Chapter 11
Translational control

- **Advantage** of translational regulation:
  Respond very rapidly to external stimuli

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1. Translational control in prokaryotes

1.1 The structure of mRNA

- The structure formed by 5'-UTR of the mRNA may obscure (隐藏) ribosome binding sites, thus reducing translation.

- The formation of stems and loops can inhibit exonuleases and give certain regions of the polycistronic mRNA a greater half-life.

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1.2 Ribosomal proteins

- The control of the translation of ribosomal protein mRNAs is the result of **autorepression**.
- If there is insufficient rRNA available for the translation product to bind to, ribosomal proteins will bind to its own mRNA and prevent further translation.

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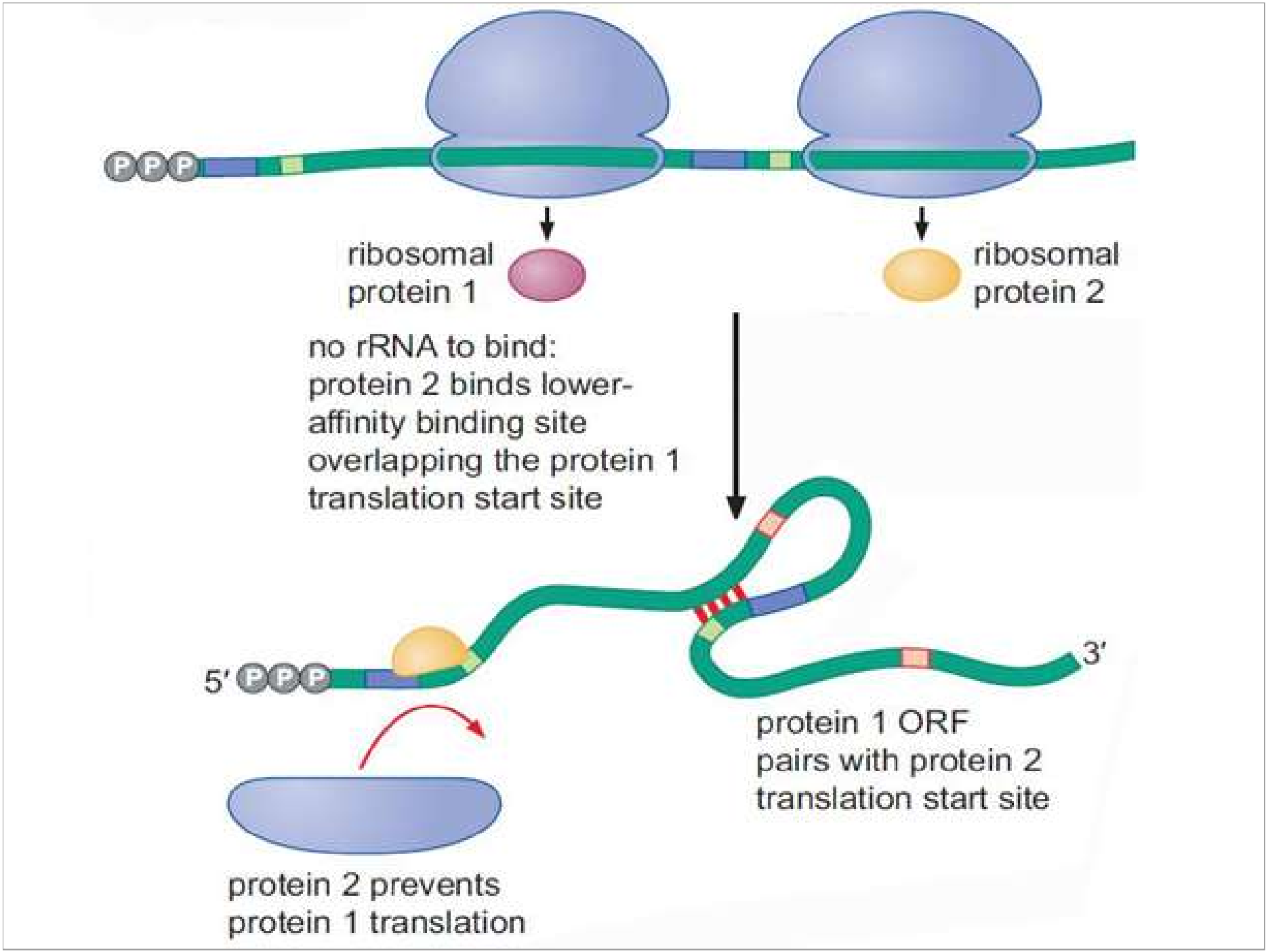
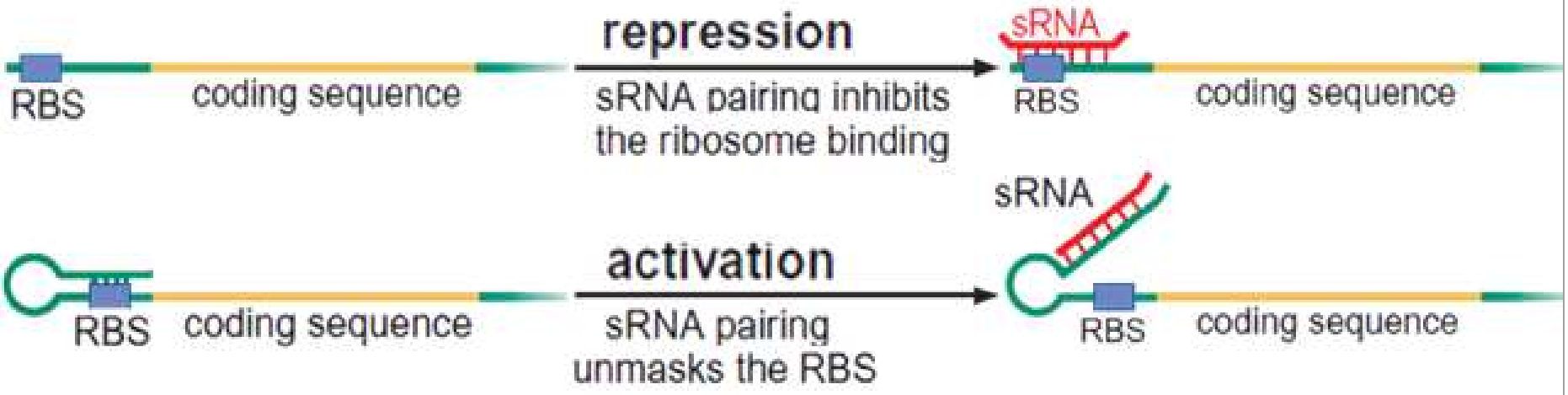

ribosomal protein 1
ribosomal protein 2
no rRNA to bind:
protein 2 binds lower-affinity binding site overlapping the protein 1 translation start site
5′
3′
protein 1 ORF pairs with protein 2 translation start site
protein 2 prevents protein 1 translation

1.3 Antisense RNA

- **Antisense RNA is a single stranded RNA that is complementary to a protein coding messenger RNA (mRNA) with which it hybridizes.**
- **Repress or active gene expression**



1.4 Riboswitches (核糖开关)

- A riboswitch is a regulatory segment of a messenger RNA molecule that binds a small molecule, resulting in a change in production of the proteins encoded by the mRNA.
- Typically turn off gene expression in response to the small molecule, but some turn it on.

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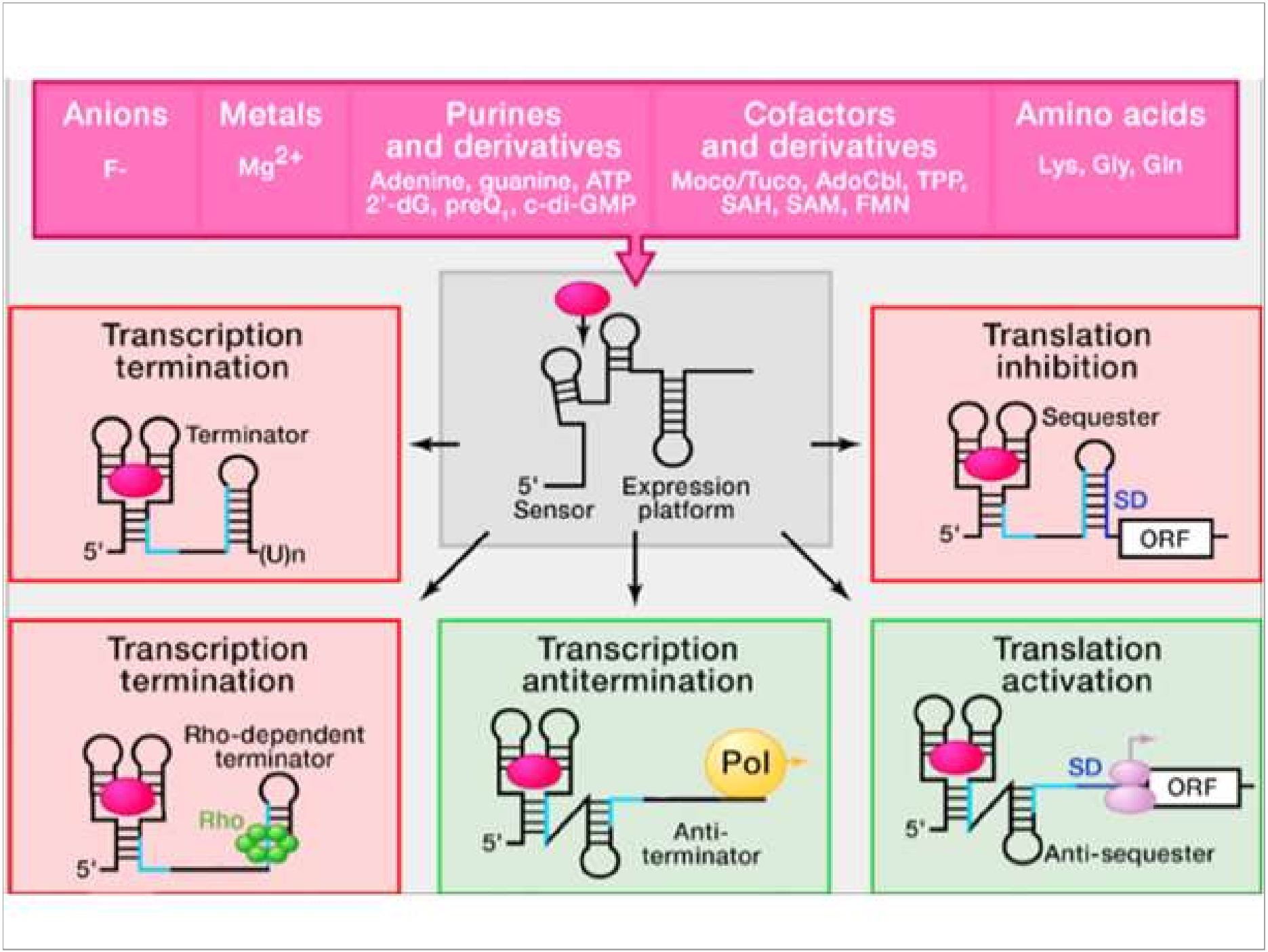
Anions
F-
Metals
Mg^{2+}
Purines and derivatives
Adenine, guanine, ATP, 2'-dG, $preQ_1$, c-di-GMP
Cofactors and derivatives
Moco/Tuco, AdoCbl, TPP, SAH, SAM, FMN
Amino acids
Lys, Gly, Gln
Transcription termination
Terminator
5'
(U)n
5'
Sensor
Expression platform
Translation inhibition
Sequester
SD
5'
ORF
Transcription termination
Rho-dependent terminator
Rho
5'
Transcription antitermination
Pol
5'
Anti-terminator
Translation activation
SD
ORF
5'
Anti-sequester

2. Translational control in eukaryotes

2.1 The structure of mRNA

- The repeats of the sequence 5'-AUUUA-3' in the 3'-noncoding region marks the mRNA for rapid degradation and thus limited translation.

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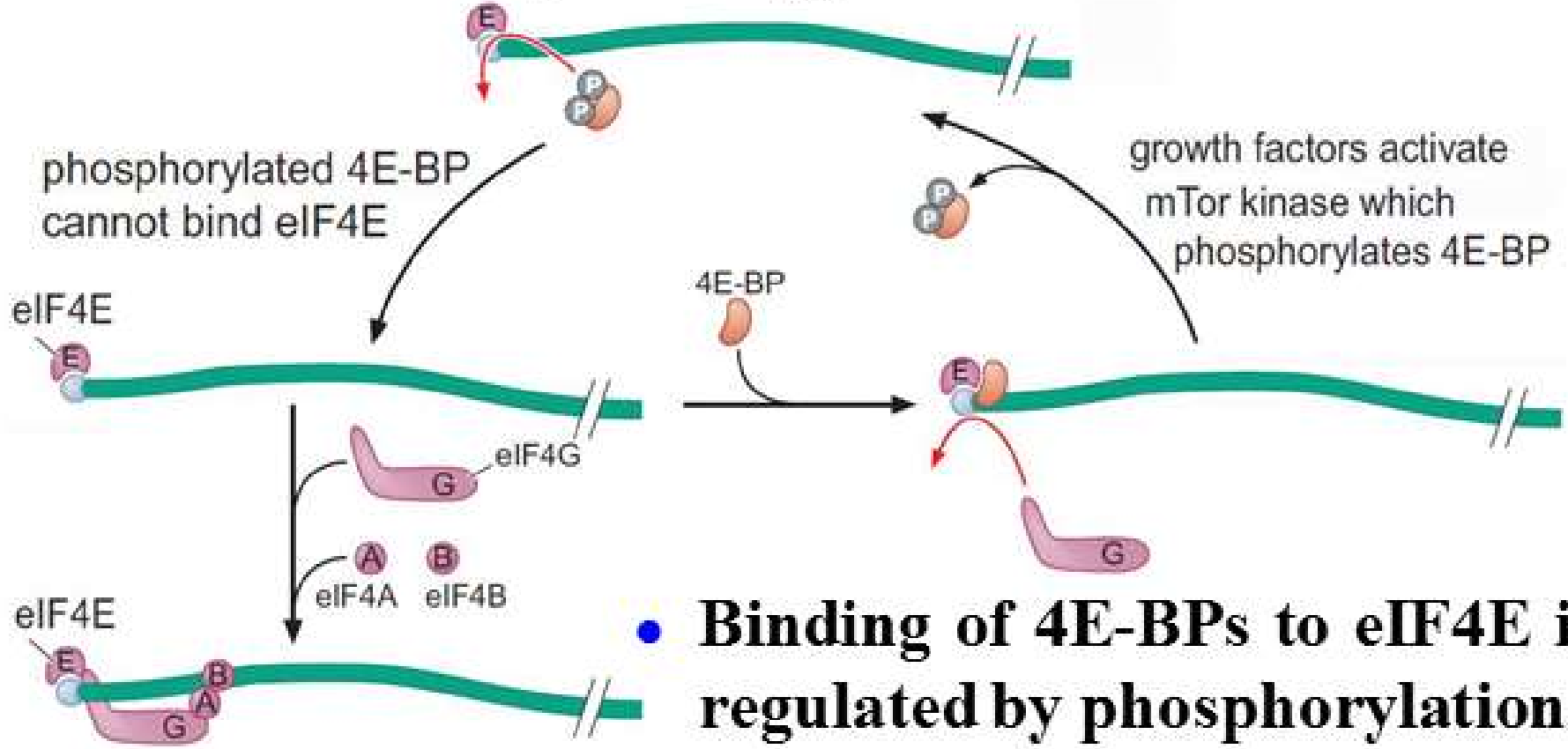
2.2 Spatial control of translation by eIF4E-binding proteins (4E-BPs)

- **4E-BPs compete with eIF4G for association with the cap-binding protein eIF4E.**



- **Binding of 4E-BPs to eIF4E is regulated by phosphorylation.**

2.3 Regulation of transferrin receptor and ferritin translation by iron

- Iron is essential for the activity of some proteins, but harmful in excess.
- Iron is transported into cells by the transferrin receptor (TfR, 转铁蛋白受体) and is stored within cells bound to ferritin (铁蛋白)
- Iron ↑ ——TfR ↓ , ferritin ↑
  Iron ↓ ——TfR ↑ , ferritin ↓

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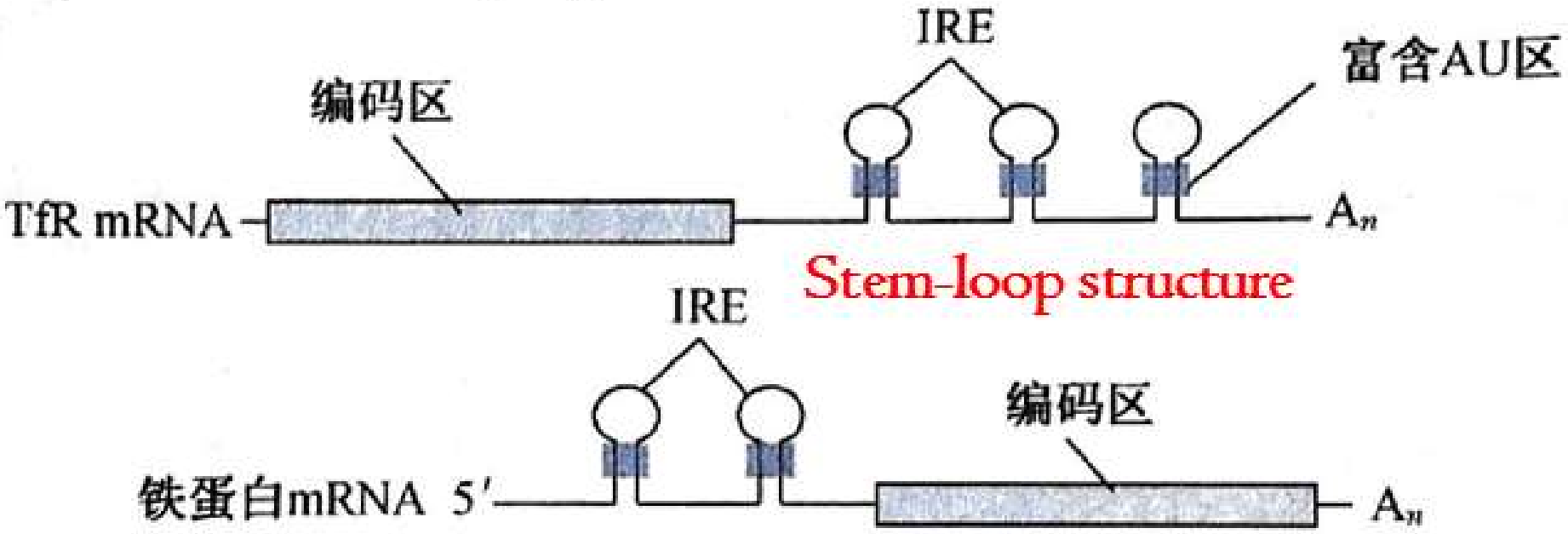
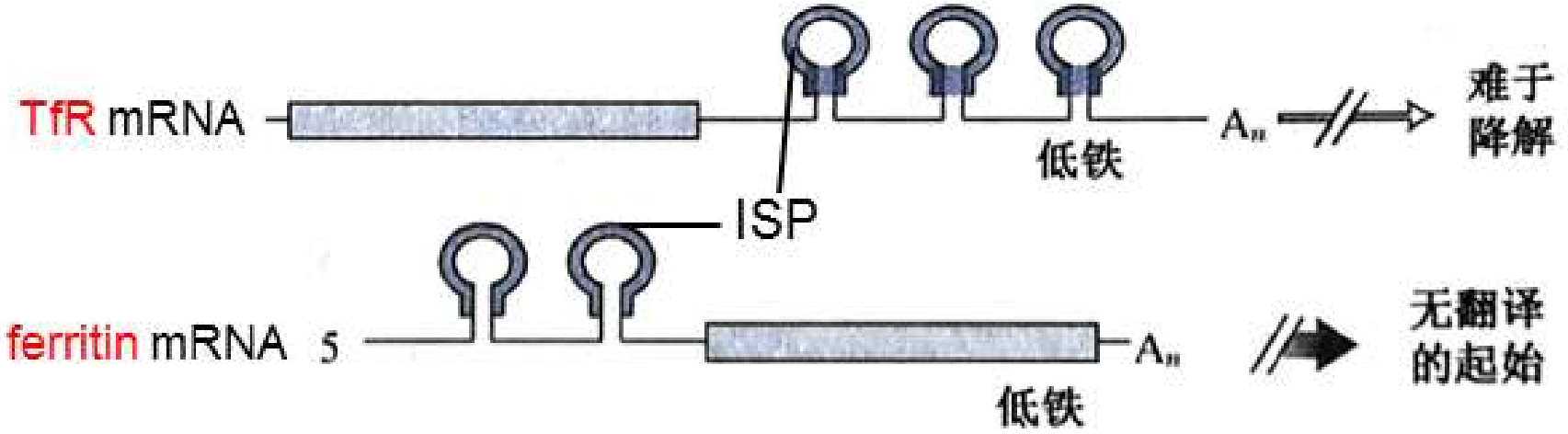
2.3.1 Iron response element (IRE, 铁应答元件)

- In the TfR mRNA, the IRE is in the 3' noncoding region.
- In the ferritin mRNA, the IRE is in the 5' noncoding region.

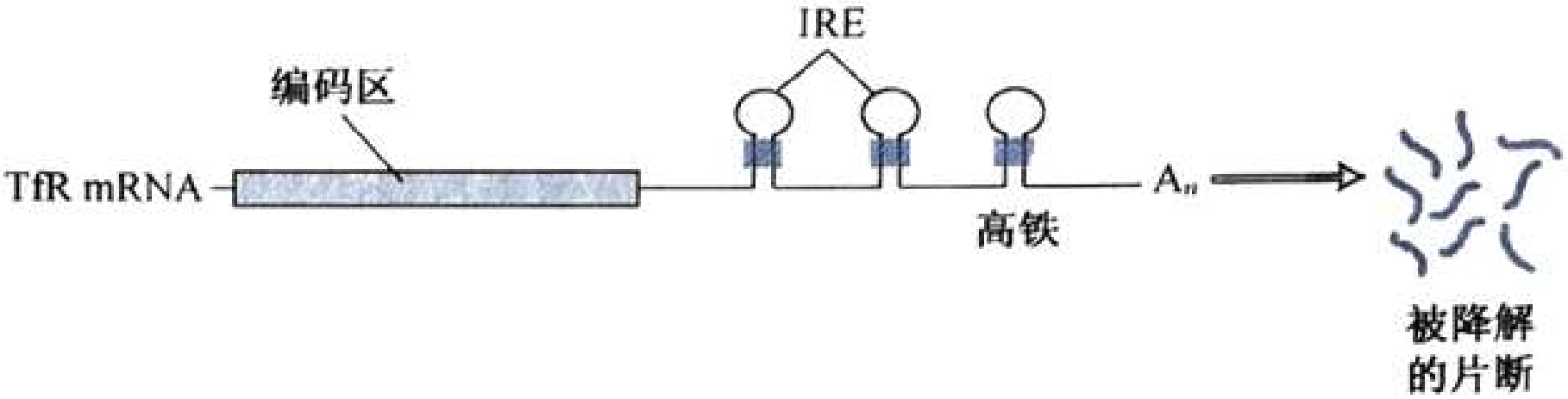


2.3.2 Iron sensing/response protein (ISP/IRP, 铁感应/应答蛋白)

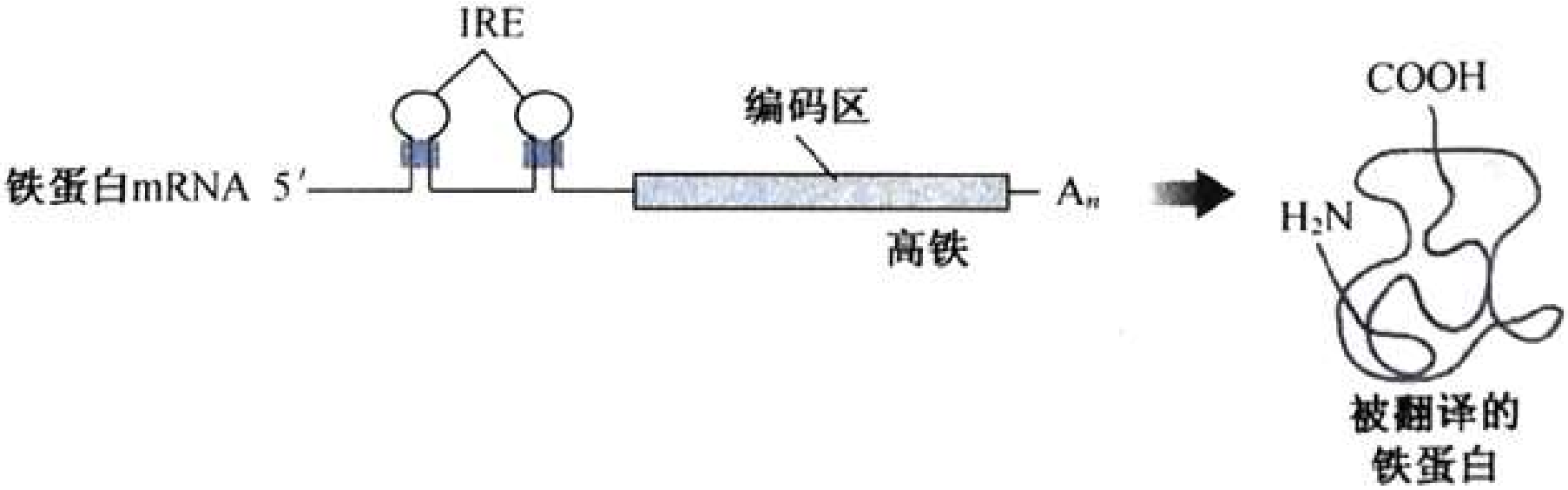
- ISP binds to the IRE when iron is scare.
- The bound ISP to the IRE stabilizes the TfR mRNA and allows more translation, while it reduces the ribosome's ability to translate the ferritin mRNA.



- ISP can not bind to the IRE when iron levels are high.
- IRE without bound of the ISP unmasks destabilizing sequences in TfR mRNA, thus reducing translation due to mRNA degradation caused by nucleases.



- **IRE without bound of the ISP cause an increase in translation of ferritin because the ribosome's progress is not hindered.**



2.4 Regulatory RNAs

Regulatory RNAs are non-coding RNAs that can regulate gene expression.

- **Regulatory RNAs in bacteria**
  - sRNA (antisense RNA, CRISPR)
  - Riboswitch ← 真核少见
- **Regulatory RNAs in eukaryotes**
  - microRNAs (miRNAs)
  - Small interfering RNA (siRNAs)
  - lncRNAs
  - ceRNAs (竞争性内源RNA)

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2.4.1 miRNAs

- microRNAs (miRNAs) are endogenous ~22-nt non-coding RNAs produced naturally in plant and animal cells by cleavage from a larger, stem-loop precursor.
- miRNAs base-pair with the specific mRNAs and silence gene expression by blocking translation or cleaving those mRNAs.

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(1) Discovery of miRNAs

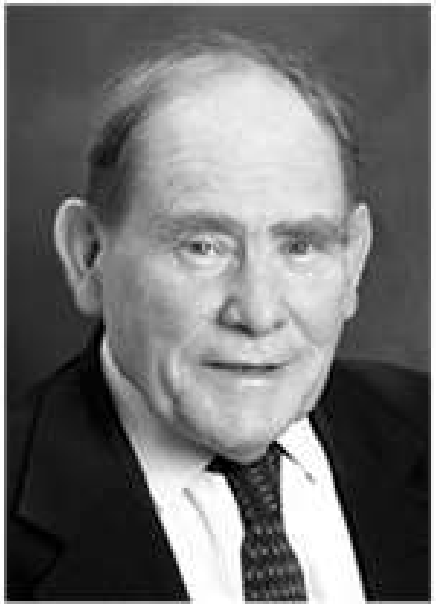
- 1989年，Victor发现线虫（*C. elegans*）*lin-4*基因可抑制另一个基因*lin-14*的表达.

Gene ID: 266860, updated on 4-Oct-2014

Gene symbol	lin-4
Gene description	ncRNA
Locus tag	CELE_F59G1.6
Gene type	ncRNA

```
1    atgcttccgg cctgttccct gagacctcaa
31   gtgtgagtgt actattgatg cttcacacct
61   gggctctccg ggtaccagga cggtttgagc
91   agat
```

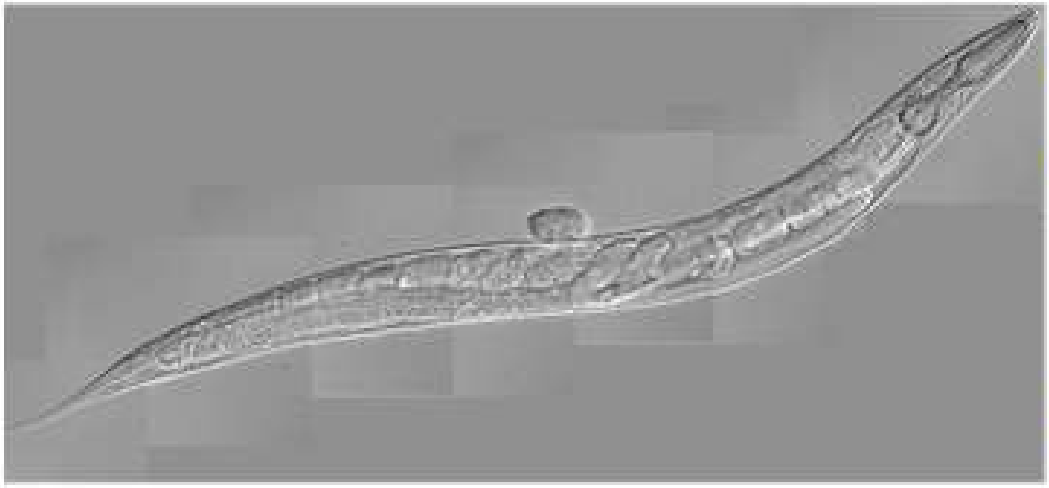
lin-4 miRNA UCCCUGAGACCUCAAGUGUGA



Sydney Brenner

Laboratory of Molecular Biology in Cambrige, UK

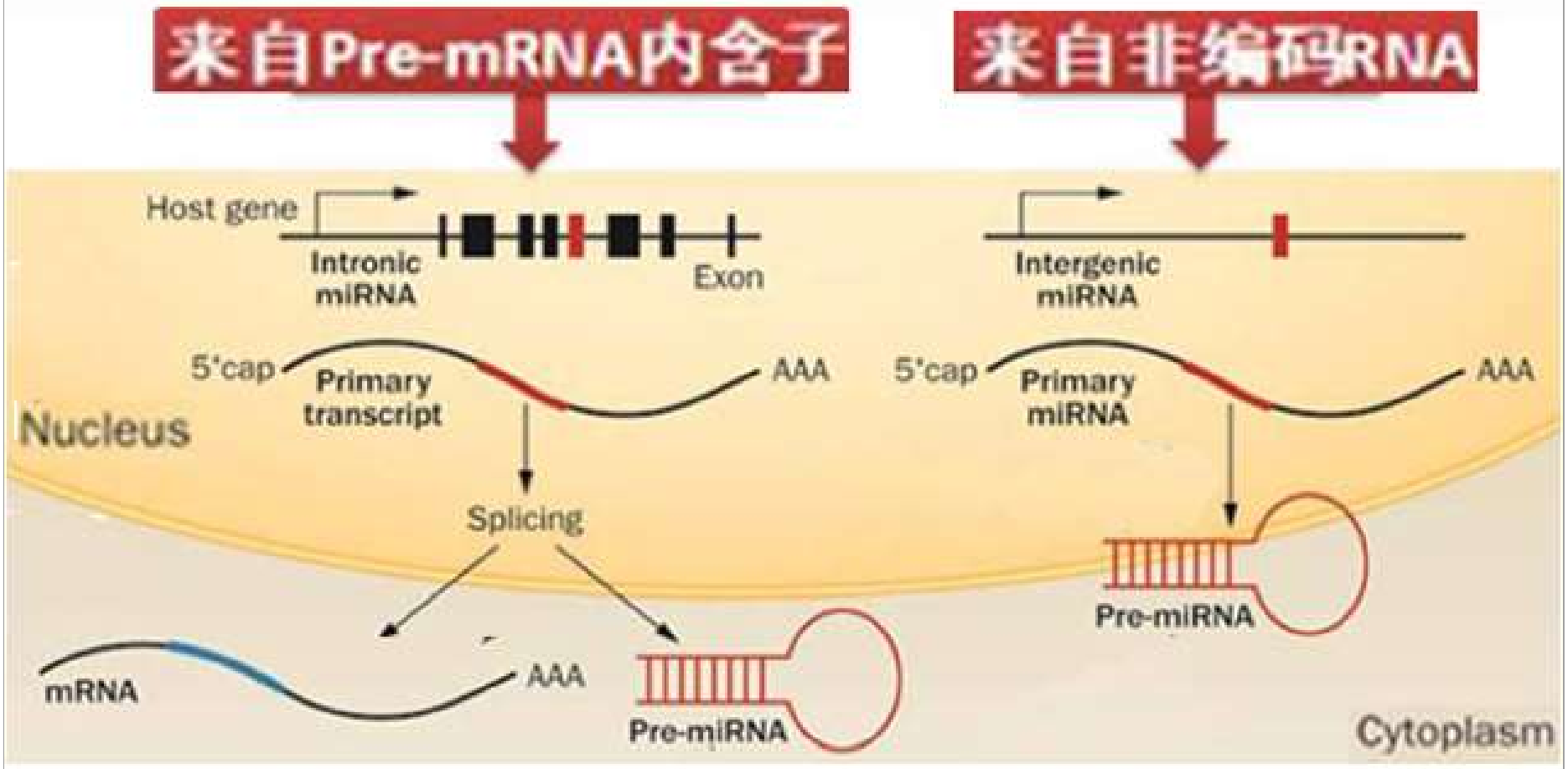
Established the nematode（线虫）(*C.elegans*) as a novel model organism (early 1960s)



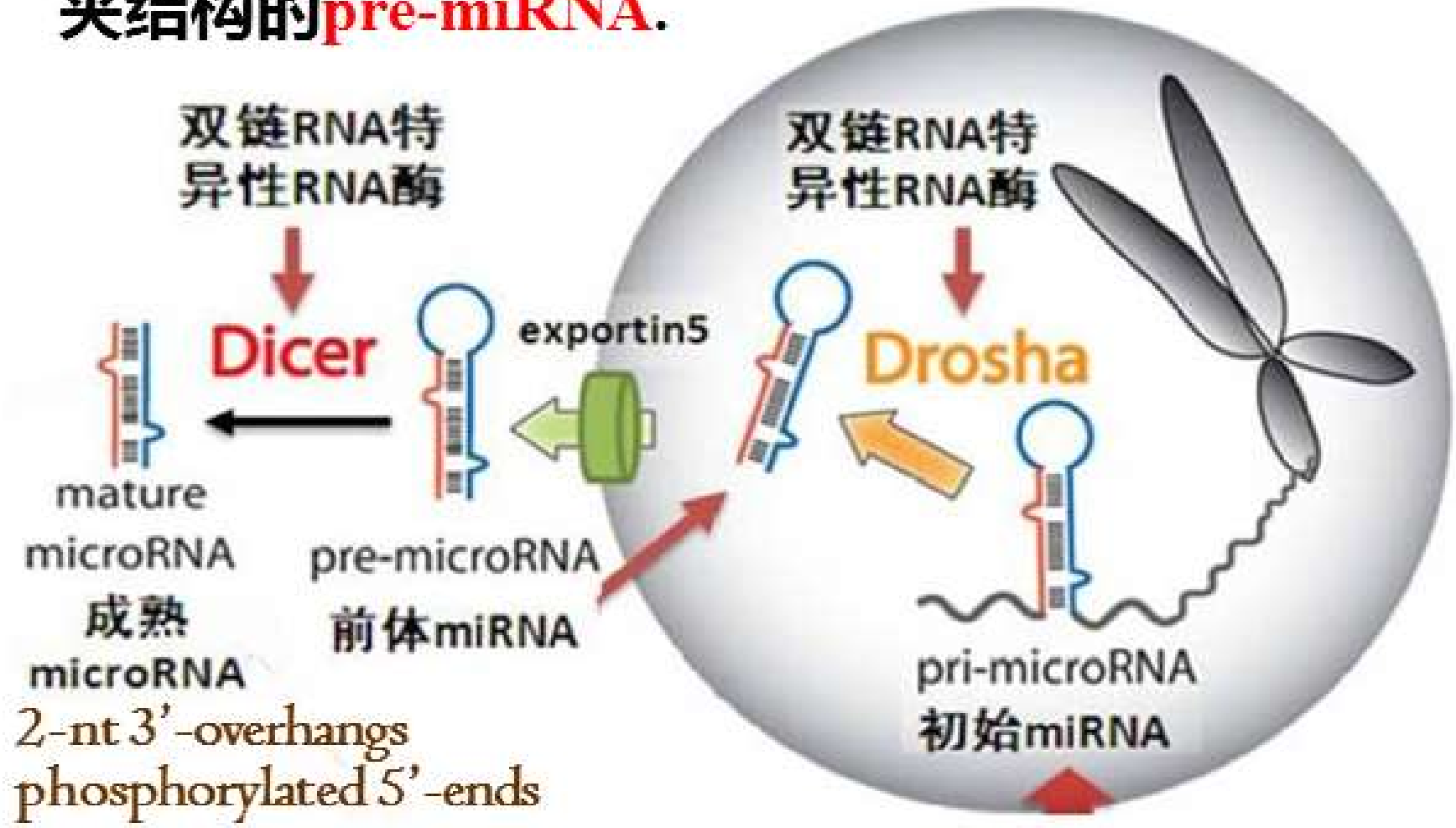
- 易观察——身体透明
- 世代周期短(胚胎至具生殖能力成虫只需3.5天)
- 易培养(25℃)、繁殖

(2) Synthesis of miRNAs

内源 endogenous



- Pri-miRNA在核内被Drosha识别并切割，形成发夹结构的pre-miRNA.



- Pre-miRNA在细胞质内被Dicer识别并切割，形成~22bp的成熟ds miRNA.

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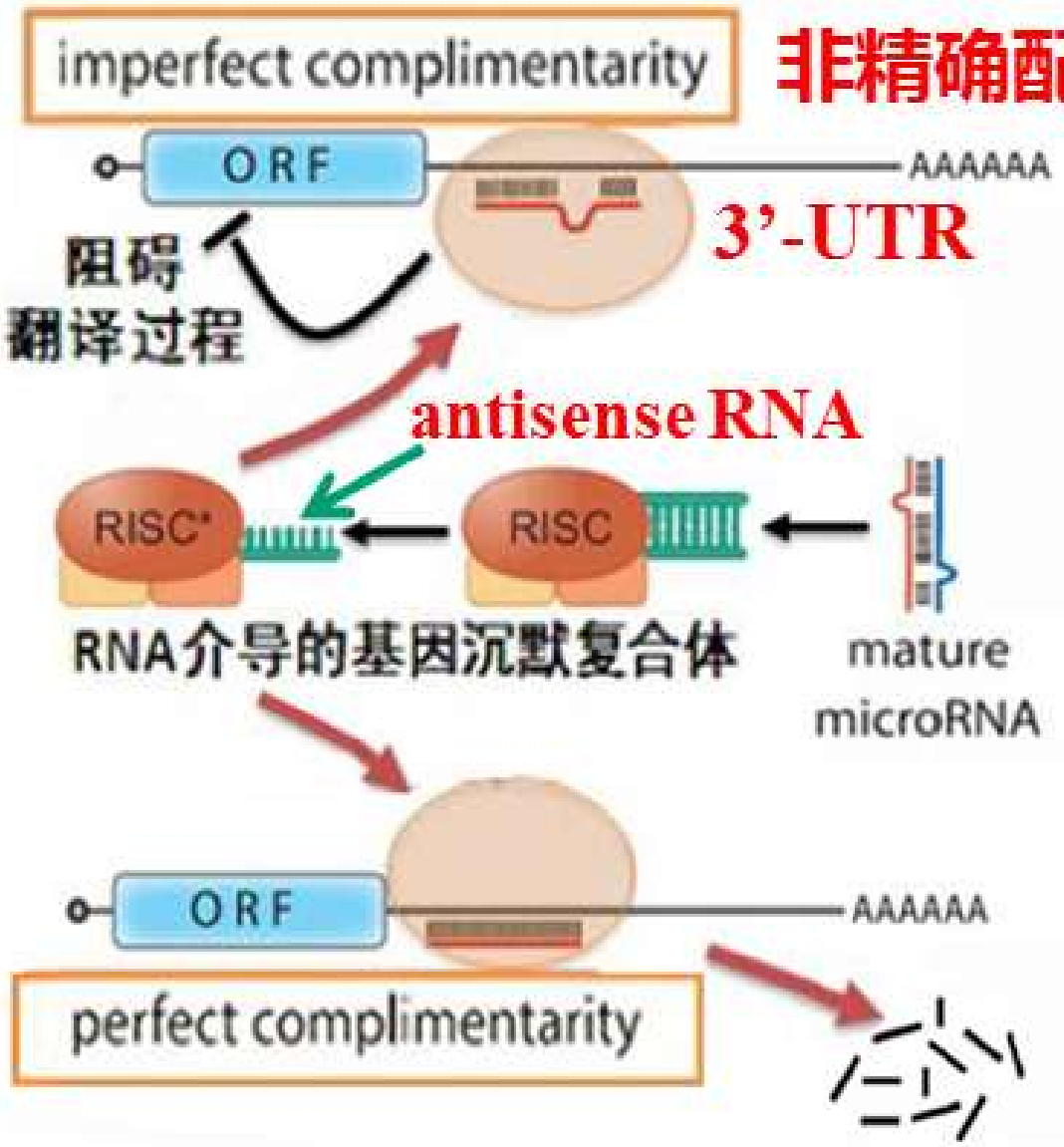
(3) Gene silencing by miRNAs

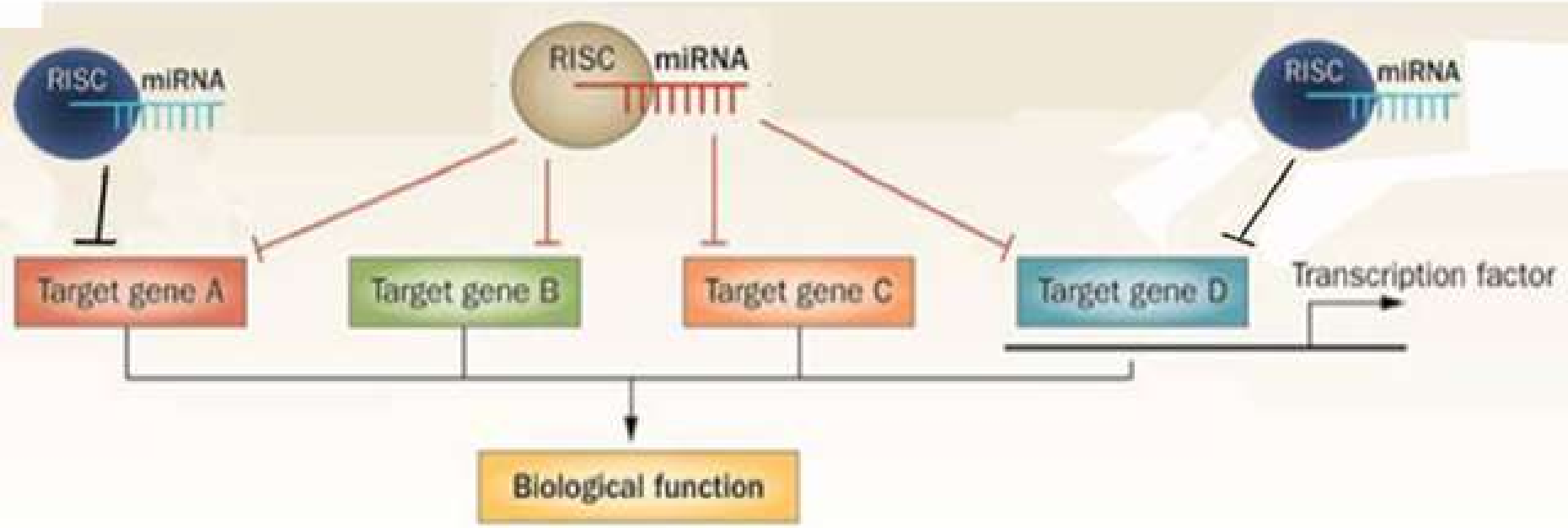


RNA-induced silencing complex, RISC

RISC = miRNA + Dicer + Dicer-associated protein + Argonaute (Ago)

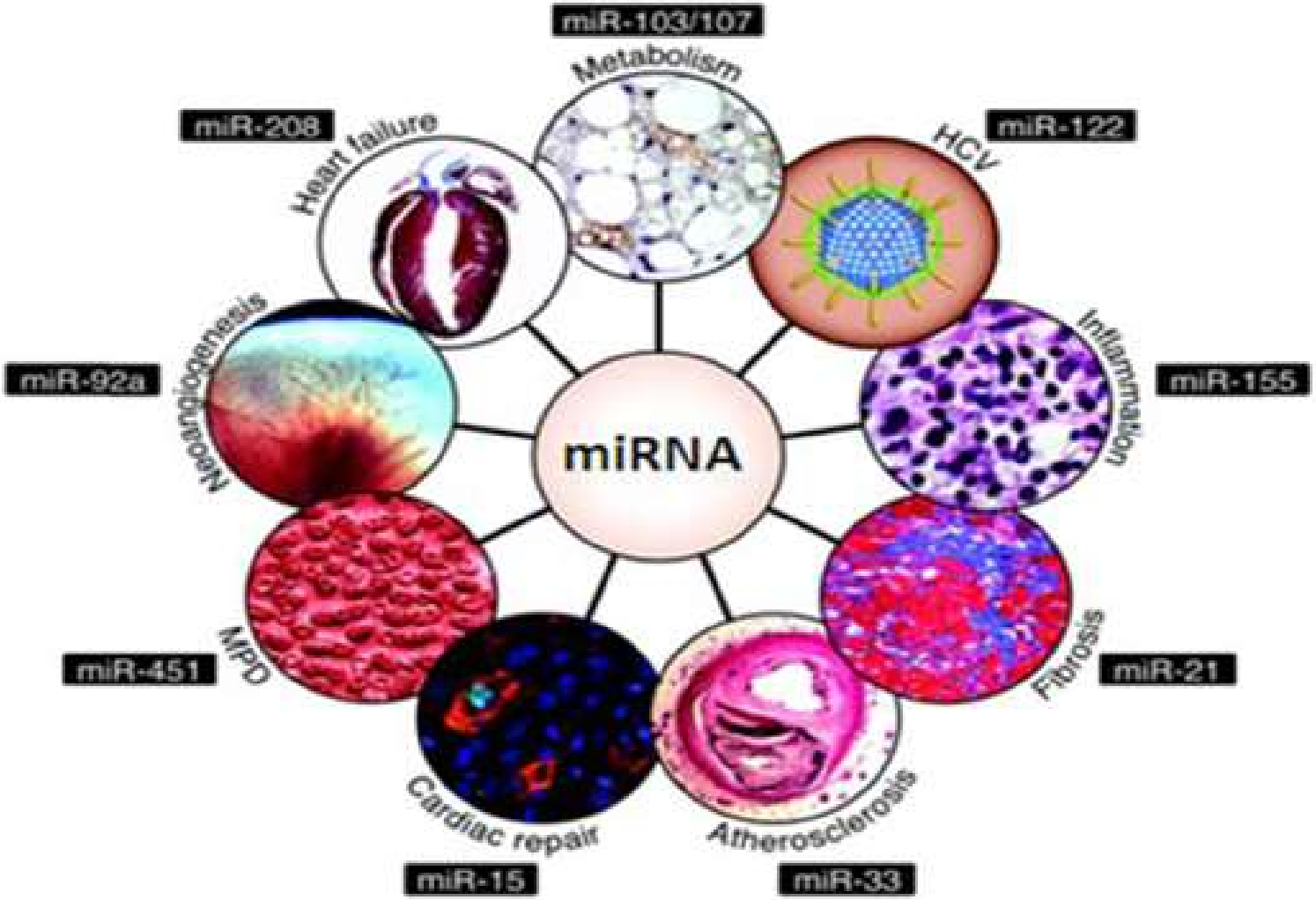
精确配对，切割靶mRNA

- 一个miRNA通常可以调控数十个基因。每个miRNA可以有多个靶基因，而几个miRNA也可以调节同一基因。



(4) Functions of miRNA

- 调控细胞周期、细胞凋亡和个体发育等。



2.4.2 Small interfering RNA (siRNA)

(1) Discovery of RNAi

1990年

Richard Jorgensen

查尔酮合酶基因

- 导入色素合成关键酶基因反而抑制色素合成基因的表达。

Cell, Vol. 81, 611–620, May 19, 1995, Copyright © 1995 by Cell Press

par-1, a Gene Required for Establishing Polarity in C. elegans Embryos, Encodes a Putative Ser/Thr Kinase That Is Asymmetrically Distributed

Su Guo and Kenneth J. Kemphues
Section of Genetics and Development
Cornell University
Ithaca, New York 14853

appear to play a role in at least the first division. Brief pulses of the microfilament-disrupting drug cytochalasin during a critical period of the first cell cycle prevent the posterior localization of the P granules (Strome and Wood, 1983; Hill and Strome, 1988). Cytochalasin pulses during

- **向细胞中注入sense RNA，可以像antisense RNA一样阻断特定基因的表达。**

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

** Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, USA*
† Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA
‡ Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA

NATURE | VOL 391 | 19 FEBRUARY 1998

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

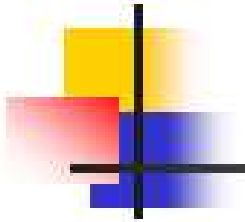
Photo: L. Cicero/Stanford

Andrew Z. Fire

Photo: R. Carlin/UMMAS

Craig C. Mello

- **Double-stranded RNA (dsRNA) blocked specific gene expression much better than either sense or antisense RNA.**



- RNA interference (RNAi) is a biological process triggered by the introduction of double-stranded RNA (dsRNA) which leads to gene silencing in a sequence-specific manner, typically by causing the destruction of specific mRNA molecules.

  RNA干扰是一种由双链RNA 所引起的序列特异性基因沉默，这种基因沉默通常由mRNA特异性降解造成。

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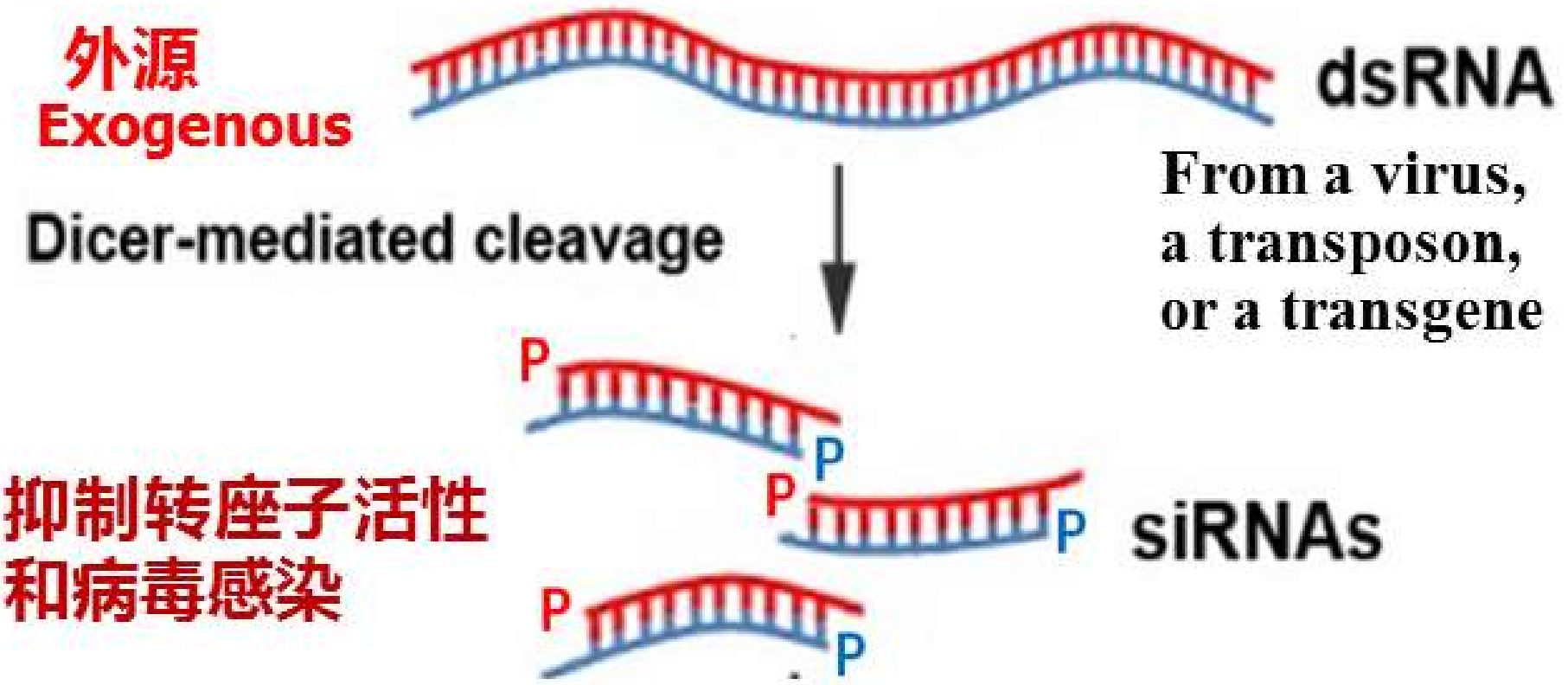
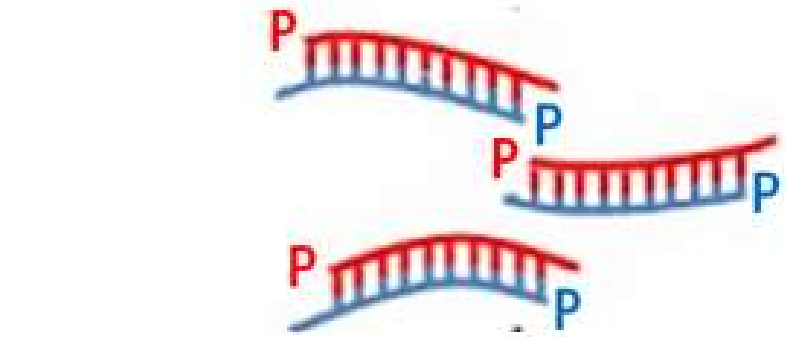
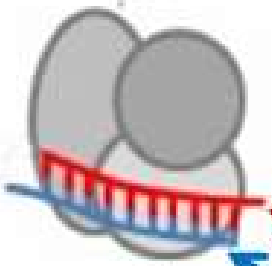
(2) Mechanism of RNAi mediated by siRNA



- Dicer 识别切割外源ds RNA为21–23 mer siRNAs.

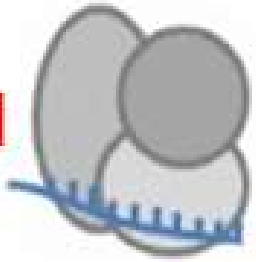


RISC assembly



RISC

RISC activation



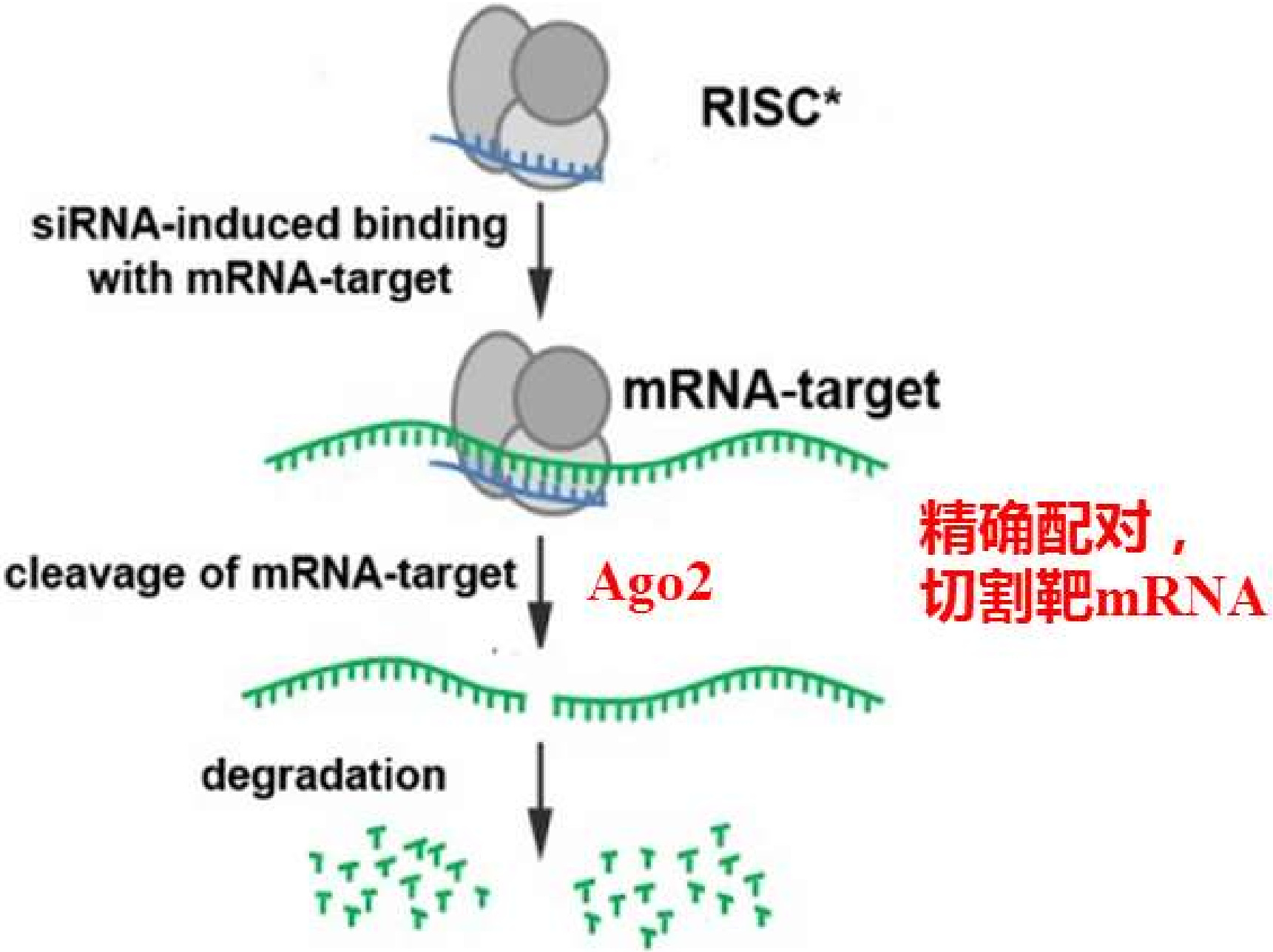
guide strand
引导链

RISC*

- RISC = siRNA + Dicer + Dicer-associated + Ago2
- ds siRNA→ss siRNA

passenger strand (sense strand) - discarded

guide strand (antisense strand)


RISC*
siRNA-induced binding
with mRNA-target
mRNA-target
cleavage of mRNA-target
Ago2
精确配对，
切割靶mRNA
degradation

miRNA与siRNA的相同之处

- 长度都约在22nt左右。
- 都依赖Dicer酶的加工，是Dicer的产物。
- 都和Argonaute等蛋白形成RISC复合物。
- 都可以对靶基因的表达起负调控作用。

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miRNA 与 siRNA 的不同之处

	miRNA	siRNA
来源		
结构		
作用位置		
作用方式		
生物学意义		

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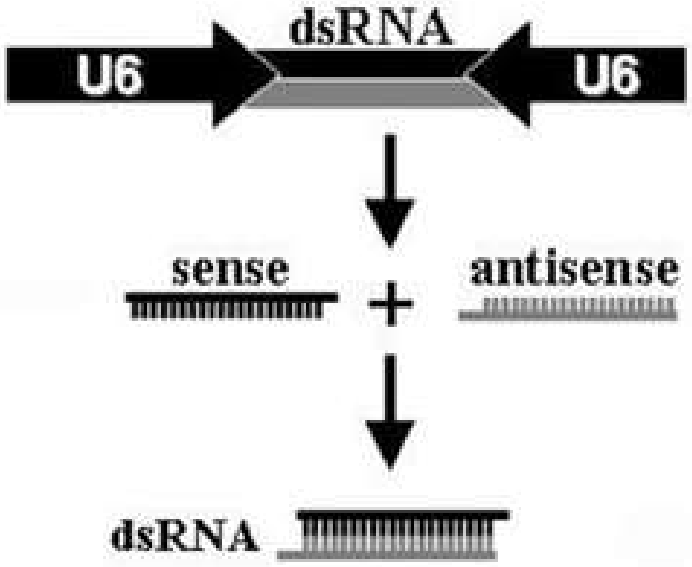
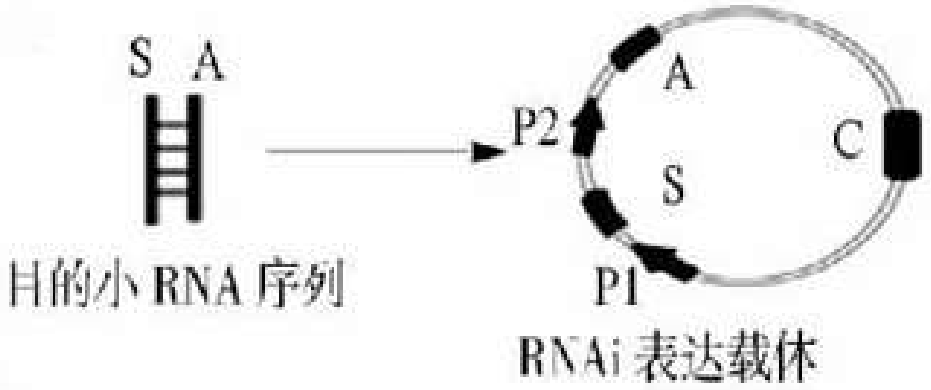
2.4.3 Application of RNAi

(1) Inactivate genes at will (随意)

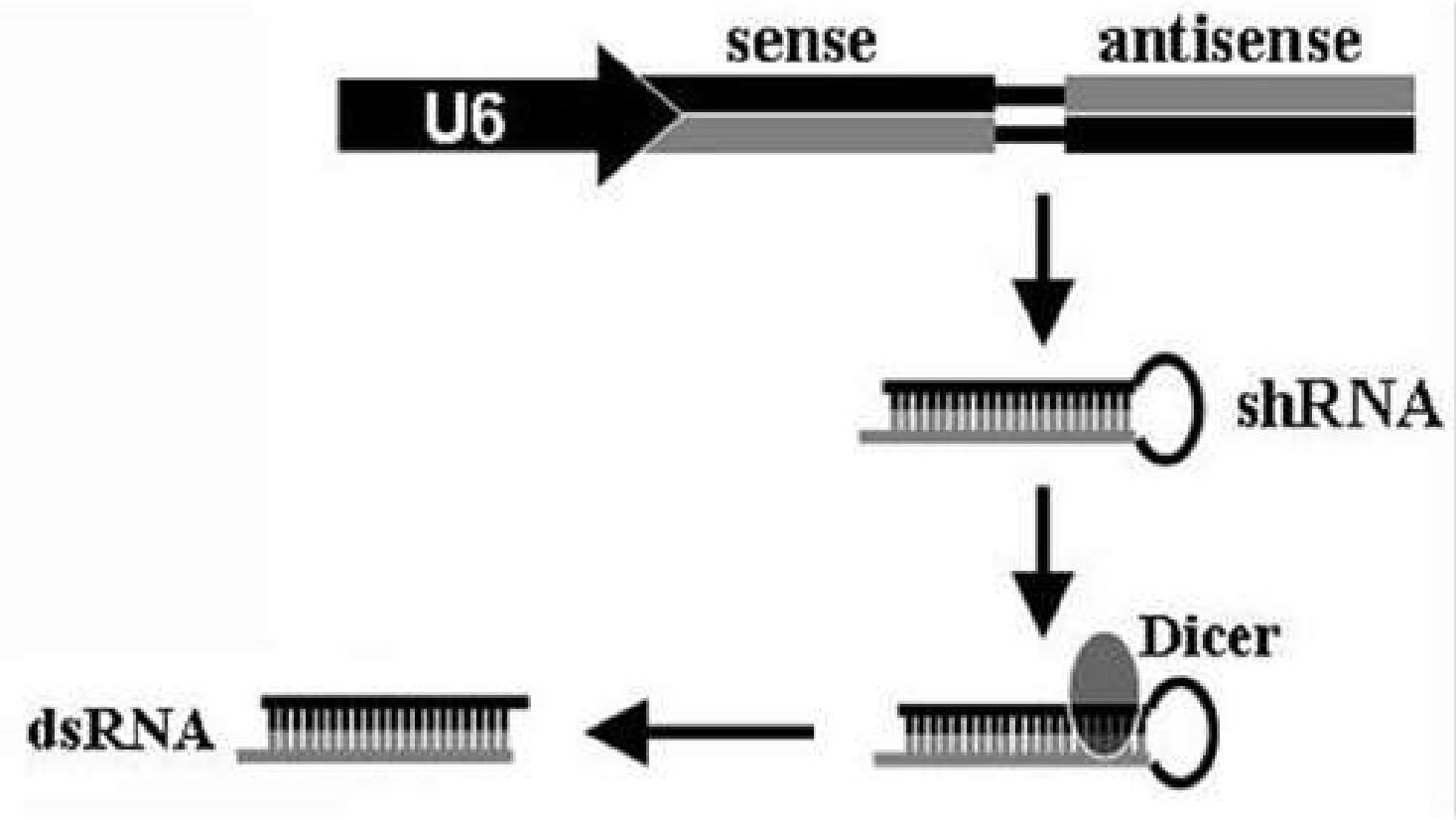
引入siRNA的方法：

- 人工合成两条siRNA
- 构建siRNA表达载体





➢ 构建shRNA表达载体



(2) Cancer control (siRNA target to oncogene)

LETTERS

nature biotechnology

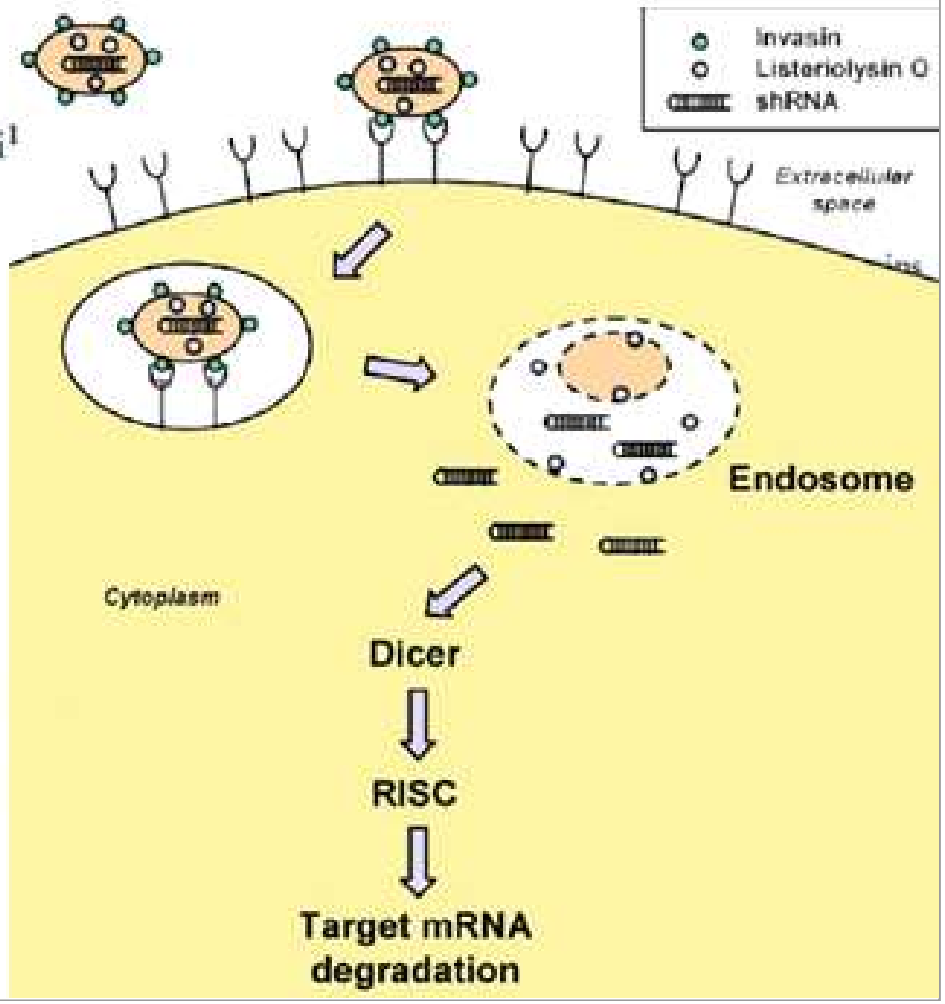
NATURE BIOTECHNOLOGY VOLUME 24 NUMBER 6 JUNE 2006

Trans-kingdom RNAi

Short hairpin RNA–expressing bacteria elicit RNA interference in mammals

Shuanglin Xiang[1,2], Johannes Fruehauf[1,2] & Chiang J Li[1]

RNA-interference (RNAi) is a potent mechanism, conserved from plants to humans for specific silencing of genes, which holds promise for functional genomics and gene-targeted therapies. Here we show that bacteria engineered to produce a short hairpin RNA (shRNA) targeting a mammalian gene induce trans-kingdom RNAi *in vitro* and *in vivo*. Nonpathogenic *Escherichia coli* were engineered to transcribe shRNAs from a plasmid containing the invasin gene *Inv* and the listeriolysin O gene *HlyA*, which encode two bacterial factors needed for successful transfer of the shRNAs into mammalian cells. Upon oral or intravenous administration, *E. coli* encoding shRNA against *CTNNB1* (catenin β-1) induce significant gene silencing in the intestinal epithelium and in human colon cancer xenografts in mice. These results provide an example of trans-kingdom RNAi in higher organisms and suggest the potential of bacteria-mediated RNAi for functional genomics, therapeutic target validation and development of clinically compatible RNAi-based therapies.



Summary

1. Mechanisms of translational control of ribosomal proteins
2. Mechanisms of translational control by the structure of mRNA, antisense RNA, riboswitches, and eIF4E-binding proteins
3. Regulation of transferrin receptor and ferritin translation by iron
4. Mechanisms of gene silencing caused by siRNA and miRNA
5. Comparison between siRNA and miRNA

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