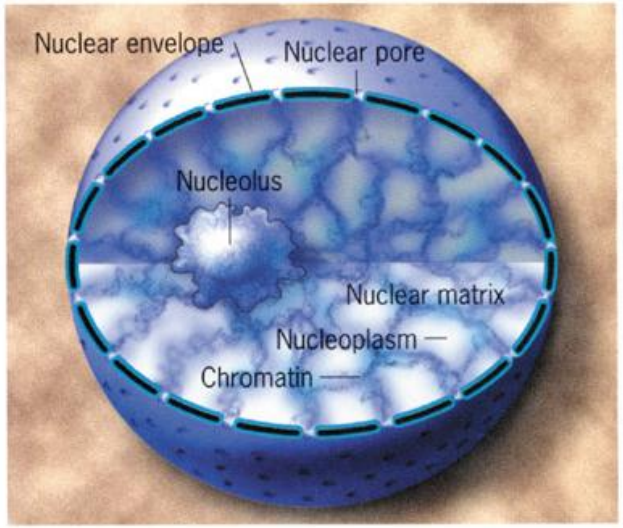
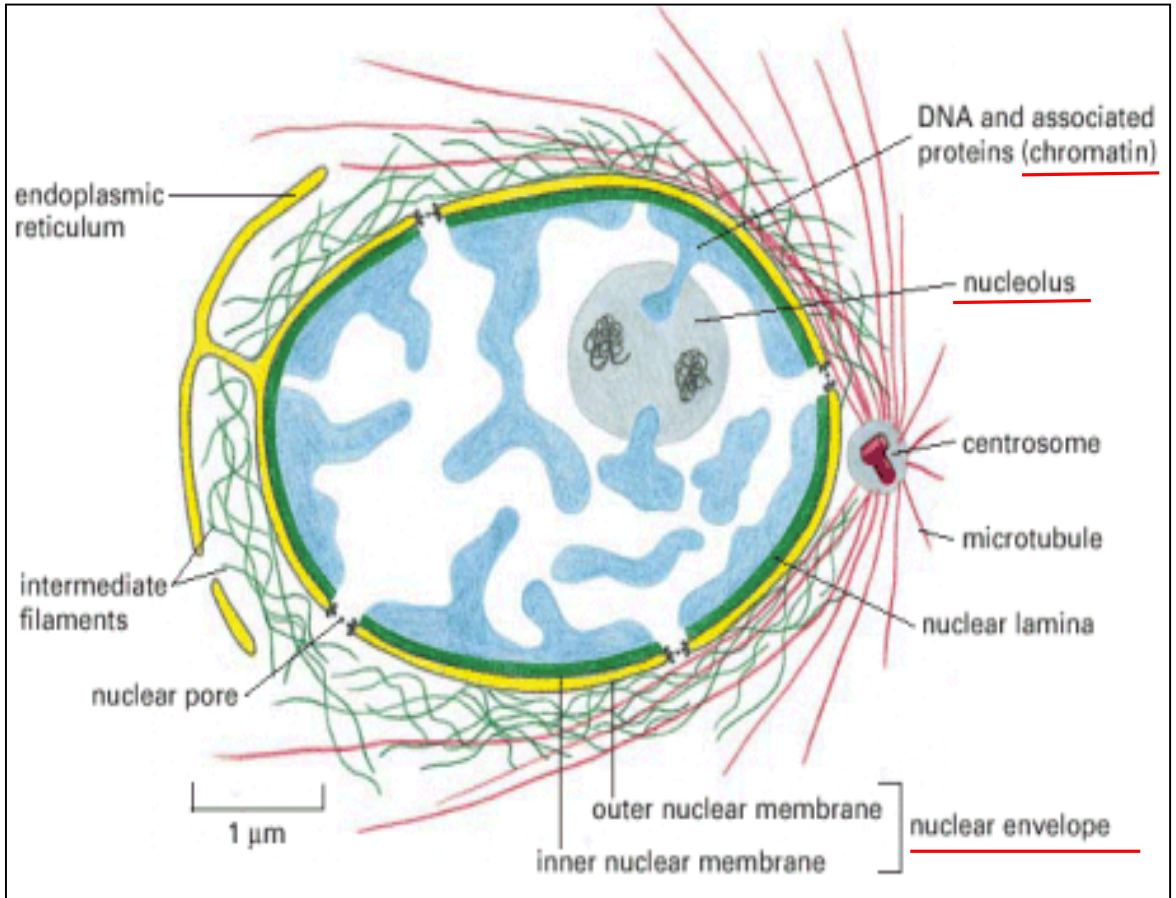
Chapter 7 Nucleus

pl. nuclei



Preface

- **Size:** 5-10 μm(high animal); 5-20 μm(High plant)
- **Volume ratio of nucleus to cytoplasm:** 1:10 (9)
- **Shape:** Spherical or oval
- **Contain:** **4 subunits**
  - Nuclear Envelope
  - Chromatin
  - Nucleolus
  - Nuclear Matrix (nuclear skeleton)



Section1. Nuclear envelope

I. Nuclear membrane

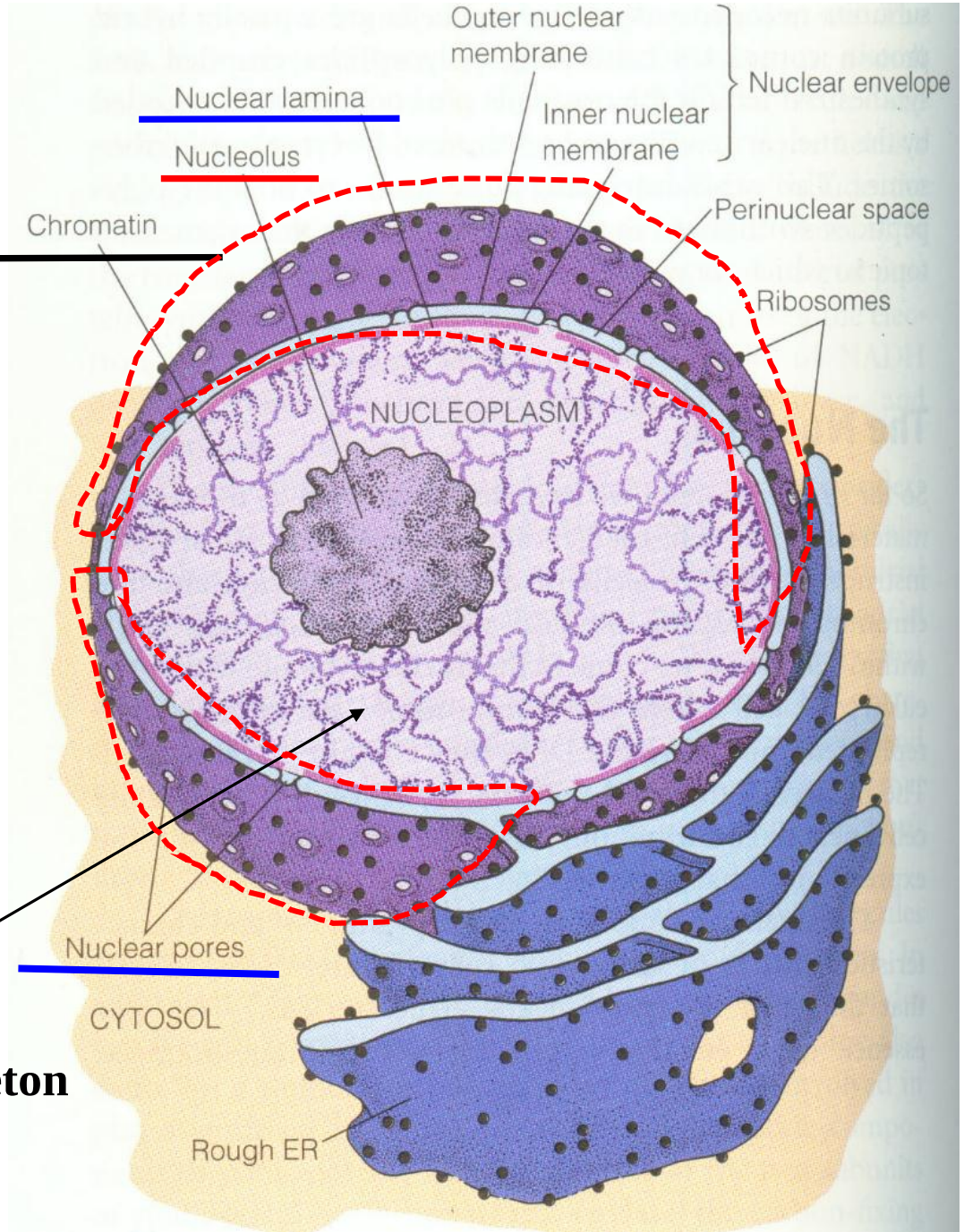
II. Nuclear pore complex (NPC)

III. Nuclear lamina

Section2. Chromatin

Section3. Chromosome

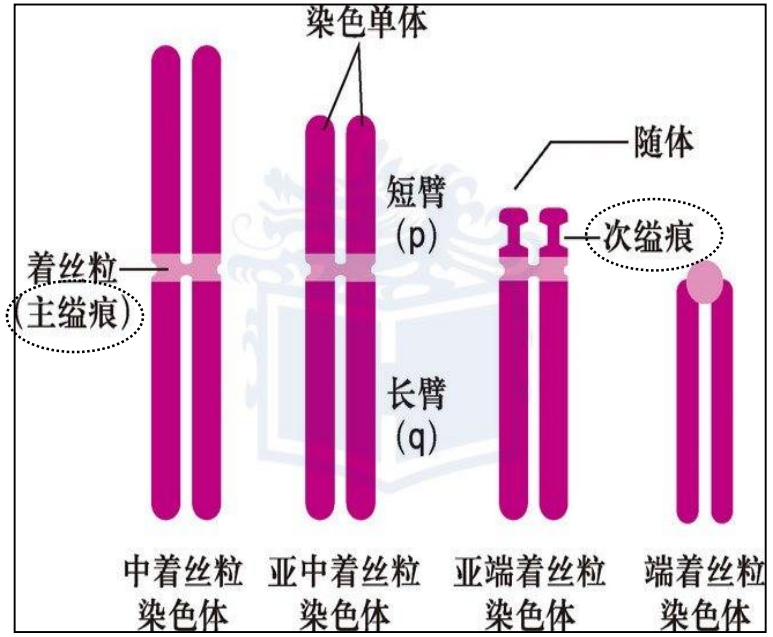
Section4. Nucleolus & Nuclear Matrix/skeleton

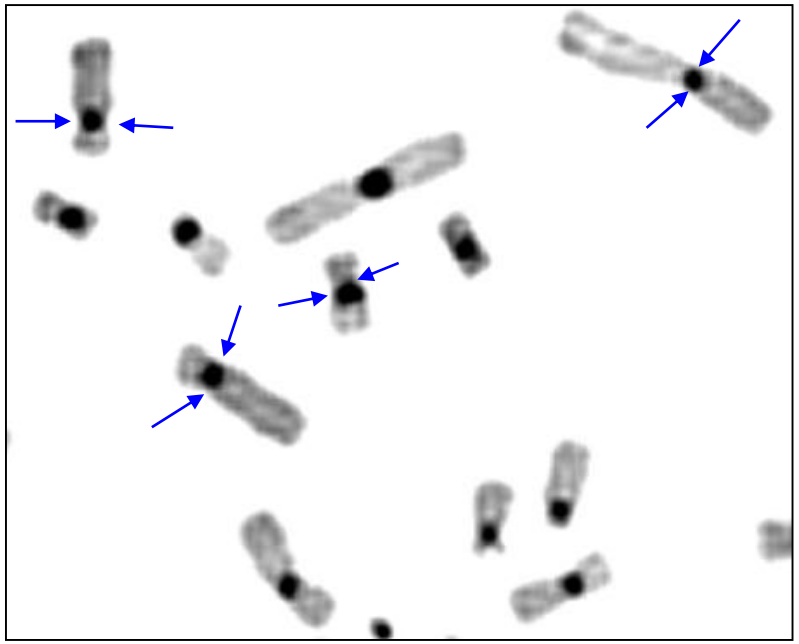


Section 3. Chromosome

I. Structure

1. Four types: see the right



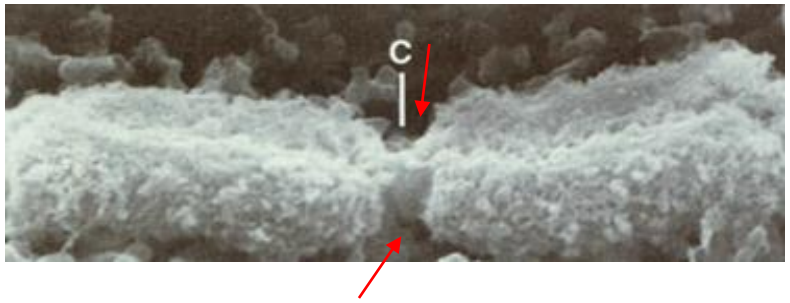


2. Main subunits:

2.1 Centromere:

Centrosome

(1) Definition: The highly constricted region of a chromosome that is the position at which the pair of chromatids are held together.



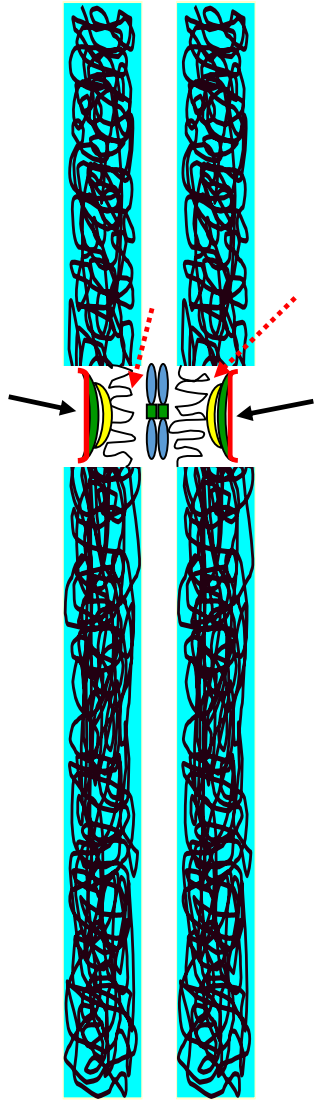
Primary constriction (主缢痕)

(2) Function:

Serve both as linker sites of sister chromatids, and as attachment sites
①
②

for microtubules of the mitotic spindle.

（有丝分裂纺锤体微管）



(3) Composition:

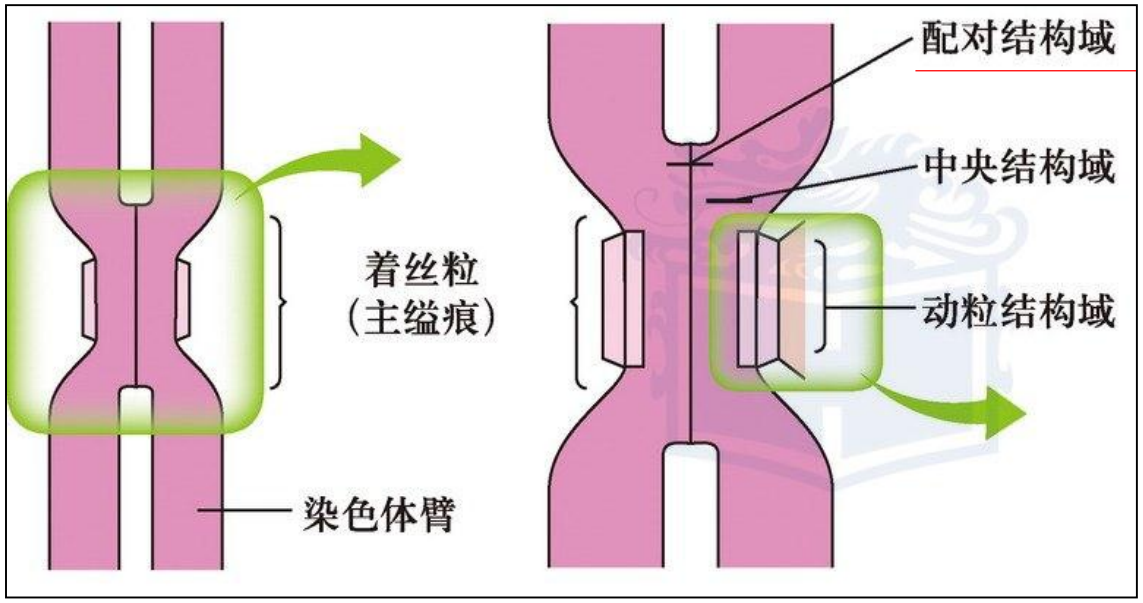
- Centromere DNA;
- Centromere associated proteins.

(4) Subunits: **3**主要**+1**次要 inner ⟶ outer

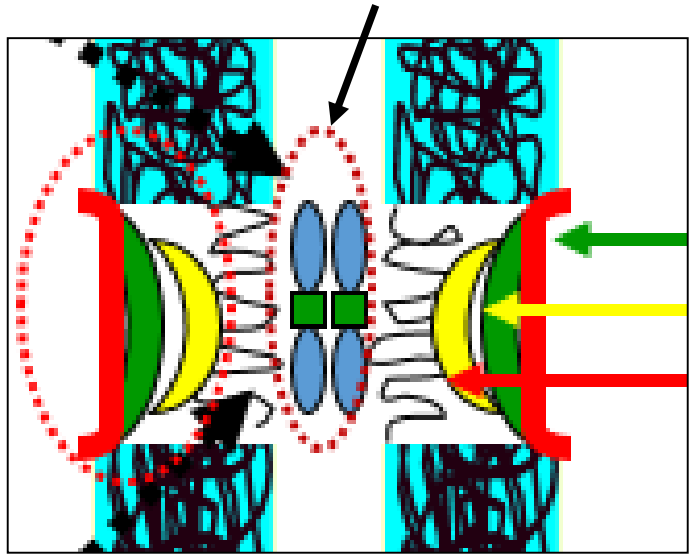
① Pairing domain:

- The interaction site for Metaphase chromatids (sister chromatids).
- Include two types of proteins:

  INCENP (inner centromere proteins); CLIP (chromatid linking proteins).
- These two types of proteins are responsible for the paring of chromatids.

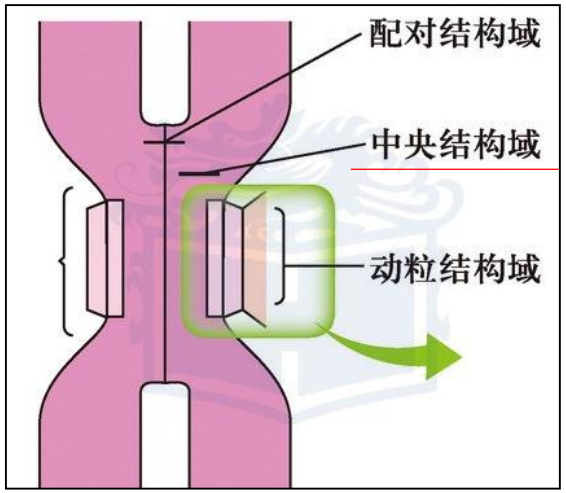


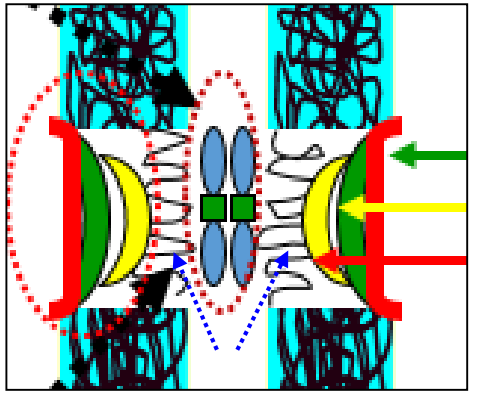
着丝粒的结构域组织



② Central domain：

- Composed of highly repeated satellite DNA.
  (Centromere DNA)

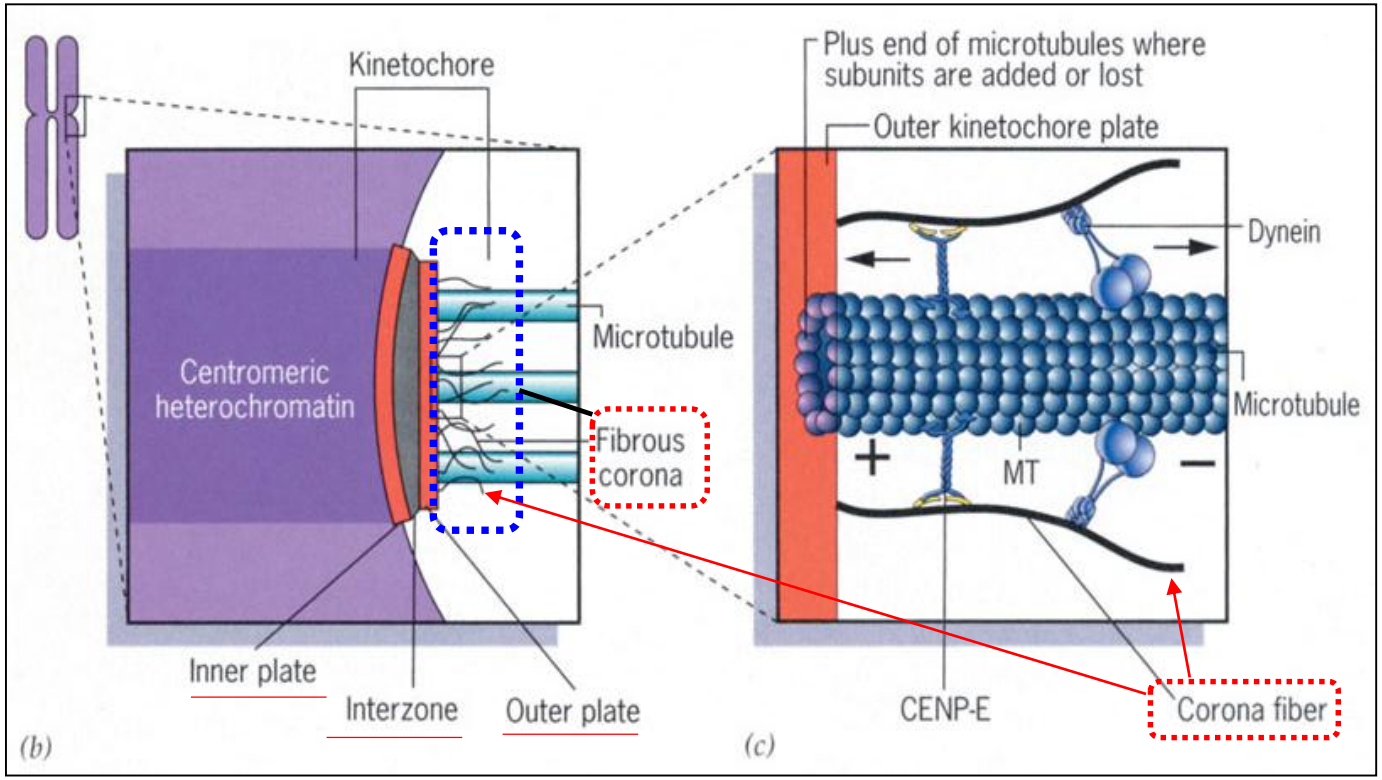




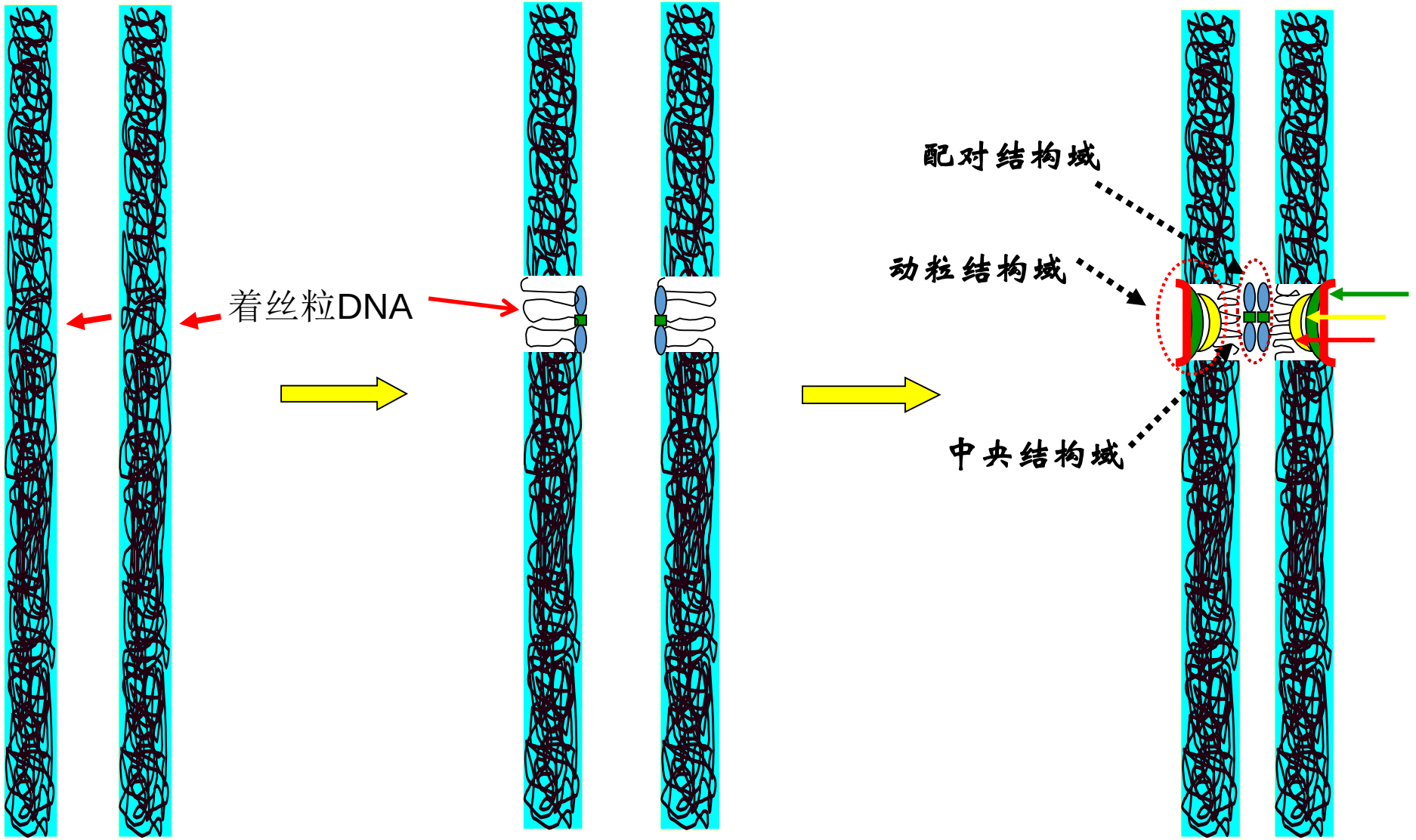
③ kinetochore domain: （动粒/着丝点）

- Inner plate（内板）
- Middle space/interzone (中间间隙)
- Outer plate（外板）
  (microtubule)

④ Fibrouscorona: corona fiber（微管蛋白）
（纤维冠）

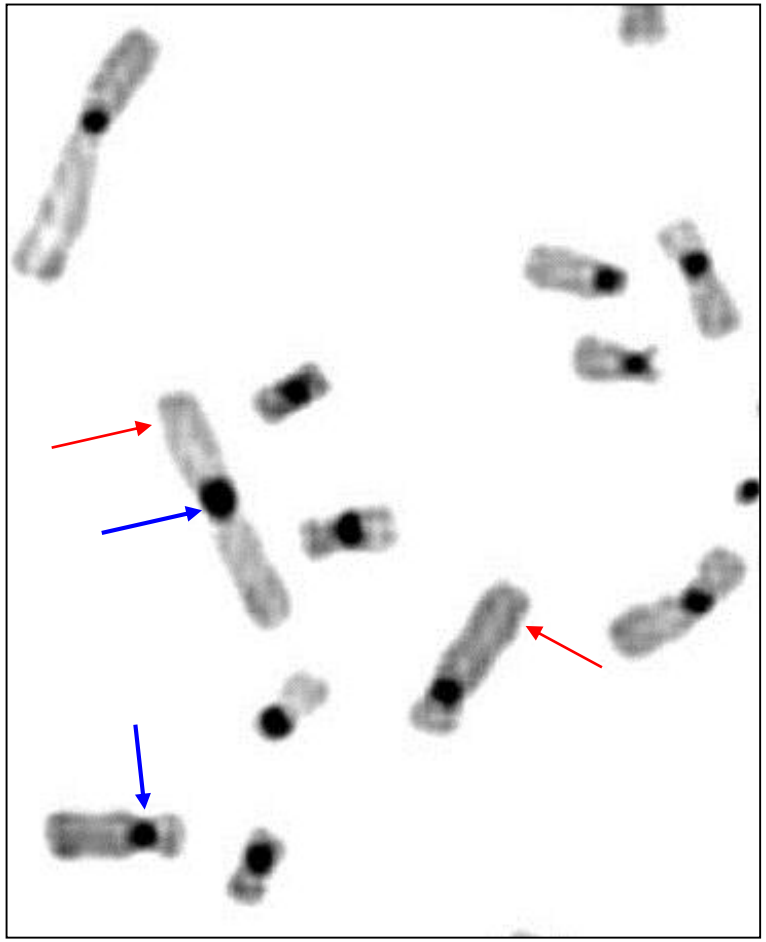


Centromere formation

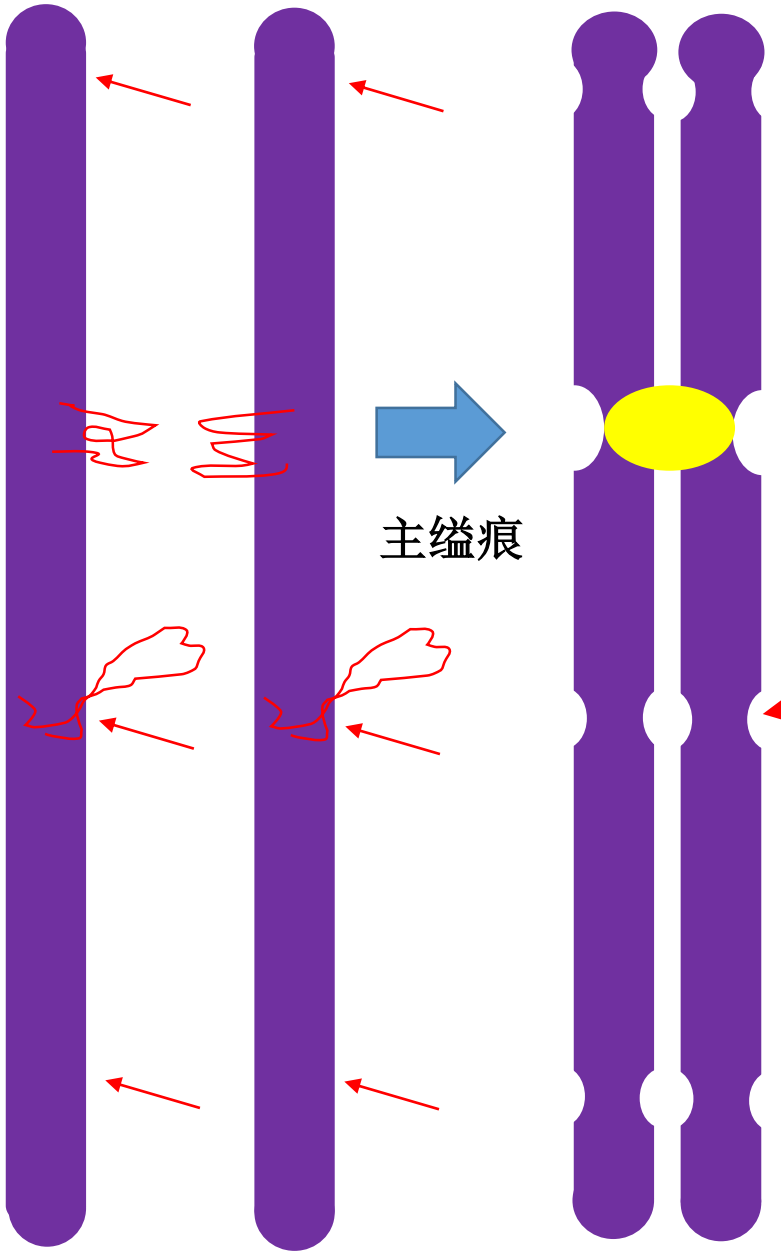


2.2 Secondary constriction: 次缢痕

P280, 282



着丝粒蛋白特异标记



由于染色体DNA上基因转录，折叠晚了。导致折叠压缩程度不高，吸附染料较少。（没有跟上大部队）

什么基因？

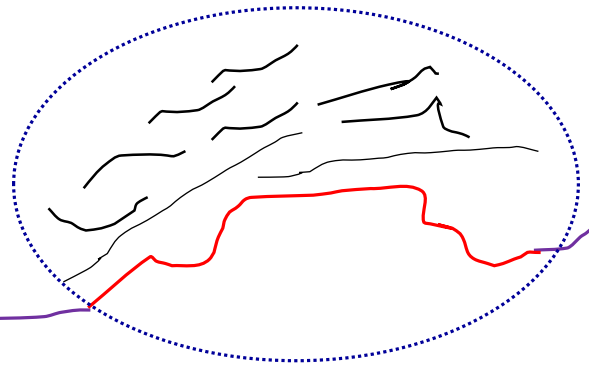
- **某些**次缢痕是rRNA(5srRNA除外)基因的所在部位。
- 则次缢痕与间期细胞核内核仁形成有关（组织者）。

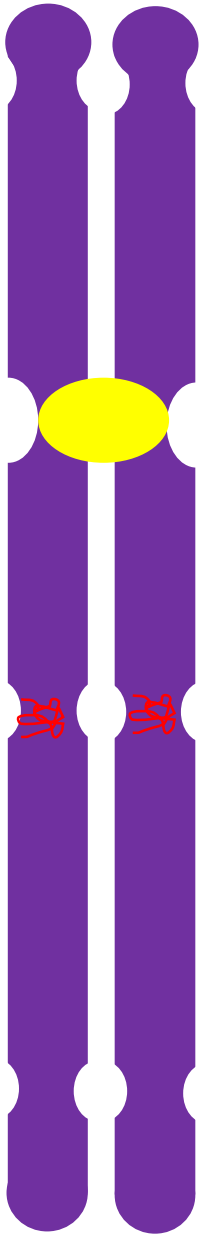
  故，这些次缢痕又称核仁组织区(nucleolar organizing region，NOR)。
- 次缢痕可作为染色体的“鉴定标记”。

- For the reason that the rRNA (excluding 5srRNA) genes are located here and they are involved in ribosomes biogenesis.
- These secondary constrictions are also called as NOR.

rRNA基因？
(5srRNA外)

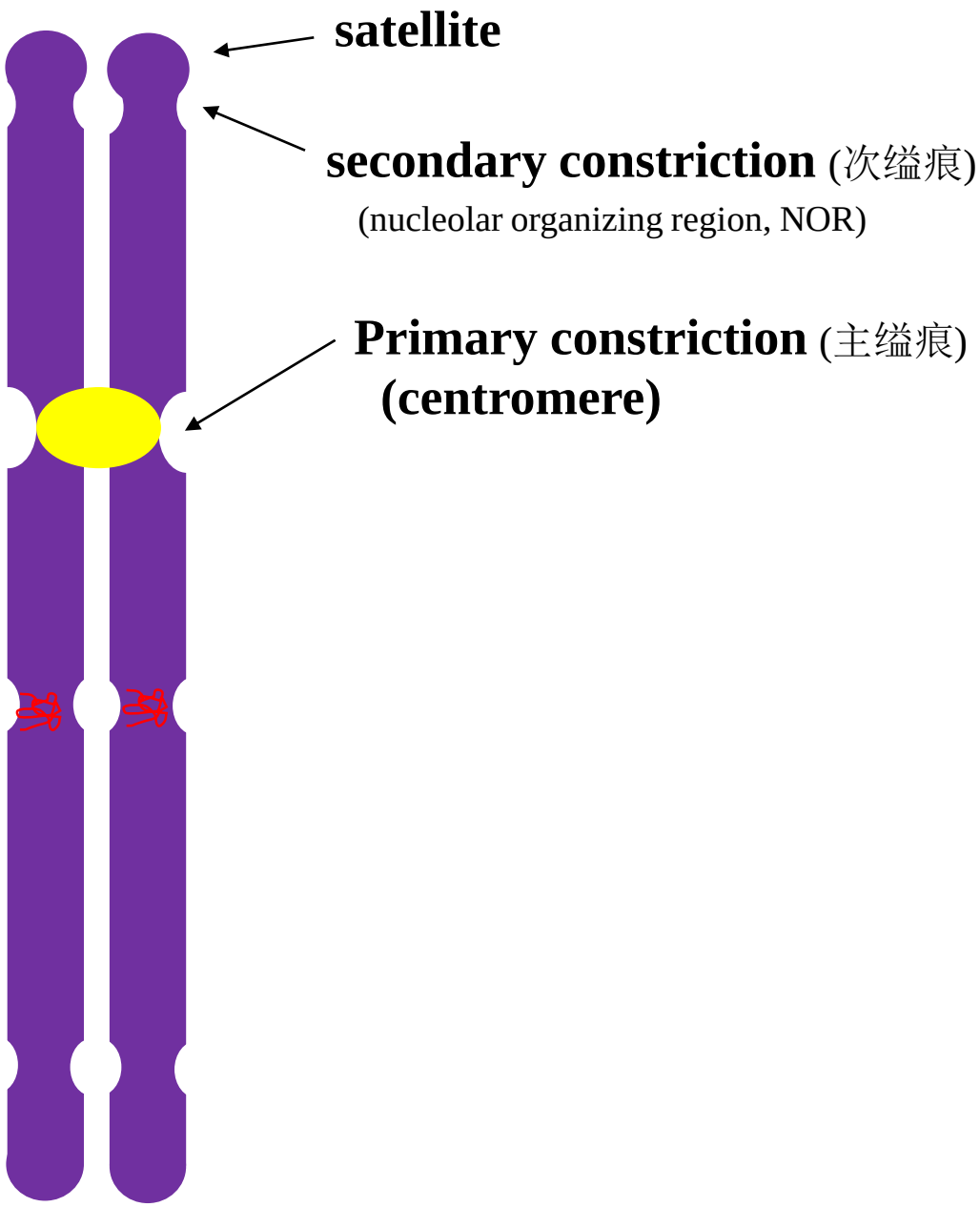
28S、18S和5.8S





2.3 Satellite: 随体

- A spherical segment at the end of a chromosome.

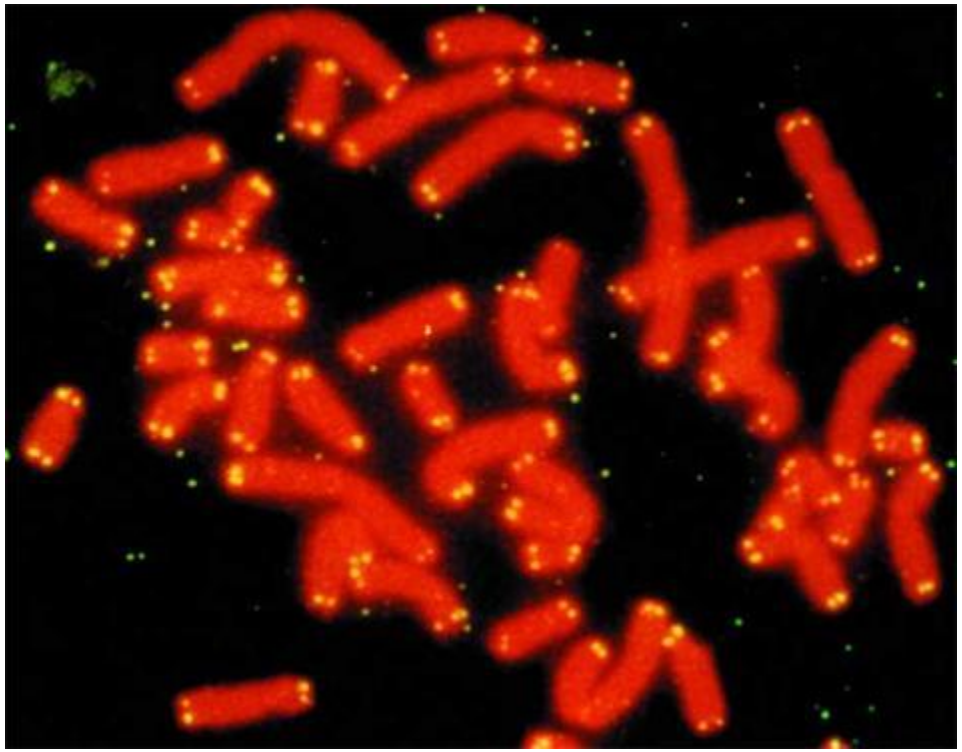


2.4 Telomere:

Composition of telomere?

- The specialized structure located at the end of chromosome.
- It is rich in short tandem repeated DNA sequences, e.g. “TTAGGG” in humans.
  （端粒DNA序列）
- Functions:
  - Ensure the replication of the ends of chromosomes. ?
  - Protect them from erosion (nuclease digestion). 腐蚀
  - Prevent them from fusion with other DNA fragments.

(维持chr.完整性和独立性)



人类染色体端粒DNA的荧光原位杂交照片

II. Three functional elements of chromosome DNA

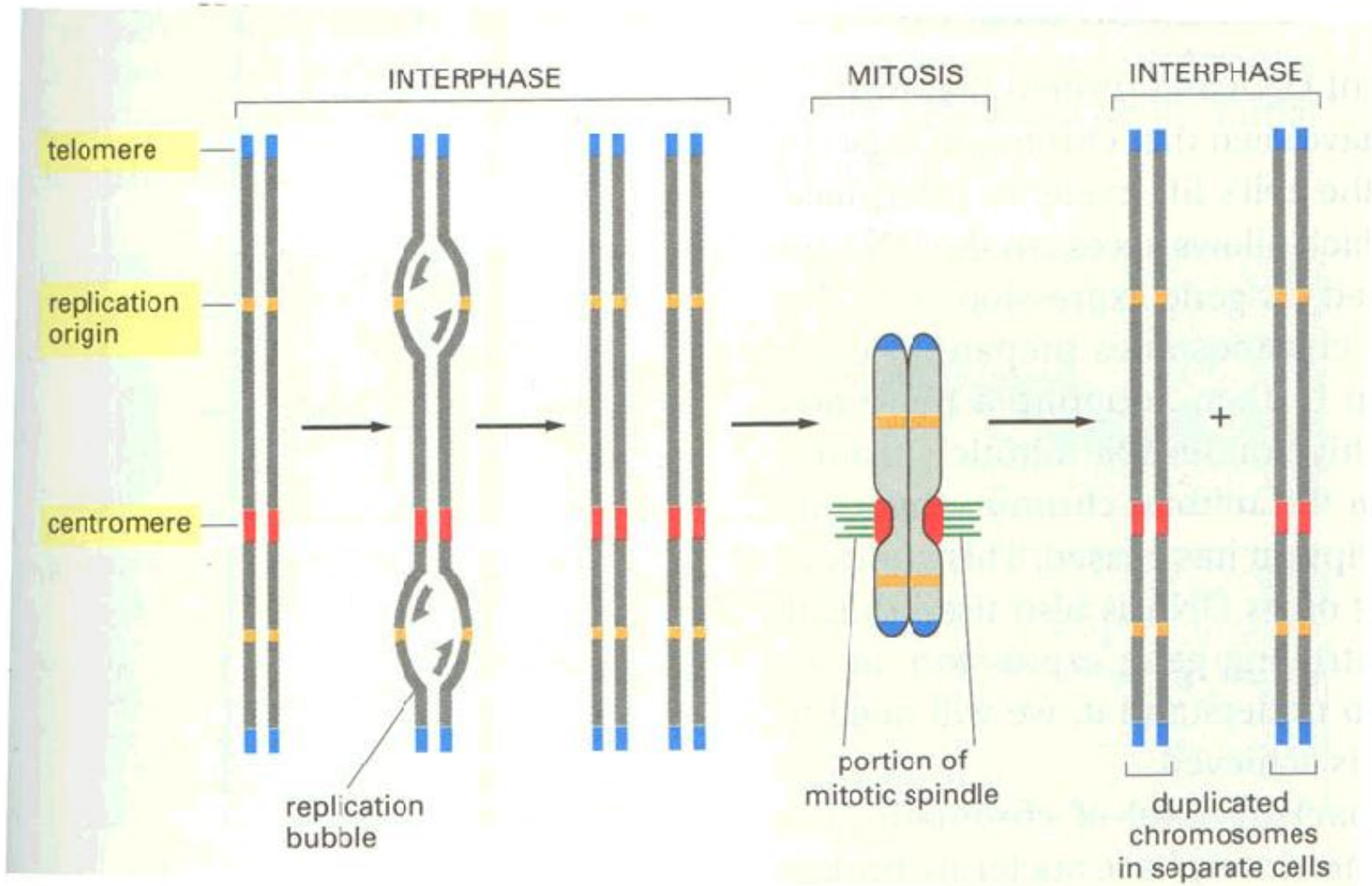
(key sequences)

（Plasmid DNA）

Telomere DNA sequence

Autonomously replicating DNA sequence

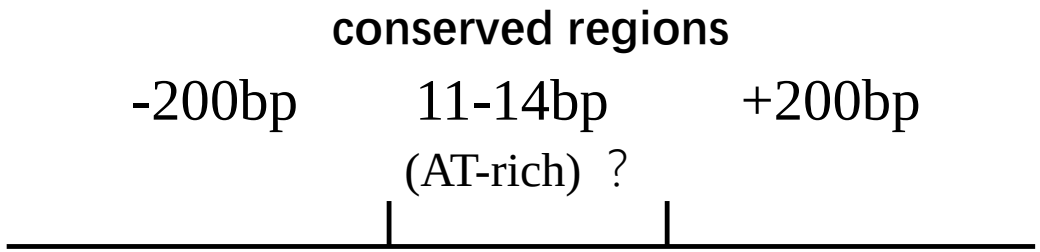
Centromere DNA sequence



1. Autonomously replicating DNA sequence (ARS): “复制起始点(质粒)”

(Ori)

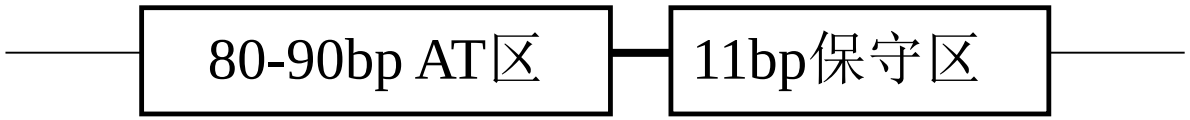
- Structure:



- Function: ARS act as an origin of replication. 启动DNA复制

2. Centromere DNA sequence (CEN):

- Structure: 2 core regions



- Function: Ensure that chromosomes are equally distributed to two daughter cells.

3. Telomere DNA sequence (TEL):

☐ Telomere , Telomere DNA and Telomerase ?

The telomere DNA sequences of a variety of eukaryotes

Organism	Telomeric repeat sequence
Yeasts	
Saccharomyces cerevisiae	$G_{1\sim3}T$
Schizosaccharomyces pombe	$G_{2\sim5}$TTAC
Protozoans	
Tetrahymena	GGGGTT
Dictyostelium	$G_{1\sim8}$A
Plant	
Arabidopsis	AGGGTTT
Mammal	
Human	TTAGGG

Special topic:

Artificial minichromosome （人造微小染色体）

1. Definition:

Using molecular cloning technology, three key sequences (ARS, CEN and TEL) of eukaryotic chromosome DNA were cloned respectively, then connected together, and got the artificial chromosomes.

2. Function:

Act as the transgenic vector. （作为转基因载体）

3. Example: Yeast Artificial Chromosome, YAC (酵母人工染色体)

YAC = CEN & ARS (Yeast) + TEL (Tetrahymena) (四膜虫)

- 1983年，A. W. Murray等人首次成功构建了包括ARS、CEN、TEL和外源DNA， 总长度为55kb的酵母人工染色体。
- YAC作载体可以克隆很大DNA分子。

Yeast Artificial Chromosome (YAC)

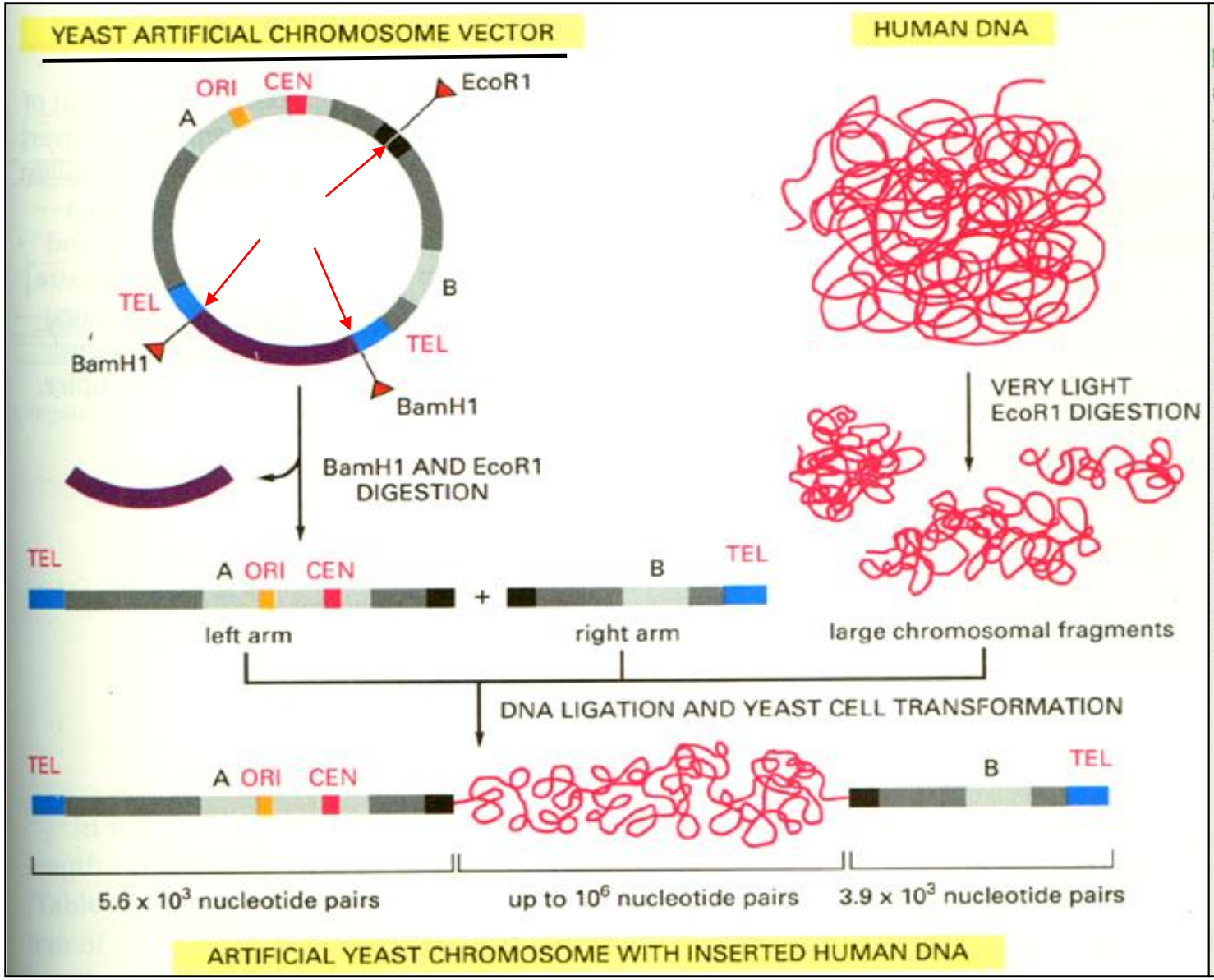


Figure 8–5 The making of a yeast artificial chromosome (YAC). A YAC vector allows the cloning of very large DNA molecules. TEL, CEN, and ORI are the telomere, centromere, and replication origin sequence elements, respectively, for the yeast *Saccharomyces cerevisiae*. BamH1 and EcoR1 are sites where the corresponding restriction nucleases cut the DNA double helix. The sequences denoted as A and B encode enzymes that serve as selectable markers to allow the easy isolation of yeast cells that have taken up the artificial chromosome. (Adapted from D.T. Burke, G.F. Carle, and M.V. Olson, *Science* 236:806–812, 1987. © 1987 AAAS.)

YAC可用于转基因和构建基因文库，容纳插入片段的能力远高于质粒.

Q: For Plasmid DNA or Prokaryote genomic DNA,

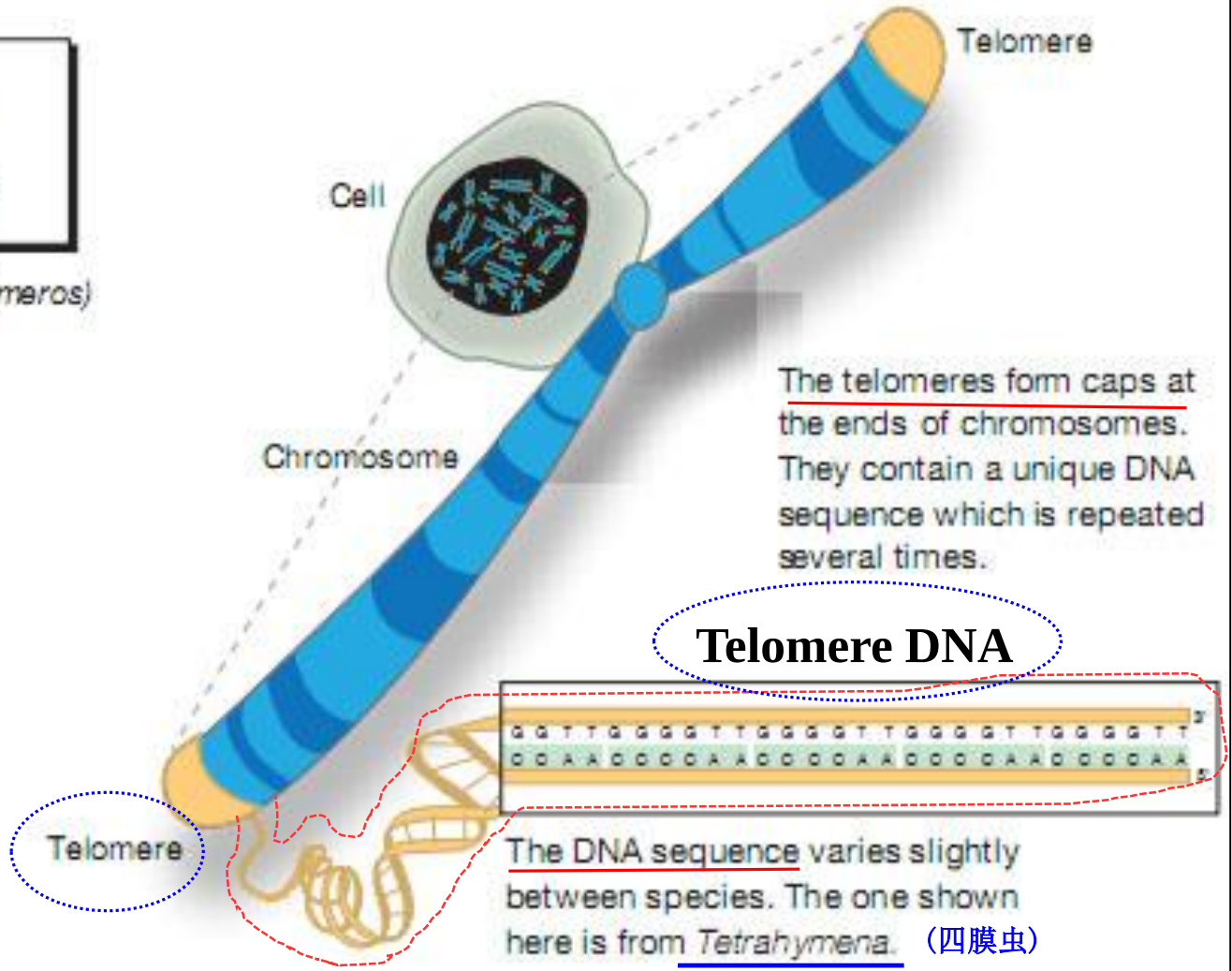
How many key DNA sequences?

Telomere & Telomere DNA

1. The mysterious telomere

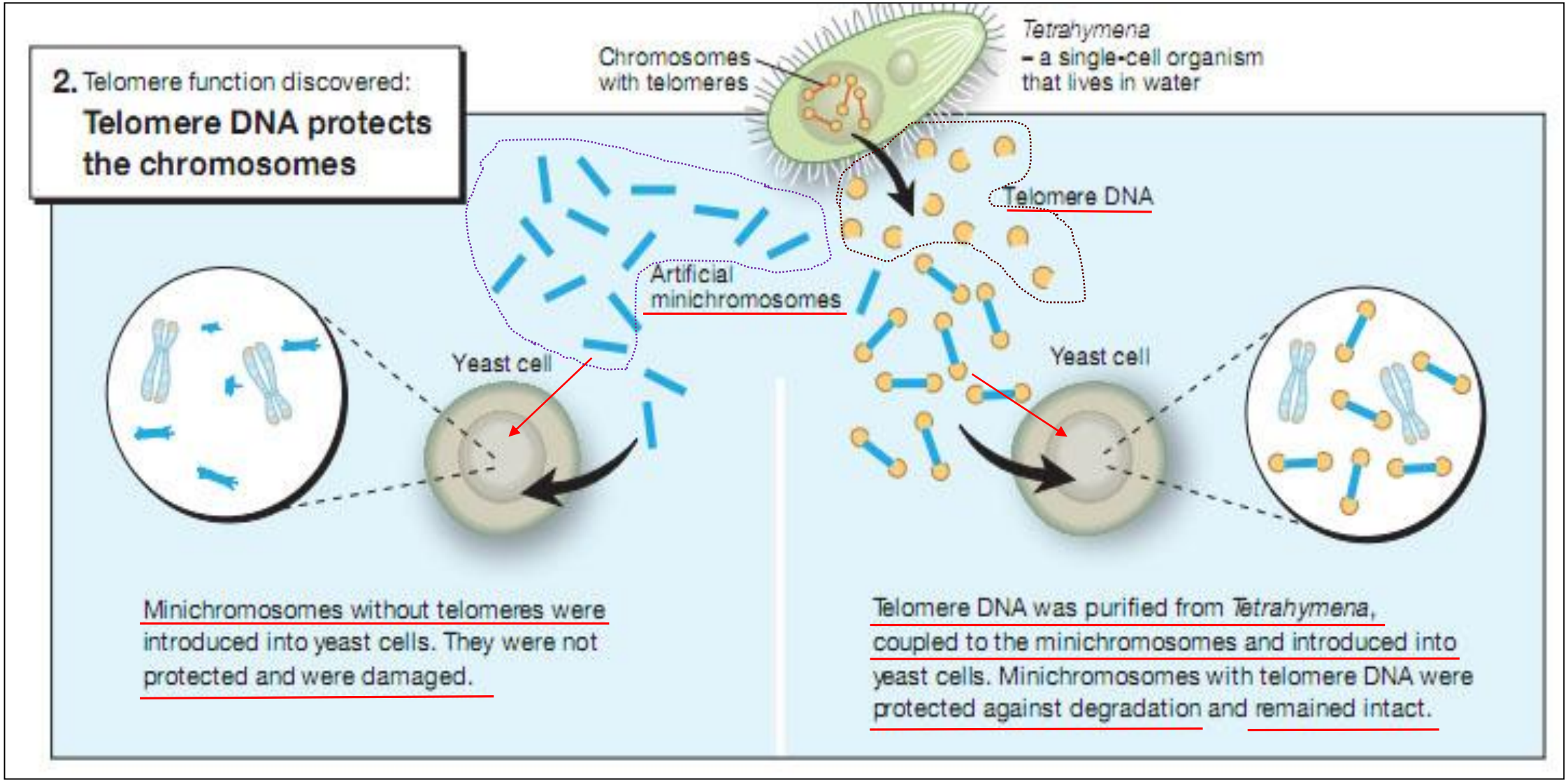
The telomeres appear to protect the chromosomes from damage. But how?

Telomere = Greek for "end" (*telos*) and "part" (*meros*)

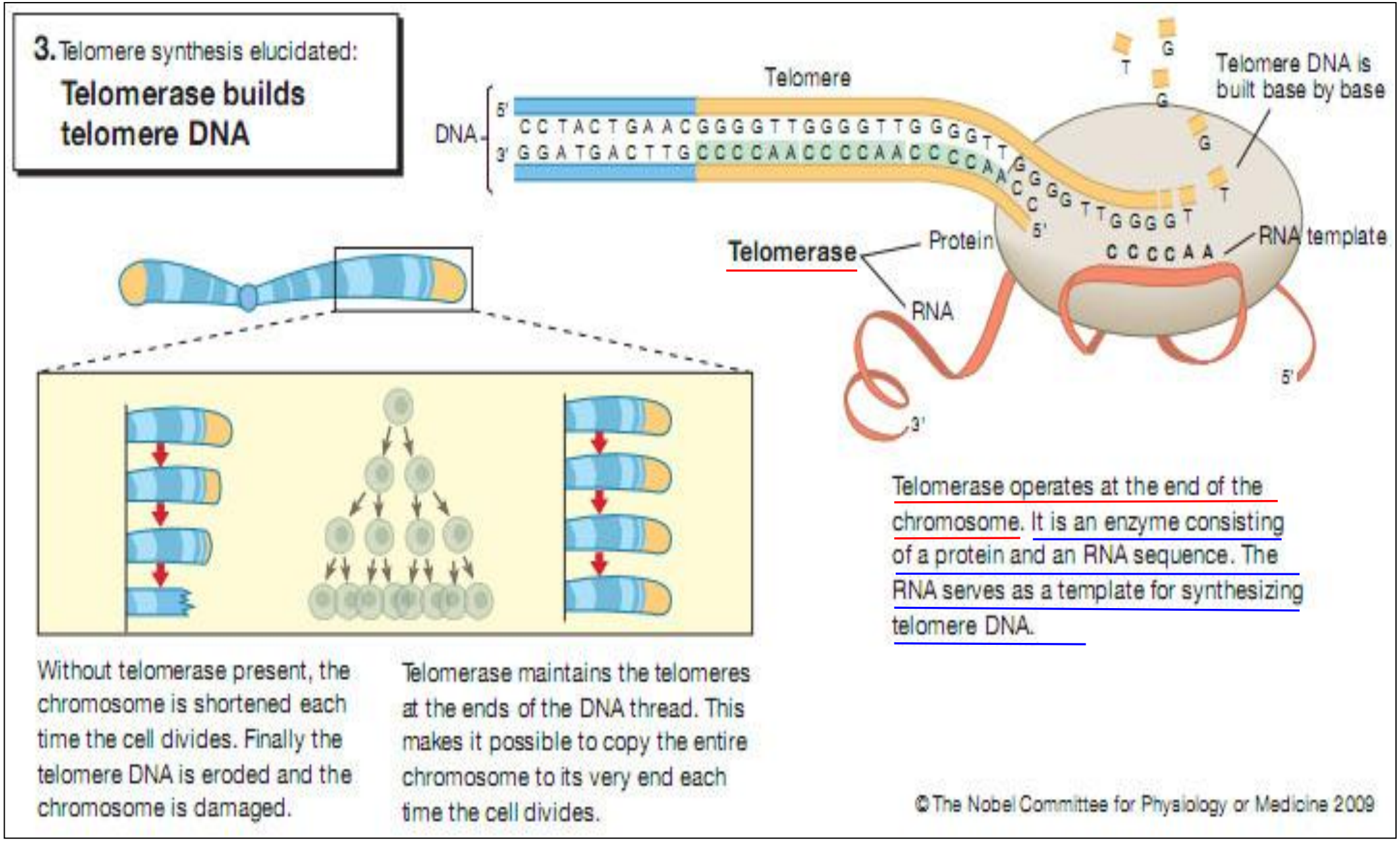


Experimental data：

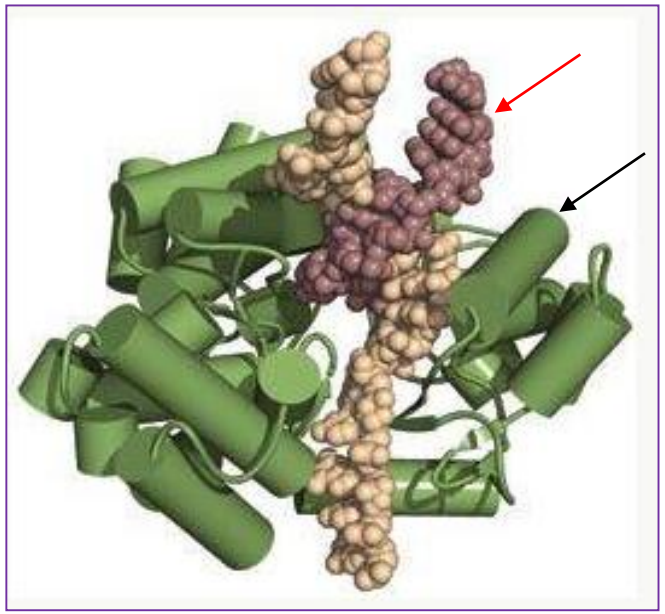
Telomere DNA is crucial for the integrity of chromosomes.



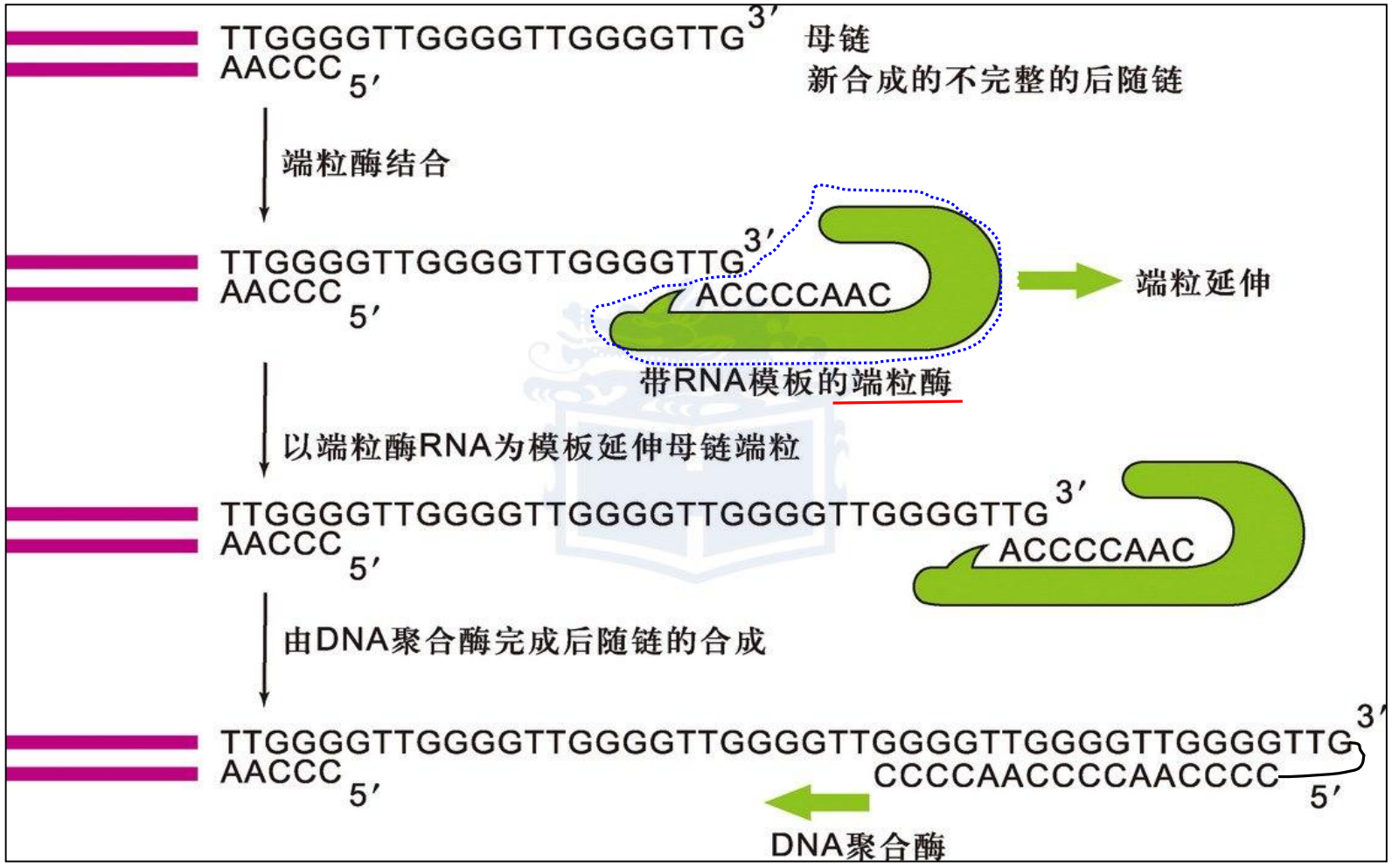
Telomerase（端粒酶）



☐ Telomerase & Telomere DNA replication



Three-dimension structure of telomerase



Special topic:

Telomere, telomerase and cellular aging, cancer cell

- Telomerase is found in germline cells, not in somatic cells.
- The telomere length of the adults is shorter than that of the youngers.
- About 90% of human tumors contain an active telomerase.
- Telomere shortening is thought to activate a suicide program.

So, telomere shortening plays a key role in protecting the body from cancer.

The Nobel Prize in Physiology or Medicine 2009

- Prize motivation: "for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase."

Elizabeth H. Blackburn
Born: 26 November 1948, Hobart, Tasmania, Australia
Affiliation at the time of the award: University of California, San Francisco, CA, USA
Prize share: 1/3

Carol W. Greider
Born: 15 April 1961, San Diego, CA, USA
Affiliation at the time of the award: Johns Hopkins University School of Medicine, Baltimore, MD, USA
Prize share: 1/3

Jack W. Szostak
Born: 9 November 1952, London, United Kingdom
Affiliation at the time of the award: Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA, Howard Hughes Medical Institute
Prize share: 1/3

III. Special chromosome:

(Giant chromosome)

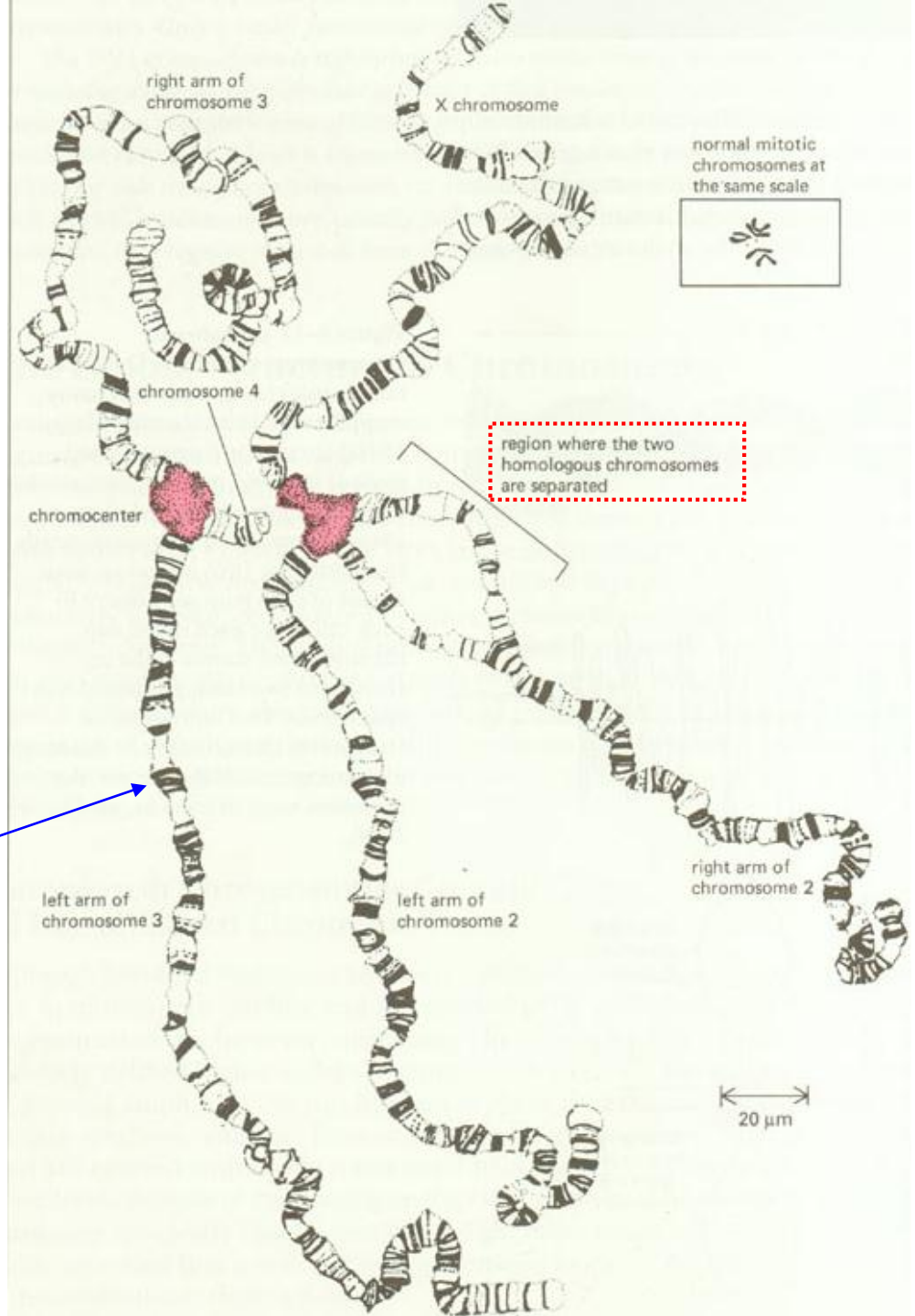
1. Polytene chromosome：

Salivary glands cells:

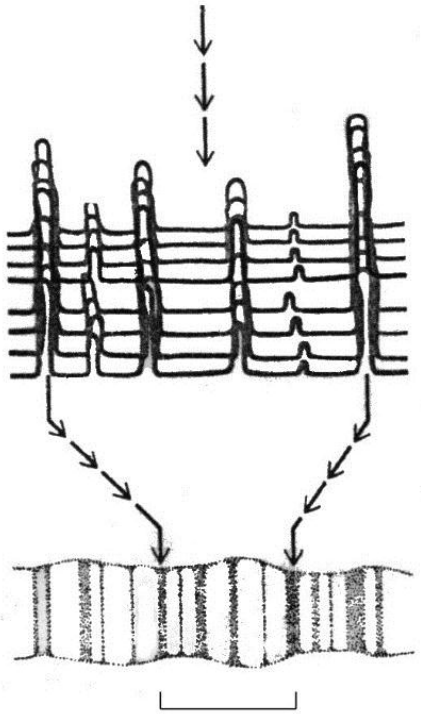
- Endomitosis & Homologous chromosome pairing

（核内有丝分裂10次 + 同源染色体配对）

2^{10} ＝ 1024条相同DNA

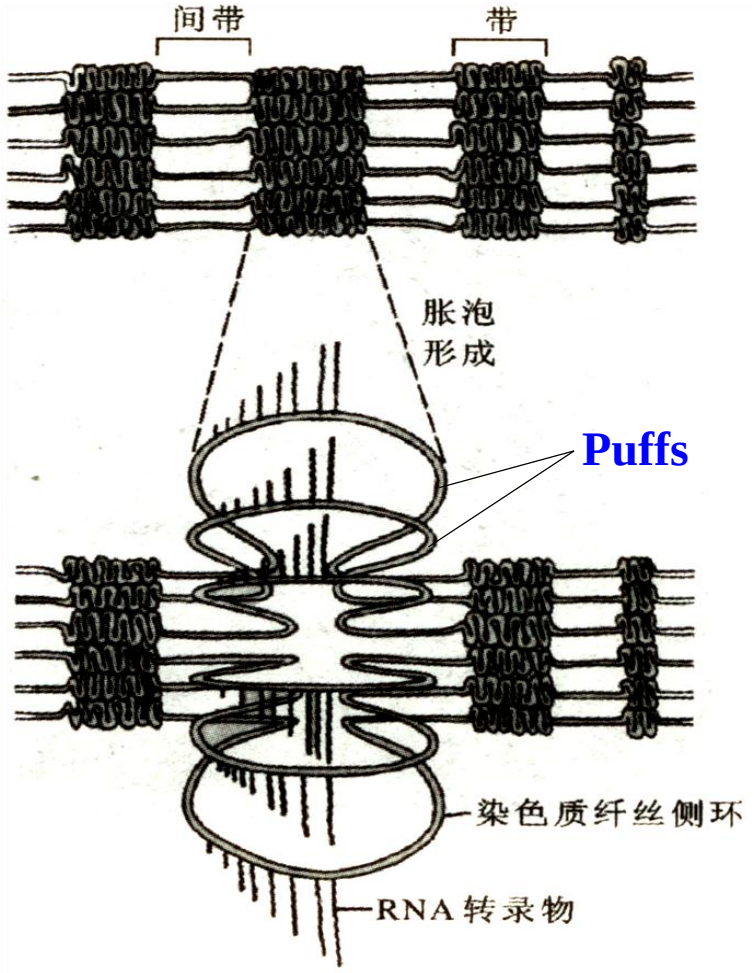


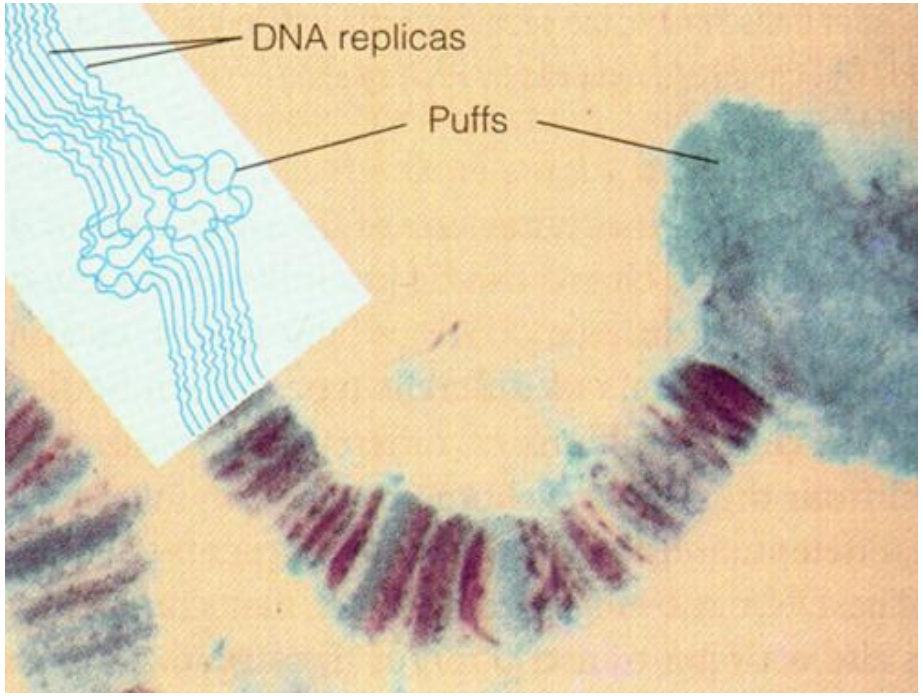
DNA 多次复制形
成多线染色体



- Bands, interbands and Puffs (Balbiani ring)
  (胀泡)

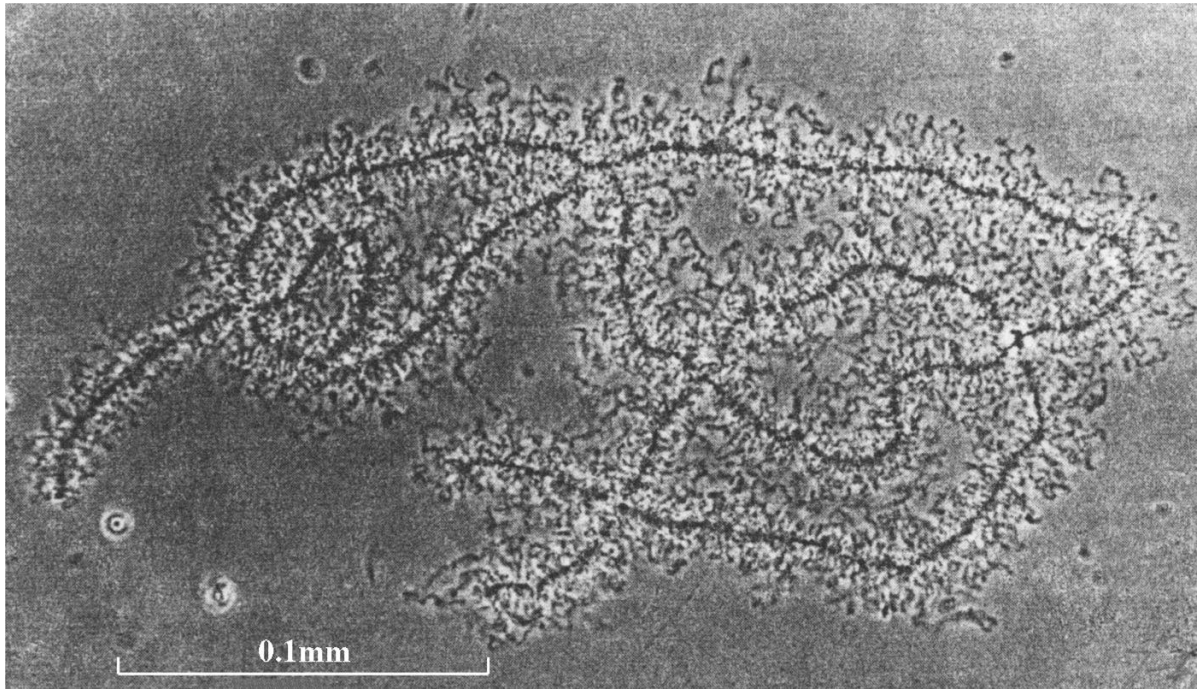
transcriptionally active chromatin.





2. Lampbrush chromosome:

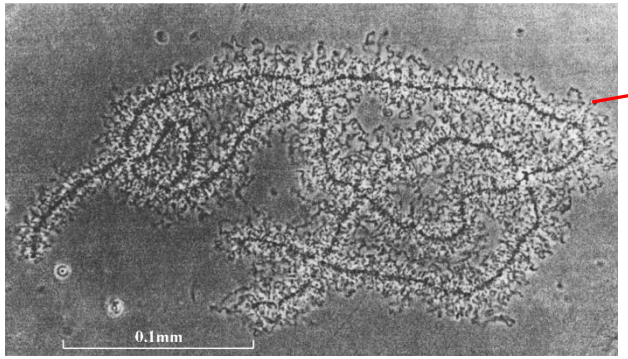
P280, 282

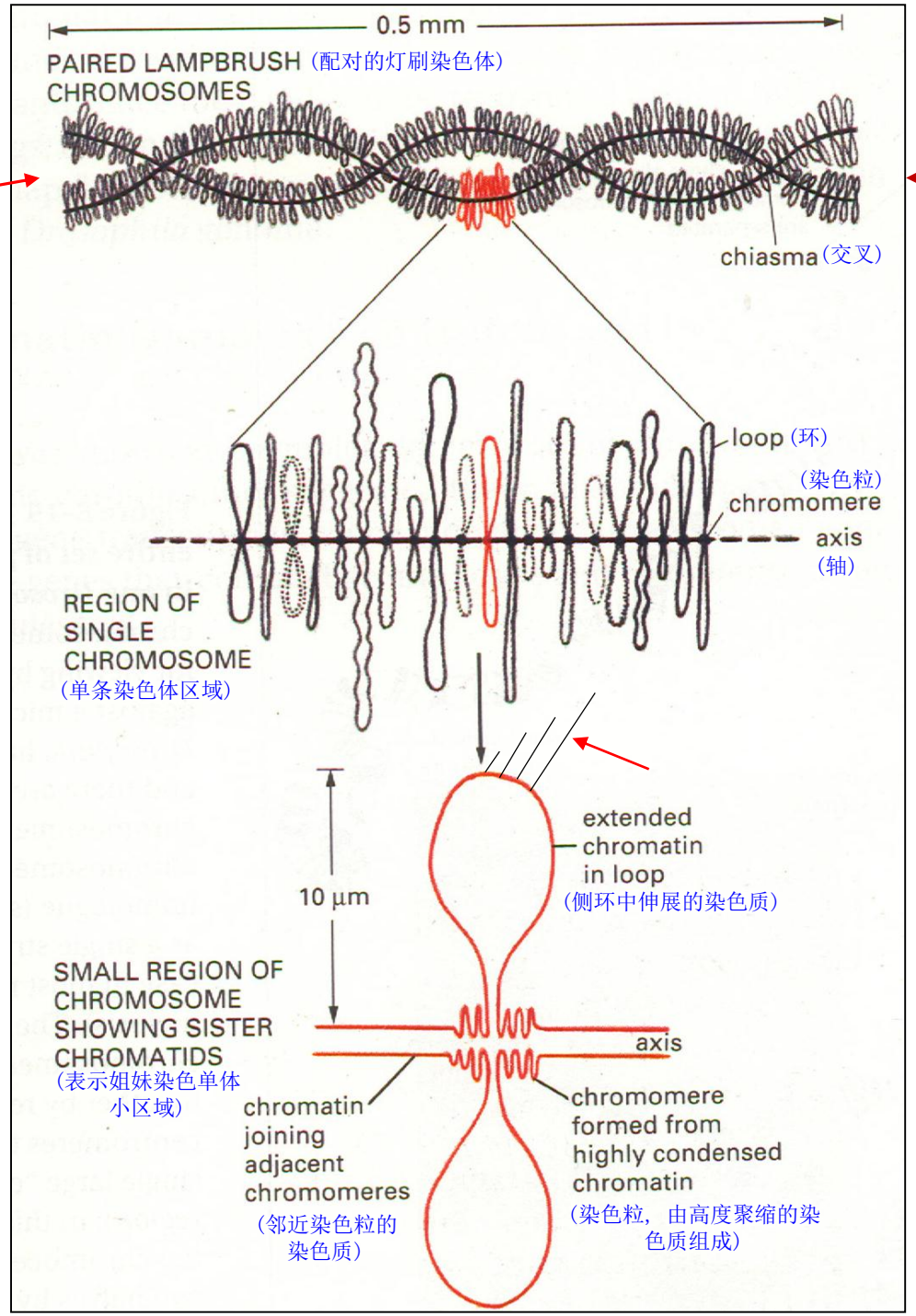


In growing amphibian oocyte

Stay in meiosis I (diplotene)

双线期





Structure

Functions:

Transcript into RNA，storage

IV. Karyotype

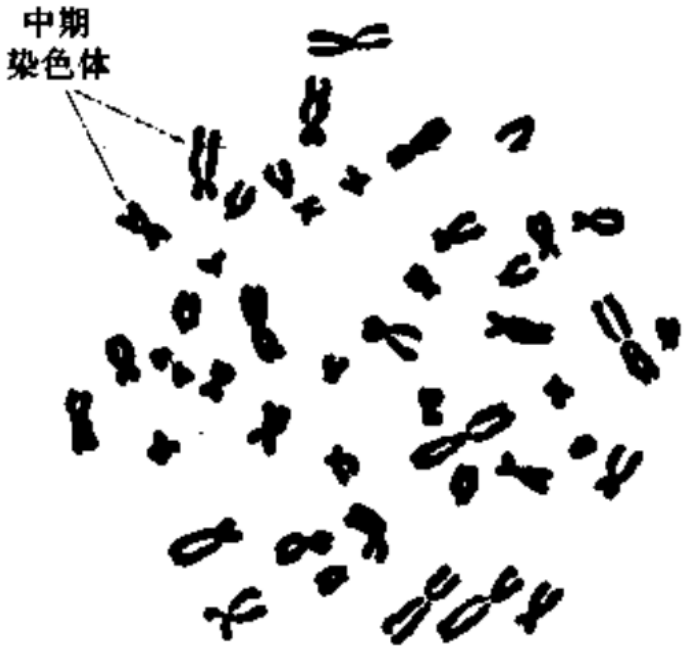
1. Three concepts

(1) Karyotype:

(核型, 即染色体组型)

■ 染色体组在分裂中期的表型, 包括Chr. 数目、大小、形态特征等。

■ 意义：杂种鉴定；相似性确定亲缘关系、遗传病等



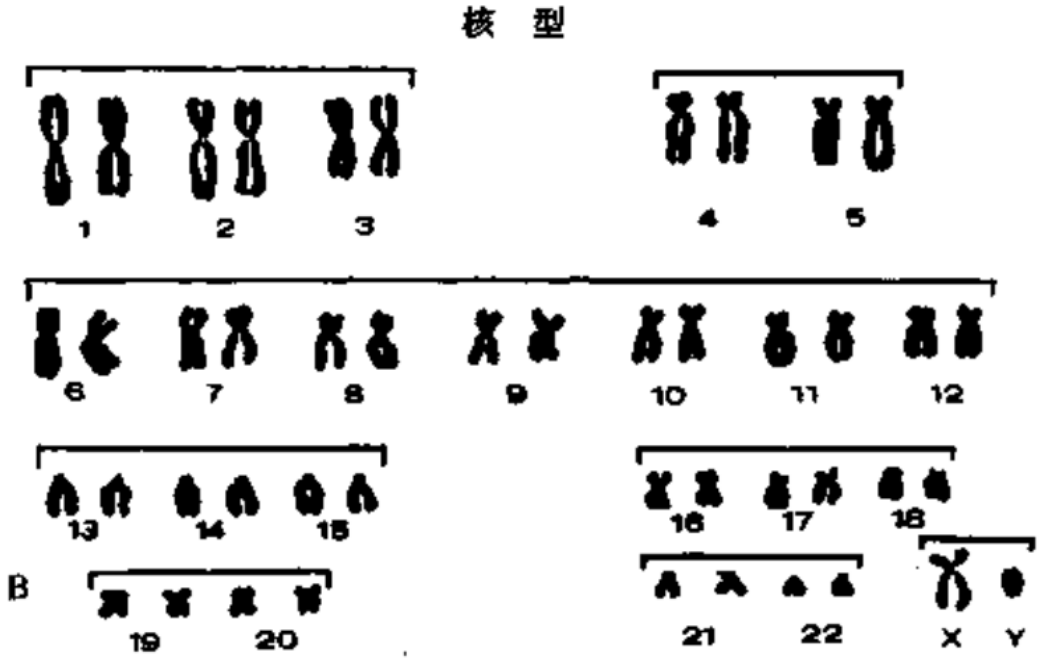
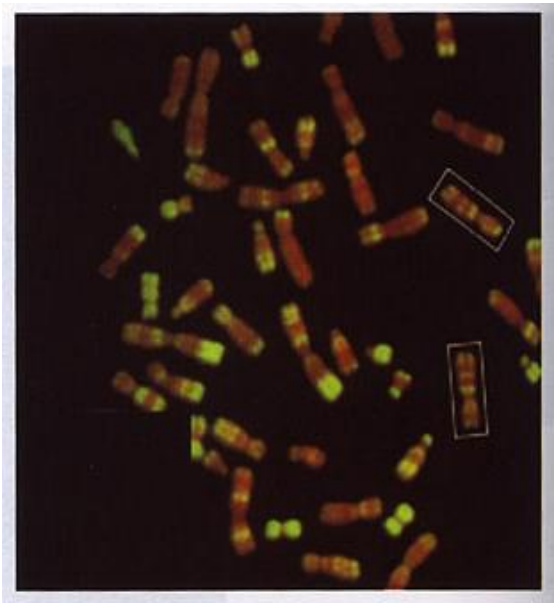


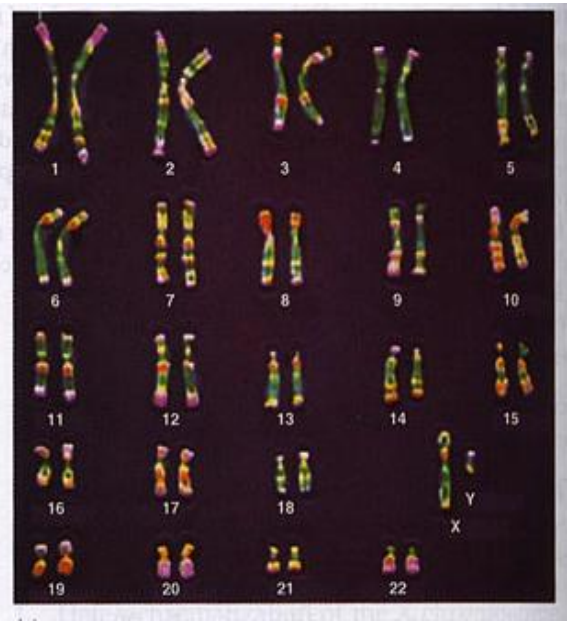
图 2－28　人体体细胞核型

A. 制备好的人体二倍体染色体组；B. 将染色体组图片经剪裁，依序排列成核型

(2) Karyotype analysis:

■ 获得每条染色体照片或图片后，在对染色体组所有染色体进行测量、计算的基础上，进行分组、排队、配对并进行形态分析的过程。

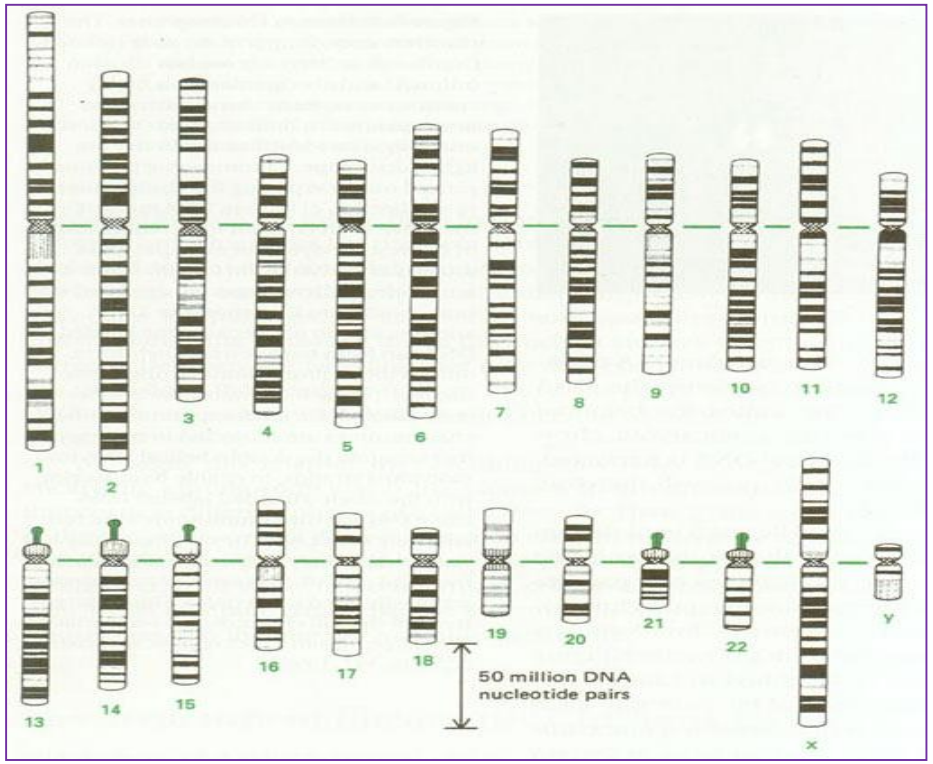




(3) Idiogram :

■ 将一个物种的染色体组逐个绘制出来，再按长短、形态等特征排列起来的图象，称为-。它可作为一个物种的标识。

■先有核型（染色体组）分析，后得到核型图、核型模式图。



2. Chromosomes preparation technique

Taking the preparation of human chromosomes as an example.

(1) Steps:

人外周血培养

↓

低　　渗

↓

固　　定

↓

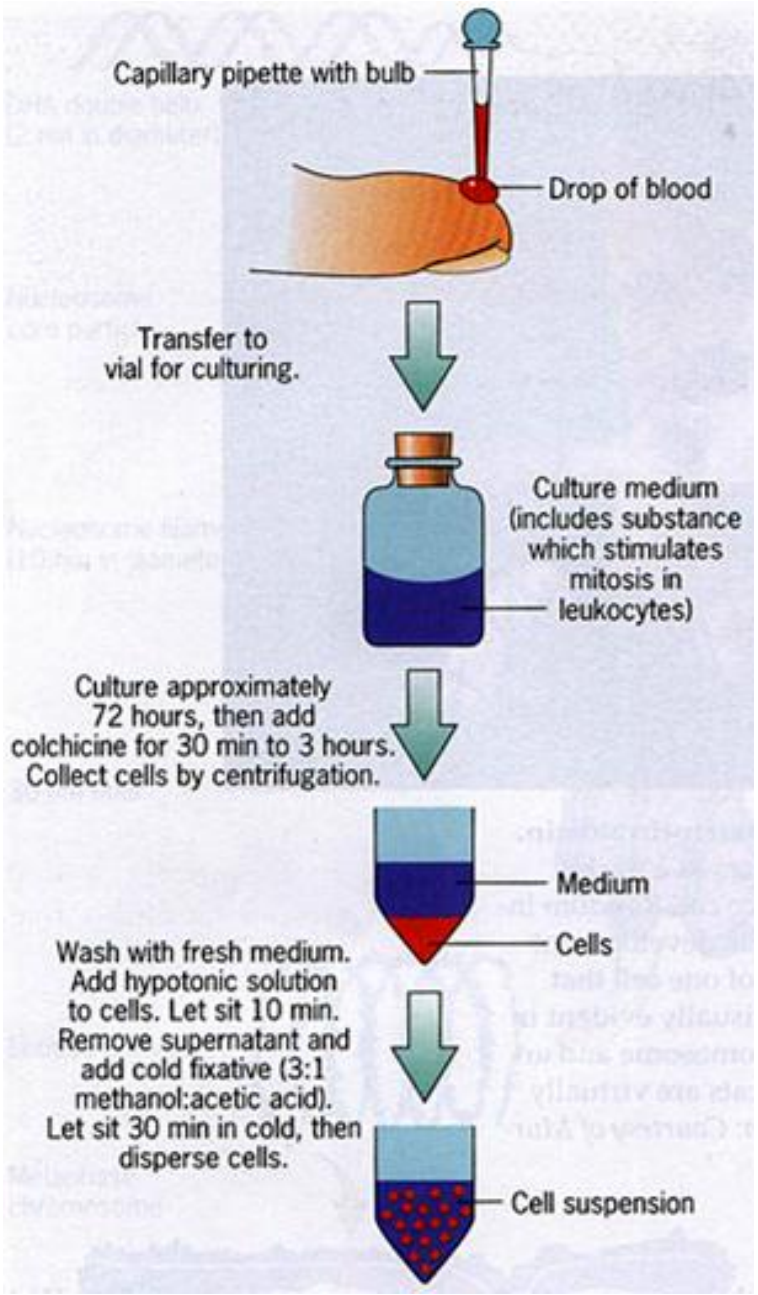
滴　　片

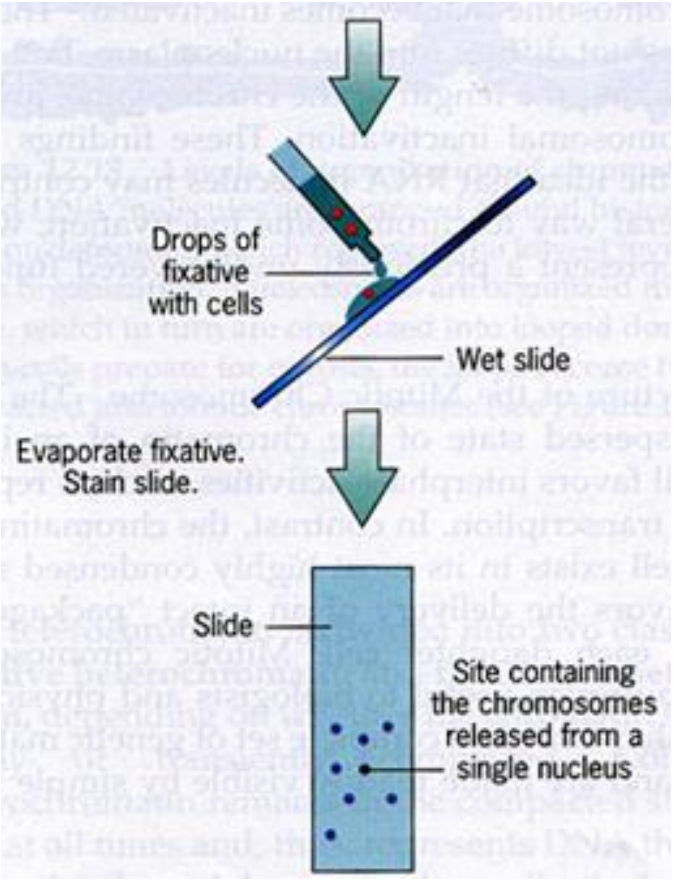
↓

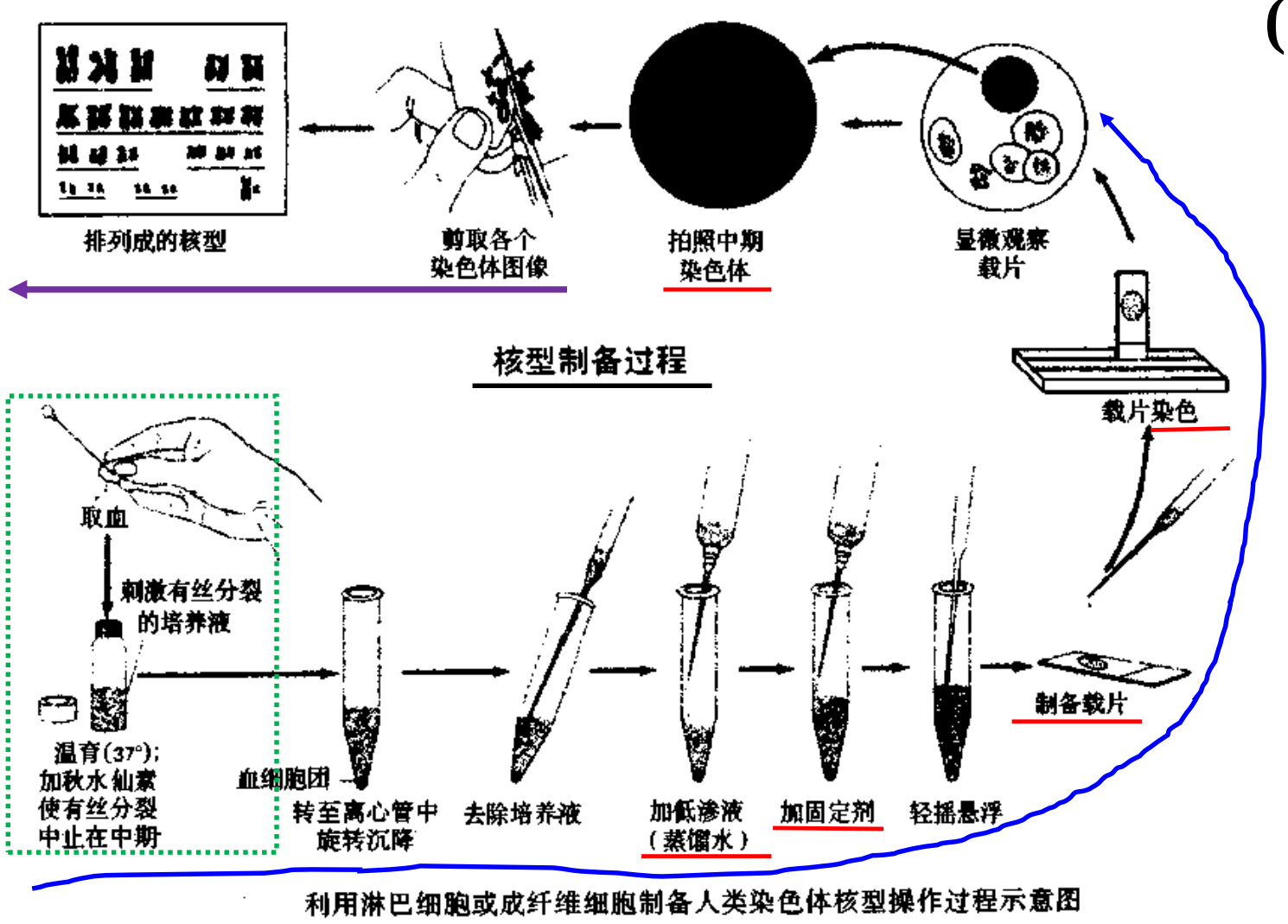
干燥与染色

↓

拍　　照







人外周血培养

(2) Involved three key techniques:

- 植物血凝素(PHA)的应用：刺激淋巴细胞分裂；
- 秋水仙素的应用： 富集中期细胞；
- 低渗技术： 细胞吸胀，便于破裂。

1952，徐道觉发明。

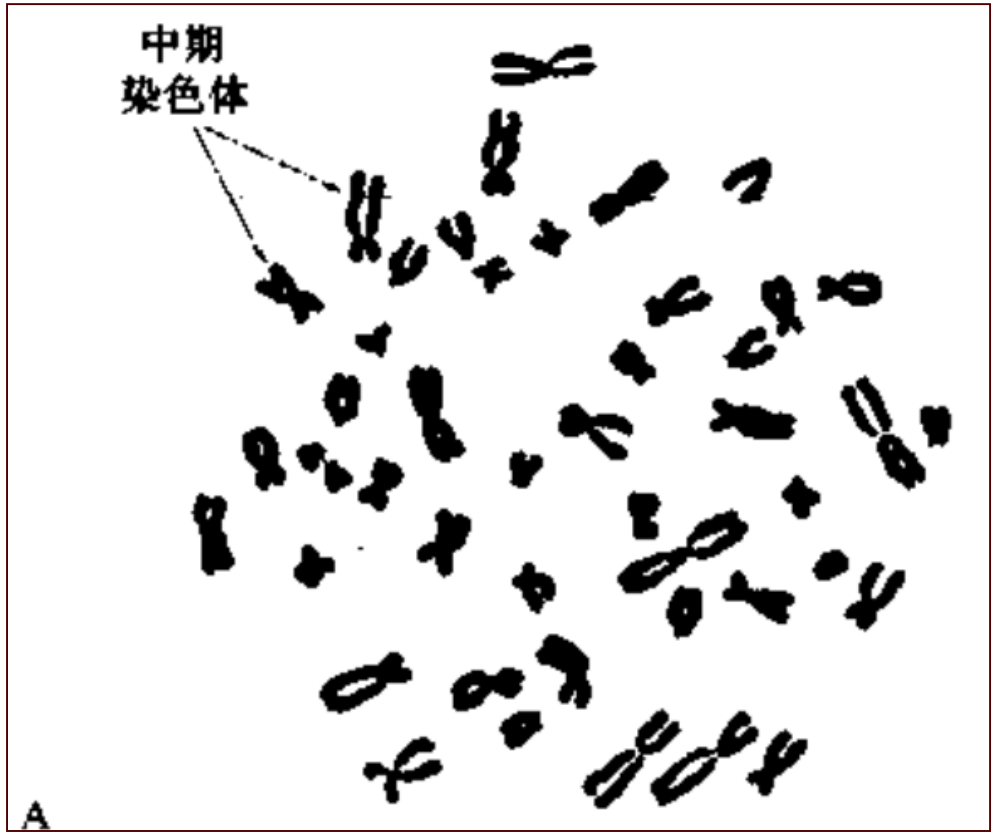
上述具体方法 (详见实验)。

Phytohemagglutinin (PHA)

hemacyte

agglutinin

Q：相同长度、臂比相同的染色体，如何确定其为同源染色体？



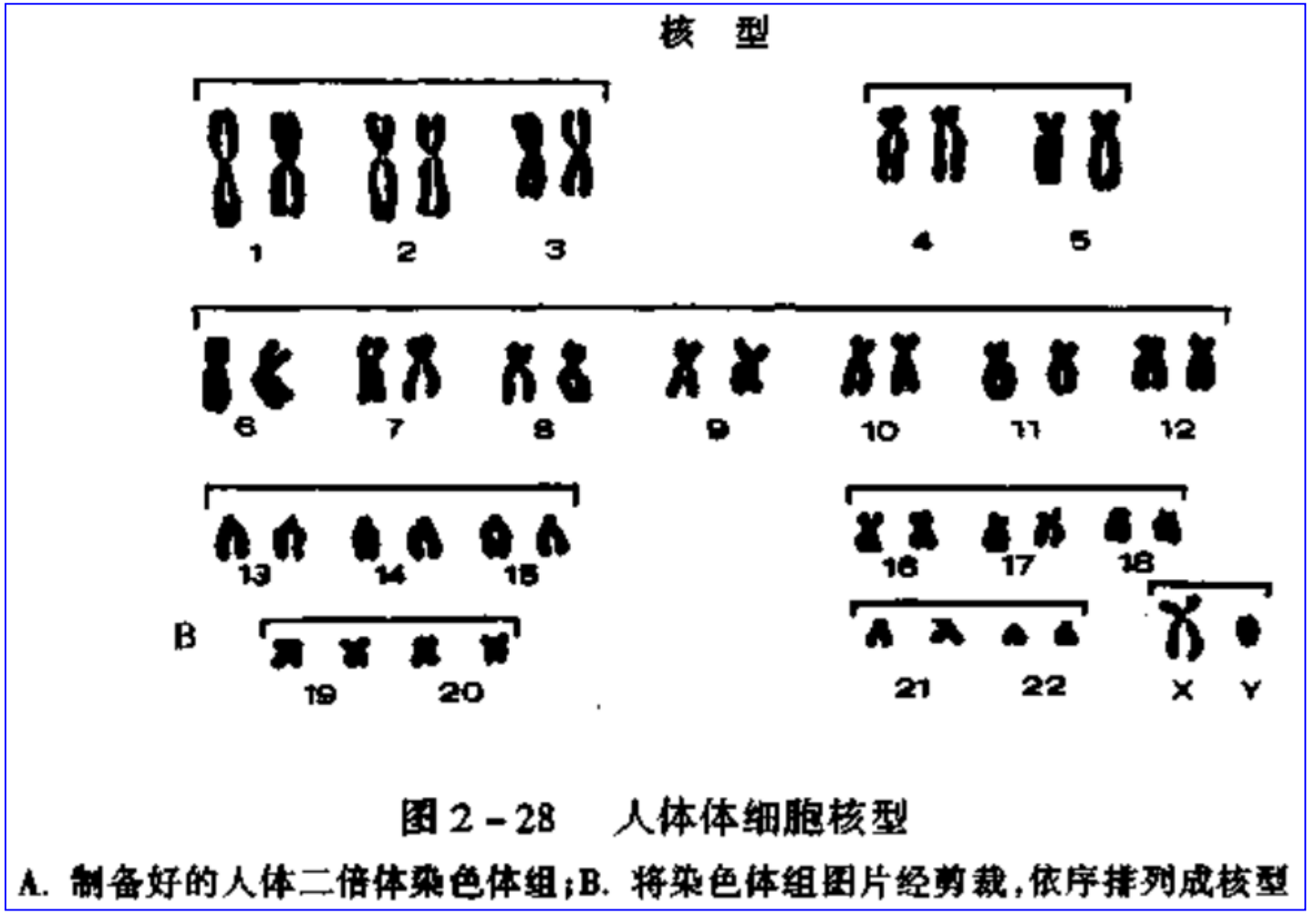


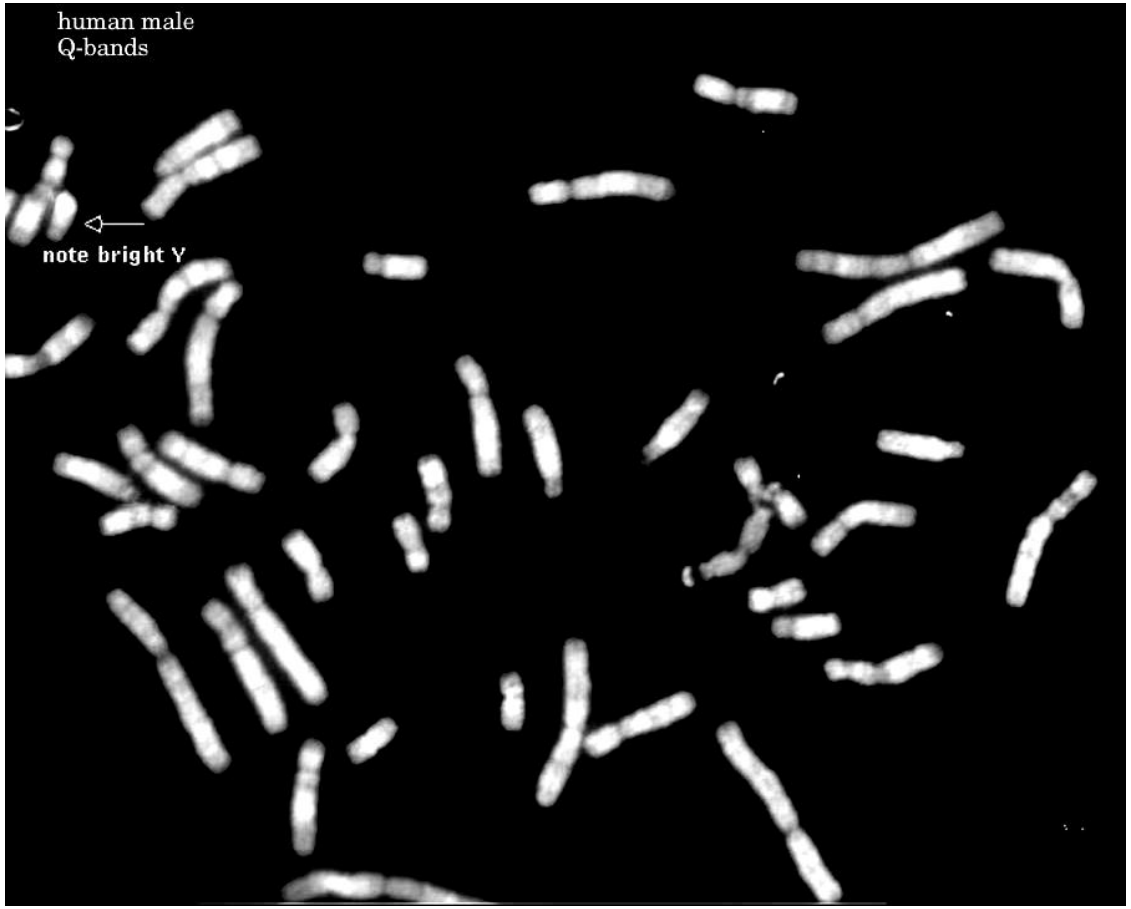
图 2－28　人体体细胞核型

A. 制备好的人体二倍体染色体组；B. 将染色体组图片经剪裁，依序排列成核型

3. chromosome banding technique:

- 利用一定的染色技术等处理分裂中期染色体，使其臂上呈现出一些特定的明暗相间的带纹。
- 染色体不同带型：见下

	带型	染料	显示染色体区域	处理方式
整条染色体显带：	Q带	喹吖因(Queyain）	AT亮带,GC暗带	
	G带	姬姆萨(Giemsa）	同上	胰酶或碱,热,尿素,去污剂 处理(使DNA暴露)
	R带	吉姆萨或吖啶橙	与Q、G 带相反	磷酸盐处理
局部染色体显带：	C带	Giemsa染色	着丝粒等结构型异染色质	酸或碱变性处理
	T带	吖啶橙	端粒带	
	N带	Ag-As染色	显示核仁组织区(NOR)	染NOR酸性蛋白质

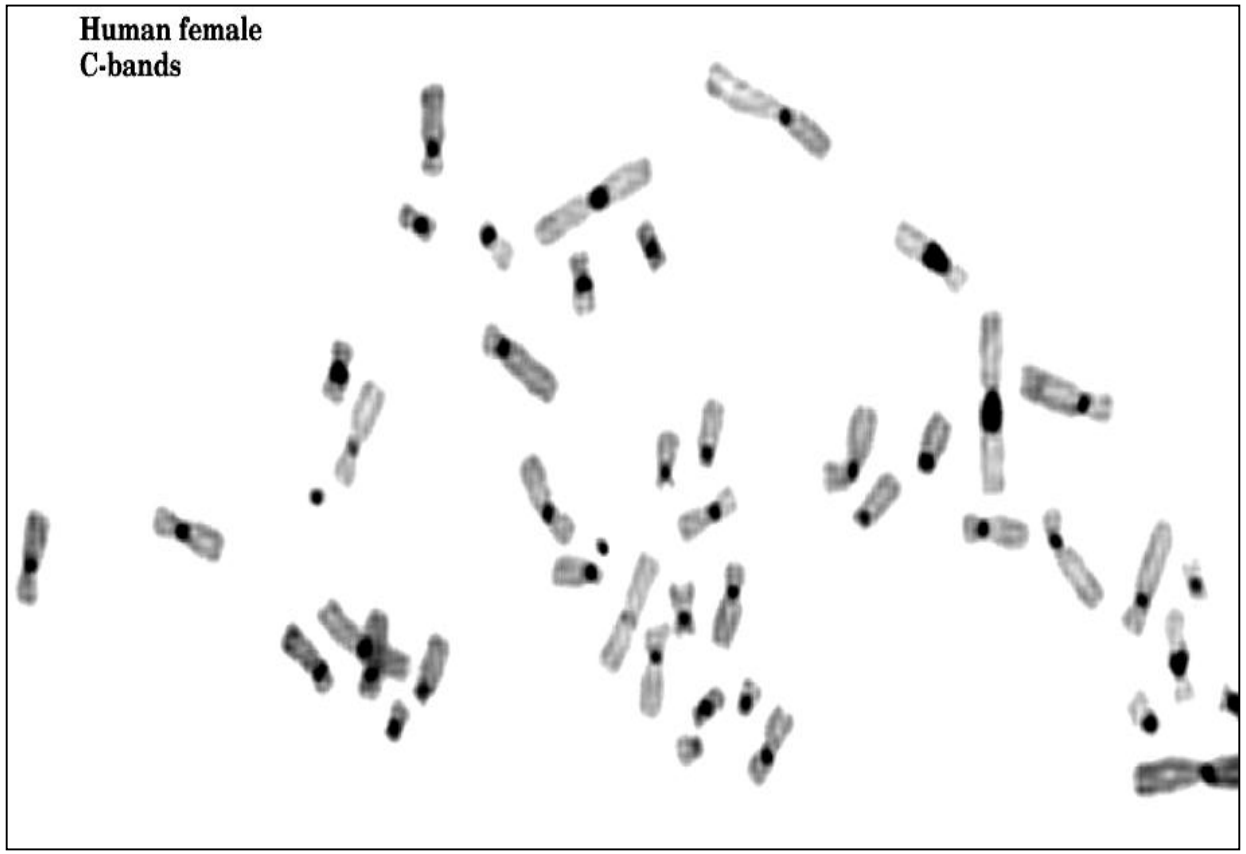


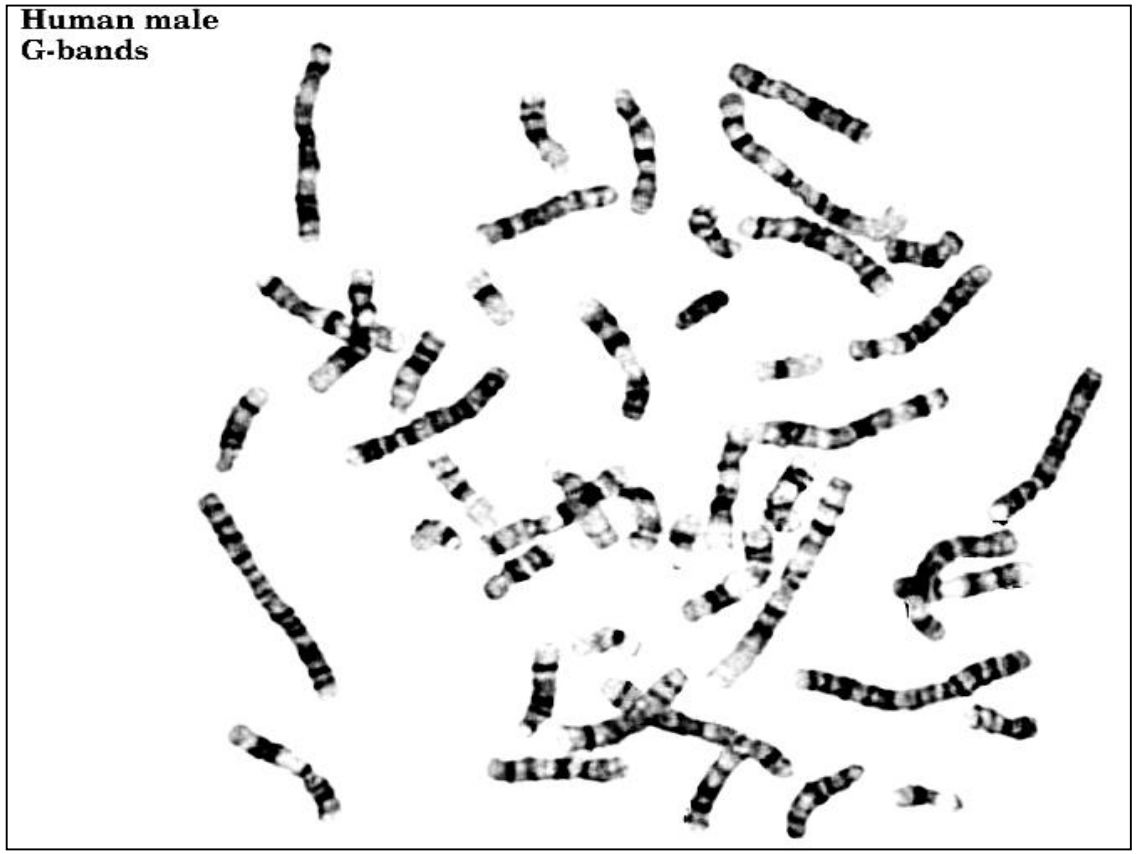
Q带　喹吖因(Queyain)　**AT亮带,GC暗带**

G带　姬姆萨(Giemsa）　同左

两类带型：

- 整条染色体显带：如Q、G和R带
- 局部 -----显带：如C、T和N带





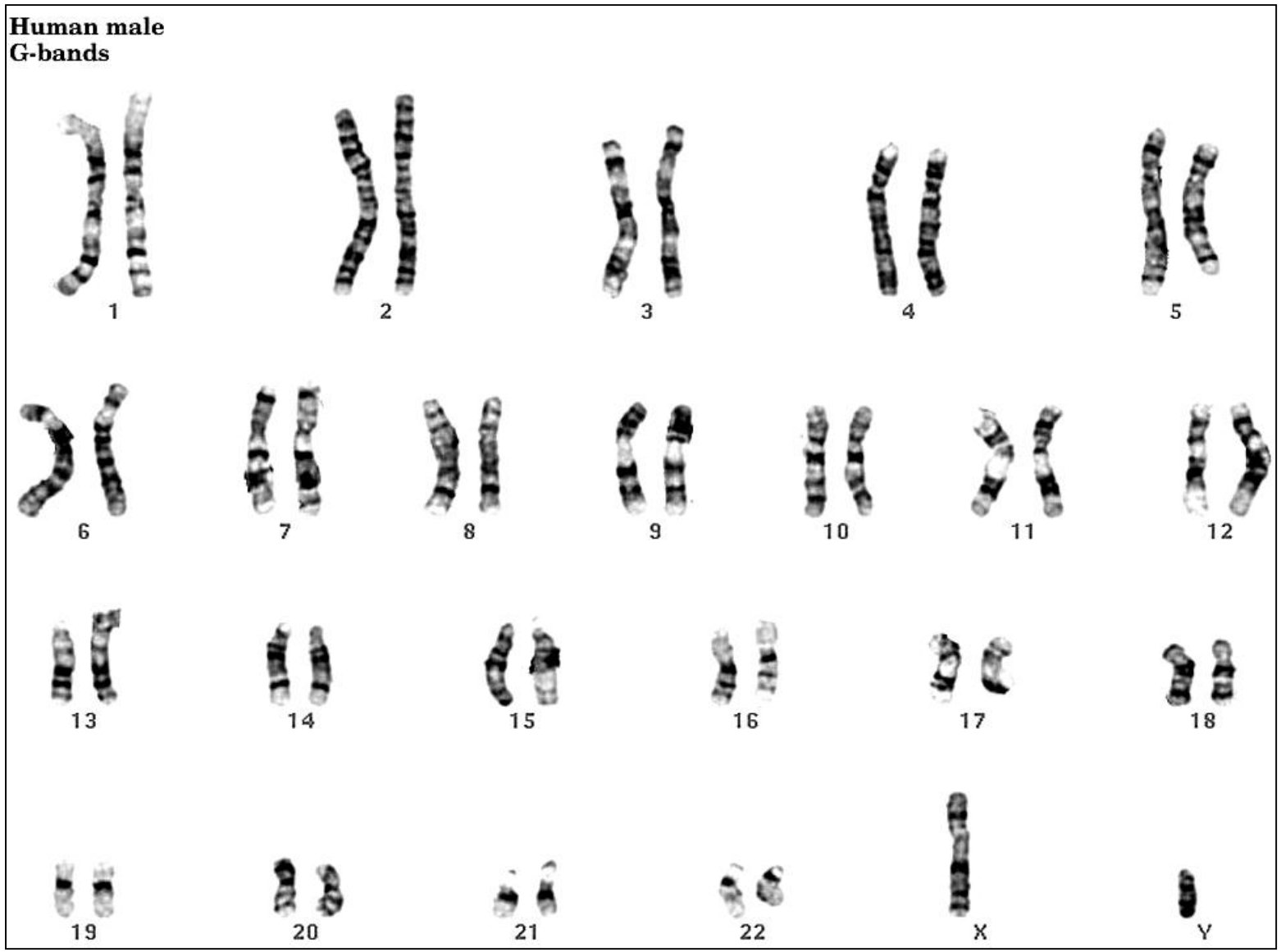
思考题

1. 染色体上DNA的压缩程度都一样?

2. 染色体上DNA都处于失活状态(不转录)?

有何证据？ P280, 282

☐ Chromosome banding facilitates Karyotype analysis



G带 姬姆萨(Giemsa）

Thank you!