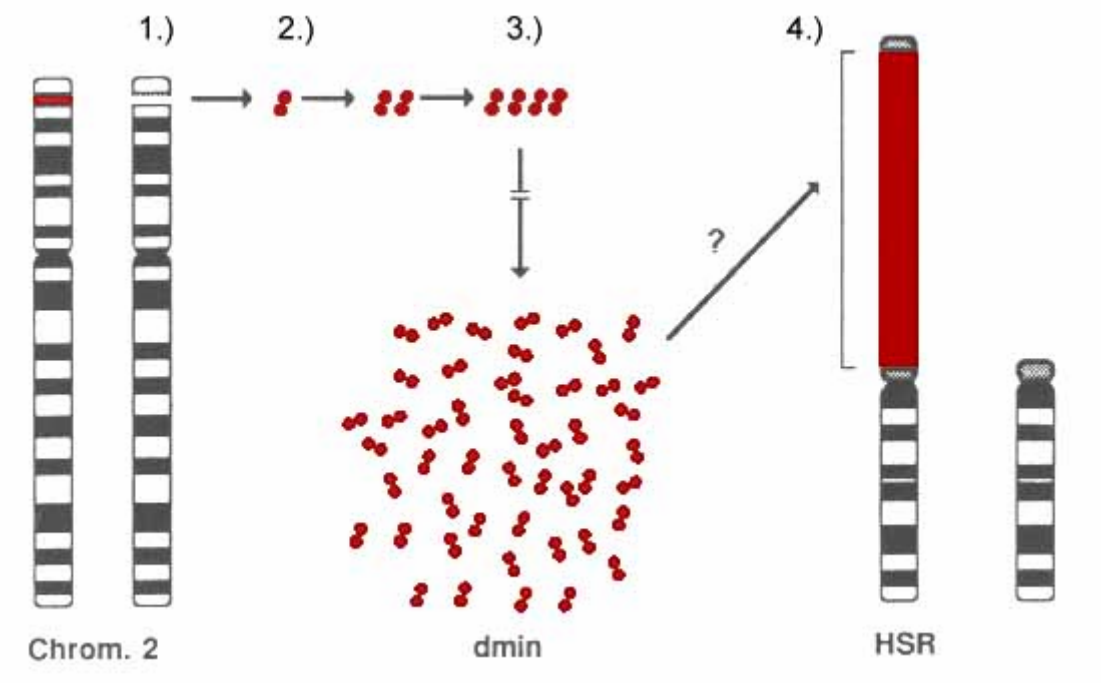
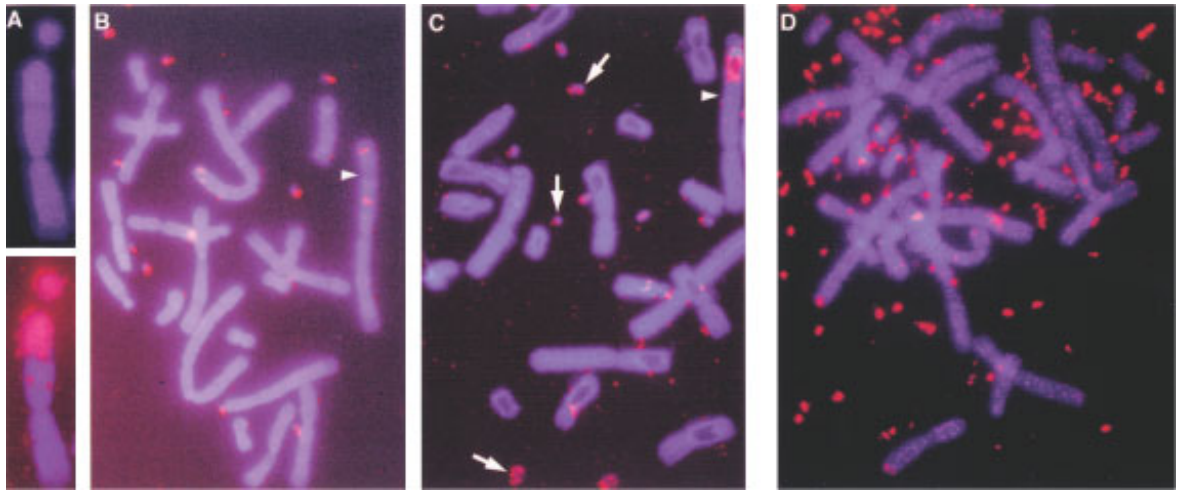
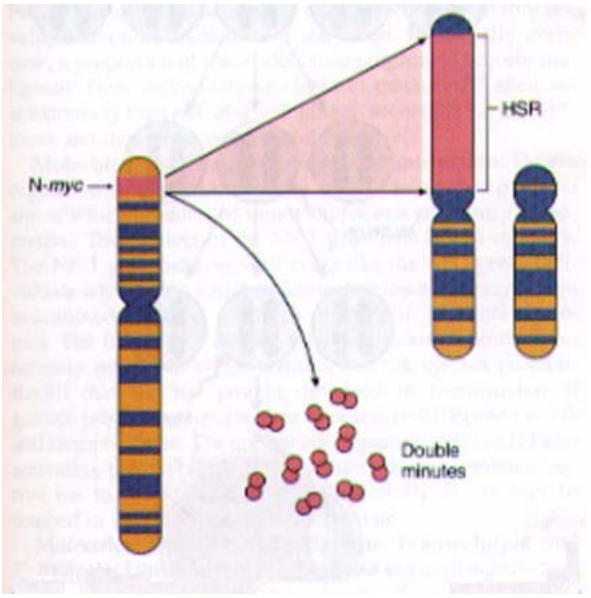
„Double minutes“ und HSR (homgeneously staining region)







HSR → dmin upon DS break induced by a homing endonuclease (I-SceI).

Arnaud Coquelle, Lorène Rozier, Bernard Dutrillaux and Michelle Debatisse
ONCOGENE, 31 October 2002, Volume 21, Number 50, Pages 7671-7679
Induction of multiple double-strand breaks within an hsr by meganucleaseI-SceI expression
or fragile site activation leads to formation of double minutes and other chromosomal rearrangements

DNA-Amplifikation in Tumorzellen

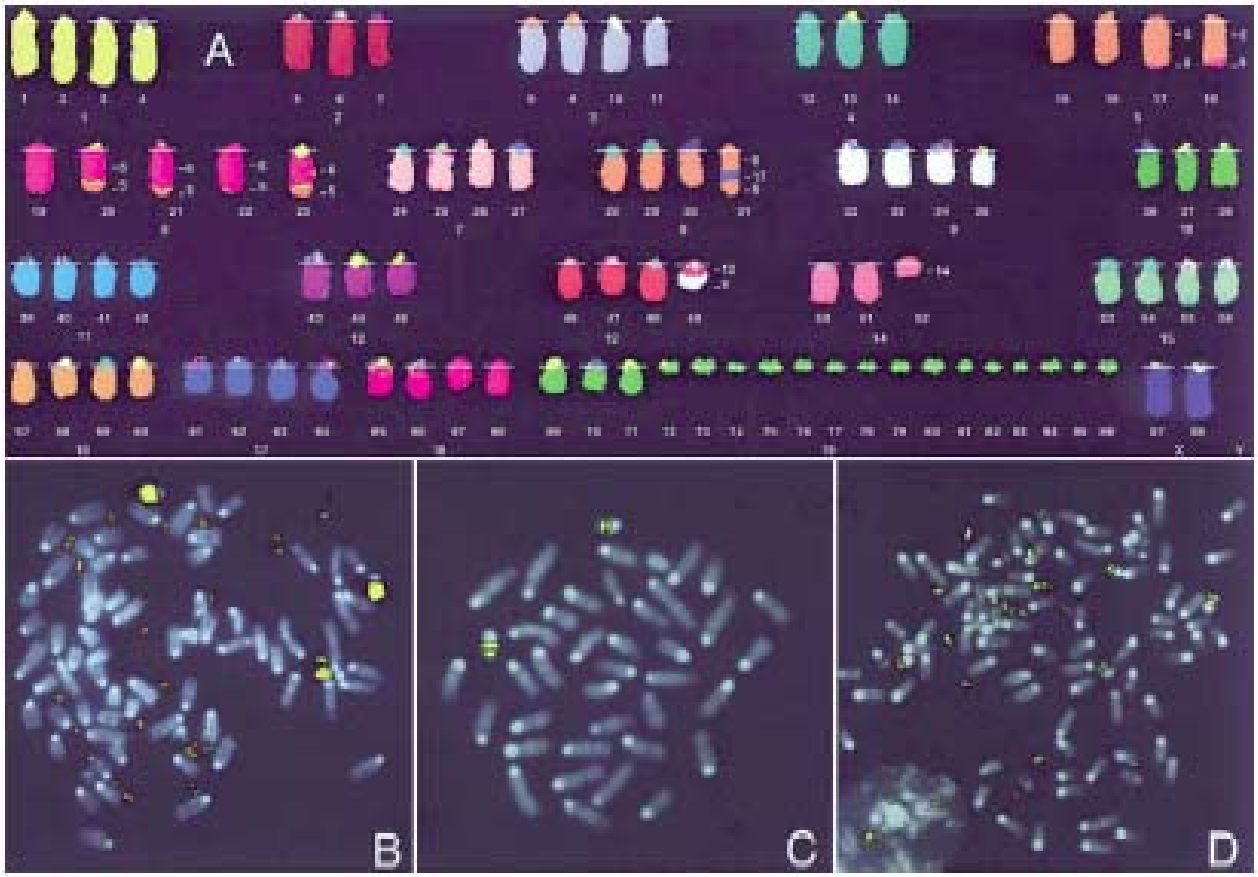
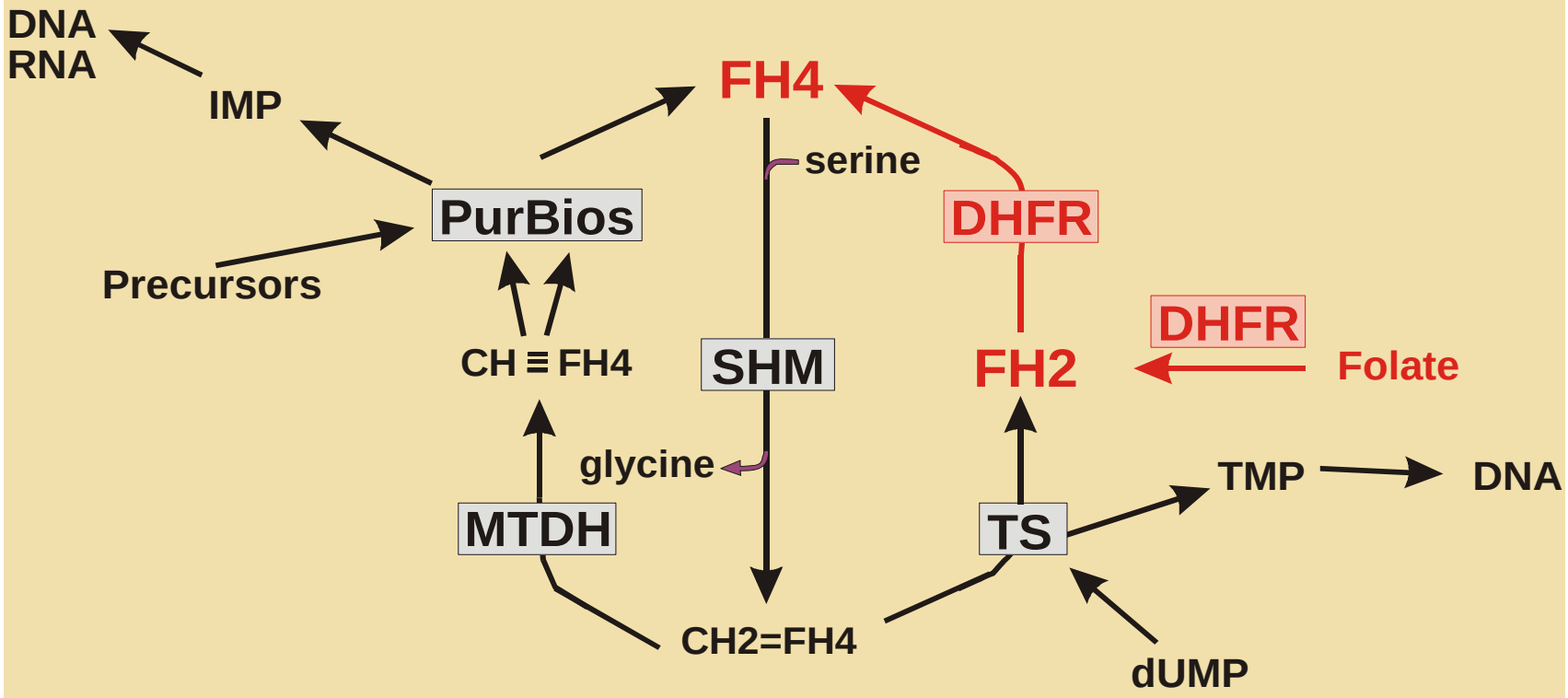
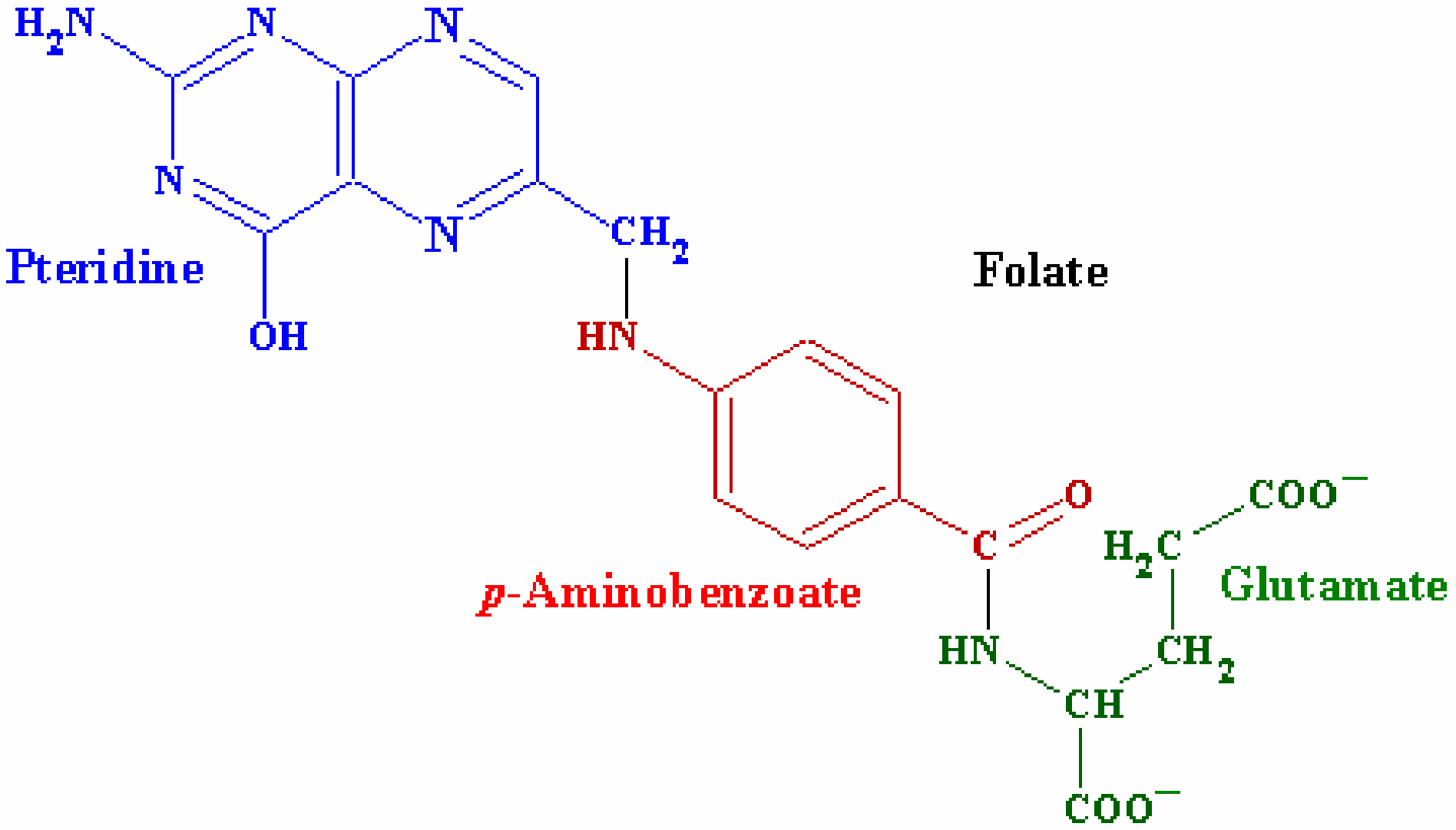


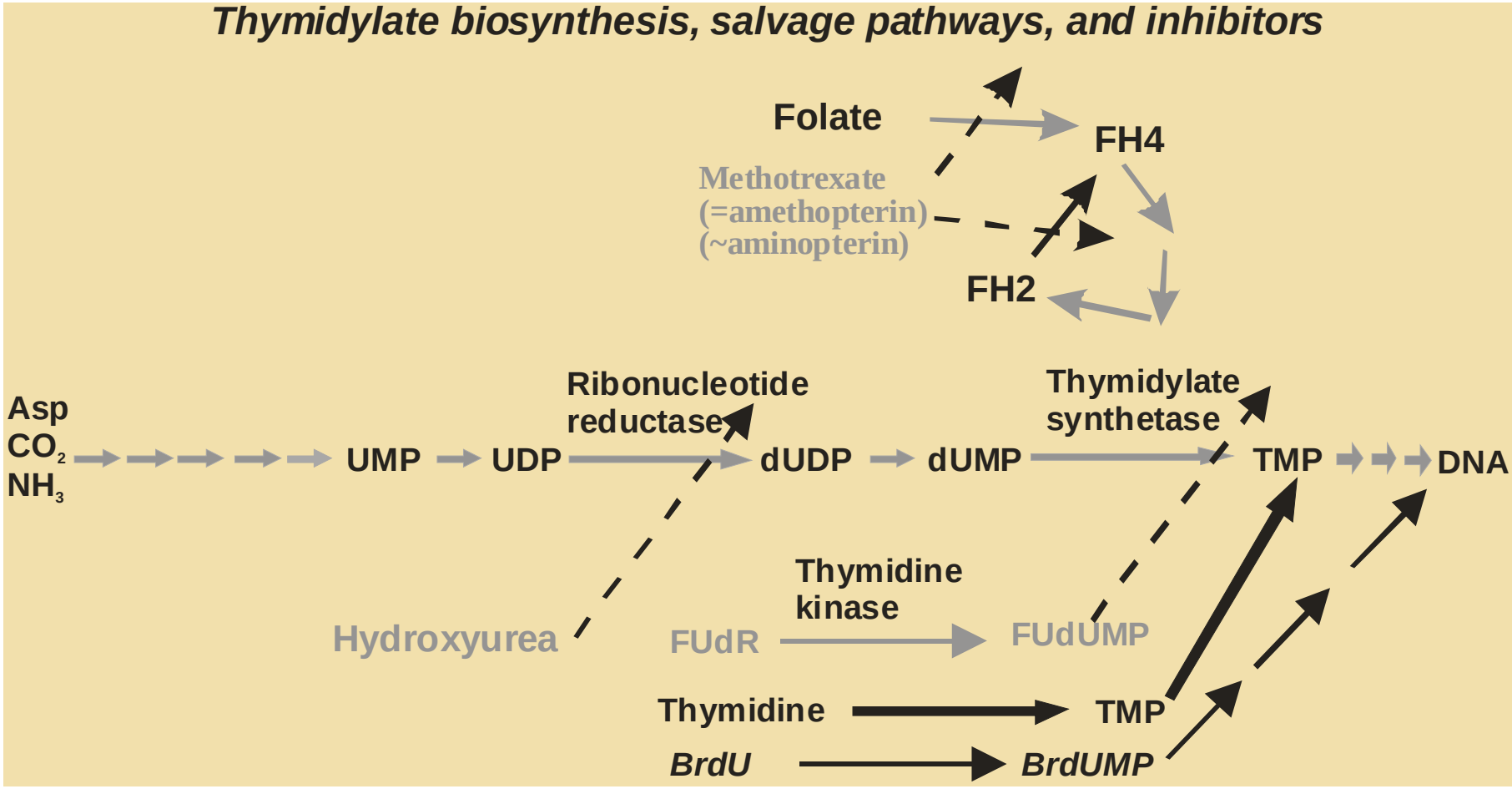
Fig. 1. SKY and FISH analysis of the HCC-myc 3 cell line. (A) Spectral classified karyotype of a hypotetraploid cell showing numerical and structural abnormalities among which are the presence of 15 pairs of double minutes derived from chromosome 19. (B) Metaphase after hybridization with chromosome 19 painting probe confirming the origin of DM. Four copies of chromosome 19 and DM are labeled with the painting probe. (C) Normal metaphase from stimulated mouse spleen cell culture hybridized with a biotinylated PCR amplified DNA probe of microdissected DM showing fluorescent signal at regions B1.1–B1.3 and D1–D2.1 of both chromosomes 19. (D) FISH with microdissected DM probe of a metaphase from HCC-myc-3 cell line showing labeling at two sites of chromosome 19 and DM.

Reduction of folate to tetrahydrofolate



DHFR:	**Dihydrofolate reductase**
SHM:	**Serine hydroxymethyltransferase**
TS:	**Thymidylate synthetase**
PurBios:	**Purine nucleotide biosynthetic pathway**
MTDH:	**Methylenetetrahydrofolate dehydrogenase**


Folate
Pteridine
p-Aminobenzoate
Glutamate


Thymidylate biosynthesis, salvage pathways, and inhibitors
Folate
FH4
Methotrexate
(=amethopterin)
(~aminopterin)
FH2
Asp
CO2
NH3
UMP
UDP
Ribonucleotide
reductase
dUDP
dUMP
Thymidylate
synthetase
TMP
DNA
Hydroxyurea
FUdR
Thymidine
kinase
FUdUMP
Thymidine
TMP
BrdU
BrdUMP

Methotrexate Resistance by DHFR Amplification

CHO cells

↓ mtx

↓ mtx

↓ mtx

Colonies of mtx resistant CHO cells

↓

Prepare Genomic DNA

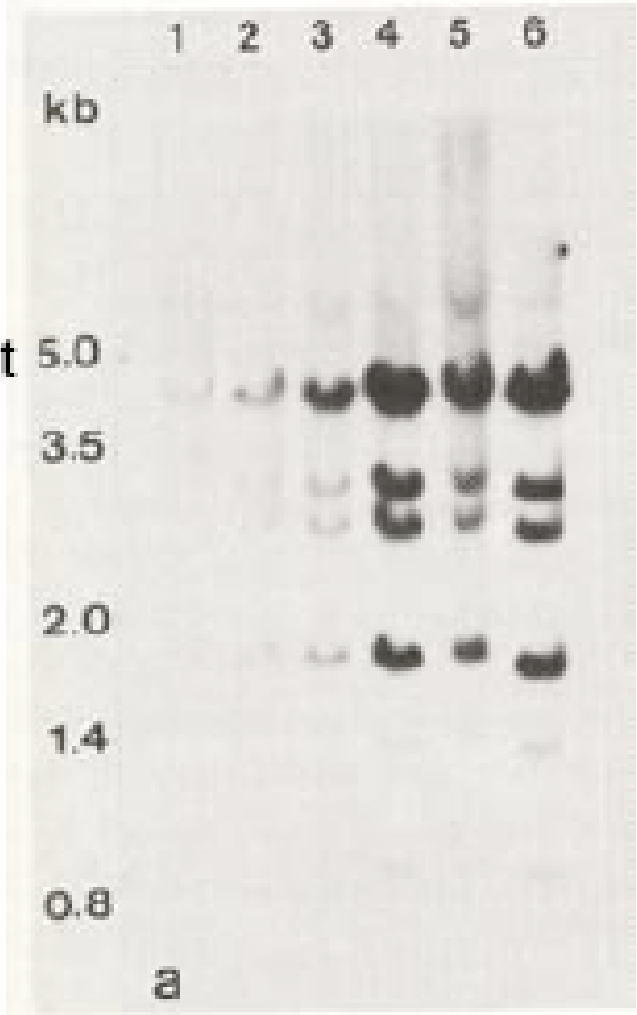
↓

EcoRI digest

↓

Southern Blot using DHFR cDNA probe

Lane 1 Parental CHO cells
Lanes 2-6 mtx resistant cells



Copy Number Per haploid Genome

Lane 1	1.0
Lane 2	3.0
Lane 3	6.7
Lane 4	39
Lane 5	17
Lane 6	53

Adapted from:
Johnston et al
PNAS 80, 3711

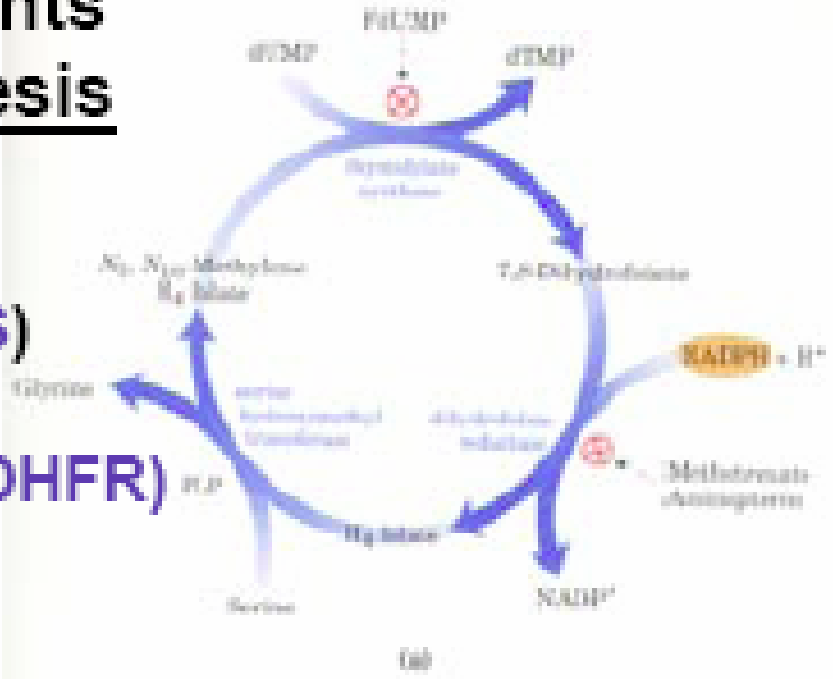
Chemotherapeutic Agents Targeting dTMP Synthesis

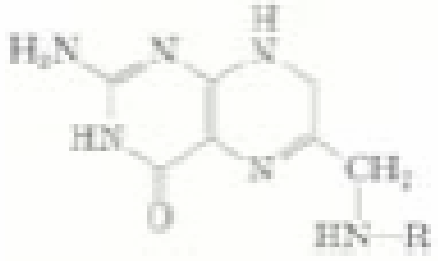
Fluorouracil → FdUMP

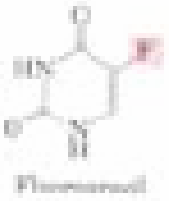
Thymidiylate Synthase (TS)

Dihydrofolate Reductase (DHFR)

Methotrexate (mtx) is a competitive inhibitor of DHFR







Adapted from:
Lehninger
"Principles of
Biochemistry"

Causes of Cancer: Replication Errors

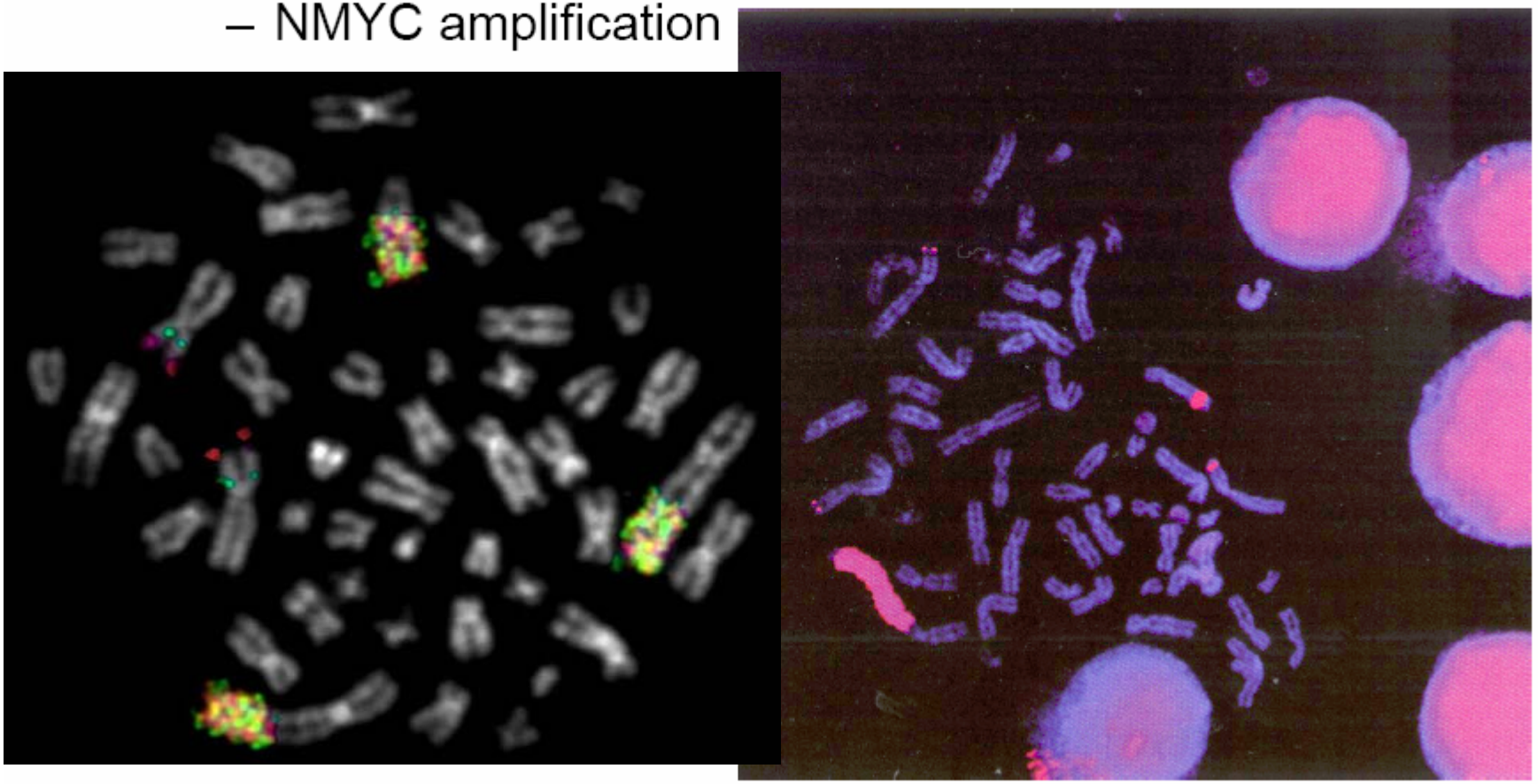
- Gene amplification
  - Over expression of oncogenes

Oncogene	Tumour	Amplification
MYC	Promyelocytic leukemia	20x
MYC	Small-cell lung carcinoma	5→30x
MYCN	Neuroblastoma	>100x
MYB	Acute myeloid leukemia	10x
MYB	Colon carcinoma	10x
EGFR	Epidermal carcinoma	30x
KRAS	Lung cancer	10x

  - Leads to…
    - Homogenously Stained Regions (HSR)
    - Double minutes (DM)

Causes of Cancer

- Homogenously Stained Regions (HSR)
  - NMYC amplification

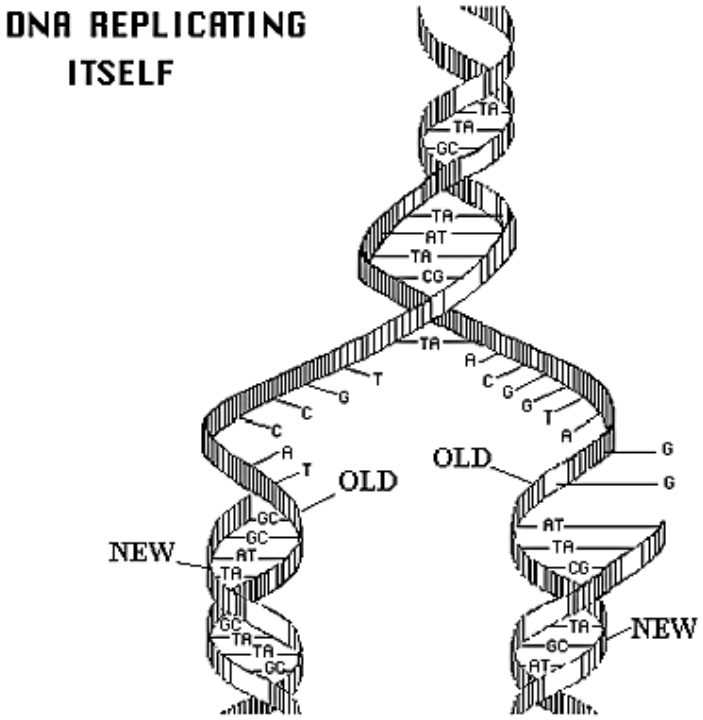


'Molecular and Genetic Analysis of Human Traits' Maroni (2001)

Andere amplifizierte Gene

Adenosine deaminase	ADA	deoxycoformycin	MTX + adenosine
Ornithine decarboxylase	ODC	difluoromethyl-ornithine	Polyamine-free
Asparagine synthetase	AS		Asparagine-free
Ribonucleoside reductase	RR	hydroxyurea	Deoxynuceloside-free
Tri-functional pyrimidine synthetic enzyme	CAD	PALA N-(phosphonacetyl)-L-aspartate	Pyrimidine-free
Thymidylate synthetase	TS	FUdr	Thymidine-free
Dihydrofolate reductase	DHFR	MTX	Gly-, TdR-, purine-free
Glutamine synthetase	GS	Methionone sulfoxime	Gln-free

DNA-Replikation
bei Pro-und Eukaryoten



Die DNA-Replikation findet in der S-Phase des Zellzyklus statt

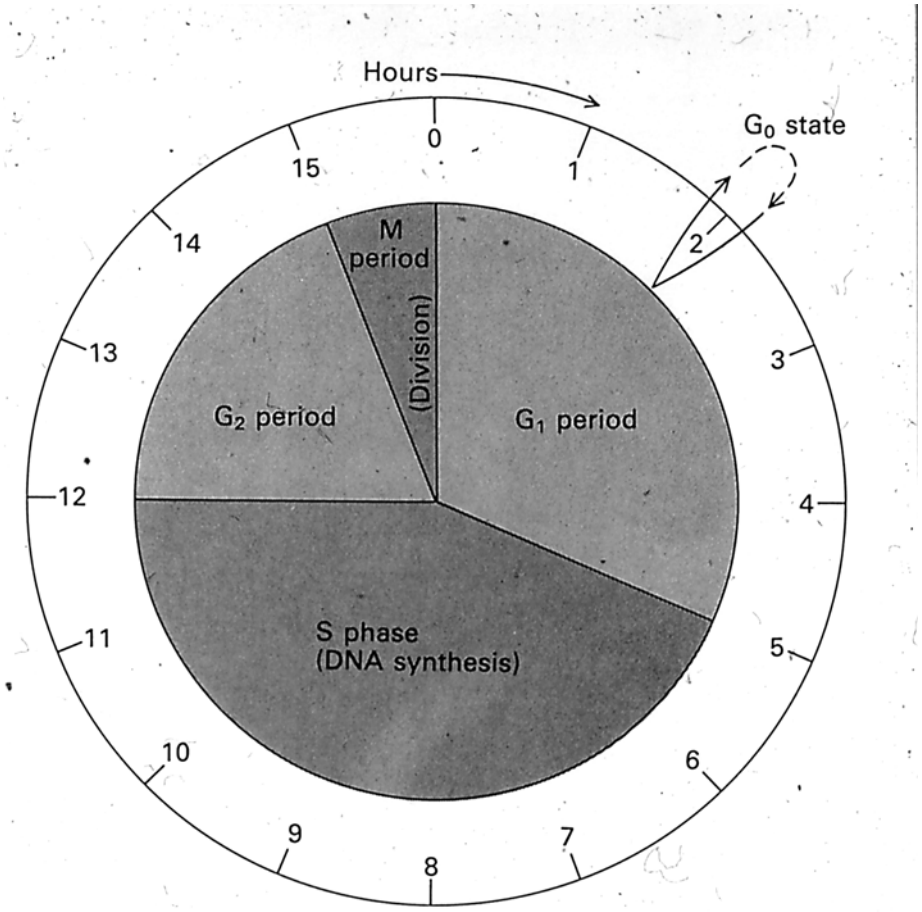


Figure 5-13 The cell cycle in a mammalian cell having a generation time of 16 h. The three periods spanning the first 15 h or so— the G_1 (first gap) period, the S (synthetic) phase, and the G_2 (second gap) period—make up the interphase, during which DNA and other cellular macromolecules are synthesized. The remaining hour is the M (mitosis) period; it is during this period that the cell actually divides.

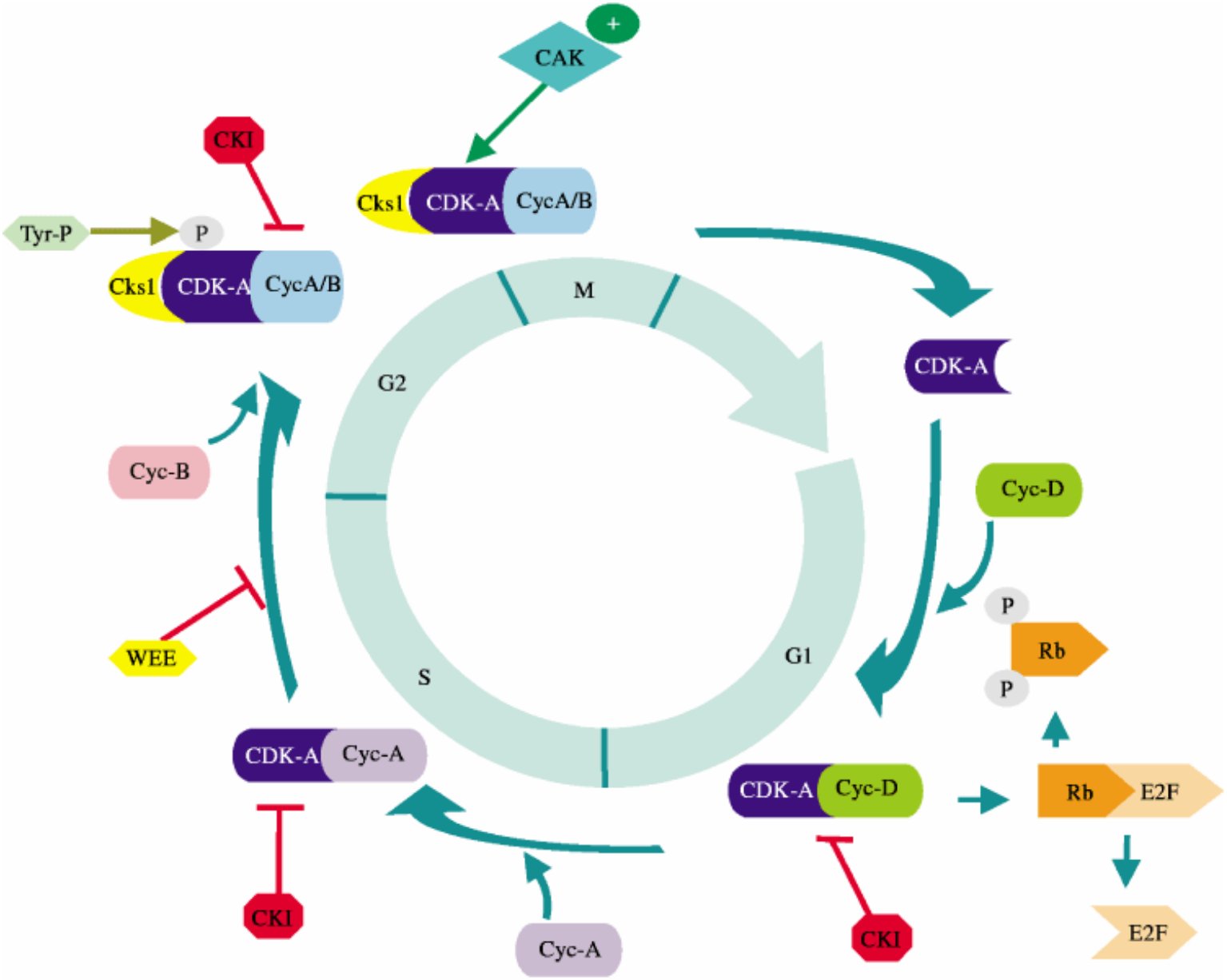
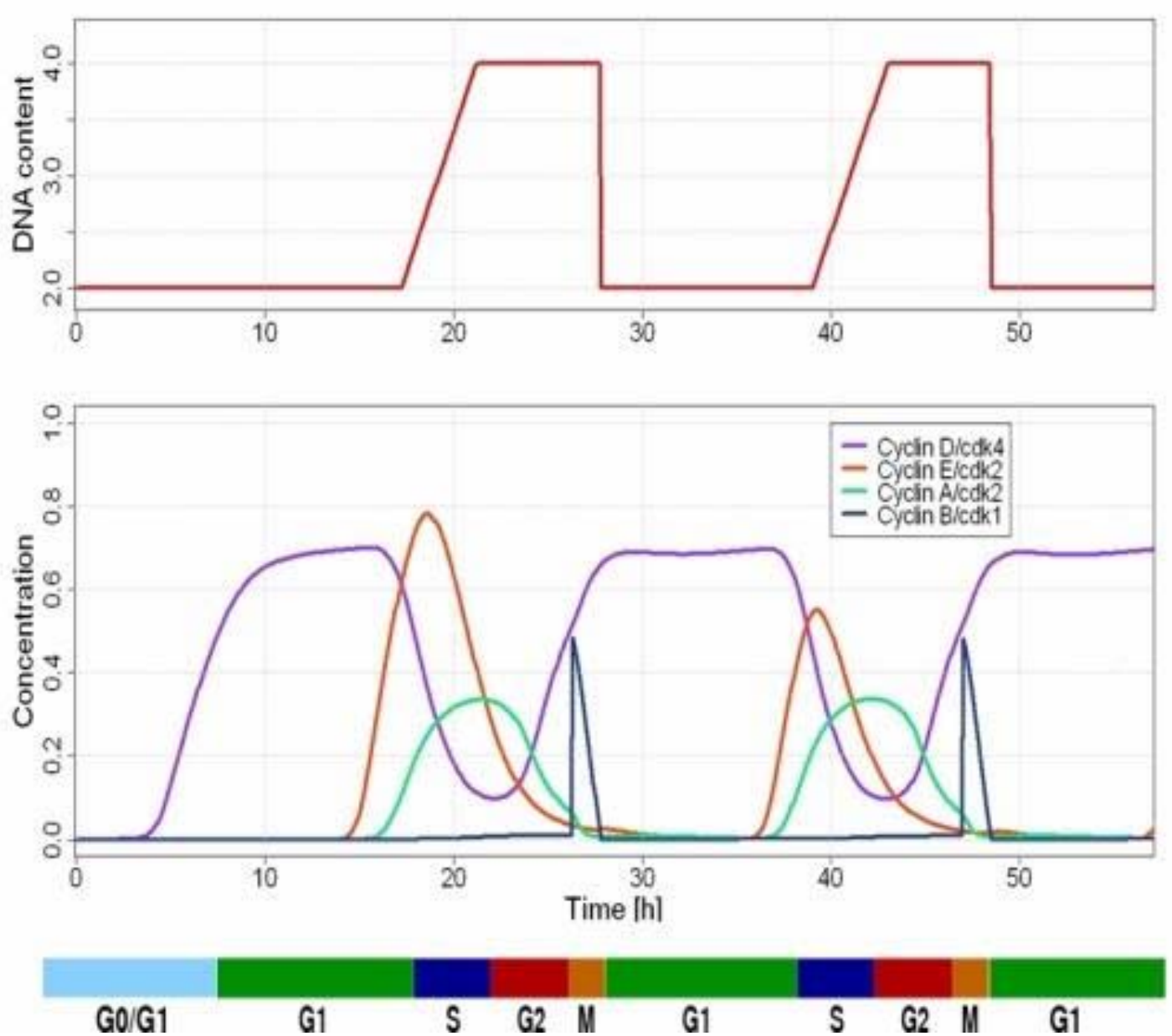
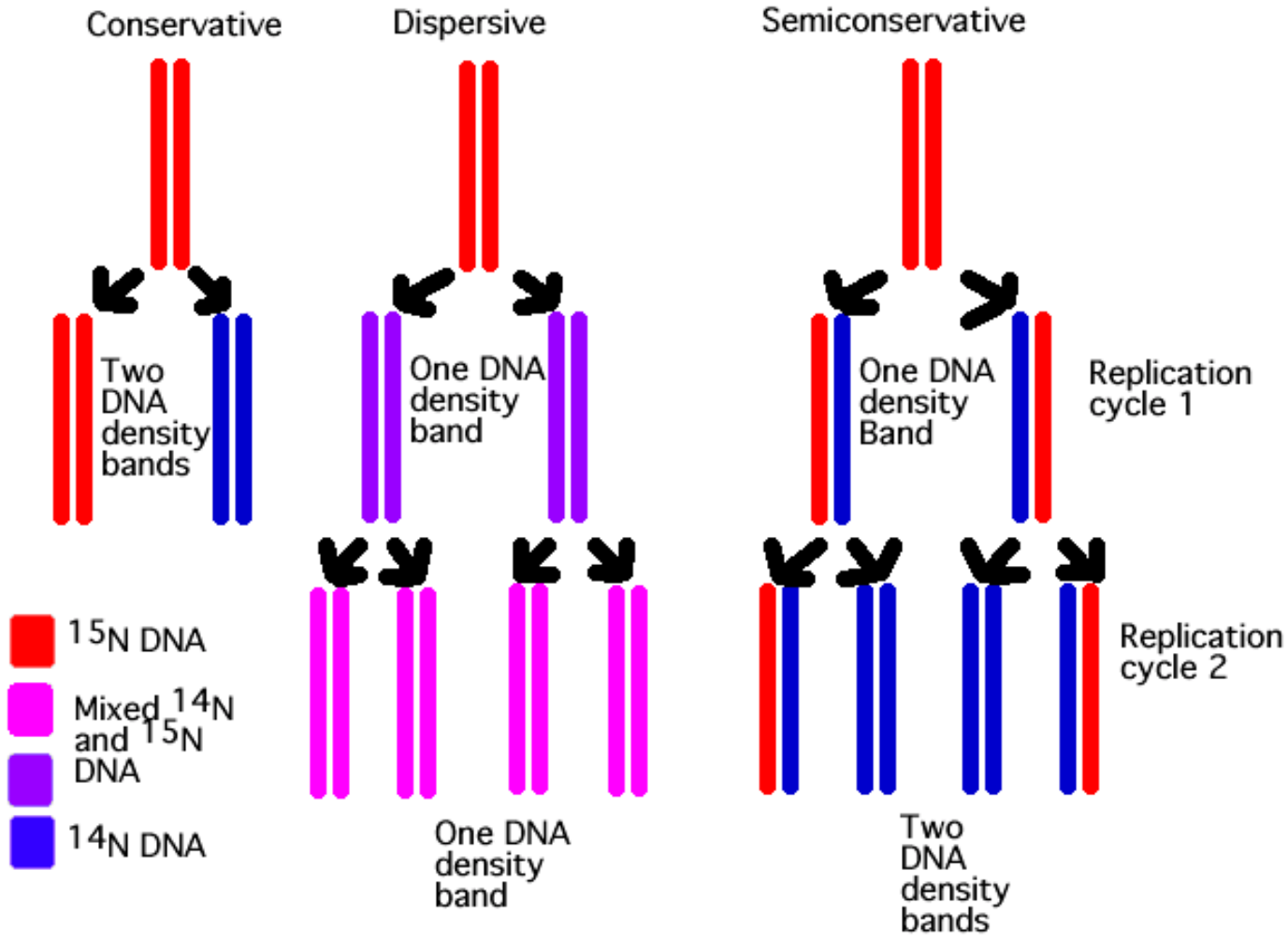


Figure 1 - Schematic representation of cell cycle control in plants. Progression through the mitotic cycle involves the successive formation, activation and subsequent inactivation of cyclin-dependent protein kinases (CDKs). The kinases bind sequentially to a series of cyclins, which are responsible for differential activation of the kinase during the cell cycle. The G1 to S transition is thought to be controlled by CDKs containing D-type cyclins that phosphorylate the retinoblastoma protein, releasing E2F transcription factors. E2F are involved in the transcription of genes needed for the G1 to S transition. The G2 to M transition is carried by CDK complexes containing CycA and CycB cyclins. CDK complexes are kept in inactive state by phosphorylation by the Wee1 kinase, and by interaction with inhibiting proteins (CKIs). At the G2 to M boundary activation of the kinase is brought about by release of the CKI protein, by positive phosphorylation (by CAK kinase), and by a still unidentified protein phosphatase.

Steuerung des Zellzyklus durch Cycline



Die DNA-Replikation verläuft semi-konservativ, bewiesen durch Meselson und Stahl, Nobelpreis 1962



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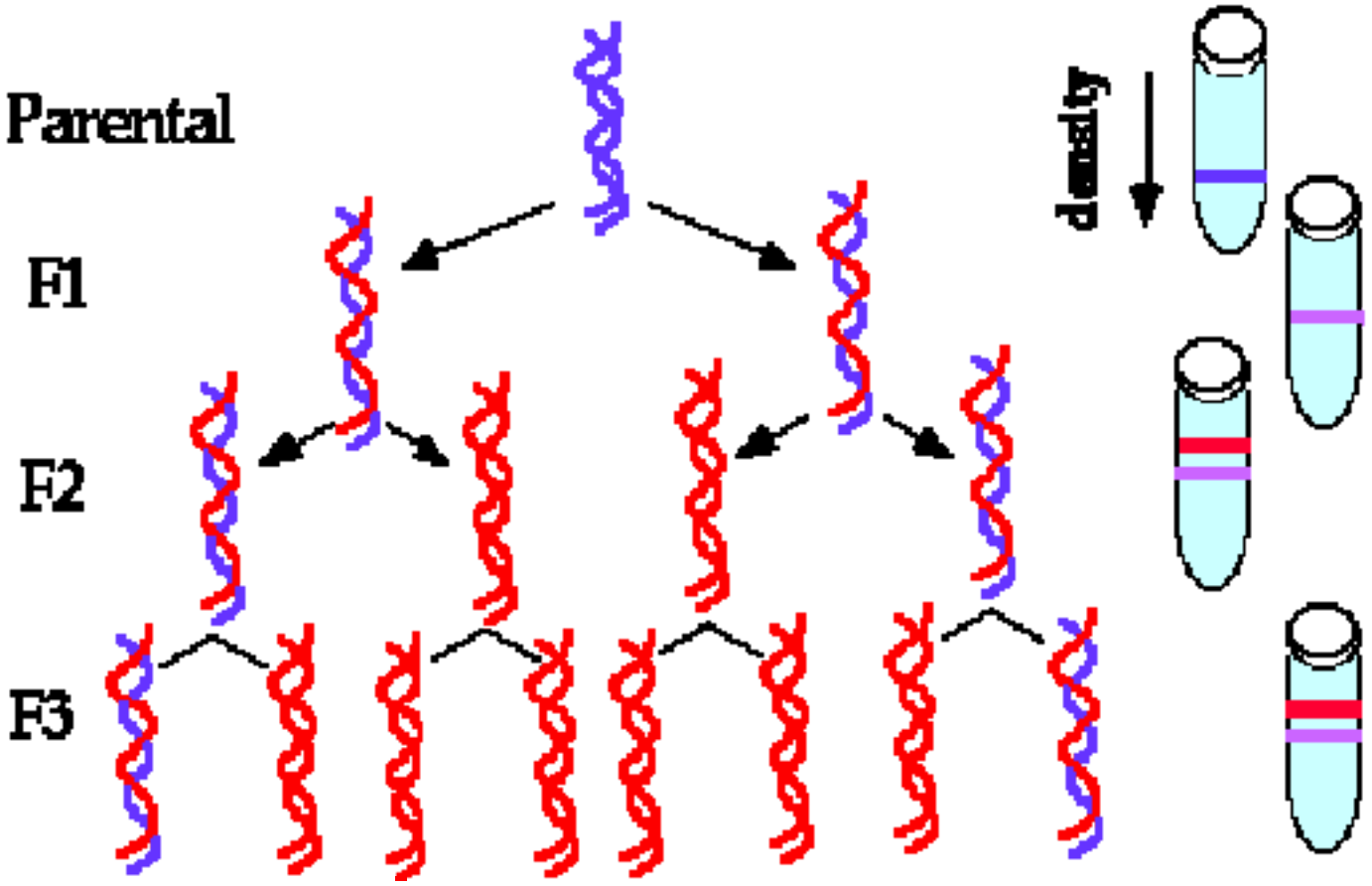
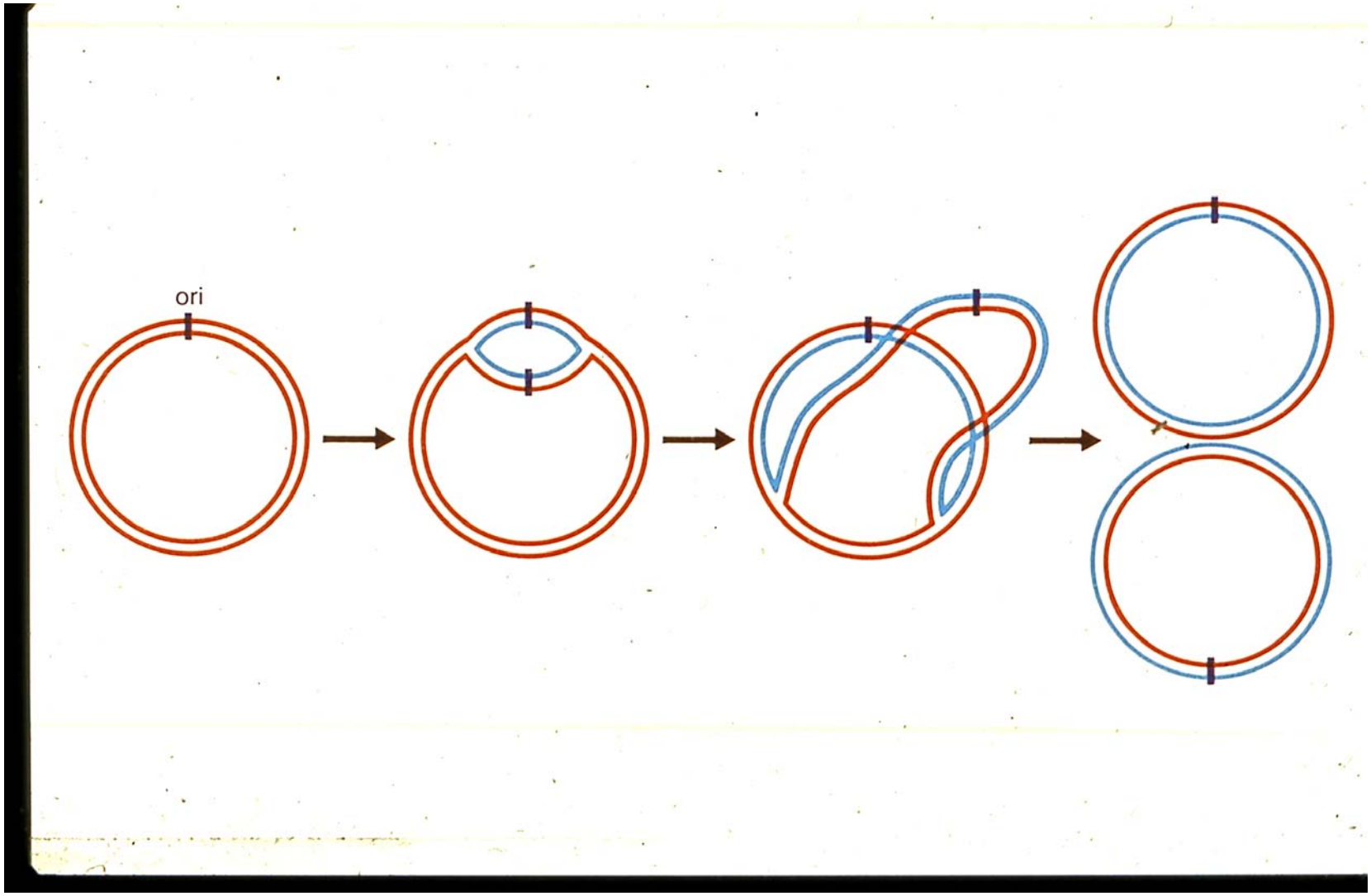
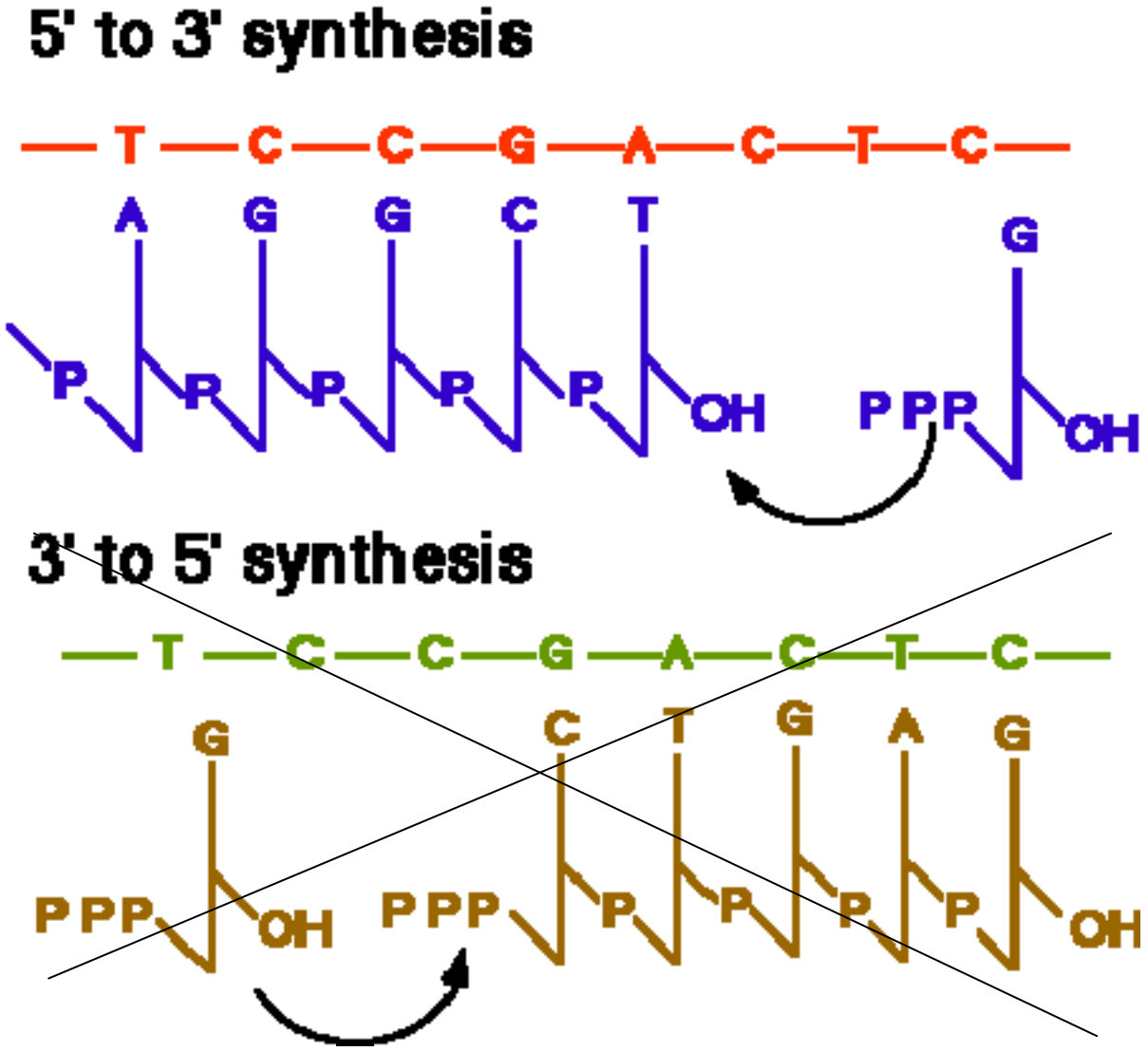


Die DNA-Replikation verläuft normalerweise „bidirektional“



Die DNA-Synthese läuft immer 5´- 3´ab



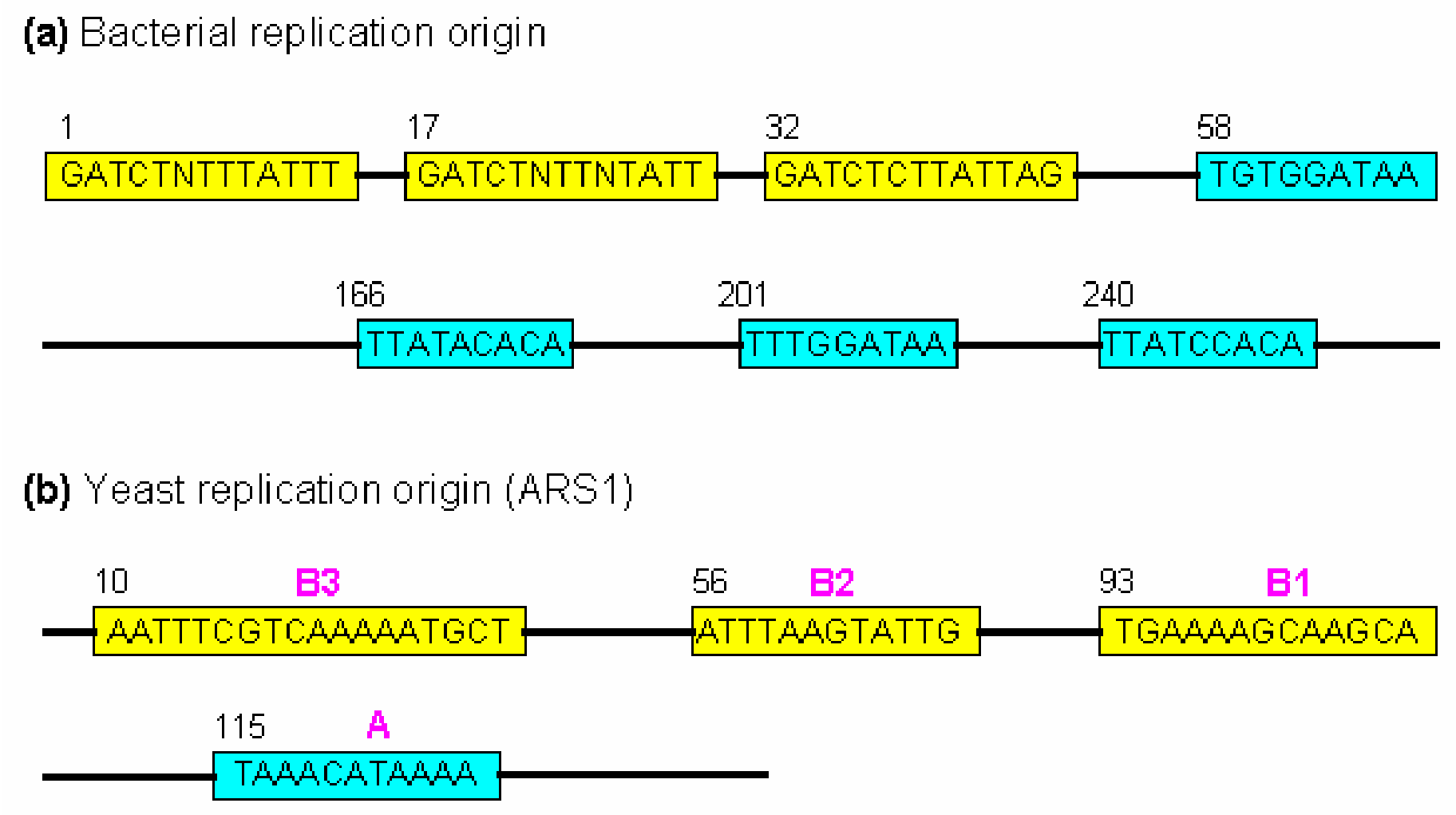
DNA-Replikation

- Ein Prozess in drei Stufen
  1. Initiation
  2. Elongation
  3. Termination

Die DNA-Replikation beginnt an einem speziellen Sequenzelement:

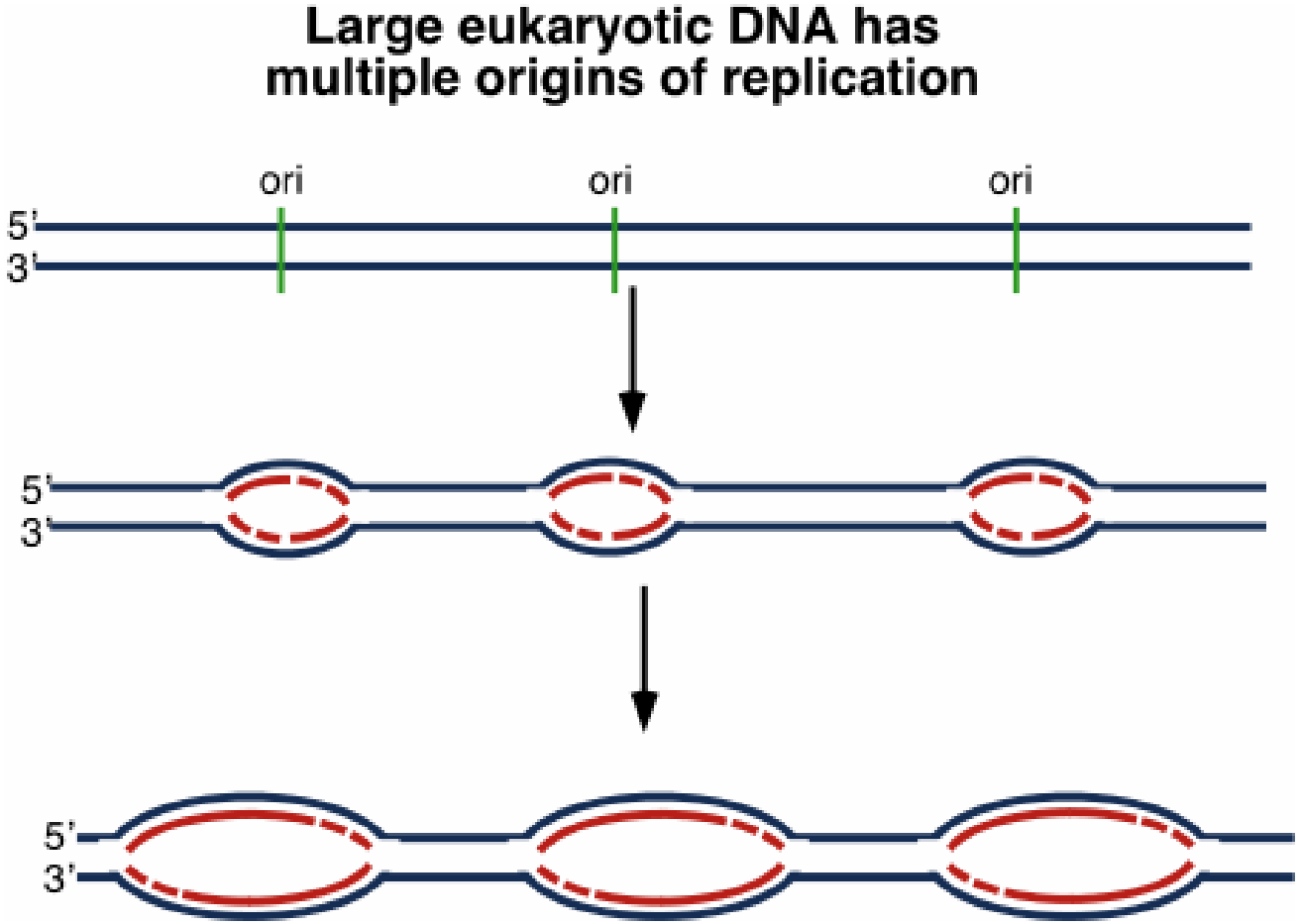
„**origin of replication**“
(ori)
(Replikationsursprung)

Sequenzen bekannter Origins

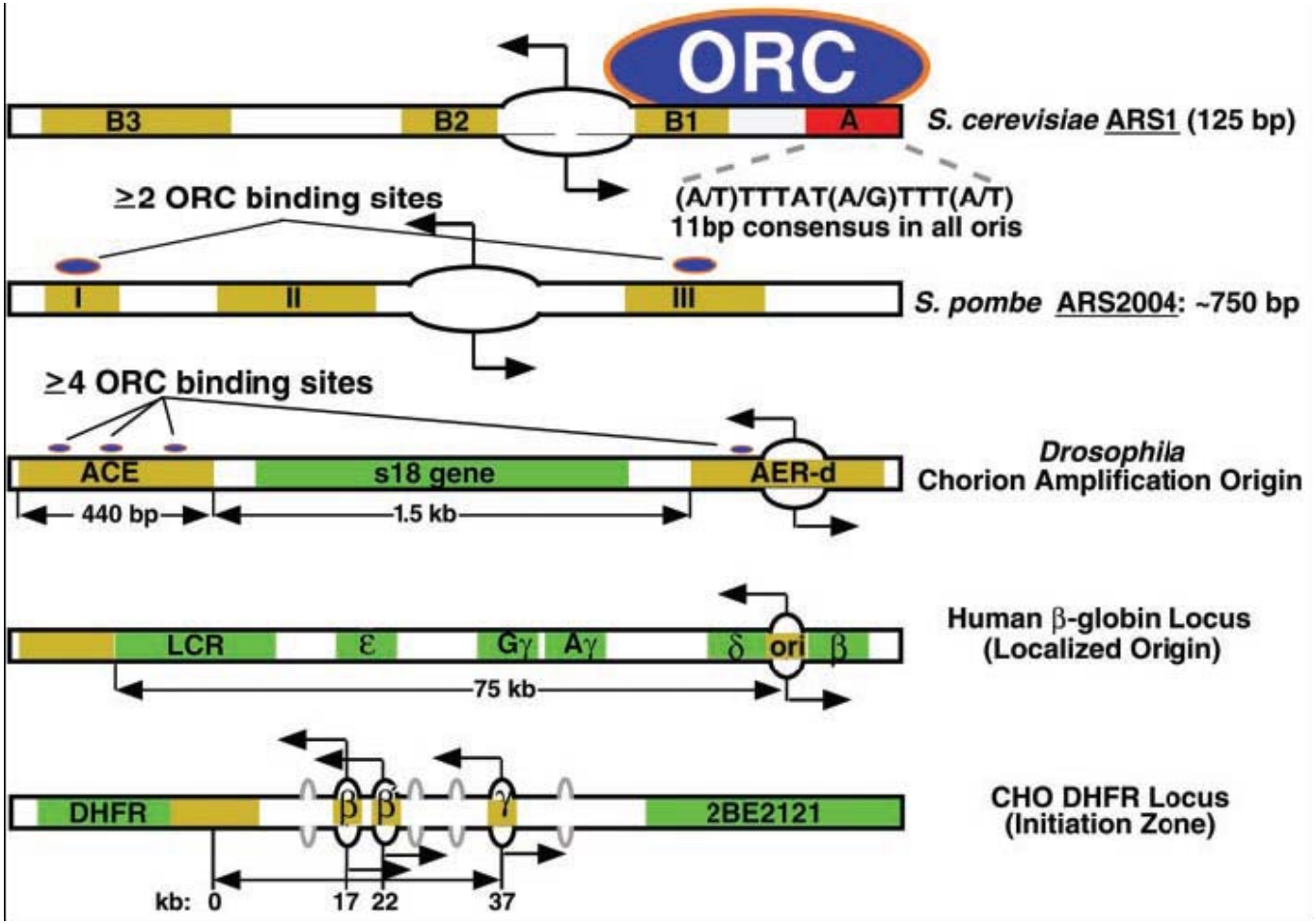


Prokaryoten besitzen meist nur einen „vegetativen“ Origin of Replication;

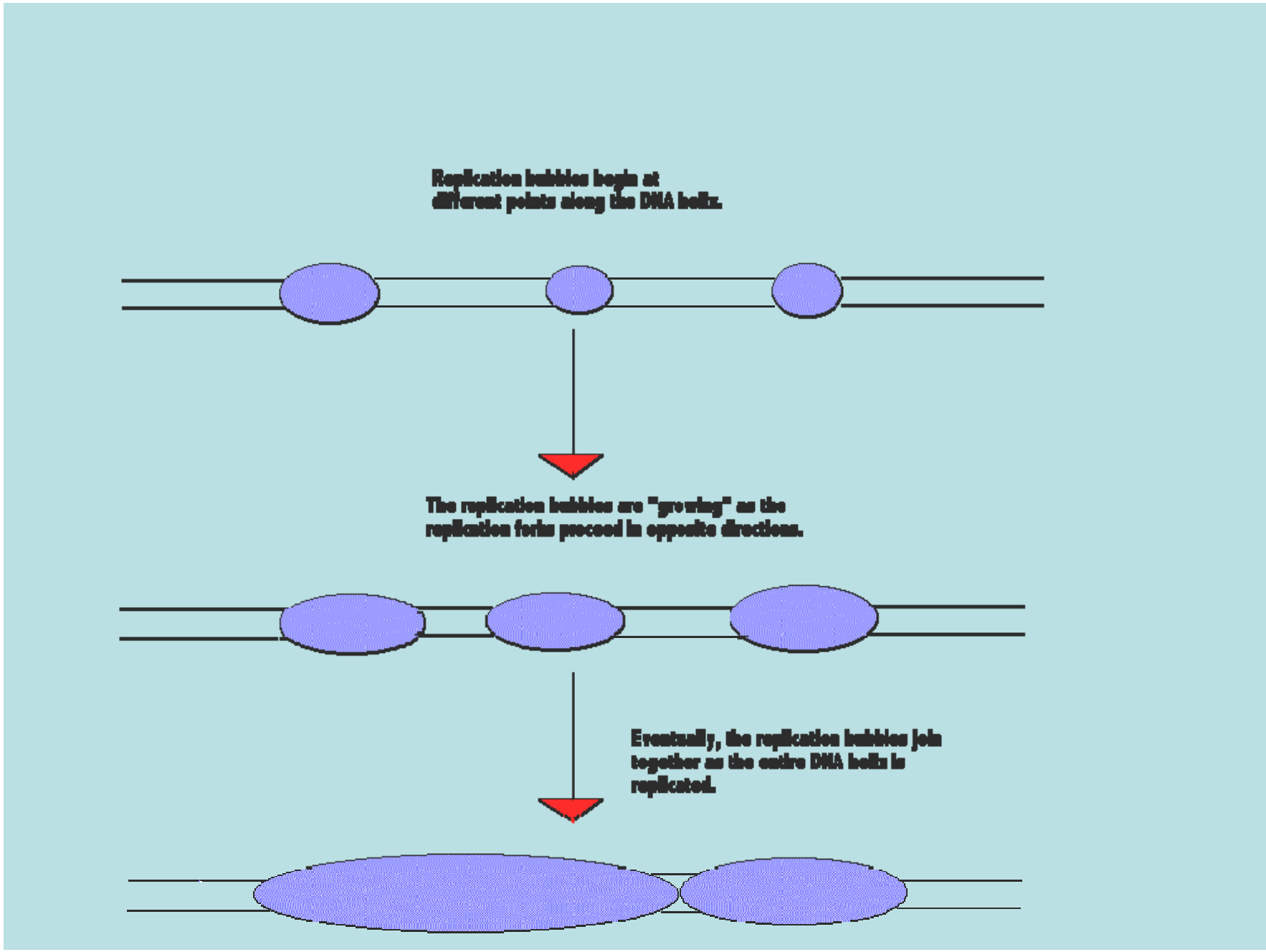
Eukaryoten haben dagegen multiple Origins



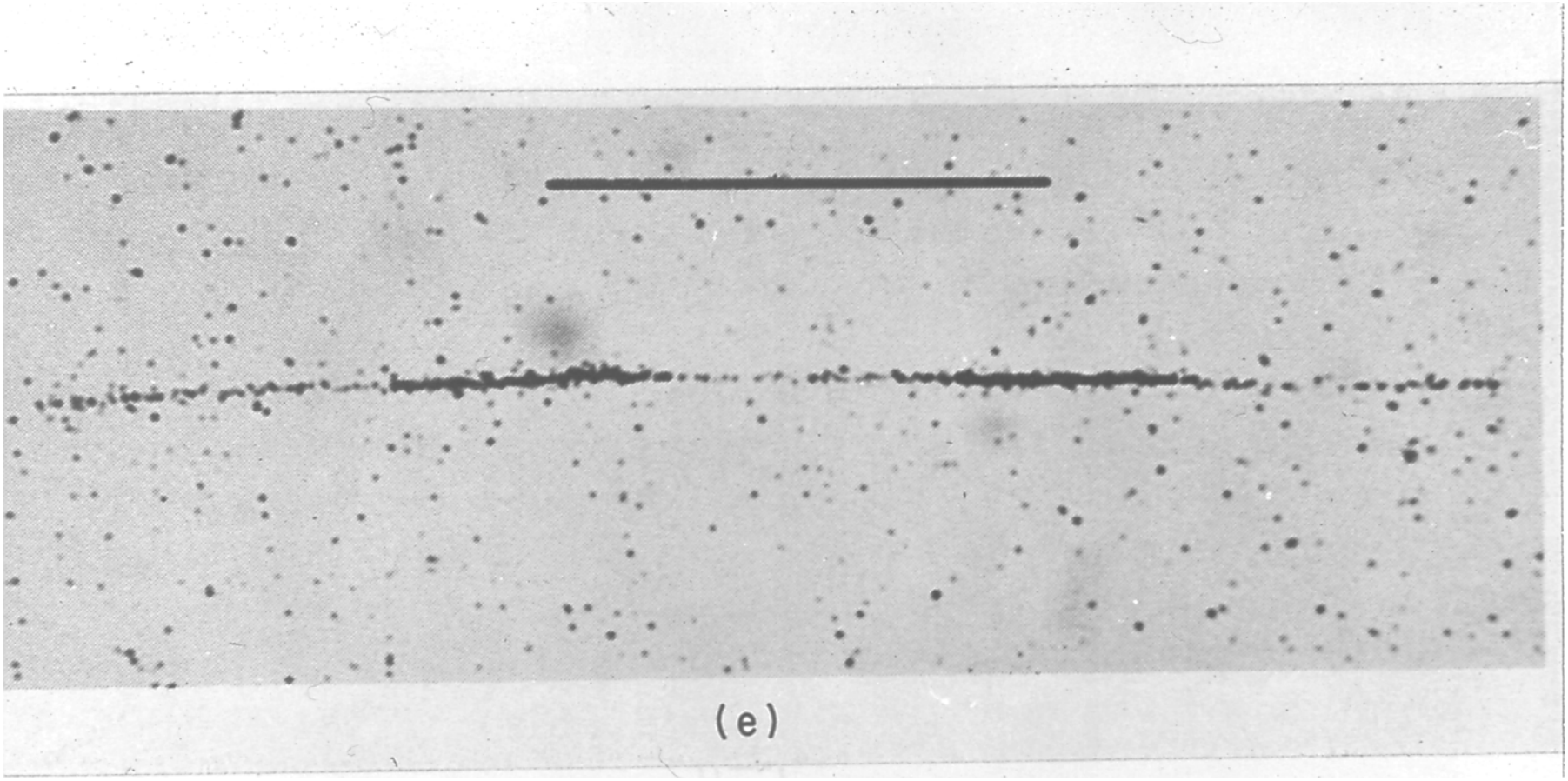
Eukaryotische Replikationsursprünge



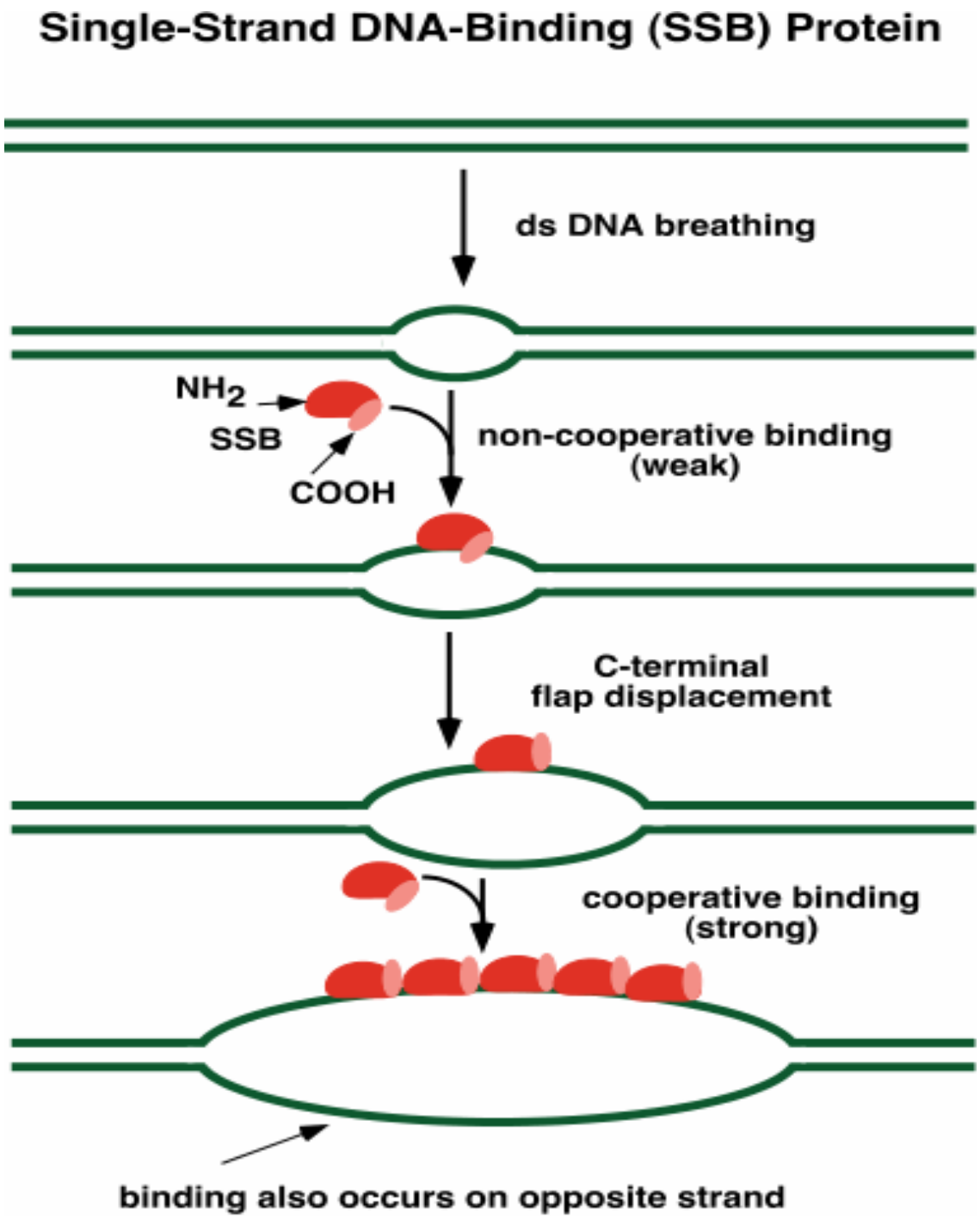
SCIENCE VOL 294 5 OCTOBER 2001

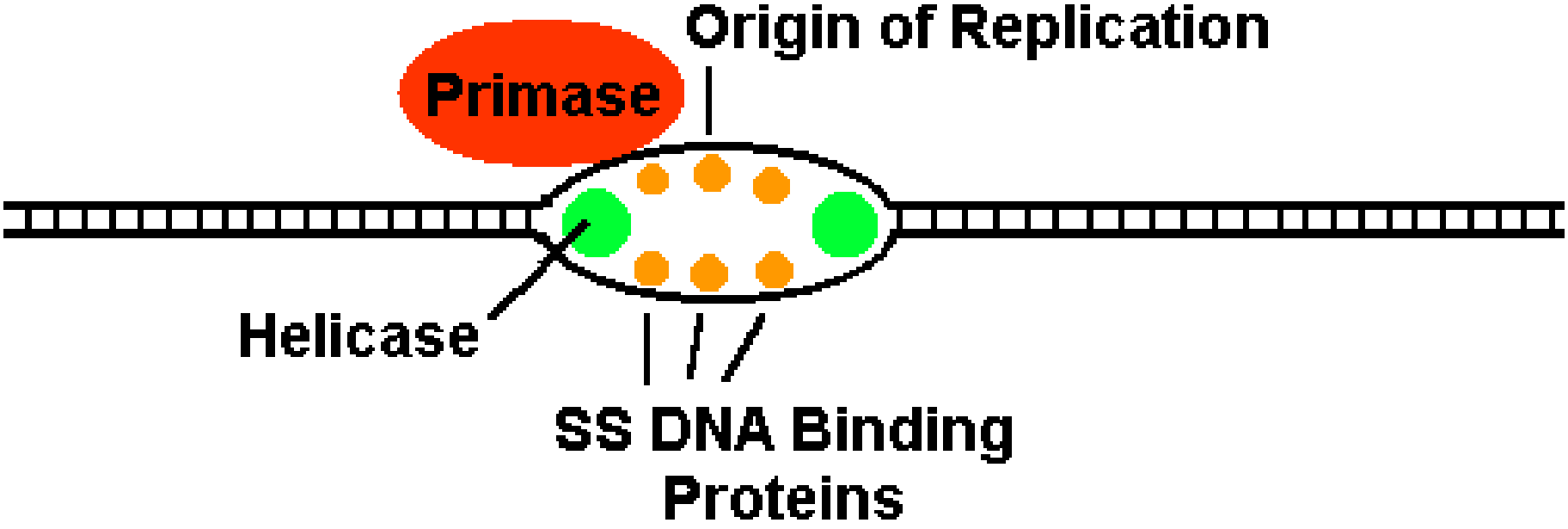

Replication bubbles begin at different points along the DNA helix.
The replication bubbles are "growing" as the replication forks proceed in opposite directions.
Eventually, the replication bubbles join together as the entire DNA helix is replicated.

Nachweis von Replikationsstartpunkten (Oris) durch Fiber-Autoradiografie

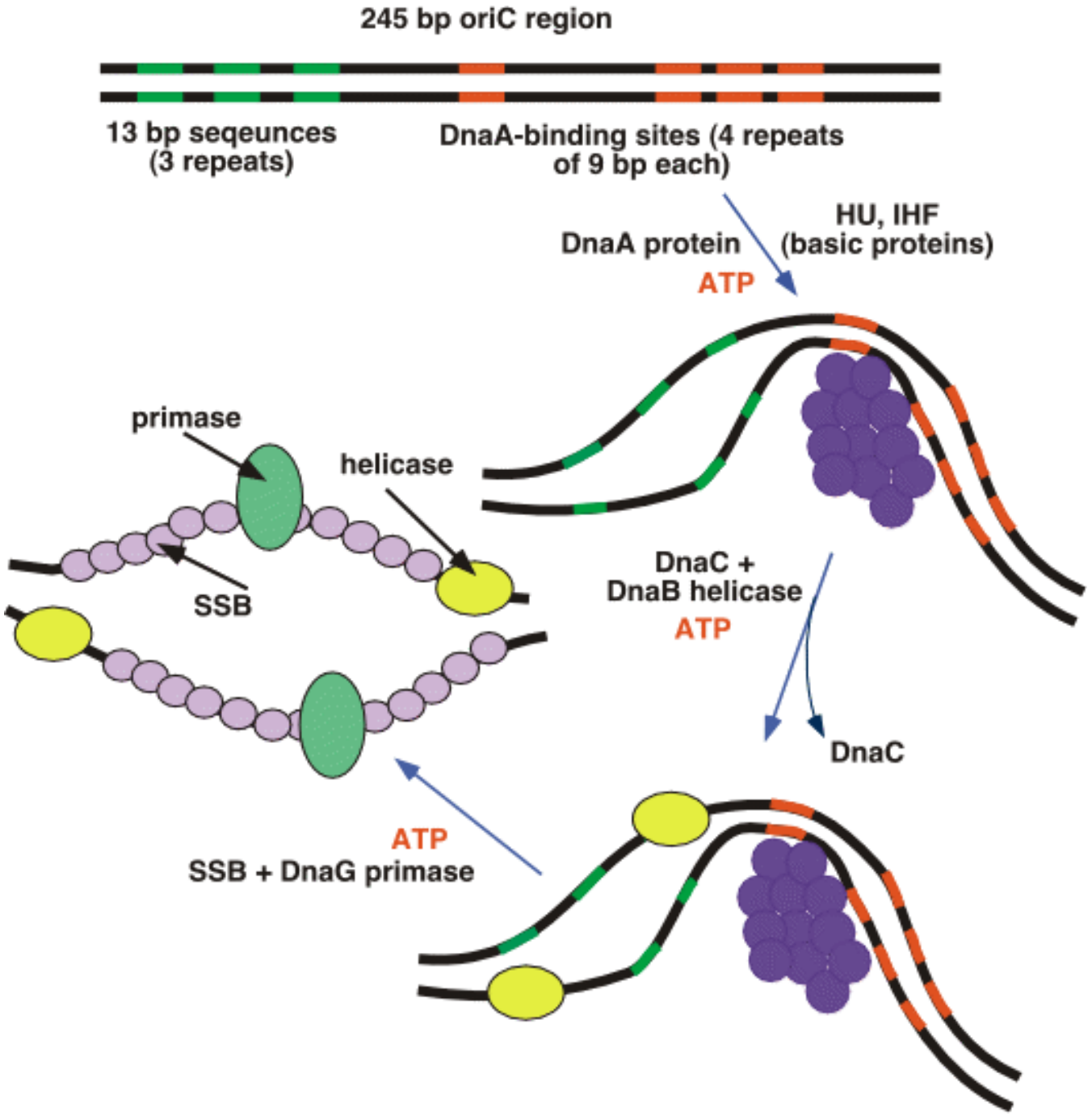


Initiation

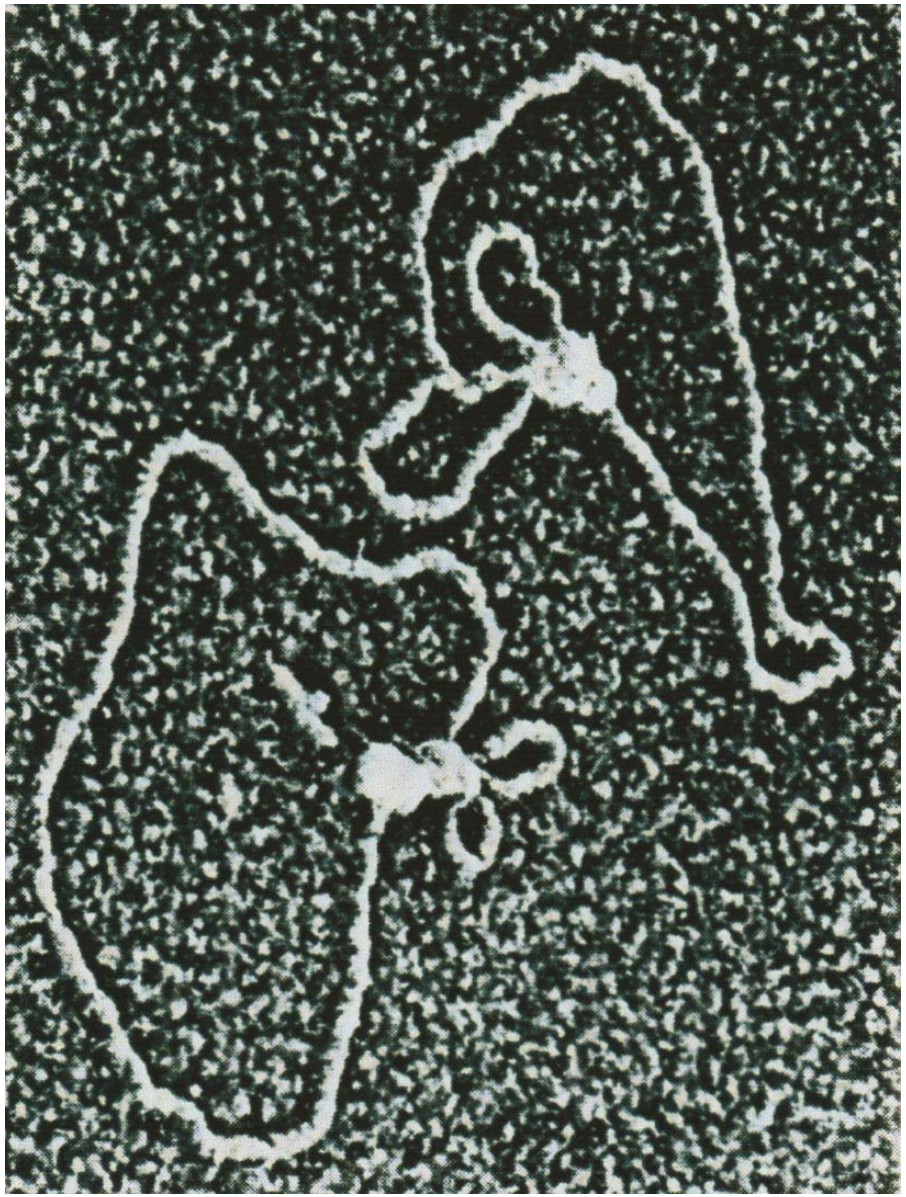

Single-Strand DNA-Binding (SSB) Protein
ds DNA breathing
NH2
SSB
COOH
non-cooperative binding
(weak)
C-terminal
flap displacement
cooperative binding
(strong)
binding also occurs on opposite strand


Origin of Replication
Primase
Helicase
SS DNA Binding
Proteins

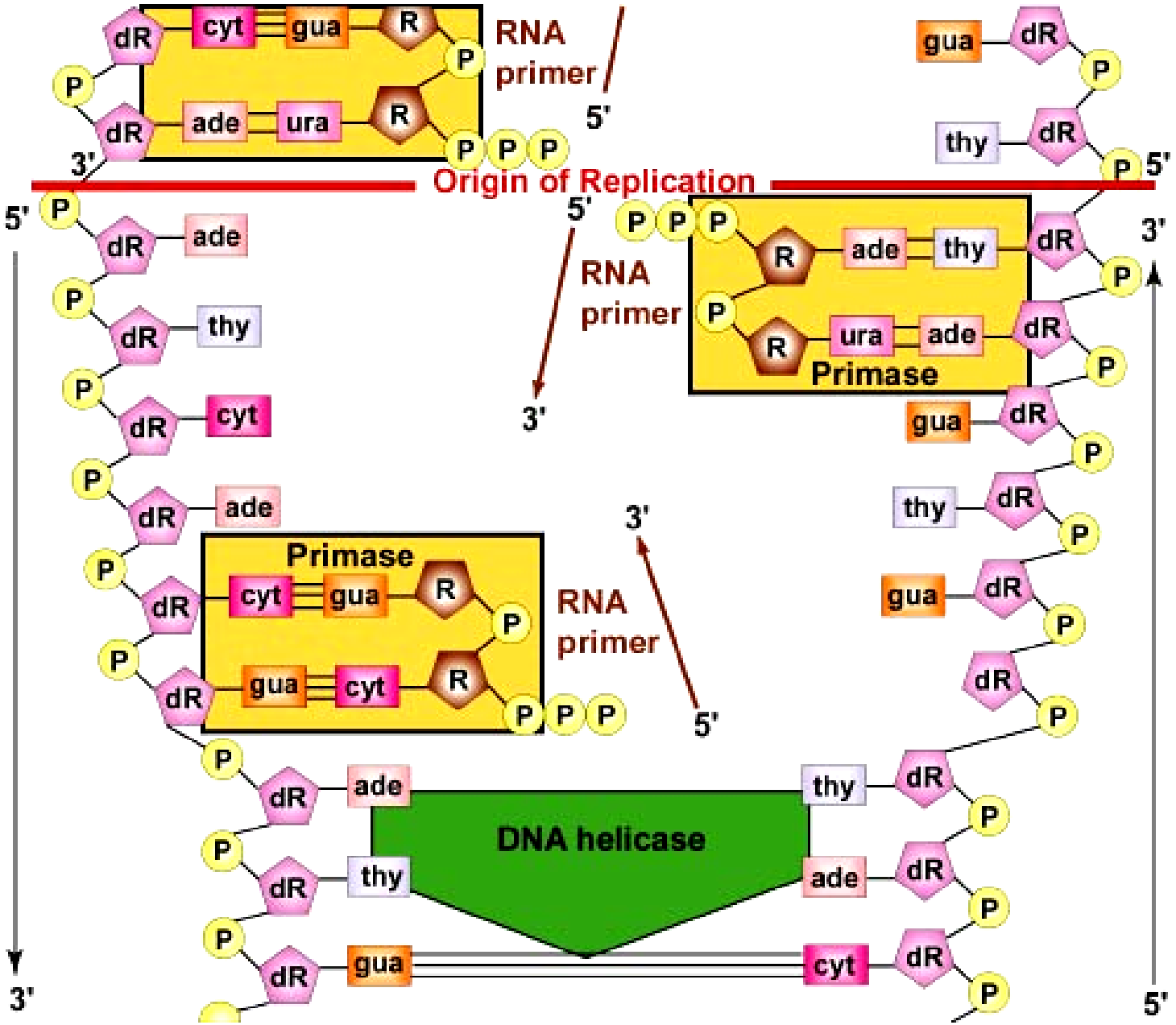
Initiation

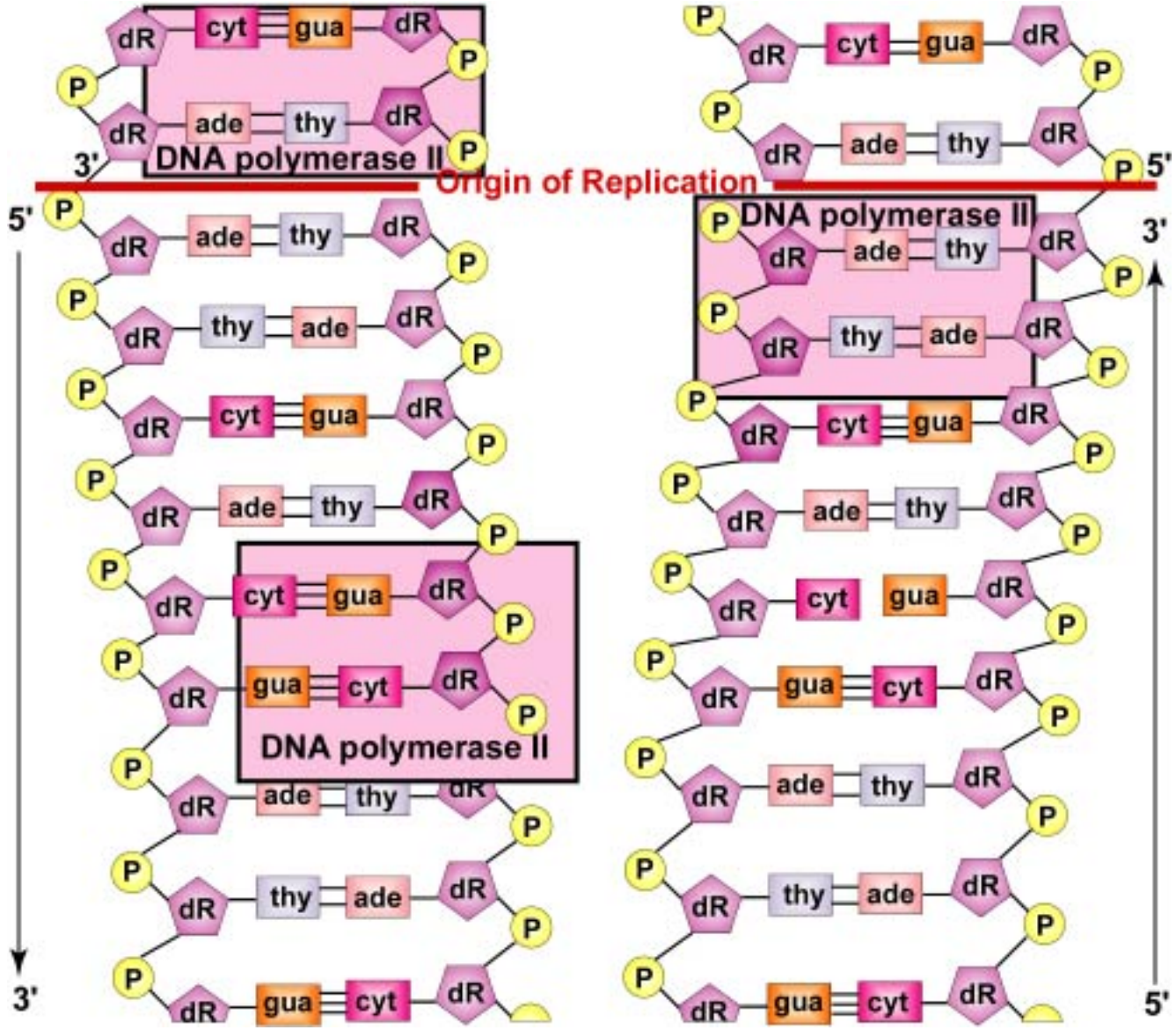

245 bp oriC region
13 bp seqeunces
(3 repeats)
DnaA-binding sites (4 repeats
of 9 bp each)
DnaA protein
HU, IHF
(basic proteins)
ATP
primase
helicase
SSB
DnaC +
DnaB helicase
ATP
DnaC
ATP
SSB + DnaG primase

Initiationskomplex bei PhiX-Phage

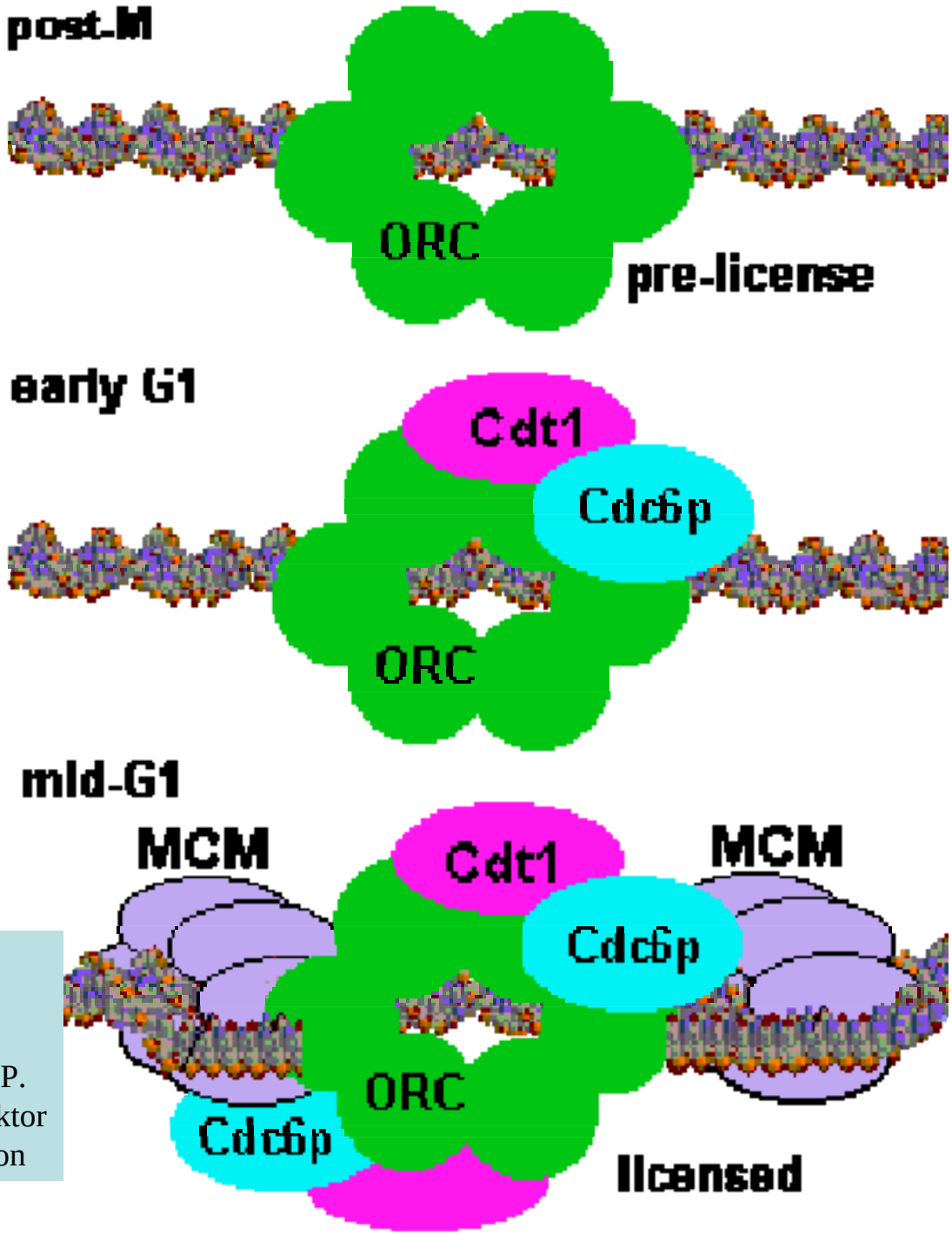


1. Syntheseschritt: Synthese des „Primer“ durch Primase




Origin of Replication
DNA polymerase II
DNA polymerase II
DNA polymerase II
5'
3'
3'
5'
cyt
gua
ade
thy
dR
P

Die **Initiation** der DNA-Replikation bei **Eukaryoten** am ori erfolgt erst nach der „Lizensierung“ durch ORC und weitere Proteine



ORC= Origin recognition complex
Cdk= Cyclin dependent kinase
Cdc= Cell division cycle Protein
Mcm= Minichromosome maintenance P.
Cdt1= Replikationsfaktor/LicensingFaktor
OBR= Origin of bidirectional replication

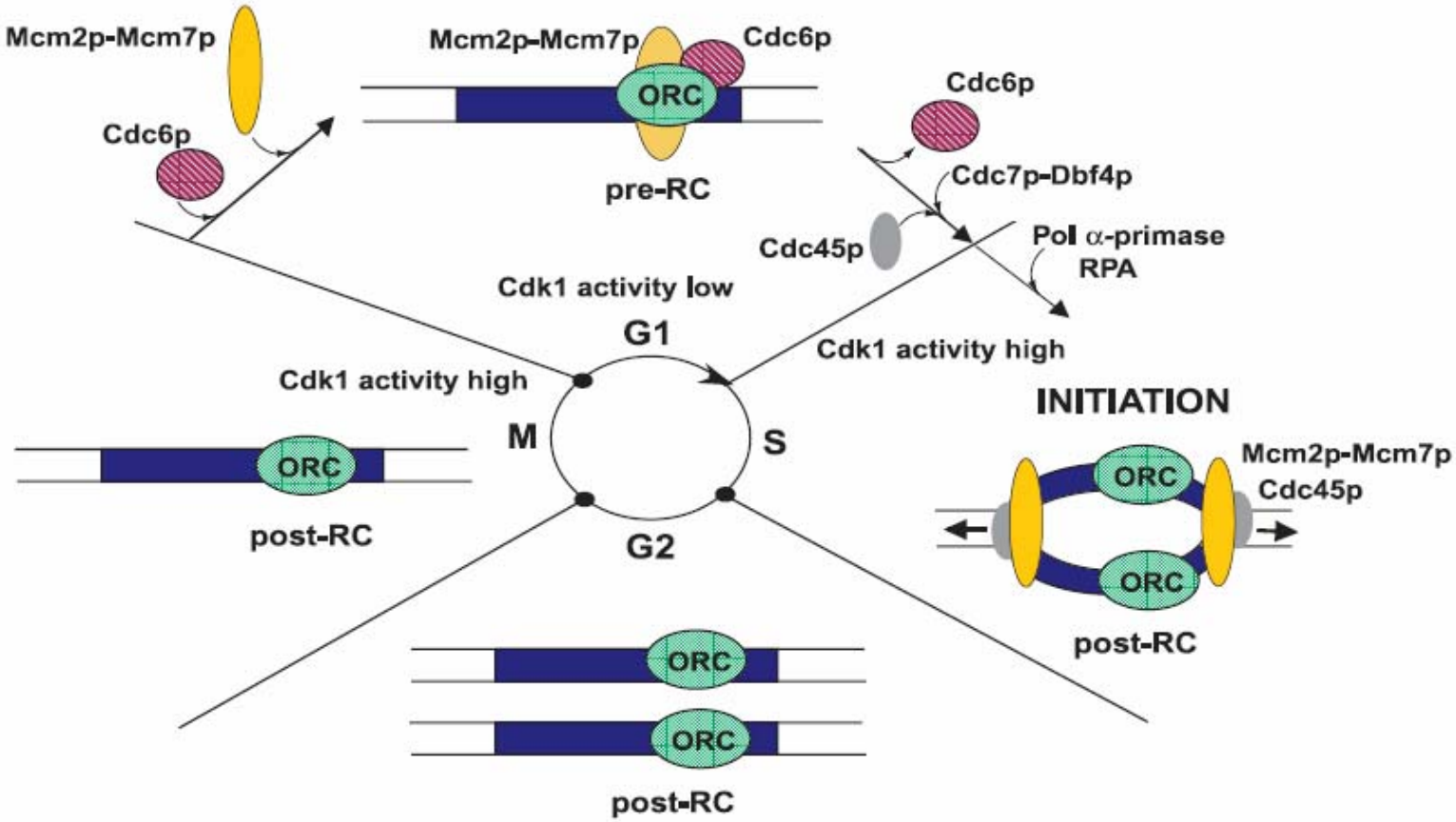
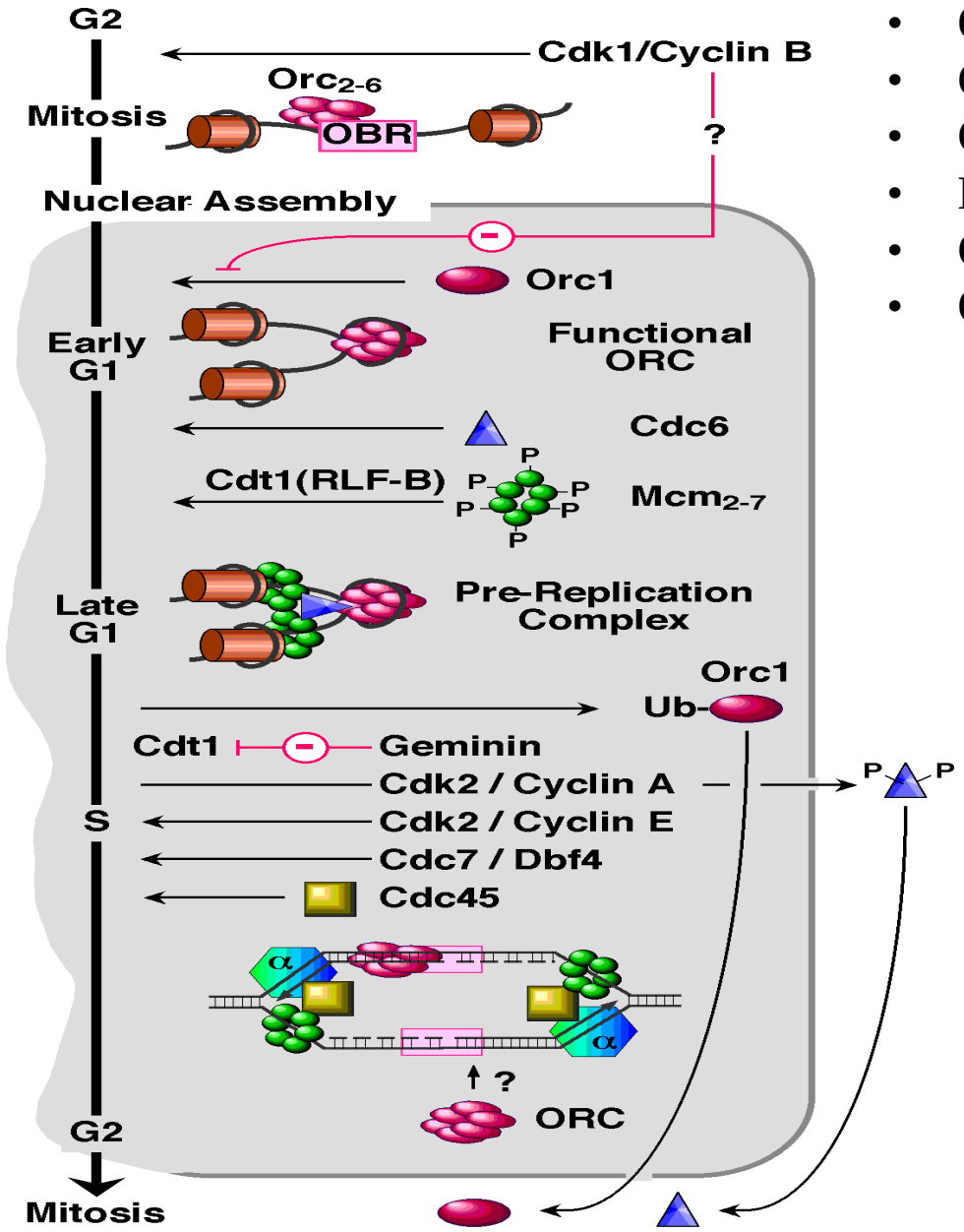


Fig. 1. Events leading to origin activation in budding yeast. Origin recognition complex (ORC) binds to an ARS element in yeast (blue rectangle). For the stepwise assembly of the pre-RC during G_1 phase when Cdk1p activity is low, ORC recruits Cdc6p, which in turn loads Mcm2p-Mcm7p. When Cdk1p activity rises at the G_1/S transition, the pre-RC is disassembled. The post-RC remains stable until the end of mitosis, and owing to high Cdk1p activity the pre-RC cannot re-associate during this time but must await the subsequent G_1 phase.



- ORC= Origin recognition complex
- Cdk= Cyclin dependent kinase
- Cdc= Cell division cycle Protein
- Mcm= Minichromosome maintenance P.
- Cdt1= Replikationfaktor
- OBR= Origin of bidirectional replication

Initiator-Proteine

Table 2. Initiation Proteins

Function	*E. coli*	Phage λ	Phage T4	SV40/Human	Yeast
Initiator protein	DnaA	λ O	none	T antigen	ORC
Loading and remodeling factor(s)	DnaC	λ P, DnaJ, DnaK	gp59	Cellular chaperone?	Cdc6 protein
DNA helicase	DnaB	DnaB	gp41	T antigen	MCM proteins?