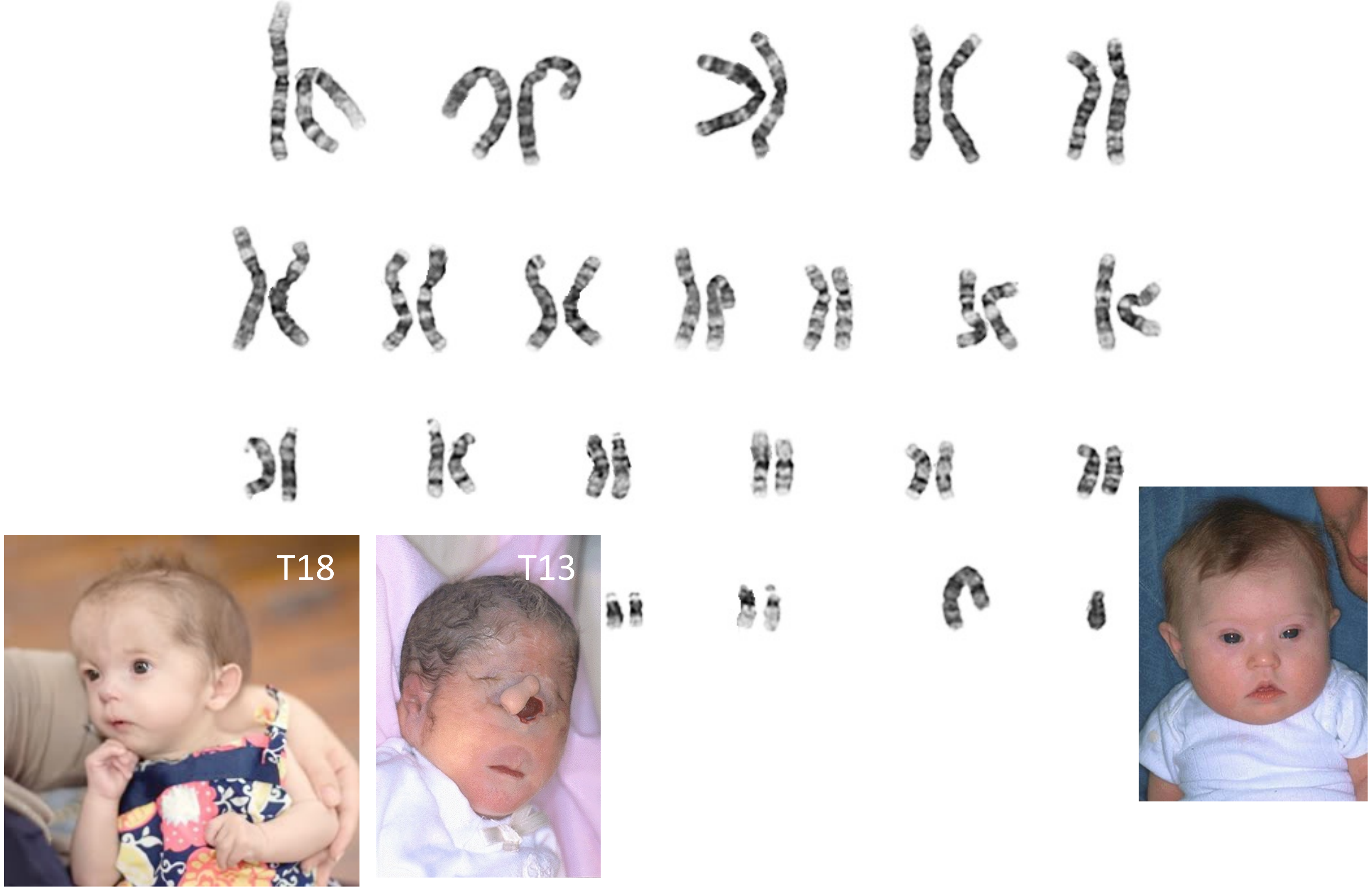
Introduction to the human genome

Chapter 2

Chapter 2

- ✓ The Human Genome Sequence
- ✓ Organization of the Human Genome
  - Single-Copy DNA Sequences
  - Repetitive DNA Sequences
  - Repetitive DNA and Disease
- ✓ Variation in the Human Genome
- ✓ Transmission of the Genome
  - The Cell Cycle
  - Mitosis
  - Meiosis
- ✓ Human Gametogenesis and Fertilization
  - Spermatogenesis
  - Oogenesis
  - Fertilization
- ✓ Medical Relevance of Mitosis and Meiosis

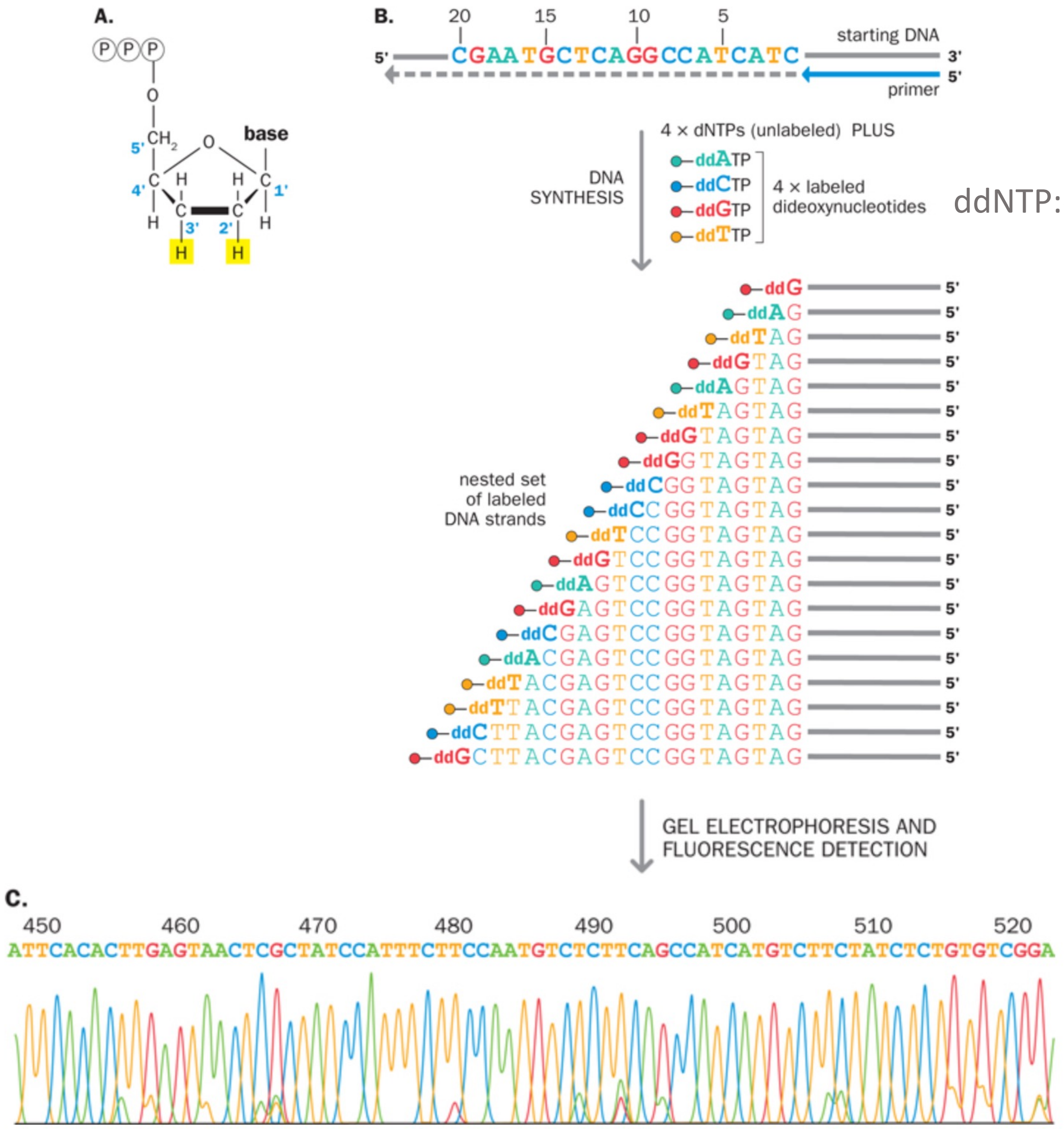
1956 : a normal cell has 46 chromosomes



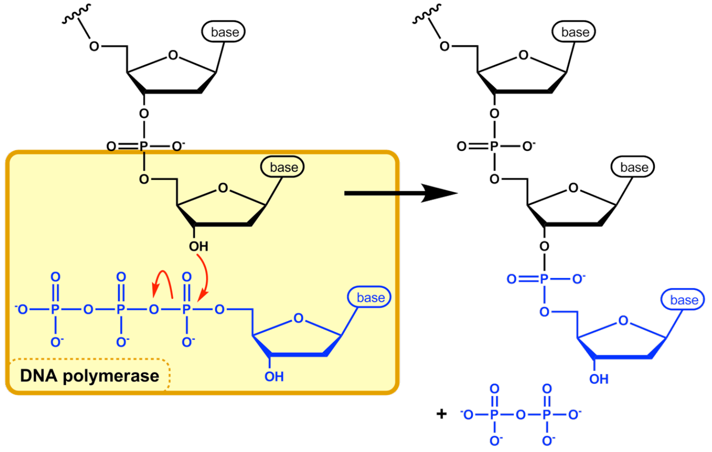
1975: Sanger-dideoxy DNA-sequencing

Human Molecular Genetics, 5th Edition (Tom Strachan & Andrew Read)

1975: Sanger-dideoxy DNA-sequencing



ddNTP:dNTP (1:100)



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Sequencing of the human genome

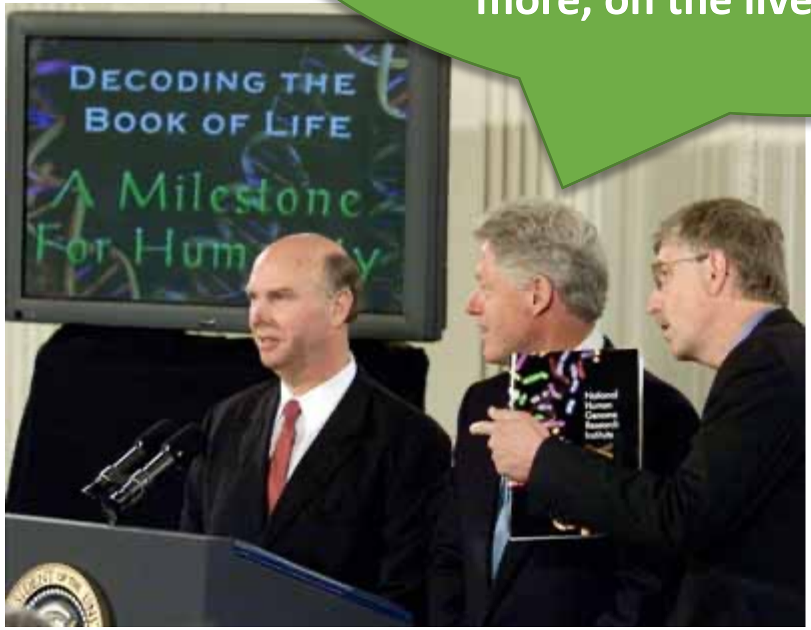
- 3.200.000.000 nucleotides
- 5.333.333 experiments (600 bases per experiment)
- 533.333 days (10 samples/run) to 55.556 (96 samples/run)
- 1461 - 152 year

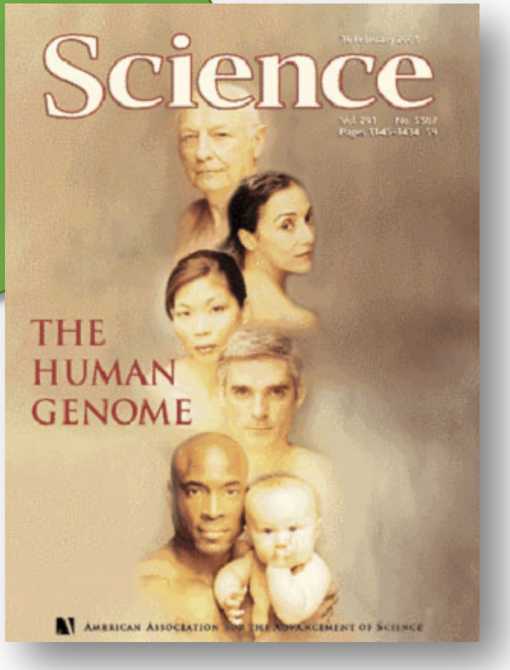
1990 - 2003 : International Human Genome Project

Today we are learning the language in which God created life.

…

[G]enome science will have a real impact on all our lives – and, even more, on the lives of our children



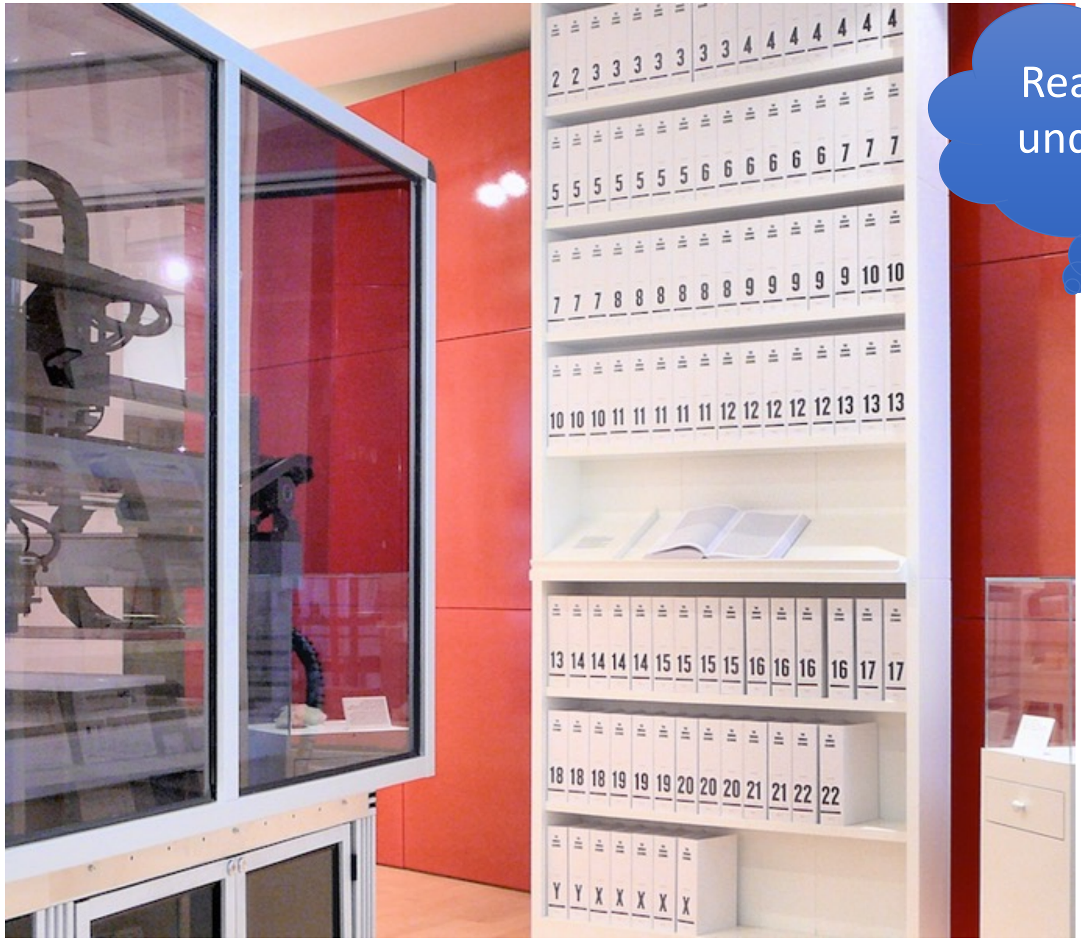


1990 - 2003 : International Human Genome Project

3.000.000.000 bp @ $ 2.700.000.000

(most advanced appliances: 96 samples, 30.000-60.000 bases per run)

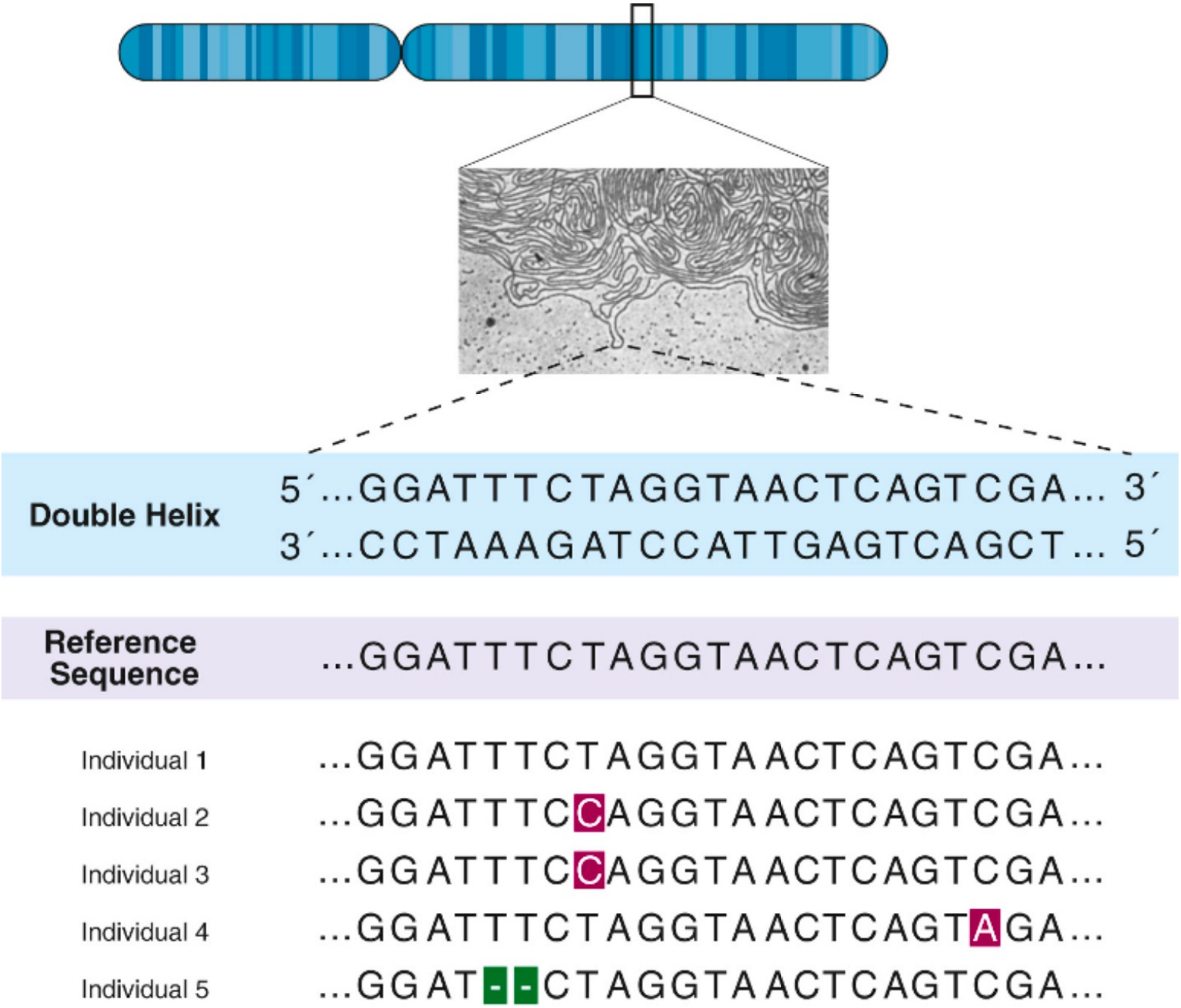
Human reference genome (n>1)



Reading is not understanding

Genome Reference Consortium

By convension, only sequences from one strand of DNA are presented



Double Helix	5´...GGATTTCTAGGTAACTCAGTCGA... 3´ 3´...CCTAAAGATCCATTGAGTCAGCT... 5´
Reference Sequence	...GGATTTCTAGGTAACTCAGTCGA...
Individual 1	...GGATTTCTAGGTAACTCAGTCGA...
Individual 2	...GGATTTCCAGGTAACTCAGTCGA...
Individual 3	...GGATTTCCAGGTAACTCAGTCGA...
Individual 4	...GGATTTCTAGGTAACTCAGTAGA...
Individual 5	...GGAT--CTAGGTAACTCAGTCGA...

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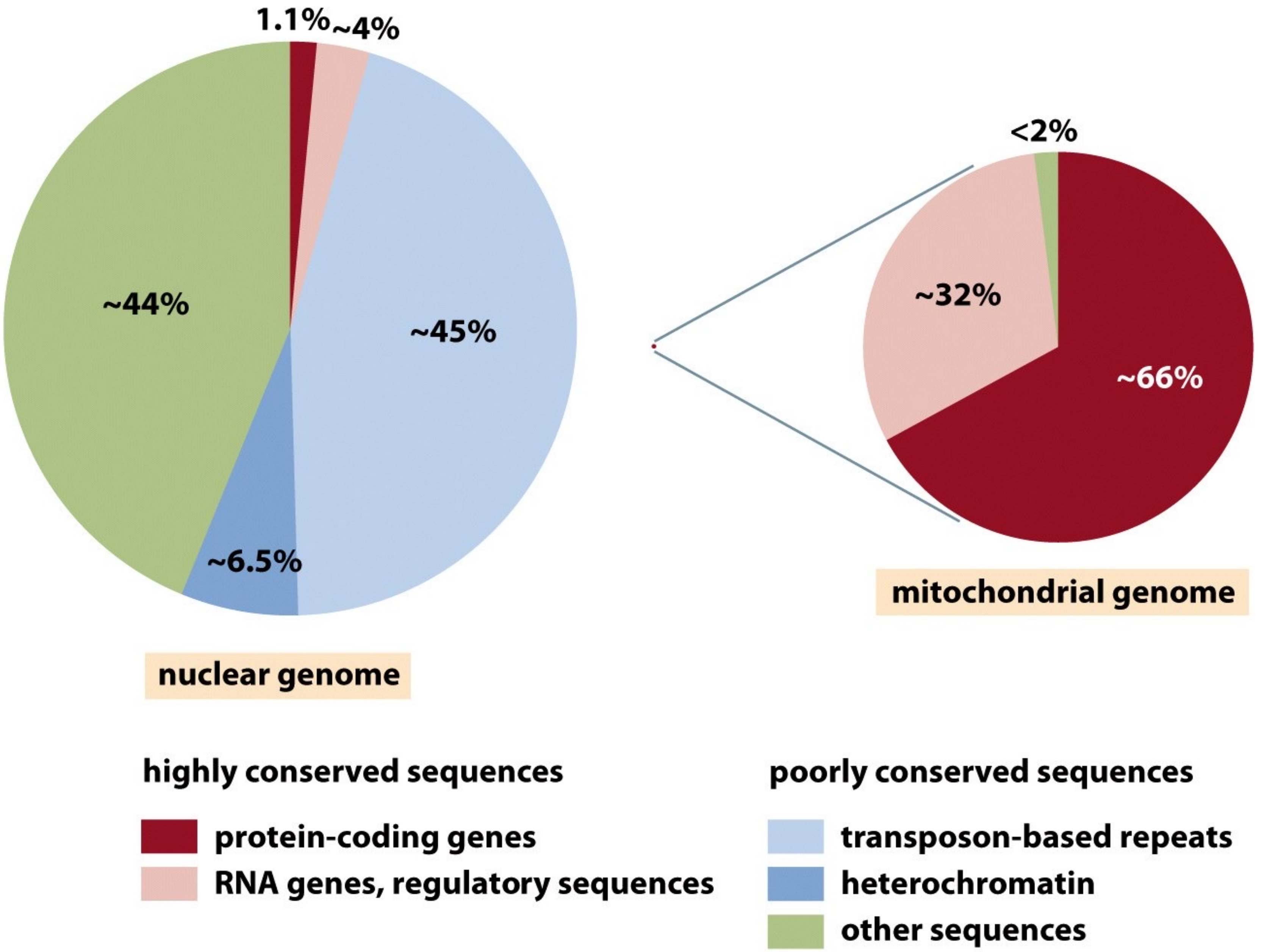
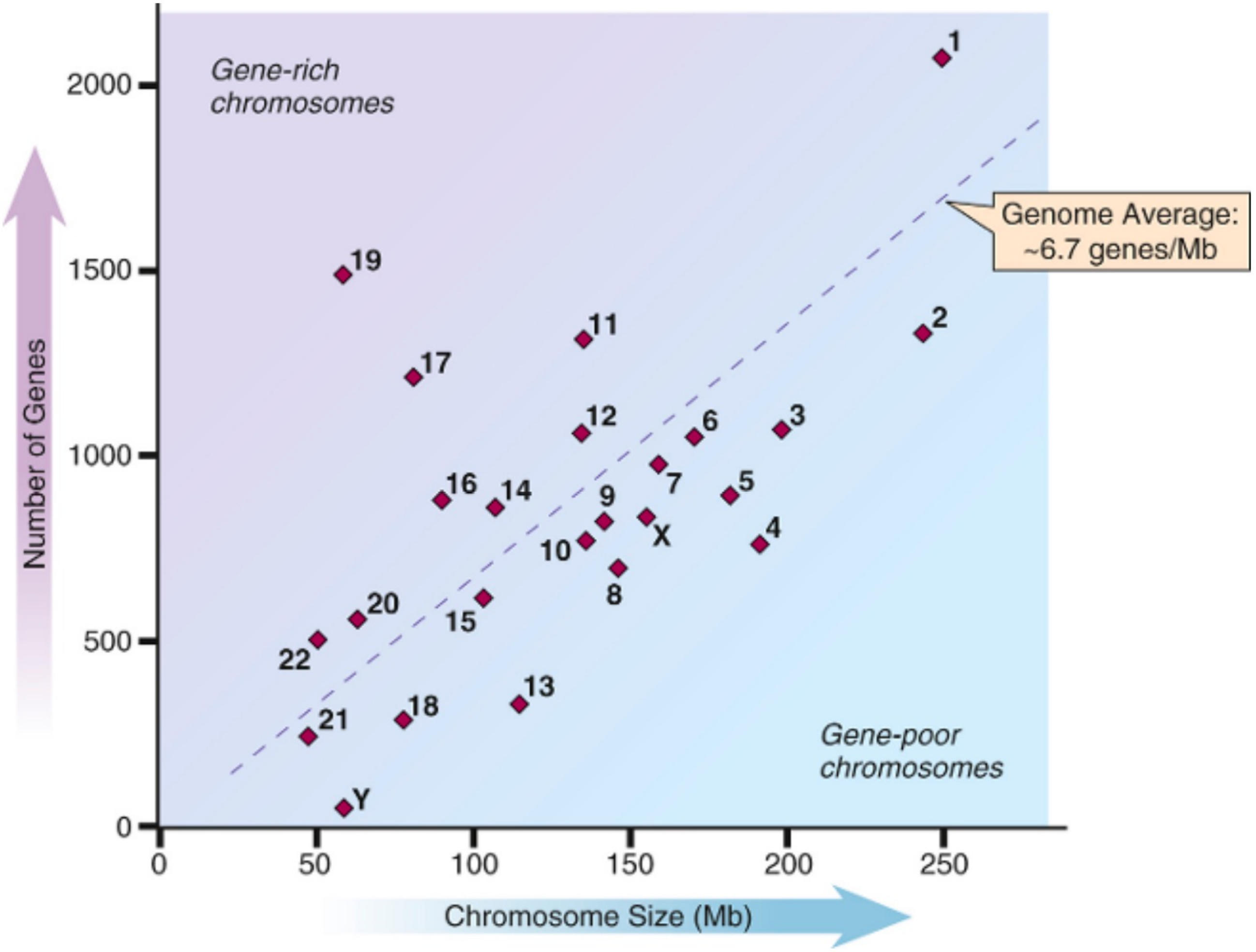
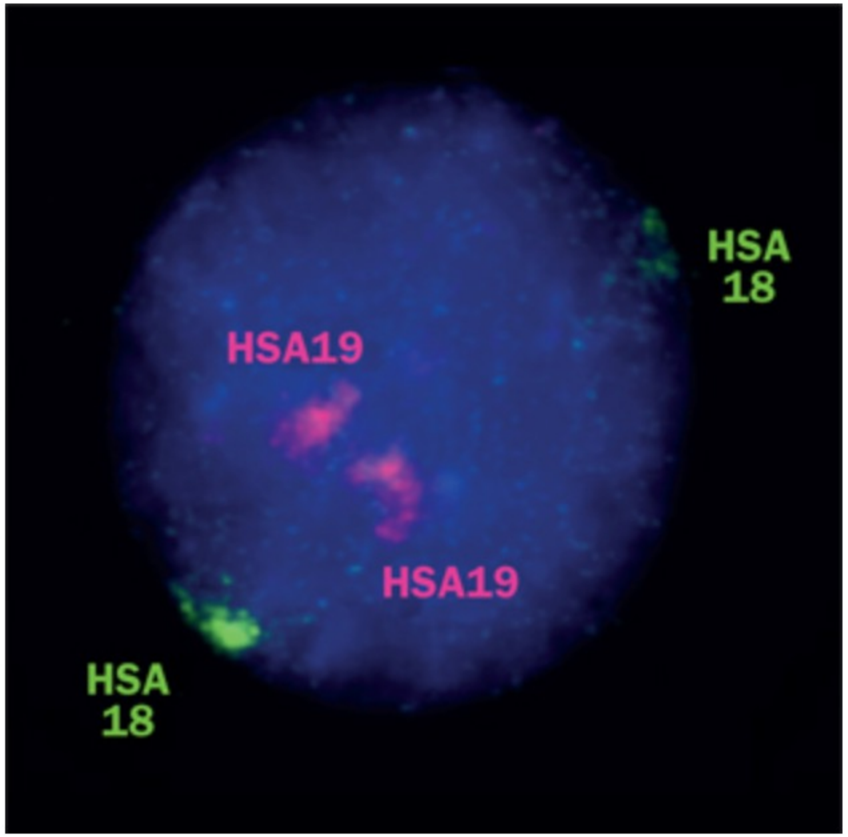


Figure 9.1 Human Molecular Genetics, 4ed. (© Garland Science)

Human Molecular Genetics, 4th Edition (Tom Strachan & Andrew Read)

Size and gene content of the 24 human chromosomes





Genetics in Medicine, 8th Edition (Thompson & Thompson)

Human Molecular Genetics, 4th Edition (Tom Strachan & Andrew Read)

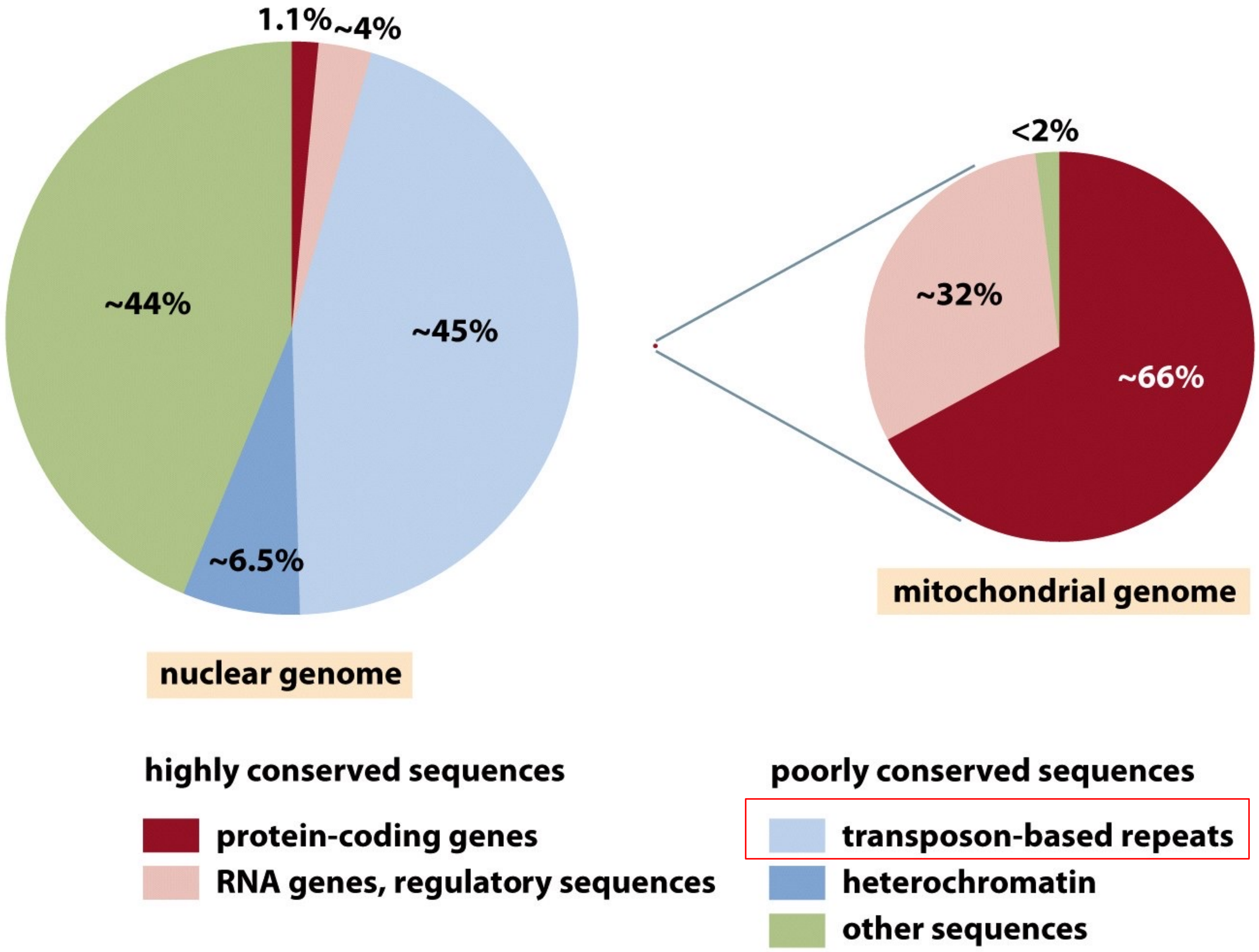


Figure 9.1 Human Molecular Genetics, 4ed. (© Garland Science)

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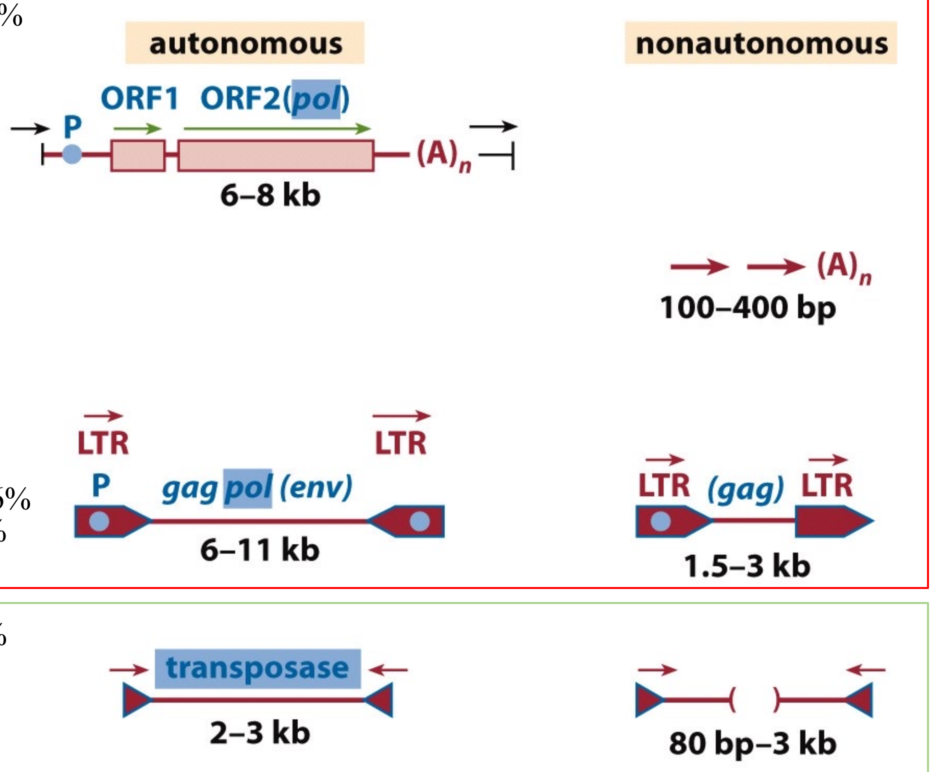
Transposons

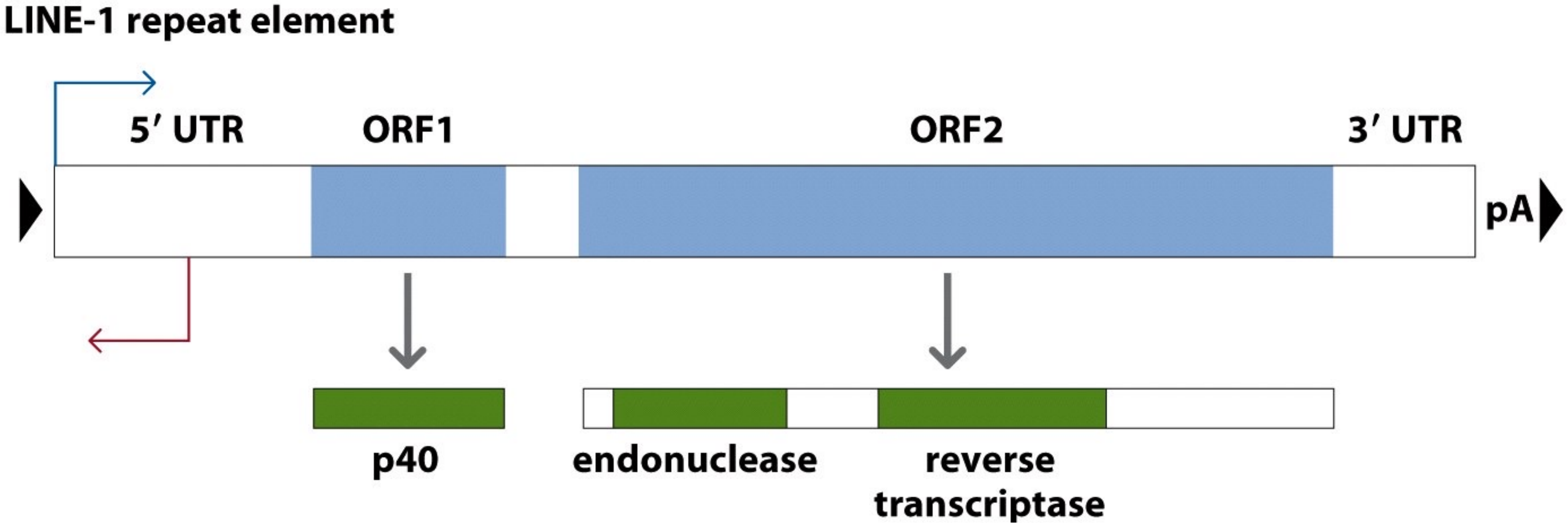
- Retrotransposons (**copy-and-paste**)

LINEs		20%
LINE-1 family	**~600,000**	
LINE-2 family	**~370,000**	
LINE-3 family	**~44,000**	
SINEs		
Alu family	**~1,200,000**	
MIR	**~450,000**	
MIR3	**~85,000**	
retrovirus-like (LTR transposons)		
HERV families	**~240,000**	4.6%
MaLR	**~285,000**	4%
DNA transposon fossils		3%
MER1 (Charlie)	**~213,000**	
MER2 (Tigger)	**~68,000**	
others (including marine, etc.)	**~60,000**	



- Transposons (**cut-and-paste** | inactive)

Human Molecular Genetics, 4th Edition (Tom Strachan & Andrew Read)



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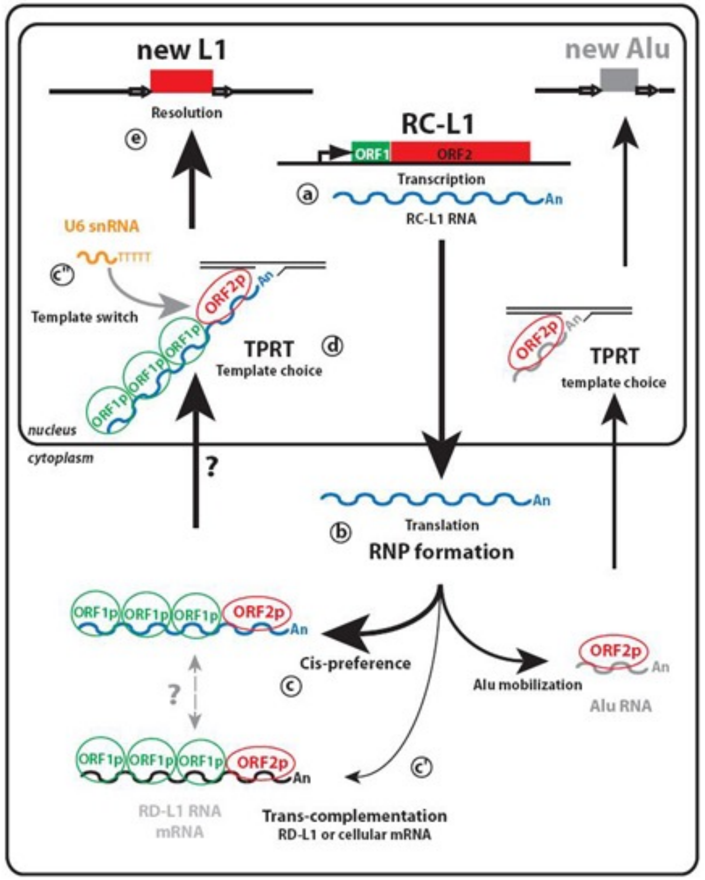
Model of L1- / Alu-retrotransposition

Model of Retrotransposition



a. Transcription of an active L1 element (RC-L1). b. Translation and Ribo Nucleo Protein complex formation. c. *cis*-preference model; the proteins bind preferentially the RNA that encoded them. c'. Possibility of *trans*-complementation of cellular RNA. d. Insertion by Target-site Prime Reverse Transcription (TPRT). c''. mobilization of snRNA by template switch upon L1 insertion. e. Resolution of the insertion.

Integration in TTTT|A (preference for AT-rich regions)

1/100 are full lengths (genome-wide average = 900bp)

80-100 full-lengths L1 (n=6000) active

Gene mutation by L1-insertion

LINE-1 products used for retrotransposition of SINEs, mRNAs (-> processed pseudogenes) and retrogenes

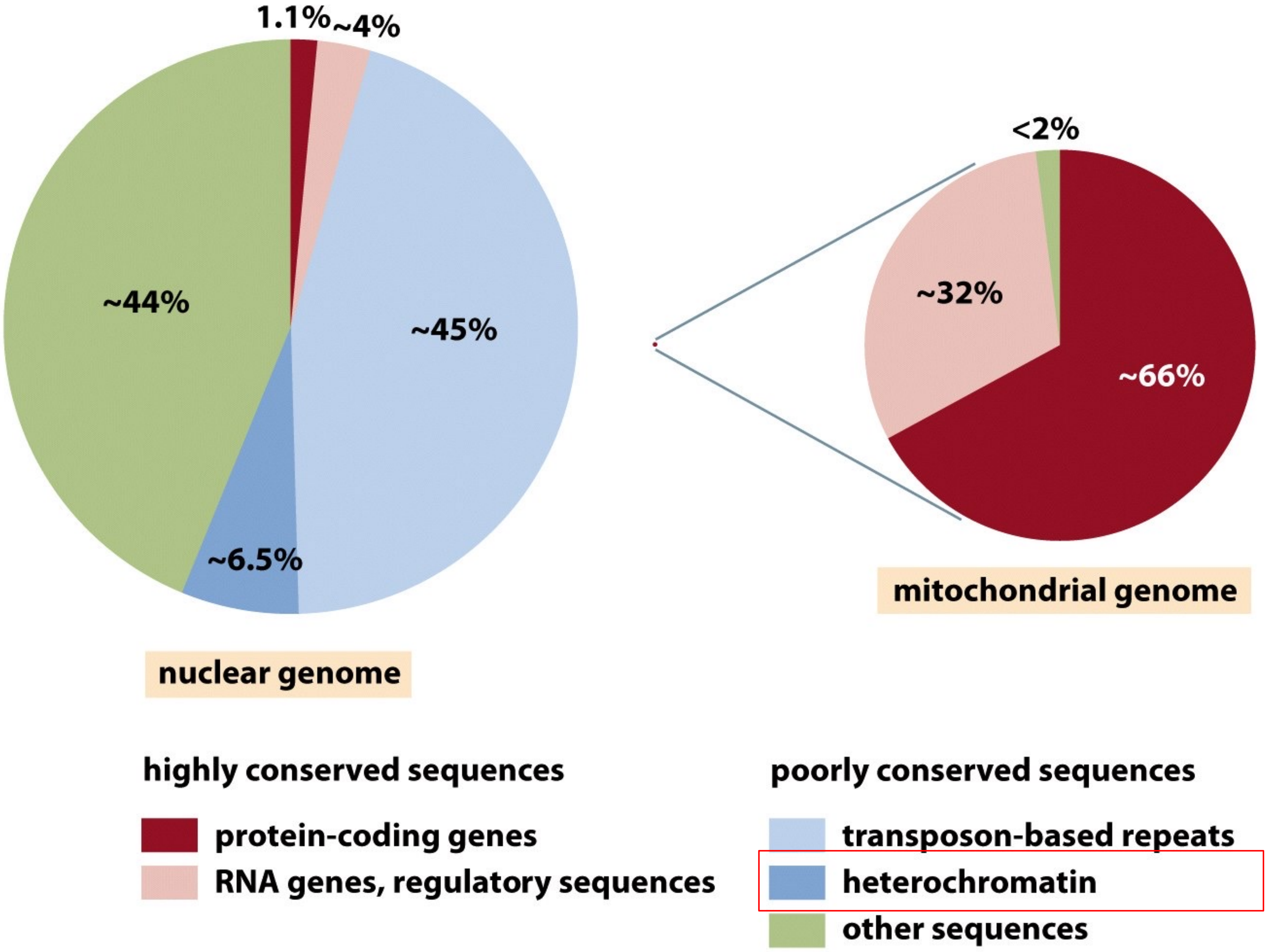
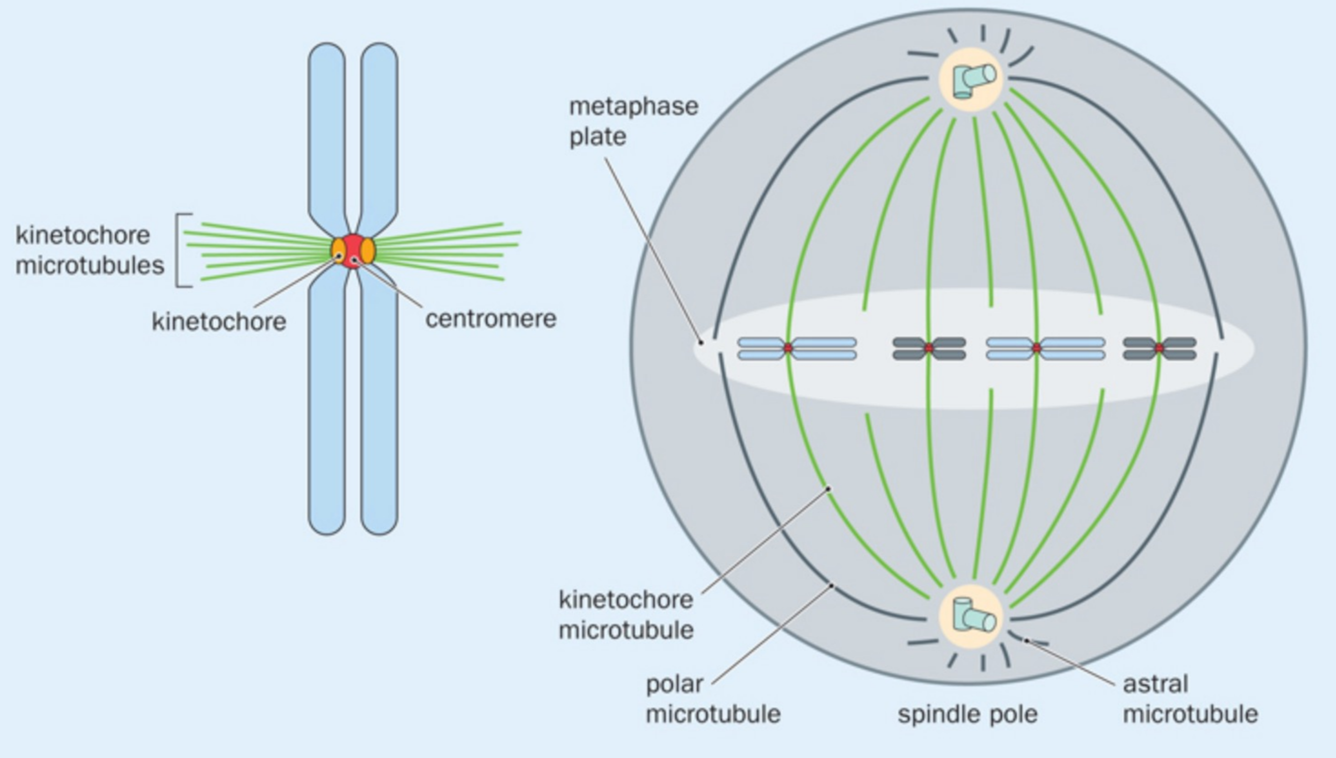


Figure 9.1 Human Molecular Genetics, 4ed. (© Garland Science)

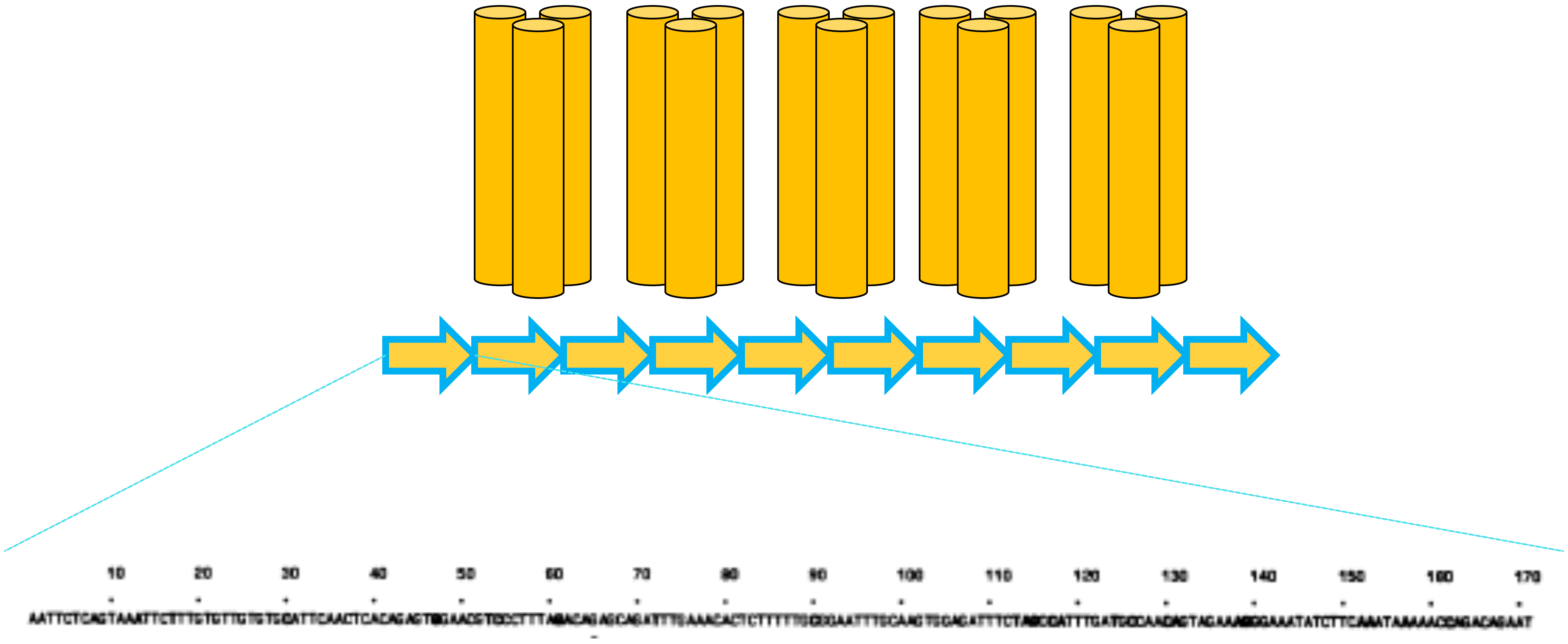
Human Molecular Genetics, 4th Edition (Tom Strachan & Andrew Read)

Centromeric and telomeric DNA



Human Molecular Genetics, 5th Edition (Tom Strachan & Andrew Read)

Centromeric DNA:
Alpha-satellite or alphoid DNA at normal human chromosomes



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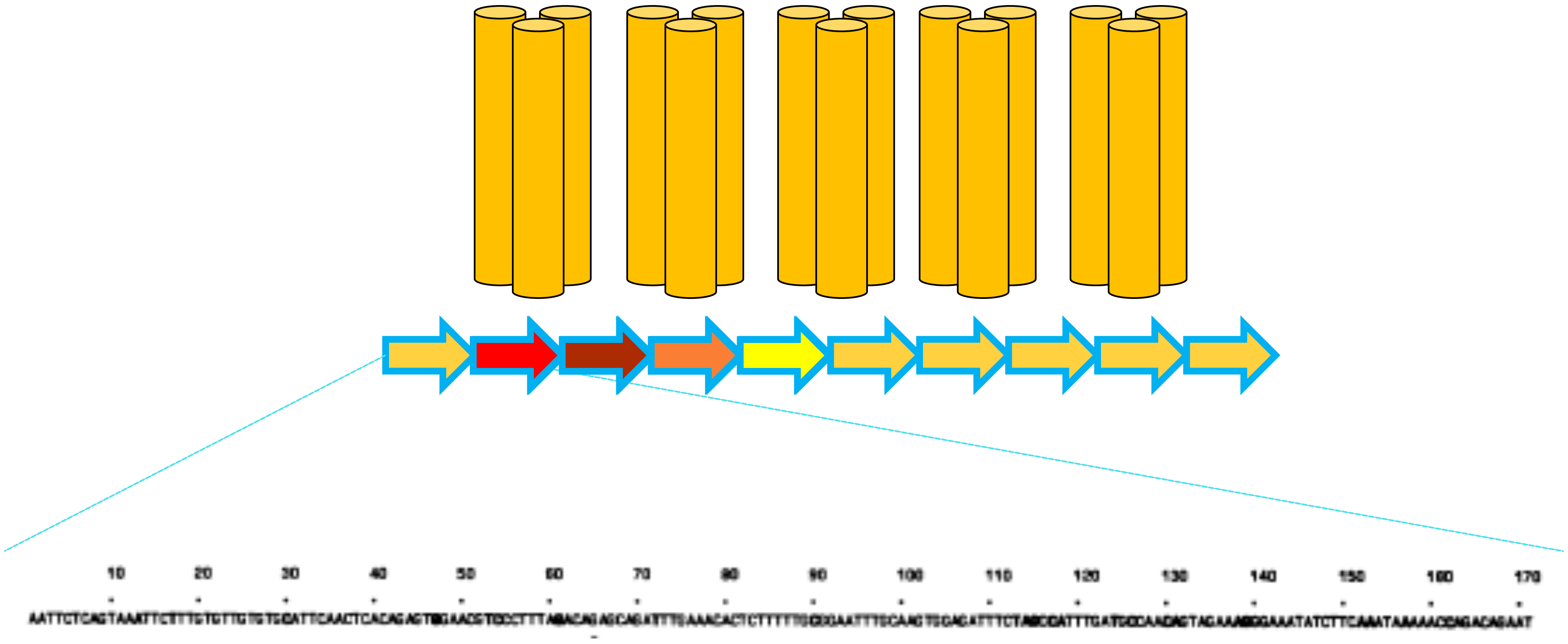

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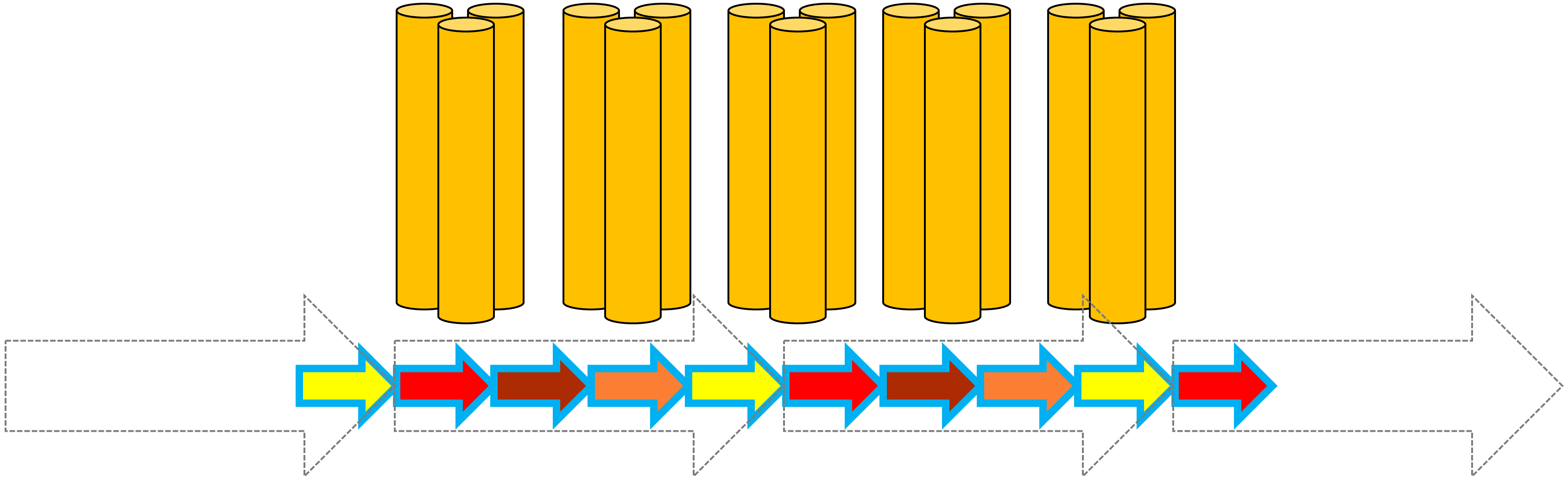


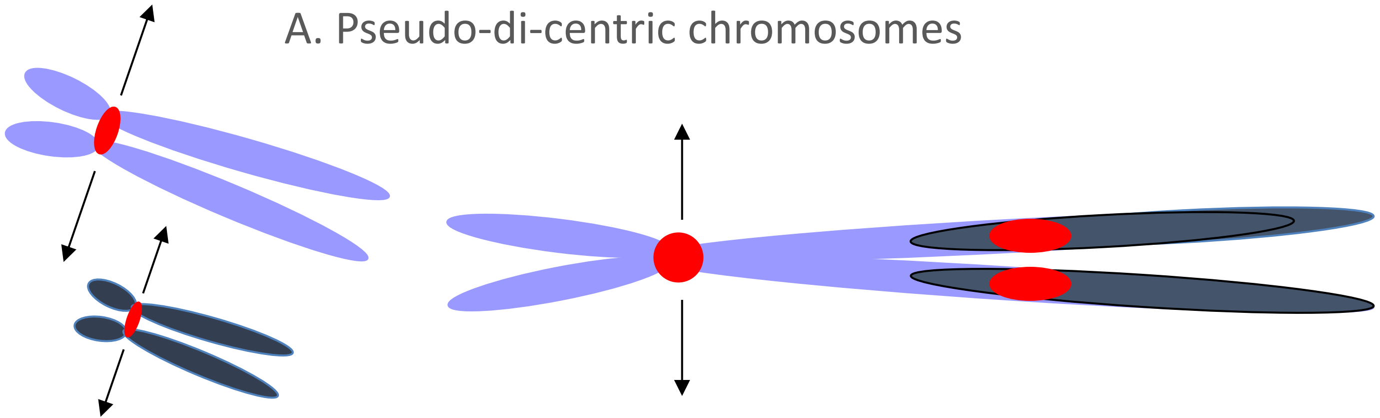
One alphoid higher order repeat can be :

- specific for one chromosome
- occurring on different chromosomes

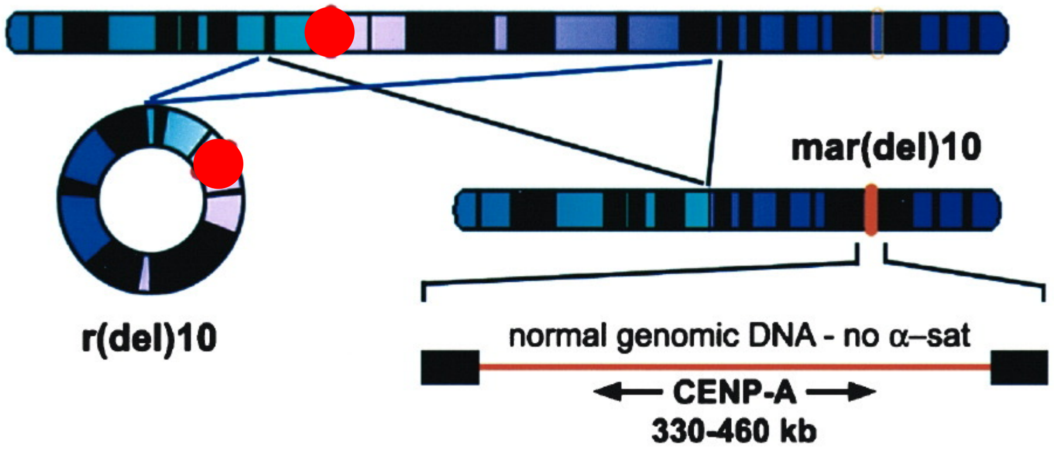
Different alphoid higher order repeats can be co-existing on the same chromosome

Alpha-satellite DNA is not sufficient nor necessary for centromere function

A. Pseudo-di-centric chromosomes



B. Neo-centromeres without alpha-satellite DNA



Centromere function is epigenetically regulated

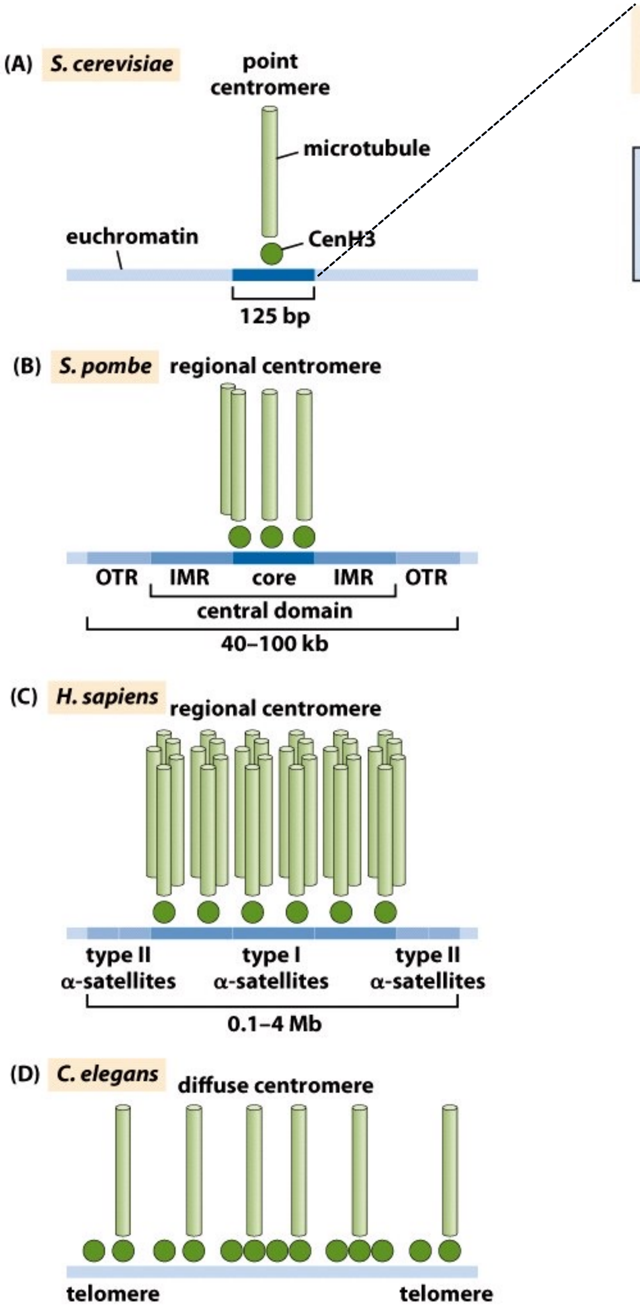


Figure 2.11 Human Molecular Genetics, 4ed. (© Garland Science)

centromere

CDE I	CDE II	CDE III
TCACATGAT AGTGTACTA	80–90 bp > 90% (A+T)	TGATTTCCGAA ACTAAAGGCTT

Centromere DNA elements

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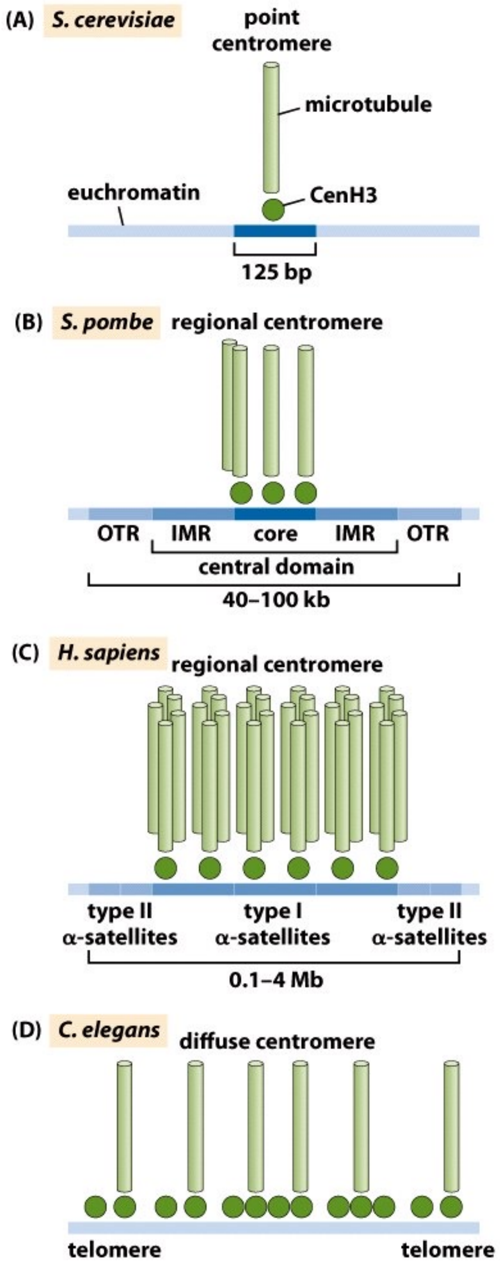
Histone H3 variant CENP-A is the best candidate to carry the epigenetic centromere mark



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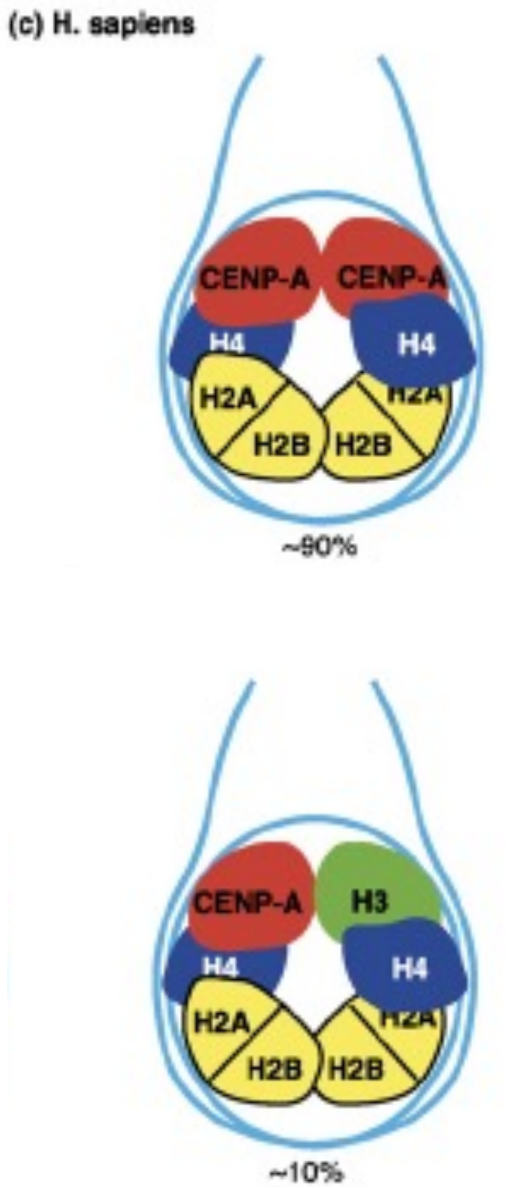
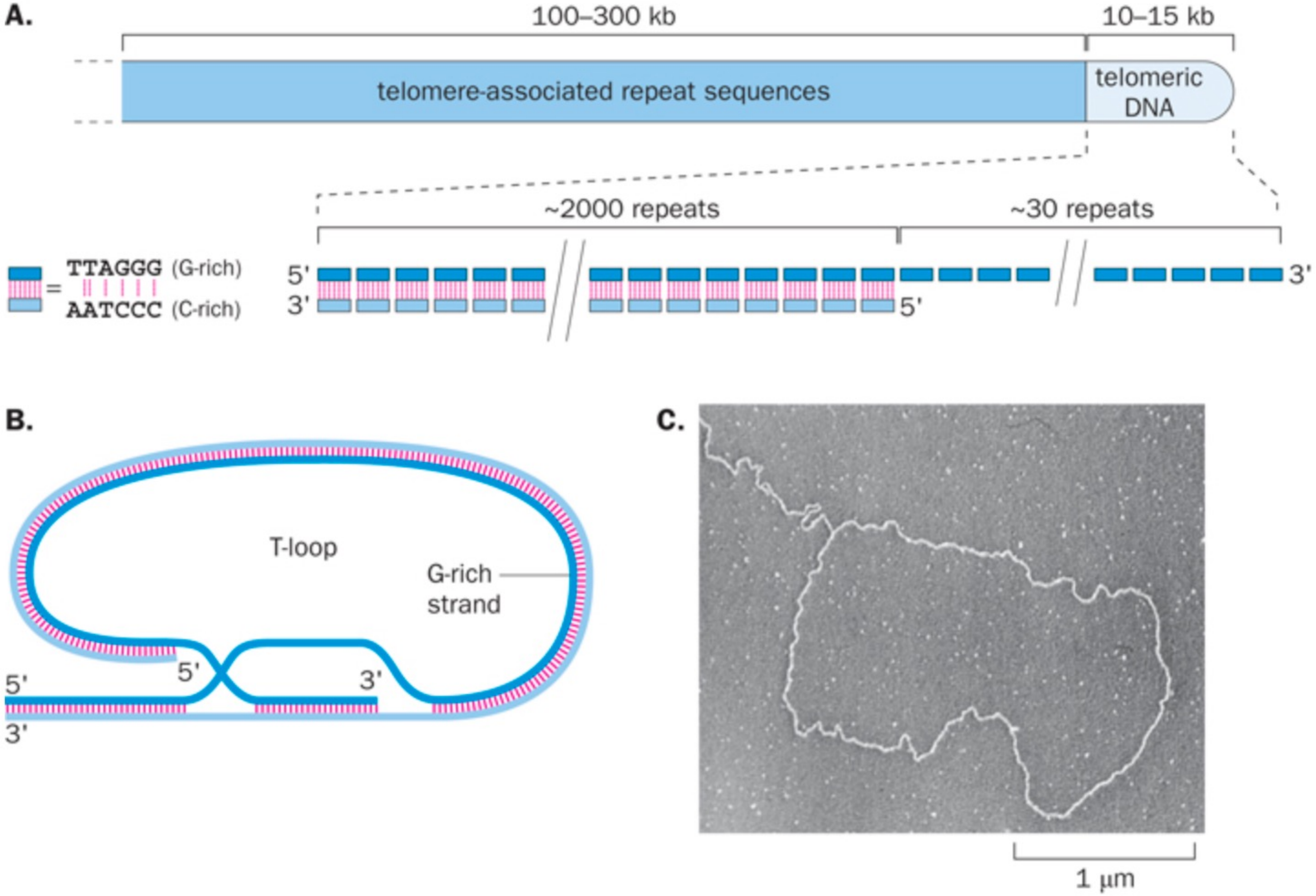


Telomeric DNA



Telomeres:

- important for maintaining structural integrity (prevents instability of ends, degradation and fusion with ends of broken chromosomes)
- required for complete replication of chromosome end
- in some cells: interaction with nuclear envelope for positioning of chromosomes in the nucleus

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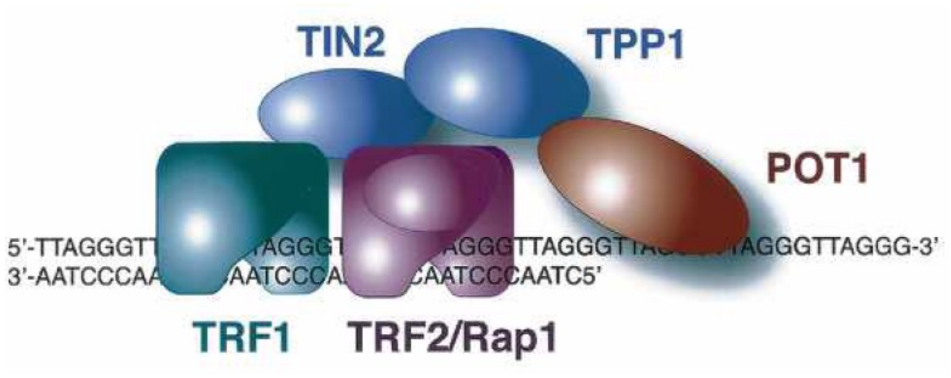
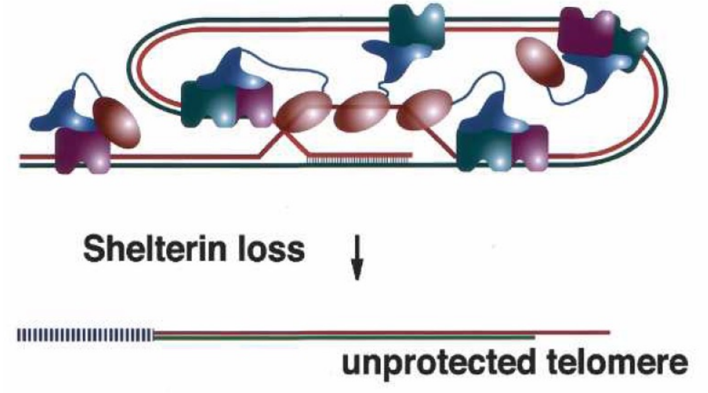
Telomeric DNA

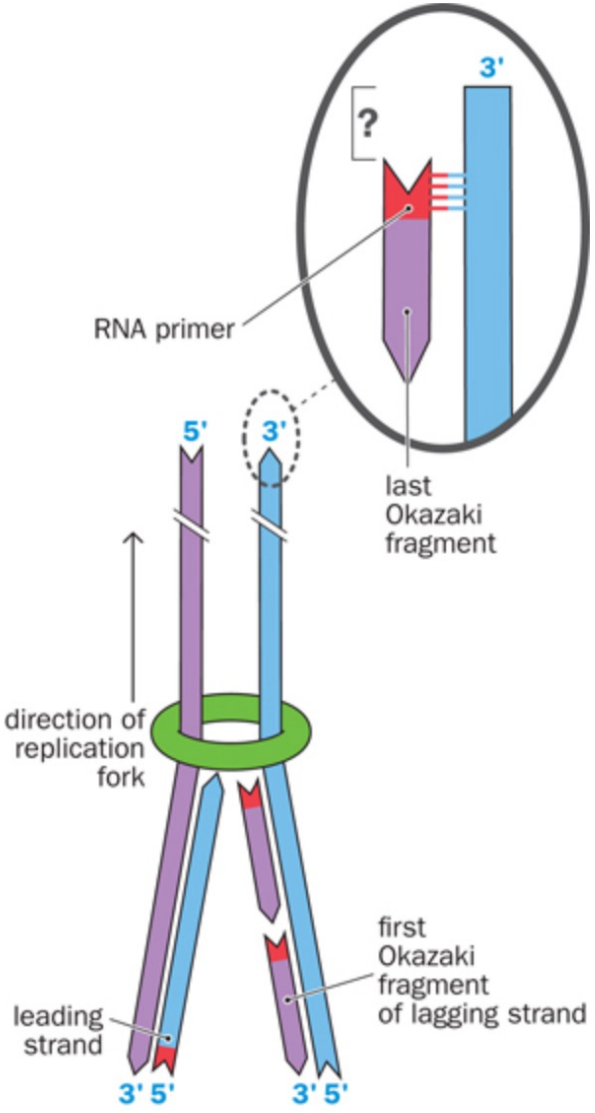
Shelterin - Telosome

Nucleoprotein complex: $(TTAGGG)_{2500}$ + shelterin





The ‘chromosome end-replication’ problem: incomplete synthesis



DNA synthesis starts at free 3’-OH of an RNA primer

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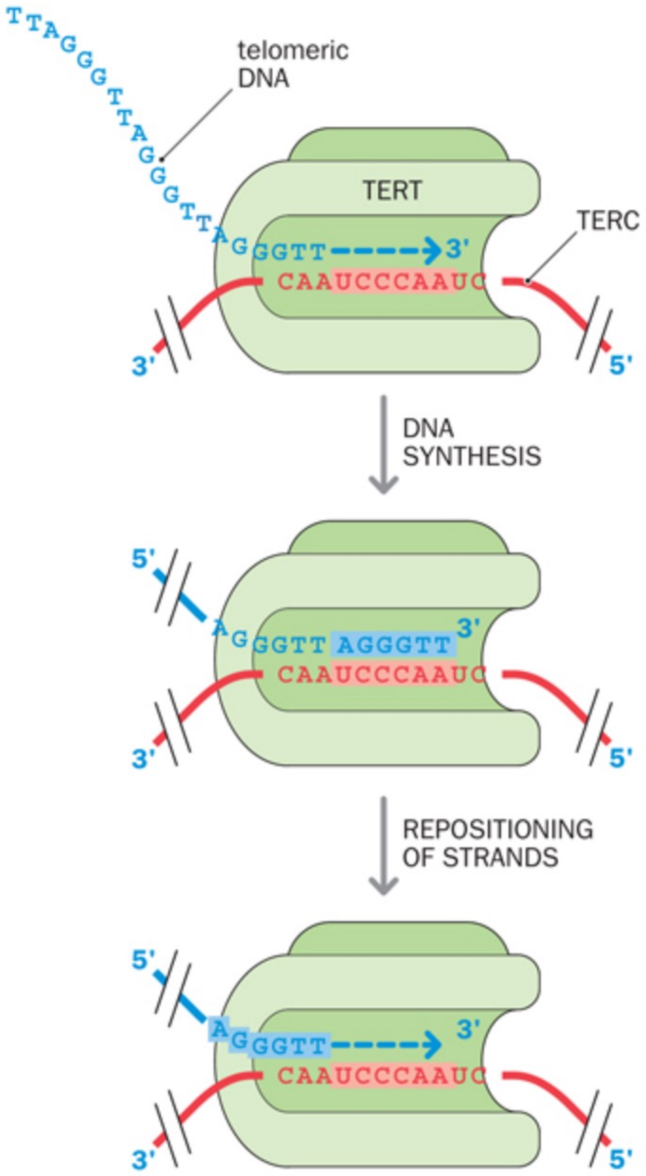
Telomerase uses a reverse transcriptase and a non-coding RNA template to make new telomeric DNA repeats



Telomerase:

- Germ cells
- Embryonic cells
- Stem cells
- Cancer cells

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Humane genetic variation

The key to:

- Understanding differences between people
- Identifying genes / variants that play a role in disease and health

single nucleotide variants
small insertions and deletions
copy number variation
structural variation
(Deletion, Duplication, Amplification, Translocation, Inversion, Retrotransposition)

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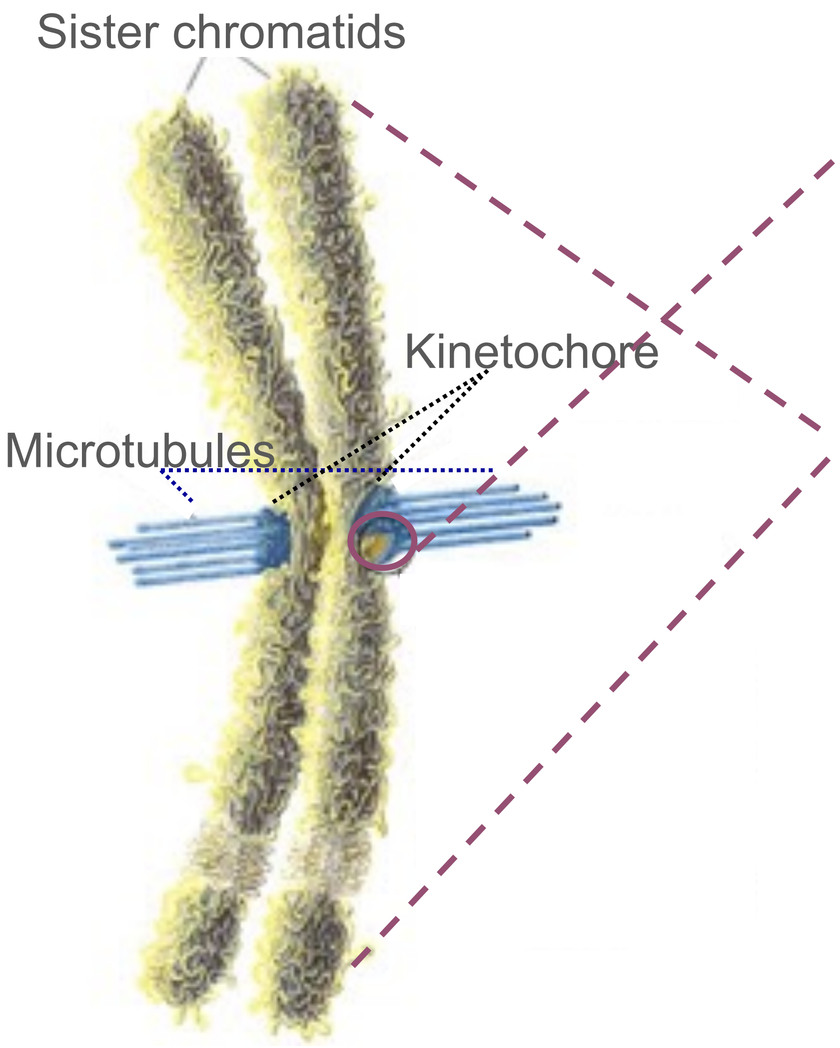
Chromosomes ensure transport and integrity of genetic information



Functional domains

Centromere

- correct segregation (capture microtubules)
- chromosome movements

Telomeres

- protect against degradation, fusion and recombination
- complete end replication
- chromosome movements
- subtelomeric gene expression

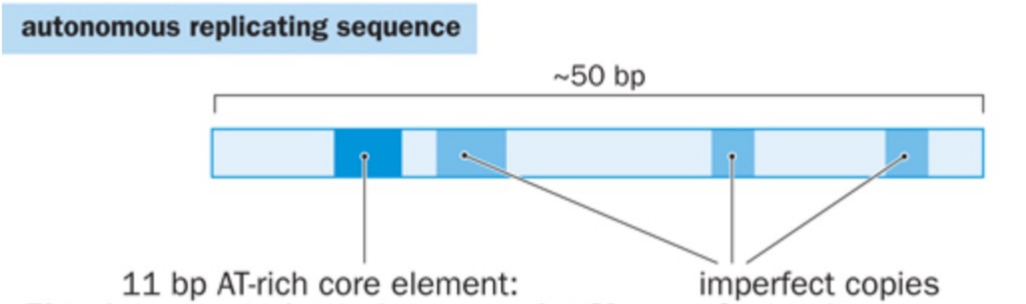
Origins of replication

- replication of the genetic information once per cell cycle

Origins of replication

DNA sequence in cis where proteins bind in preparation for DNA replication

S. cerevisiae



ARS provides a binding site for:
ORC (multi-protein origin of replication complex) + TF

Mammalia

DNA is replicated from multiple initiation sites per chromosome, with an average of **one initiation site per 40-80 kb DNA**

Structural motives can be important: probable replication origins often have guanine-rich DNA sequences with the potential to form G-quadruplexes, a four-stranded DNA structure with Hoogsteen binding between the guanines

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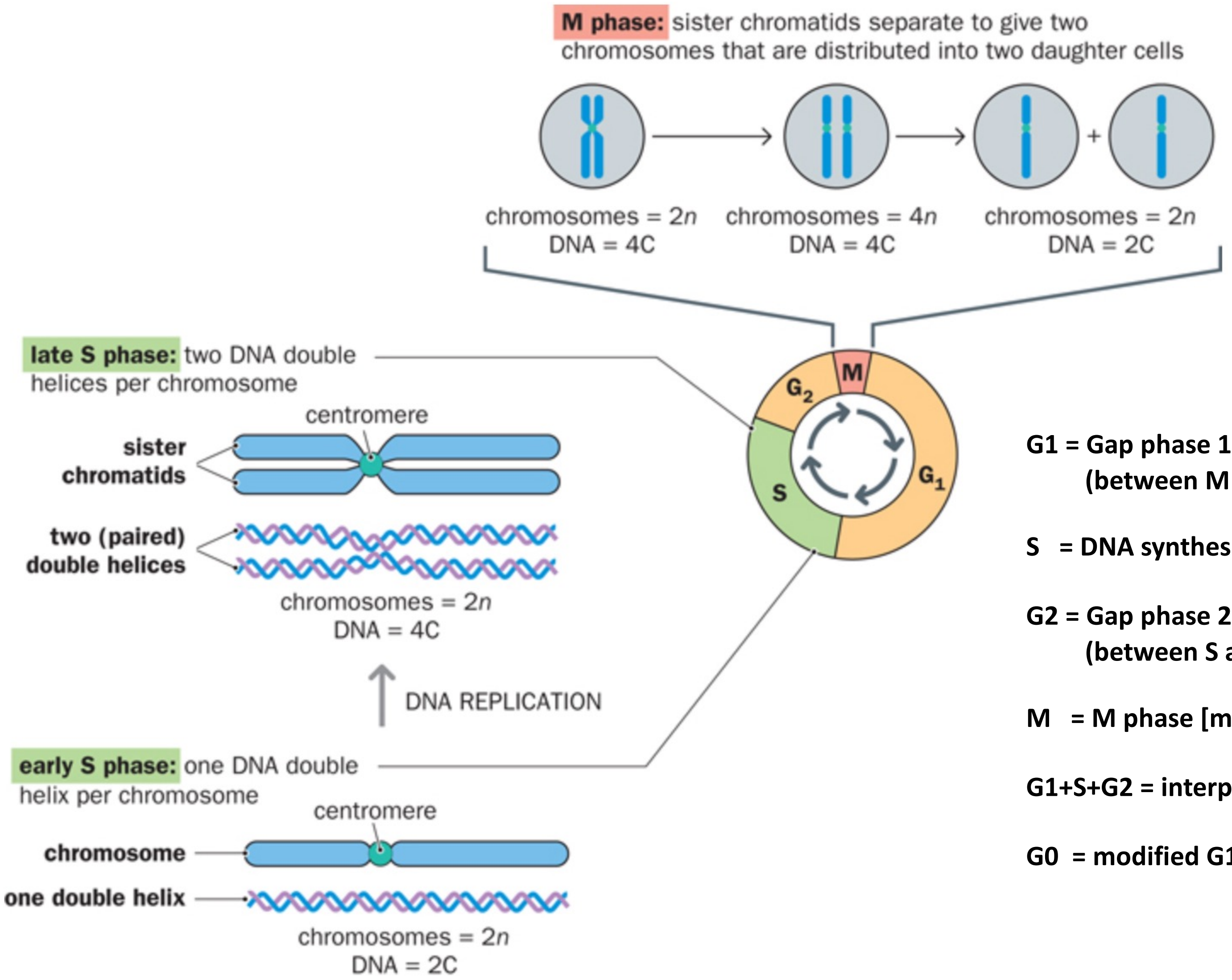
Doubling the number of chromosomes and the DNA content prior to mitosis during the cell cycle



G1 = Gap phase 1
(between M and S)

S = DNA synthesis

G2 = Gap phase 2
(between S and M)

M = M phase [mitosis + cytokinesis]

G1+S+G2 = interphase

G0 = modified G1-phase in non-dividing cells

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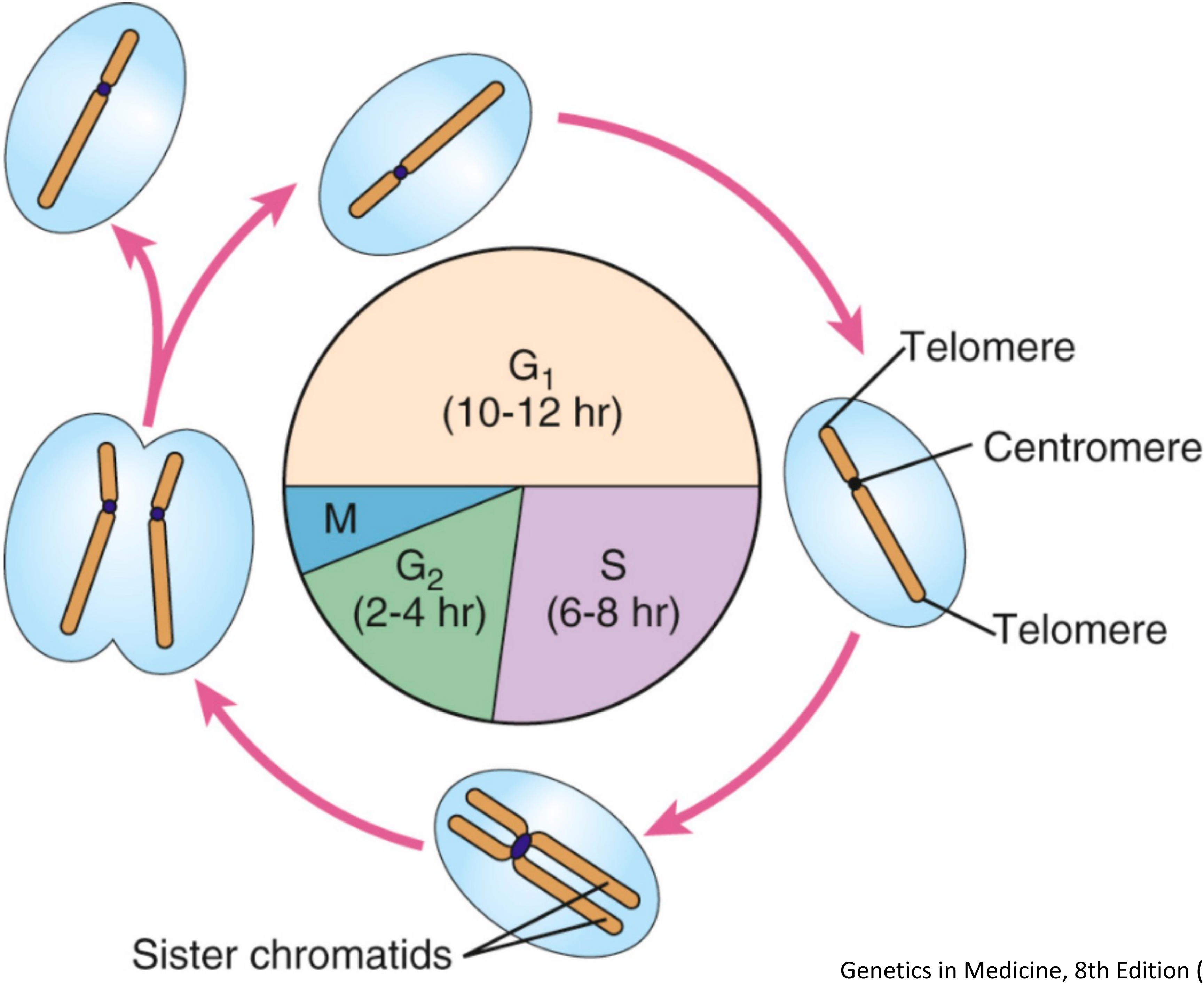

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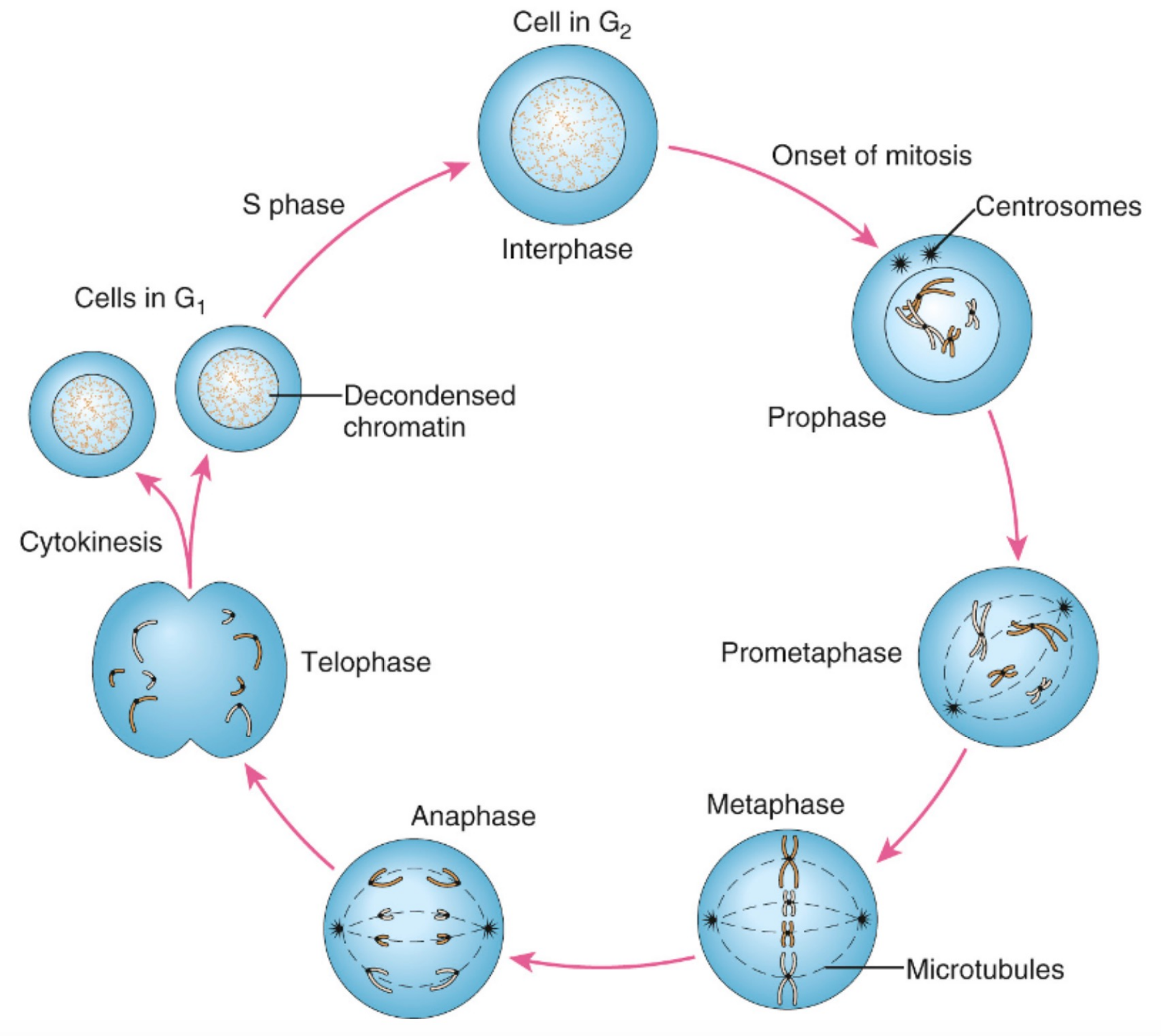
Mitosis (nuclear division) and cytokinesis (cell division)



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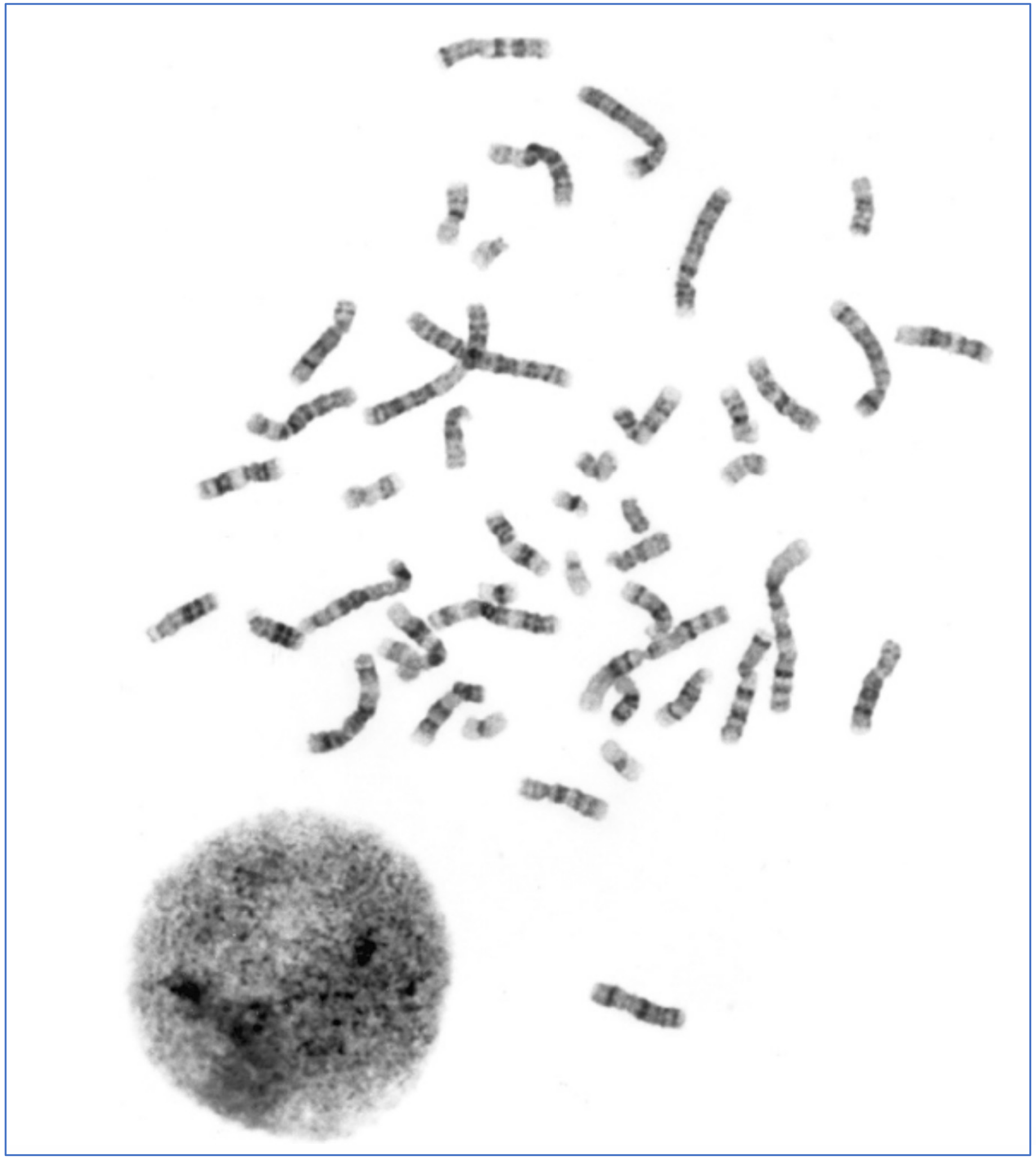

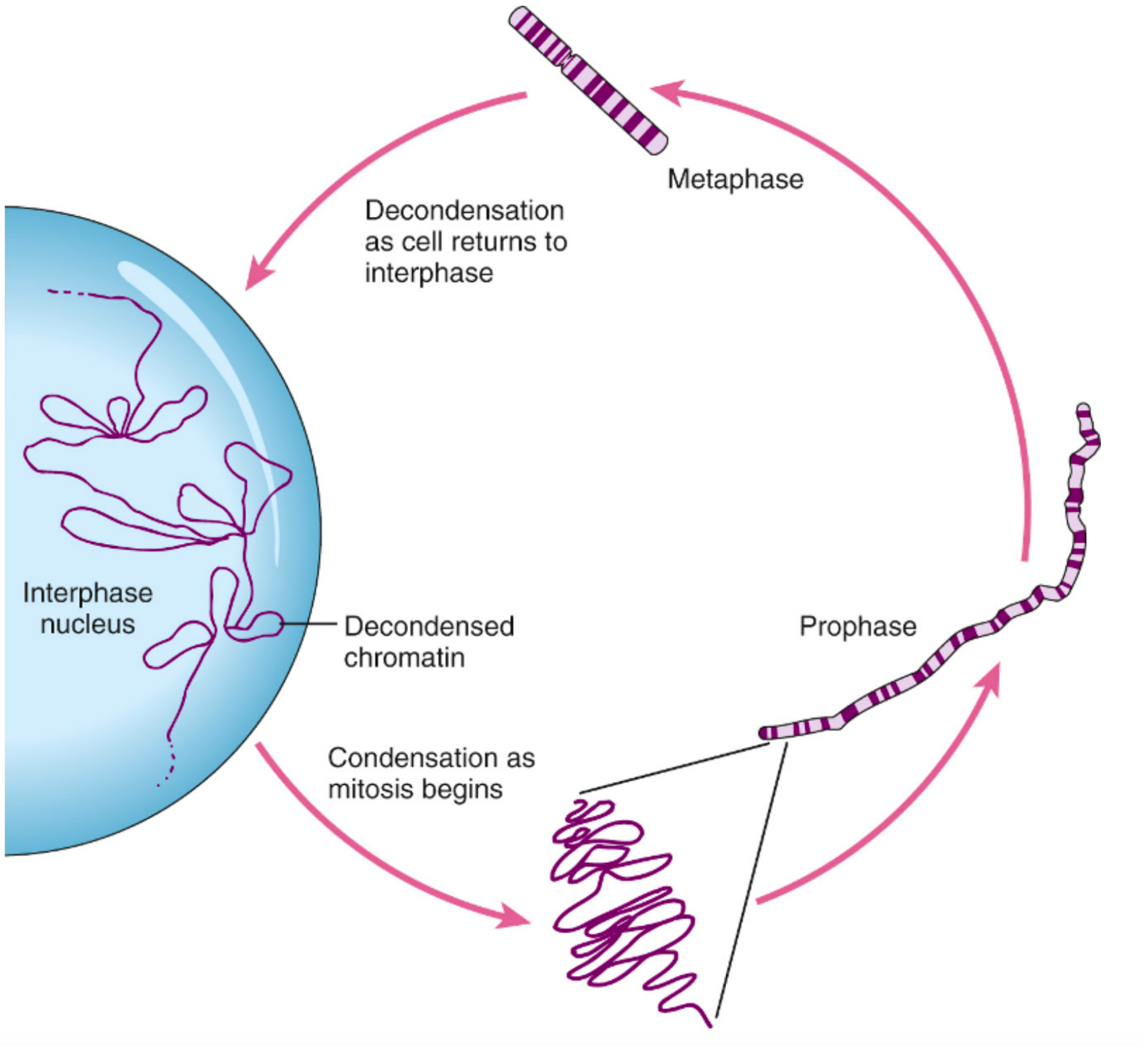

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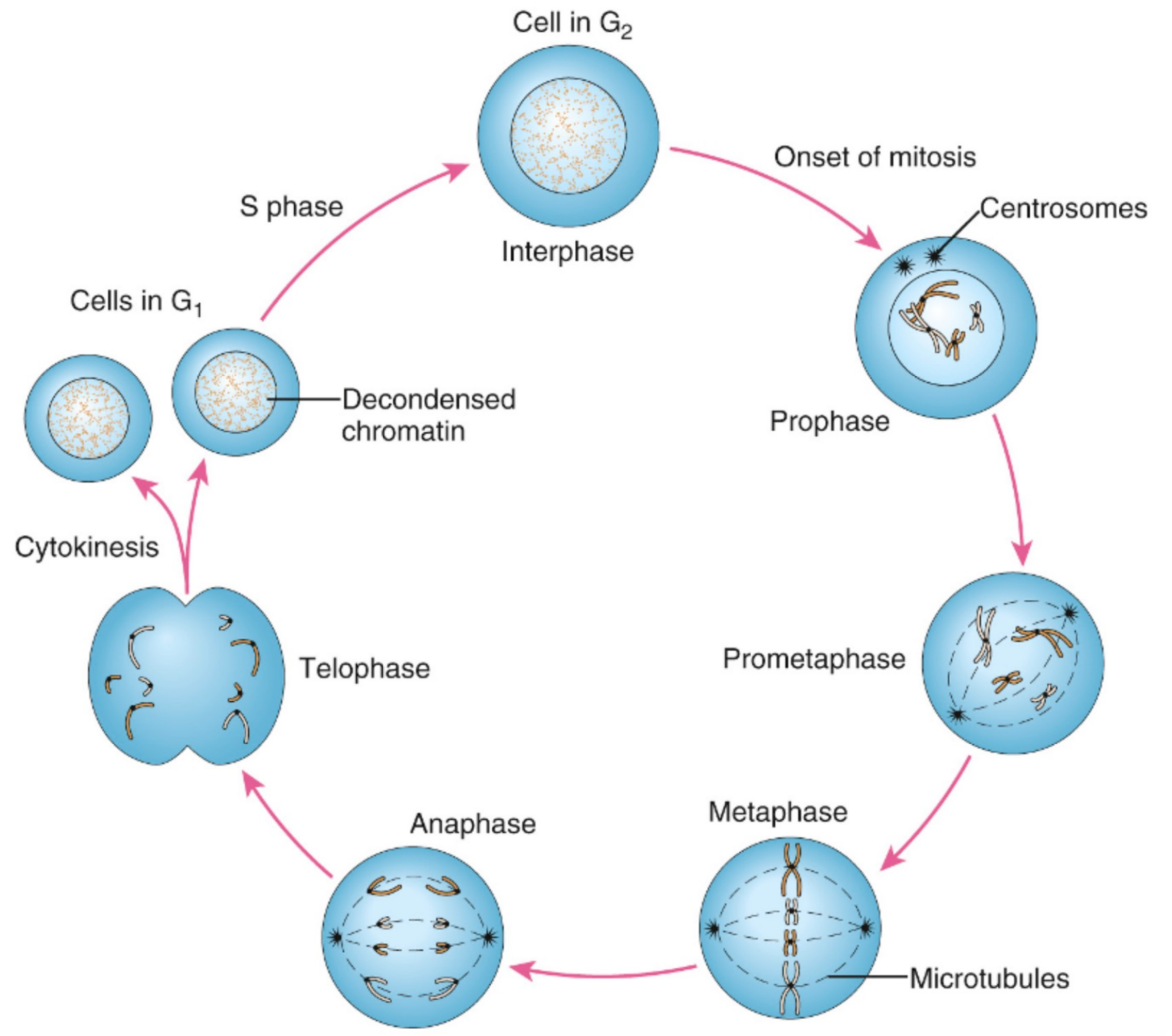

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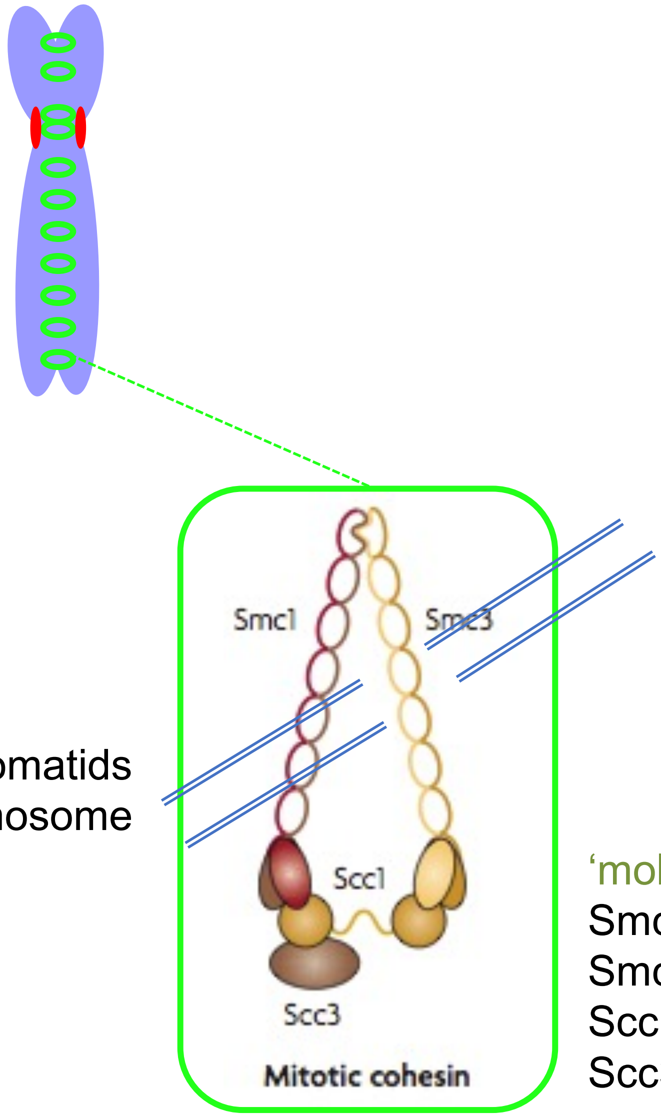
Molecular glue between replicated DNA-molecules = cohesin complex

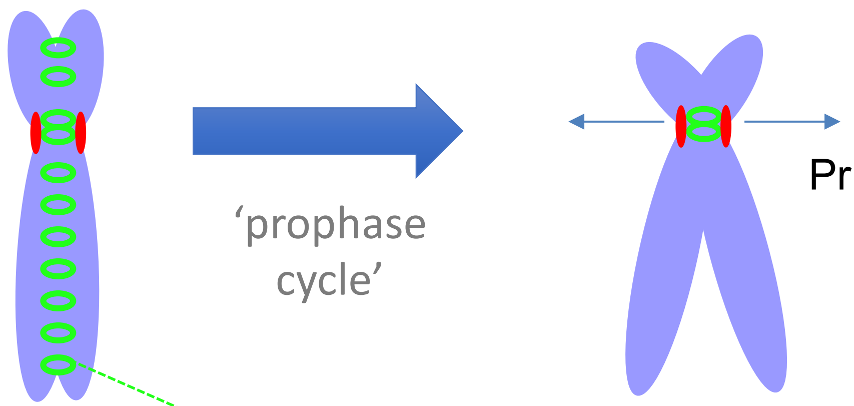
Prophase



Sister chromatids
of a chromosome

‘molecular glue’ between replicated DNA-molecules (sister chromatids)
Smc1: Structural maintenance of chromosomes protein 1
Smc3: Structural maintenance of chromosomes protein 3
Scc1: Sister chromatid cohesion protein 1
Scc3: Sister chromatid cohesion protein 3

Loss of sister chromatid cohesion during mitosis

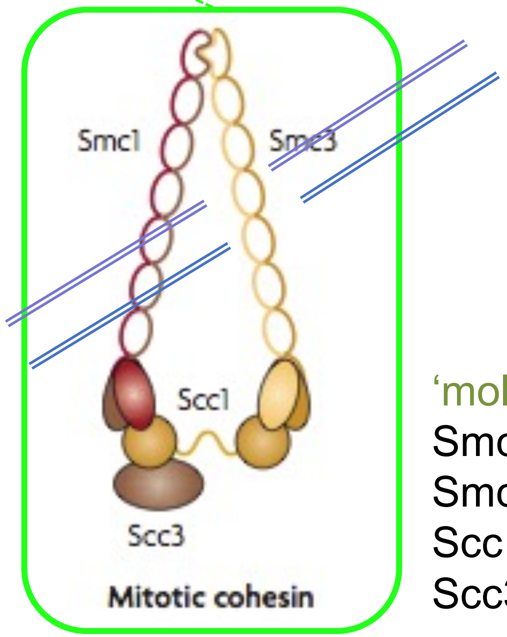


Prophase

Metaphase

‘prophase cycle’

Primary constriction



Sister chromatids of a chromosome

‘molecular glue’ between replicated DNA-molecules (sister chromatids)

Smc1: Structural maintenance of chromosomes protein 1
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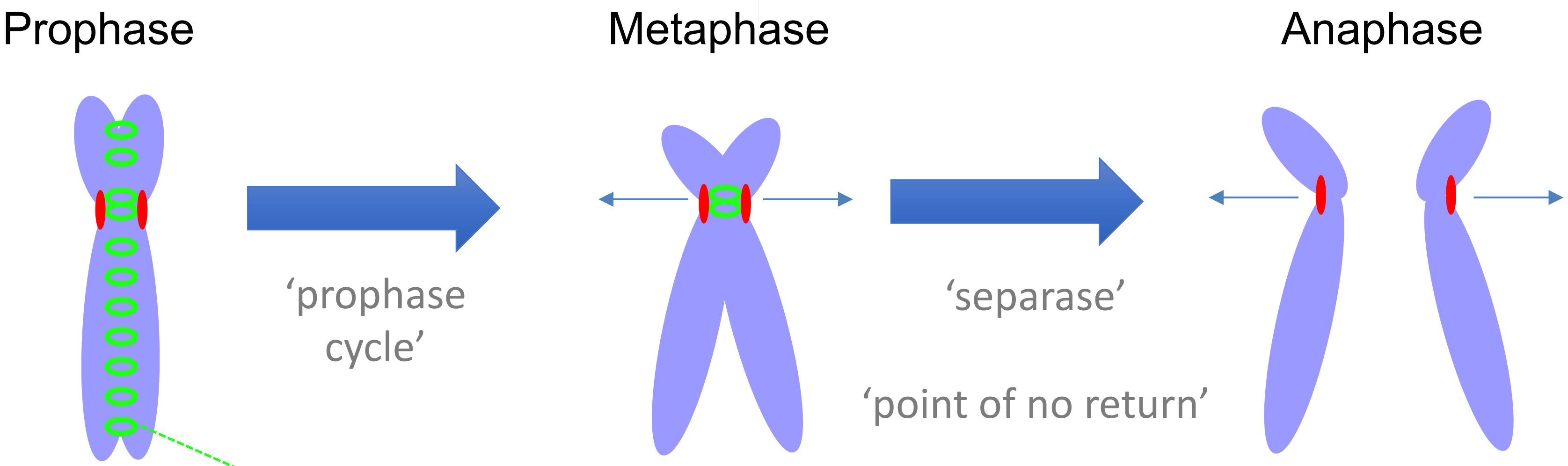


Prophase

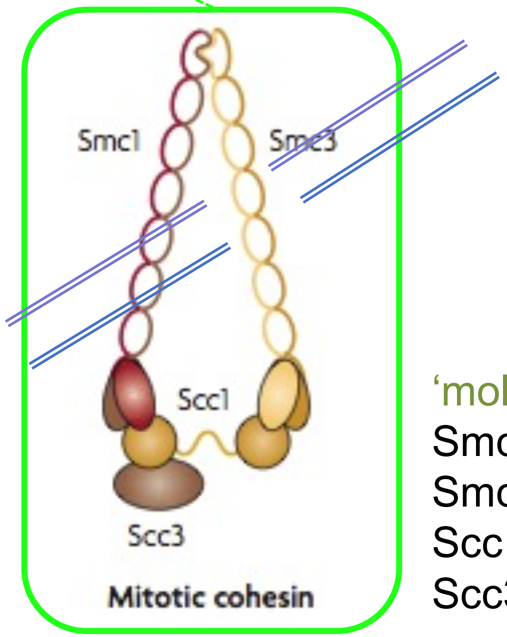
Metaphase

Anaphase

‘prophase cycle’

‘separase’

‘point of no return’



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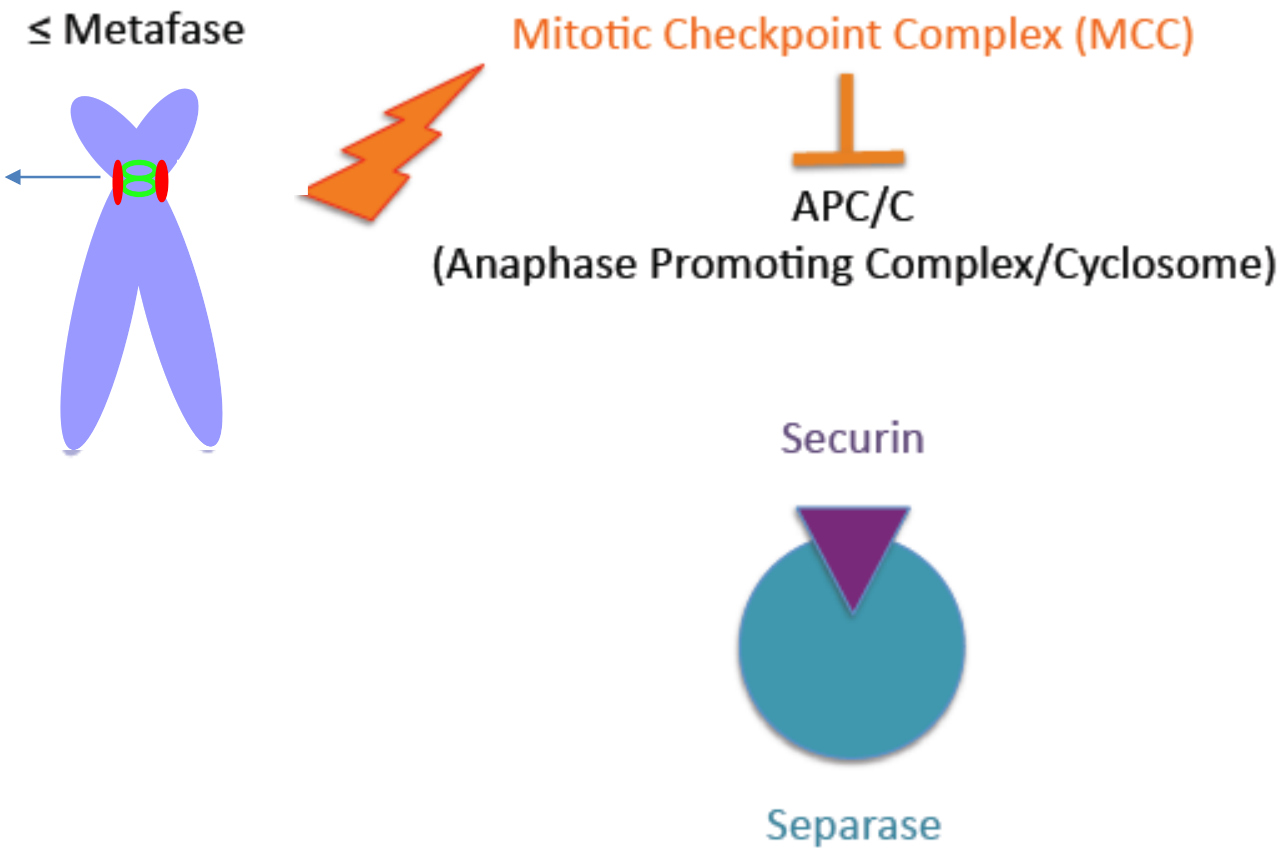
Spindle Assembly Checkpoint



≤ Metafase

Mitotic Checkpoint Complex (MCC)

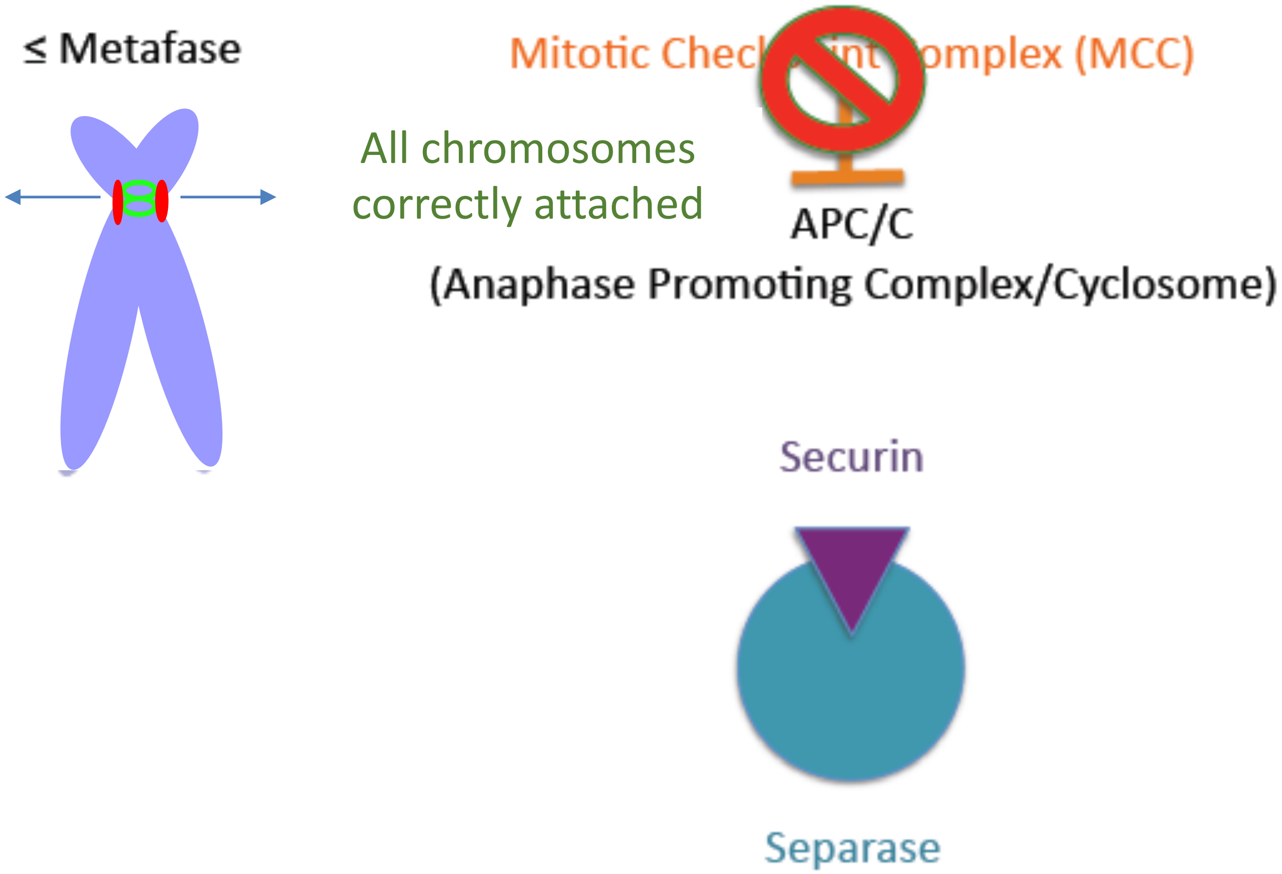
APC/C

(Anaphase Promoting Complex/Cyclosome)

Securin

Separase

Spindle Assembly Checkpoint



≤ Metafase

All chromosomes
correctly attached

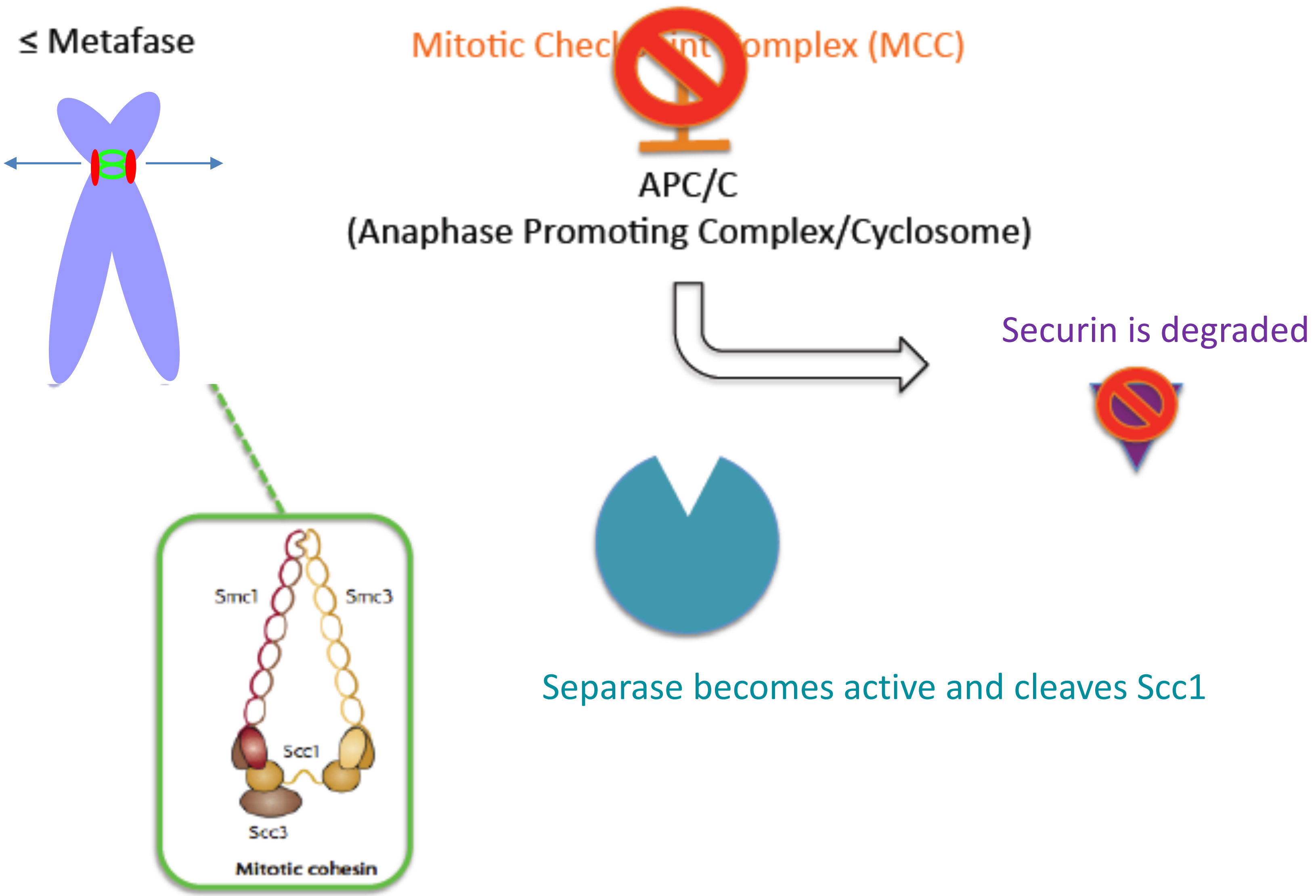
Mitotic Checkpoint Complex (MCC)

APC/C
(Anaphase Promoting Complex/Cyclosome)

Securin

Separase

Spindle Assembly Checkpoint



≤ Metafase

Mitotic Chec int omplex (MCC)

APC/C
(Anaphase Promoting Complex/Cyclosome)

Securin is degraded

Separase becomes active and cleaves Scc1

Hoofdstuk 2

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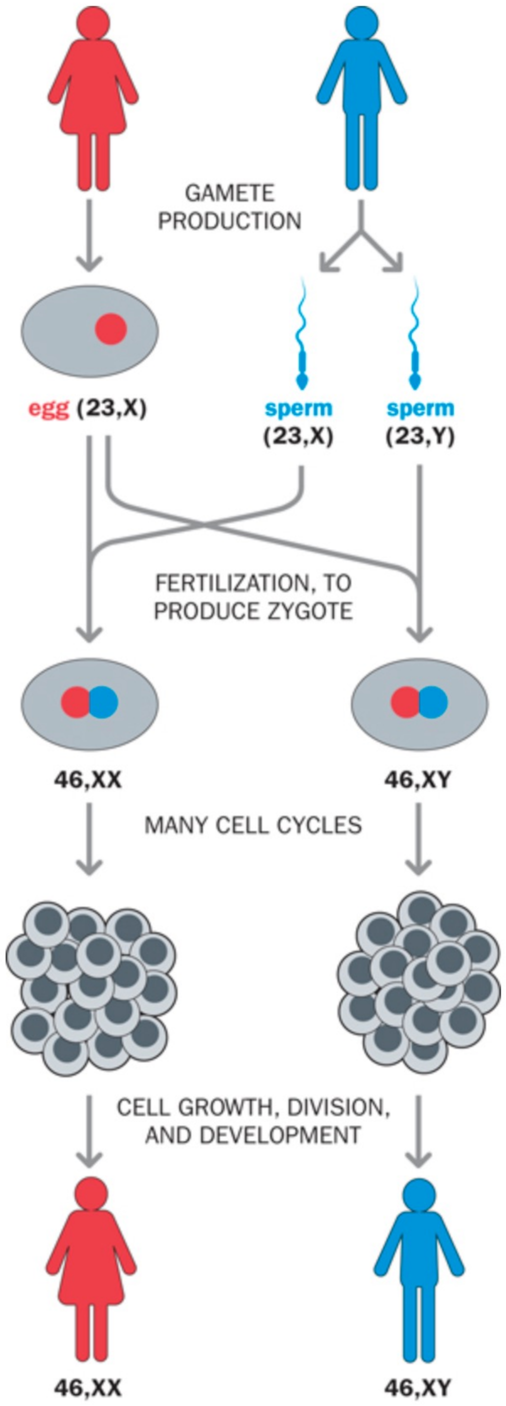
- ✓ Human Gametogenesis and Fertilization
  - Spermatogenesis
  - Oogenesis
  - Fertilization
- ✓ Medical Relevance of Mitosis and Meiosis

The human life cycle, seen from a chromosomal angle



<u>Meiosis</u>:

1 diploid cell (2n)
-> man: 4 haploid gametes (1n)
-> woman: 1 haploid gamete + polar bodies

<u>Mitosis</u>:

1 diploid cell (2n) -> 2 diploid daughter cells (2n)

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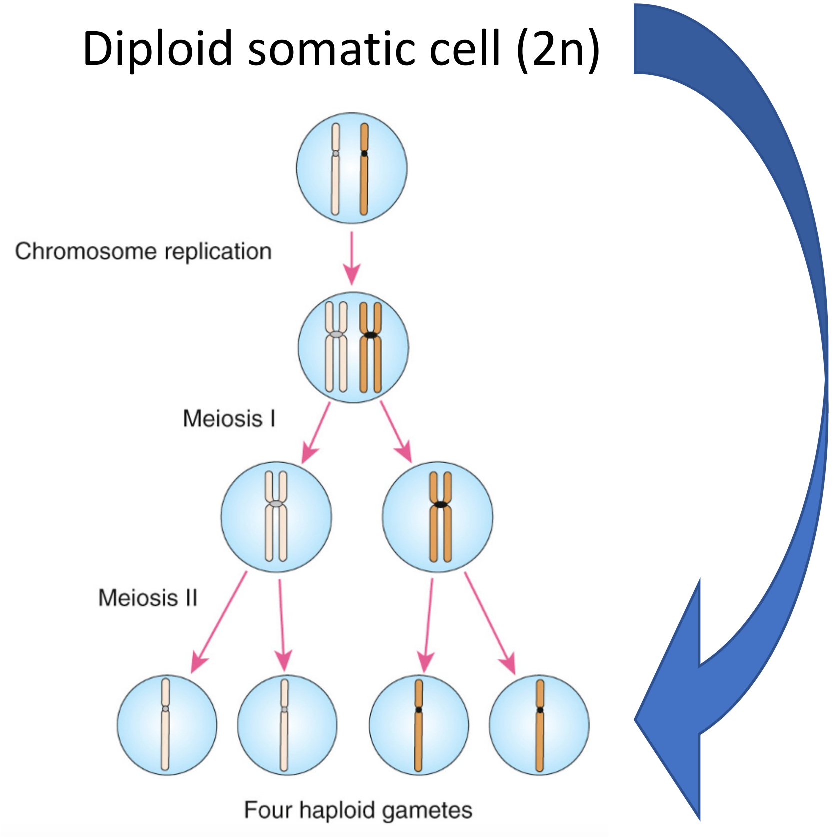
Meiosis: ‘to reduce’

Diploid somatic cell (2n)



Haploid gametes
4 x (n)

Chromosomes replicated once

Crossovers -> genetic diversity
-> correct homologue segregation

meiosis I: reductional division (disjunction)

- separation of homologous autosomes
- separation of sex chromosomes

Random assortment

meiosis II: equational division

- separation of sister chromatids (~mitosis)

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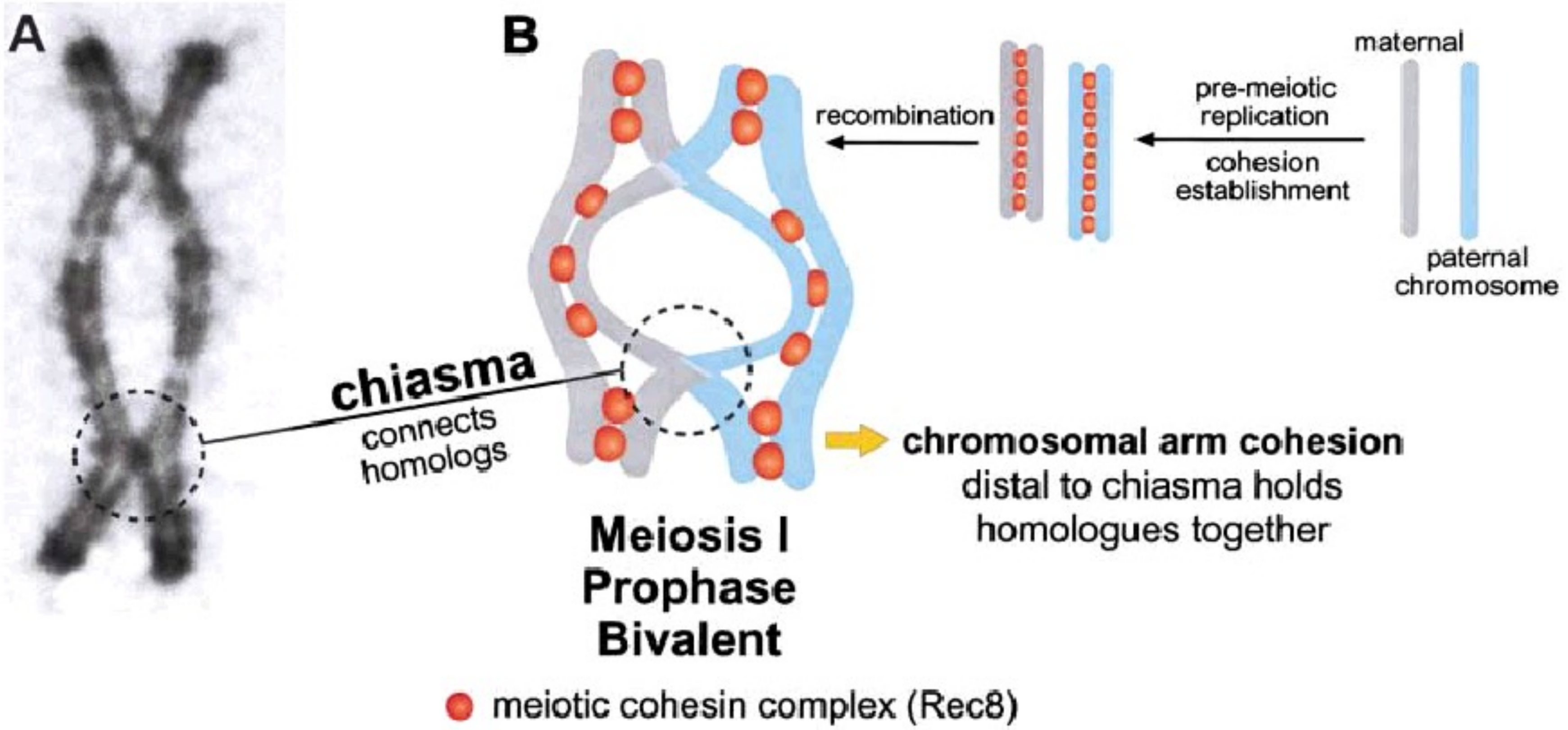
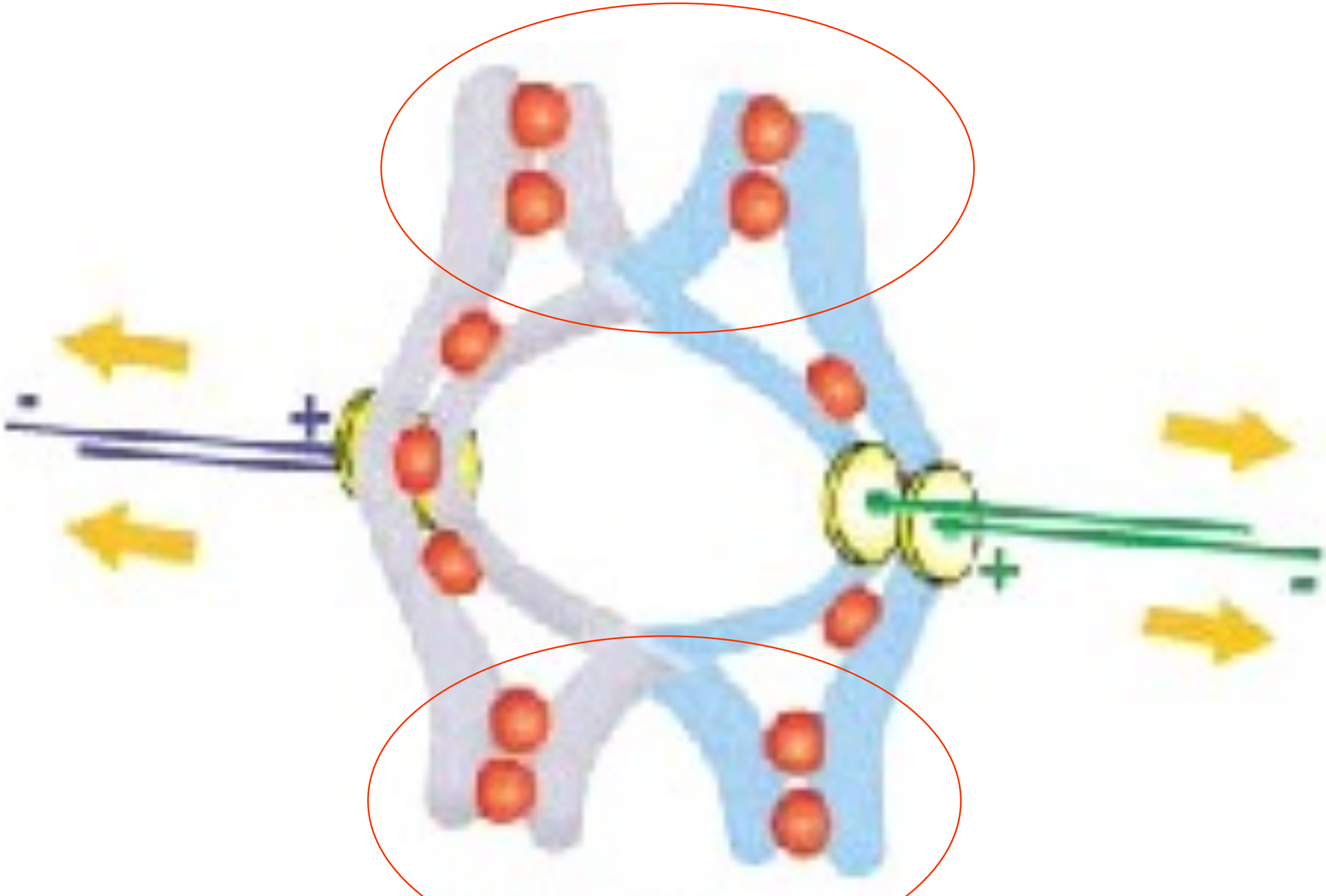
Prophase I

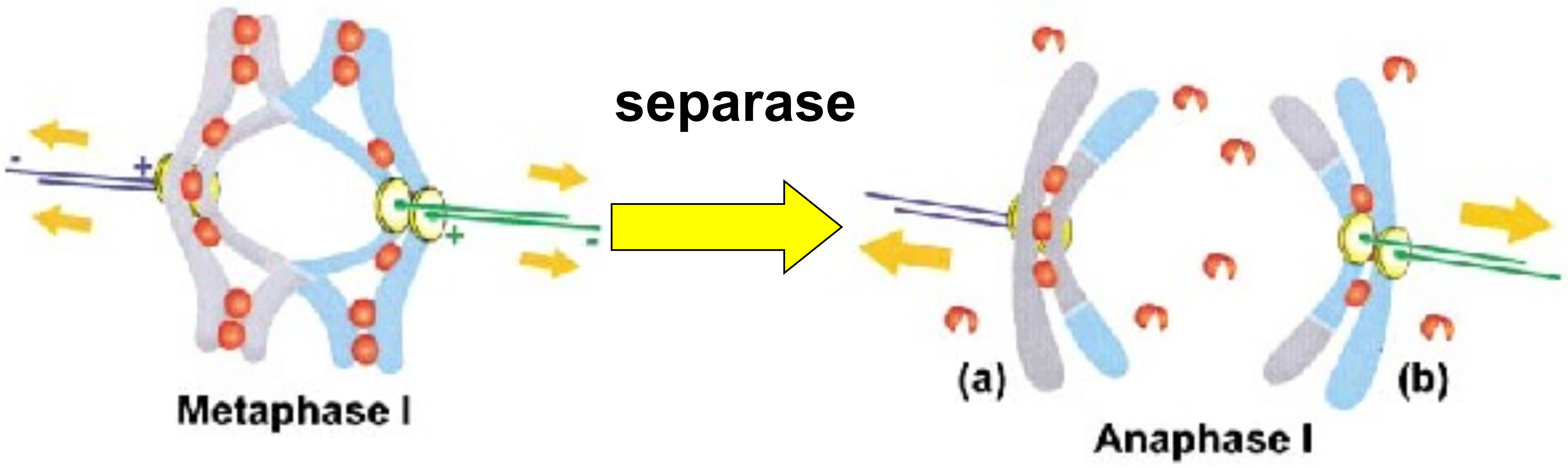
Premeiotic S-fase





Metaphase I

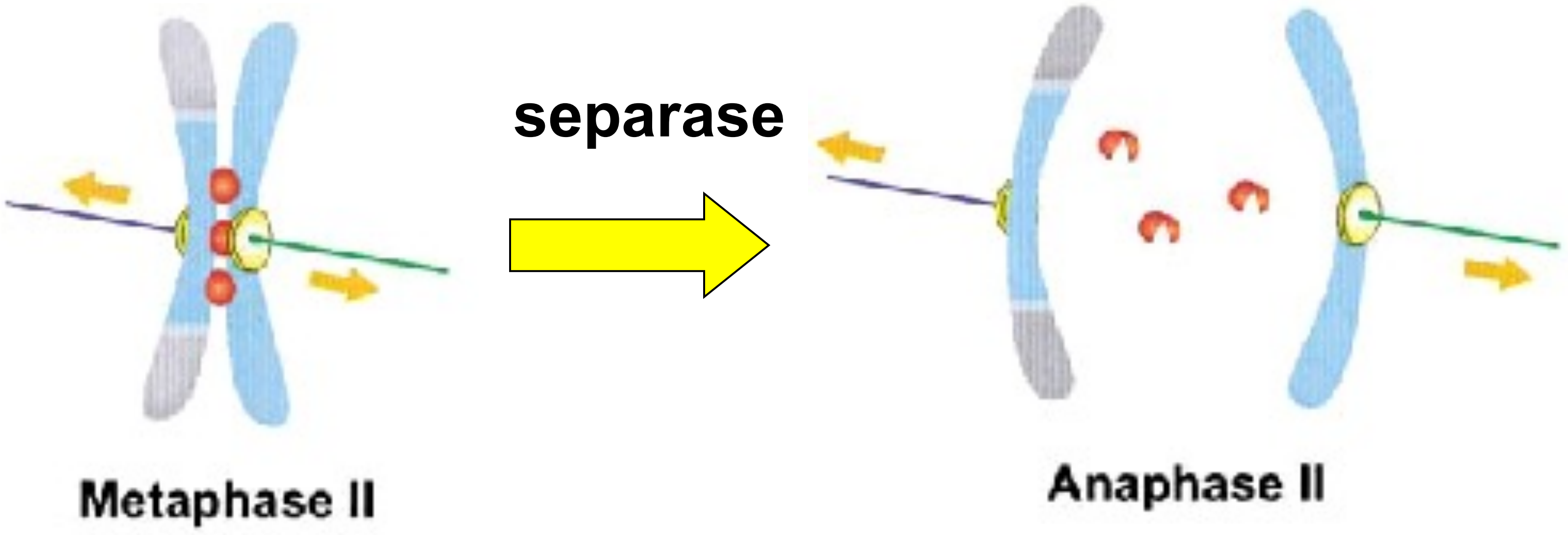
First meiotic division



separase

Cohesin rings at the chromosomal arms are opened, but remain intact at the centromeres

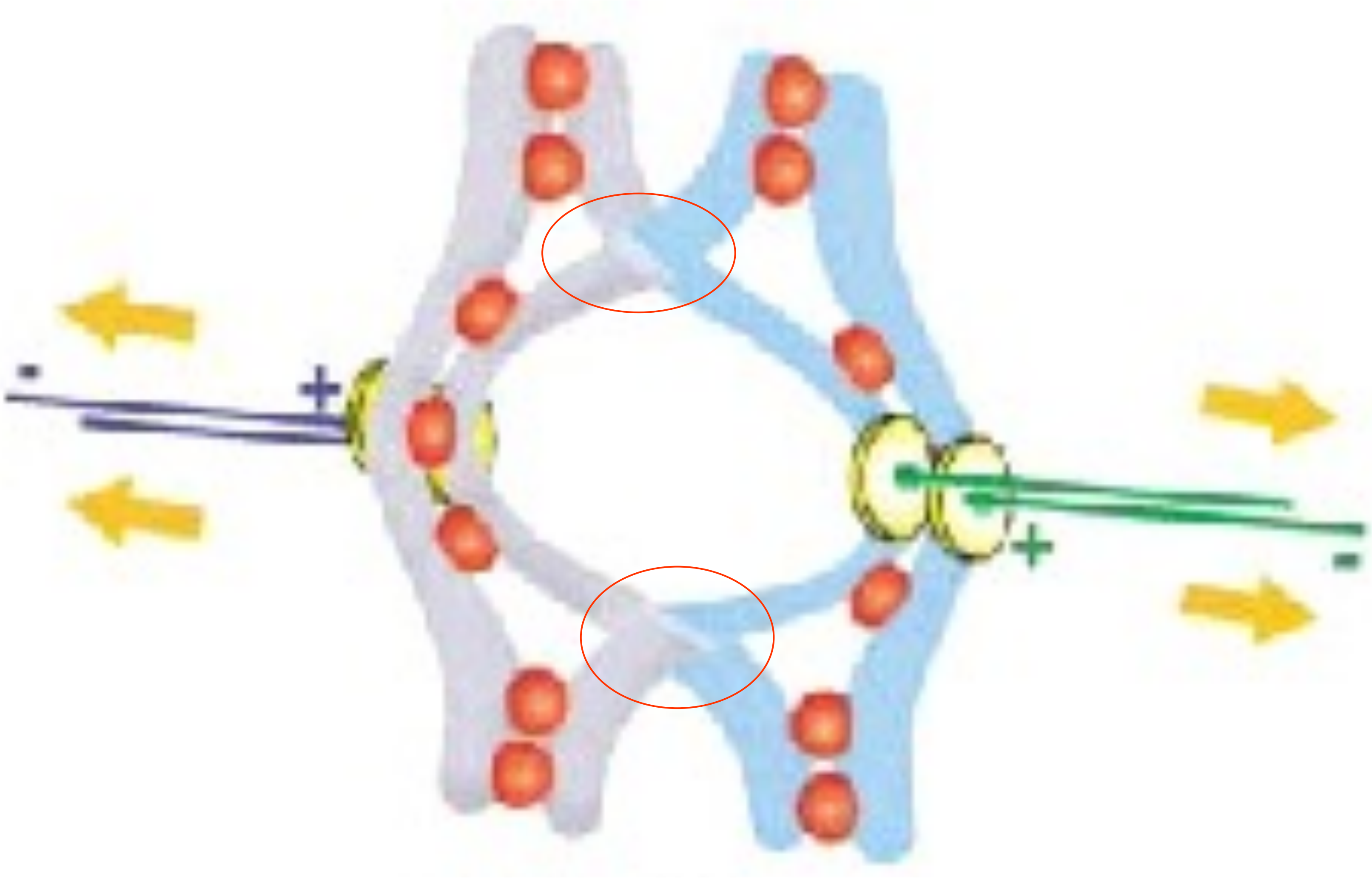
Second meiotic division



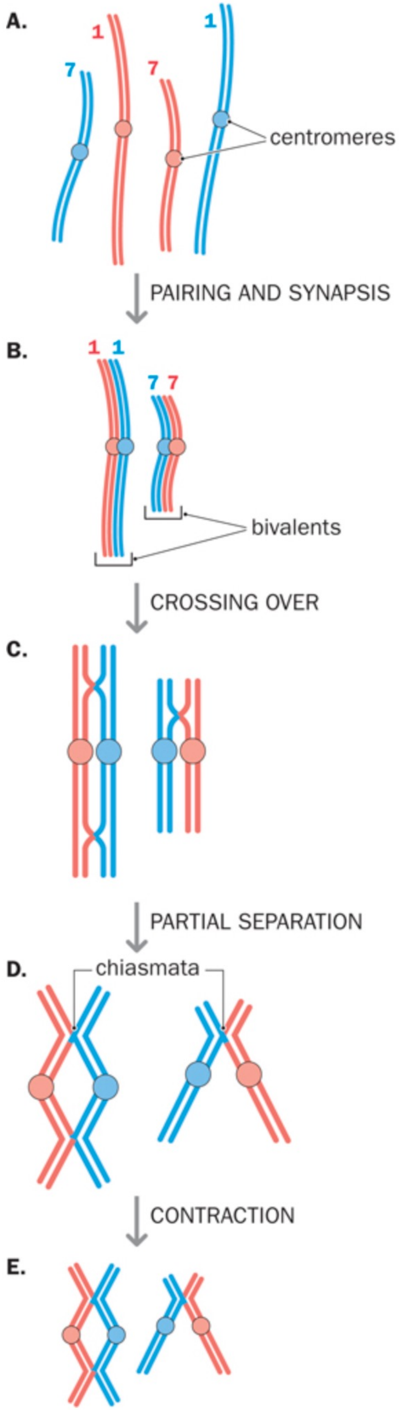
separase

Cohesin rings at centromeres are opened

Formation of chiasma(ta) by homologous recombination



± 55 chiasmata per cell in human male meiosis
± 90 chiasmata per cell in human female meiosis



The five stages during prophase of meiosis I

A: leptotene (chr condensation, chr unpaired, dsDNA breaks [DSB])

B: zygotene (repair of DSB in progress; pairing of homologues to bivalents;
synapsis through synaptonemal complex)

C: pachytene (synapsis complete; crossing-over complete; formation of chiasmata)

D: diplotene (partial separation of homologues by breakdown of the synaptonemal complex, held together by chiasmata)

E: diakinesis (chromosome condensation and transition to metaphase I)

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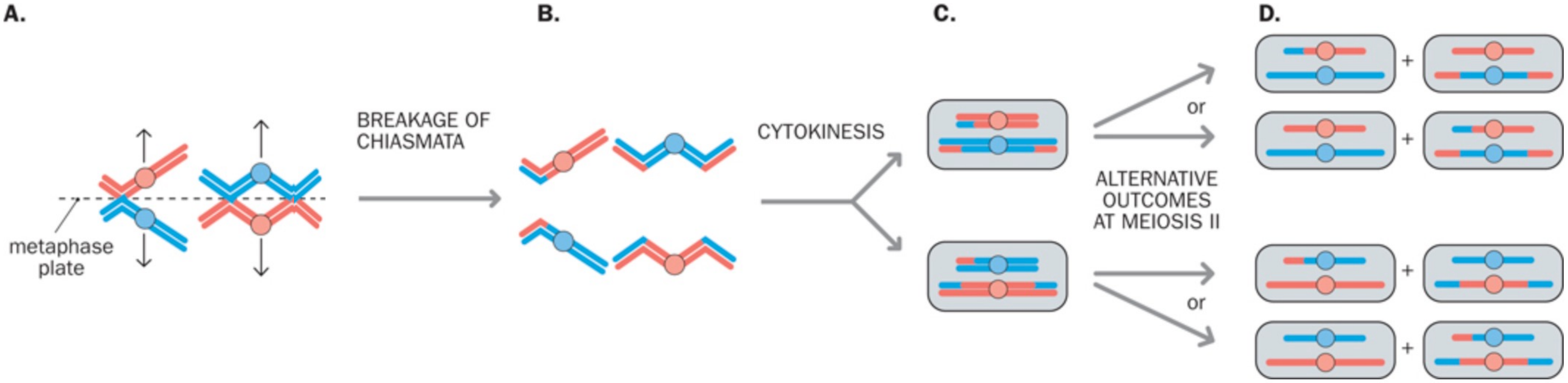
From metaphase I to gametes



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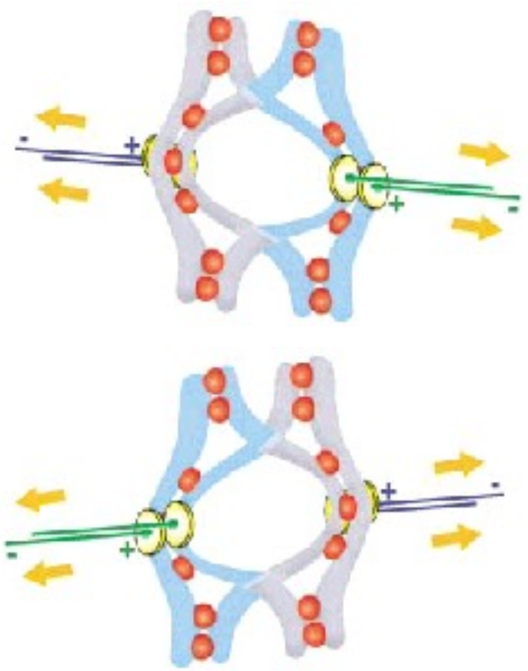
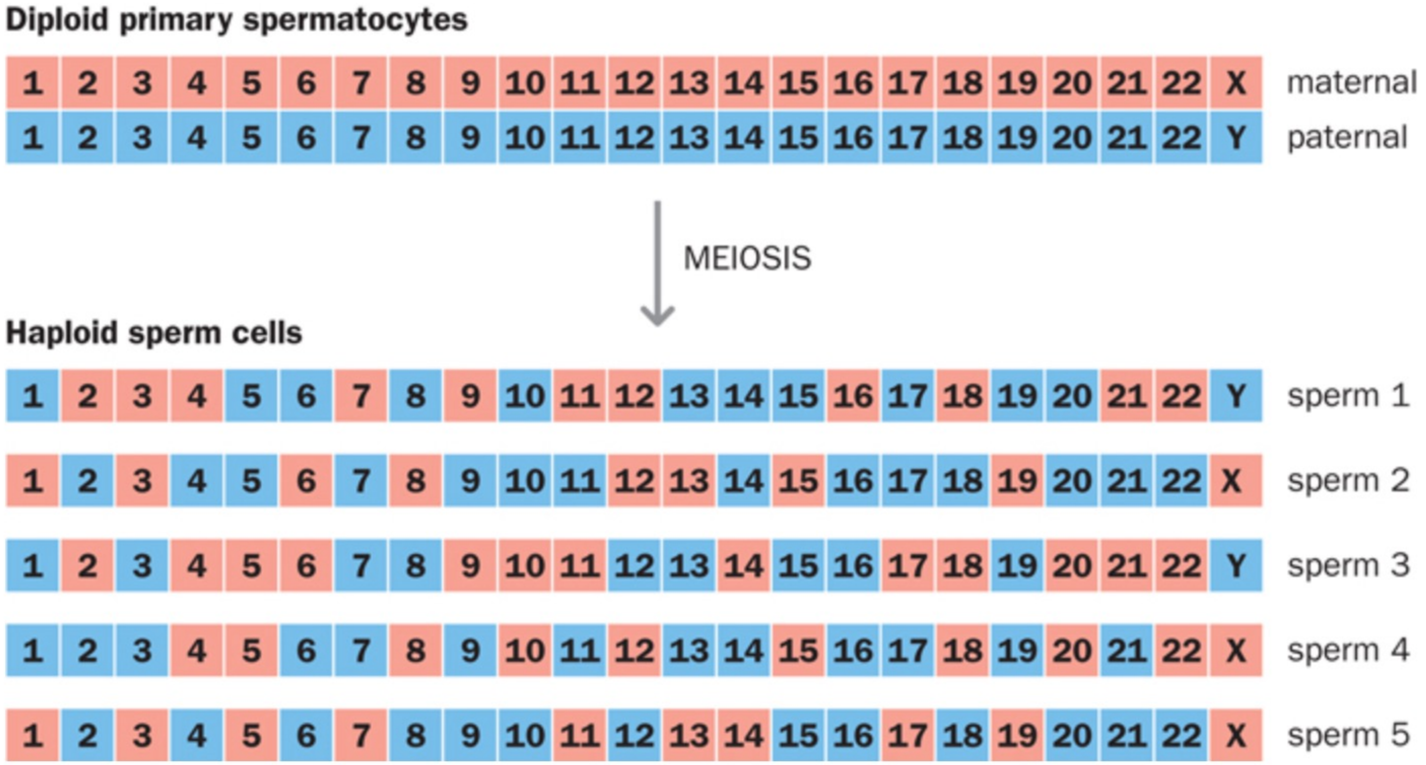
Source of genetic diversity: **Independent assortment** + homologous recombination





Diploid primary spermatocytes

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	maternal
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Y	paternal

Haploid sperm cells

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Y	sperm 1
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	sperm 2
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Y	sperm 3
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	sperm 4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	sperm 5

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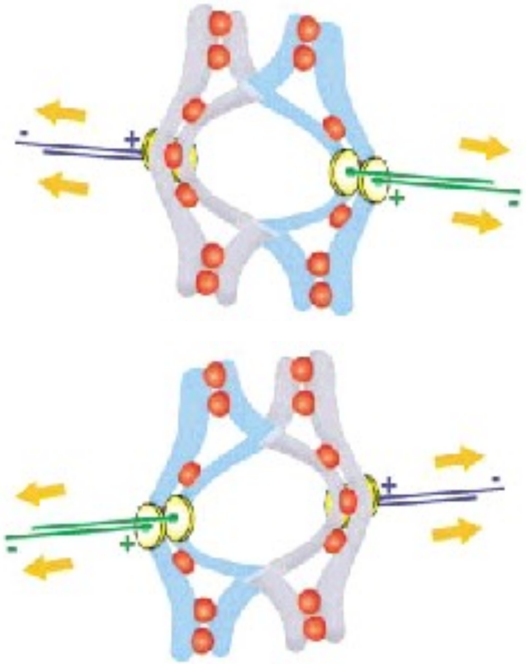
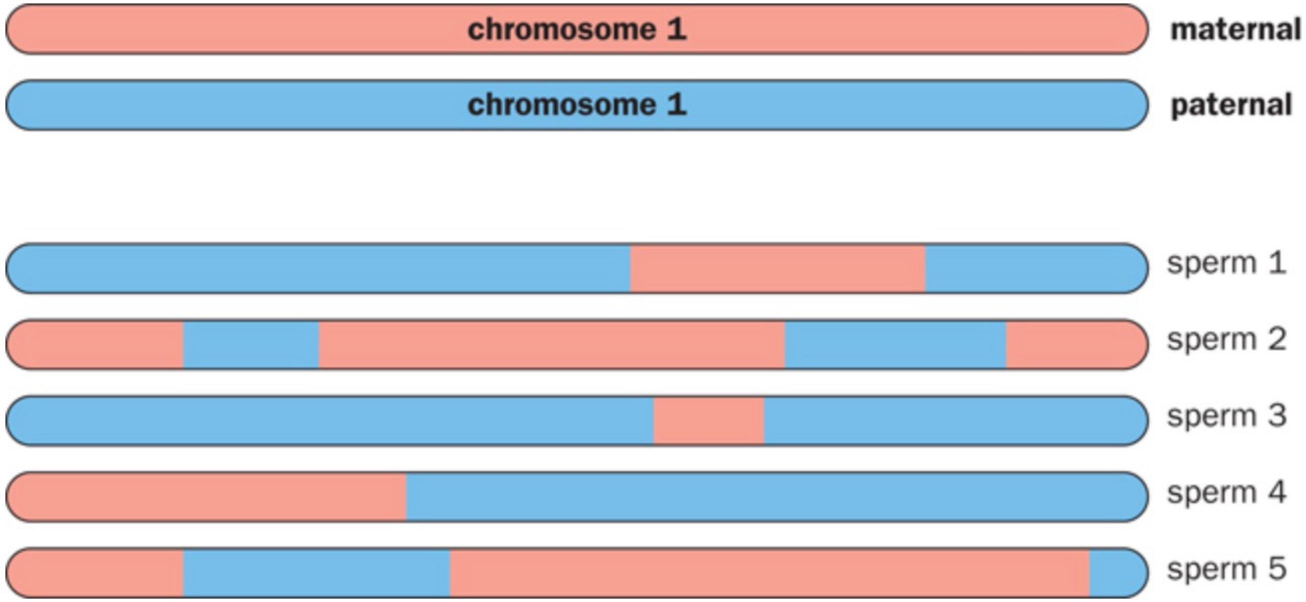
Source of genetic diversity: Independent assortment + **homologous recombination**





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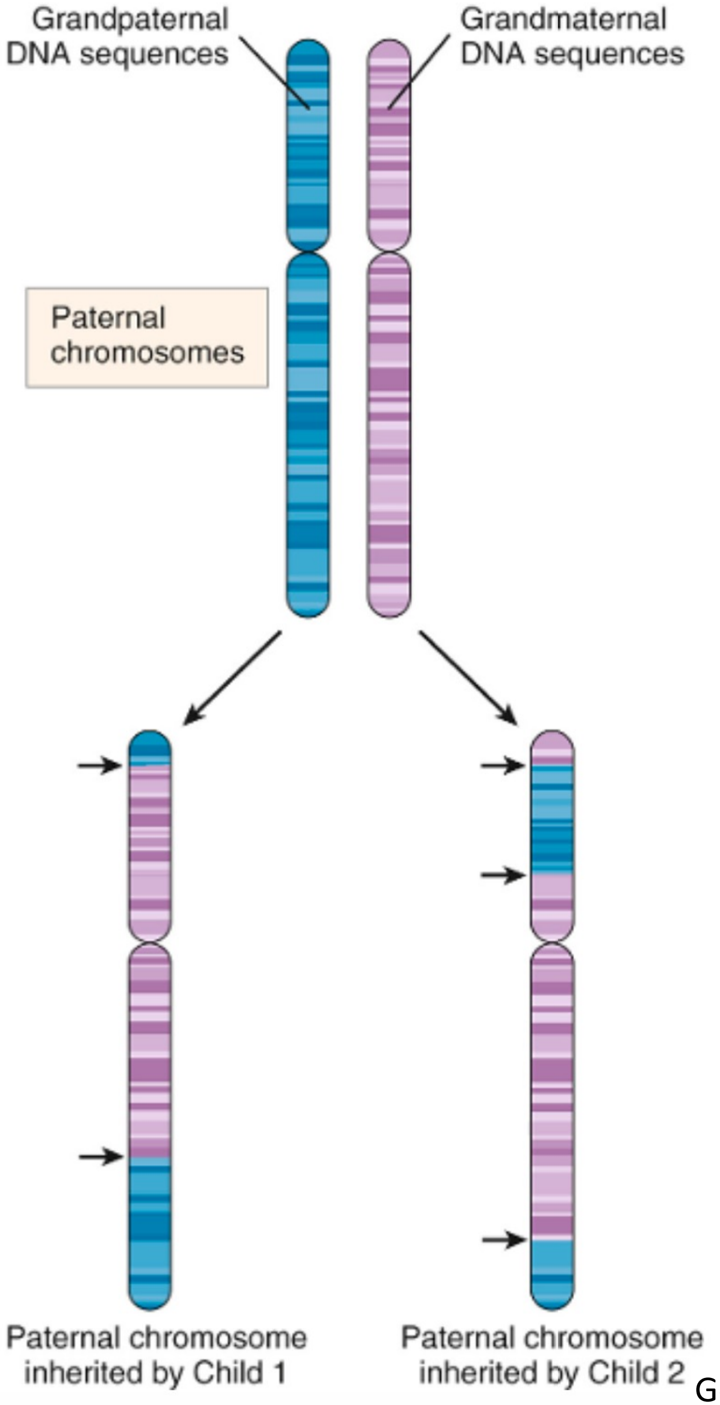

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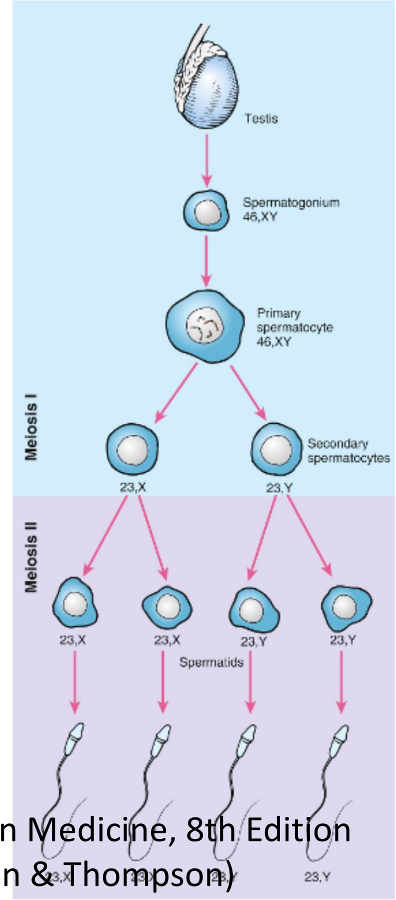
Chapter 2

- ✓ The Human Genome Sequence
- ✓ Organization of the Human Genome
  - Single-Copy DNA Sequences
  - Repetitive DNA Sequences
  - Repetitive DNA and Disease
- ✓ Variation in the Human Genome
- ✓ Transmission of the Genome
  - The Cell Cycle
  - Mitosis
  - Meiosis
- ✓ Human Gametogenesis and Fertilization
  - Spermatogenesis
  - Oogenesis
  - Fertilization
- ✓ Medical Relevance of Mitosis and Meiosis

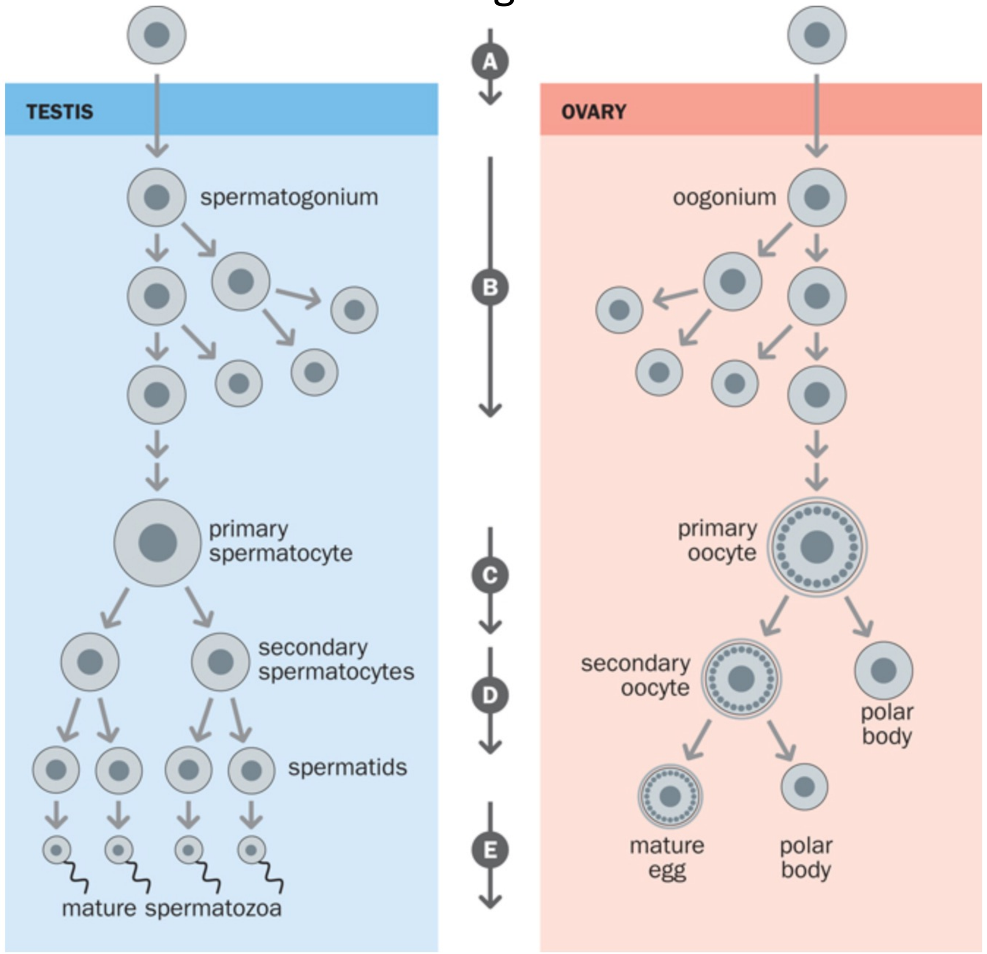
General overview of gametogenesis in ovary and testis



2n

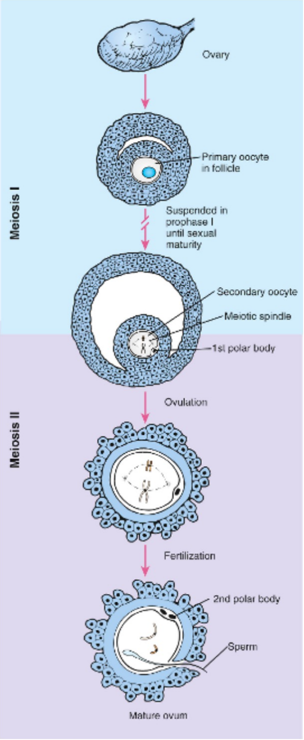
n

Primordial germ cells



2n

n



C: meiosis I

D: meiosis II

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