# History of Cytogenetics

## True chromosome number established in 1956

(before that, the number of chromosomes wasn't fully understood for humans)

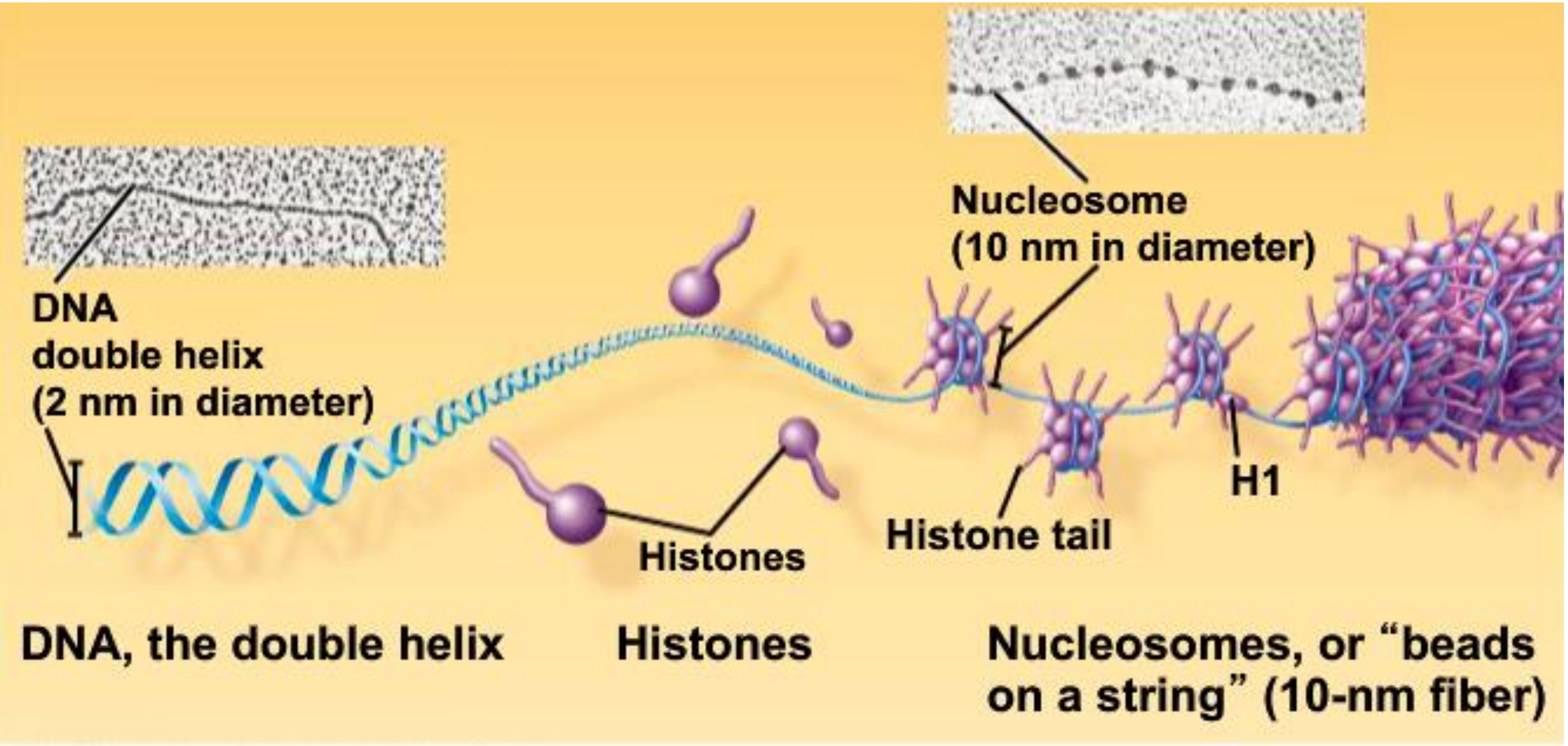
*" From their vantage through the microscope, the cytogeneticists' view of the genome is still unrivalled in its scope, detail and color."*

***Barbara J. Trask, 2002***

## Before starting, there are fundamental concepts that must be known:

- **Cytogenetics** is the study of chromosomes and diseases related to chromosomal anomalies.
- Humans have 23 pairs of chromosomes, totaling 46. For every pair, we inherit one chromosome from our father and one from our mother, ensuring that we carry a genetic mix of both parents.
- DNA: The hereditary molecule composed of two antiparallel strands forming a double helix that carries genetic information.
- Gene: A segment of DNA that encodes a functional product, either a protein or RNA.
- Chromosome: A highly organized DNA–protein structure containing many genes.
- **Human genome** is the entire sequence of the DNA in humans.
- ✓ It was initially achieved in the early 2003–2004, but in reality, that was most of the human genome, not the entire.
- ✓ Certain regions in the human genome were yet to be sequenced.
- ✓ The entire human genome, from telomere to telomere, came out a few years ago.
- o This tells you how rapidly this field is evolving and how much new knowledge is changeable.
- **Chromatin** is the diffused form of the chromosome.

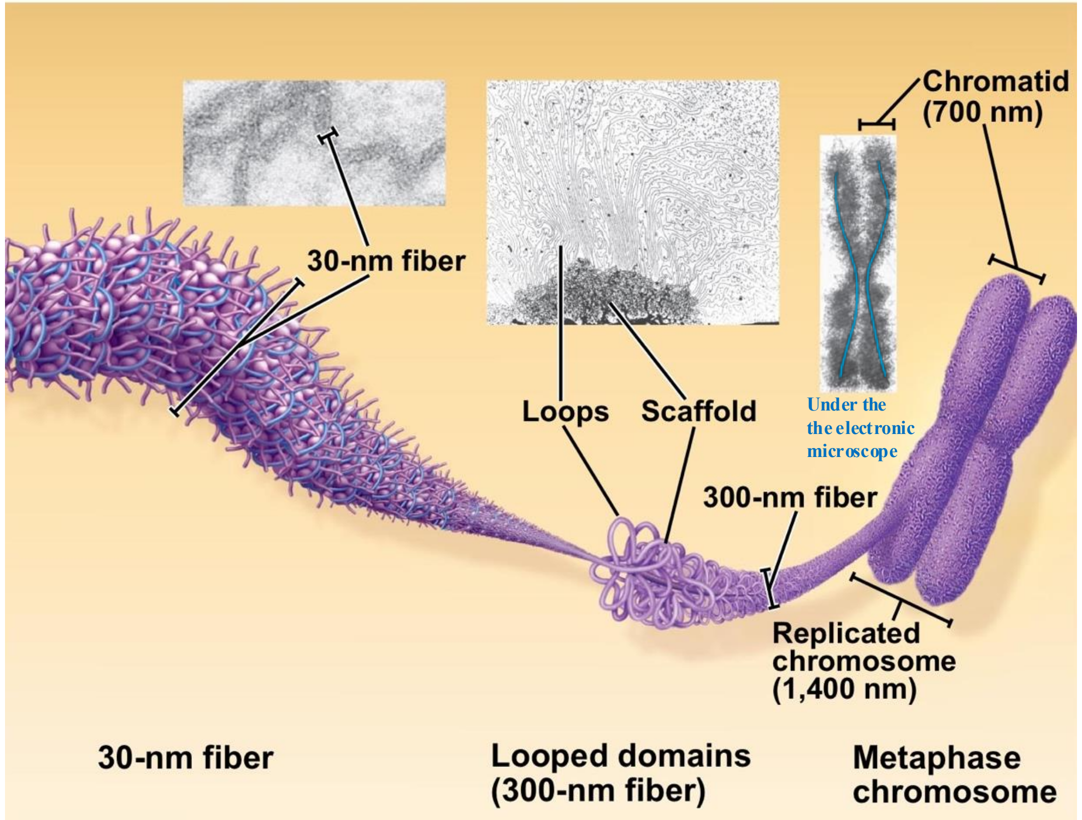
# *Chromosome structure*






- Anti-parallel double-stranded DNA is wrapped around proteins called **histones**.
- The histones are grouped together along the DNA, and the DNA wrapped around them forms a unit called a **nucleosome**.

# *Chromosome structure*



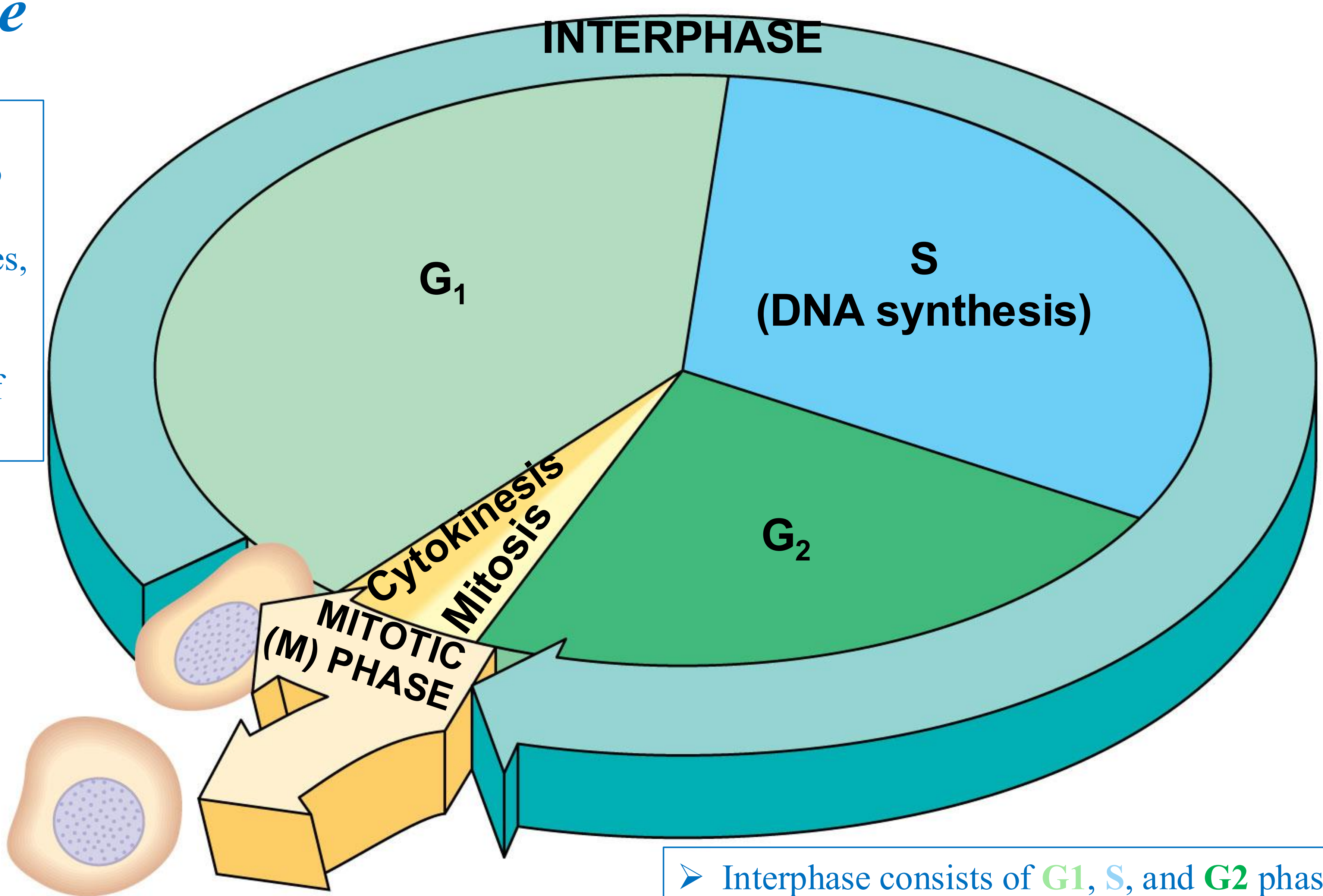

- ➢ These nucleosomes are assembled into a structure called a **fiber**.
- ➢ The fibers are arranged on a protein scaffold, and this fiber gives rise to a **chromatid**.
- ➢ There are then ***identical sister chromatids.***

- o Each chromatid is one (single) double-stranded DNA molecule combined with proteins.
- o We have one long double-stranded DNA molecule, and the sister chromatid contains a second DNA molecule with an identical sequence.

✓ **Hence, we call them sister chromatids.**

# *Cell Cycle*

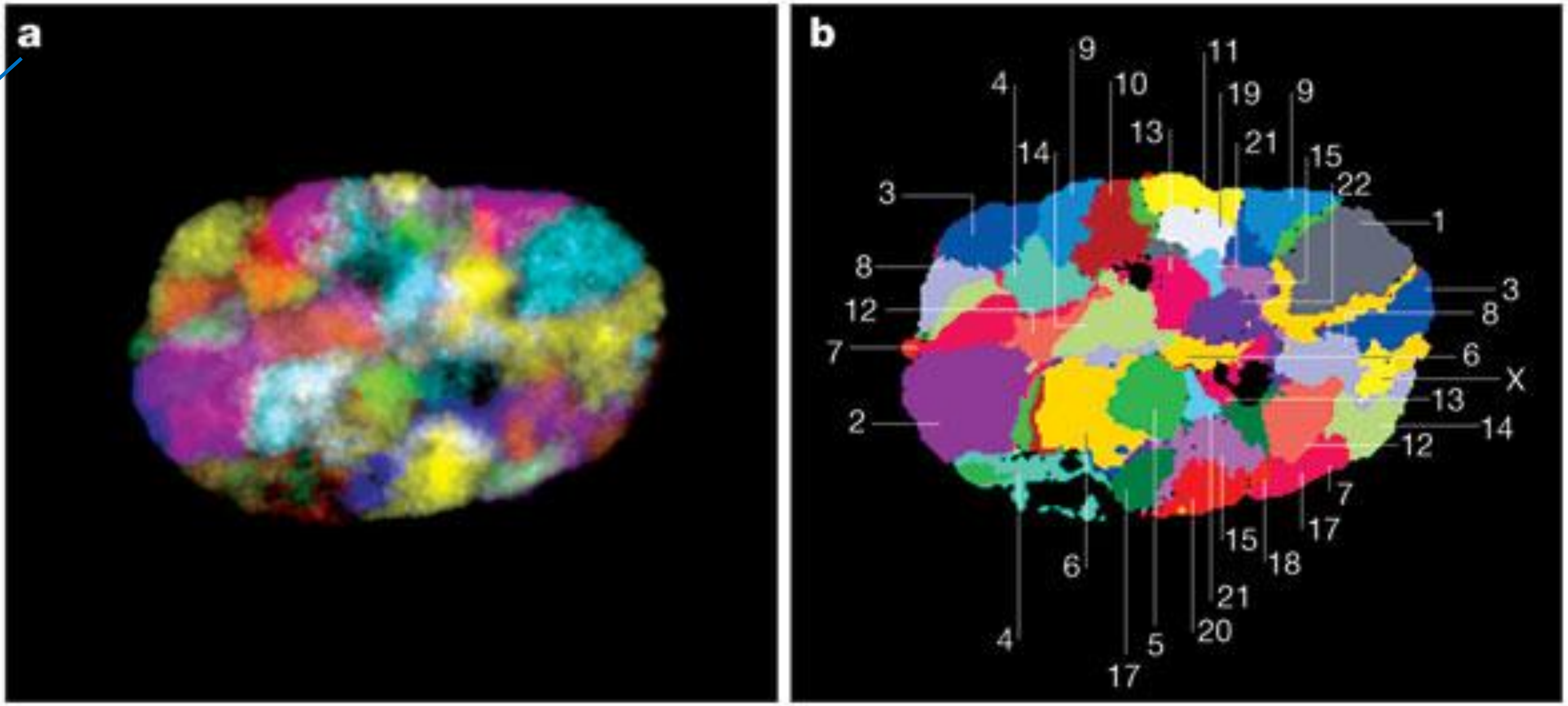
When the cell divides, it needs to divide all of its contents: organelles, membranes, cytosol, and the genetic material of the nucleus.






- Interphase consists of G1, S, and G2 phases.
- The end phase, called the mitotic (M) phase, includes mitosis, which is the division of the nucleus and cytokinesis.

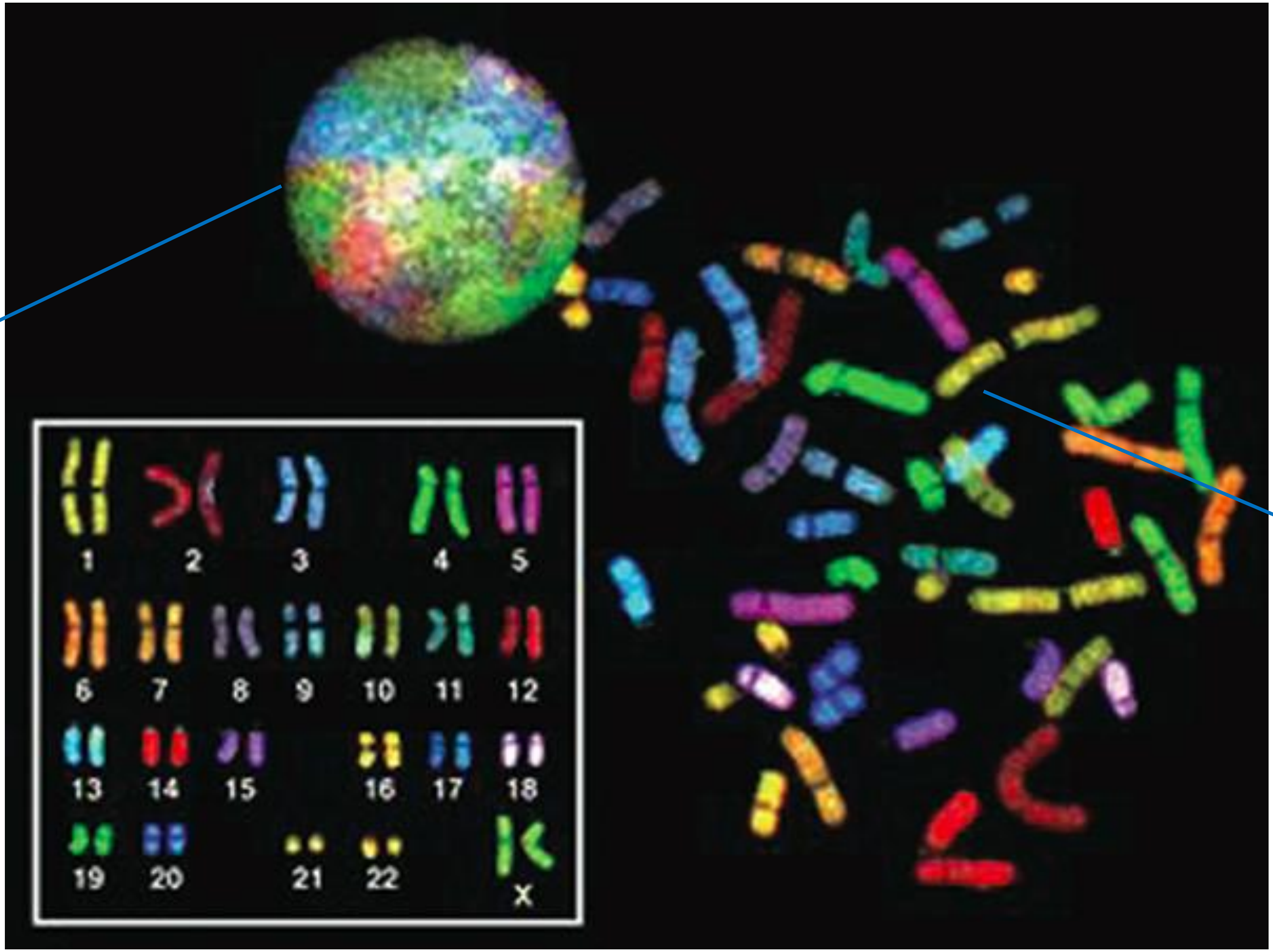
This is an actual image of the genetic material in the nucleus during the ***interphase***, where each chromosome's chromatin appears in a different color.






- During *interphase*, if you look at the nucleus, you will not see chromosomes; instead, you will see **chromatin**.
- **Chromosomes** only exist during the *mitotic phase*.
- Each chromosome occupies a distinct territory in the nucleus.

This is how the nucleus looks during the ***interphase*** (diffused chromosomes).




This is how the nucleus looks during the ***mitotic phase*** (condensed chromosomes).

- ➢ The compact structure of chromosomes, occupying a minimal region and forming distinct entities for each chromosome, is present only during the *M phase*.

- **Why are chromosomes discrete, highly condensed, and present as separate units during the mitotic stage of the cell cycle, whereas during interphase they are diffused, occupy specific regions, and are less condensed?**

- ✓ During ***interphase***, in **G1**, the cell is growing, so it needs more proteins to produce more organelles and other macromolecules. These are generated through transcription and translation from the DNA template; therefore, the DNA has to be decondensed (loose) and diffused so that transcription factors and RNA polymerase can access the DNA content.

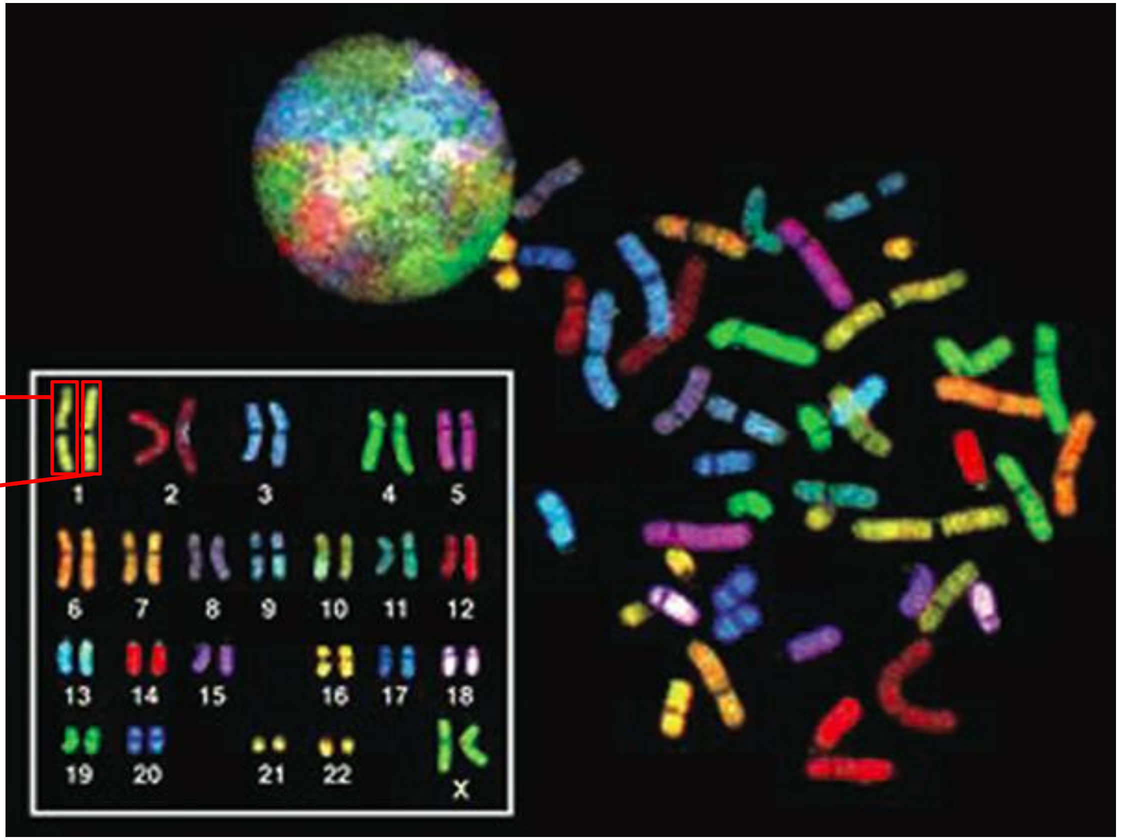
- ✓ During the **S** phase, where DNA replication occurs, DNA polymerase and other proteins and enzymes, such as helicase and topoisomerase, also need access to the DNA so that it can be replicated. The same applies in **G2.**

- ✓ On the other hand, during ***mitosis***, what matters is the proper and accurate separation of each chromosome into daughter cells. Here, if there are extra or missing chromosomes, this will lead to diseases such as cancer or hereditary diseases.

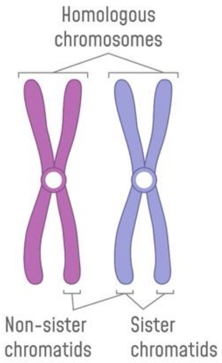
- ➢ Chromosomes are ideally aligned, after which they are arranged according to their size.
- ➢ The largest chromosome is **chromosome 1**, and the smallest is **chromosome 22**.

This is a zoomed-out view of two sister chromatids

Two sister chromatids




Together, they are called ***homologous chromosomes***. One is inherited from each parent.

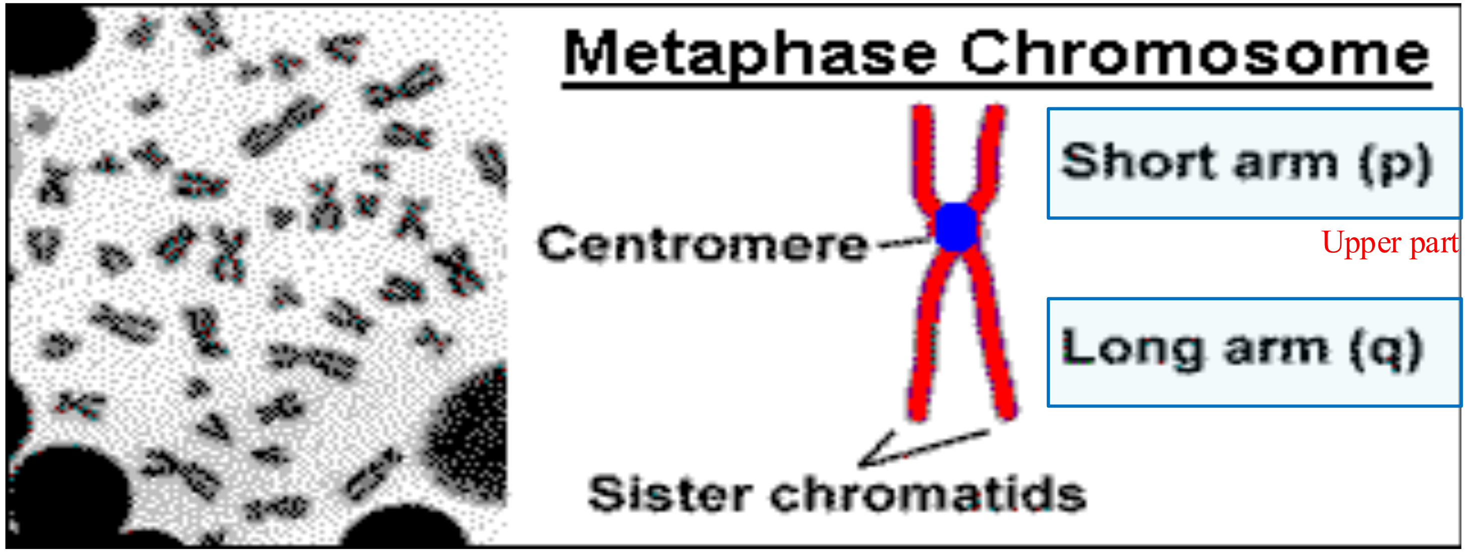


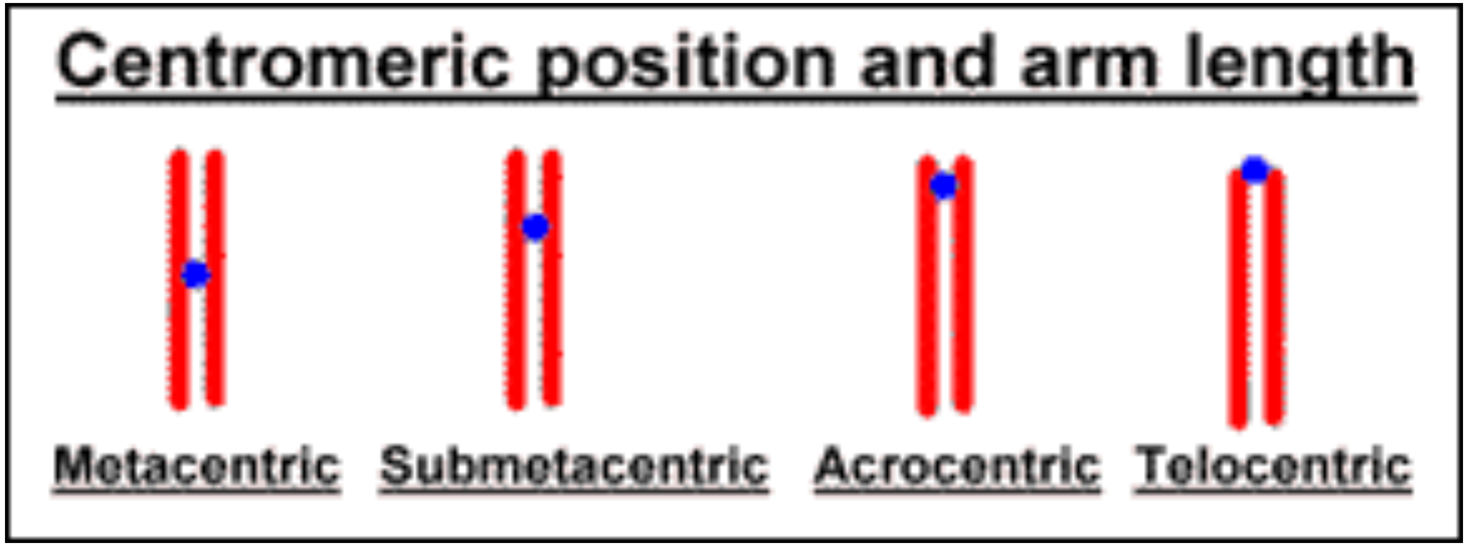

Extra

# *Nomenclature of chromosomes*

- Sister chromatids are joined together at a region called the **centromere**.

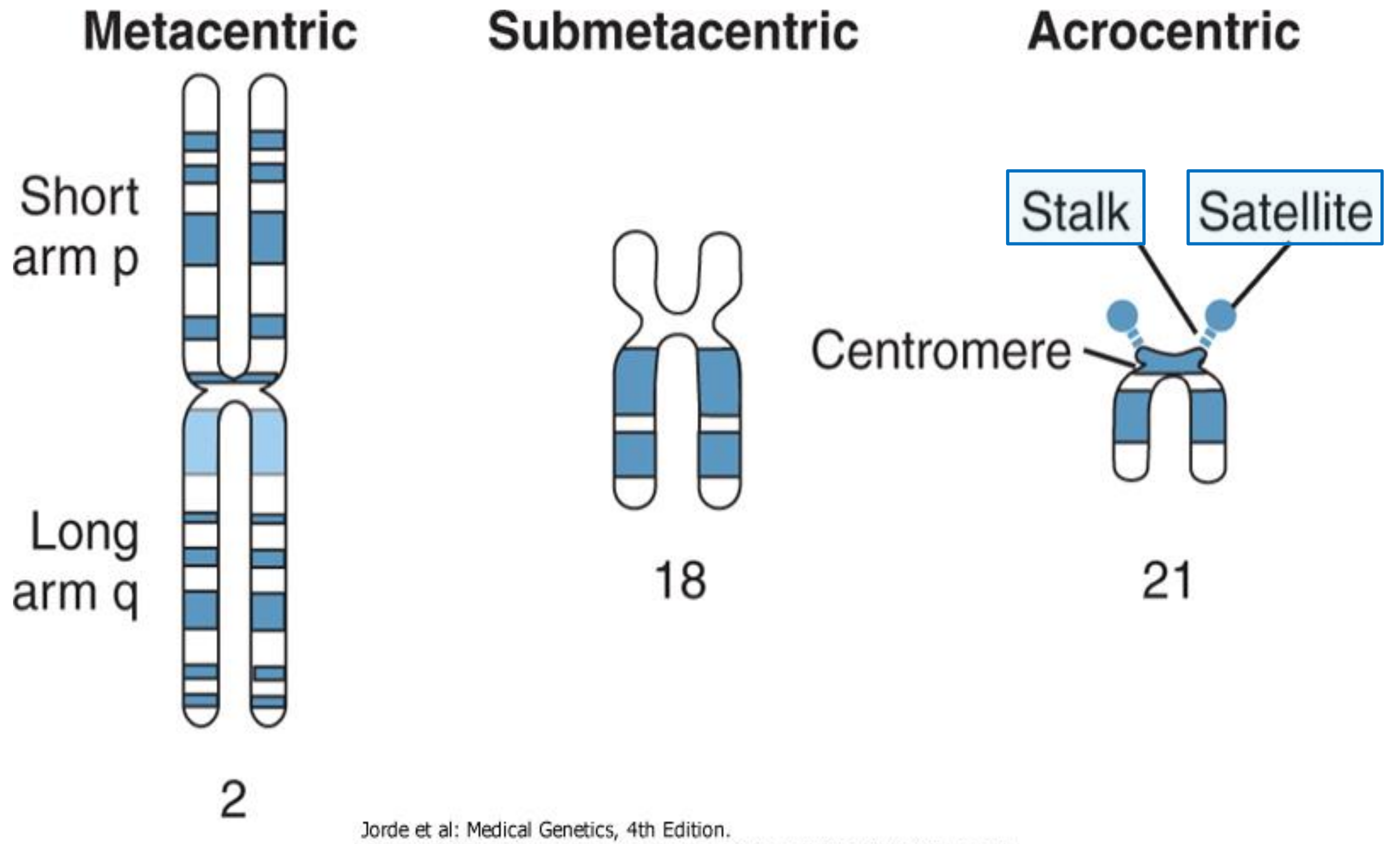
- Depending on the position of the centromere, chromosomes are classified as follows:
  - ✓ **Acrocentric**
  - ✓ **Metacentric**
  - ✓ **Submetacentric**
  - ✓ **Telocentric**: This is ***not*** a normal chromosome structure in humans, though it may exist in other species.



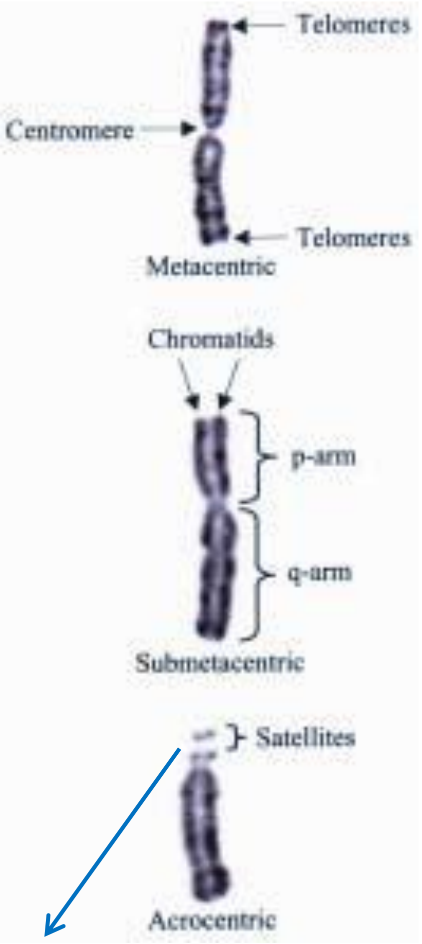



# *Chromosome Shape*

- ✓ **Metacentric- centromere is located in the middle of chromosome** (It's hard to tell the p arm from the q arm)
- ✓ **Submetacentric- centromere is displaced from the center**
- ✓ **Acrocentric – centromere is placed near the end**




Jorde et al: Medical Genetics, 4th Edition.

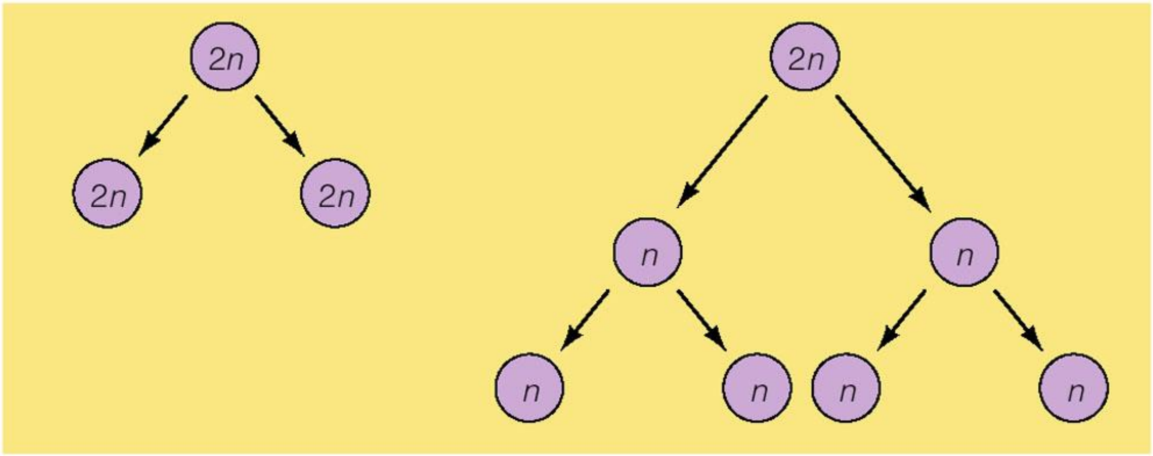

The p arm is so small that under 1000x magnification in light microscopy, there is almost nothing visible there.

# *Human chromosomes*

- DNA and associated proteins are organized into chromosomes
- Human somatic cells are **diploid** and have 22 pairs of **autosomes** AND 1 set of **sex** chromosomes (XX or XY)= total of 46
  - ✓Females XX
  - ✓Males XY
- Germ cells are haploid and contain 22 chromosomes plus 1 sex chromosome (X or Y)

- Initially, **germ cells** have two sets of chromosomes, one from each parent.
- **Gametes**, which are the egg and sperm, have 23 chromosomes. 22 autosomes and 1 sex chromosome.

| Mitosis | Meiosis |
|---|---|

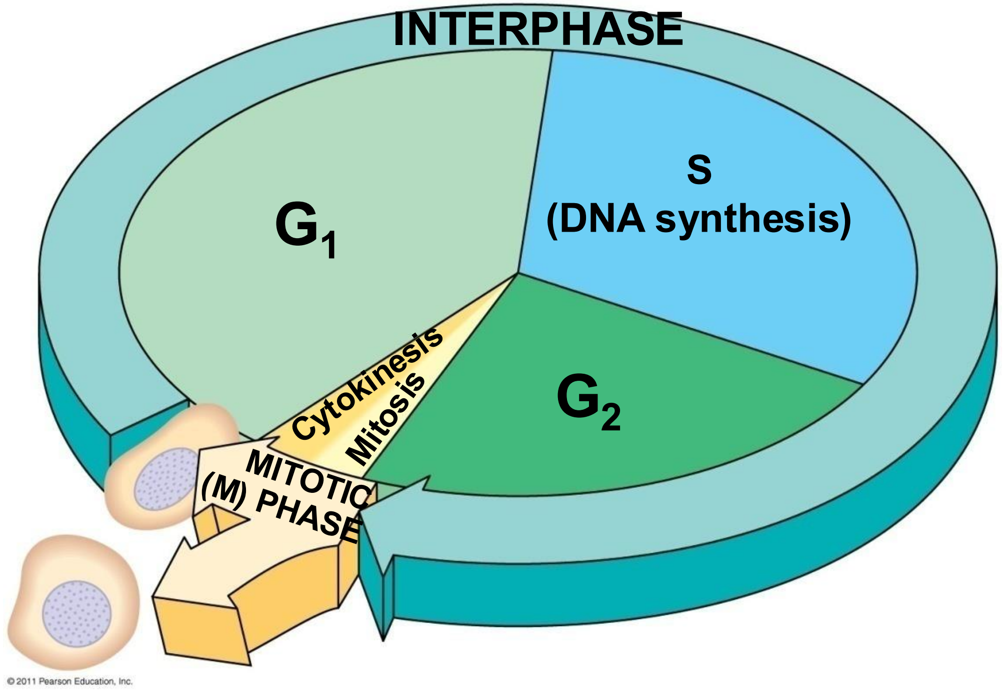




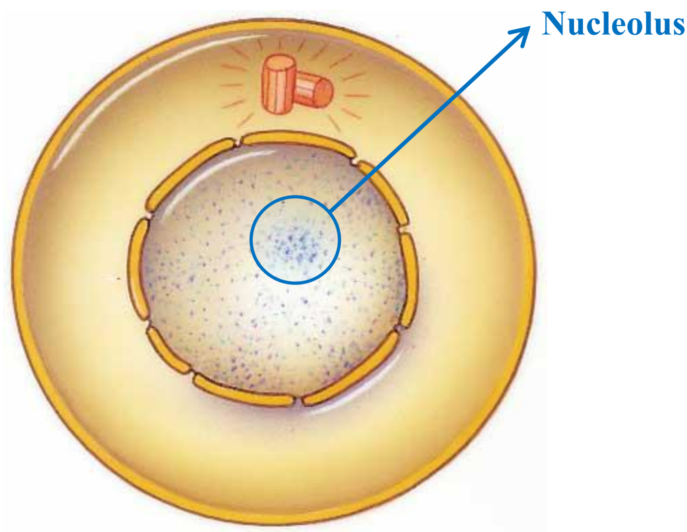

- ➢ The fertilized egg (zygote) receives 1n (23 chromosomes) from the father and 1n (23 chromosomes) from the mother; when fertilization occurs, this results in 2n.
- ✓ The zygote, once formed, undergoes massive rounds of mitotic division.
- ➢ ***Mitosis*** produces daughter cells that have an identical number of chromosomes, and the DNA sequence in both daughter cells is the same as in the parental cell.
- ➢ ***Meiosis*** is the reduction phase in which the number of chromosomes is reduced by half, from 46 to 23 in the daughter cells.
- ➢ In meiosis, the DNA sequence in the gametes is similar but not identical.
- ✓ That is why the evolutionary advantage of sexual reproduction is higher than reproduction that is not based on this mechanism, because meiosis generates genetic variation.

# *Interphase*

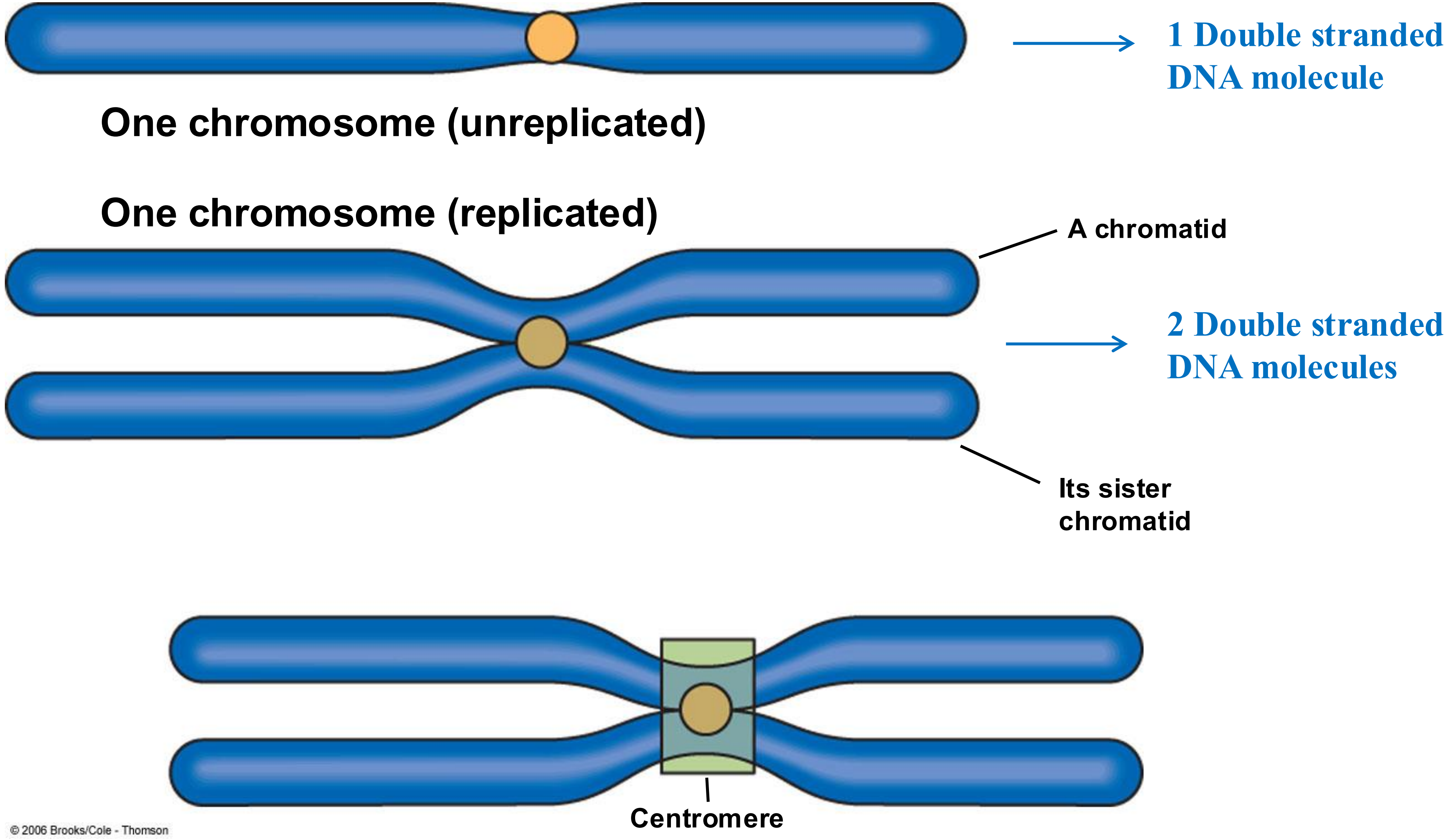
- **Gap 1** (G1) – many cytoplasmic organelles are constructed; RNA, protein and other molecules are synthesized; cell almost doubles in size
- **Synthesis** (S) – DNA is replicated and chromosomes duplicate, forming 2 sister chromatids attached at the centromere
- **Gap 2** (G2)– more cell growth; mitochondria divide; spindle precursors form




**Interphase**
***18-24 hours***



According to the semi-conservative model of DNA replication, each strand of the original molecule serves as a template for the synthesis of a new complementary strand.




- Chromosomes replicate during the **S phase**. While attached to the centromere, the replicated chromosomes are called ***sister chromatids***. Once the sister chromatids are separated and no longer joined by a centromere, they are no longer referred to as sister chromatids; instead, each individual strand becomes a chromosome in its own right.

# *Mitosis*

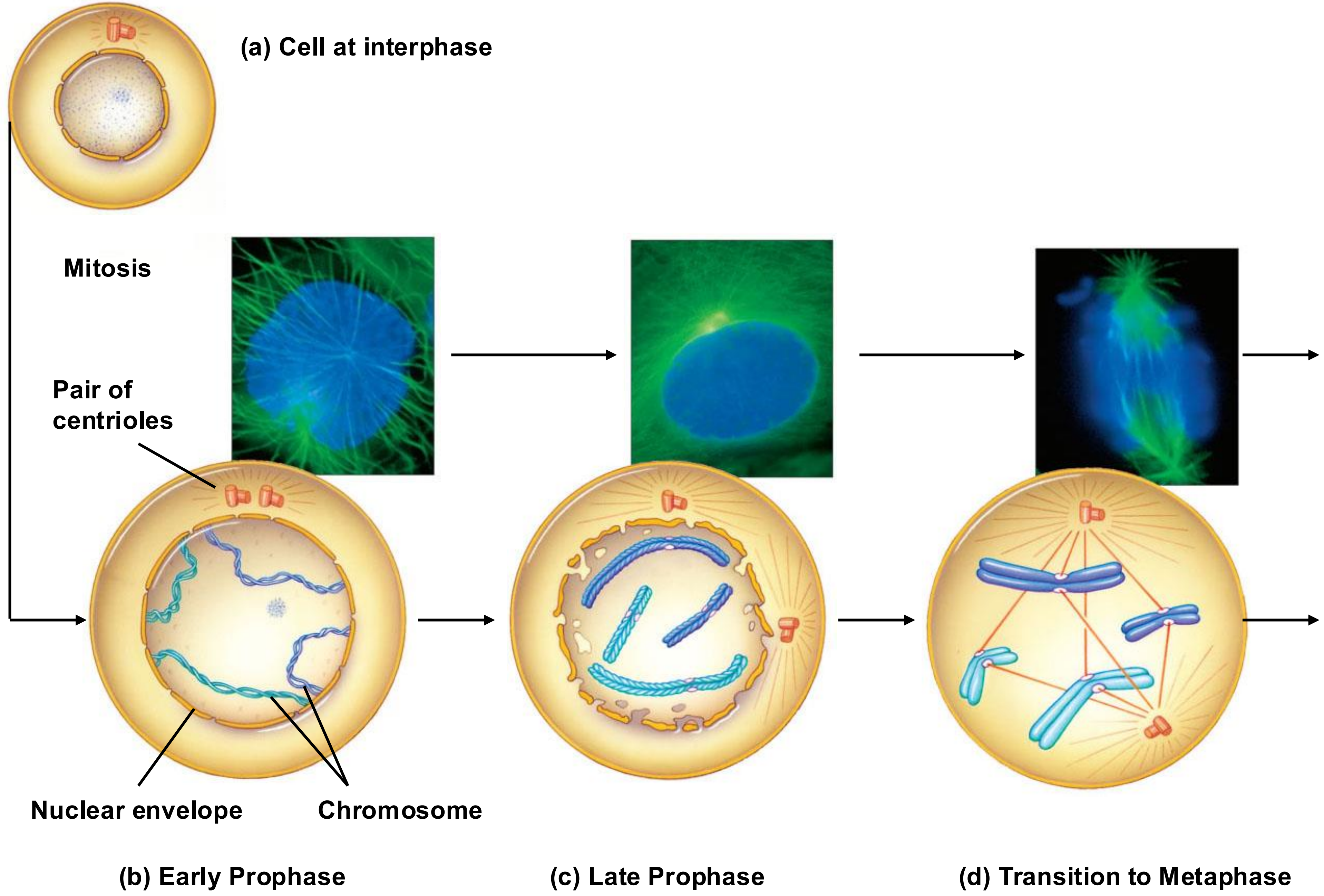
- Produces identical daughter cells

✓ Daughter cells are identical only from a genetic perspective. From a cytoplasmic perspective, they may not be completely identical. Although the cytoplasm is relatively homogeneous, it still exhibits some heterogeneity, which may lead to unequal distribution of organelles such as mitochondria (or other cytoplasmic organelles) or proteins between daughter cells.

  – (46 chromosomes) – **as the parental cell**

✓ If the parental cell has a numerical chromosomal abnormality, the daughter cells produced by mitosis will carry the same abnormality in chromosome number.

- It must be accurate for cells to function properly
- Continuous process but divided into distinct steps:
  – Prophase
  – Metaphase
  – Anaphase
  – Telophase



(a) Cell at interphase
Mitosis
Pair of centrioles
Nuclear envelope
Chromosome
(b) Early Prophase
(c) Late Prophase
(d) Transition to Metaphase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➢ When the cell divides by separating (disjoining) the sister chromatids, the chromosome number in the daughter cells remains the same.

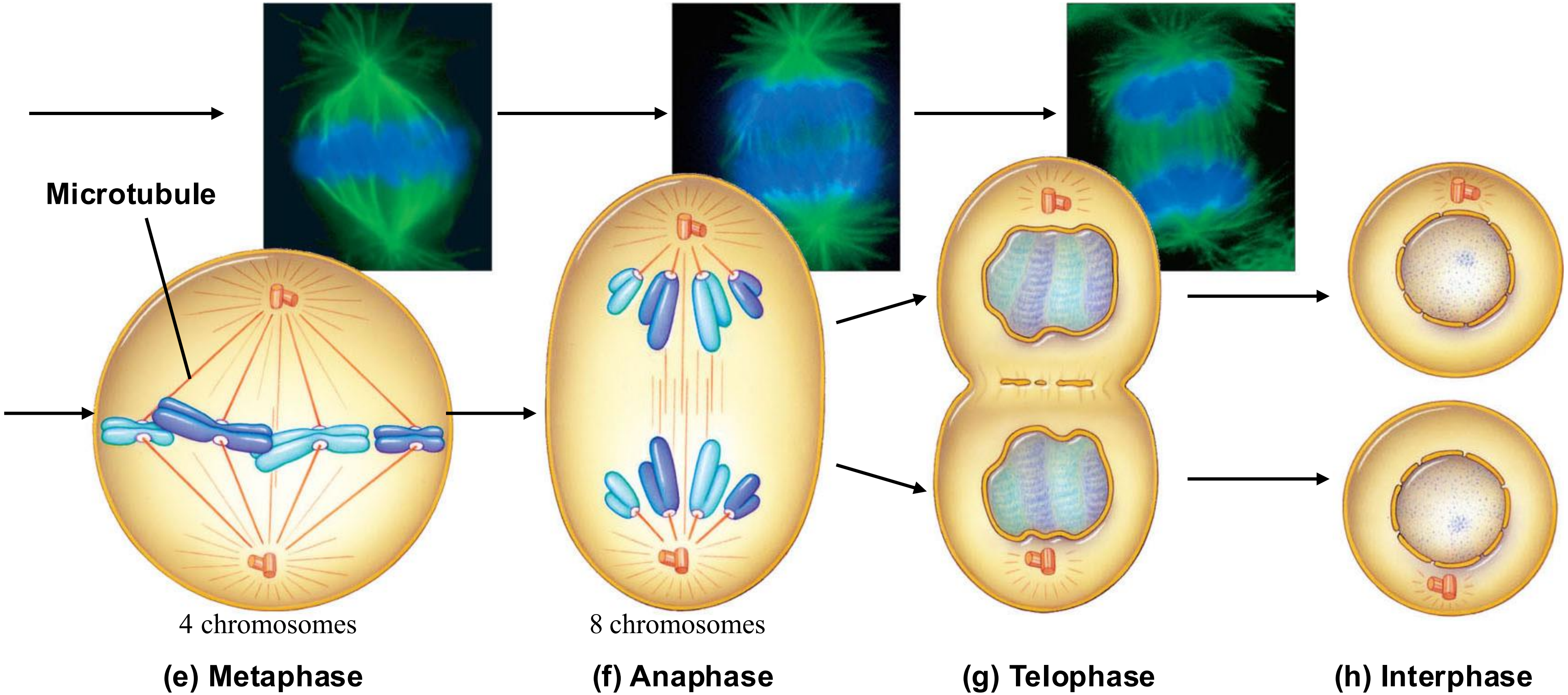


FIGURE **2.8** Stages of mitosis. Only two pairs of chromosomes from a diploid (2*n*) cell are shown here. The photographs show mitosis in a mouse cell; the DNA is stained blue and the microtubules (spindle fibers) are stained green.

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## ➢ *Prophase*

- During prophase, it could sometimes be divided into subcategories like early and late.
- The chromosomes start to condense during prophase and become visible entities.
- The nuclear envelope starts to get degraded.
- A pair of **centrioles** happens, then each pair migrates to the opposite poles of the cell.
- These centrioles contain microtubules, also known as **spindle fibres**, which attach to the centromere of the chromosome.

❖ Notice that the centromere is attached to a spindle fibre from both sides (poles) of the centrioles.

## ➢ *Metaphase*

- During metaphase, chromosomes align **individually** in the cell on the metaphase plate.
- Each chromosome is bound from both poles of the cell through spindle fibres to the centrioles, as mentioned before.
- Some of those spindle fibres overlap (seen more clearly in anaphase) and are not necessarily attached to the centromere of the chromosome.

## ➢ *Anaphase*

- During anaphase, sister chromatids separate and migrate to the opposite poles of the cell.
- The microtubules start pulling the sister chromatids in opposite directions, **disjoining** them from each other, and each one is considered a chromosome by itself. ***Failure to disjoin sister chromatids can cause diseases***.
- The overlapping microtubules help **elongating** the cell (the cell becomes more elongated).

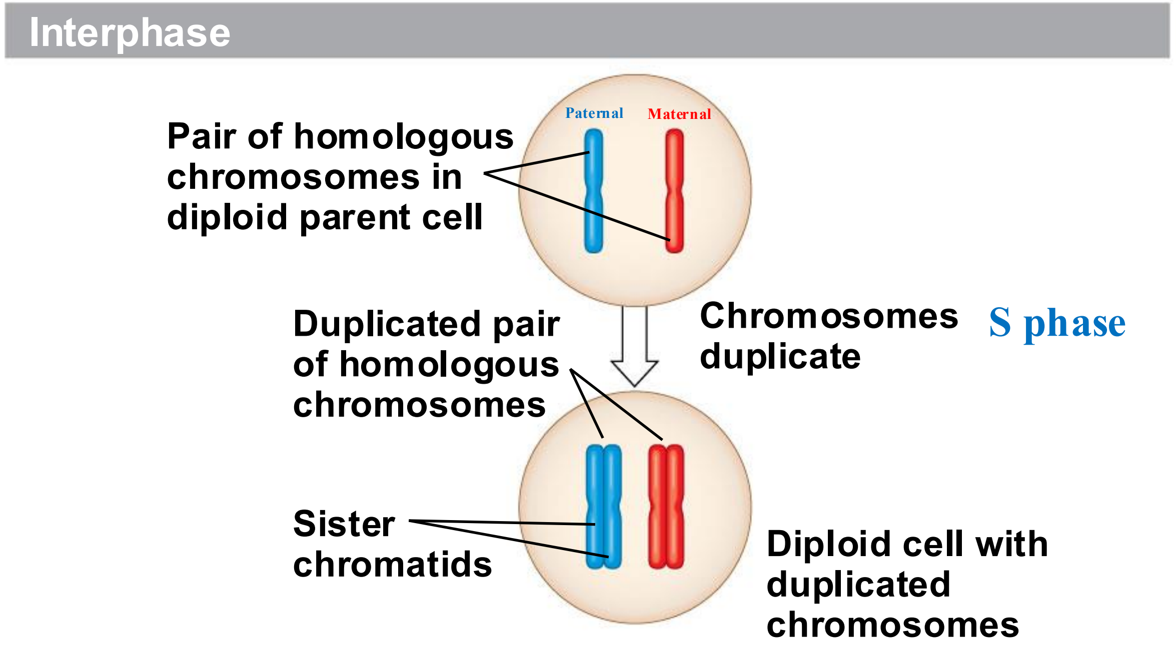
## ➢ *Telophase*

- Telophase happens simultaneously with ***cytokinesis***, which is the division of the cytoplasm between the two nuclei that formed during telophase.
- The chromosomes are diffused in the form of chromatin, and the nuclear envelope appears again.
- Eventually, two daughter cells with **identical DNA material** are formed.

# *The Stages of Meiosis*

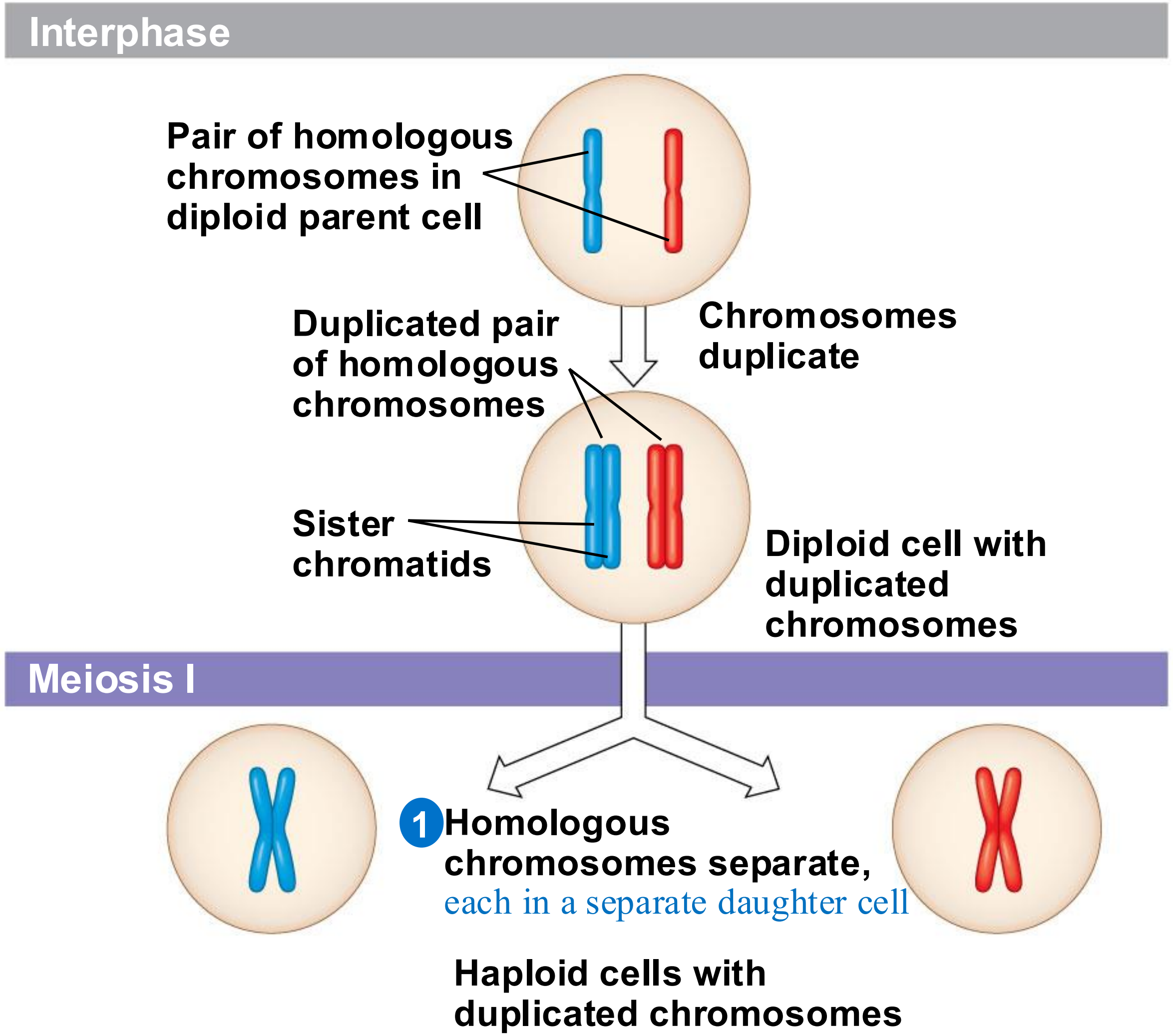
- After chromosomes duplicate, two divisions follow
  - Meiosis I (reductional division): homologs pair up and separate, resulting in two haploid daughter cells with replicated chromosomes
  - Meiosis II (equational division) sister chromatids separate
- The result is four haploid daughter cells with unreplicated chromosomes

Figure 13.7-1



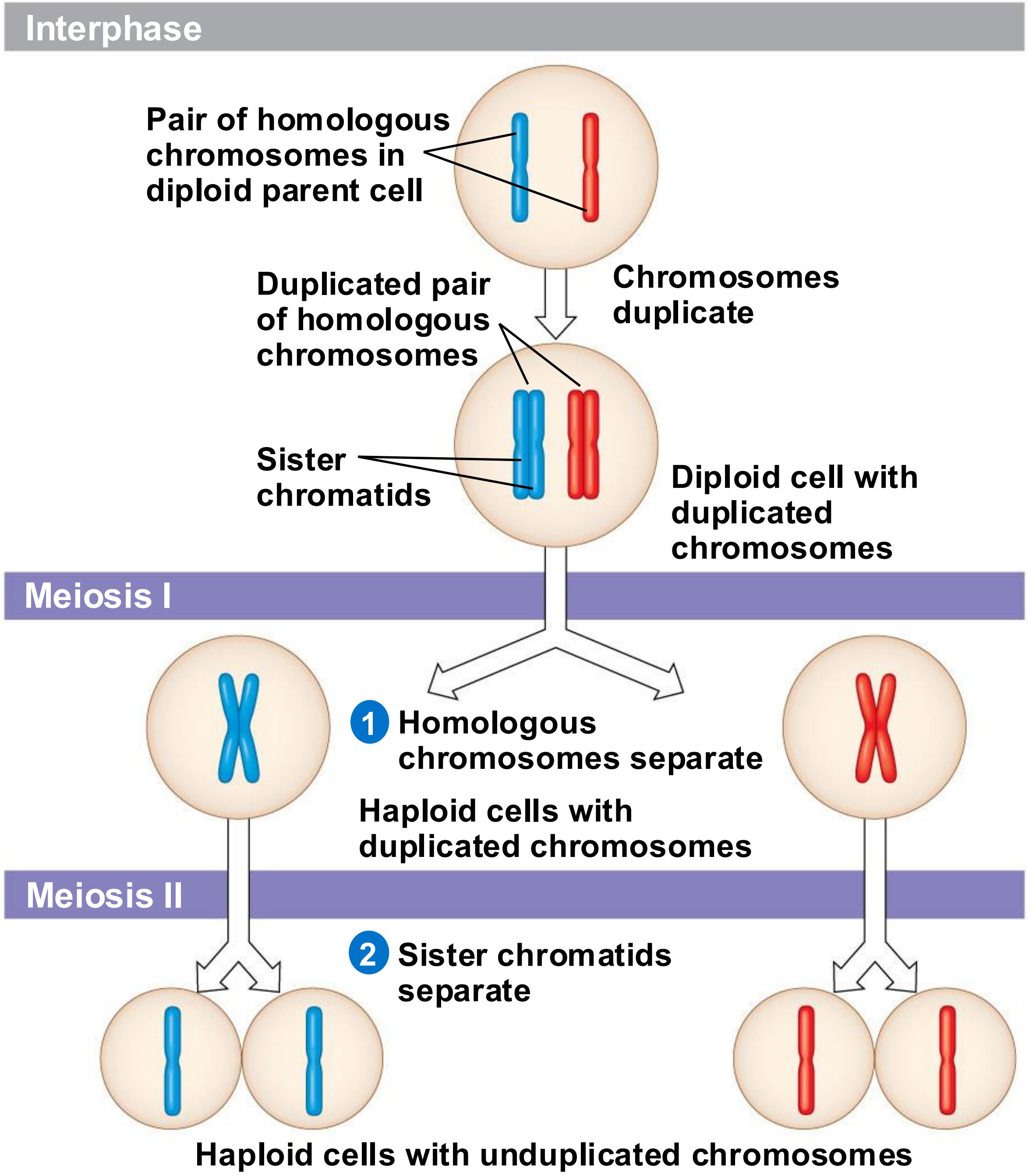

- The genes in homologous chromosomes are the same (similar), but the sequence is not necessarily identical.

Figure 13.7-2



Interphase
Pair of homologous chromosomes in diploid parent cell
Chromosomes duplicate
Duplicated pair of homologous chromosomes
Sister chromatids
Diploid cell with duplicated chromosomes
Meiosis I
1 Homologous chromosomes separate, each in a separate daughter cell
Haploid cells with duplicated chromosomes

Figure 13.7-3

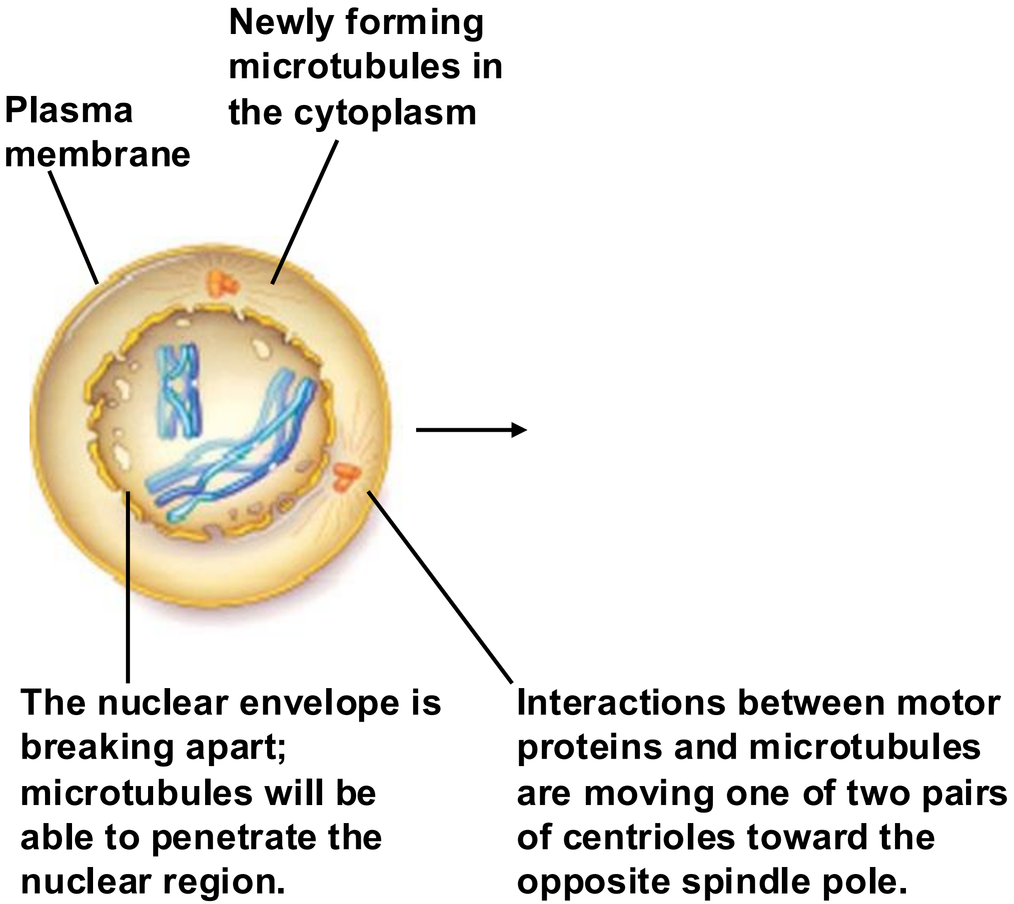





The reduction in the number of chromosomes happens in ***meiosis I.***

The number of chromosomes will remain the same in ***meiosis II***.

# *Meiosis I*




(a) **Prophase I:**

Recombination (crossing over) happens only during prophase I. (Will be discussed)

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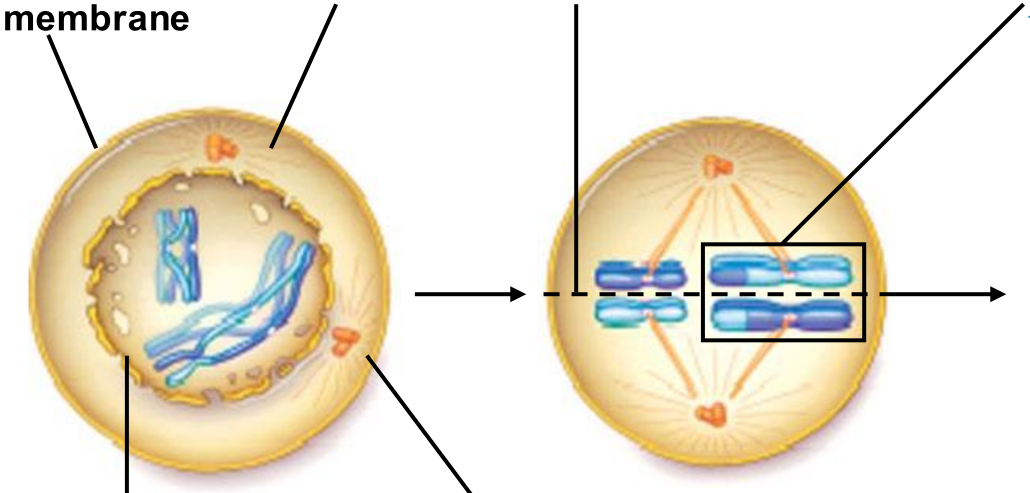
# *Meiosis I*




The spindle fibers will attach the centromere of only one chromosome, and on the opposite pole of the cell, the they will attach the other chromosome.

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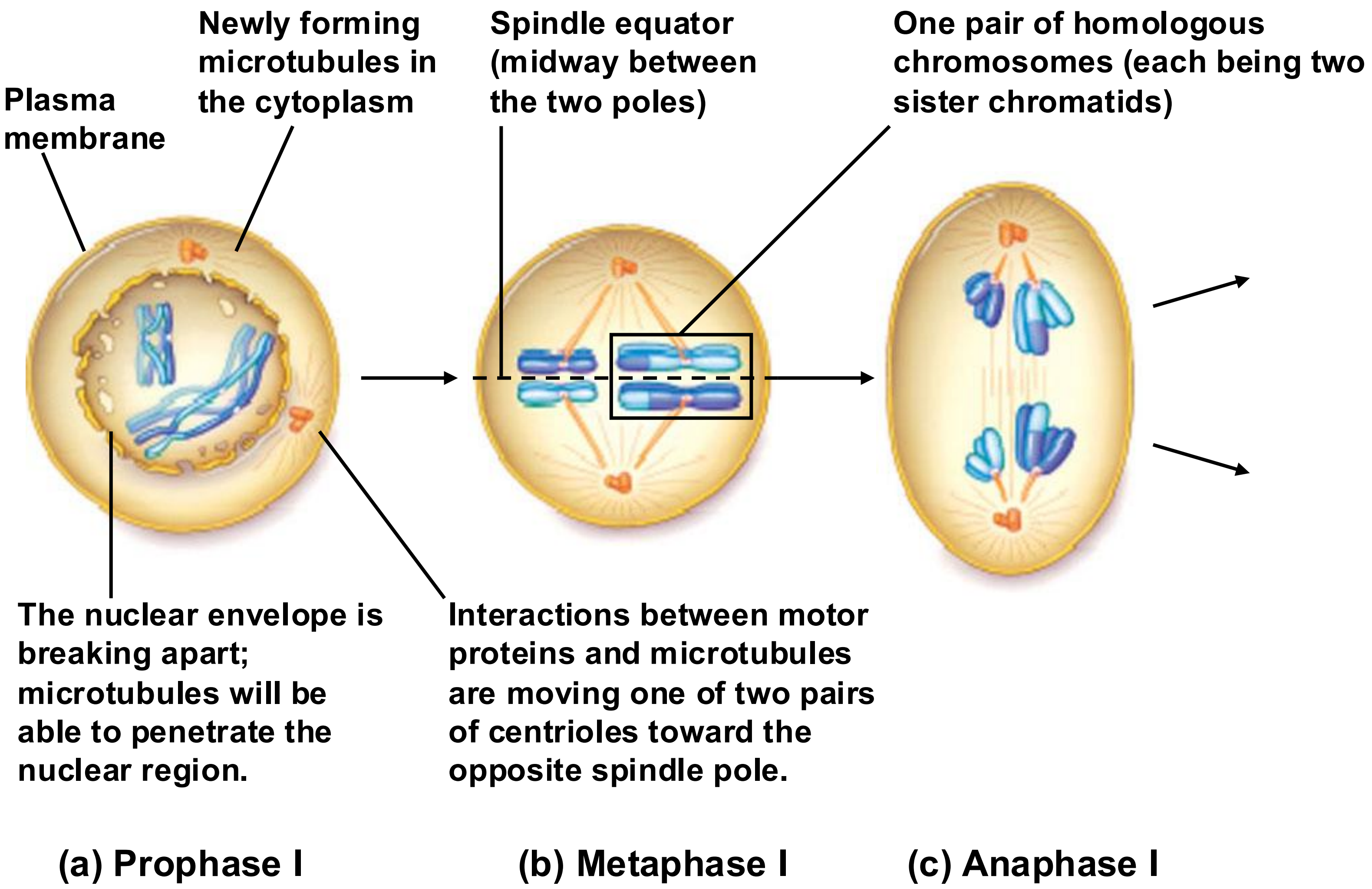
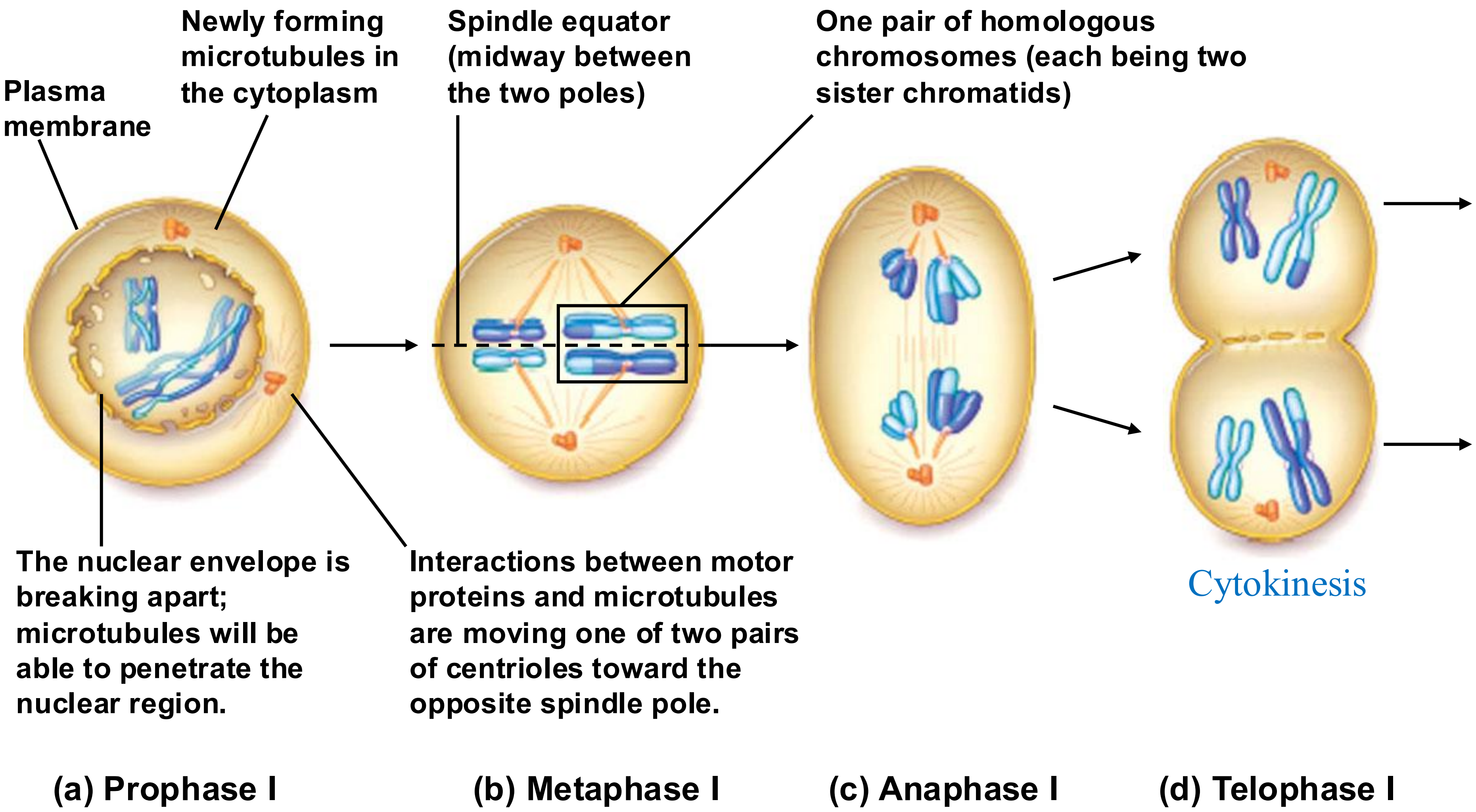
# *Meiosis I*




The homologous chromosomes will separate, with each one moving to a pole of the daughter cells.

# *Meiosis I*

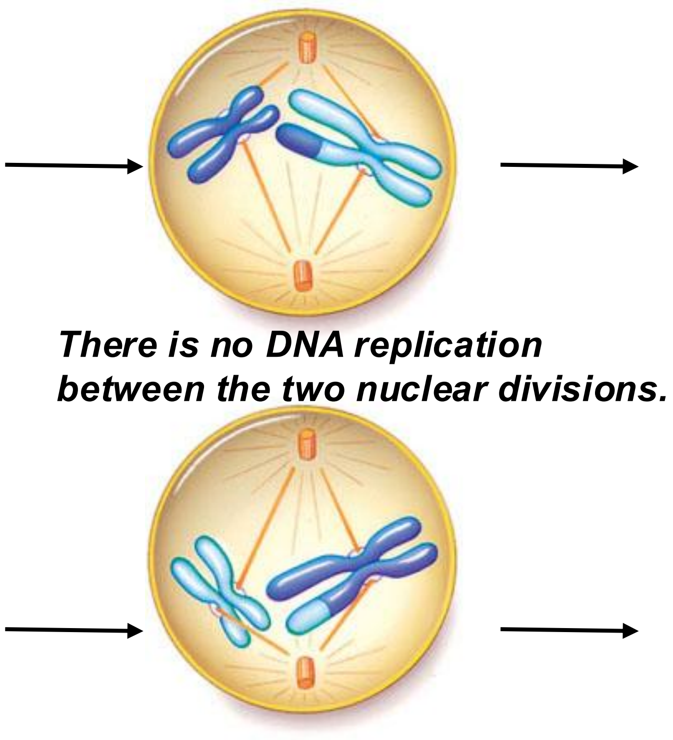



***Meiosis I*** results in **two** daughter cells with **half** the number of chromosomes.

FIGURE **2.13** The stages of meiosis. In this form of cell division, homologous chromosomes physically associate to form a chromosome pair. Members of each pair separate from each other at meiosis I. In meiosis II, the centromeres of unpaired chromosomes divide, producing four cells, each with the haploid (*n*) number of chromosomes.

# *Meiosis II* similar to mitosis

- Meiosis II proceeds without interphase. No DNA replication occurs between Meiosis I and Meiosis II.




(e) Prophase II

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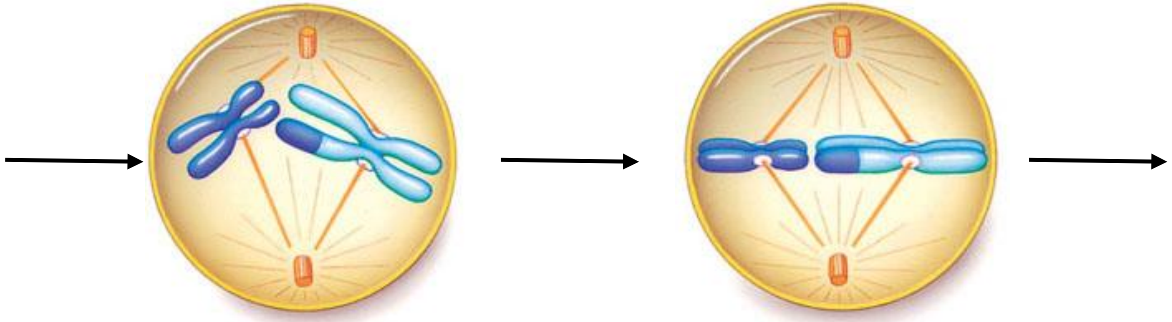
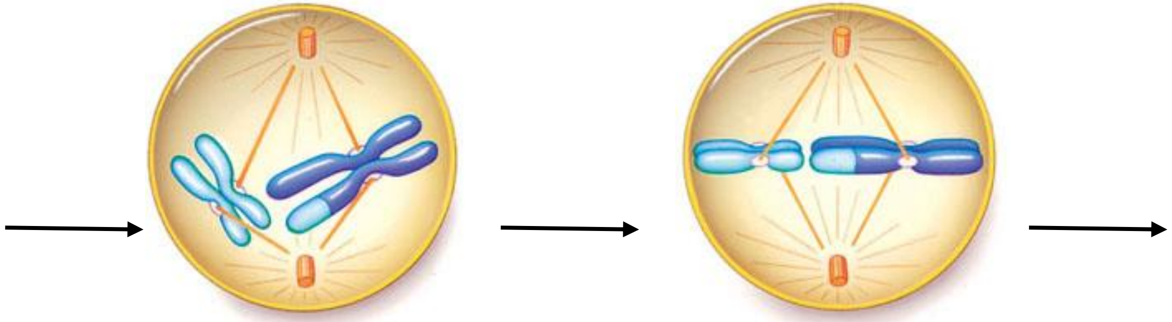
*Meiosis II* similar to mitosis



*There is no DNA replication between the two nuclear divisions.*



**(e) Prophase II** **(f) Metaphase II**

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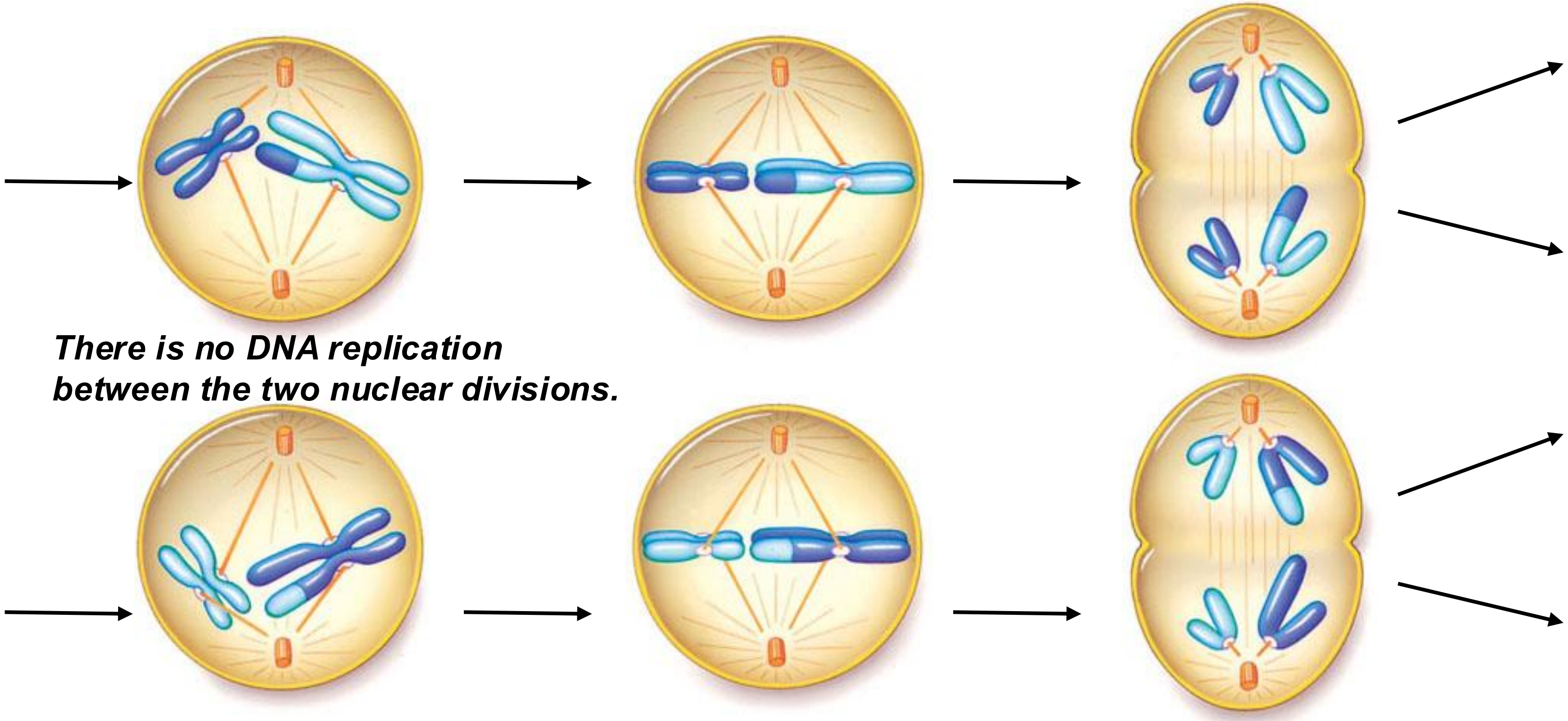
***Meiosis II*** similar to mitosis




**(e) Prophase II** **(f) Metaphase II** **(g) Anaphase II**

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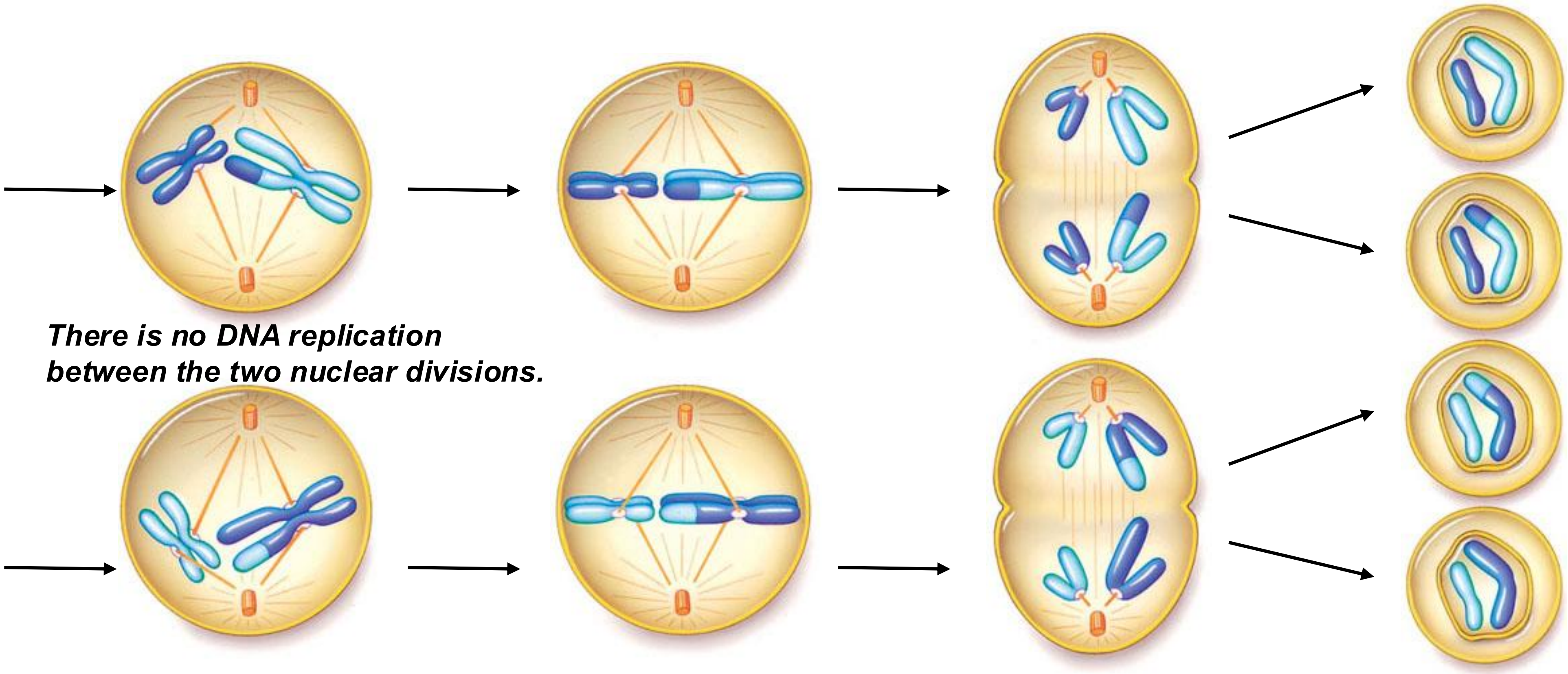
# ***Meiosis II*** similar to mitosis




***Meiosis II*** ends up with **4** daughter cells with **half** the number of chromosomes of the parental cell.

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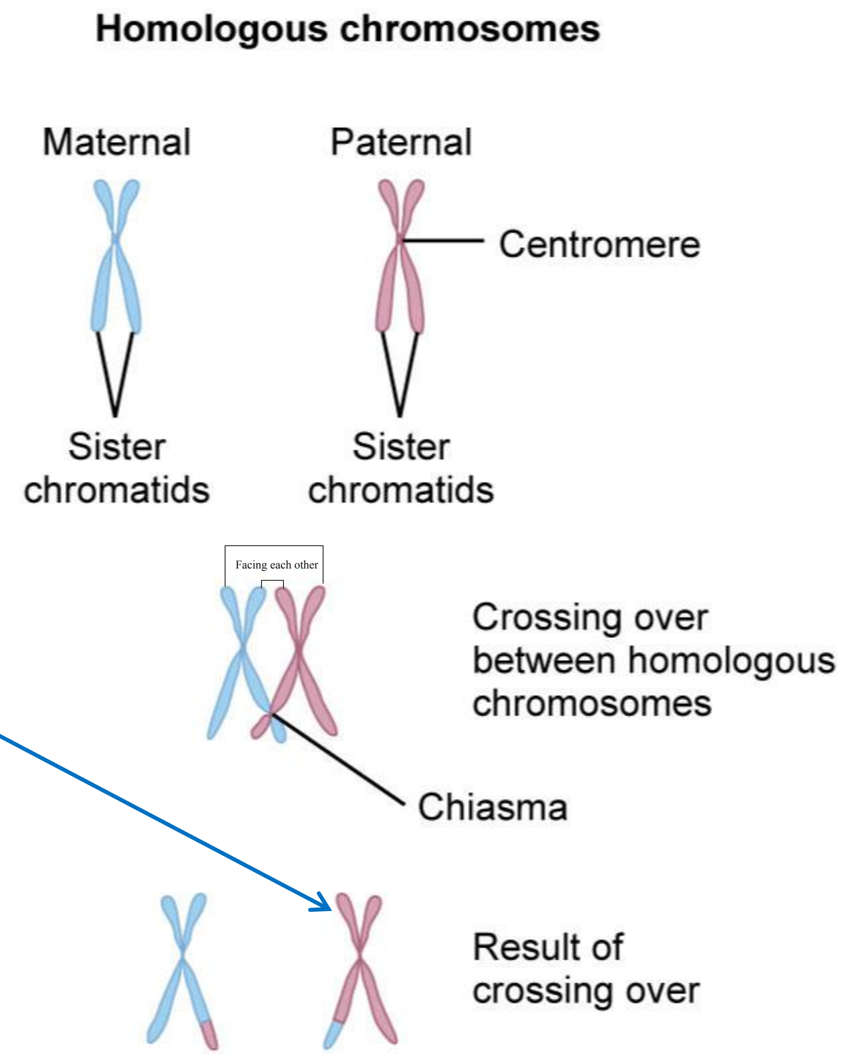
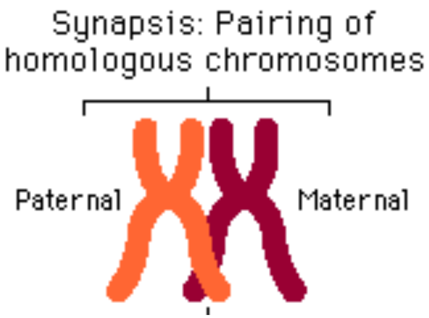
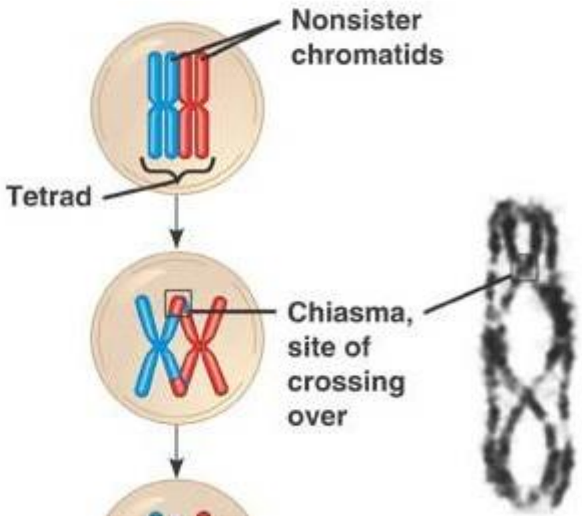
- During **prophase I**, homologous chromosomes overlap, and this overlap is called ***chiasma***.
- After the overlap occurs, **crossing over** takes place.
- ***Crossing over (recombination)*** is the exchange of genetic material between the <u>non-sister chromatids of homologous chromosomes</u>, which eventually results in a maternal chromosome carrying part of the paternal genetic material and vice versa.
- This crossed-over chromosome is not identical to the paternal chromosome because it contains DNA sequences that are identical to those of the mother rather than the father.
- This process is a **normal** physiological process that occurs during sexual reproduction.
- If it occurs between non-homologous chromosomes, it can give rise to hereditary genetic diseases, including numerical or structural chromosomal abnormalities.



# *Prophase I*

- Leptotene
  - Replicated chromosomes align and begin to **condense**
  - Chromosomes will be on different regions in the cell
- Zygotene
  - homologous chromosomes pair along entire length (**synapsis**) and close to each other
  - Chromosomes appear on a shape of 4 chromatids (tetrad)
  - synaptonemal complex forms
- Pachytene
  - Synapsis is complete and each pair of homologues is called a **tetrads** (**bivalent**)
  - **Crossing over** occurs (recombination at **chiasmata**)
- Diplotene
  - Homologous chromosomes separate some but remain bound at chiasmata
    - usually 2 chiasmata/chromosome, more frequent in females during oogenesis)
- Diakinesis
  - Further chromosome condensation; tetrads viable
  - Chromosomes are packed, shorter and smaller in size.



- **Why are the homologous chromosomes still close and not completely separated even after recombination has happened?**

- ✓ In **Metaphase I**, chromosomes align in the form of pairs of homologous chromosomes. Therefore, we would not separate them after **crossing over**. Instead, they remain close or not completely separated so they can align properly in the next stage, since disjoining occurs at the chromosome level rather than between sister chromatids.

**See the next slide**

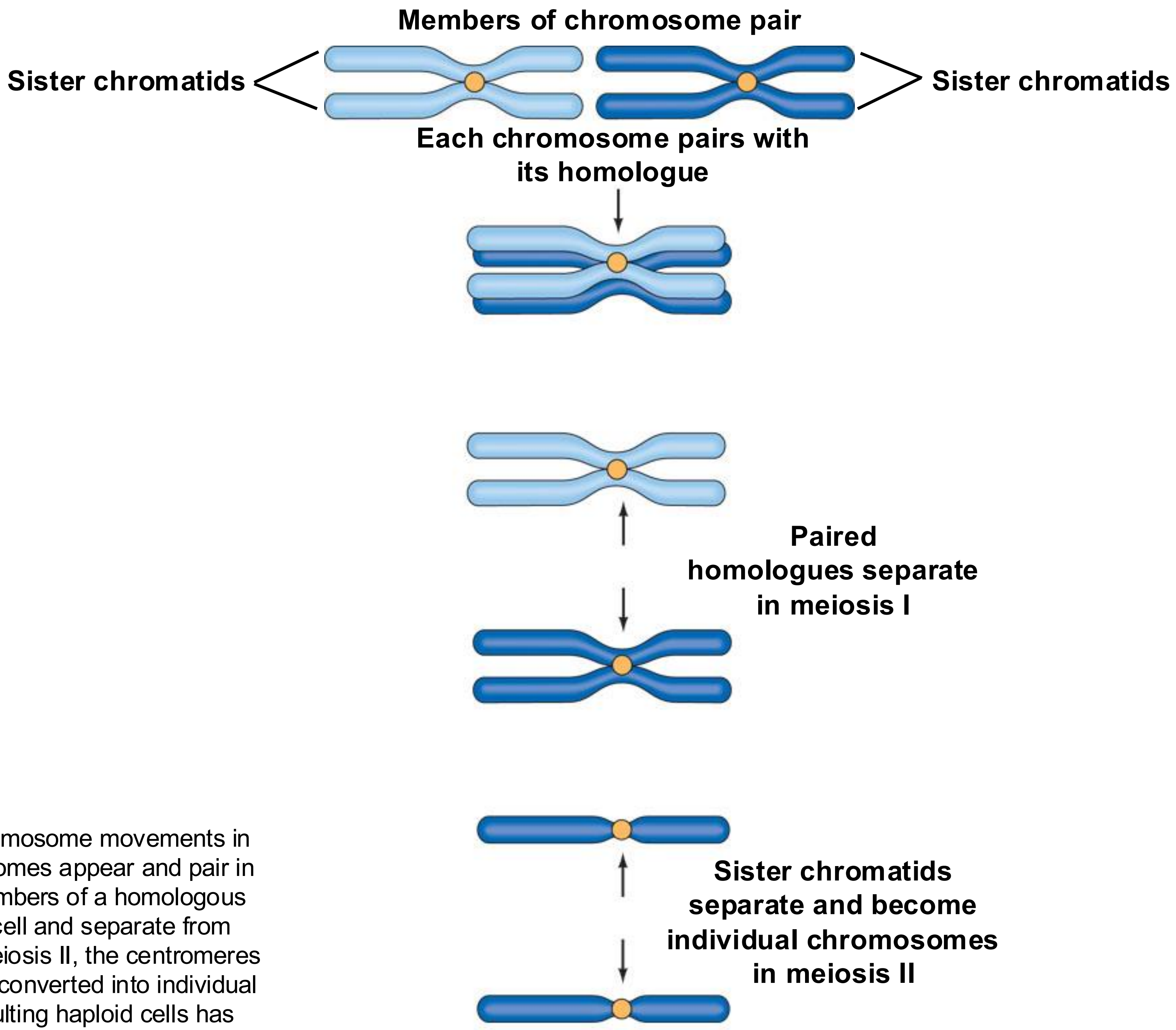



FIGURE **2.14** Summary of chromosome movements in meiosis. Homologous chromosomes appear and pair in prophase I. At metaphase I members of a homologous pair align at the equator of the cell and separate from each other in anaphase I. In meiosis II, the centromeres split, and sister chromatids are converted into individual chromosomes. Each of the resulting haploid cells has one set of chromosomes.

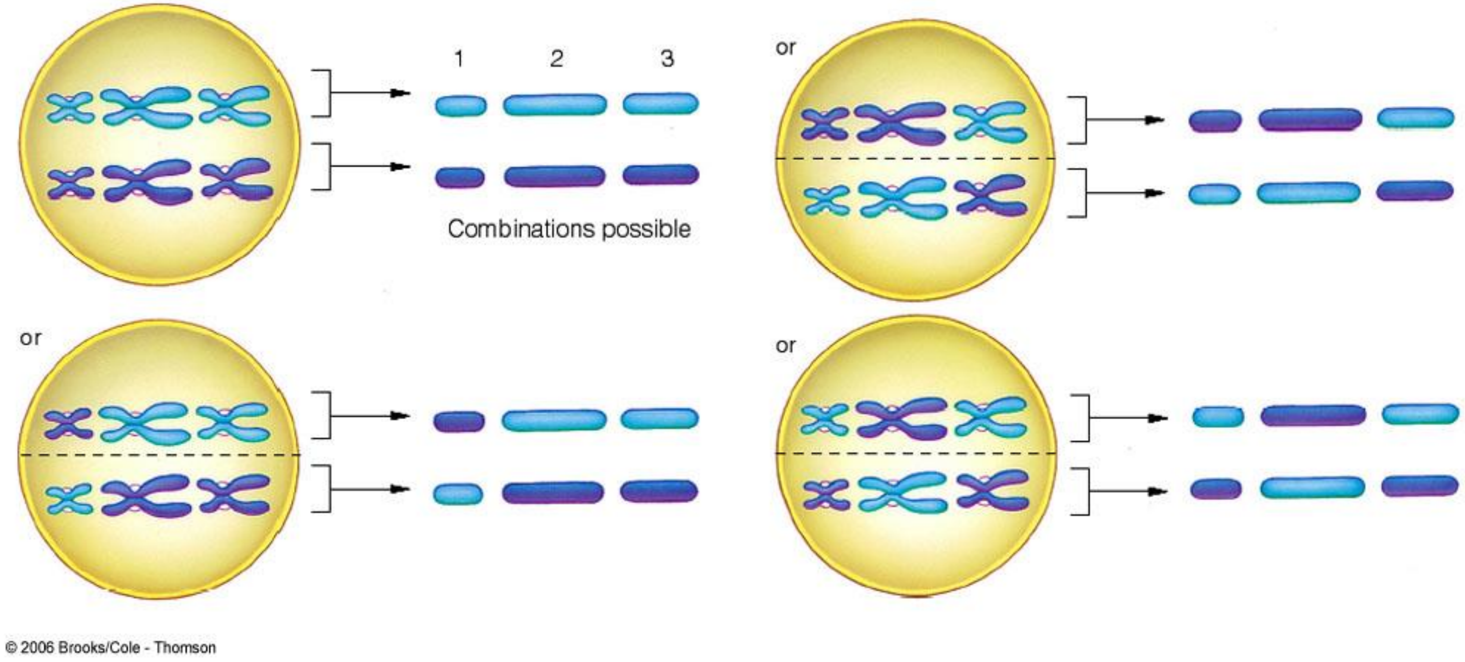
# *Genetic consequences of meiosis*

- Reduction of chromosome number
- Diploid to haploid (essential for gametes)
- Random assortment of maternal and paternal chromosomes
  - genes on different chromosomes
  - maternal/paternal chromosomes
  - Number of possible chromosomal combinations = $2^{23}$ or 8,388,608
  - Recombination between chromosome pairs increases the possible combinations
- Segregation of alleles
- Recombination/crossing-over
  - Allows new combinations of genes to be produced
  - Important for normal chromosome disjunction
  - Ensures genetic diversity

# *Chromosome combinations: independent assortment*

Paternal
**Maternal**

- ➢ There is no rule that states that, during Metaphase, paternal chromosomes must align on one side and maternal chromosomes on the other; their orientation is random.
- ➢ This principle is explained by **Mendel's law of independent assortment.**
- ✓ Therefore, if a cell contains 6 chromosomes (3 homologous pairs), meiosis can produce 8 different combinations of daughter cells when it undergoes meiosis.




❖ **Number of possible chromosomal combinations = 2^n**

✓ **n= number of pairs of homologous chromosomes**