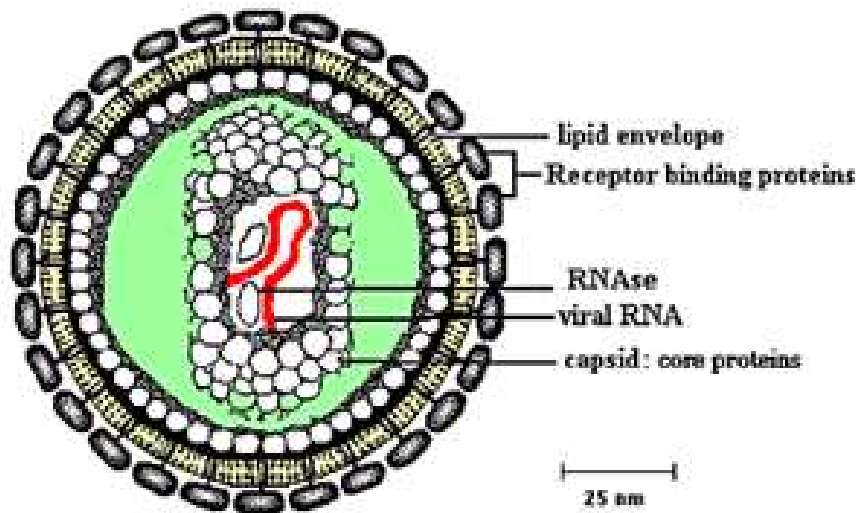


# Part V Molecular biology of tumors

## Chapter 12

### Tumor viruses and Oncogenes



- Tumor, tumor viruses and oncogenes
- Tumor suppressor genes
- Apoptosis



# 1. Tumor, tumor viruses and oncogenes

## 1.1 Definition and types of tumor

- **Tumor** is a newgrowth formed by clonal **abnormal proliferation** from a certain local tissue cell **losing its normal regulation of growth** at the genetic level.

**肿瘤**是局部组织的某一个细胞在基因水平上**失去对其生长的正常调控**，导致其**克隆性异常增生**而形成的**新生物**。

## 良性肿瘤

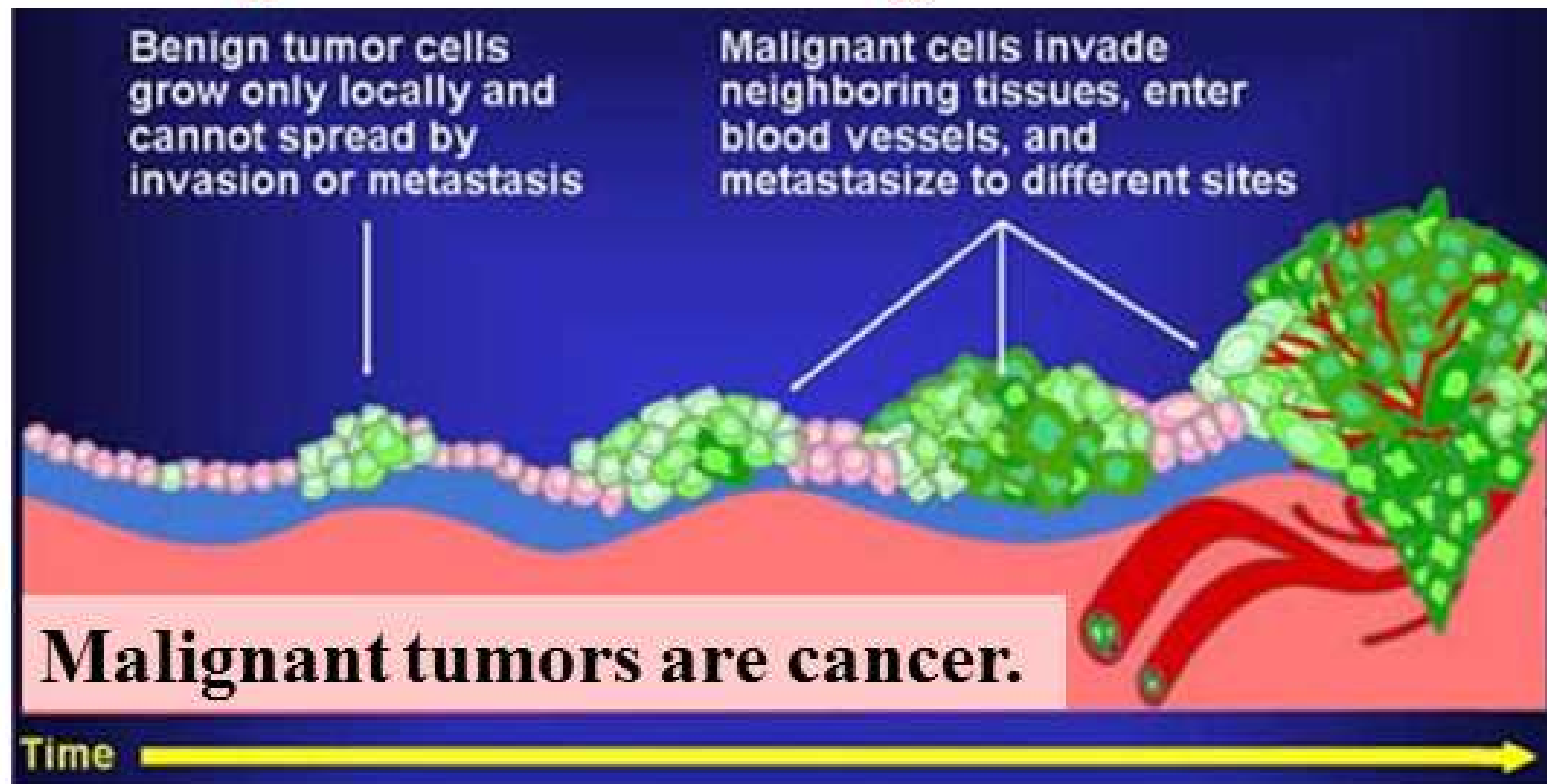
### Benign tumor

Benign tumor cells grow only locally and cannot spread by invasion or metastasis

## 恶性肿瘤

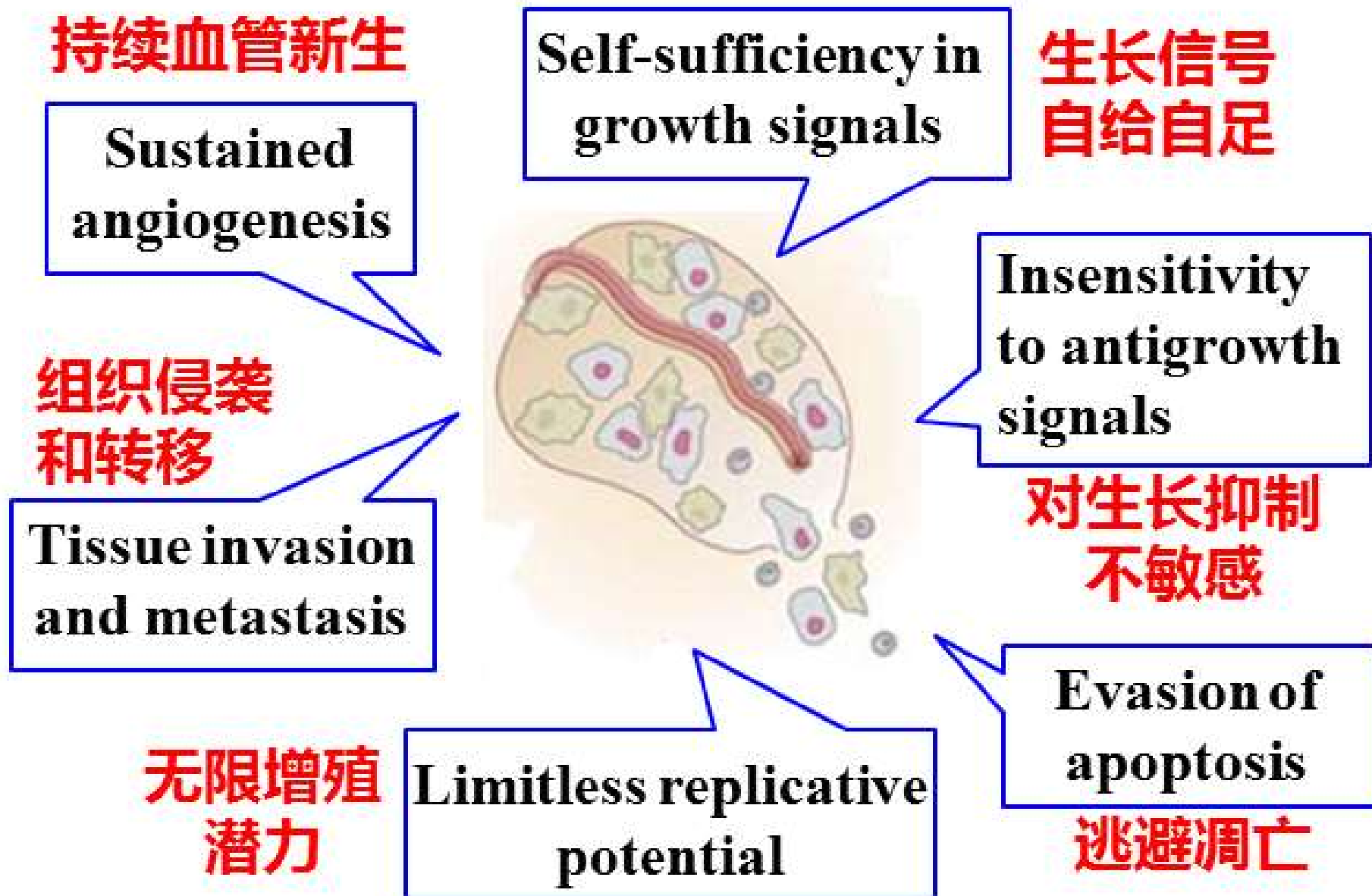
### Malignant tumor

Malignant cells invade neighboring tissues, enter blood vessels, and metastasize to different sites

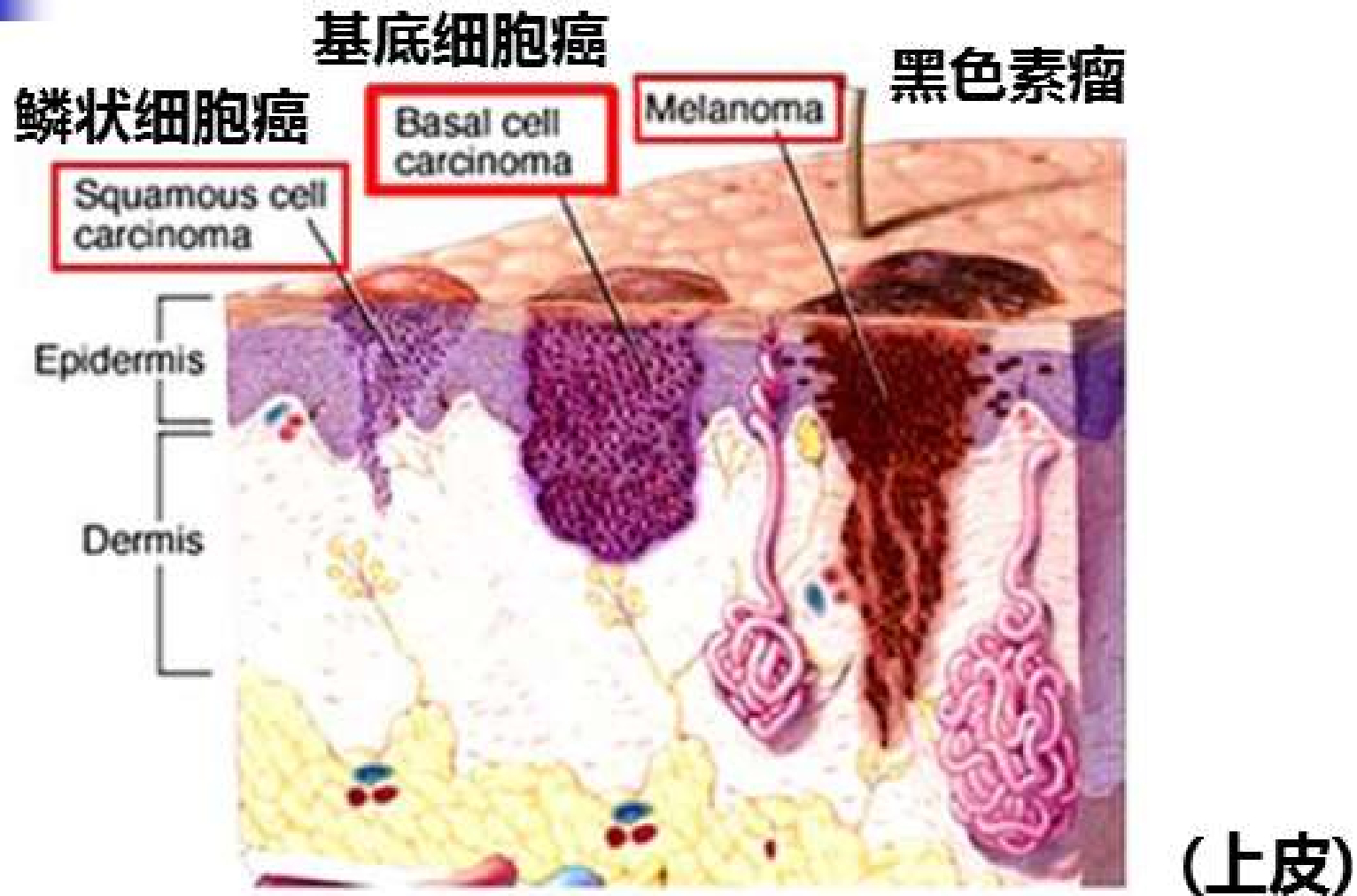


**Cancer** is a disease in which abnormal cells divide without control and are **able to invade other tissues**. **癌症**是失去分裂控制的异常细胞**侵入其他组织**的一种疾病。

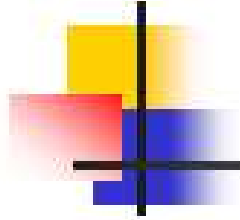
## 1.2 Basic characteristics of malignant tumor



## 1.3 Some major types of cancer

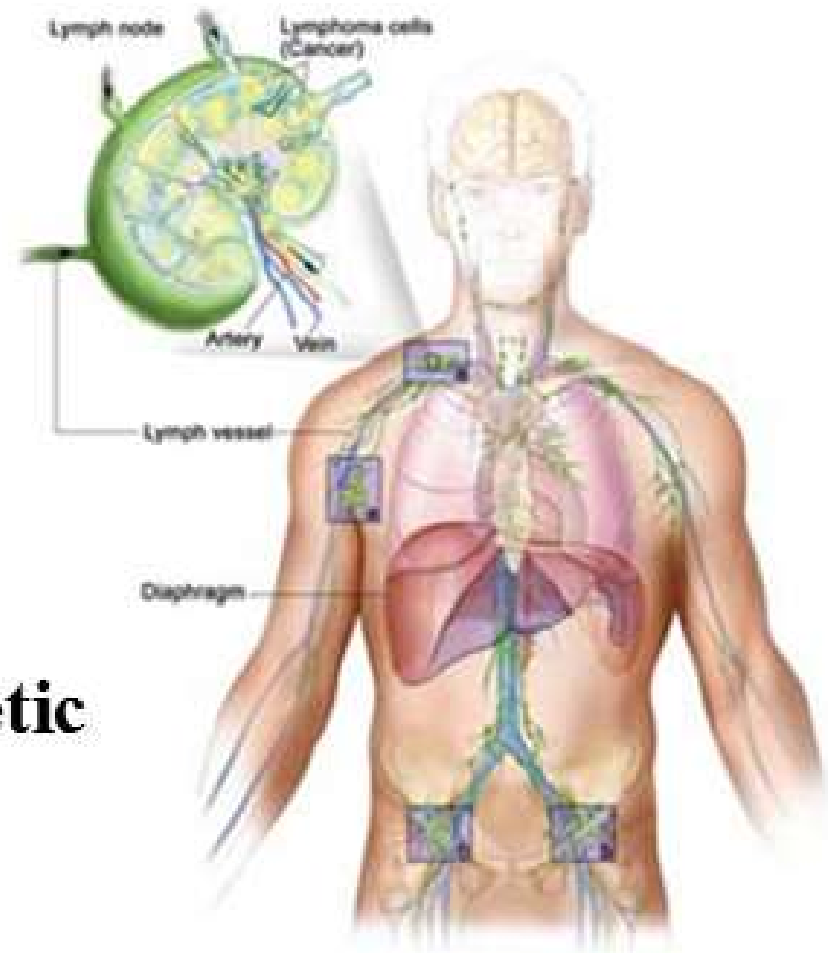


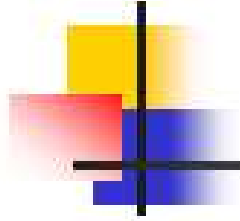
**(1) Carcinoma (癌): cancer arising from epithelium**



**(2) Lymphoma and myeloma (淋巴瘤和骨髓瘤): cancer of immune system**

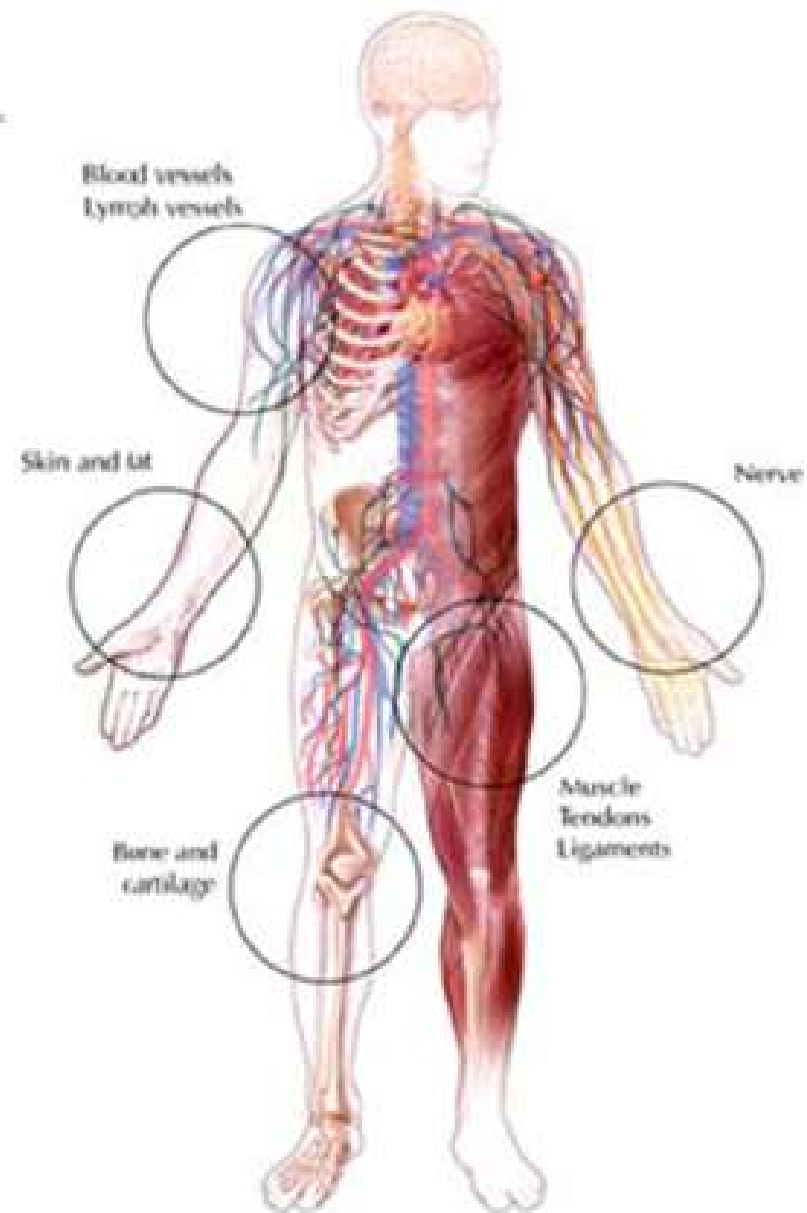
**(3) Leukemia (白血病): cancer of hematopoietic (造血的) cells**



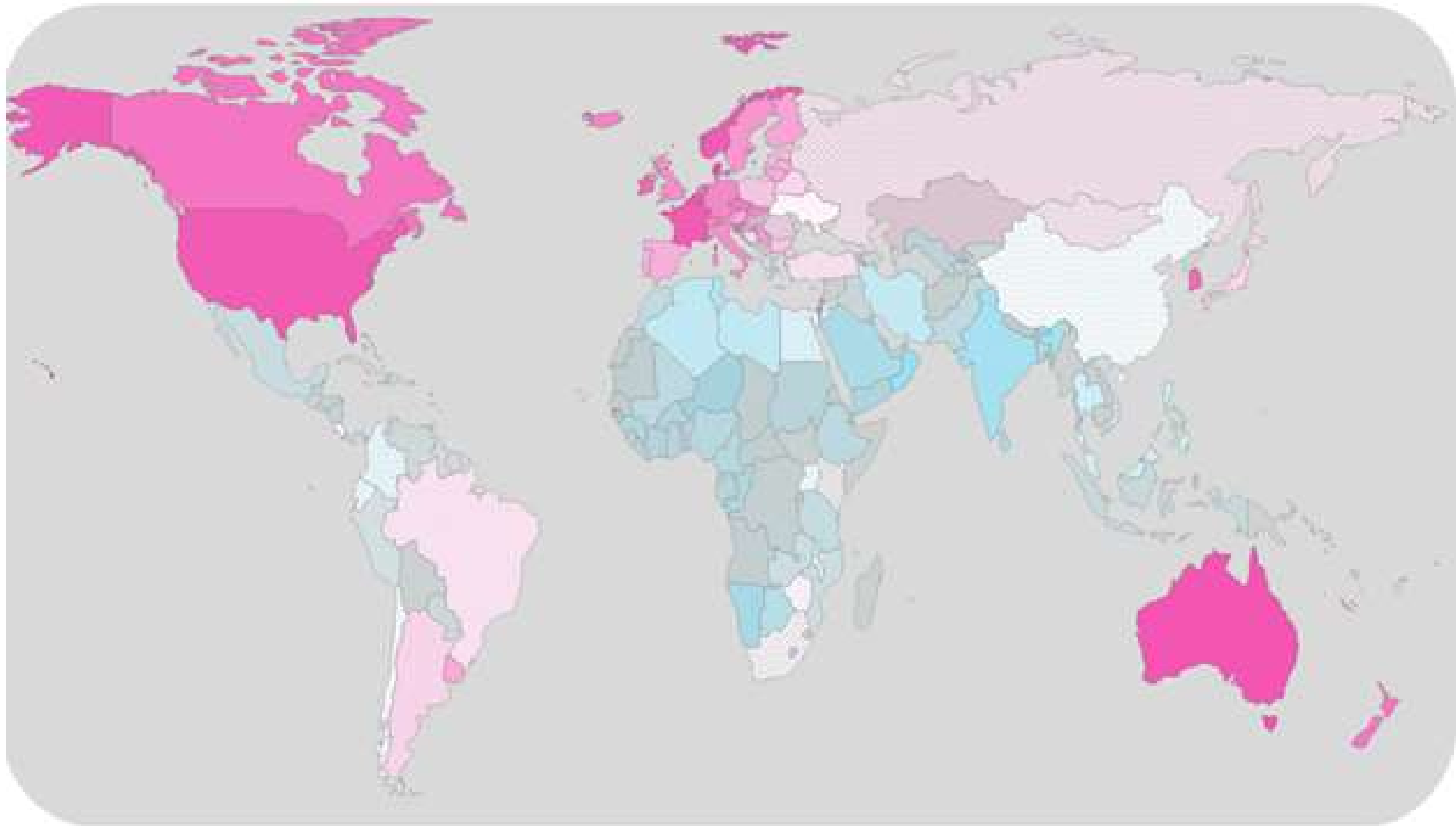


**(4) Sarcoma(肉瘤):**  
**cancer arising from**  
**connective tissue (结**  
**缔组织), bone and**  
**muscle.**

**(5) Glioma (神经胶质**  
**瘤): cancer of brain**  
**glial cells**



## Cancer incidence — Worldwide



<http://www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer/incidence#heading-Zero>



## 1.4 Tumor viruses and oncogenes

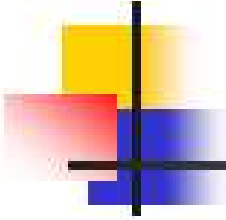
### 1.4.1 Tumor viruses

1910年，P. Rous就发现含有肉瘤病毒（一种反转录病毒）的鸡肉瘤无细胞滤液能在鸡体内诱发新的肉瘤，提出**病毒致癌学说**，于**1966年**获得了**诺贝尔医学和生理学奖**。

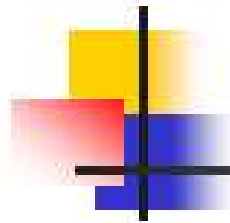


劳斯  
Peyton Rous

**Discovery of tumor-inducing viruses**



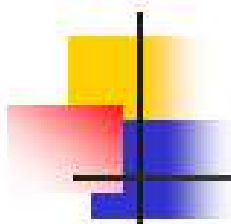
- **Tumor viruses** are animal viruses that enable the sensitive host to **produce tumor** or **transfer** normal culture cells to the cancer cells.
- Tumor viruses can be classified in two broad categories depending on the nucleic acid in the viral genome.
  - { RNA tumor viruses      **retroviruses**
  - { DNA tumor viruses



## 1.4.2 Oncogenes

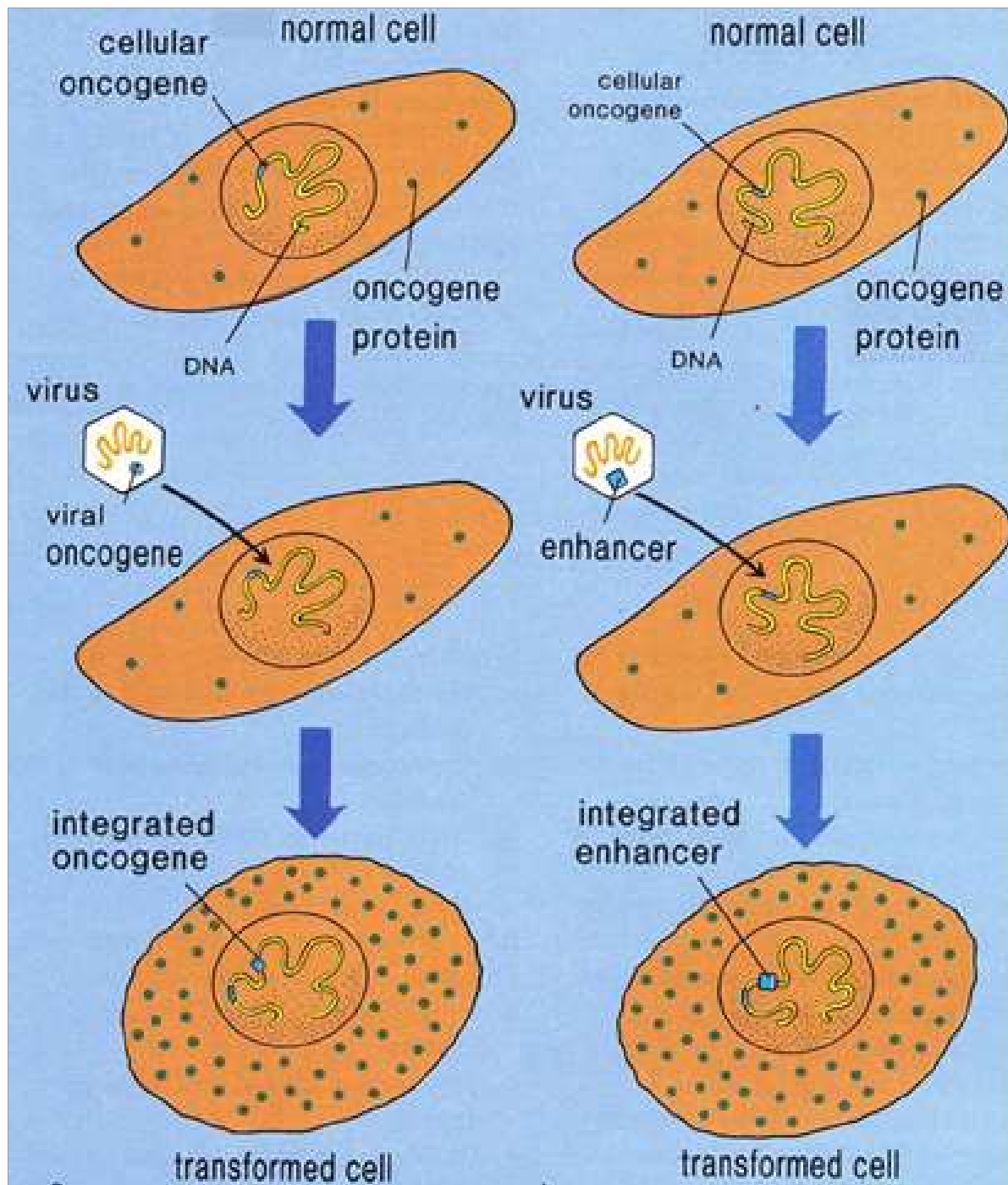
**Oncogenes** are genes whose expression causes cells to become cancerous (癌化).

- **Viral oncogenes (*v-onc*, 病毒癌基因)** are oncogenes carried by tumor viruses.
- **Cellular oncogens (*c-onc*, 细胞癌基因, 细胞转化基因)** are oncogenes in the cells.
  - A **proto-oncogene (*p-onc*, 原癌基因)** is a **normal gene** that can become an oncogene due to mutations or increased expression.



### 1.4.3 The relationship between retroviruses and oncogenes

- (1) 癌基因最先是在反转录病毒中发现的。
- (2) 每一种病毒癌基因，在动物细胞基因组中都有同源序列（即细胞癌基因）。
- (3) 细胞癌基因并非来自病毒，而是来自动物细胞（**病毒癌基因来源于细胞癌基因**）。
- (4) （原）癌基因整合到病毒中以后转变为病毒癌基因。
- (5) **反转病毒普遍带有癌基因**，反转病毒感染宿主后能使宿主产生肿瘤。

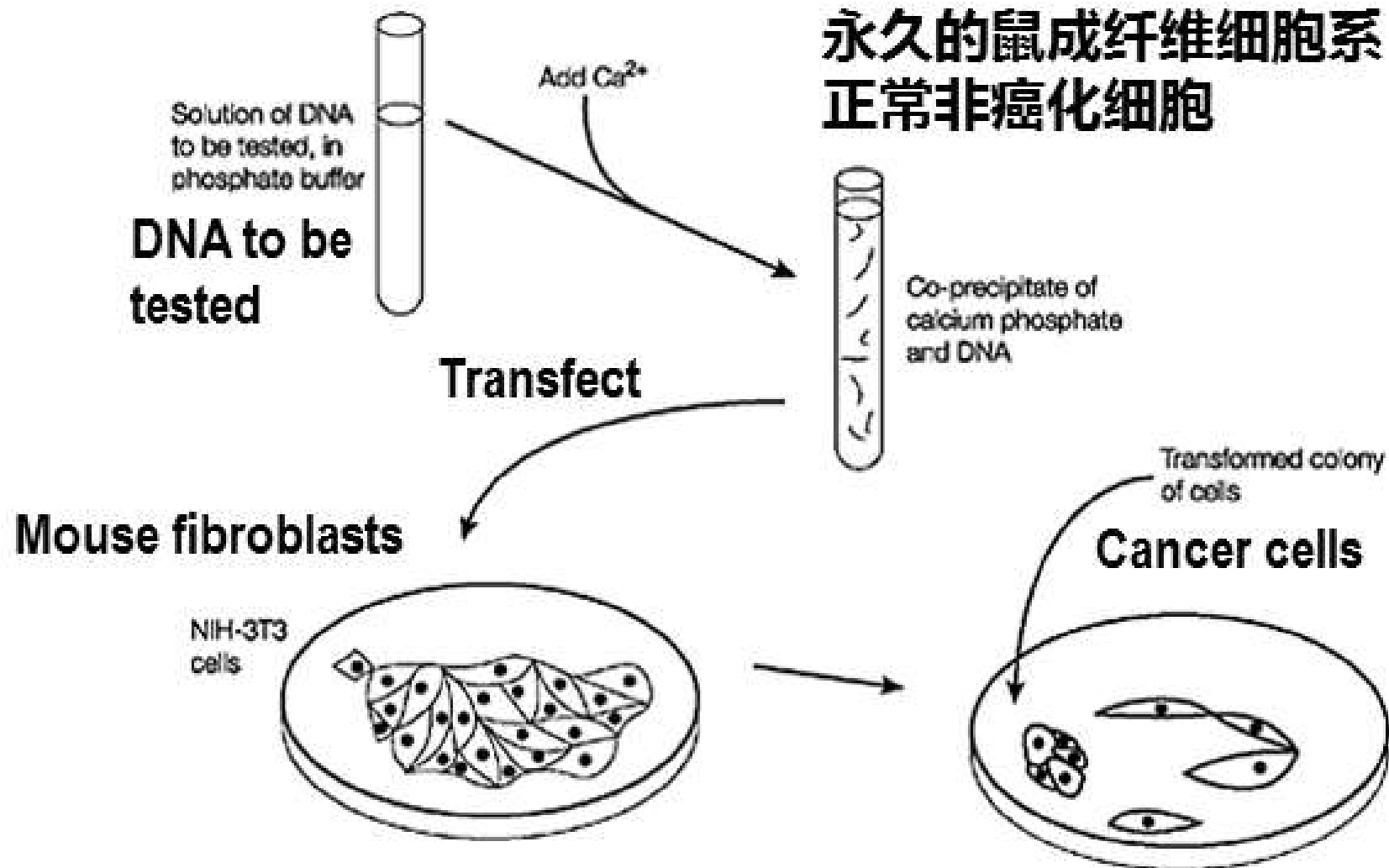


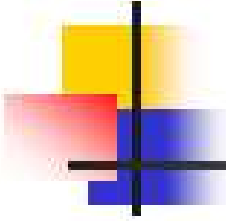
①急性反转录病毒通过转导高活性的**病毒癌基因**到宿主基因组中，使宿主细胞转化；

②慢性反转录病毒通过整合到宿主正常原癌基因附近，使其被病毒中的**强启动子或增强子**所激活，原癌基因转变为癌基因。

## 1.5 Isolation of oncogenes

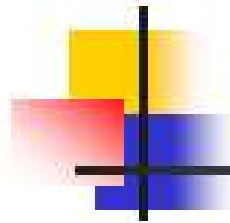
### DNA 转染 (transfect) NIH-3T3 细胞





## Advantages of NIH-3T3 assay

- Suitable for **screening** large numbers of samples;
- Much more **quickly and simple** than that *in vivo* (体内) tests;
- The NIH-3T3 cells are good at taking up and expressing foreign DNA;



## Disadvantages of NIH-3T3 assay

- Some oncogenes may be specific for particular cell types;
- Large genes may be missed because they are less likely to be transfected intact (完整的);
- The NIH-3T3 cells are **not "normal" cells** since they are a permanent cell line;
- The assay depends upon the transfected gene acting in a genetically dominant (显性) manner and so will **not detect many tumor suppressor genes.**



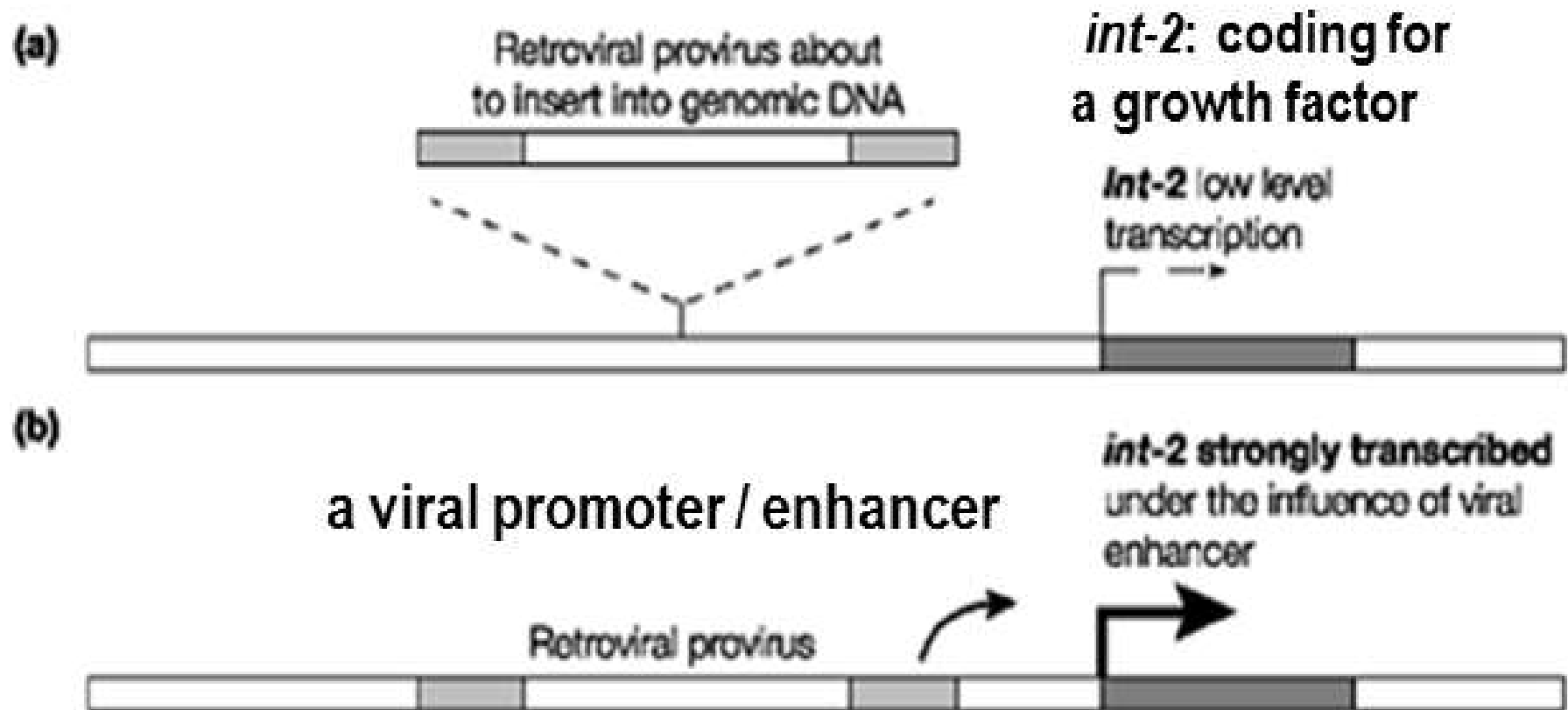
# Nude mouse (裸鼠)



## 1.6 Difference between *onc* and *p-onc*

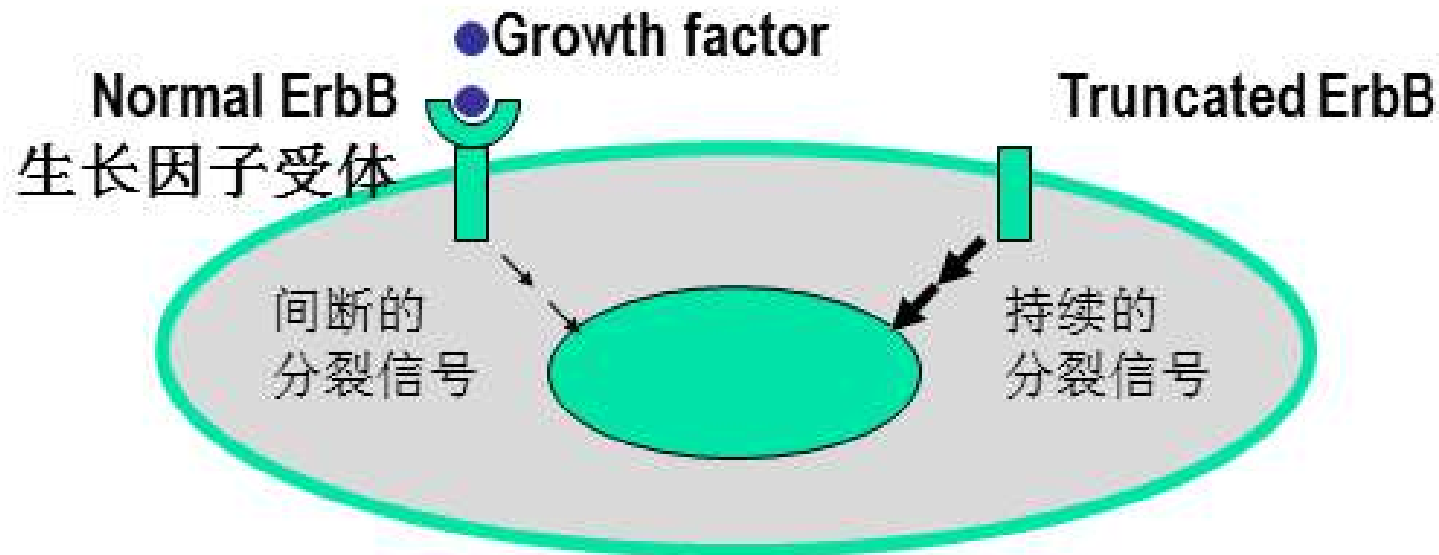
### 1.6.1 Quantitative difference (量的区别)

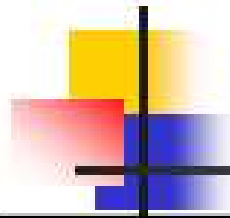
- **Overproduction** of a normal gene product



## 1.6.2 Qualitative differences (质的区别)

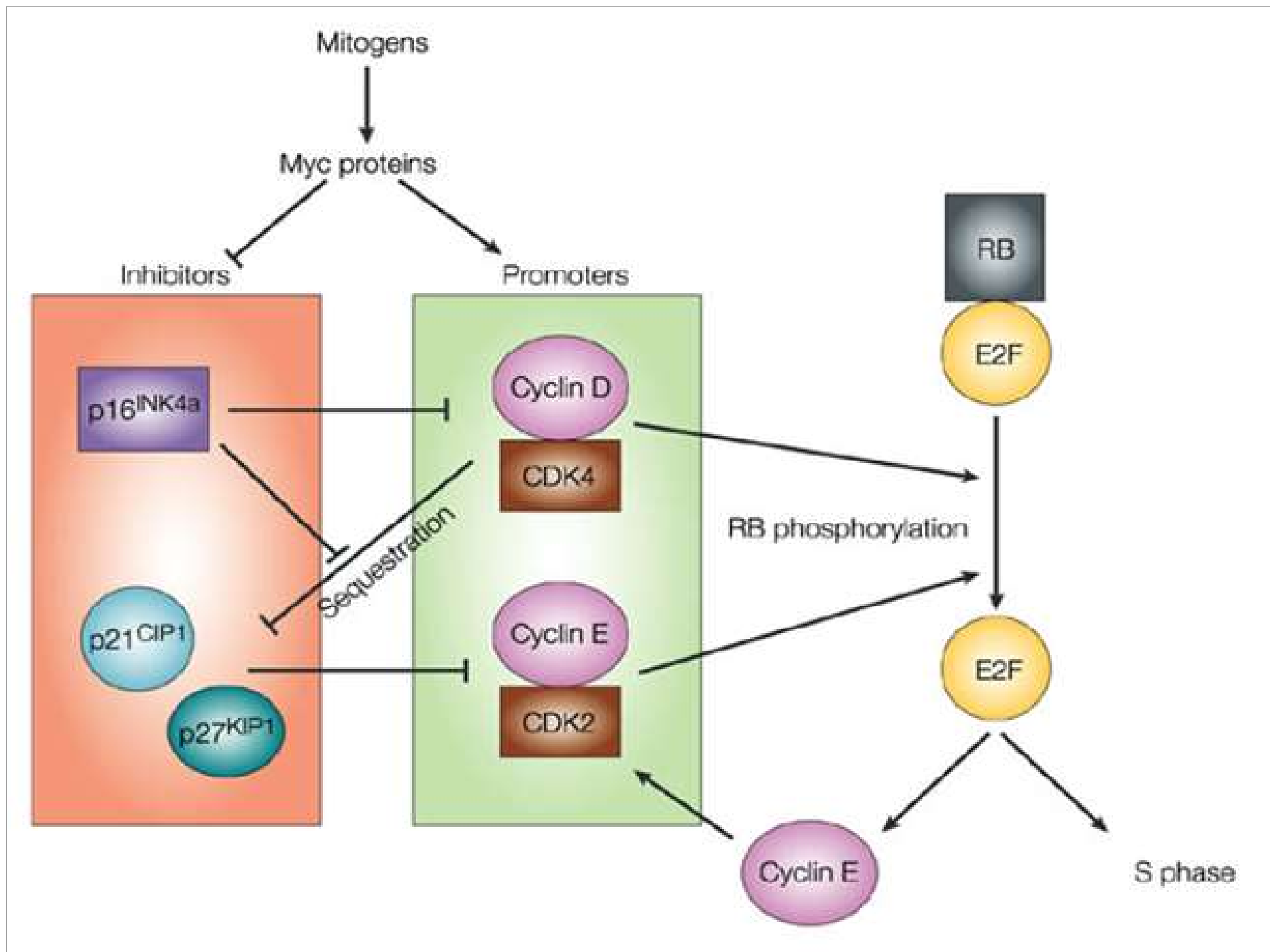
- The coding sequence is altered, so the protein product is functionally different, usually hyperactive (过分活跃).



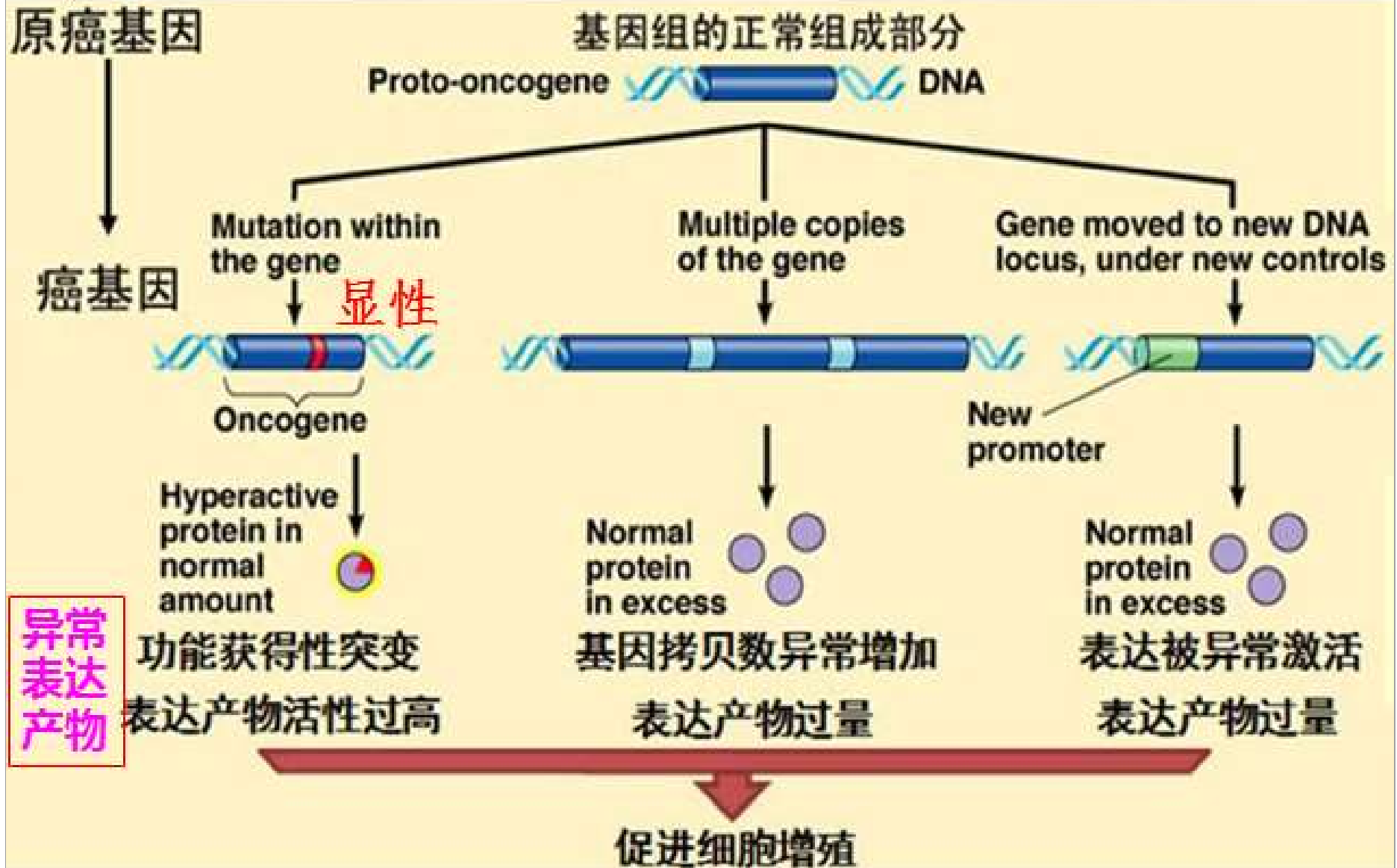


## 1.7 Categories of proto-oncogenes

Categories	Examples	Functions
Growth factors	<i>sis</i> (PDGF)	Induces cell <b>proliferation</b>
Membrane receptor tyrosine kinases	EGFR, PDGFR, VEGFR, CSF-1R	Transduce signals for <b>cell growth</b> and differentiation
Regulatory GTPase	<i>ras</i> (G-protein)	
Cytoplasmic kinases	<i>src</i> -family, <i>raf</i> , <i>cyclin</i>	Involved in organism development, cell cycle regulation, <b>cell proliferation</b> , migration, differentiation, survival, and apoptosis
Transcription factors (Nuclear oncogenes)	<i>myc</i> , <i>fos</i> , <i>jun</i> , <i>erbA</i>	Regulate transcription of genes that induce <b>cell proliferation</b>

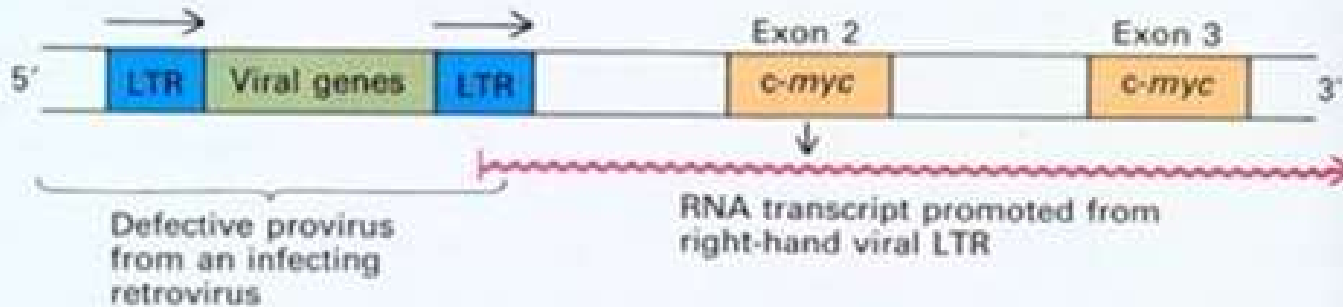


## 1.8 Carcinogenic mechanisms of oncogenes

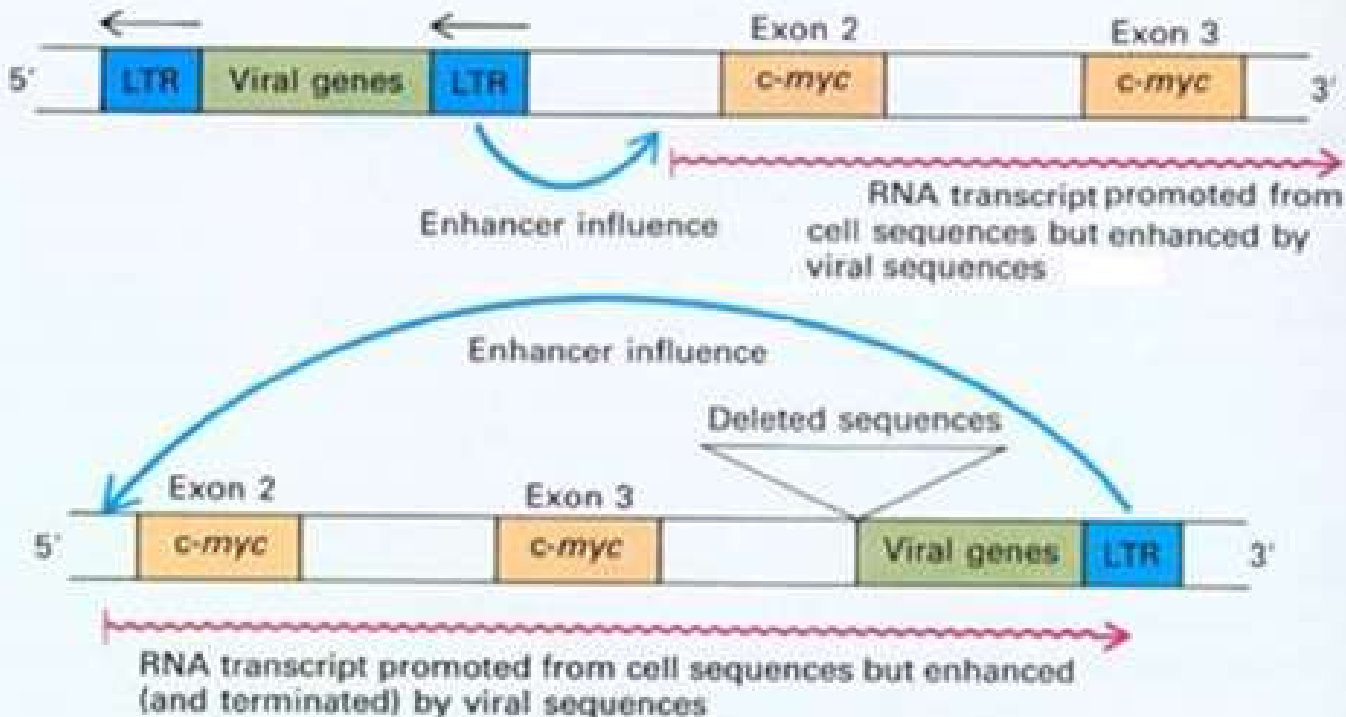


## 1.8.1 Insertion of a strong promoter or enhancer

(a) Promoter insertion



(b) Enhancer insertions



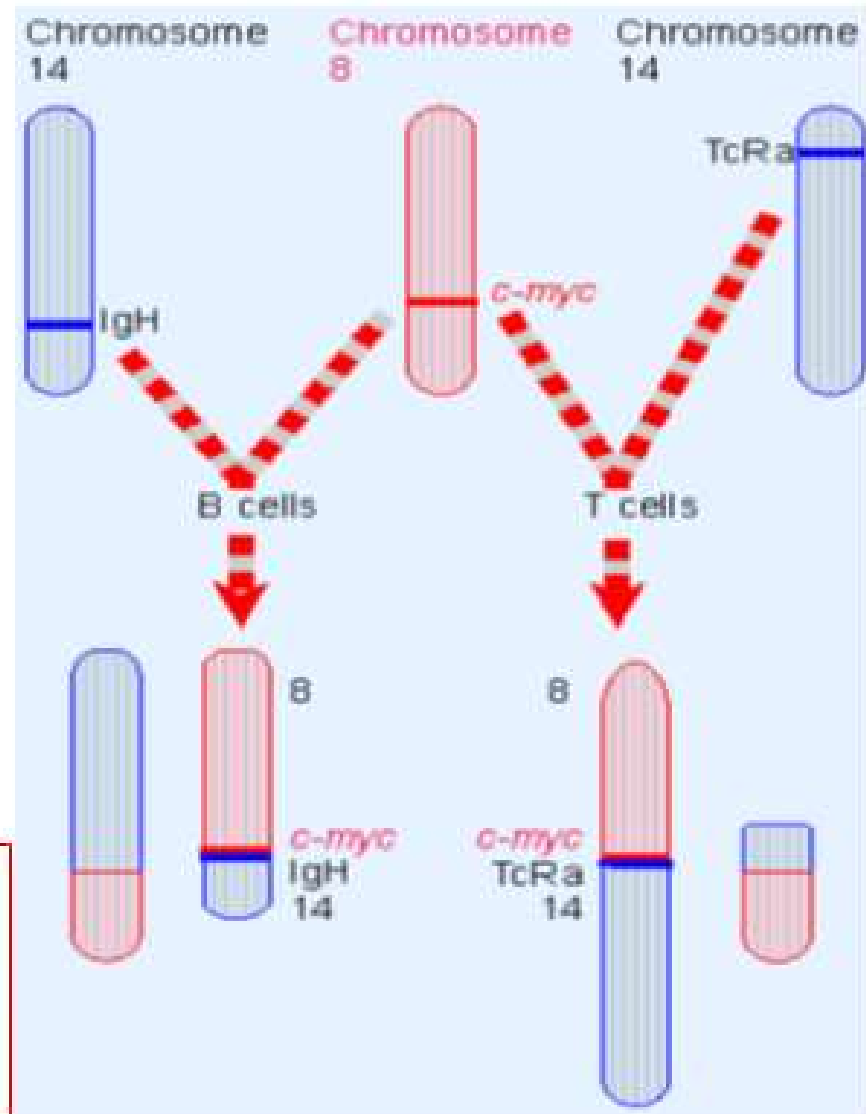
过量的  
正常表  
达产物

## 1.8.2 Gene translocation

- 染色体易位，B细胞中IgH基因转到 *myc* 的基因座，受免疫球蛋白增强子的控制，*c-myc*过量表达。
- 在T细胞中则易位到TCR基因座。

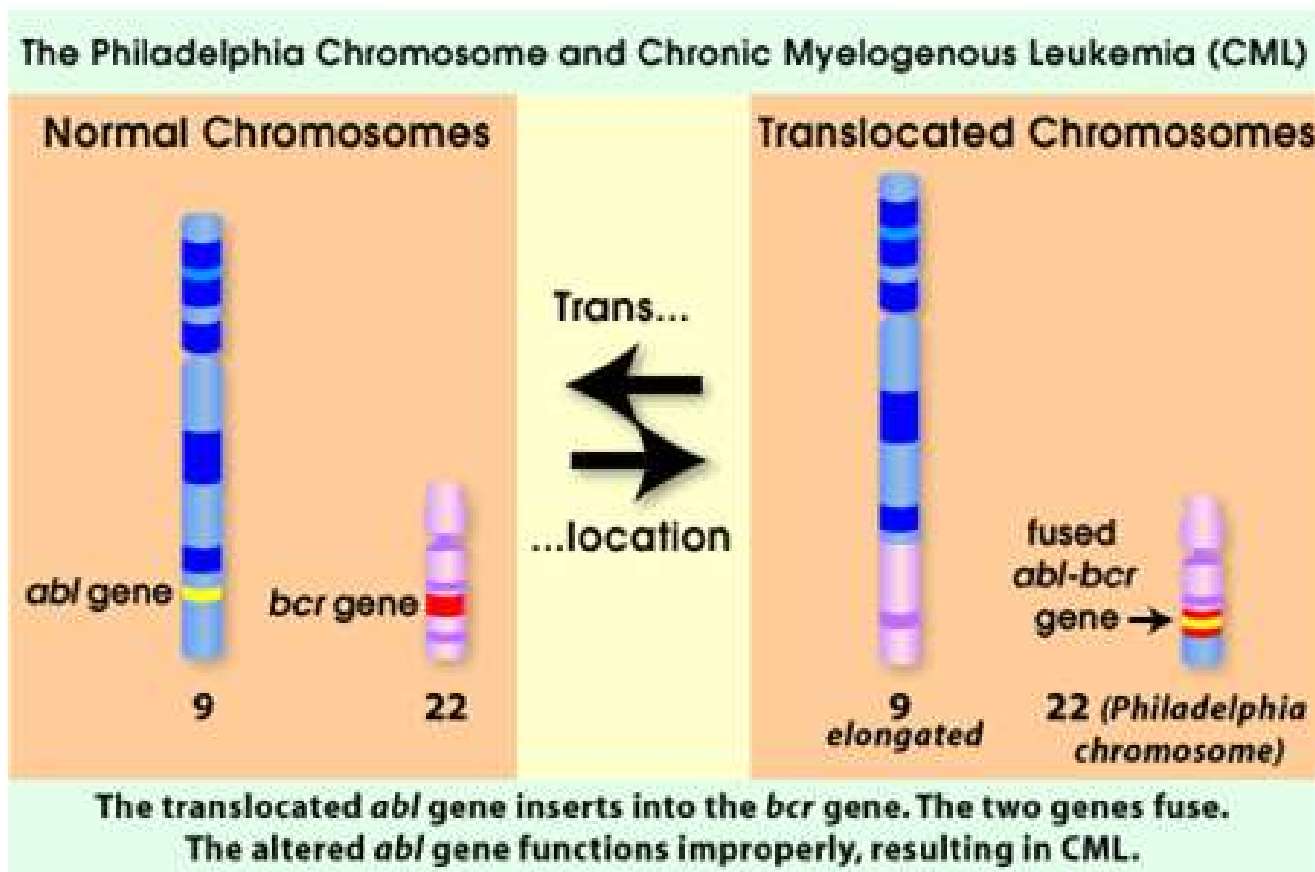
Lymphoma

过量的  
正常表  
达产物



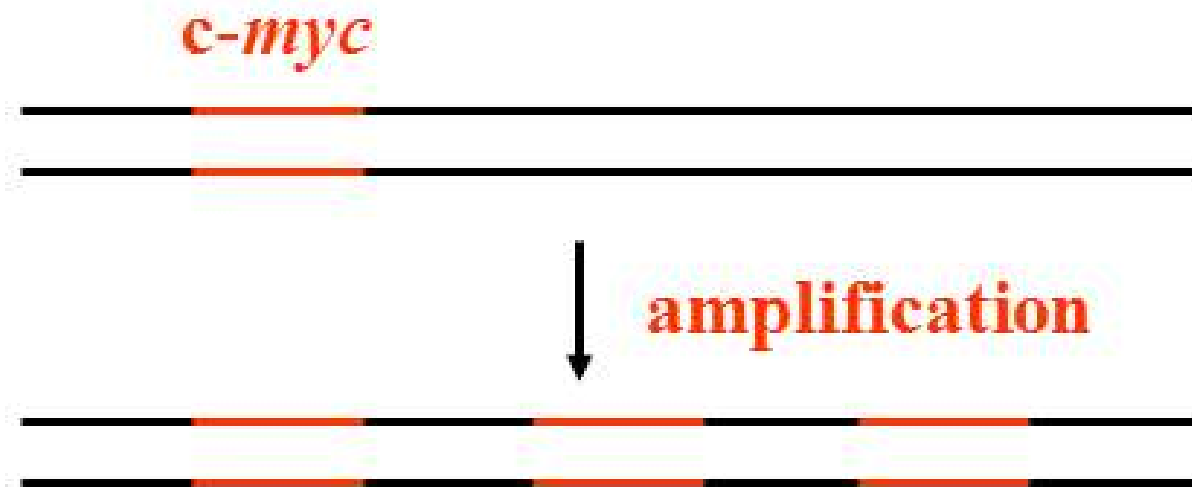


- 染色体易位使位于9q34的*abl*基因转移到第22号染色体上，重排形成*abl-bcr*融合基因。蛋白质产物也由p145变成p210，其酪氨酸蛋白激酶活性大为增加，扰乱了细胞内正常的信号传导途径，并抑制凋亡。导致慢性粒细胞白血病的发生。



异常  
表达  
产物

### 1.8.3 *P-onc* amplification in the genome

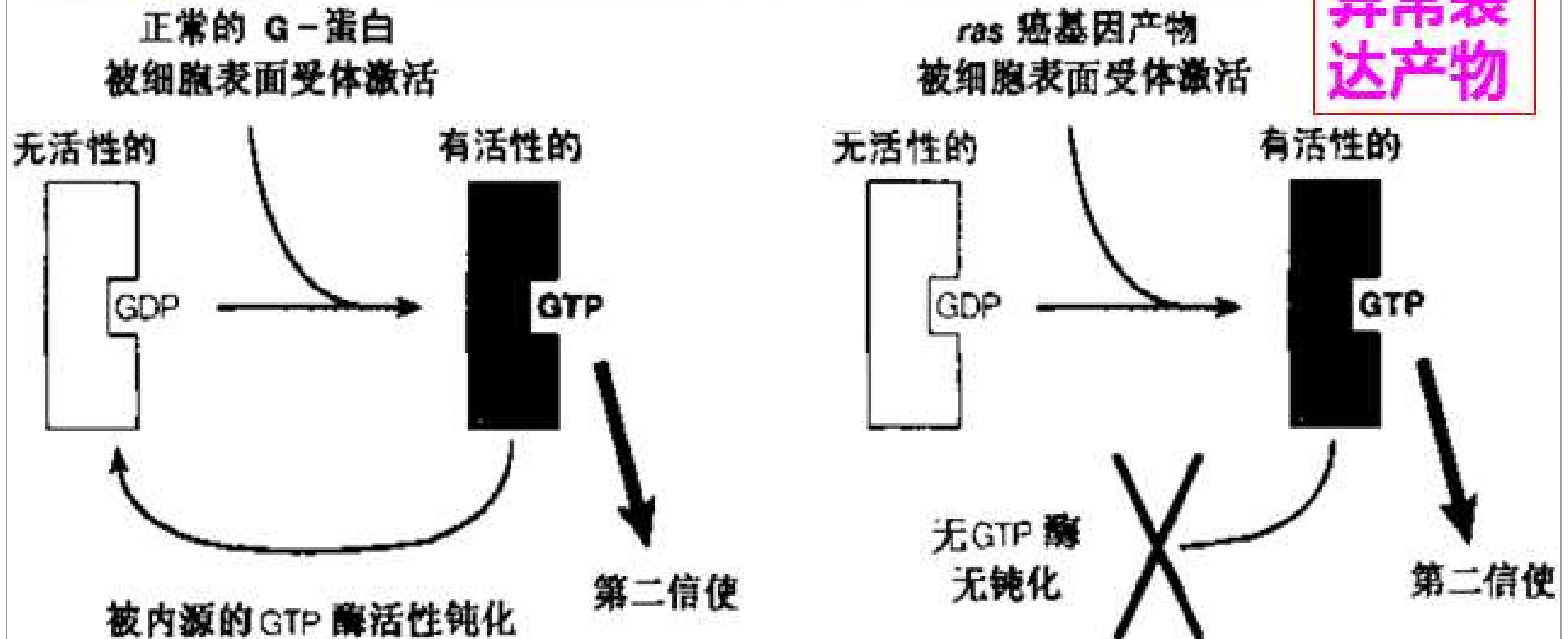


过量的正常  
表达产物

**Expression of *c-myc* is increased obviously.**

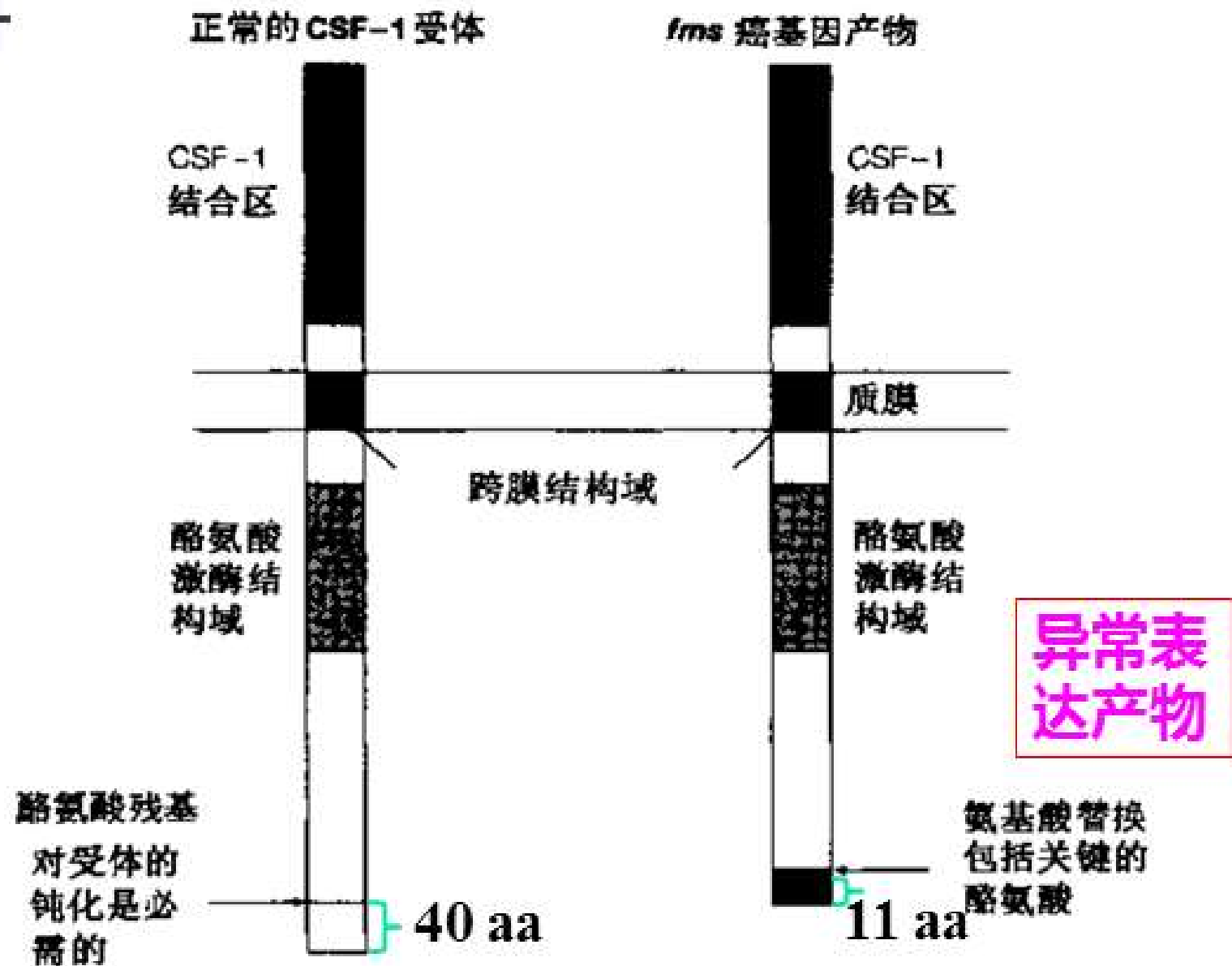
## 1.8.4 Point mutation

异常表达产物



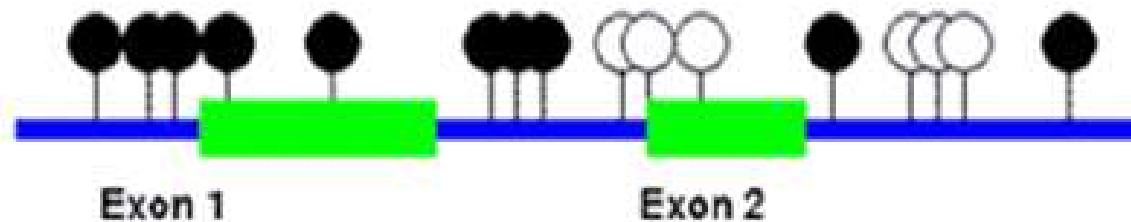
	1	2	3	4	5	6	7	8	9	10	11	12	13		188	189
正常人 <i>rasH</i>	Met	Thr	Glu	Tyr	Lys	Leu	Val	Val	Val	Gly	Ala	Gly	Gly		Leu	Ser
	ATG	ACG	GAA	TAT	AAG	CTG	GTG	GTG	GTG	GGC	GCC	GGC	GGT	.....	CTC	TCC
活化的 <i>EJrasH</i>	Met	Thr	Glu	Tyr	Lys	Leu	Val	Val	Val	Gly	Ala	GTC	Gly	.....	Leu	Ser
												Val				

## 1.8.5 Deletion mutation



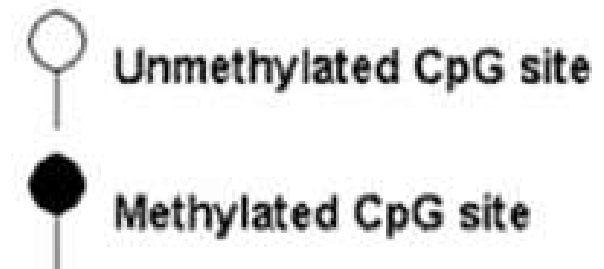
## 1.8.6 Hypomethylation (低甲基化) of *p-onc*

Normal cells

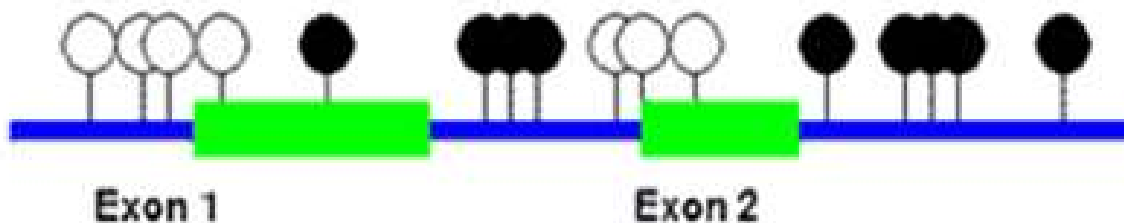


mRNA expression (-)

DNA甲基化  
阻抑基因转录



Cancer cells



mRNA expression (+)



## 1.9 Co-operation between oncogenes

- The transformation of a normal cell into a fully malignant cancer cell is **a multi-step** process involving alterations in the expression of several genes.
- Usually, a variety of **pairs of oncogenes** are able to achieve together what neither can achieve singly.

**Co-operation: growth factor-related oncogene + nuclear oncogene**



## 2. Tumor suppressor genes

- **Tumor suppressor genes** (also called anti-oncogenes) are those genes whose products **inhibit excessive growth and proliferation** of cells, and then suppress tumor formation.

**肿瘤抑制基因/抑癌基因**是一类抑制细胞过度生长和增殖从而遏制肿瘤形成的基因。



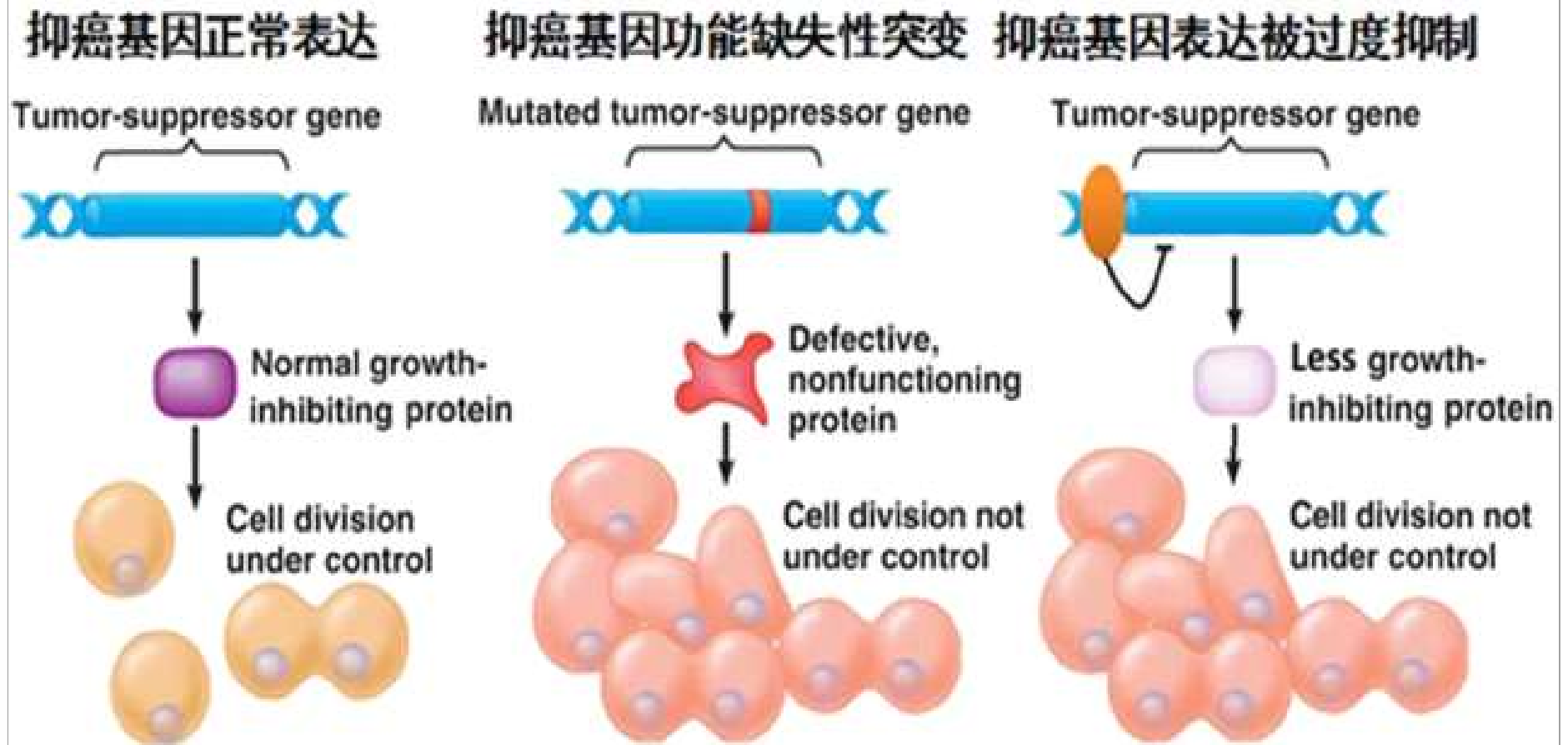
## 2.1 Differences between tumor suppressor genes and oncogenes

- Tumor suppressor genes act in a fundamentally different way from oncogenes:

	Proto-oncogenes	Suppressor genes
Function	promote cell division	inhibit cell division
Cancerous	mutated active	mutated inactive



# Inactivation of tumor suppressor gene leads to cancer



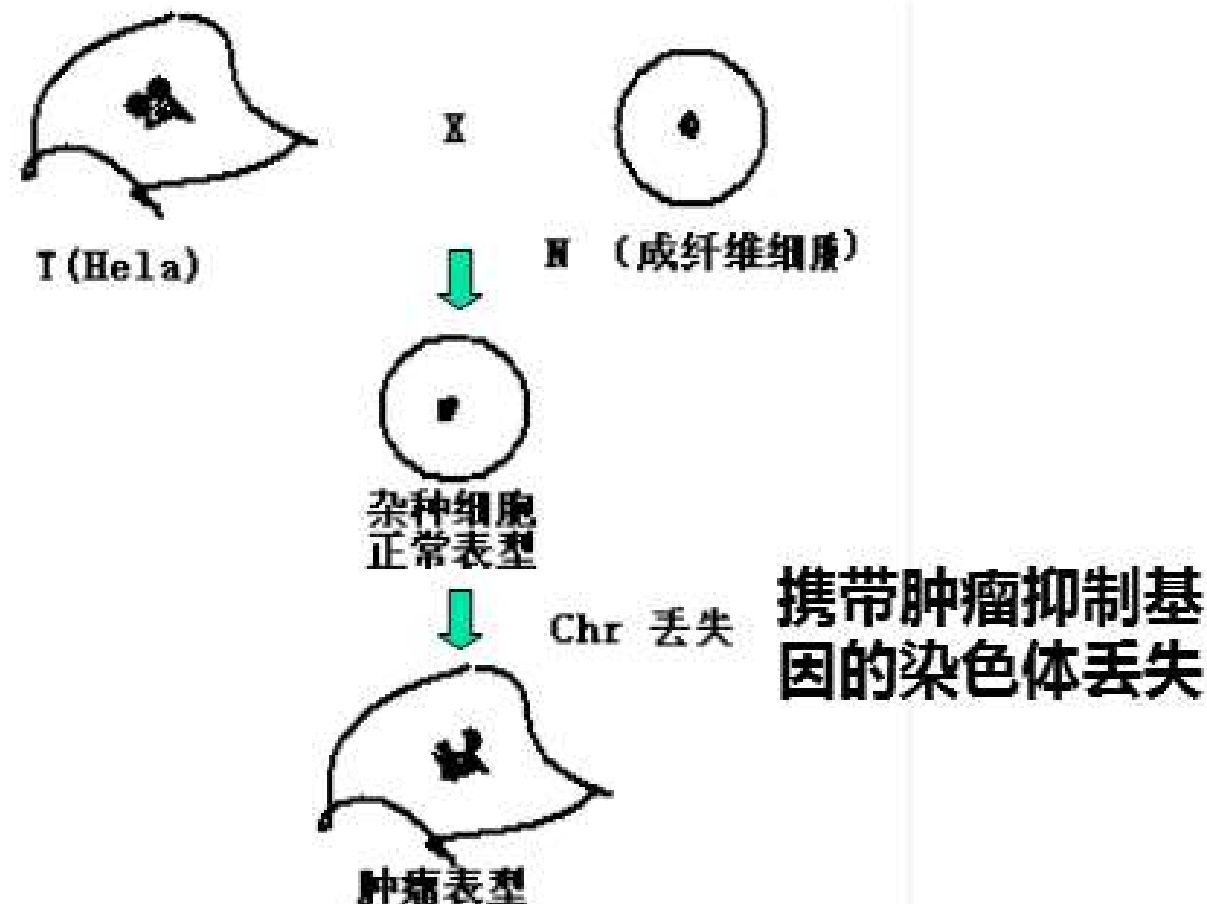
- Mutant tumor suppressors alleles are **usually recessive (隐性)** whereas mutant proto-oncogene alleles are typically dominant (**显性**).

	allele 1	allele 2	
Proto-oncogene	Mutant	Wild-type	→ tumor
Anti-oncogene	Mutant	Wild-type	→ normal
Anti-oncogene	Mutant	Mutant	→ tumor

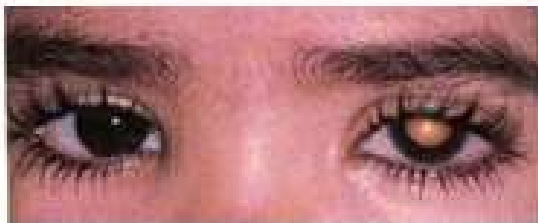
**Tumor suppressor gene is also called **recessive oncogene**.**

## 2.2 Evidence for tumor suppressor genes

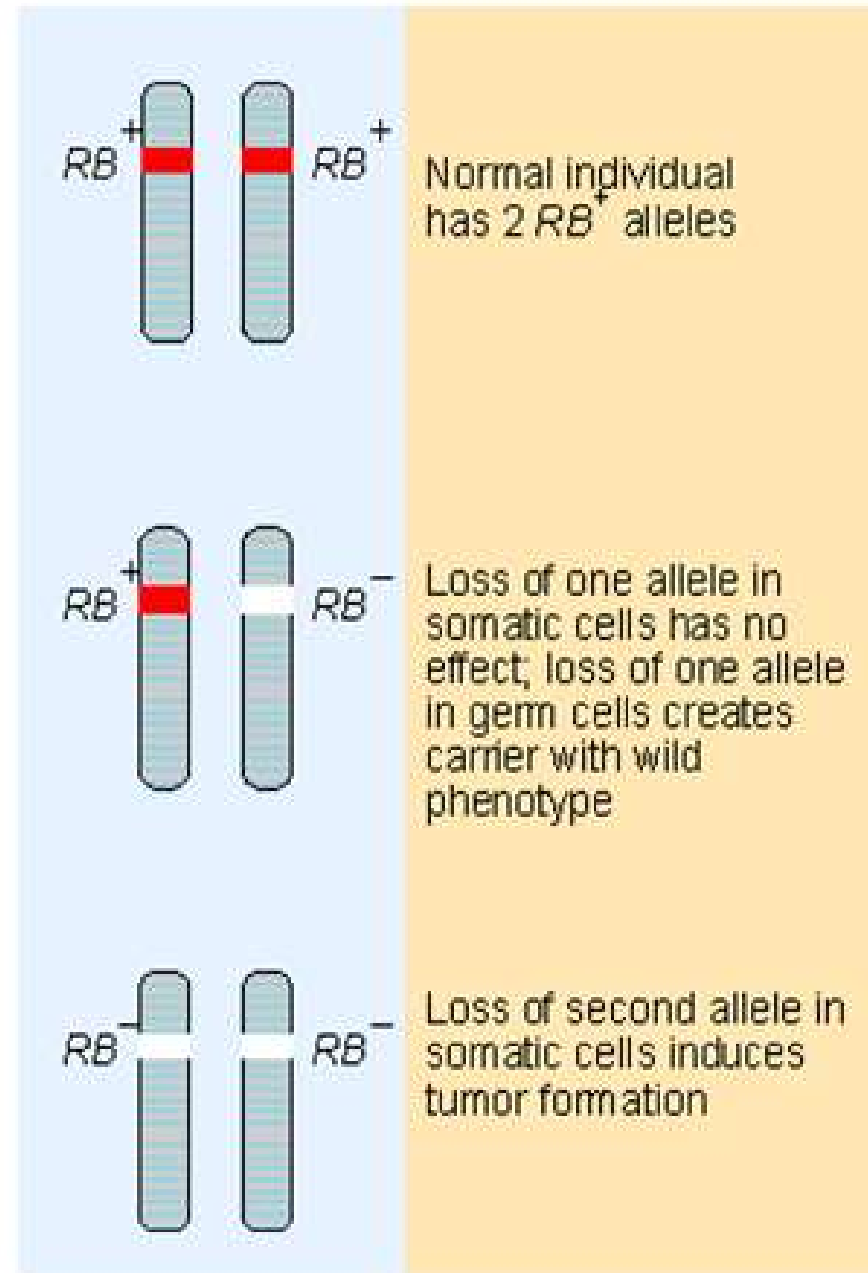
(1) A normal cell was fused with a cancerous cell, the resulting hybrid cell was invariably noncancerous.



**(2) Examination of the inheritance of certain familial cancers (such as Retinoblastoma成视网膜细胞瘤) result from recessive mutations.**

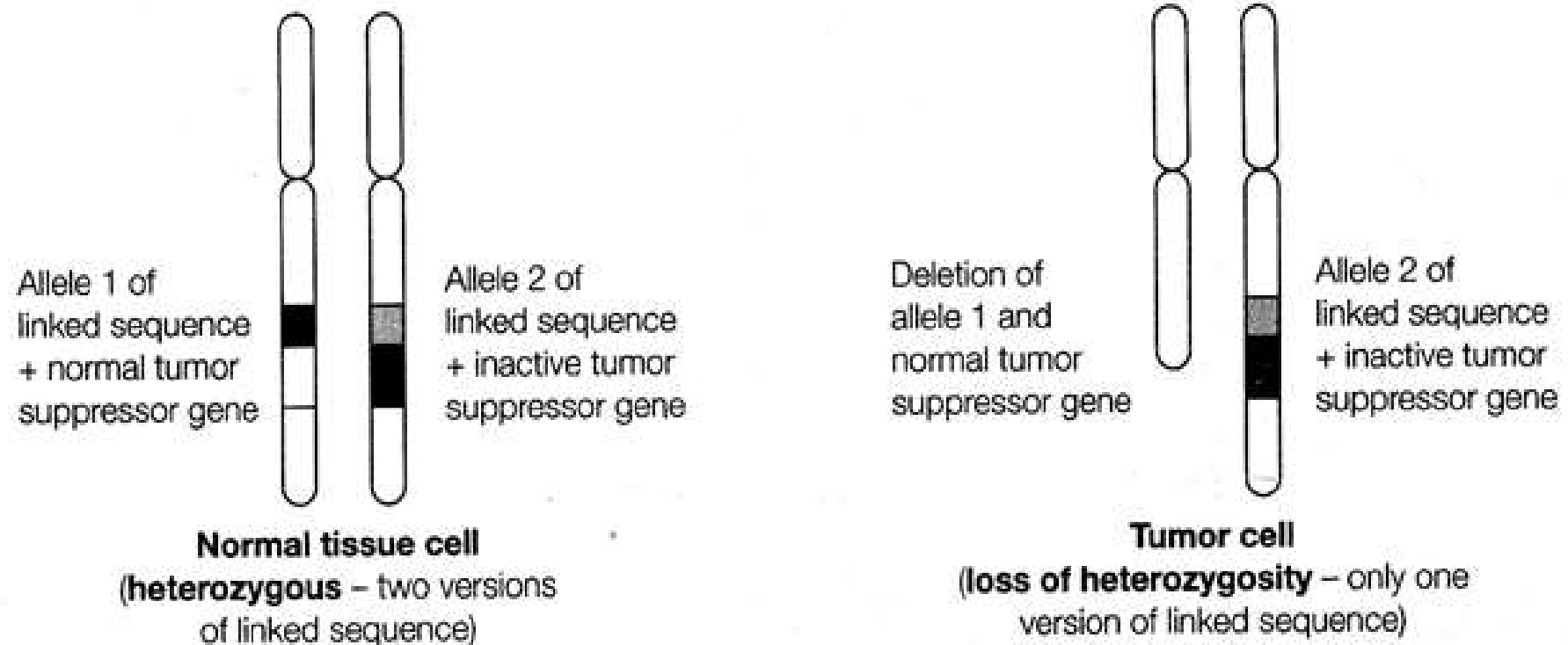


Familial RB: two eyes  
Sporadic RB: one eye



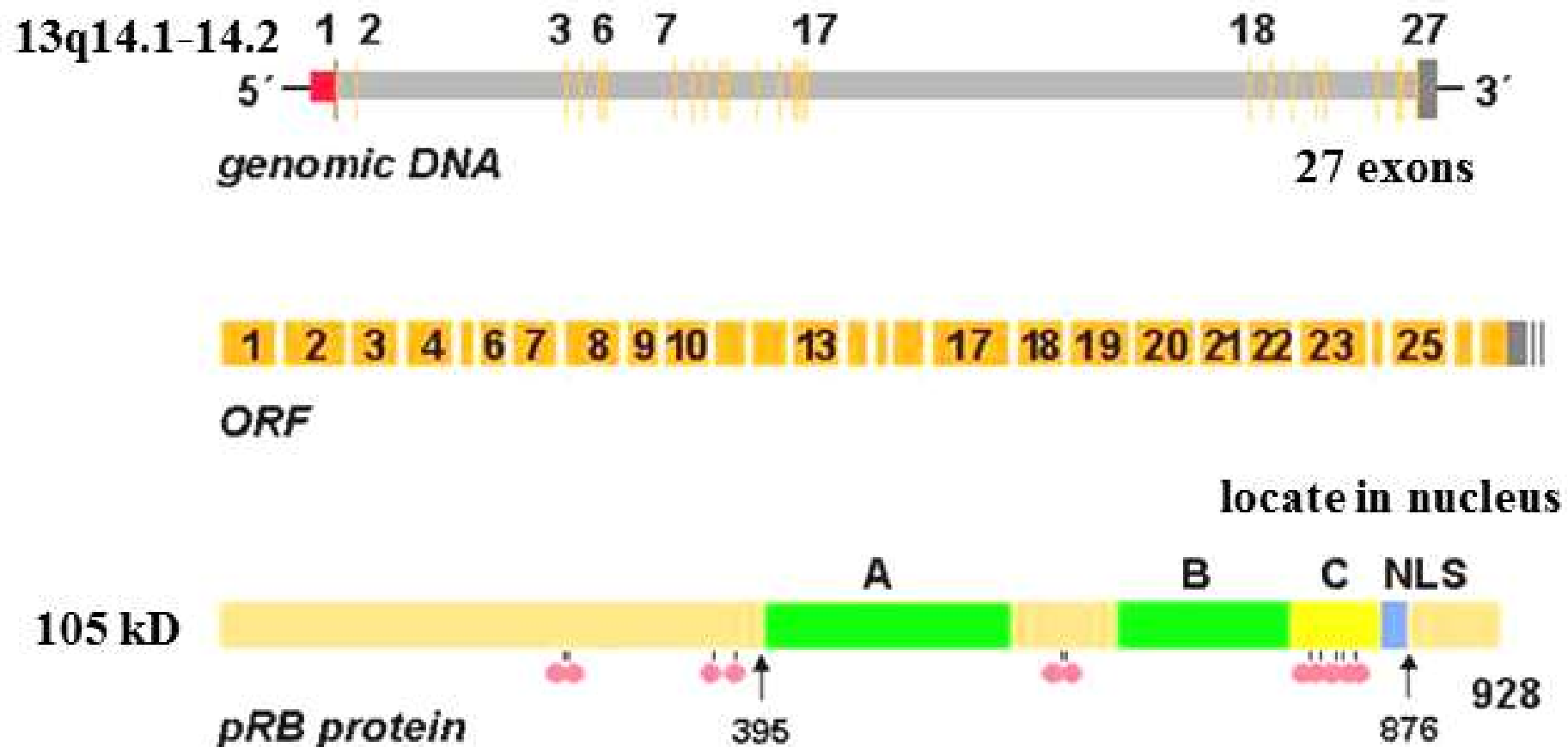
**(3) In many cancer cells, there has been a consistent loss of characteristic regions of certain chromosomes.**

**--loss of heterozygosity(杂合性丢失).**

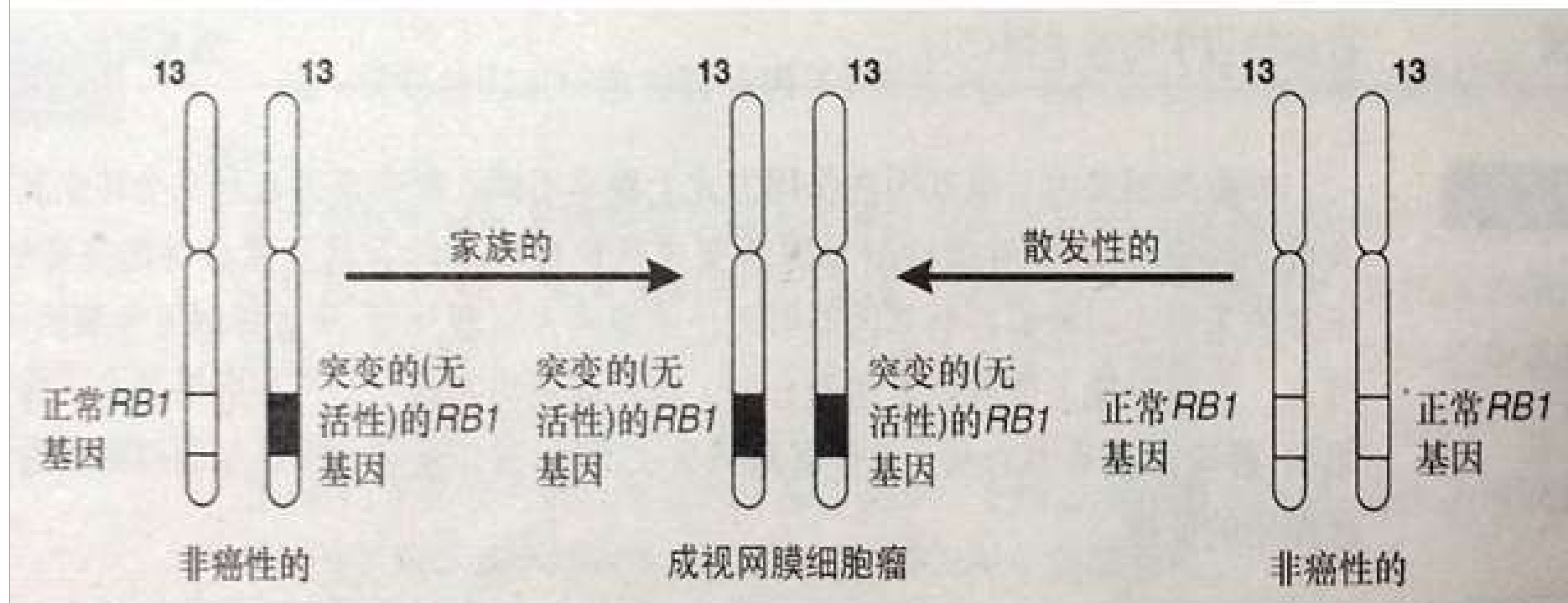


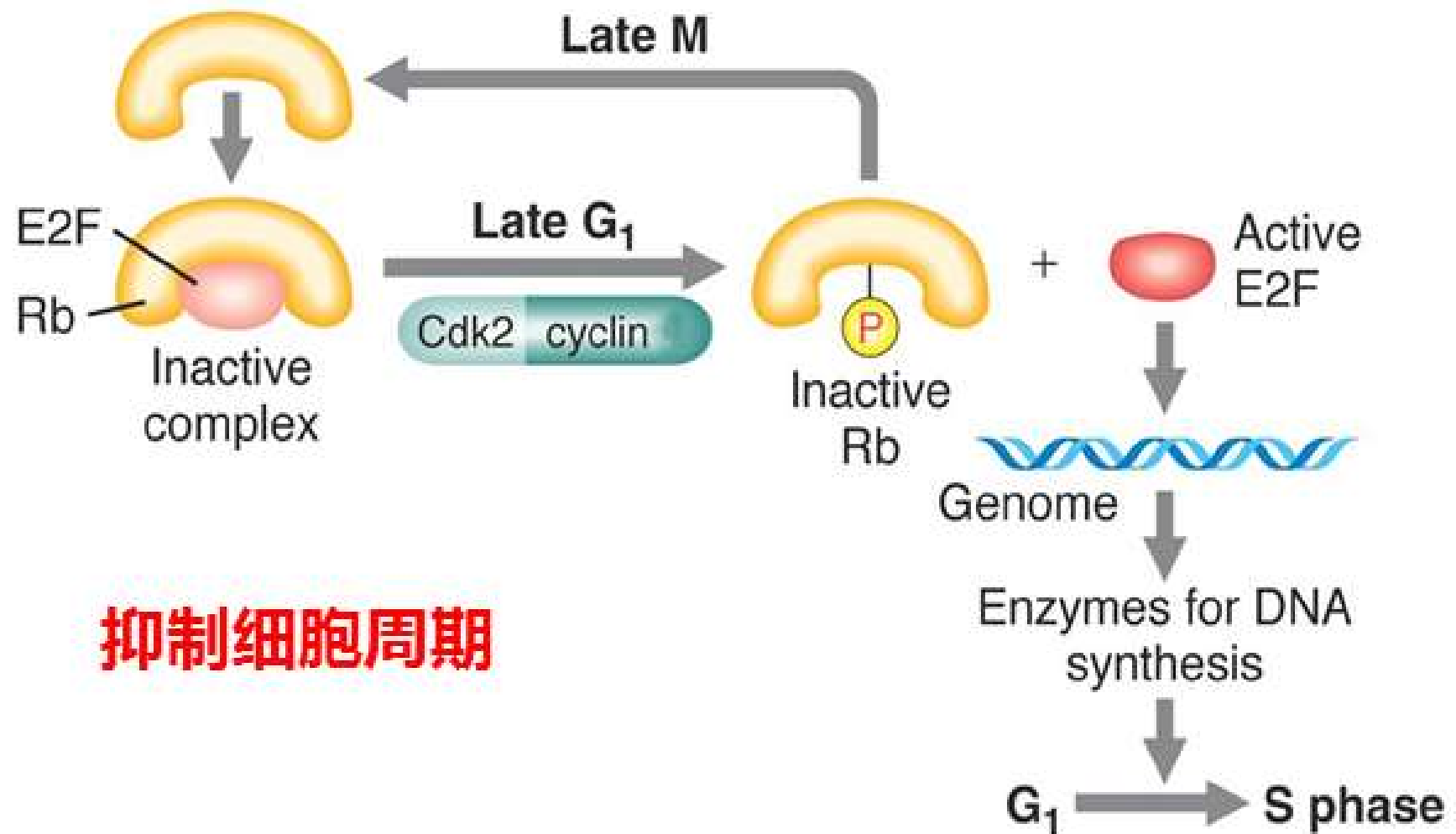
## 2.3 RB1

- The *RB1* was **the first tumor suppressor gene** to be isolated.

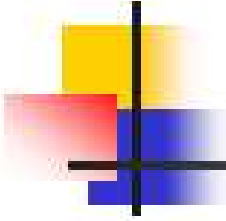


- ***RB1* mRNA was found to be absent or abnormal in retinoblastoma cells.**



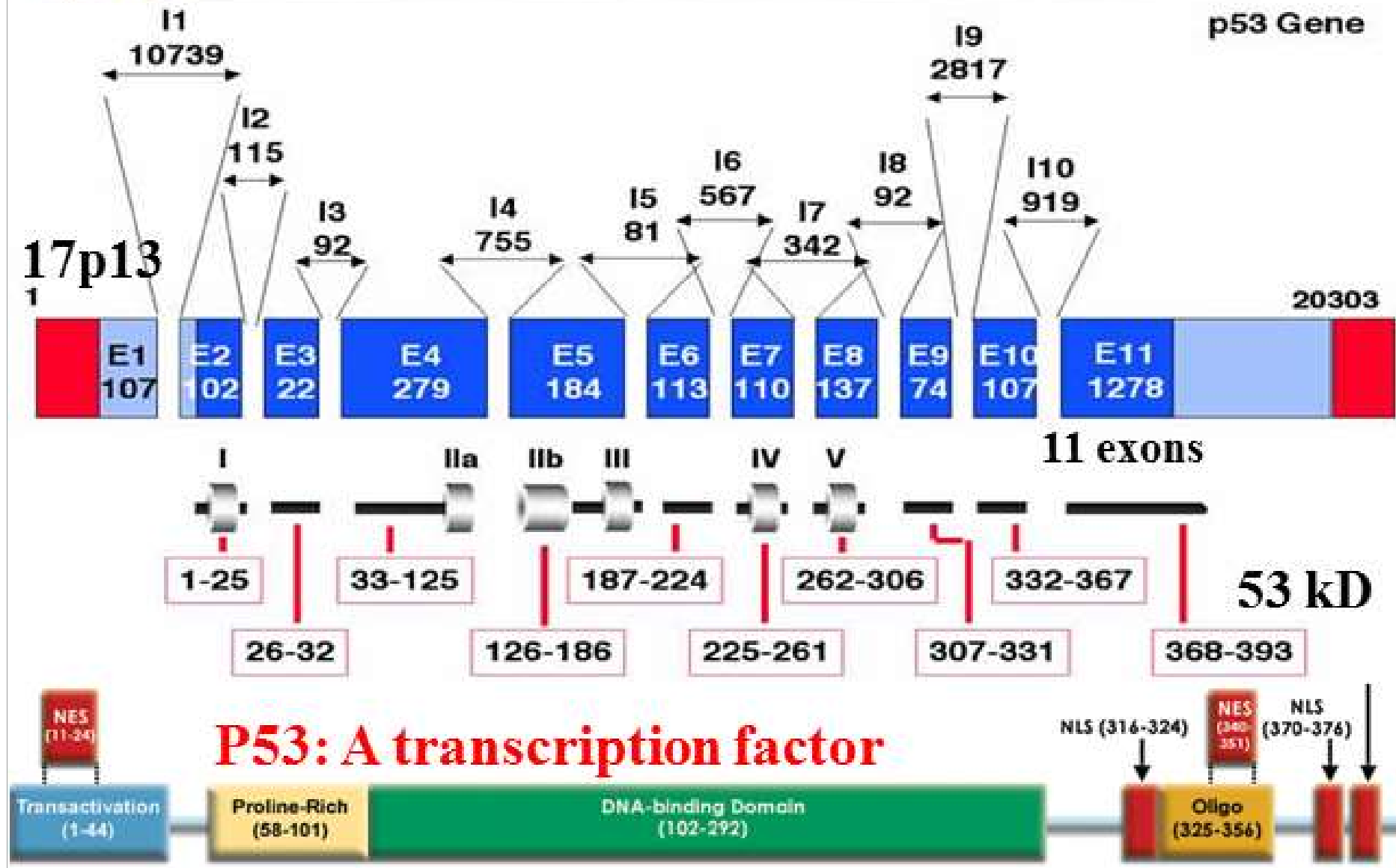




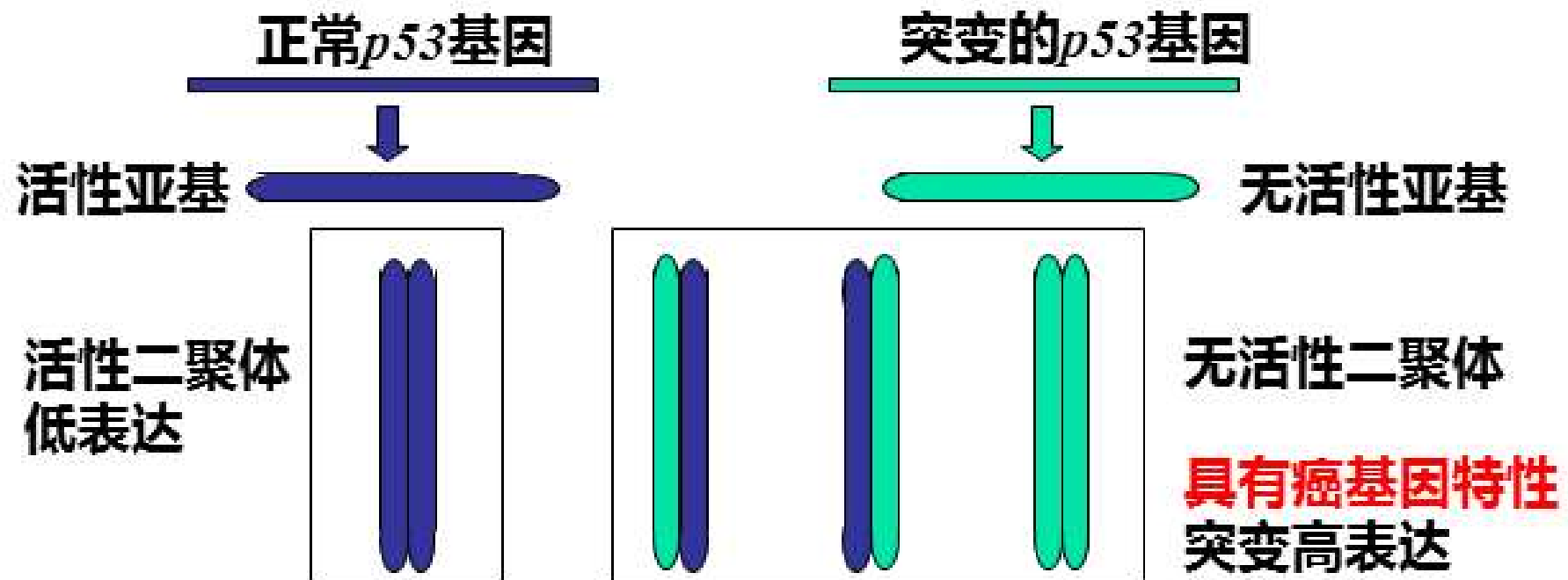


- ***RB1* gene codes for a phospho-protein that binds to DNA, and has been shown to inhibit the transcription of *c-onc* such as *myc* and *fos*.**  
**抑制某些原癌基因的转录**
- ***RB1* mutations have also been detected in breast and lung tumors.**

## 2.4 P53



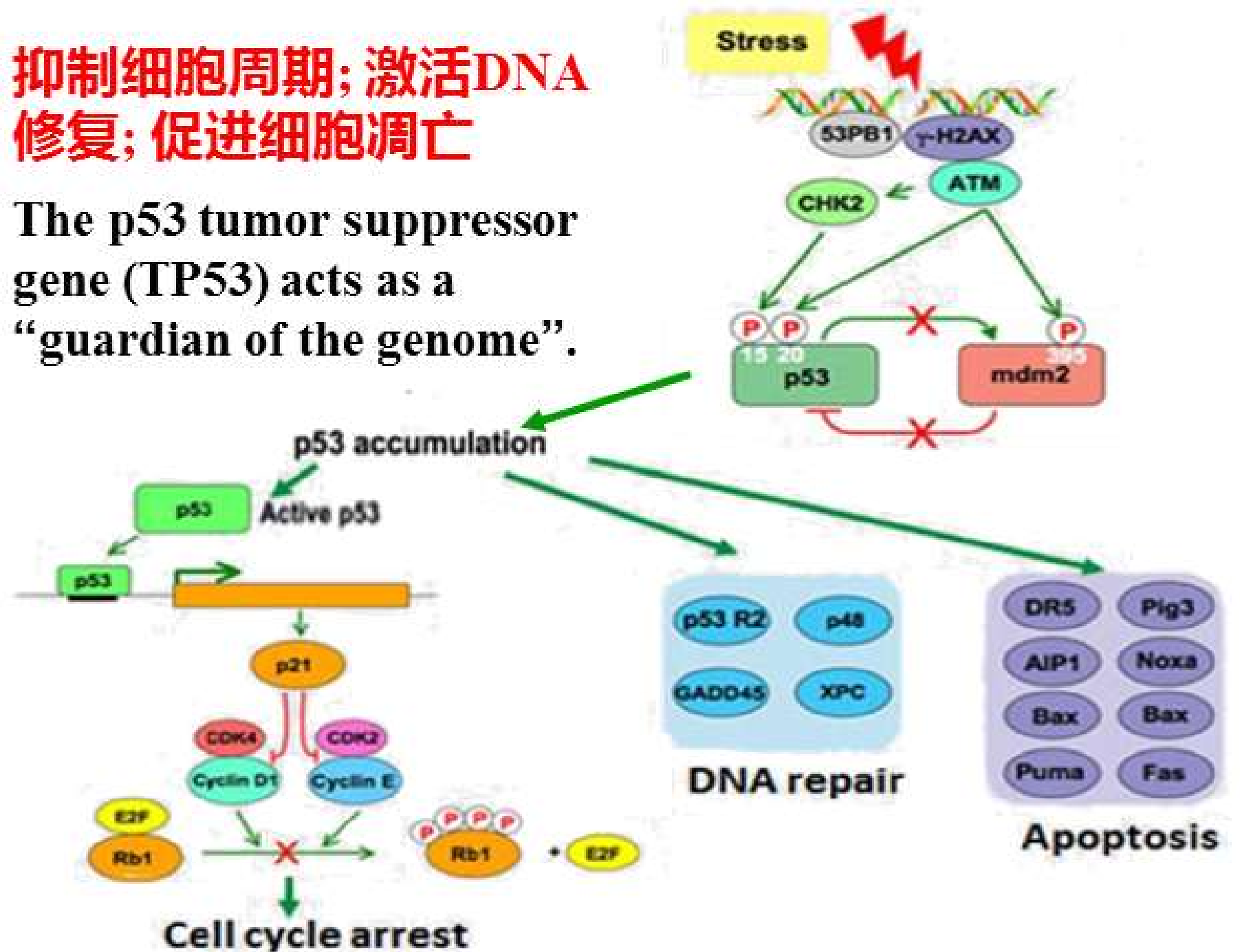
- *P53* is the tumor suppressor gene that is **mutated in the largest number of different types of tumor.**



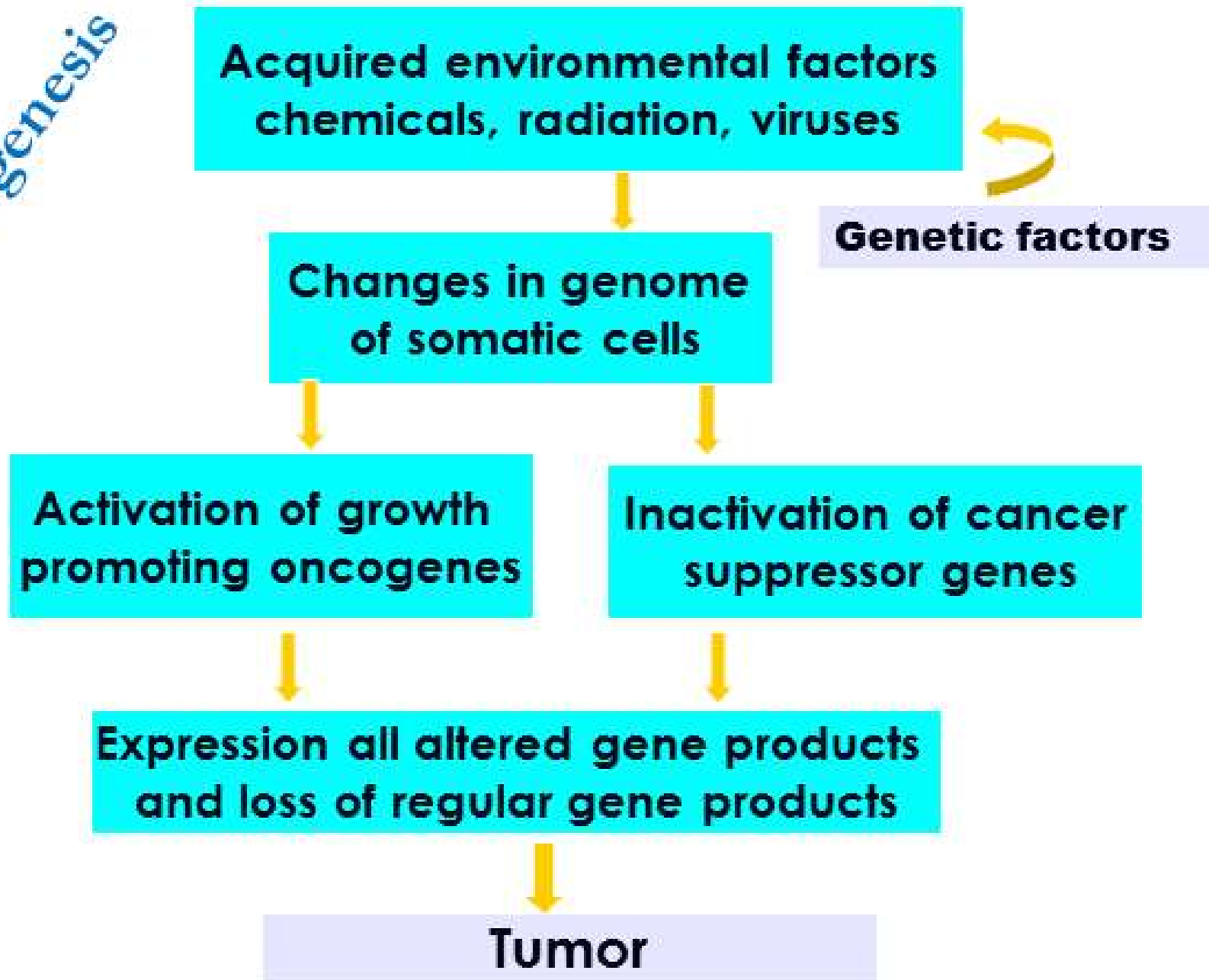
- **Properties of mutant p53 proteins:**
  - (1) **dominant-negative effect (显性负效应)**
  - (2) **loss of function**
  - (3) **mutants exhibit oncogenic properties**

抑制细胞周期; 激活DNA  
修复; 促进细胞凋亡

The p53 tumor suppressor  
gene (TP53) acts as a  
“guardian of the genome”.



*Pathogenesis*



## ~5% of cancers are inherited

### Somatic mutations

- Occur in *nongermline* tissues
- Cannot be inherited



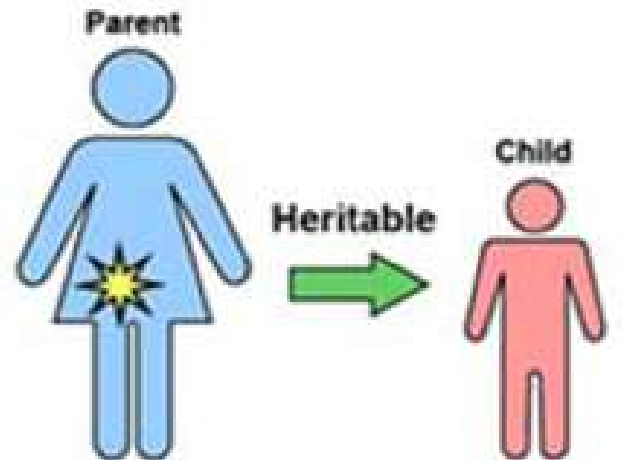
Nonheritable

Mutation in tumor only  
(for example, breast)

肿瘤主要是体细胞  
DNA的改变引起的

### Germline mutations

- Present in egg or sperm
- Can be inherited
- Cause cancer family syndrome



Mutation in  
egg or sperm

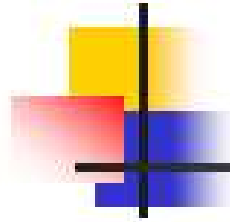
All cells  
affected in  
offspring

遗传疾病主要是生殖细  
胞DNA的改变引起的

## 正常细胞的生长增殖由两大基因调控

- 正调控信号：促进细胞生长和增殖，并且阻止其发生终末分化——原癌基因。
- 负调控信号：促进细胞成熟，向终末分化，最后凋亡（**apoptosis**）——抑癌基因。

两种信号保持着动态平衡，对正常细胞的生长、增殖、死亡精确地调控，当平衡被破坏，正调控信号基因功能过盛或负调控信号基因失活，导致细胞增殖调控的混乱而使细胞恶变。



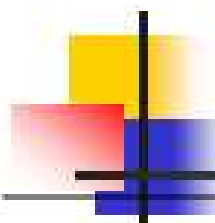
### 3. Apoptosis (凋亡)

---

**Apoptosis** refers to the autonomic and orderly death of cells controlled by genes, also known as programmed cell death.

**凋亡**是指由基因控制的细胞自主有序的主动死亡过程，也叫程序性细胞死亡。

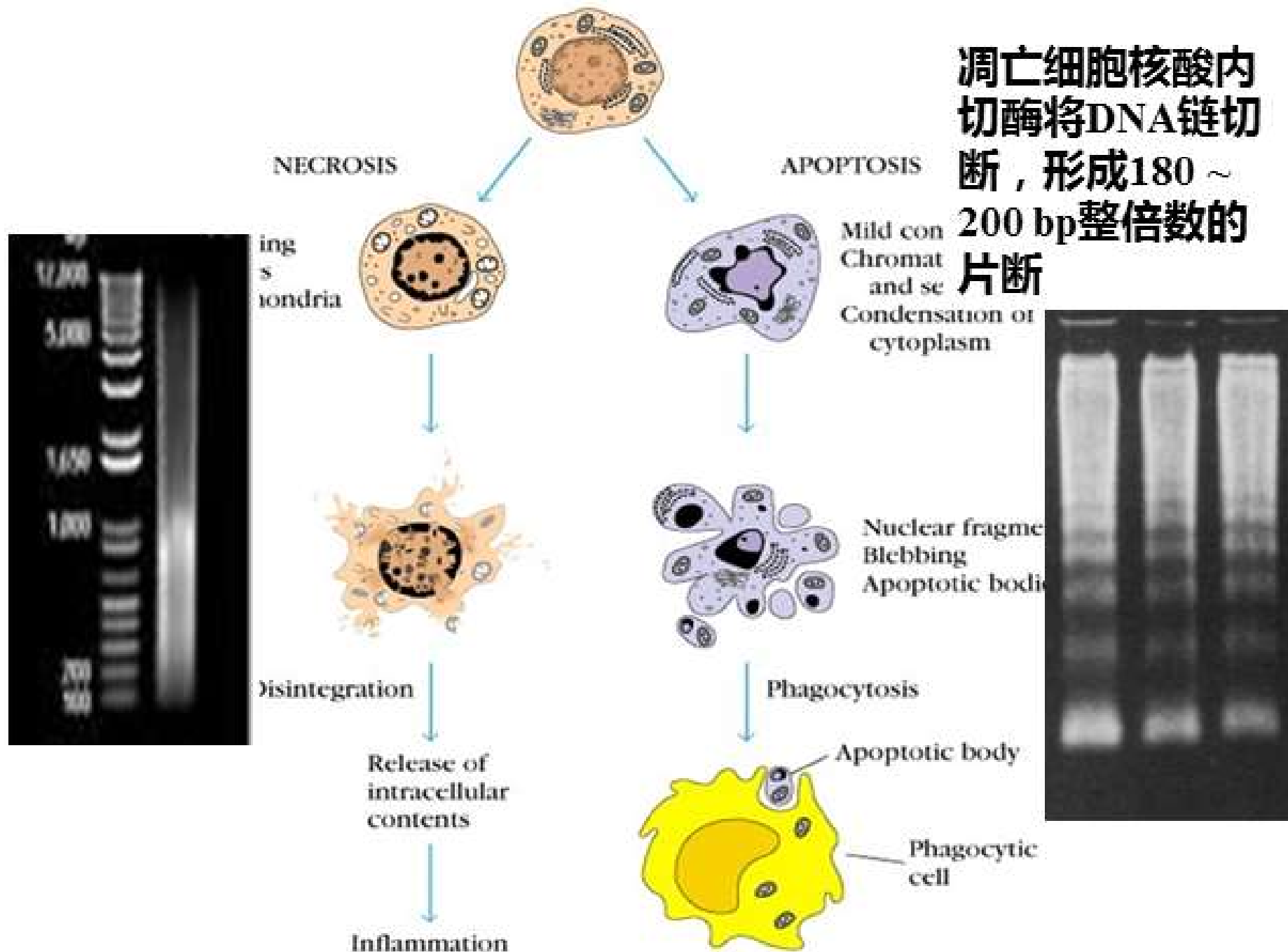




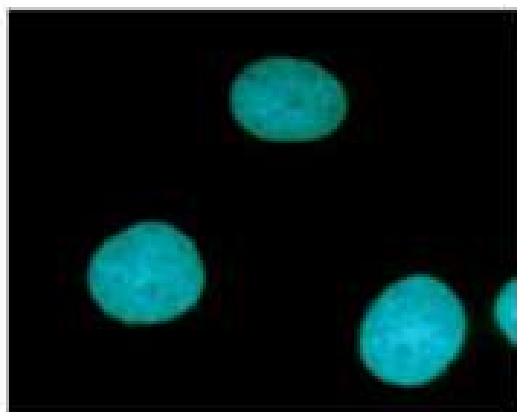
## 3.1 Differences between apoptosis and necrosis

	坏死	凋亡
性质	病理性、非特异性	生理性或病理性，特异性
诱导因素	强烈刺激，随机发生	较弱刺激，非随机发生
生化特点	被动过程，无新蛋白质合成，不耗能	主动过程，有新蛋白质合成，耗能
<b>DNA电泳</b>	弥散性降解，电泳呈均一DNA片状	DNA片段化(180—200 bp)，电泳呈“梯”状条带
形态变化	细胞结构全面溶解、破坏、细胞肿胀	胞膜及细胞器相对完整细胞皱缩， <b>核固缩</b>
炎症反应	溶酶体破裂，局部炎症反应	溶酶体相对完整，局部无炎症反应
凋亡小体	无	有
基因调控	无	有

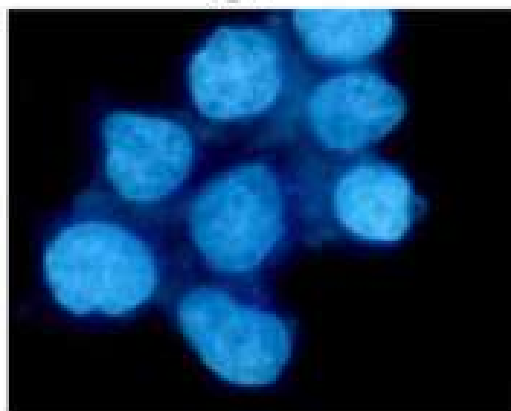
凋亡细胞核酸内切酶将DNA链切断，形成180 ~ 200 bp整倍数的片段



control



stage I



stage IIa



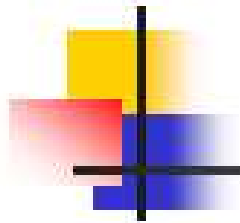
stage IIb



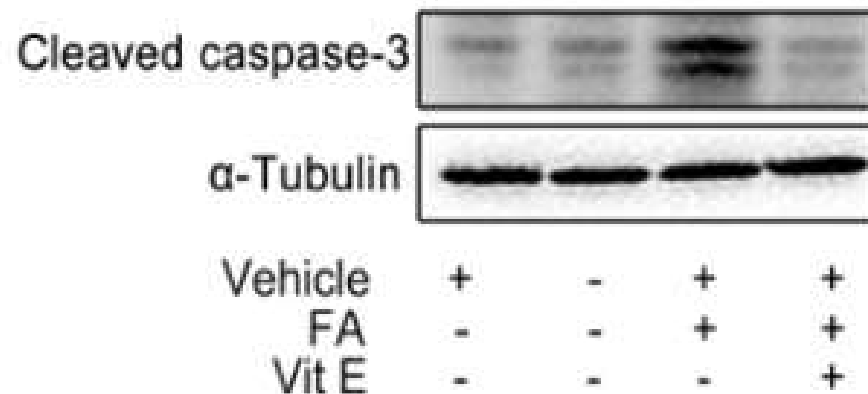
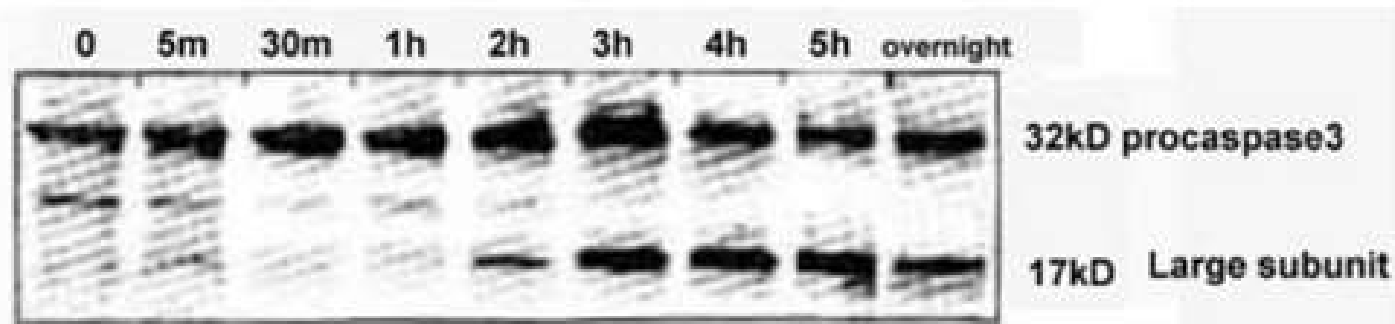
I 期的细胞核呈波纹状(rippled)或呈折缝样(creased)，部分染色质出现浓缩状态；II a期细胞核的染色质高度凝聚、边缘化；II b期的细胞核裂解为碎块，产生凋亡小体。

DAPI 染色

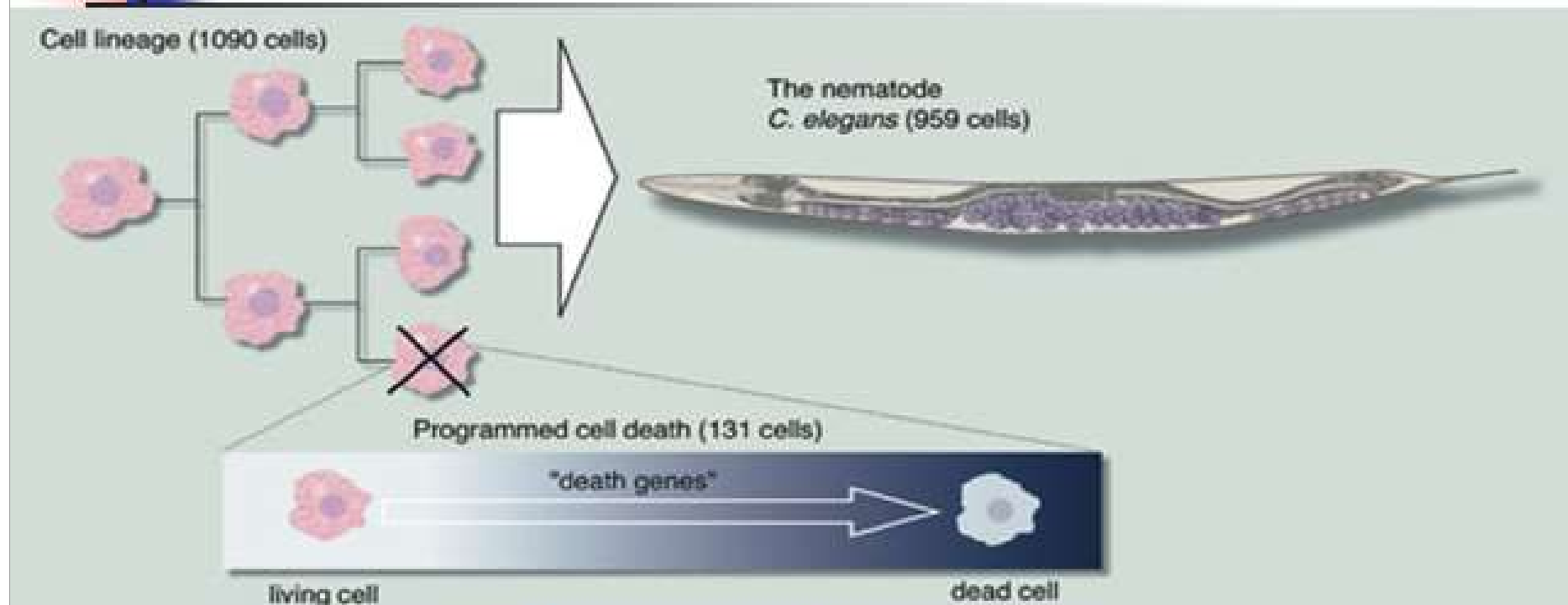
图1 HeLa细胞凋亡过程中核染色质的形态学变化



采用Western blot方法，用抗caspase-3 17 KD和12 KD片段的抗体检测其表达量，从而判断细胞的凋亡程度。



## 3.2 Apoptosis in *C.elegans*



Regulator

Adapter

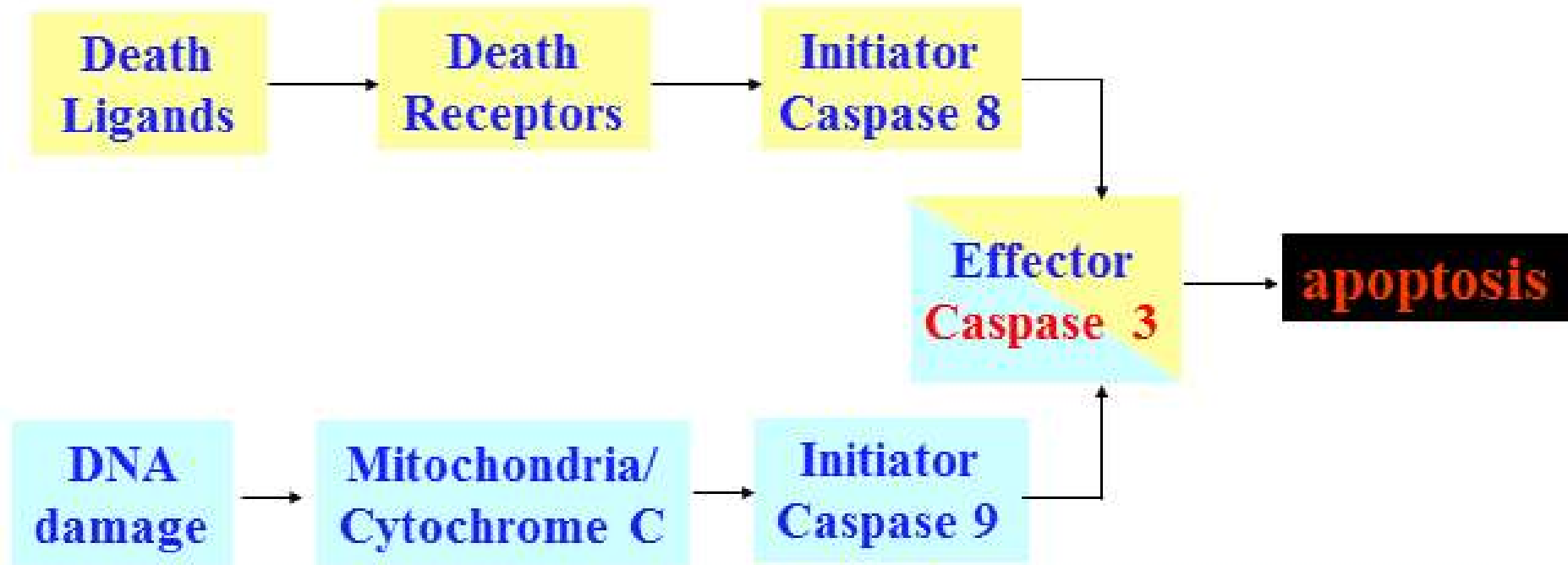
Effector

*C. elegans* CED-9 ┤ CED-4 → CED-3 → Death

Vertebrates Bcl-2 ┤ Apaf-1 → Casp9 → Casp3 → Death

### 3.3 Regulation of Apoptosis

“Extrinsic Pathway”—Death receptor pathway (死亡受体途径)

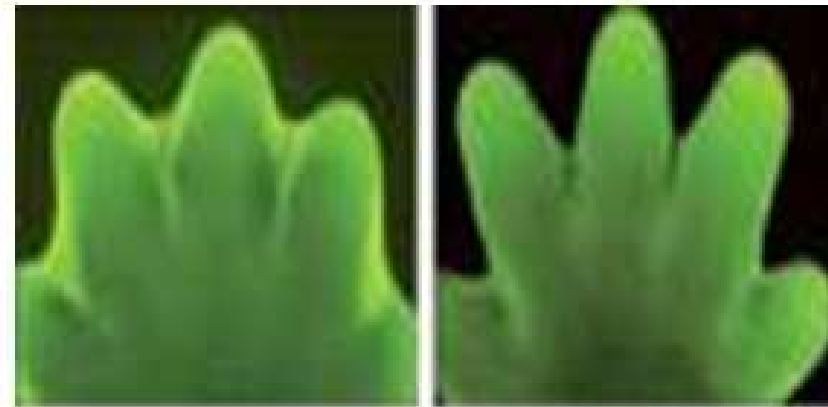
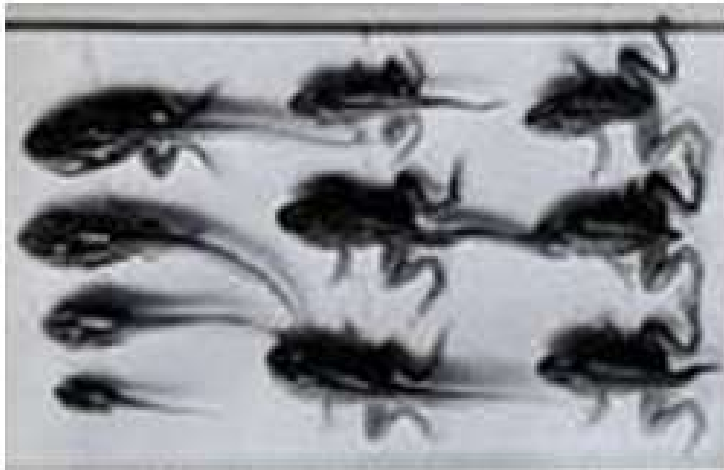


“Intrinsic Pathway”—mitochondrial pathway (线粒体途径)

**Bcl-2** suppresses apoptosis, while **Bax** and **P53** promotes apoptosis.

## 3.4 Normal functions of apoptosis

- **Playing an important role in the formation, maintenance and molding of normal tissues.**
- **Removal of damaged or dangerous cells**





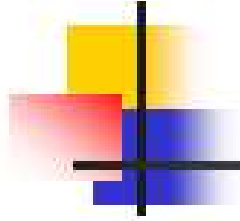
## 3.5 Apoptosis in disease and cancer

细胞凋亡与疾病的关系——无论凋亡过度或凋亡不足都可以导致疾病的发生。

- **细胞凋亡过度**：心肌缺血，心力衰竭，神经退行性疾病，病毒感染等
- **细胞凋亡不足**：肿瘤

凋亡抑制基因(如*Bcl-2*基因)过高表达,促凋亡基因(如*p53*基因、*Bax*基因)突变或缺失,导致细胞增殖过度,而细胞凋亡不足,引起肿瘤发生。





# Summary

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- 1. Concepts of tumor, oncogenes, proto-oncogenes and tumor suppressor genes**
- 2. Differences between *onc* and *p-onc*, *onc* and tumor suppressor genes**
- 3. Categories of (products of) oncogenes**
- 4. Mechanisms of cancerous induced by oncogenes and tumor suppressor genes**
- 5. Differences between apoptosis and necrosis**
- 6. The relationship between apoptosis and human disease**