Corso di

Metodologie diagnostiche di Biochimica e di Biologia molecolare

modulo di Biologia molecolare

A.A. 2012/2013

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LEZIONE 9:

REGOLAZIONE POST-TRASCRIZIONALE DELL'ESPRESSIONE GENICA. MICRORNA E RNA INTERFERENCE

Lezione 9

(5 novembre 2012)

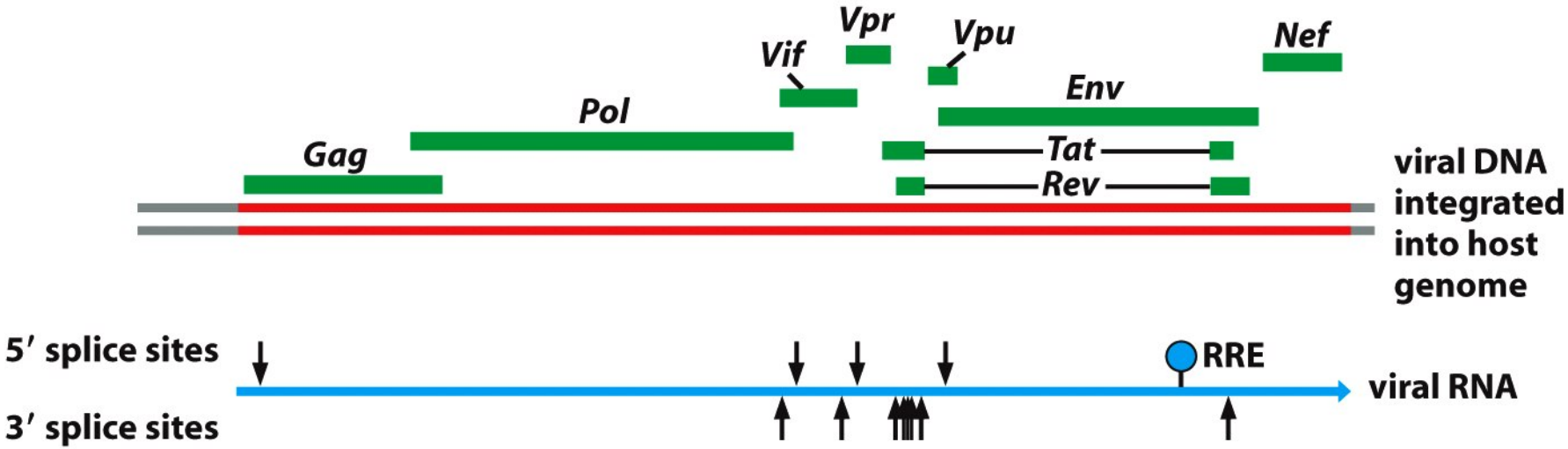
- La localizzazione dell'mRNA
- I meccanismi di controllo post-trascrizionale dell'mRNA
- RNA interference
- microRNA

Allison cap. 4, 9, 13

Alberts cap. 6

Watson cap. 7, 8

Il trasporto dal nucleo può essere regolato



Il genoma compatto di HIV

Figure 7-102 *Molecular Biology of the Cell* (© Garland Science 2008)

Il trasporto dal nucleo può essere regolato

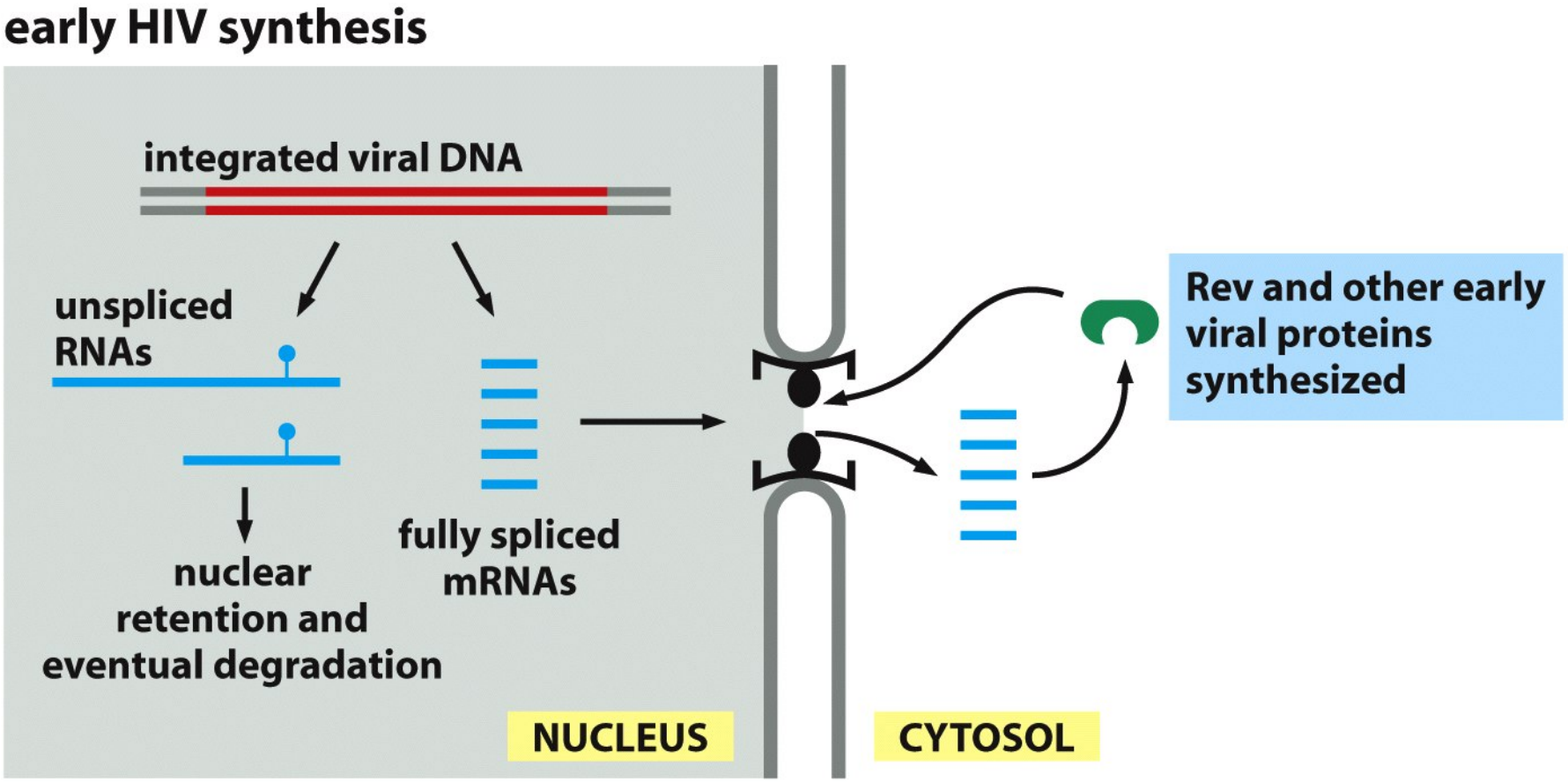


Figure 7-103a *Molecular Biology of the Cell* (© Garland Science 2008)

Il trasporto dal nucleo può essere regolato

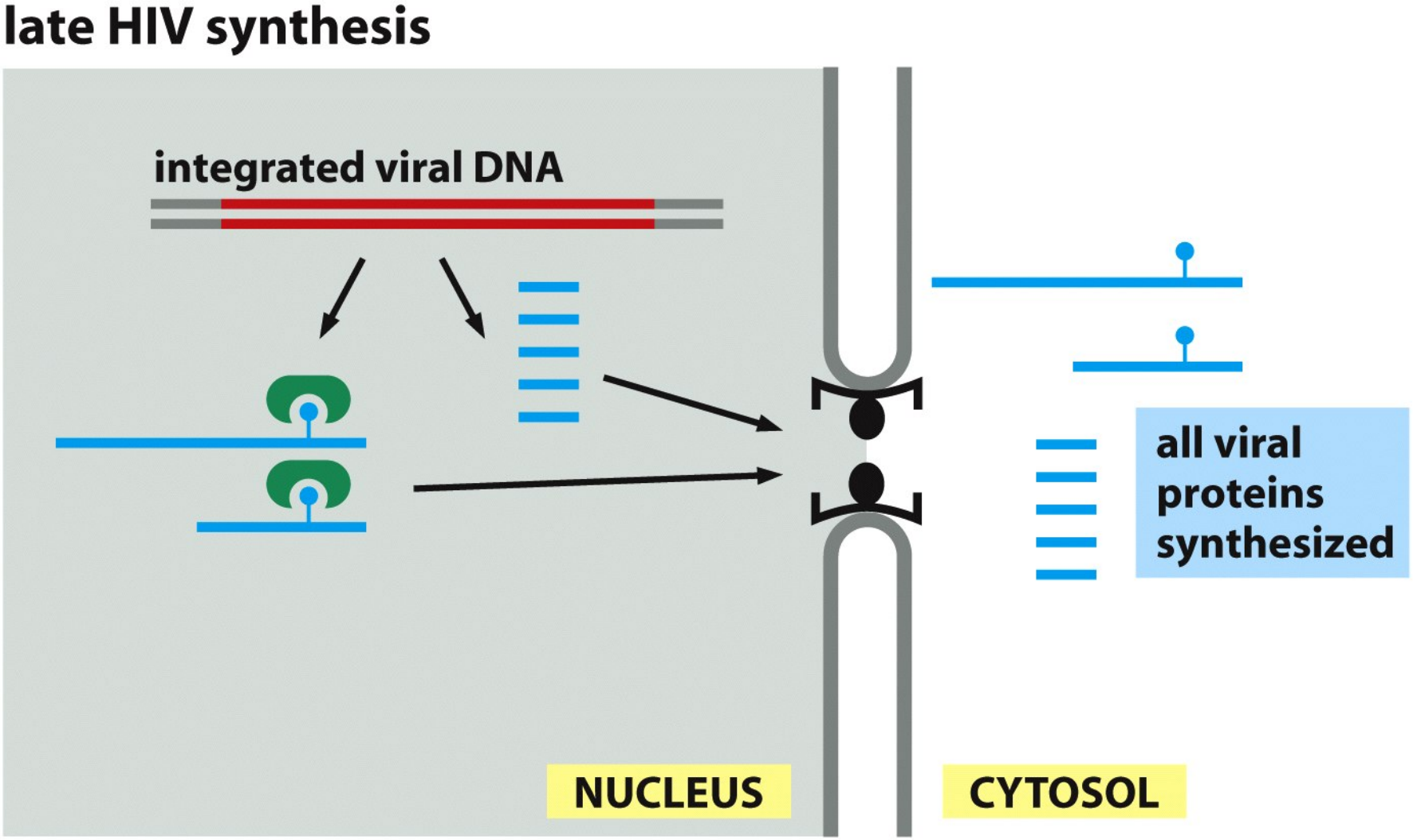


Figure 7-103b *Molecular Biology of the Cell* (© Garland Science 2008)

Alcuni mRNA sono localizzati in regioni specifiche del citoplasma (3'UTR)

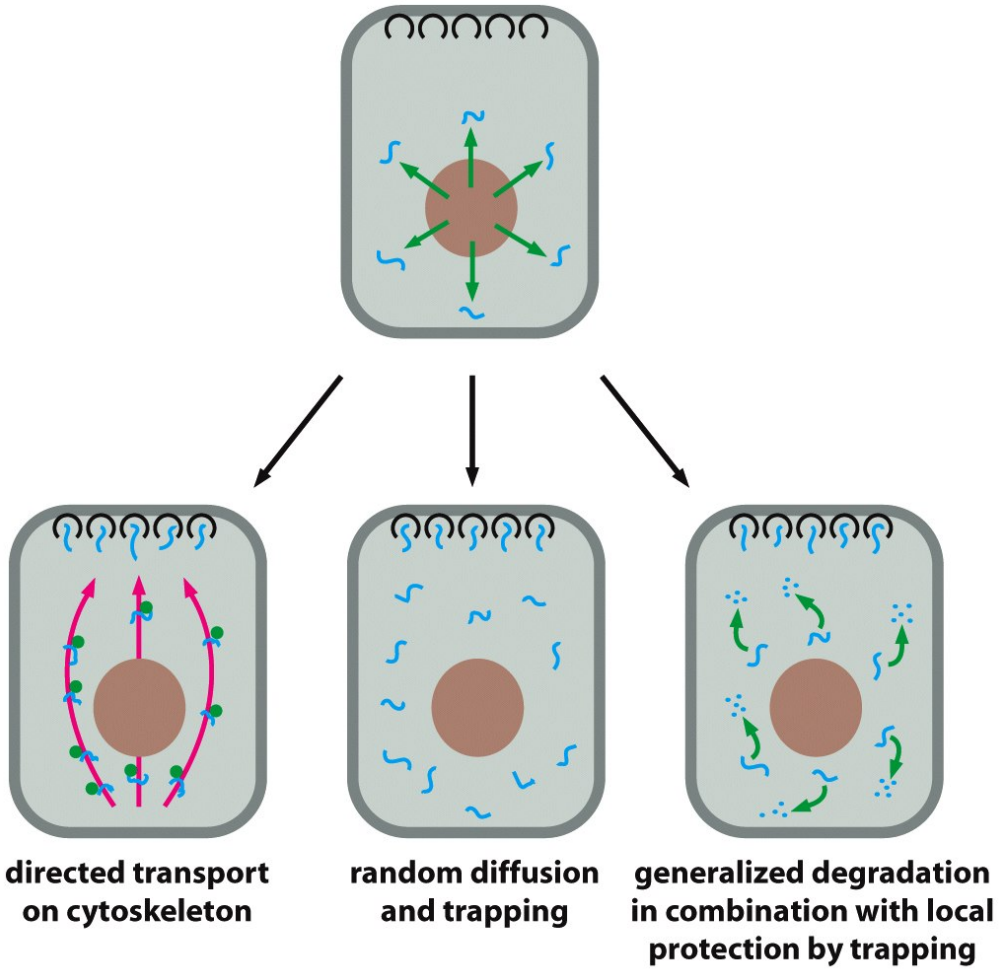
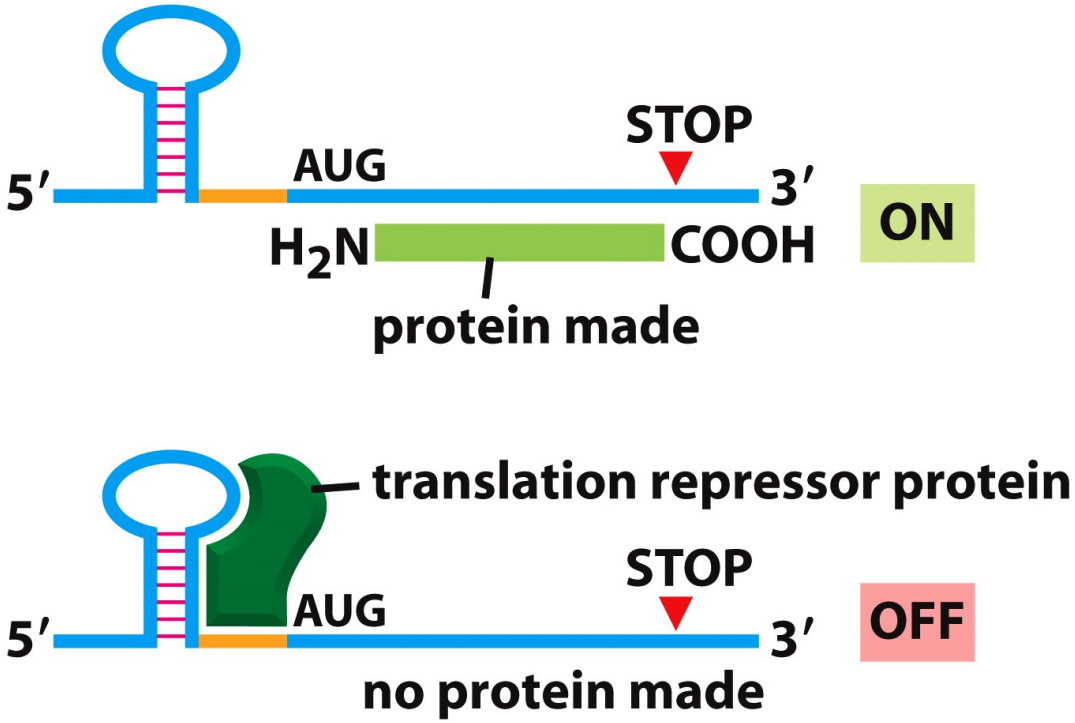


Figure 7-104 *Molecular Biology of the Cell* (© Garland Science 2008)

Le regioni non tradotte al 5’ e 3’ degli mRNA ne controllano la traduzione

Nei batteri



Es: proteine ribosomali controllano la propria traduzione

Figure 7-106a *Molecular Biology of the Cell* (© Garland Science 2008)

Termosensori

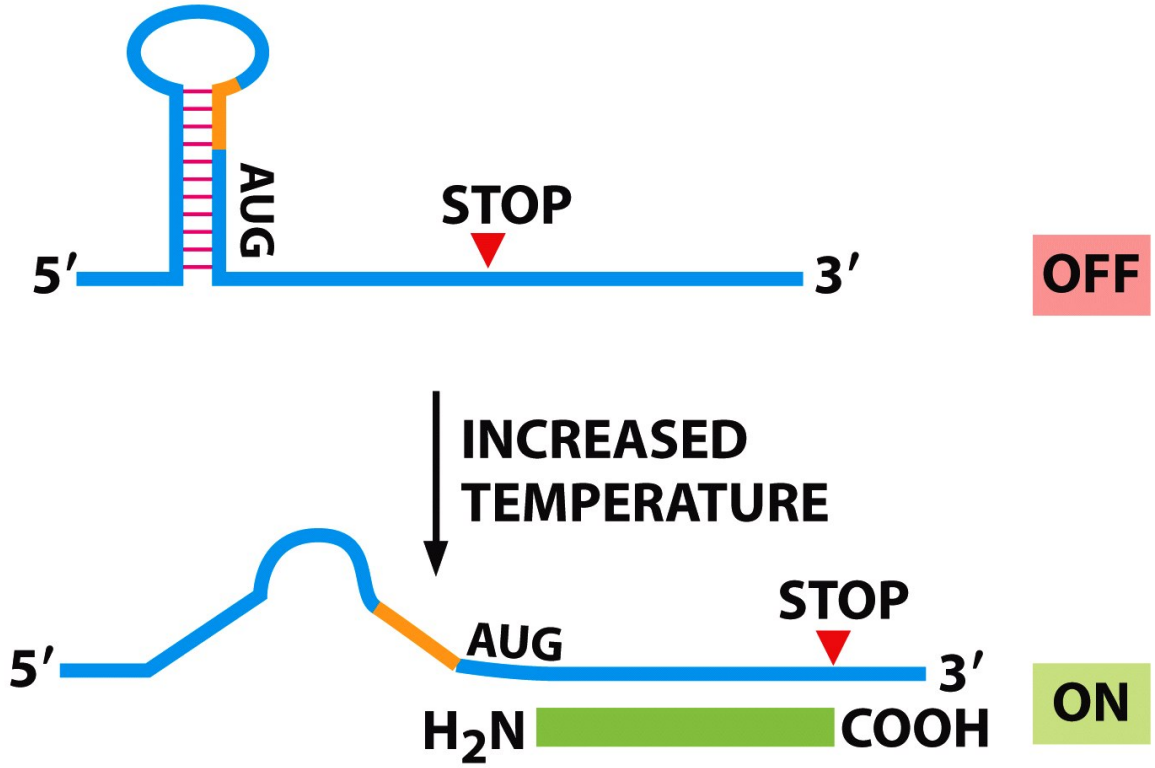


Figure 7-106b *Molecular Biology of the Cell* (© Garland Science 2008)

Ribointerruttori (*riboswitches)*

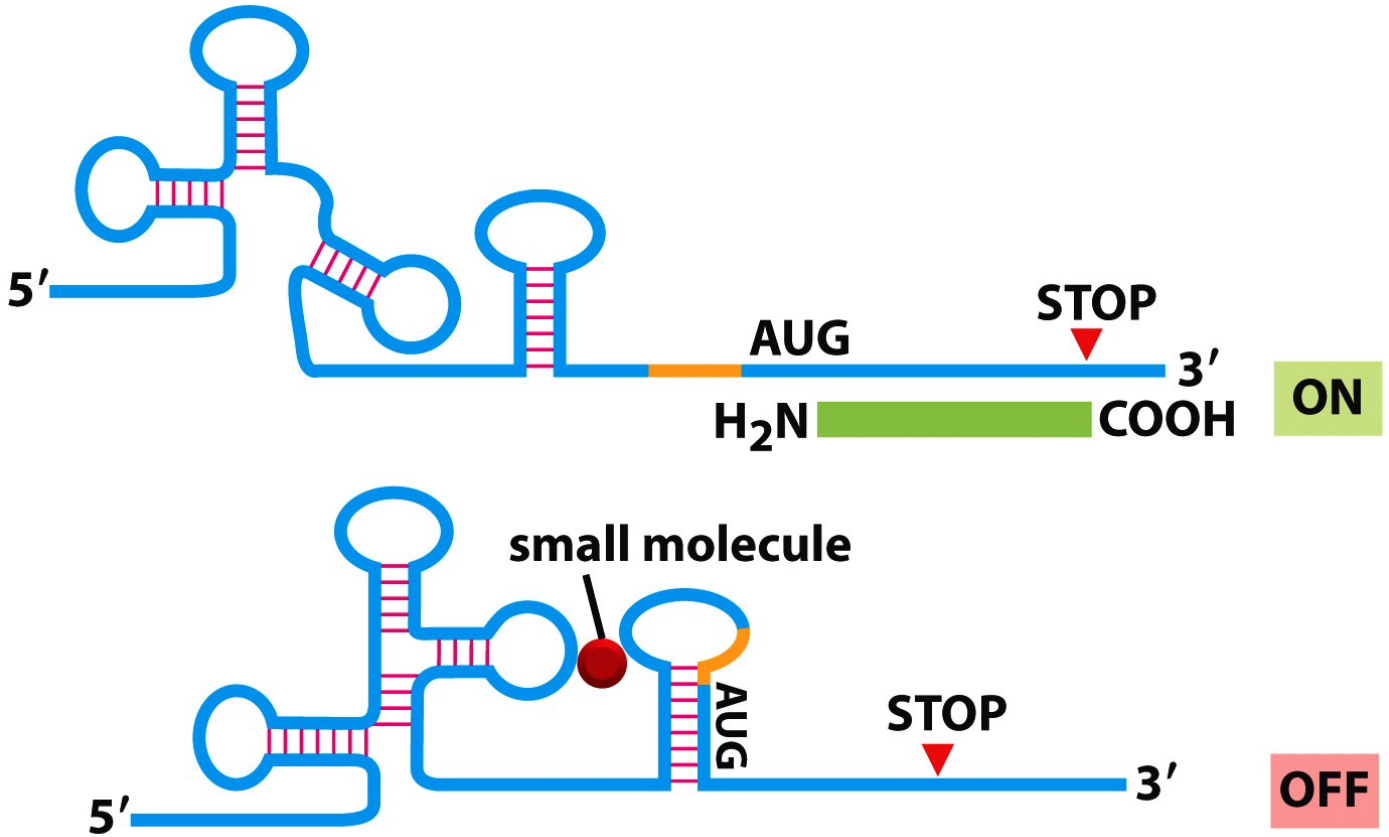
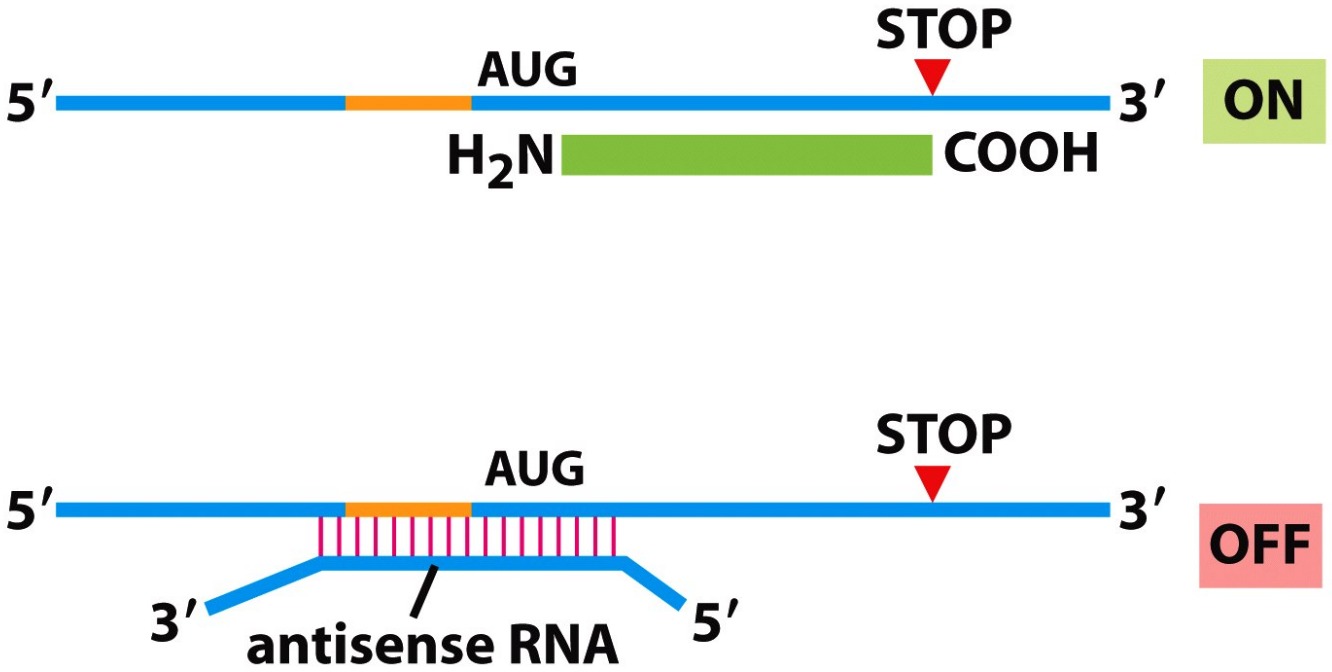


Figure 7-106c *Molecular Biology of the Cell* (© Garland Science 2008)

RNA antisenso



Es: proteine di deposito del ferro nei batteri

Figure 7-106d *Molecular Biology of the Cell* (© Garland Science 2008)

La fosforilazione di un fattore di inizio regola in modo globale la sintesi proteica

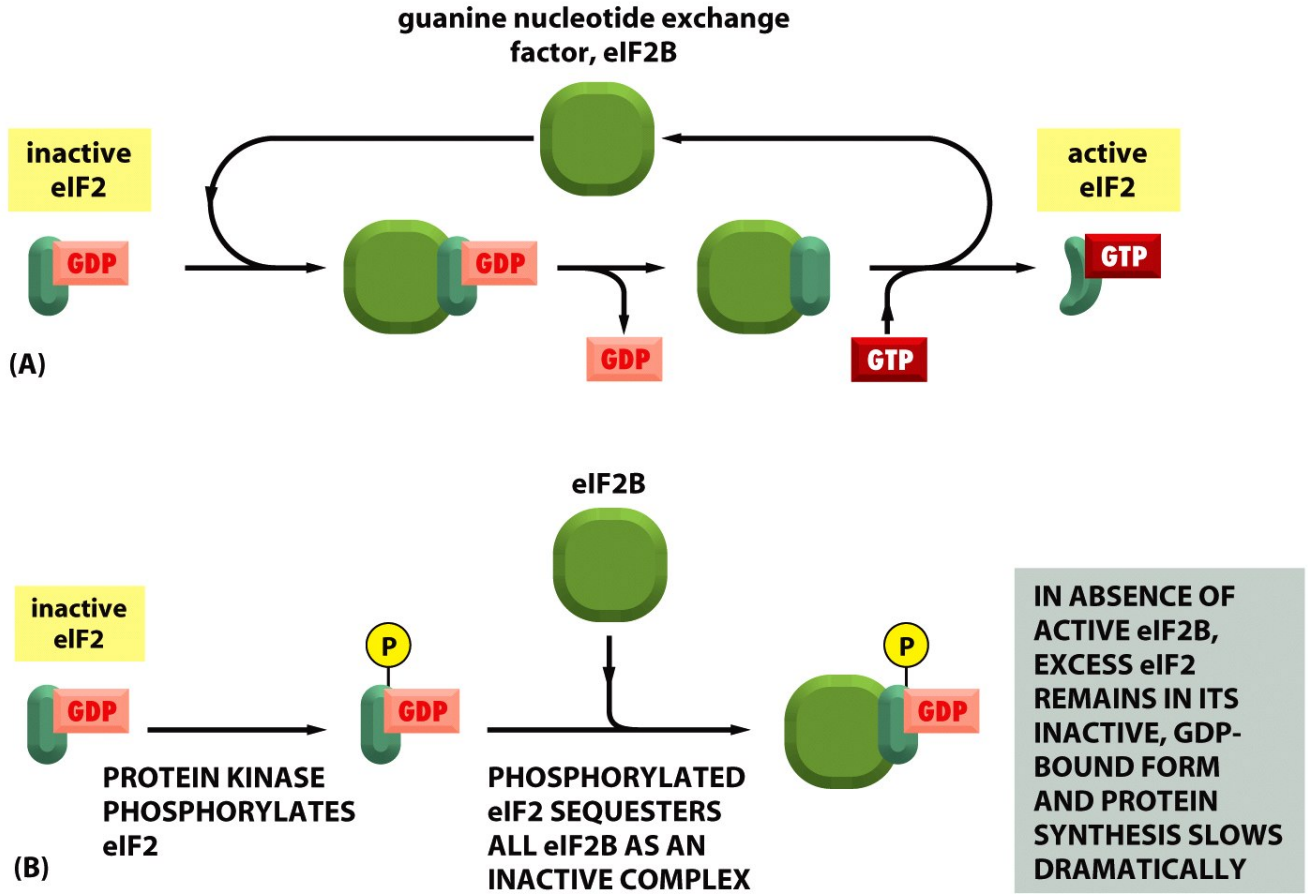


Figure 7-107 *Molecular Biology of the Cell* (© Garland Science 2008)

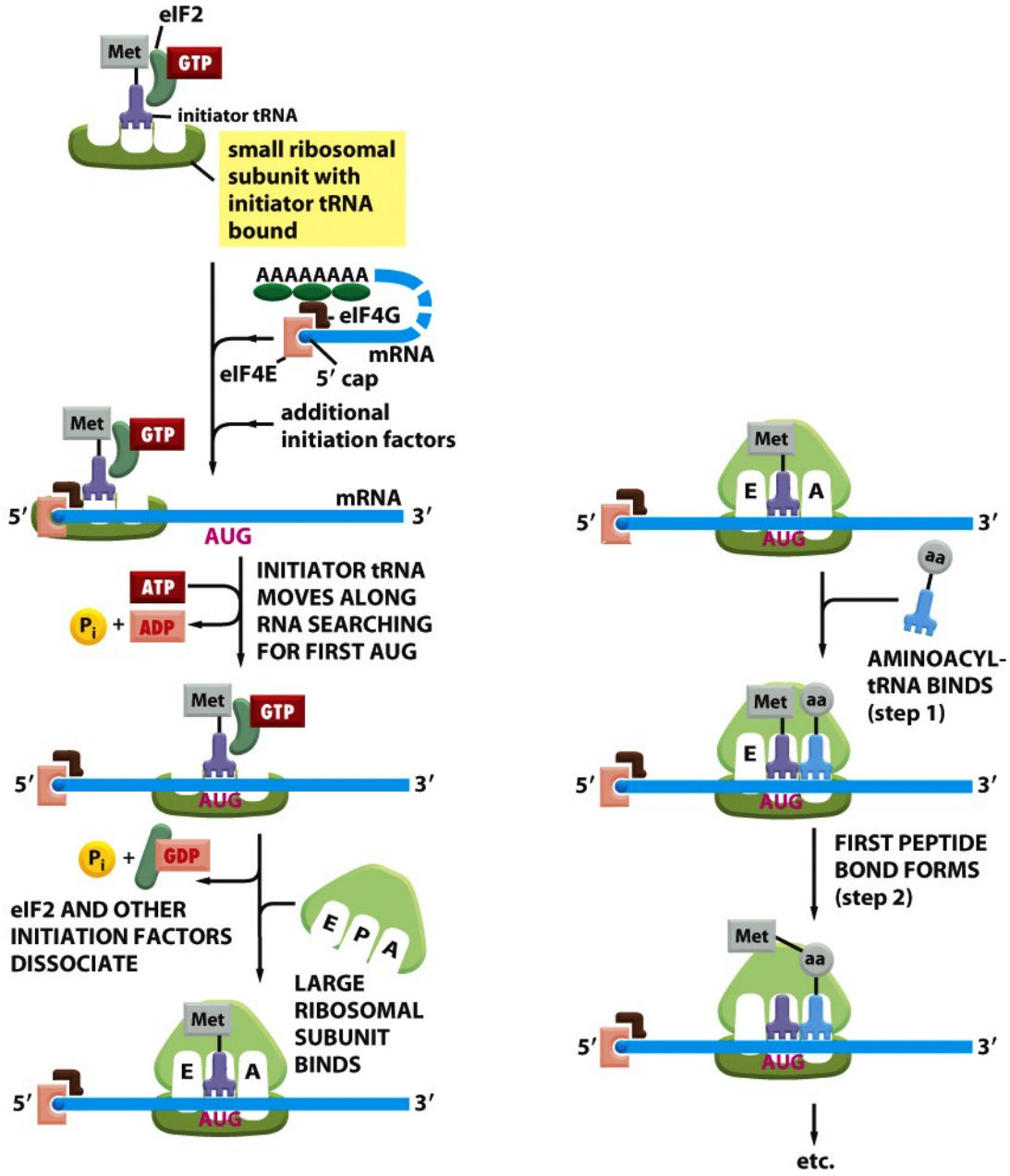


Figure 6-72 *Molecular Biology of the Cell* (© Garland Science 2008)

Negli eucarioti il complesso Met-tRNAi è prima caricato nella subunità ribosomale minore insieme ad altre proteine chiamate **fattori di inizio eucariotici**, **eIF**

Solo il Met-tRNAi è capace di legarsi alla subunità minore senza che sia presente il ribosoma completo, e si lega direttamente al sito P

Quindi la subunità minore si lega al 5' del mRNA che viene riconosciuta in virtù del suo cappuccio e dei fattori eIF4E e eIF4G

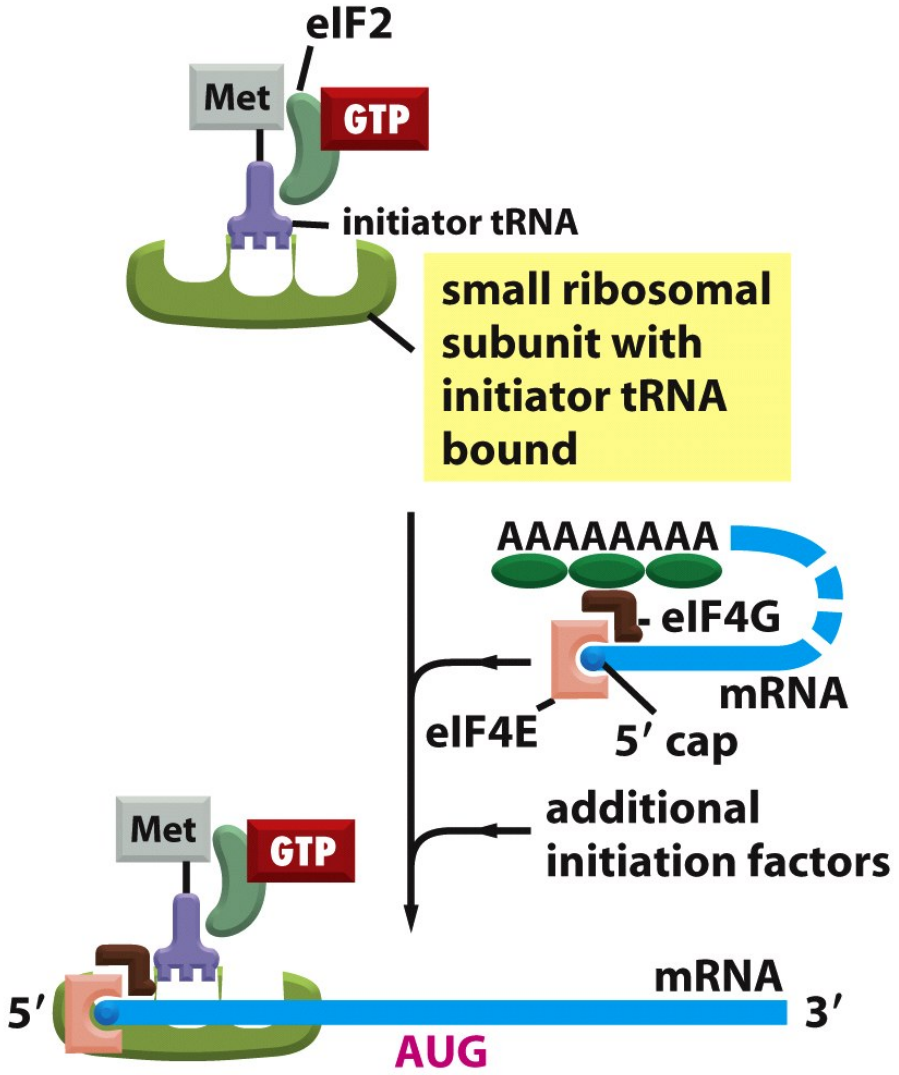


Figure 6-72 (part 1 of 5) *Molecular Biology of the Cell* (© Garland Science 2008)

La fosforilazione di un fattore di inizio regola in modo globale la sintesi proteica

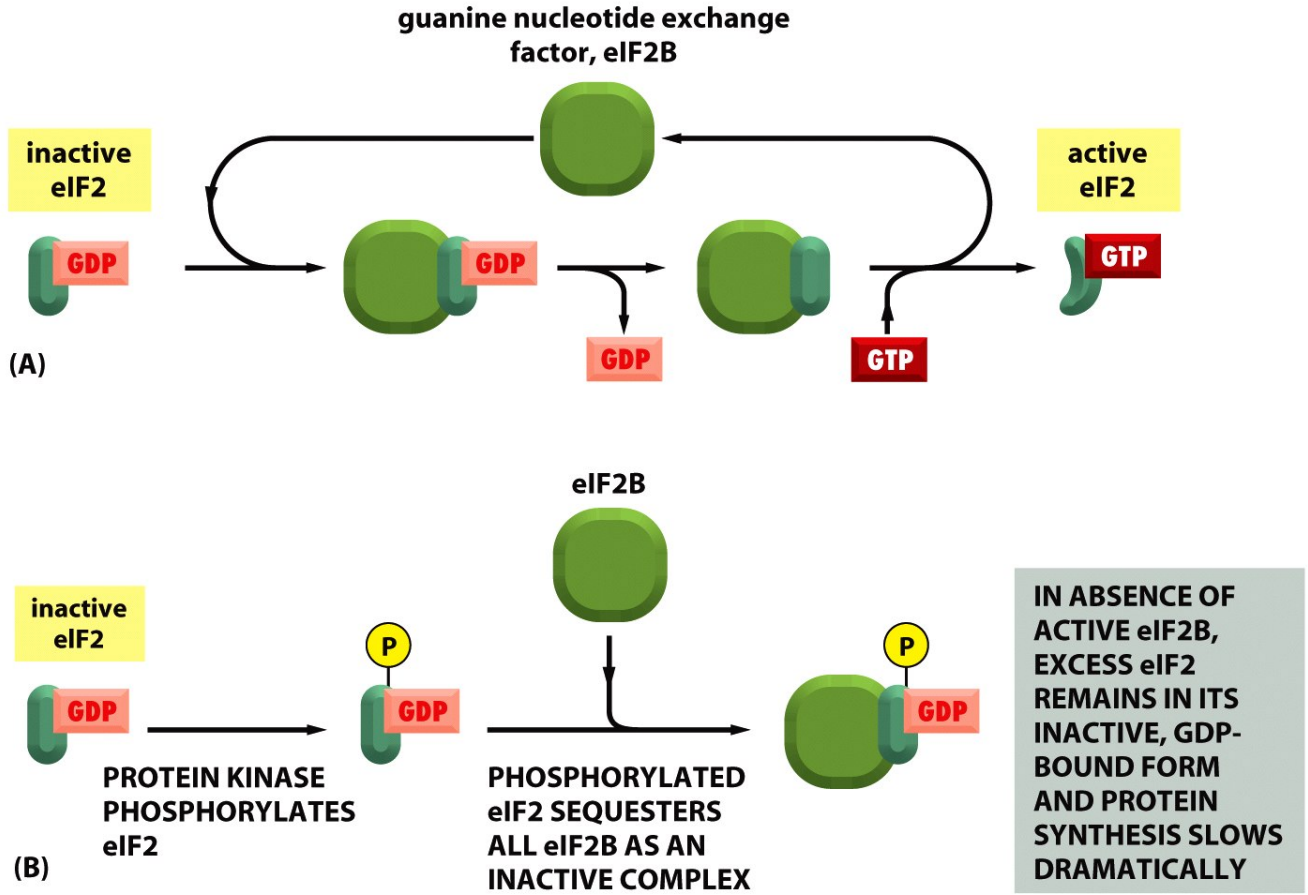


Figure 7-107 *Molecular Biology of the Cell* (© Garland Science 2008)

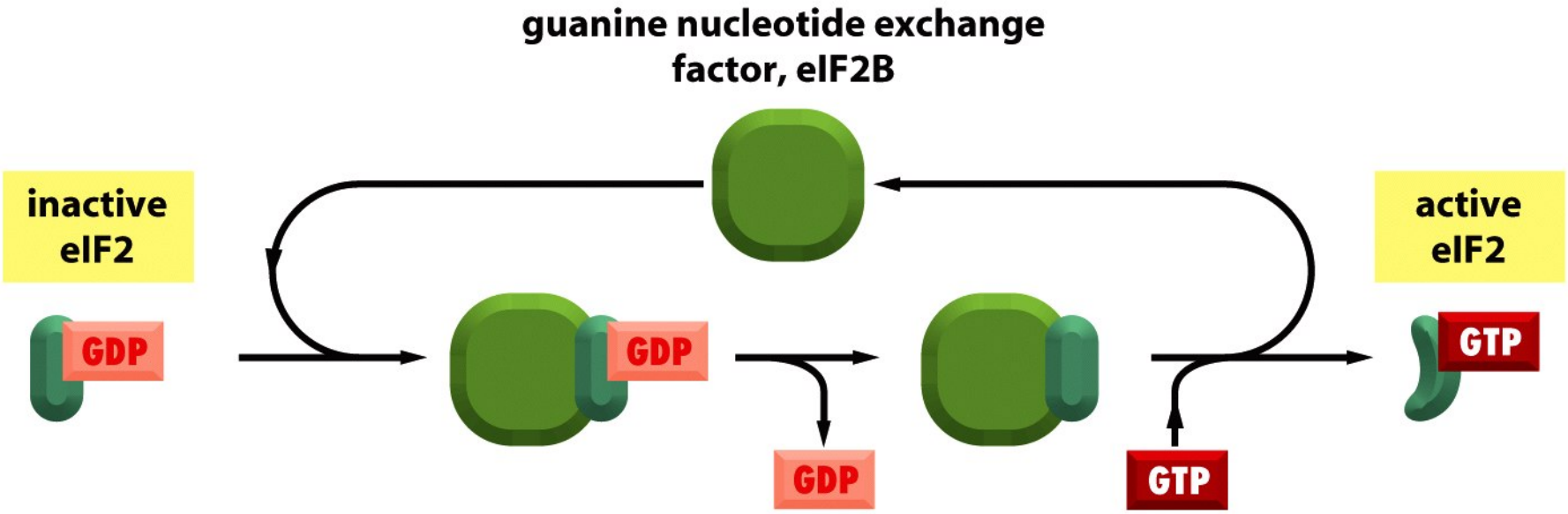


Figure 7-107a *Molecular Biology of the Cell* (© Garland Science 2008)

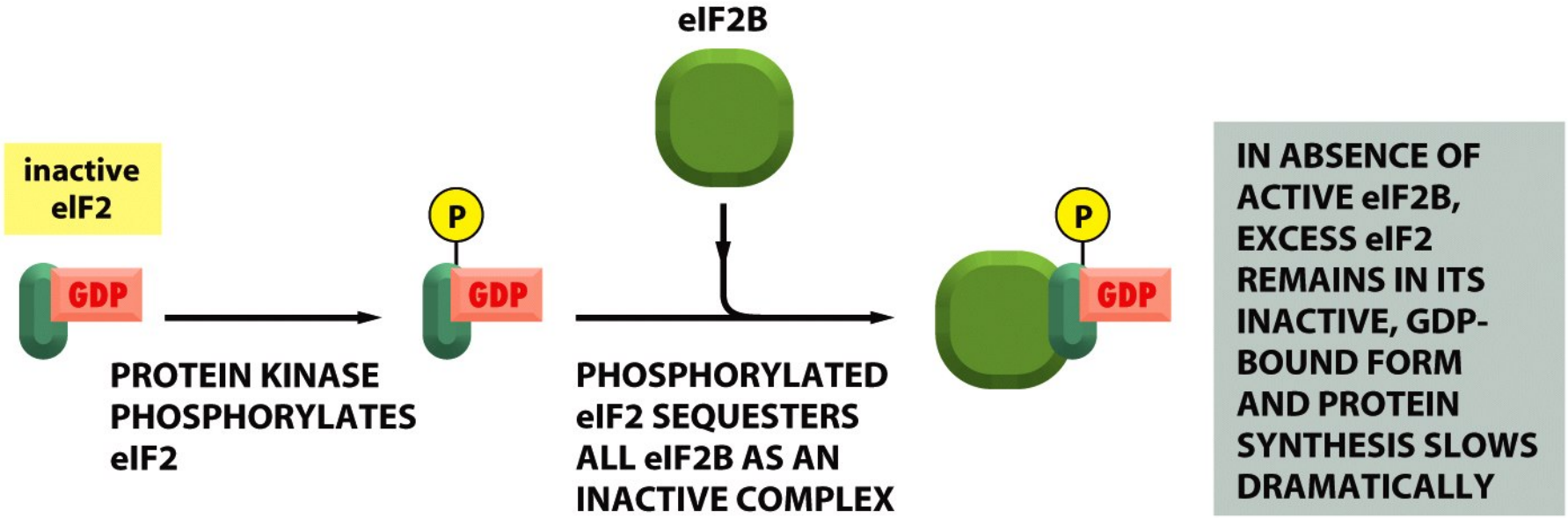
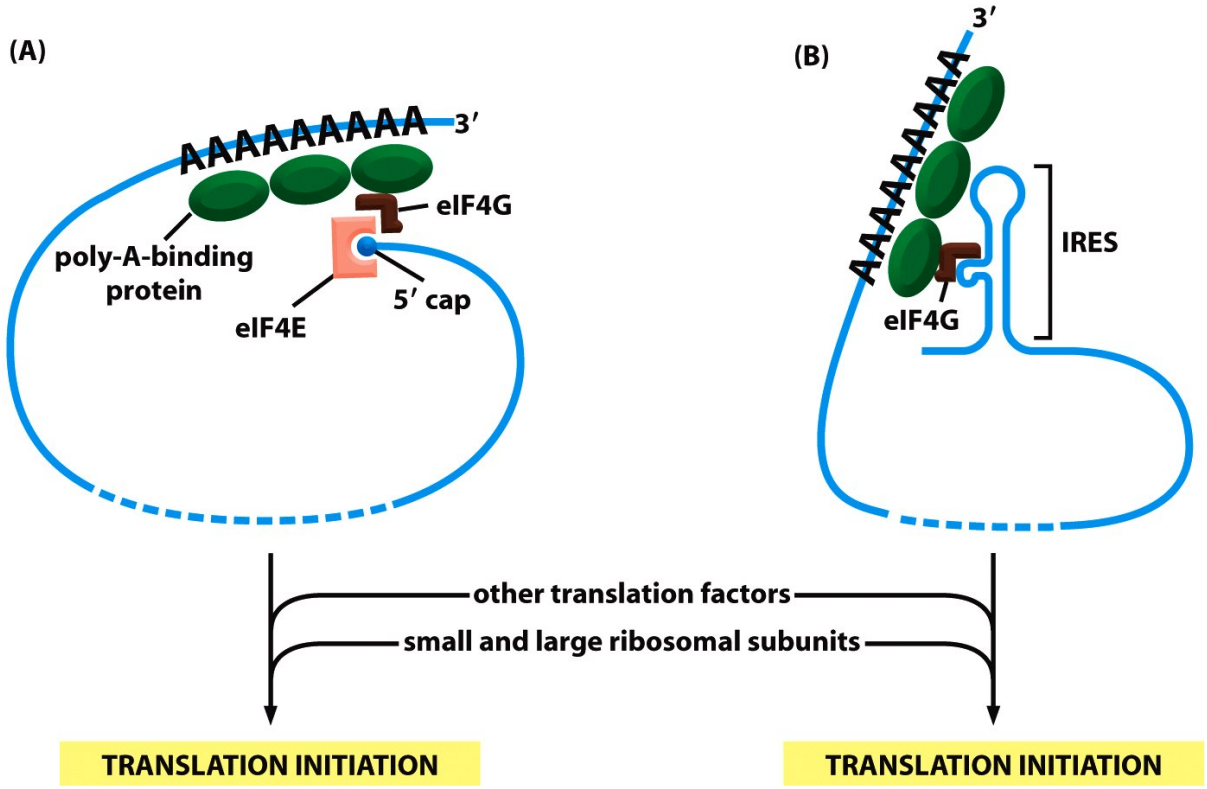


Figure 7-107b *Molecular Biology of the Cell* (© Garland Science 2008)

L’inizio della traduzione può essere regolata da sequenze nel 5’UTR



IRES: sito interno di ingresso dei ribosomi

Figure 7-108 *Molecular Biology of the Cell* (© Garland Science 2008)

Negli eucarioti il complesso Met-tRNAi è prima caricato nella subunità ribosomale minore insieme ad altre proteine chiamate **fattori di inizio eucariotici**, **eIF**

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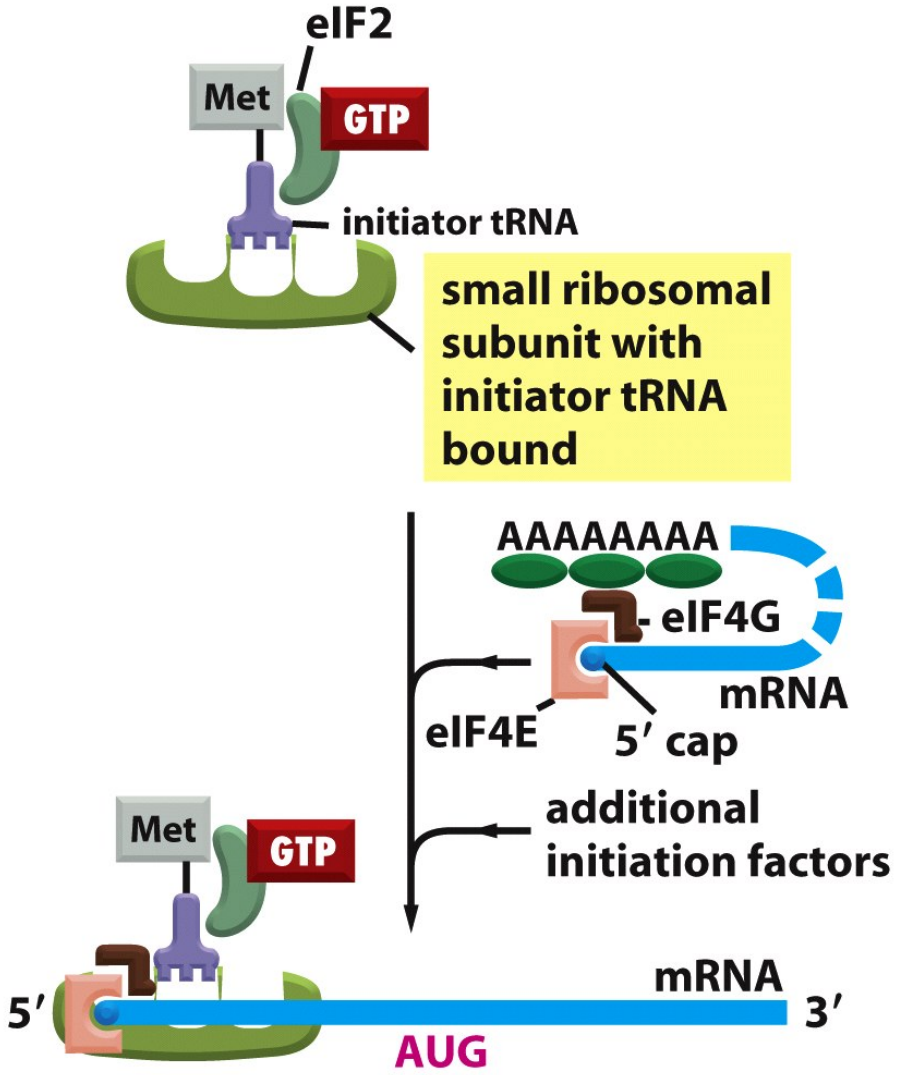
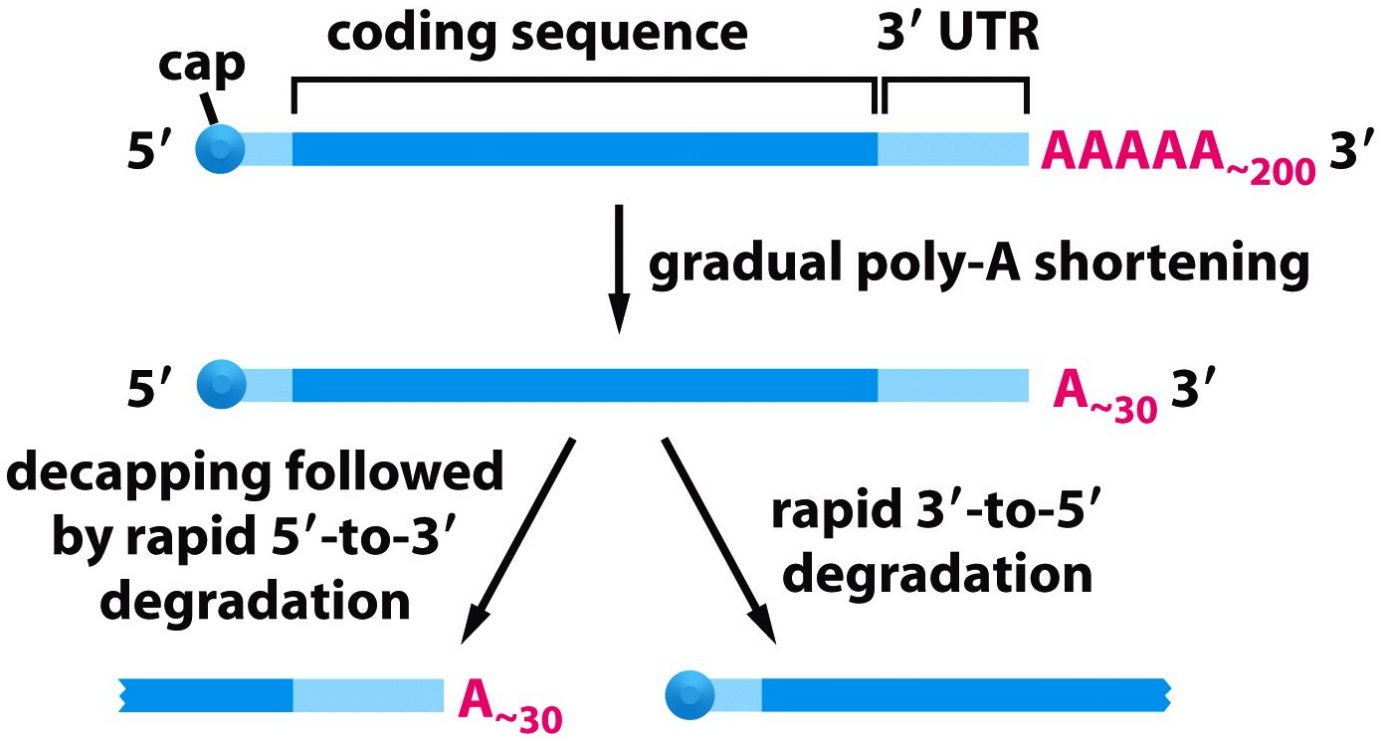


Figure 6-72 (part 1 of 5) *Molecular Biology of the Cell* (© Garland Science 2008)

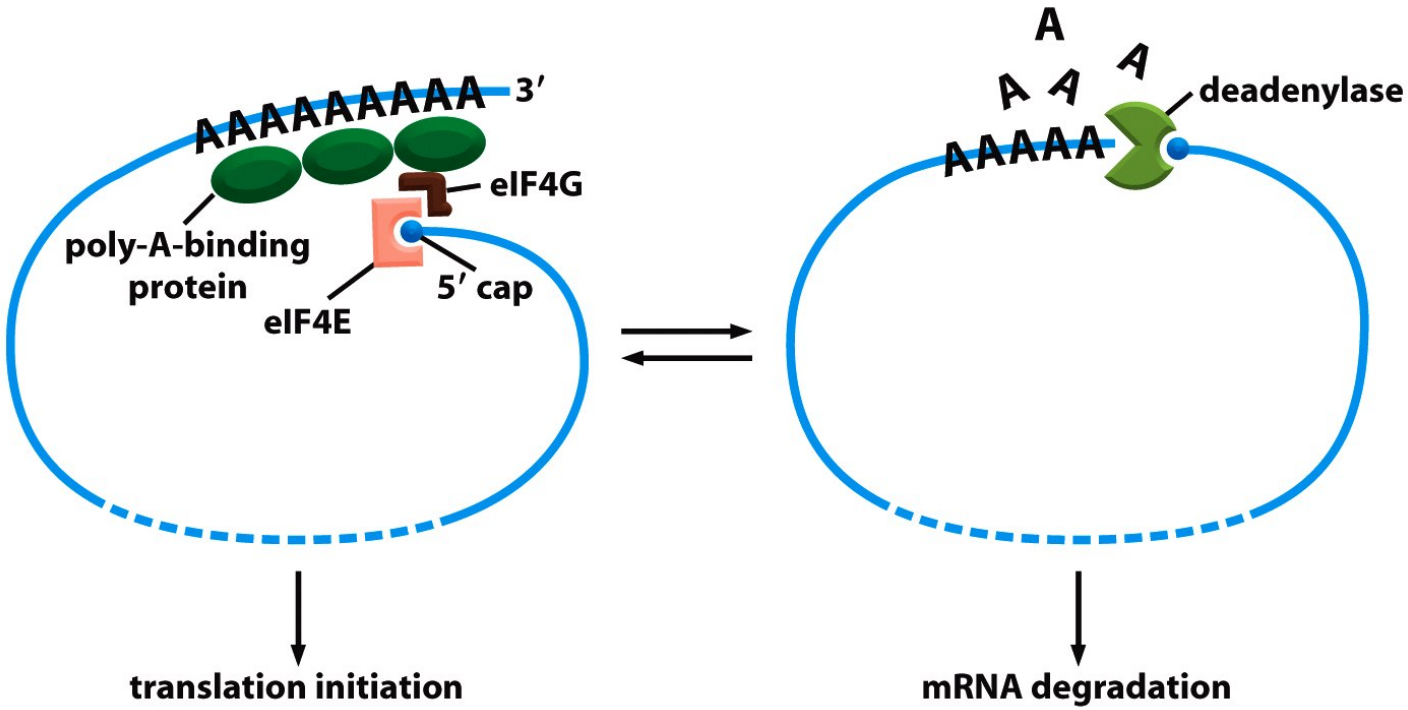
L’espressione dei geni può essere controllata da un cambiamento nella stabilità nell’mRNA



Due meccanismi di degradazione di mRNA eucariotici

Figure 7-109 *Molecular Biology of the Cell* (© Garland Science 2008)

Degradazione dell'mRNA dipendente da deadenilazione



Competizione tra traduzione dell'mRNA e degradazione dell'mRNA

Figure 7-110 *Molecular Biology of the Cell* (© Garland Science 2008)

Due controlli post-trascrizionali mediati da ferro

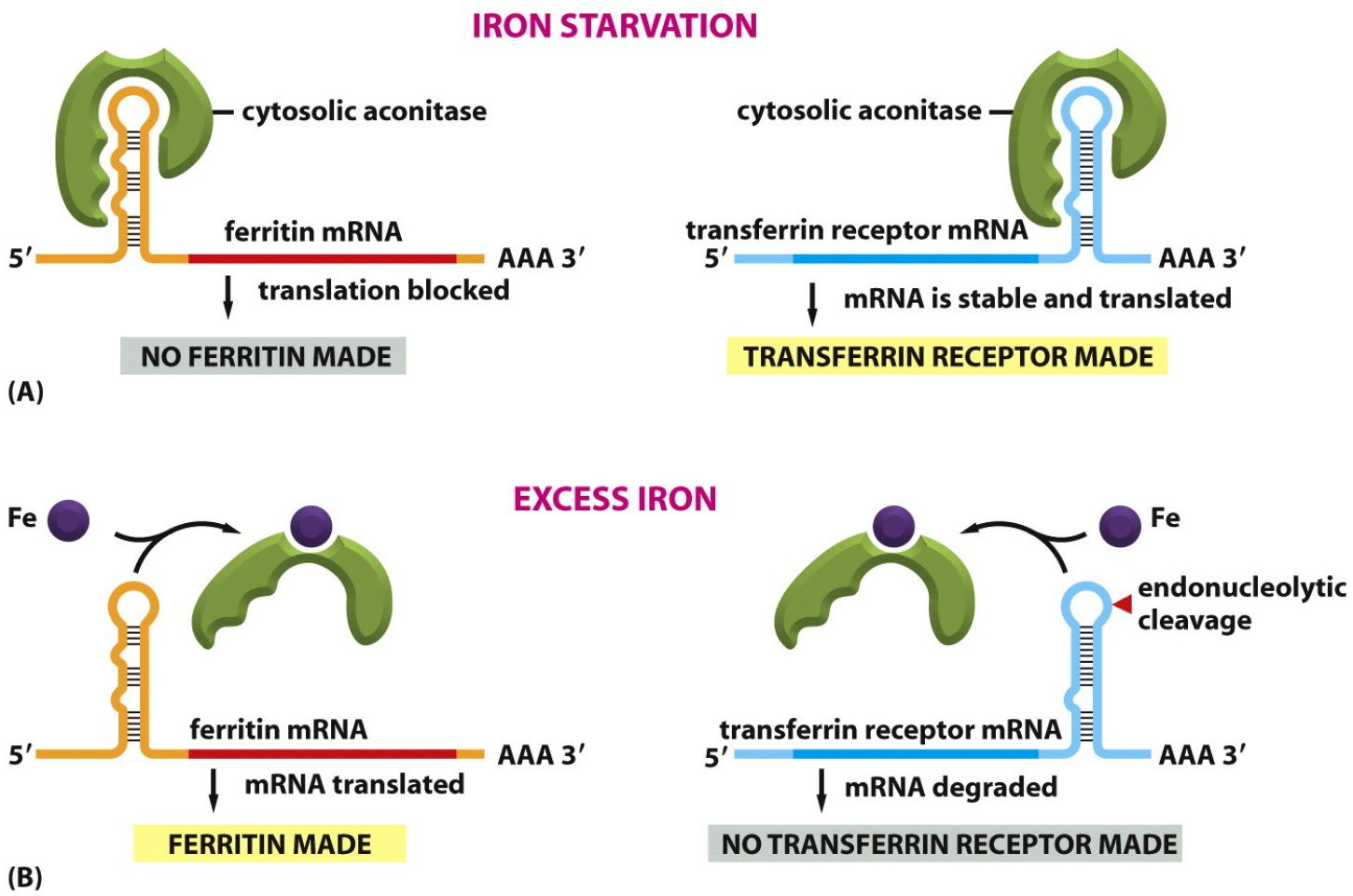


Figure 7-111 *Molecular Biology of the Cell* (© Garland Science 2008)

Piccoli RNA non codificanti regolano molti geni eucariotici: micro RNA e RNAi

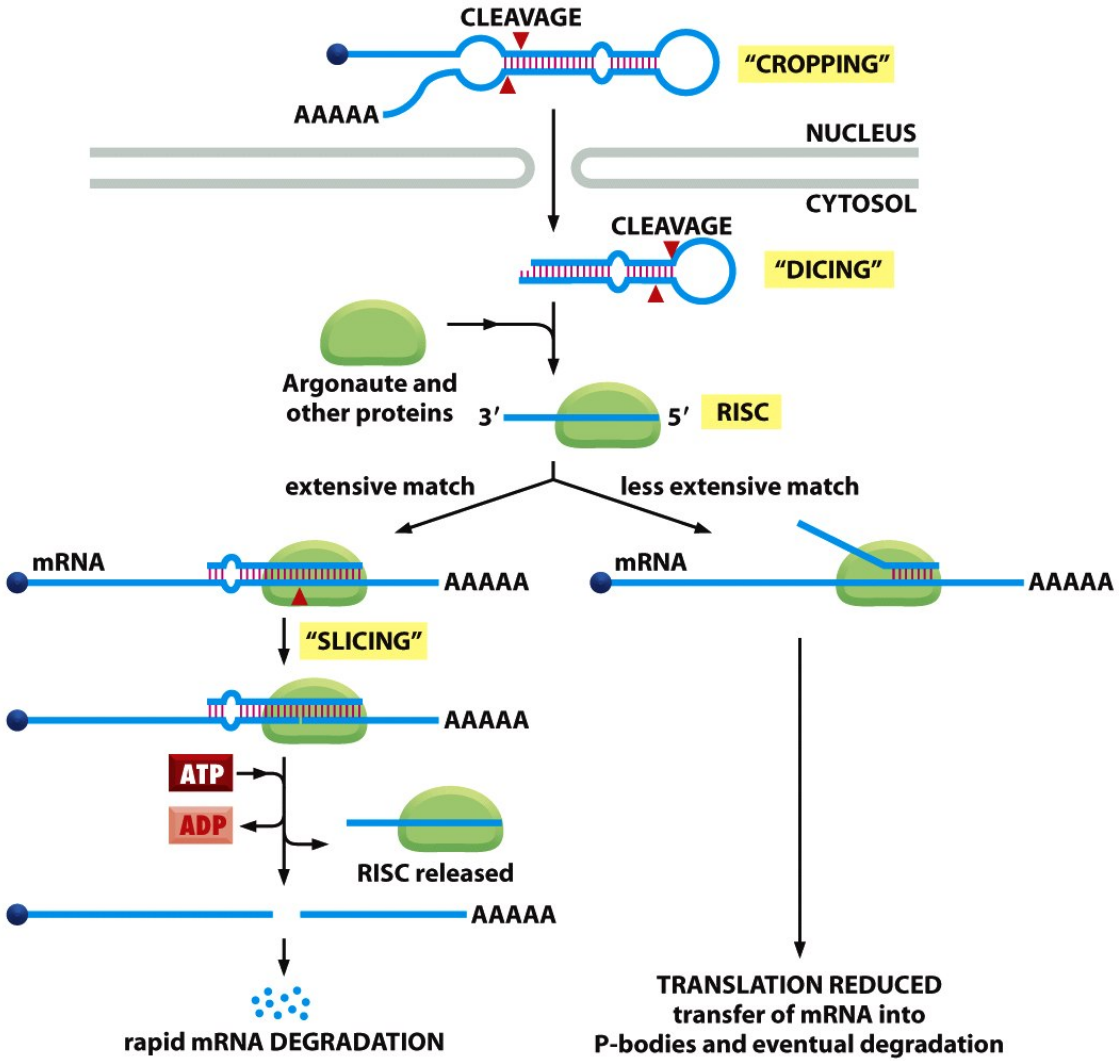


Figure 7-112 *Molecular Biology of the Cell* (© Garland Science 2008)

Breakthrough Online
For an expanded version of this section, with references and links, see www.sciencemag.org/content/vol298/issue5602/#special

Breakthrough of the Year

#1 The Winner

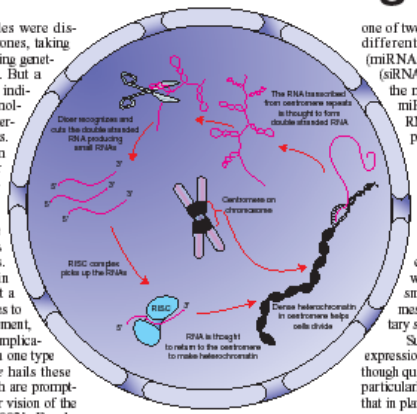
Just when scientists thought they had deciphered the roles played by the cell's leading actors, a familiar performer has turned up in a stunning variety of guises. RNA, long upstaged by its more glamorous sibling, DNA, is turning out to have star qualities of its own.

Small RNAs Make Big Splash

For decades, RNA molecules were dismissed as little more than drones, taking orders from DNA and converting genetic information into proteins. But a string of recent discoveries indicates that a class of RNA molecules called small RNAs operate many of the cell's controls. They can turn the tables on DNA, shutting down genes or altering their levels of expression. Remarkably, in some species, truncated RNA molecules literally shape genomes, carving out chunks to keep and discarding others. There are even hints that certain small RNAs might help chart a cell's destiny by directing genes to turn on or off during development, which could have profound implications for coaxing cells to form one type of tissue or another. *Science* hails these electrifying discoveries, which are prompting biologists to overhaul their vision of the cell and its evolution, as 2002's Breakthrough of the Year.

These astonishing feats are performed by short stretches of RNA ranging in length from 21 to 28 nucleotides. Their role had gone unnoticed until recently, in part because researchers, focused on the familiar larger RNA molecules, tossed out the crucial small ones during experiments. As a result, RNA has long been viewed primarily as an essential but rather dull molecule that ferries the genetic code from the nucleus to the ribosomes, the cell's protein factories, and helps assemble amino acids in the correct order during protein synthesis.

Signs that RNA might be more versatile came in the early 1990s, when biologists determined that some small RNAs could quash the expression of various genes in plant and, later, animal cells. But they didn't appreciate the molecules' true powers until 1998. That's when Andrew Fire of the Carnegie Institution of Washington in Baltimore, Maryland, Craig Mello of the University of Massachusetts Medical School in Worcester, and their colleagues injected stretches of double-stranded RNA into worms. Double-stranded RNA forms when a familiar single strand kinks back in a hairpin bend, putting two complementary sequences alongside each other. To the researchers' surprise, double-stranded RNA dramatically inhibited genes that had helped generate the RNA in the first place. This inhibition, which was later seen in flies and other organisms, came to be known as RNA interference (RNAi). It helped prove that RNA molecules were behind some gene silencing.



Life cycle. With a helping hand from proteins RISC and Dicer, small RNAs are born. We now know that these molecules keep DNA in line and ensure a cell's good health.

Another crucial step came last year, when Gregory Hannon of Cold Spring Harbor Laboratory in New York and his colleagues identified an enzyme, appropriately dubbed Dicer, that generates the small RNA molecules by chopping double-stranded RNA into little pieces. These bits belong to one of two small RNA classes produced by different types of genes: microRNAs (miRNAs) and small interfering RNAs (siRNAs). SiRNAs are considered to be the main players in RNAi, although miRNAs, which inhibit translation of RNA into protein, were recently implicated in this machinery as well.

To bring about RNAi, small RNAs degrade the messenger RNA that transports a DNA sequence to the ribosome. Exactly how this degradation occurs isn't known, but scientists believe that Dicer delivers small RNAs to an enzyme complex called RISC, which uses the sequence in the small RNAs to identify and degrade messenger RNAs with a complementary sequence.

Such degradation ratchets down the expression of the gene into a protein. Although quashing expression might not sound particularly useful, biologists now believe that in plants, RNAi acts like a genome "immune system," protecting against harmful DNA or viruses that could disrupt the genome. Similar hints were unearthed in animals this year. In labs studying gene function, RNAi is now commonly used in place of gene "knockouts": Rather than delete a gene, a laborious process, double-stranded RNA is applied to ramp down its expression.

The year's most stunning revelations emerged in the fall, in four papers examining how RNA interference helps pilot a peculiar—and pervasive—genetic phenomenon known as epigenetics. Epigenetics refers to changes in gene expression that persist across at least one generation but are not caused by changes in the DNA code.

In recent years, researchers have found that one type of epigenetic regulation is caused by adjustments in the shape of complexes known as chromatin, the bundles of DNA and certain fundamental proteins that make up the chromosomes. By changing shape—becoming either more or less compact—chromatin can alter which genes are expressed. But what prompts this shape-shifting remained mysterious.

This year, scientists peering closely at RNAi in two different organisms were startled to find that small RNAs responsible for RNAi wield tremendous control over chromatin's form. In so doing, they can permanently shut down or delete sections of DNA by mechanisms not well understood, rather than just silencing them temporarily.

That news came from several independent groups. In one case, Shiv Grewal, Robert Martienssen, and their colleagues at Cold Spring Harbor Laboratory compared fission yeast cells lacking RNAi machinery with normal cells. When yeast cells divide, their chromosomes untangle and migrate to opposite sides of the cell. The researchers already knew, broadly, that this chapter of cell division is governed by a tightly wrapped bundle of chromatin, called heterochromatin, around the centromere—the DNA region at the chromosome's "waist." The biologists found that their mutant cells, which were missing the usual small RNAs, couldn't properly form heterochromatin at their centromeres and at another DNA region in yeast that controls mating. This suggests that without small RNAs, cell division goes awry. The scientists theorized that in healthy yeast cells, small RNAs elbow their way into cell division, somehow nudging heterochromatin into position to do the job. That exposes DNA to different proteins and dampens gene expression.

Meanwhile, David Allis and his colleagues at the University of Virginia Health System in Charlottesville, along with Martin Gorovsky of the University of Rochester in New York and others, were focusing on a different organism, a single-celled ciliate called *Tetrahymena*. Biologists treasure *Tetrahymena* because it stores the DNA passed to offspring in a different nucleus from the one containing DNA expressed during its lifetime, making it easy to distinguish one gene set from the other. The researchers found that in *Tetrahymena*, small RNAs trigger deletion or reshuffling of some DNA sequences as a cell divides. RNAi appeared to be targeting structures analogous to heterochromatin, only this time strips of DNA were discarded or moved elsewhere. The mechanism remains unclear, however.

The two sets of experiments might help explain why small RNAs exist in the first place. In both the yeast and *Tetrahymena*, small RNAs' frenetic activity is focused on genome regions, such as centromeres, that contain repetitive DNA resulting from transposons. Transposons are bits of DNA that can jump around the genome and insert themselves at different locales; at times, they jam transcription machinery and cause disease. It appears possible—although still largely hypothetical—that small RNAs evolved very early in life's history to help protect the genome against instability.

This is just one of many areas that remain to be explored. Researchers are still trying to sort out how the well over 100 different miRNAs function and which species contain which ones. There are hints that they behave differently in plants and animals. And some recent work suggests that miRNAs exert more control over gene expression than previously believed. Also a focus of research are the proteins, such as Dicer, that are critical cogs in the RNAi machinery.

Researchers are also probing RNAi's possible role in development and disease. RNAi has been implicated in guiding meristems, the plant version of stem cells, so some biologists believe that it might help establish the path taken by human and other mammalian stem cells as they differentiate into certain tissues. If so, RNAi could prove an essential tool in manipulating stem cells. And if small RNAs influence cell division in humans as they do in yeast and *Tetrahymena*, minor disruptions in the machinery could lead to cancer.

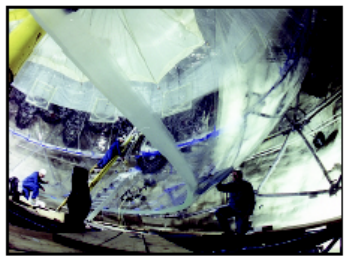
The extraordinary, although still unfulfilled, promise of small RNAs and RNAi has split the field wide open and put RNA at center stage. Having exposed RNAs' hidden talents, scientists now hope to put them to work. —JENNIFER COUZIN

ELLIS/DAVIES C. ALLIS/M. GOROVSKY

The Runners-Up

Science applauds discoveries ranging from the dawn of time to the dawn of our species.

#2 Neutrinos nailed. Neutrinos, mysterious and misunderstood, are finally getting the respect they deserve. For years, neutrinos were the terra incognita on the particle chart. Electrons, muons, taus, and quarks had all been analyzed for years, their properties measured and dissected. But neutrinos? Nobody knew even whether they had mass until a few years ago. They were essentially unknowns.



Positive ID. A huge sphere of heavy water caught fugitive neutrinos as they changed from one flavor to another.

No longer. In the last decade, physicists finally proved that neutrinos have mass, and since then, a flurry of experiments has begun to flesh out the elusive neutrinos' properties.

This year, the Sudbury Neutrino Observatory (SNO), a 1000-ton sphere of heavy water deep inside a nickel mine in Sudbury, Ontario, put the final nail in the coffin of the solar neutrino paradox. The nuclear reactions in the sun should produce a large number of electron neutrinos, but all observations had shown that only about one-third of the expected number were actually reaching Earth.

If neutrinos have mass, they can change flavors—from electron neutrinos into tau or mu neutrinos, for example—and that could explain the missing electron neutrinos. SNO showed, once and for all, that this is the case. In April, scientists at SNO announced that they had measured the abundances of all three types of neutrinos—electron, mu, and tau—by detecting when they split apart atoms of deuterium. When they added up the solar electron, mu, and tau neutrinos streaming through the detector, the total matched the number that should be created by nuclear reactions. Electron neutrinos change flavor during their journey to Earth.

As a bonus, the SNO measurements allowed scientists to drastically limit the "mixing angles" that define the neutrinos' flavor-changing abilities and, in December, the KamLAND experiment in Japan restricted the limits even further—with nuclear reactor–created antineutrinos instead of solar neutrinos. Although physicists still don't know how much neutrinos weigh, the evanescent

Interferenza a RNA e micro RNA

The Nobel Prize in Physiology or Medicine 2006

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

Photo: L. Cicero

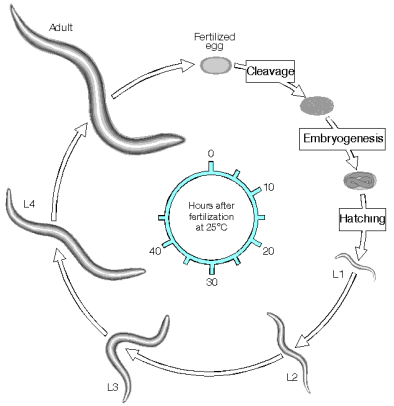
Andrew Z. Fire

1/2 of the prize

Photo: J. Mottern

Craig C. Mello

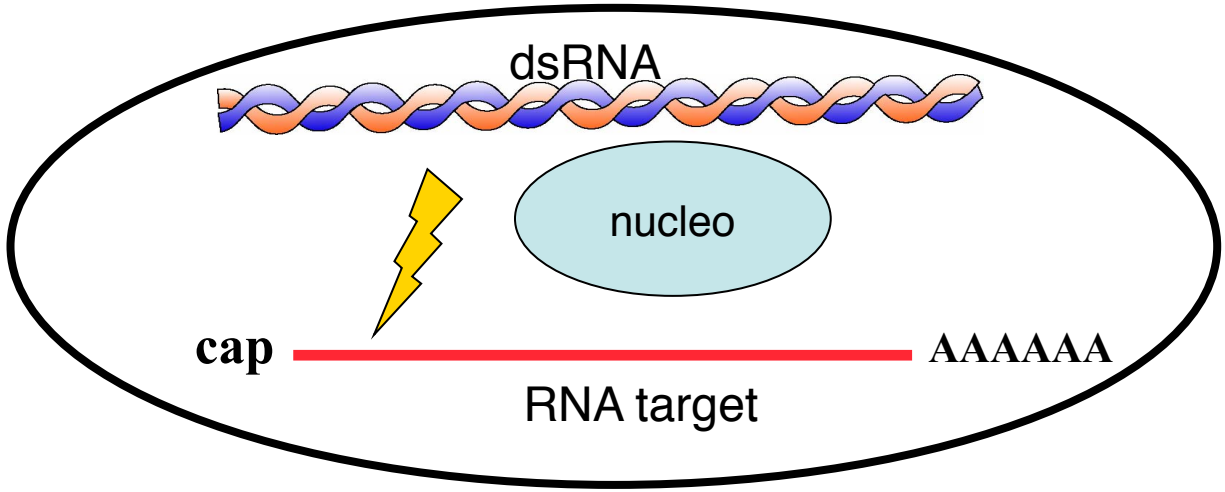
1/2 of the prize



Ambros, V. A (1989).
Arasu P, Wightman B, Ruvkun G. (1991).

Napoli et al., (1990)
Jorgensen et al., (1996)

L'RNA interference (RNAi) è un processo naturale di silenziamento post-trascrizionale dell'espressione genica iniziato da RNA a doppio filamento (dsRNA) di sequenza omologa al gene target.



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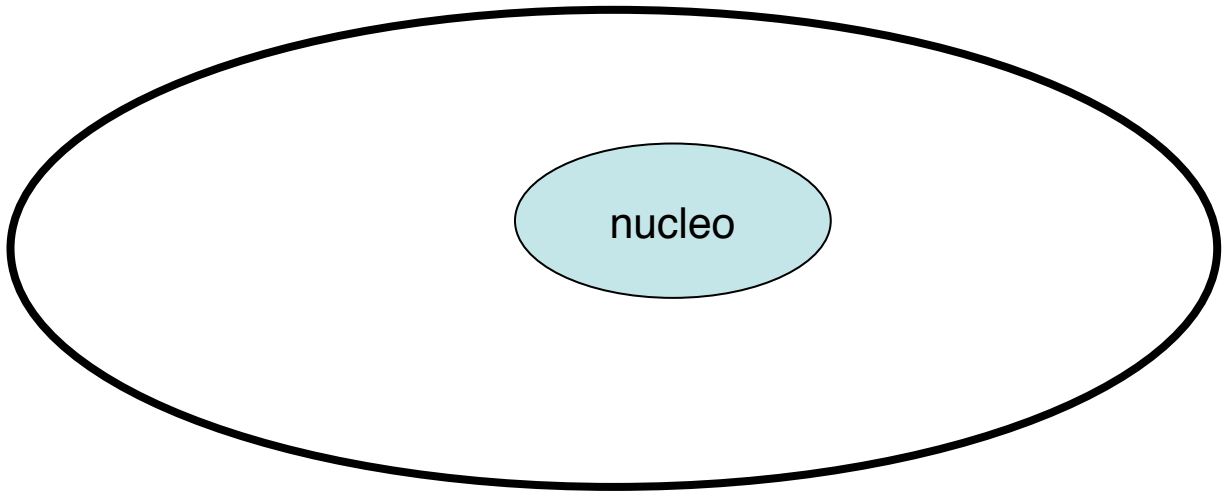
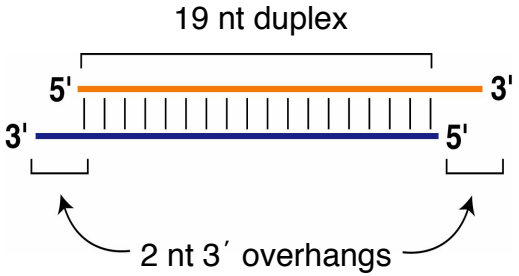


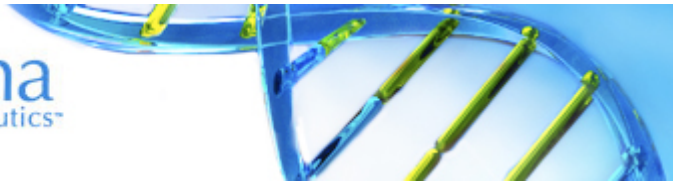
Uso terapeutico dell' RNA interference



Alnylam PHARMACEUTICALS

Respiratory syncitial virus, Fase II
Diabetic Macular Edema, Fase II
Acute kidney injury, Fase I
Tumore epatico, Fase I
Tumori solidi, Fase I

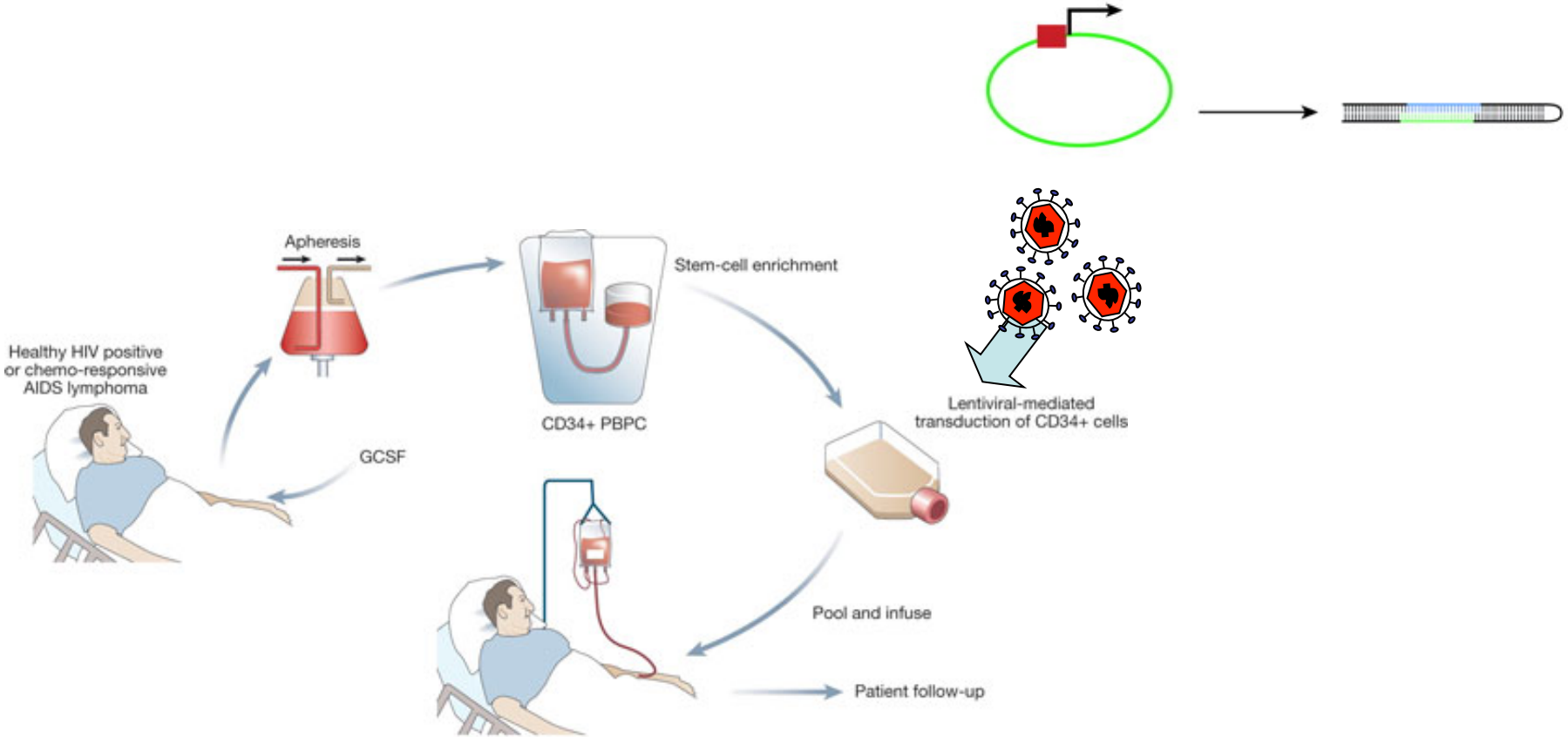
Sirna Therapeutics



Age-related Macular Degeneration, Fase II

Uso terapeutico dell' RNA interference

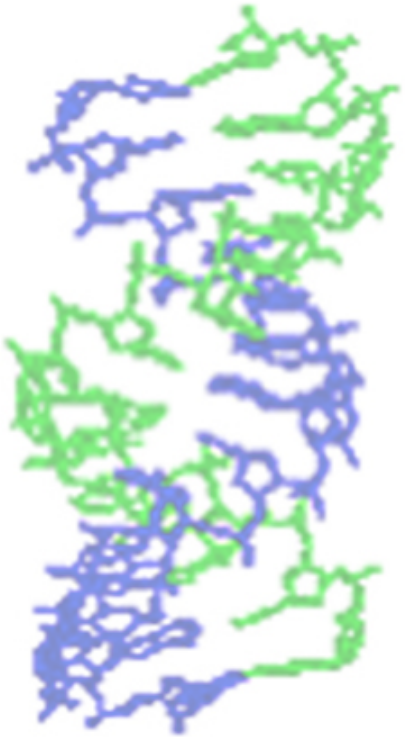
Trial clinico per uso di RNA interference contro HIV (Benitec/City of Hope)
Fase II



RNA interference

- Cosa è l'RNA interference
- Come è stata scoperta
- Come funziona
- Qual é il suo ruolo
- micro RNA
- RNAi e fenomeni epigenetici

RNA a doppio filamento



dsRNA (A form)

(dsRNA =“double stranded RNA”)

RNAi + PTGS

RNA interference (RNAi)

Post-transcriptional gene silencing (PTGS)

Virus-induced gene silencing (VIGS)

Homology-dependent silencing

Quelling

Cosuppression

Cosuppression

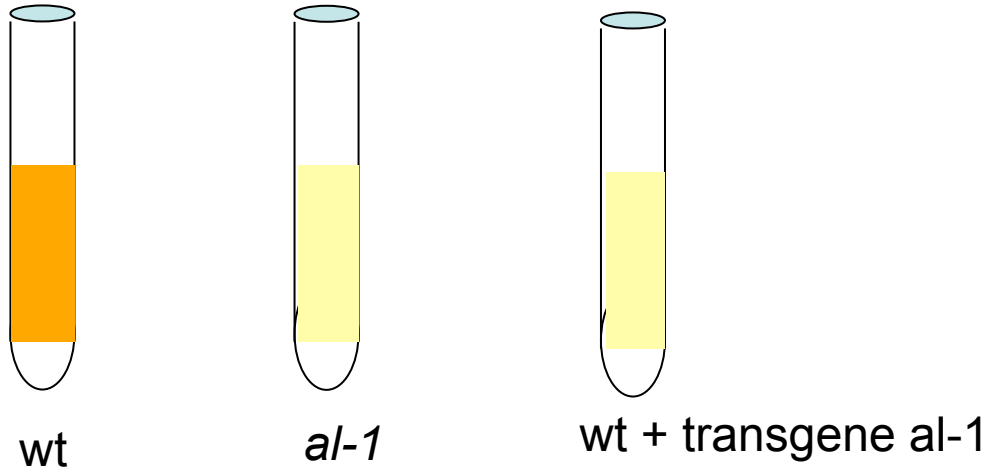
La sovraespressione del gene CHS (Chalcone synthase) in petunia porta alla perdita della pigmentazione del fiore

Napoli et al., (1990)
Jorgensen et al., (1996)

L' introduzione di un transgene causa la soppressione sia del gene introdotto che di quello endogeno.

Quelling

Nel fungo filamentoso *Neurospora crassa* l'introduzione di un transgene causa il silenziamento del gene endogeno omologo albino-1 (al-1) codificante per un gene del pathway biosintetico di pigmenti carotenoidi.

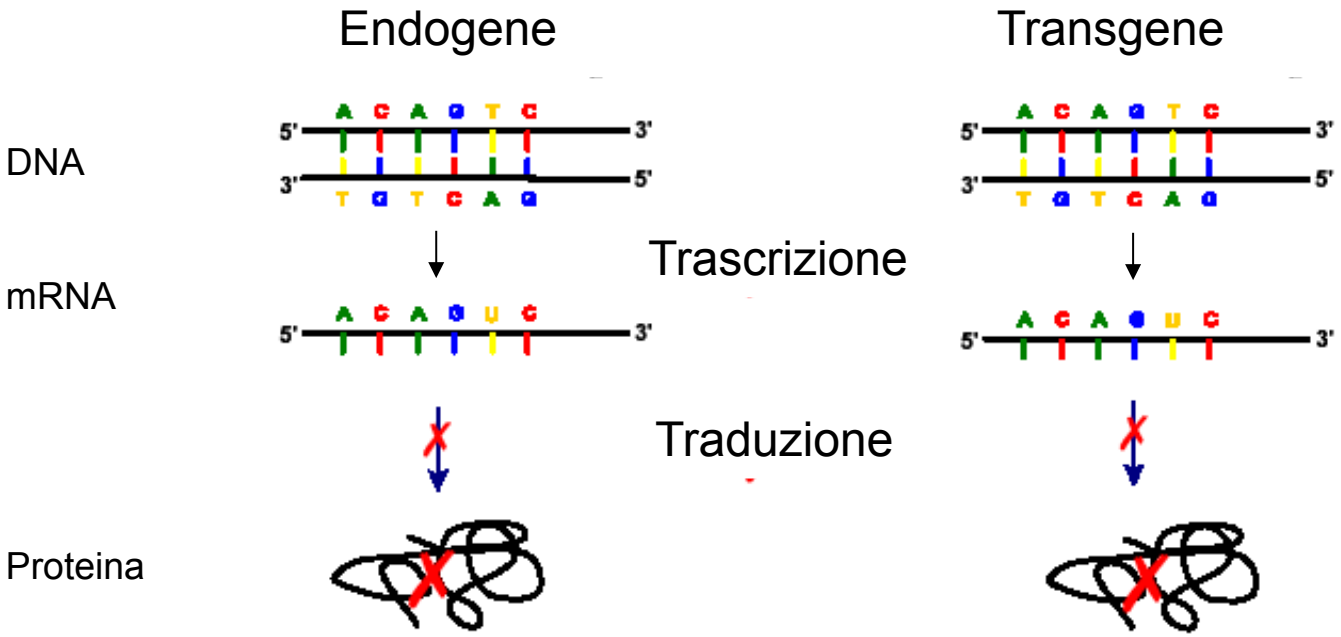


Romano e Macino (1992)
Cogoni et al., (1996)

L' introduzione di un transgene causa la soppressione sia del gene introdotto che di quello endogeno.

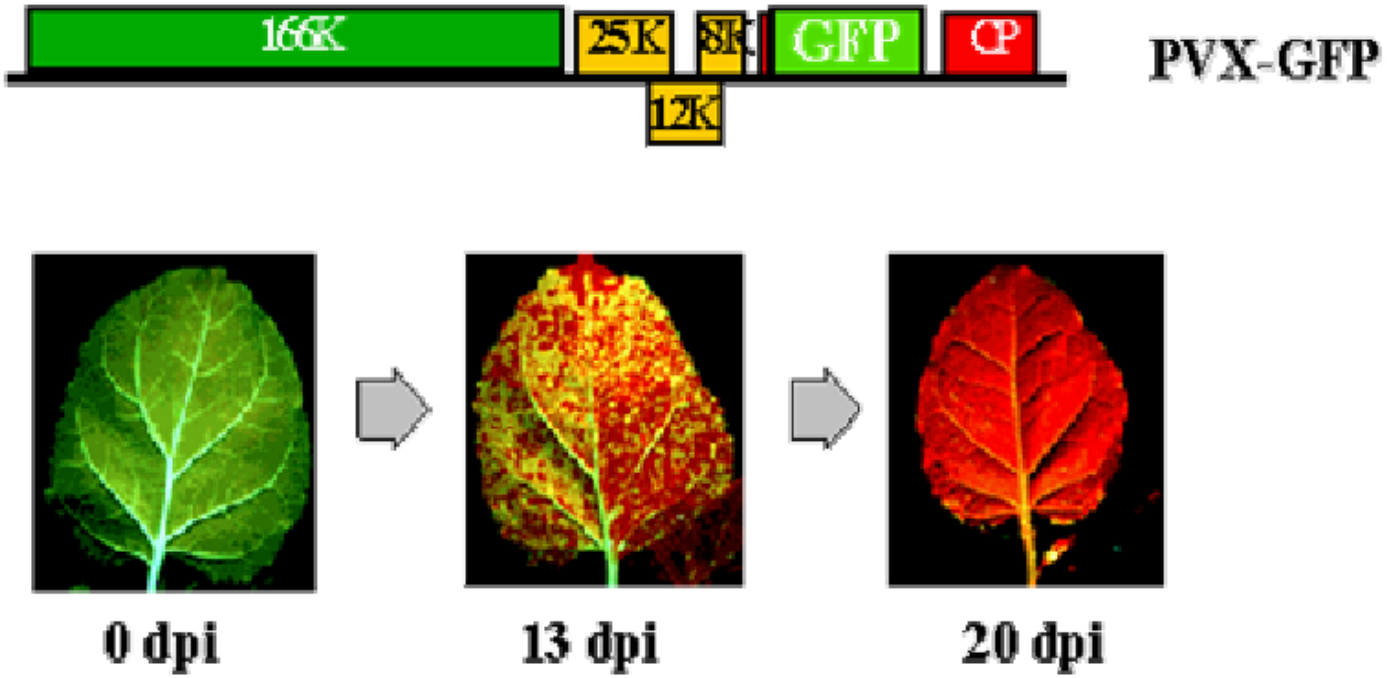
Post-transcriptional gene silencing (PTGS)

Il silenziamento del gene avviene **a livello post-trascrizionale**: I trascritti vengono prodotti ma sono rapidamente degradati nel citoplasma e non si accumulano



Virus-induced gene silencing (VIGS)

L'infezione da parte di un virus puo' silenziare i geni virali e quelli endogeni che con questi abbiano omologia



Ruiz et al., (1998)

RNA interference nei Nematodi

Guo e Kemphues (1995), usano RNA antisenso per studiare la funzione del gene *par-1.* Come atteso, l'iniezione dell'antisenso abolisce l'espressione di *par-1*.

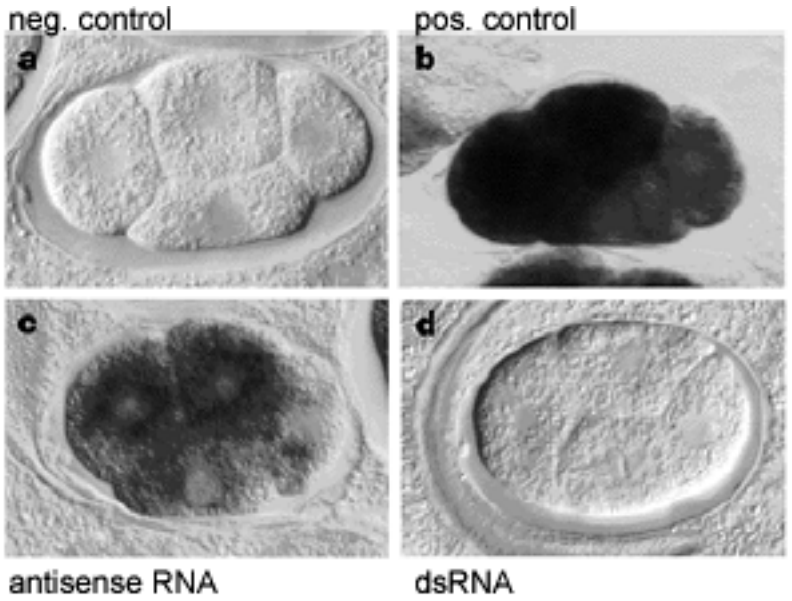
Curiosamente, pero', anche l'iniezione del controllo senso abolisce l'espressione di *par-1*!!!!

Fire e Mello (1998), iniettano per primi dsRNA in *Caenorhabditis elegans* ed ottengono un silenziamento molto piu' efficace che con il solo antisenso.

RNA interference nei Nematodi

Inibizione dell'espressione di mex3 in *Caenorhabditis elegans* mediante RNAi

- Iniezione di RNA nelle gonadi di adulti
- Analisi di embrioni a stadio a quattro cellule: ibridazione in situ



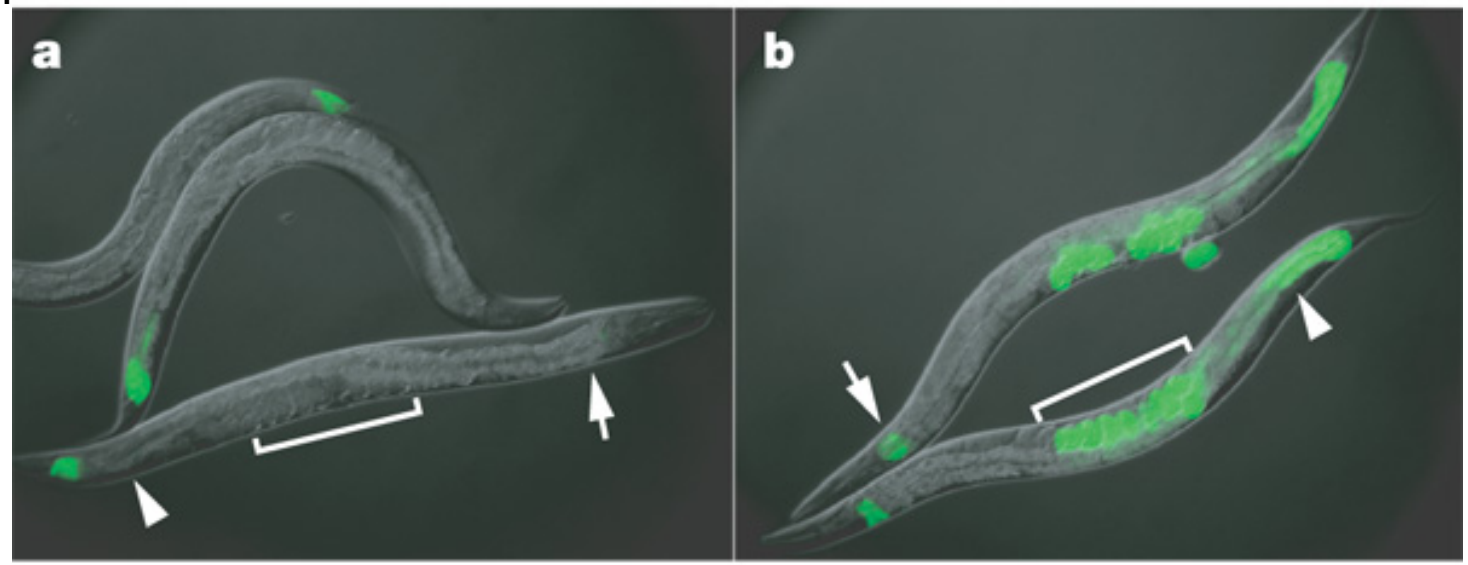
Fire et al., (1998)

Nature **391**, 806 - 811 (19 February 1998)

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‡

Experimental introduction of RNA into cells can be used in certain biological systems to interfere with the function of an endogenous gene[1,2]. Such effects have been proposed to result from a simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA transcripts. RNA interference has been used in the nematode *Caenorhabditis elegans* to manipulate gene expression[3,4]. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stochiometric interference with endogenous mRNA and suggesting that there could be a catalytic or amplification component in the interference process.

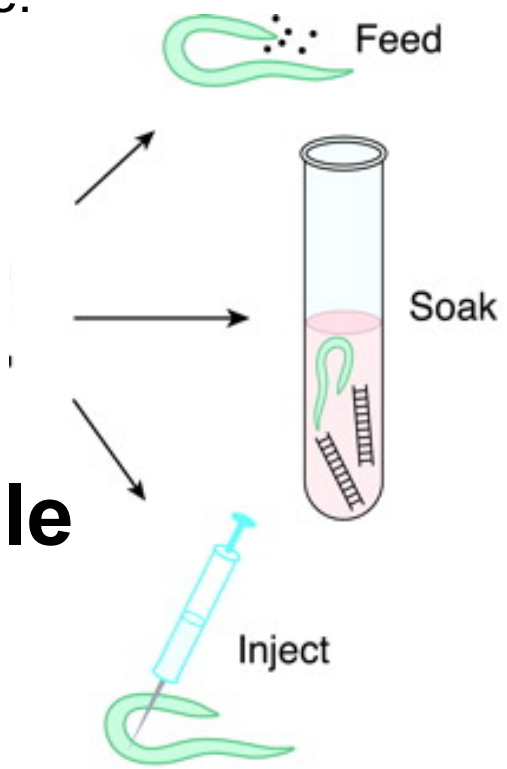


RNAi in *C. elegans*. Silencing of a green fluorescent protein (GFP) reporter in *C. elegans* occurs when animals express GFP dsRNA (a) but not in animals that are defective for RNAi (b).

RNA interference nei Nematodi

Modalita' di somministrazione:

- **"Feeding"**
- **"Soaking"**
- **Iniezione**



Studi di genetica funzionale

Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference

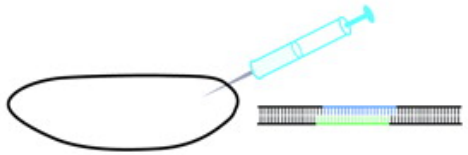
Andrew G. Fraser*†, Ravi S. Kamath*†, Peder Zipperlen*†, Manuxa Martinez-Campos*, Marc Sohrmann‡ & Julie Ahringer*

*Wellcome/CRC Institute, University of Cambridge, Tennis Court Road, CB2 1QR Cambridge, UK
‡The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK
†These authors contributed equally to this work

NATURE | VOL 408 | 16 NOVEMBER 2000 | www.nature.com

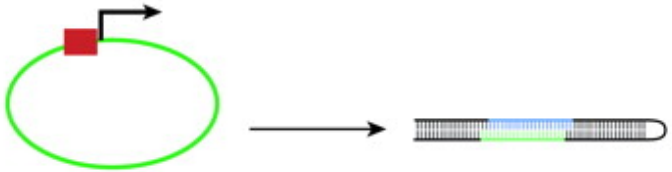
RNA interference in *Drosophila*

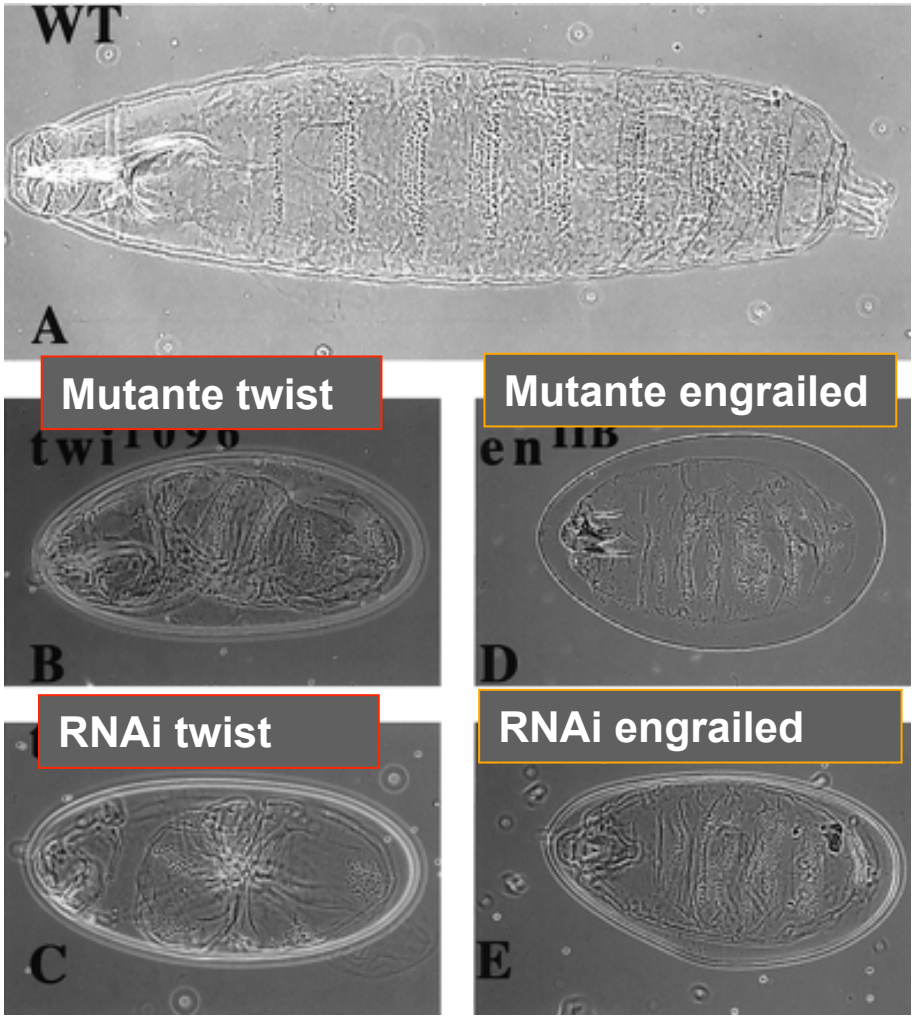
Microiniezione di dsRNA
in embrioni



..oppure

Espressione *in vivo* di
sequenze ripetute
invertite omologhe al
gene da silenziare



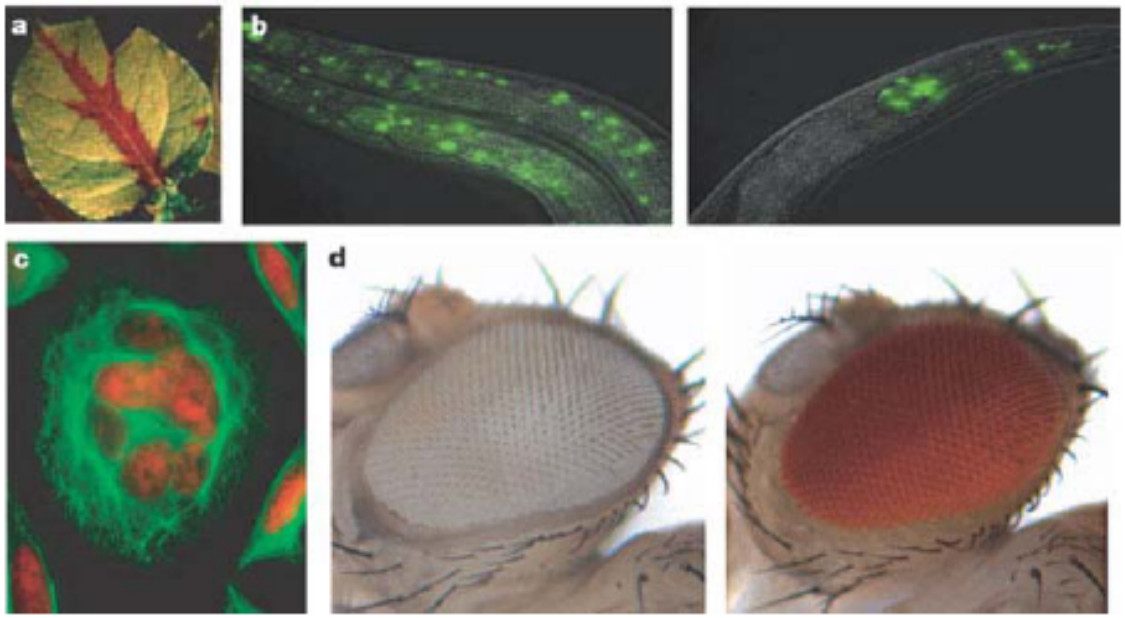


Misquitta e Paterson (1999)

RNAi: uno strumento per inibire l'espressione genica *in vivo*

Piante

C. elegans



Hannon, G (2002) Nature 418, 244-251

Cellule HeLa

Drosophila

RNAi: uno strumento per inibire l'espressione genica *in vivo*

- *C. elegans* (Fire et al., 1998)
- *Drosophila* (Carthew et al., 1998)
- Planaria (Newmark et al., 1998)
- Tripanosoma (Ullu et al., 1998)
- Idra (Lohmann et al., 1999)
- Zebrafish (Wargelius et al., 1999)
- Topo (Wianny & Zernicka-Goetz, 2000)
- "cosuppression" in piante
- "quelling" in *Neurospora*

Cosuppression, PTGS, Quelling, VGS
apparivano inizialmente come processi differenti.

L'identificazione in diversi organismi di mutanti difettivi in questi processi
e la caratterizzazione di RNA molto piccoli ha permesso
di formulare una teoria unificatrice.

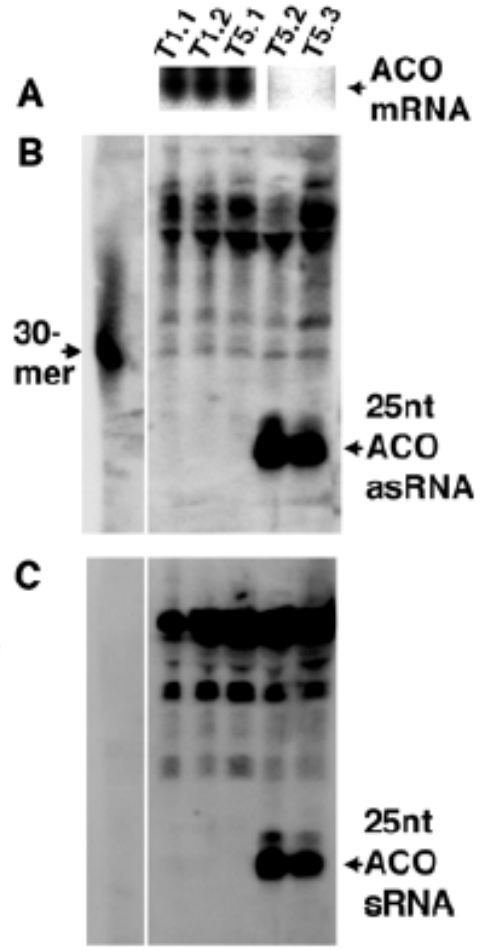
Come funziona l'RNAi?

A livello post-trascrizionale……..

in due fasi fondamentali!

La presenza di piccoli RNA correla con l'RNA interference

- In piante in cui sta avvenendo PTGS sono presenti RNA di circa 25 nt, che sono assenti in piante non silenziate
- Questi piccoli RNA sono complementari sia al senso che all'antisenso del gene silenziato

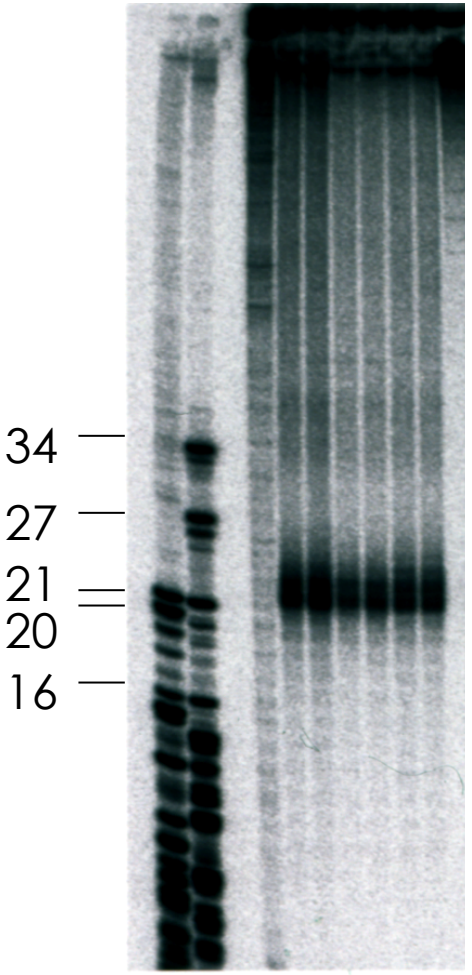


Hamilton e Baulcombe (1999)

Prima fase:

processamento del dsRNA in frammenti di 21-23 nt

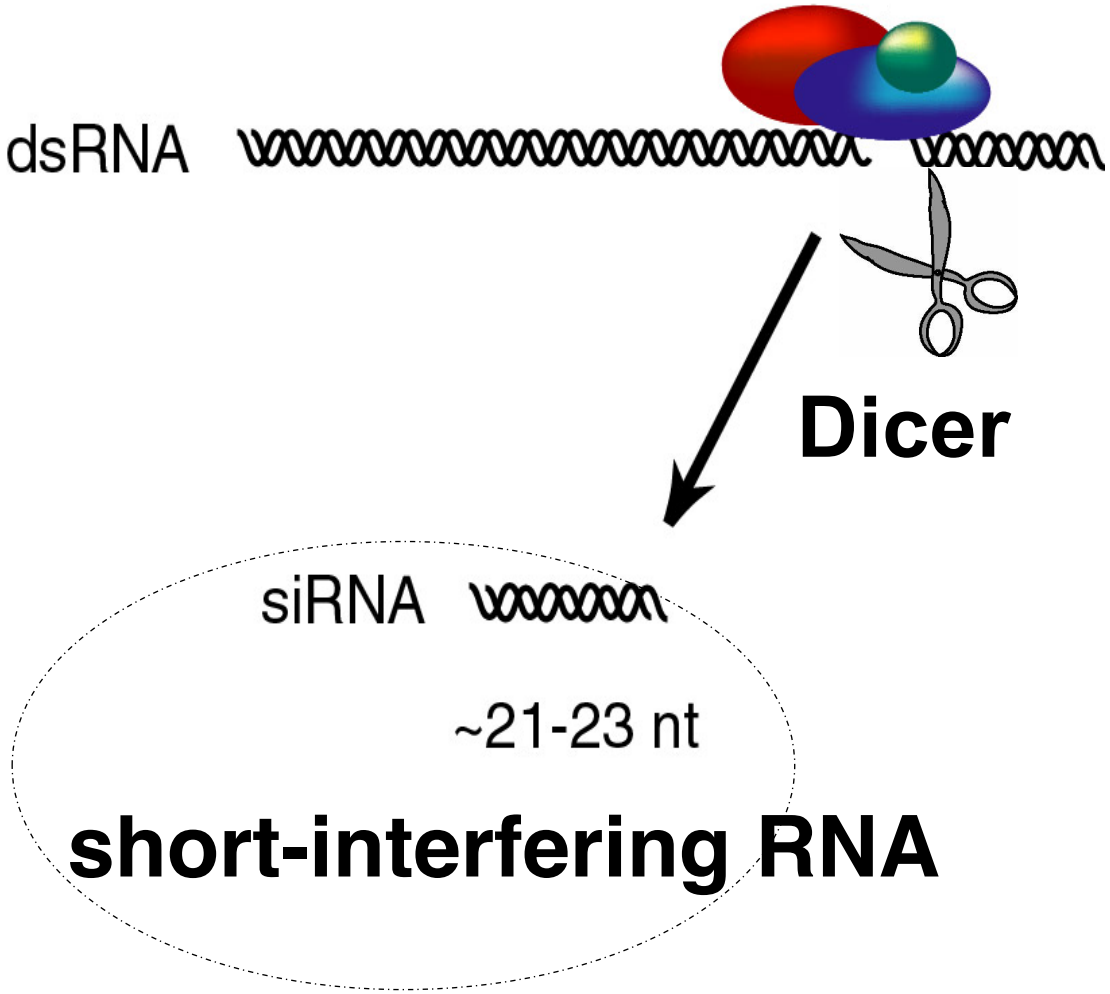
- dsRNA incubato con lisati di embrioni di *Drosophila* viene processato a specie di 21-23 nucleotidi
- Il processo richiede ATP



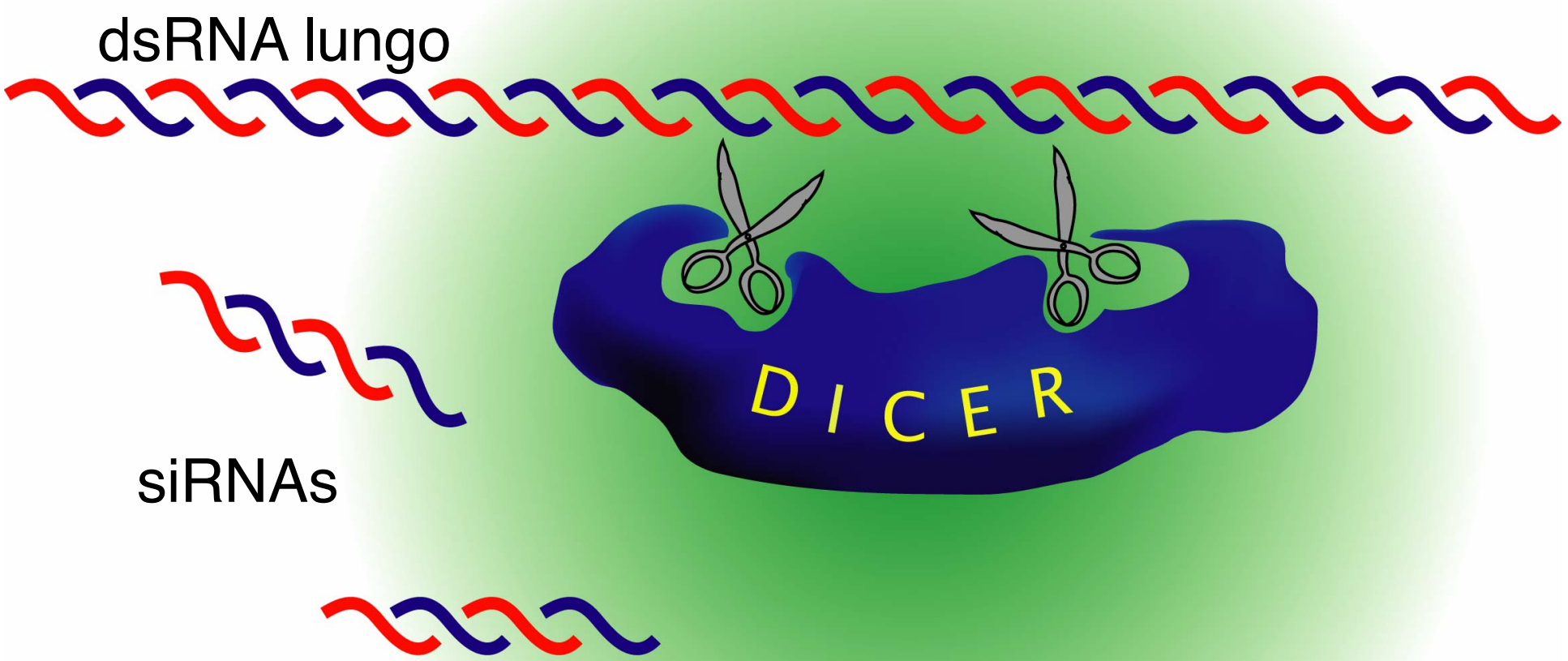
Zamore et al., (2001)

Prima fase:

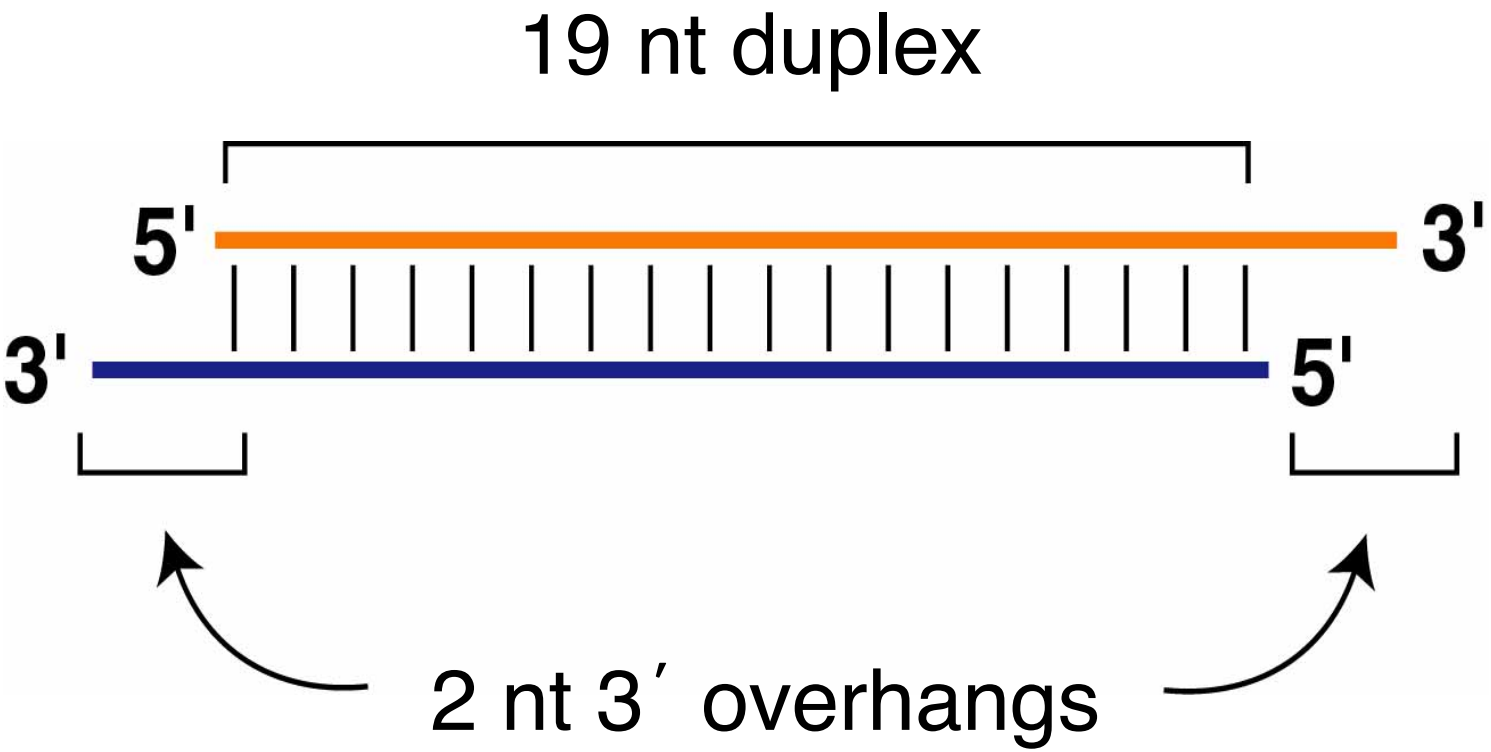
processamento del dsRNA in frammenti di 21-23 nt



Dicer contiene due dominii di tipo RNAsi III

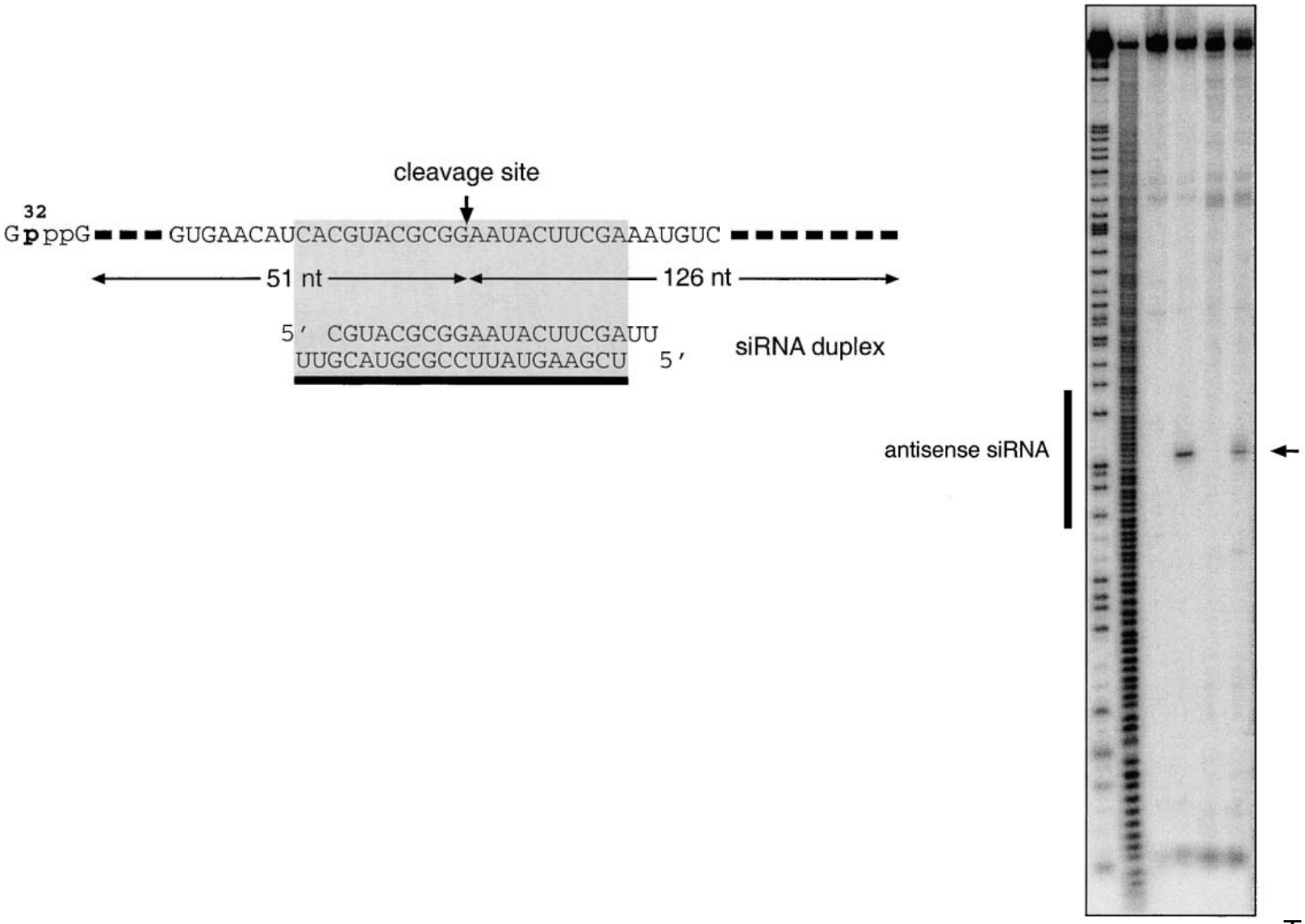


Gli siRNA hanno una struttura definita



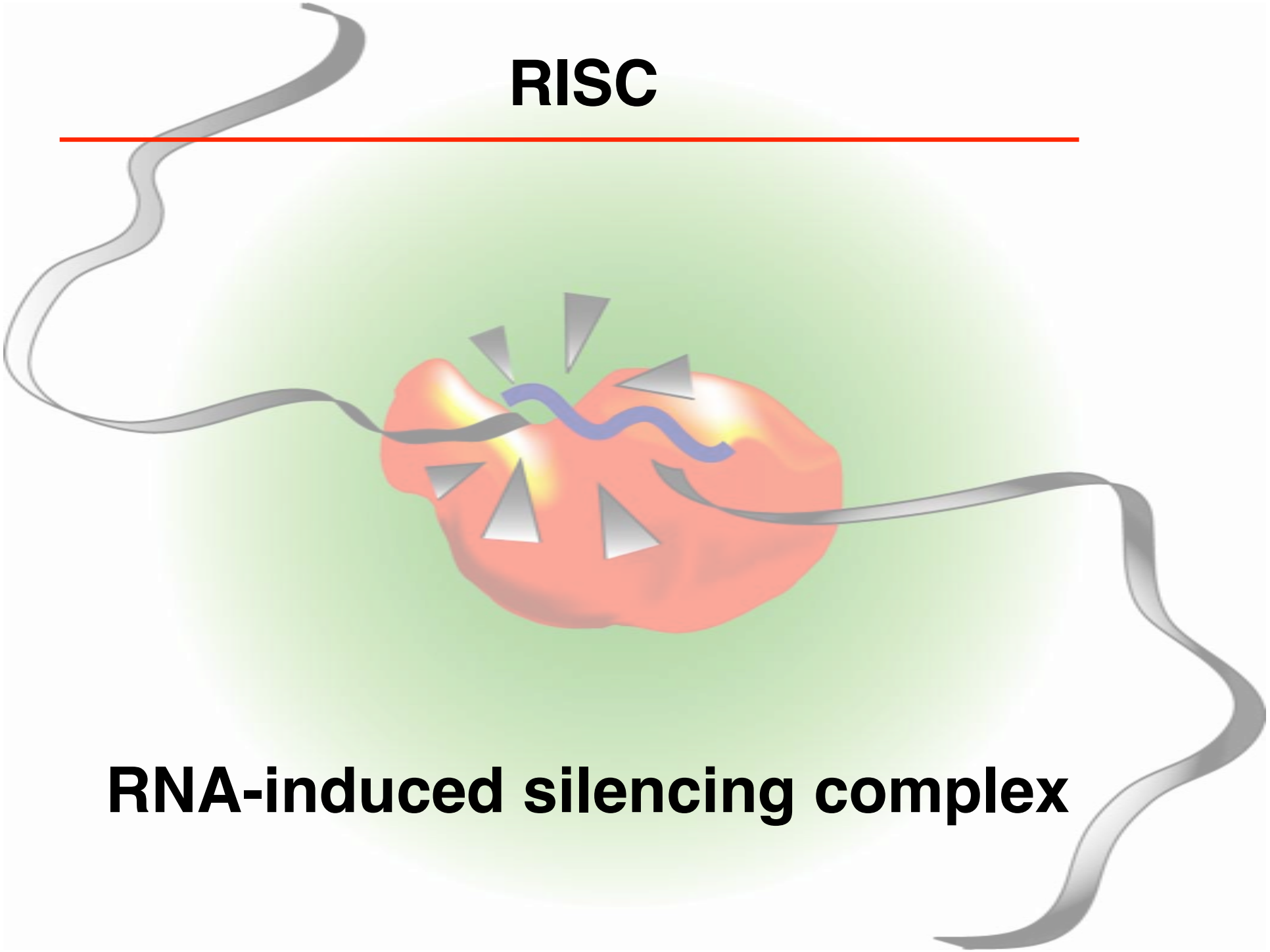
Seconda fase:

Il filamento antisenso degli siRNA guida il taglio



Tuschl (2002)

RISC

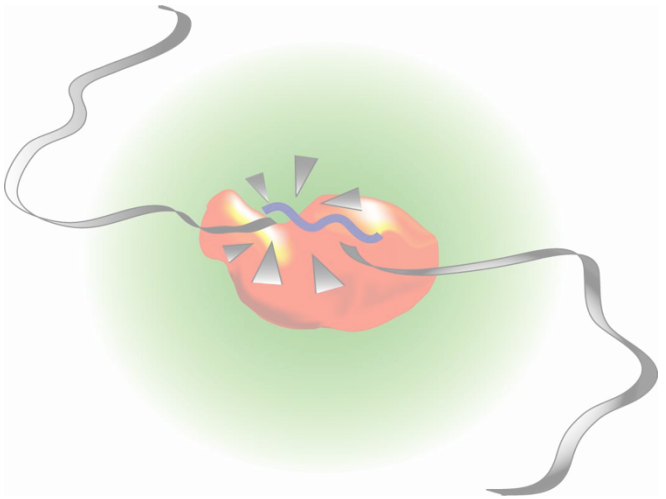


RNA-induced silencing complex

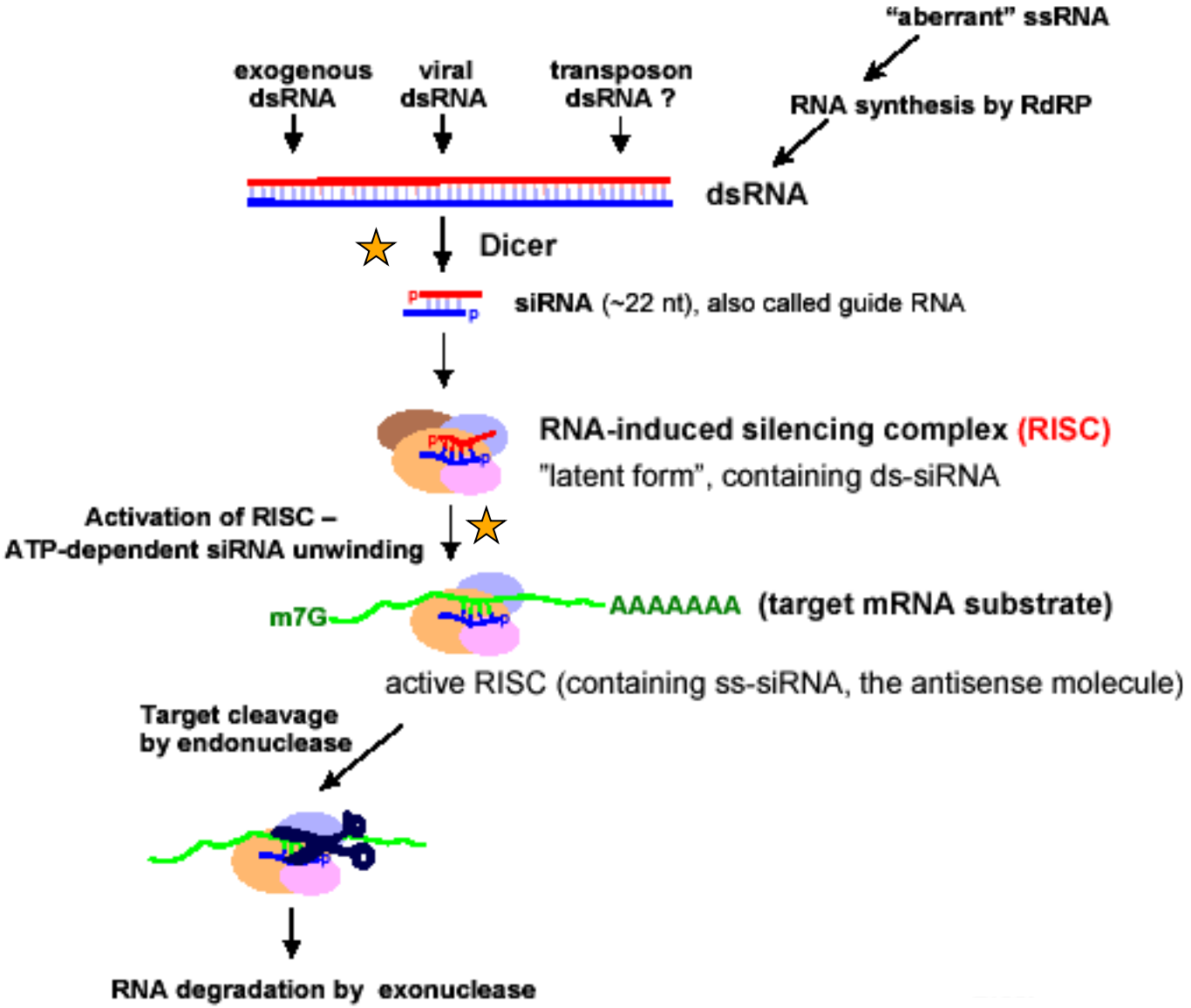
Seconda fase:

Il filamento antisenso degli siRNA guida il taglio

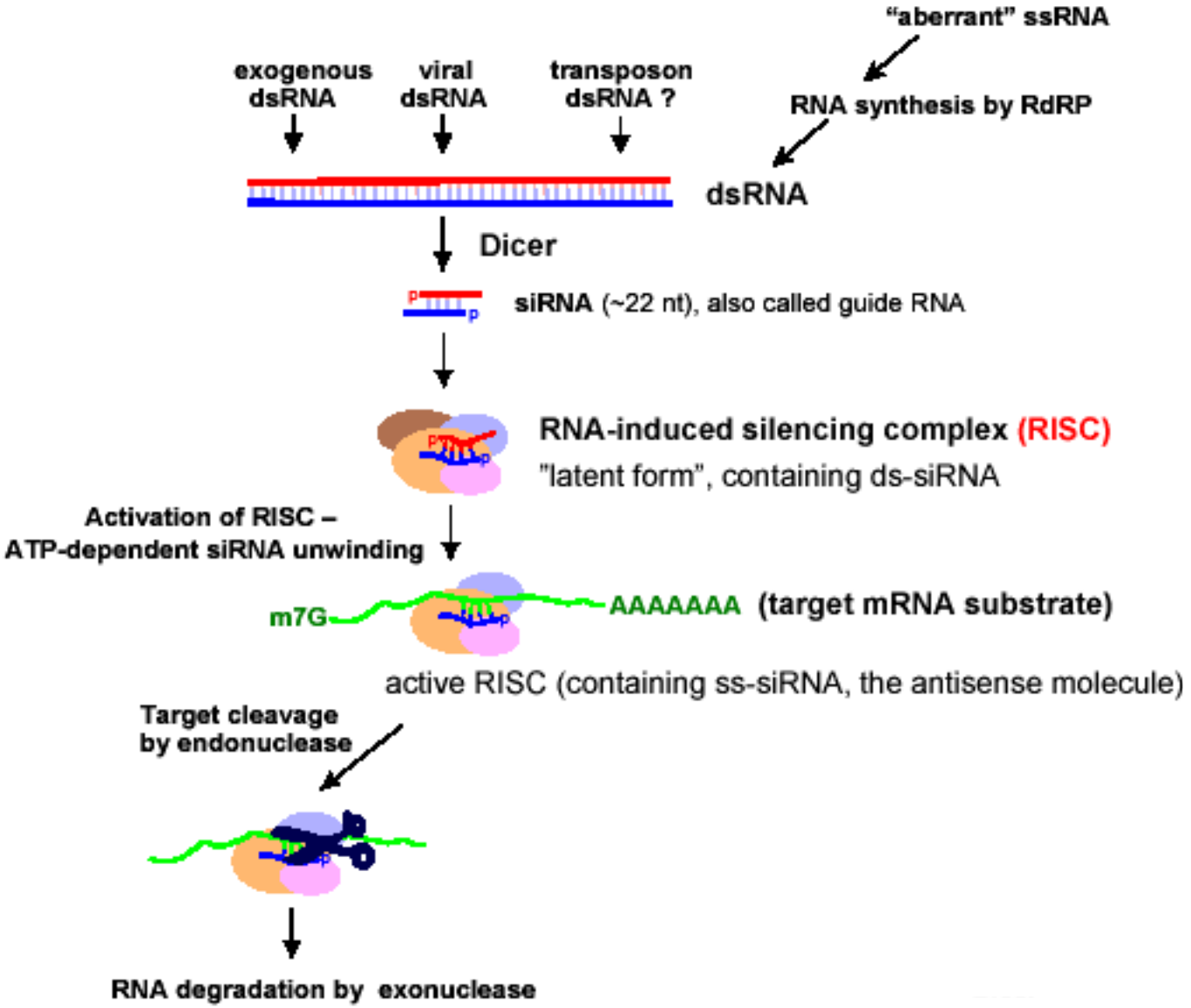
- Il duplex siRNA lega RISC, un complesso multiproteico contentente anche delle nucleasi
- L'attivazione di RISC e' ATP-dipendente e richiede lo svolgimento del duplex siRNA



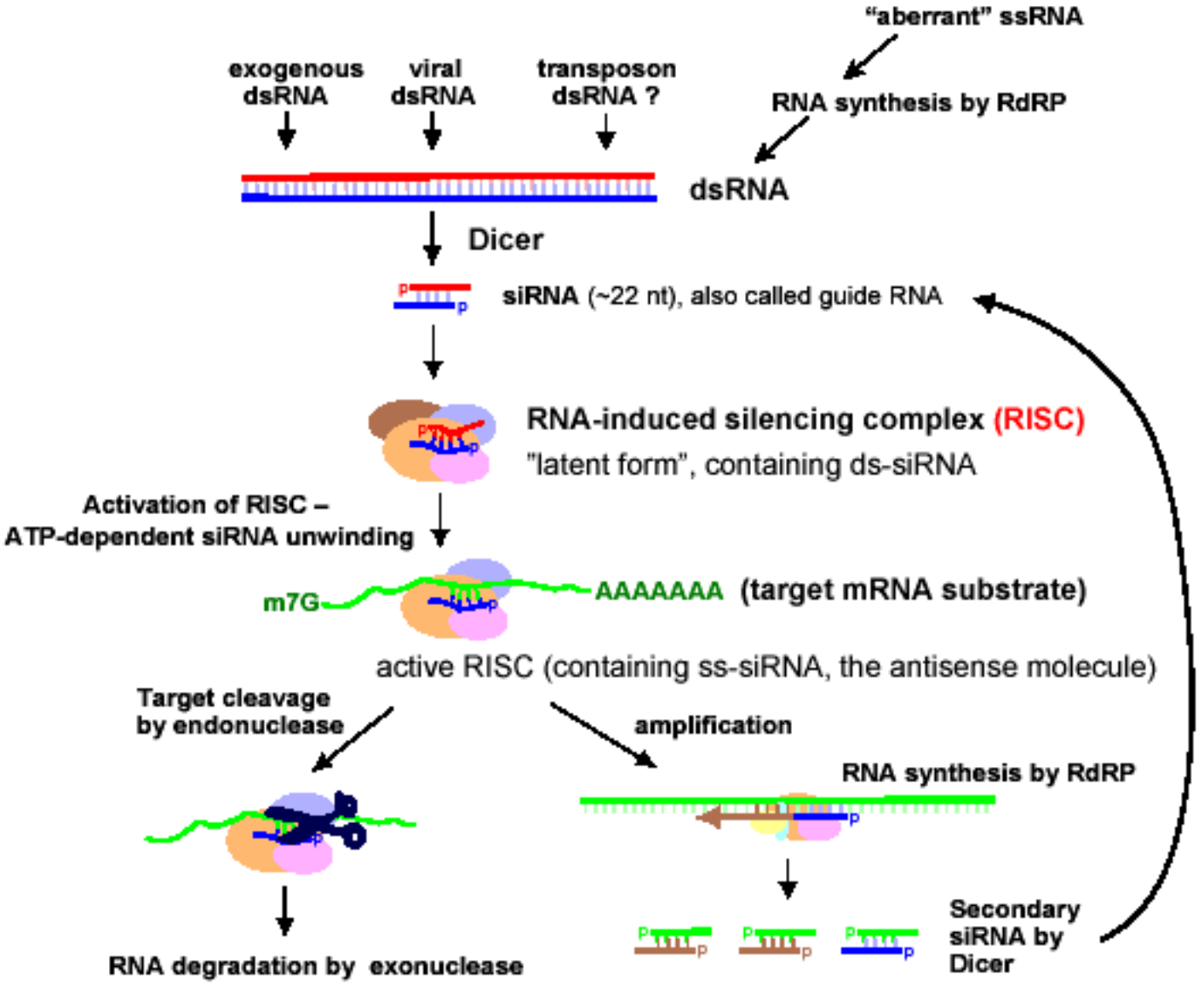
Meccanismo dell' RNAi



Meccanismo dell' RNAi



Meccanismo dell' RNAi



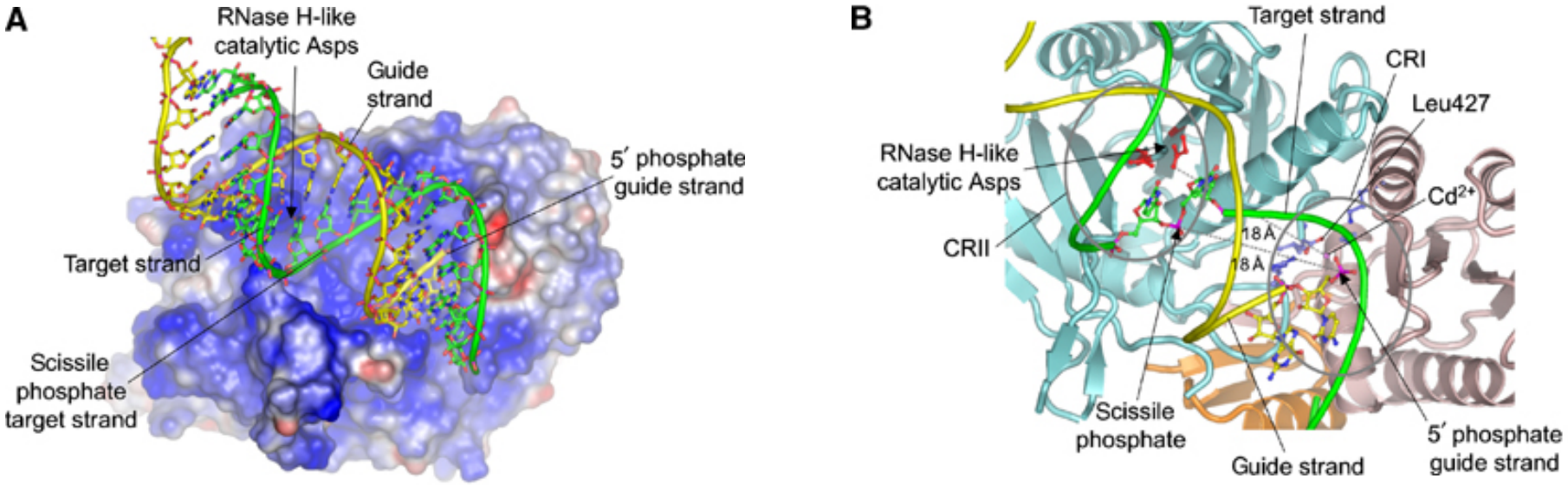
La famiglia ARGONAUTE (Ago)

In tutti gli organismi esistono varie proteine Ago:

8 uomo
10 *Arabidopsis*
5 *Drosophila*
27 *C. elegans*
1 *S.pombe (no miRNA, si siRNA)*
1 *Archea (no miRNA, no siRNA)*

ma solo alcune sono associate a RISC

Ago e' lo "slicer" del RISC



- *in vitro* e' in grado di tagliare RNA target in presenza di siRNA

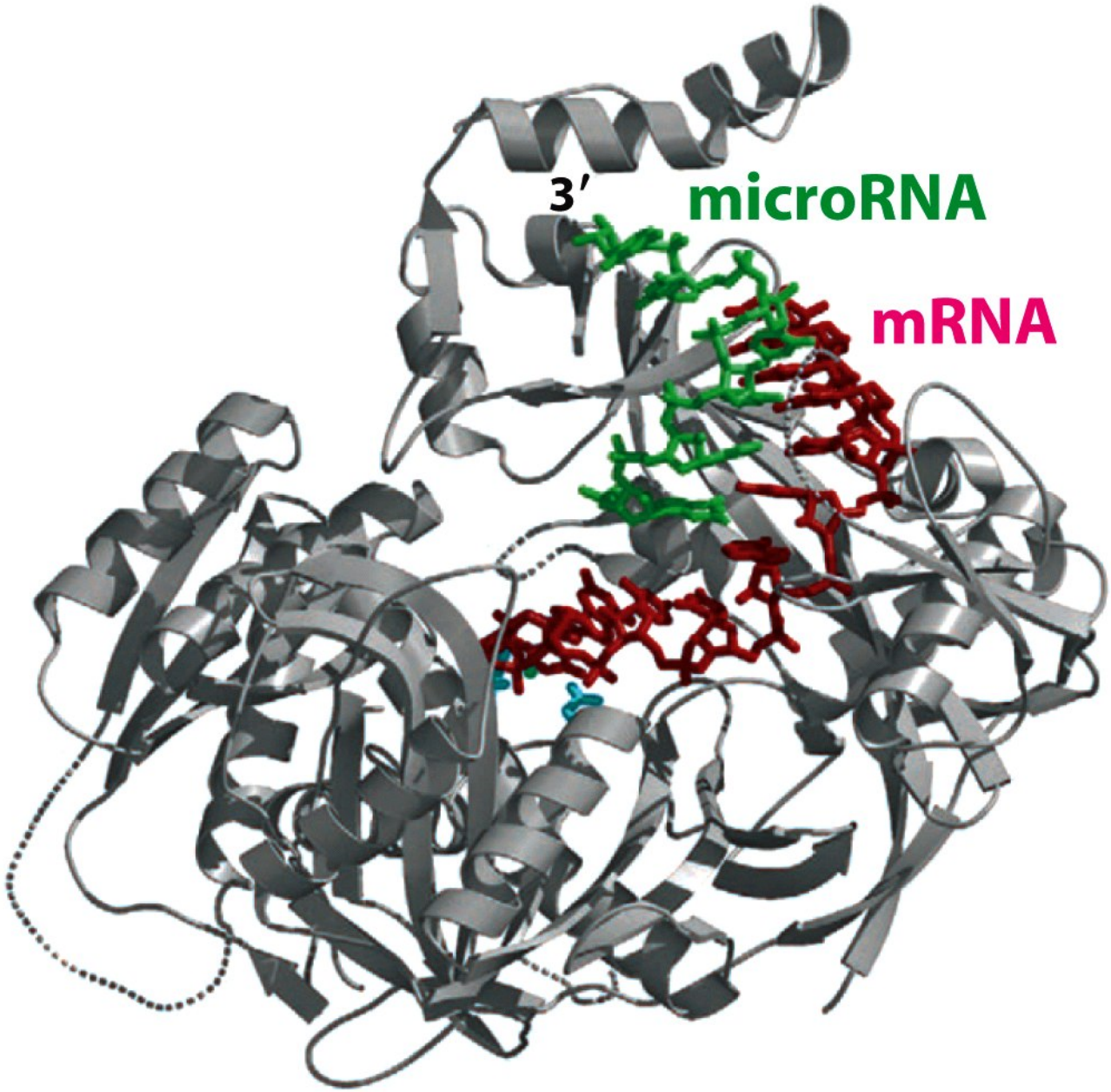
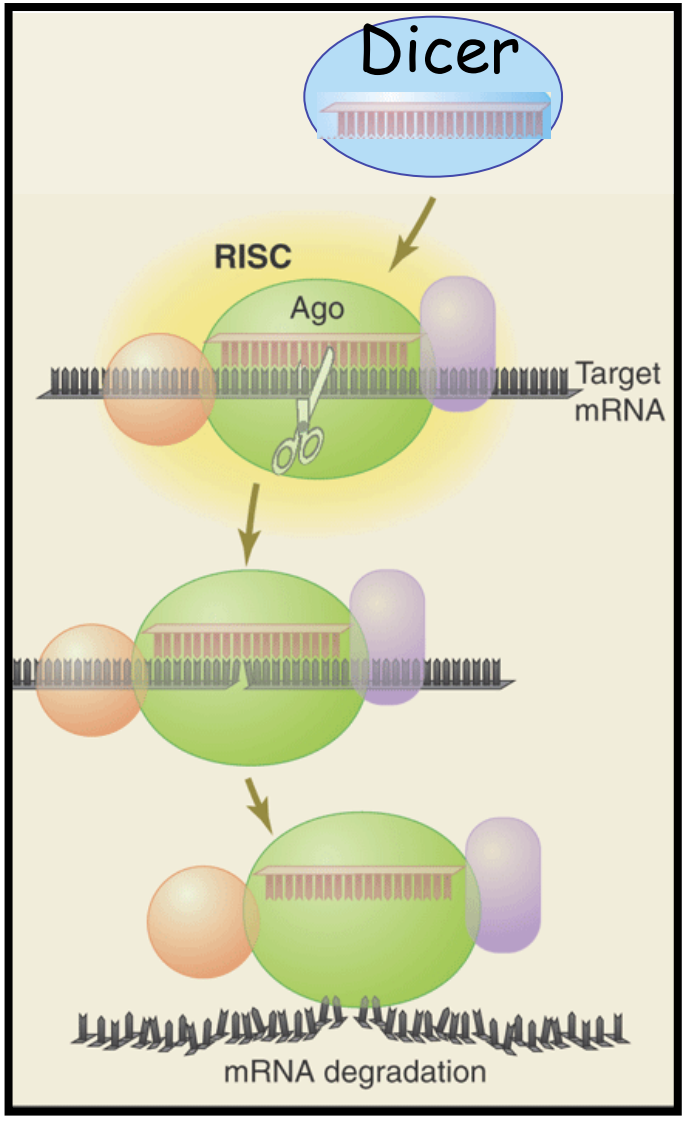


Figure 7-113 *Molecular Biology of the Cell* (© Garland Science 2008)

Ago e' il core catalitico del RISC



Qual è il ruolo biologico di RNAi?

Funzioni di RNAi

- Controllo di acidi nucleici parassiti esogeni (=virus)

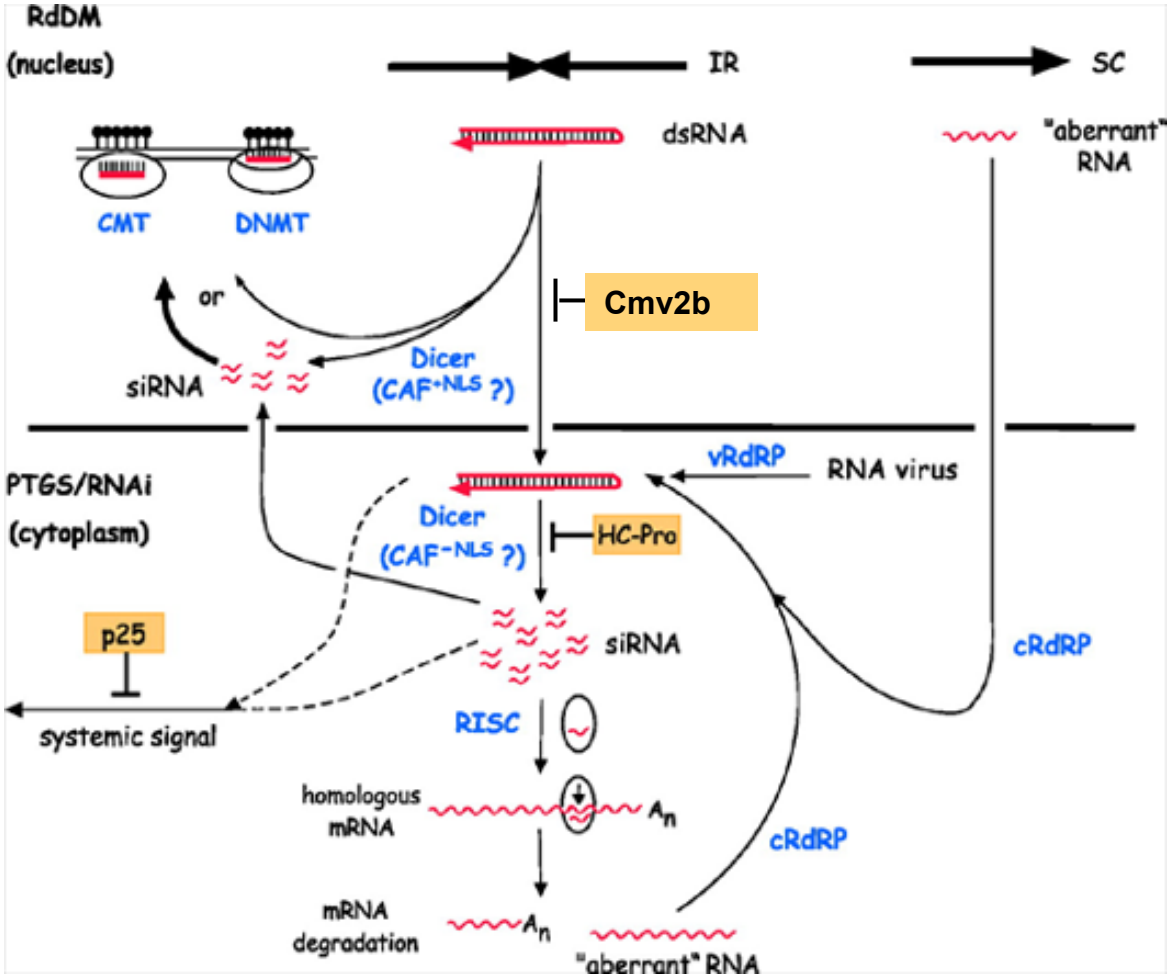
- Controllo di acidi nucleici parassiti endogeni (=trasposoni)

- Regolazione temporale dello sviluppo mediante repressione della traduzione (e controllo della stabilita' di mRNA): **stRNA** (**miRNA**)

- Regolazione dello stato della cromatina

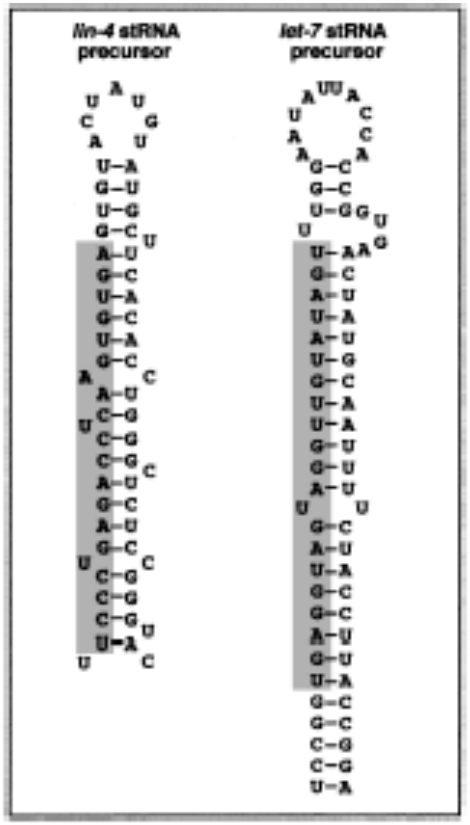
- ?

Soppressori virali di RNAi



Piccoli RNA che inibiscono la traduzione

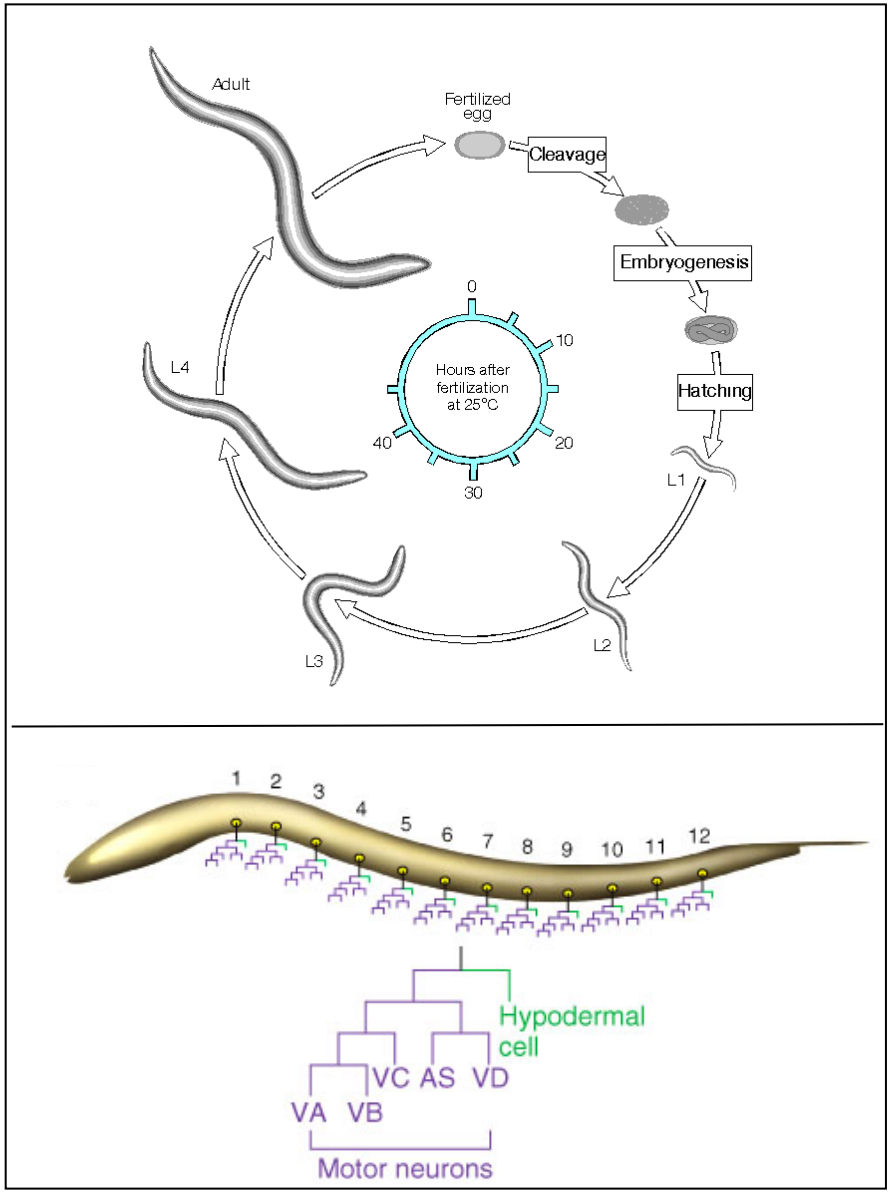
I genomi di *C. elegans*, *Drosophila*, uomo e piante codificano per micro RNA (miRNA)=small temporal RNA (stRNA)



Lo small temporal RNA lin-4

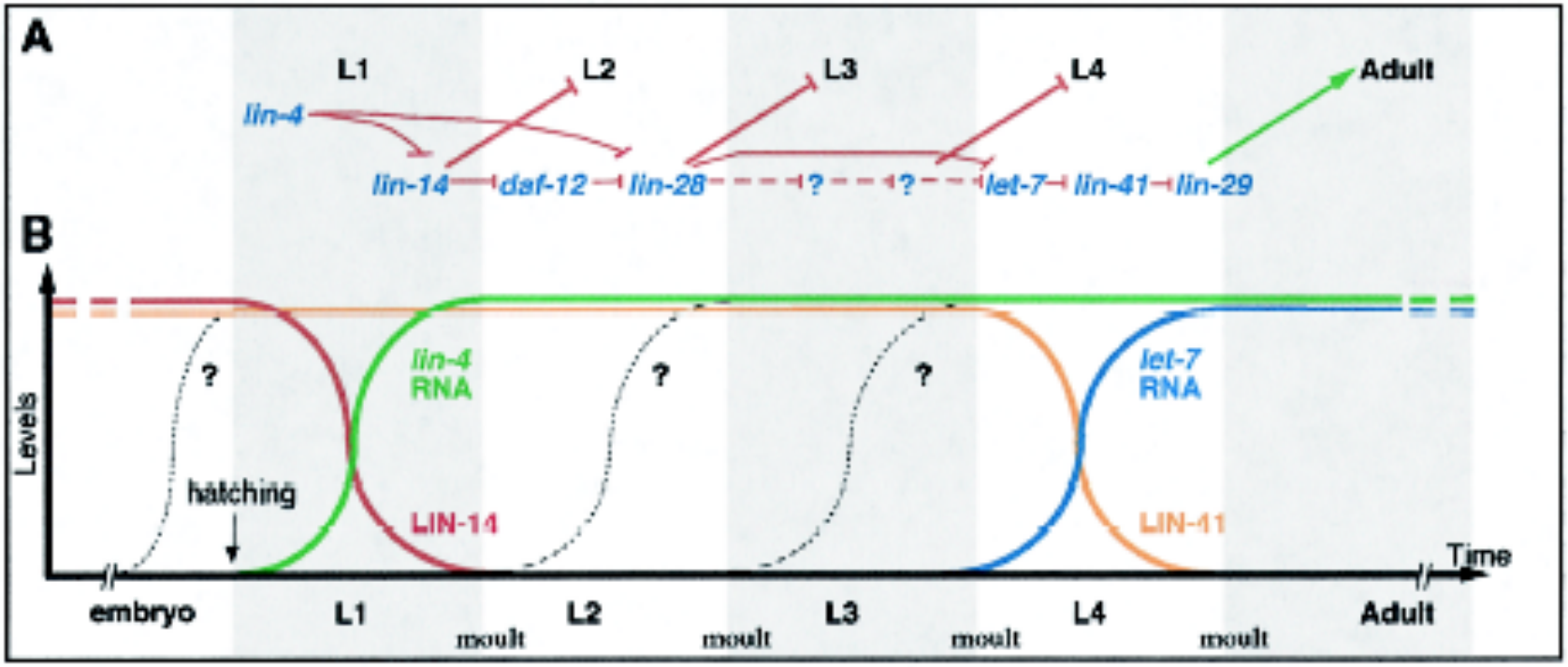
•In *C.elegans* le linee cellulari hanno caratteristiche distinte nei 4 stadi larvali

•Screening genetici per difetti nel controllo temporalle dello sviluppo embrionale avevano identificato i loci *lin-4* e *lin-14* con effetti opposti sullo stadio L1



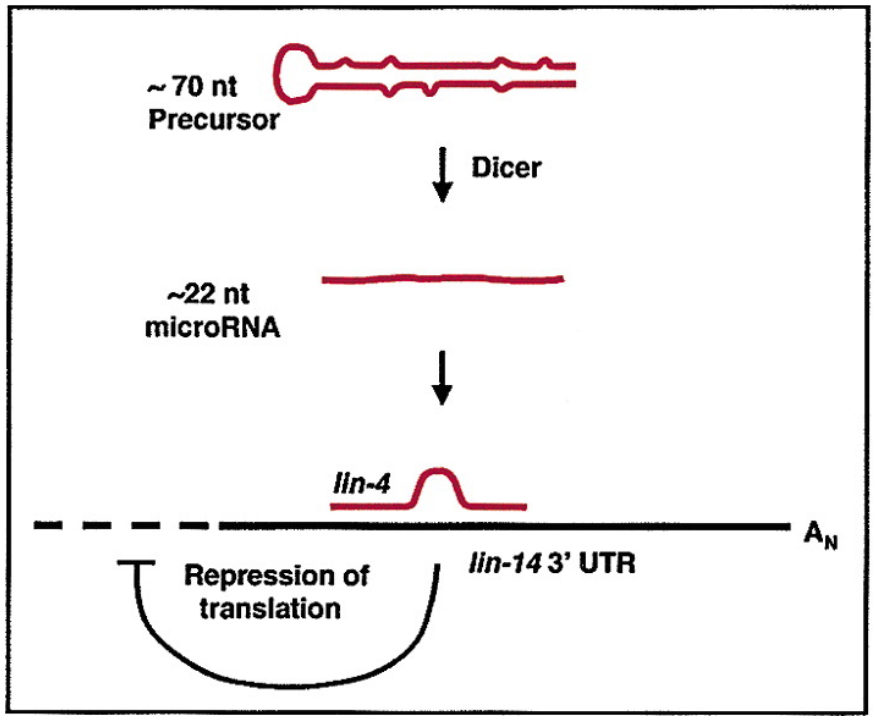
Ambros, V. A **1989**. *Cell*
Arasu P, Wightman B, Ruvkun G. **1991**. *Gen. Dev.*

Piccoli RNA che inibiscono la traduzione



Gli stRNA lin4 e let7 controllano le fasi dello sviluppo in *C. elegans* reprimendo la traduzione di lin-14 e lin-41.

Piccoli RNA che inibiscono la traduzione

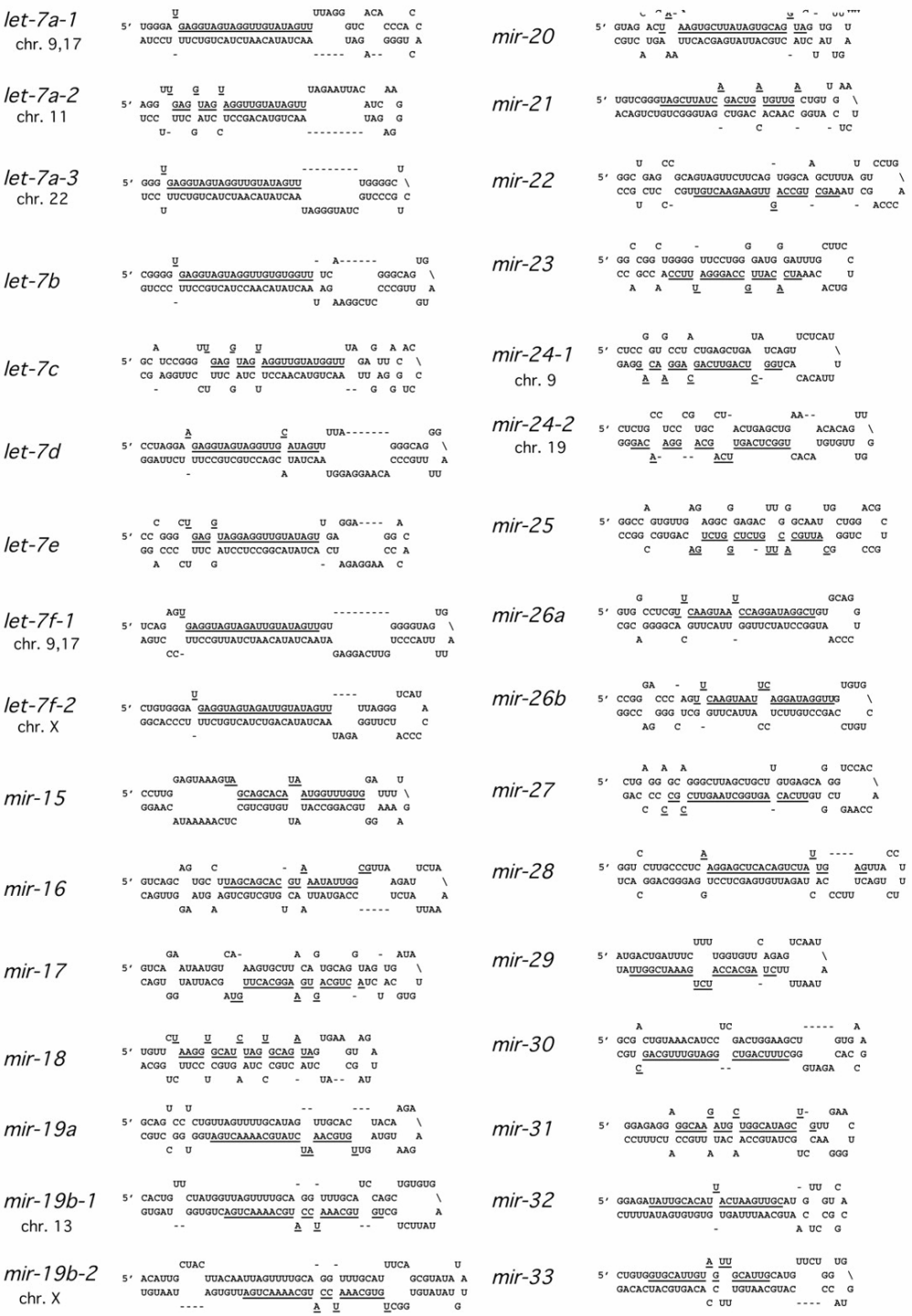


……oggi

- **centinaia di miRNA in tutti gli organismi (metazoi e piante)**

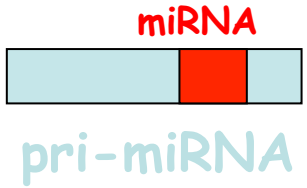
78	*D.melanogaster*
116	*C.elegans*
232	*H.sapiens*
112	*A.thaliana*

- **miRNA codificati da virus (EBV, Herpes virus, HIV)**


let-7a-1
chr. 9,17
let-7a-2
chr. 11
let-7a-3
chr. 22
let-7b
let-7c
let-7d
let-7e
let-7f-1
chr. 9,17
let-7f-2
chr. X
mir-15
mir-16
mir-17
mir-18
mir-19a
mir-19b-1
chr. 13
mir-19b-2
chr. X
mir-20
mir-21
mir-22
mir-23
mir-24-1
chr. 9
mir-24-2
chr. 19
mir-25
mir-26a
mir-26b
mir-27
mir-28
mir-29
mir-30
mir-31
mir-32
mir-33

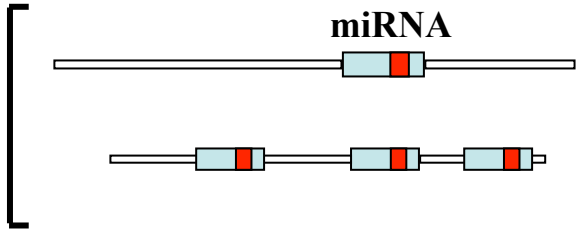
miRNA umani

Organizzazione genomica

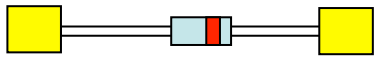



```
c    ccuccu   -     a    cc u              gaguug       cau
 cugg      gca gugcc cgcu  g guauuugacaagcu      gacacuc   g
 ||||      ||| ||||| ||||  | ||||||||||||||      |||||||   u
 gacc      cgu cacgg guga  c cauaaacuguuuga      cugugag   g
-    ---auu   a     c    ac c             ------       aug
```

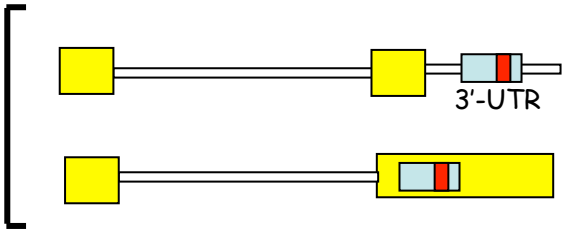
microRNA intergenici



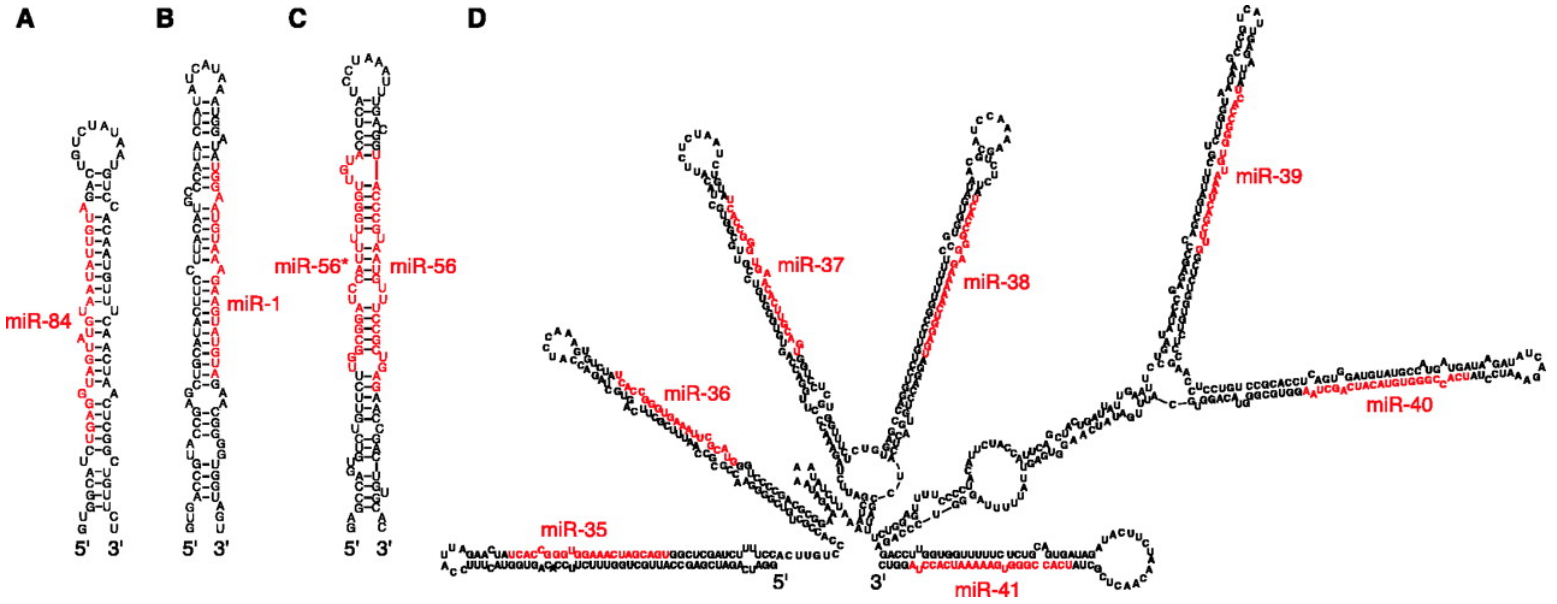
microRNA intronici



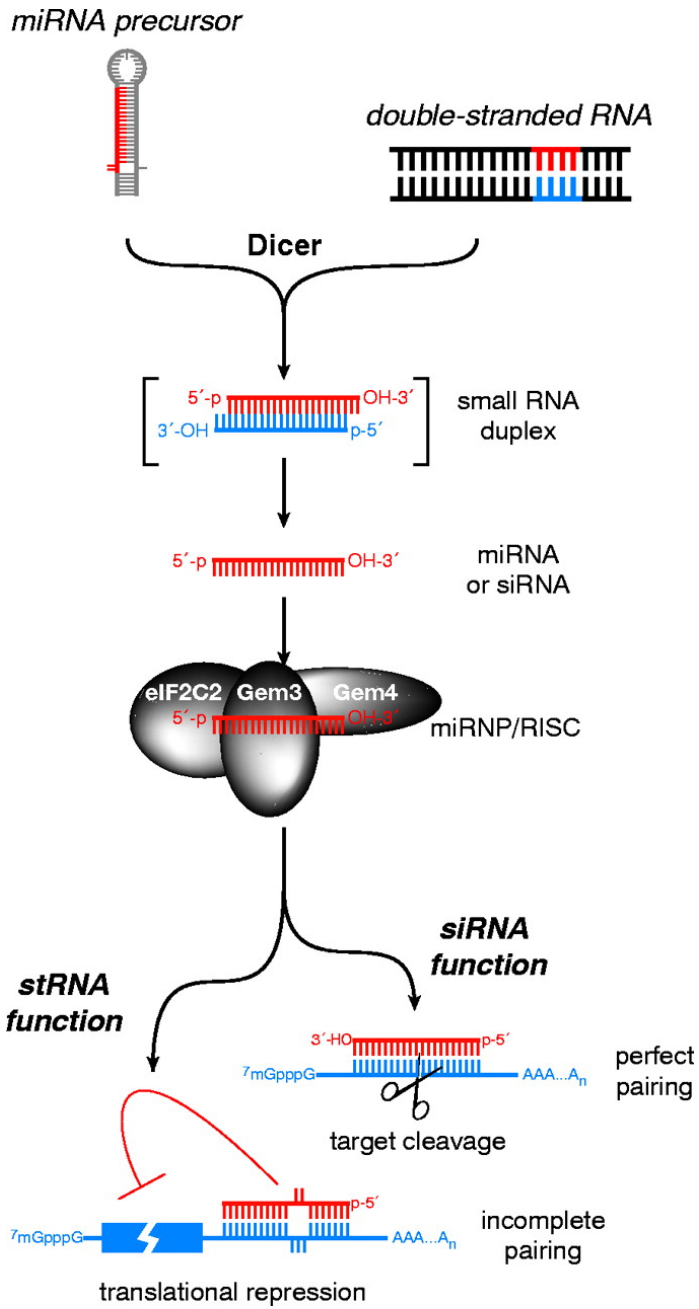
microRNA codificati in esoni o 3'-UTR



Piccoli RNA che inibiscono la traduzione

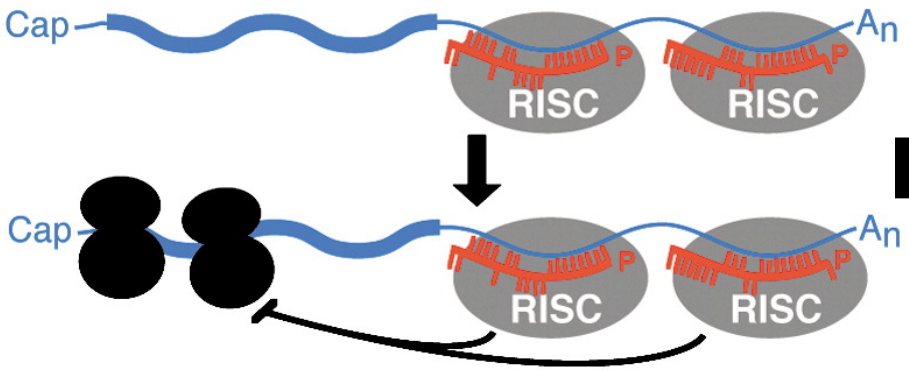


Lau et al., (2001)


miRNA precursor
double-stranded RNA
Dicer
5′-p
OH-3′
3′-OH
p-5′
small RNA duplex
5′-p
OH-3′
miRNA or siRNA
eIF2C2
Gem3
Gem4
5′-p
OH-3′
miRNP/RISC
siRNA function
stRNA function
3′-HO
p-5′
7mGpppG
AAA...An
perfect pairing
target cleavage
p-5′
7mGpppG
AAA...An
incomplete pairing
translational repression

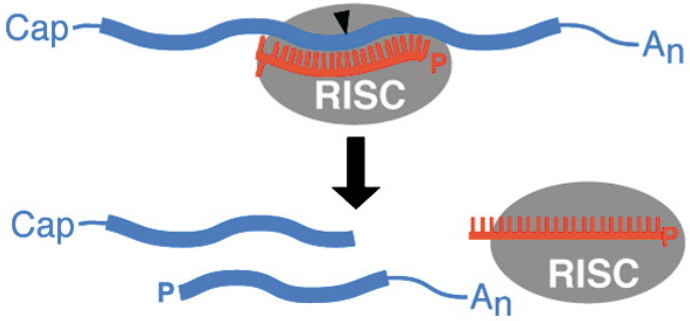
Meccanismi post-trascrizionali

Complementarieta' imperfetta



Repressione traduzionale

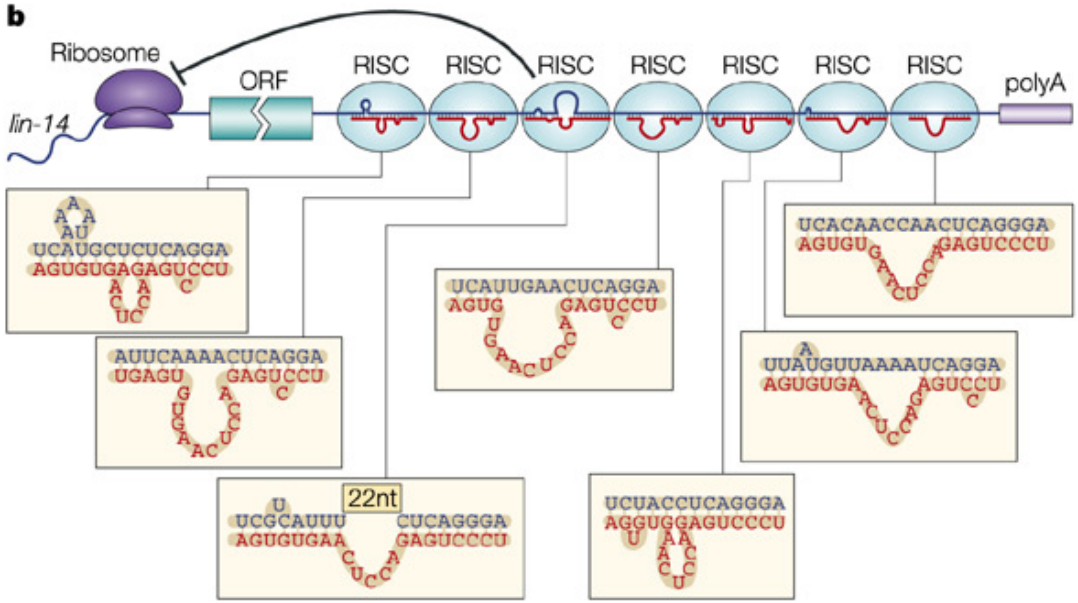
Complementarieta' perfetta



Degradazione dell'mRNA

Repressione traduzionale

- l'mRNA e' caricato normalmente sui poliribosomi
- calano solo i livelli di proteina e non quelli dell'mRNA



lin 4 miRNA

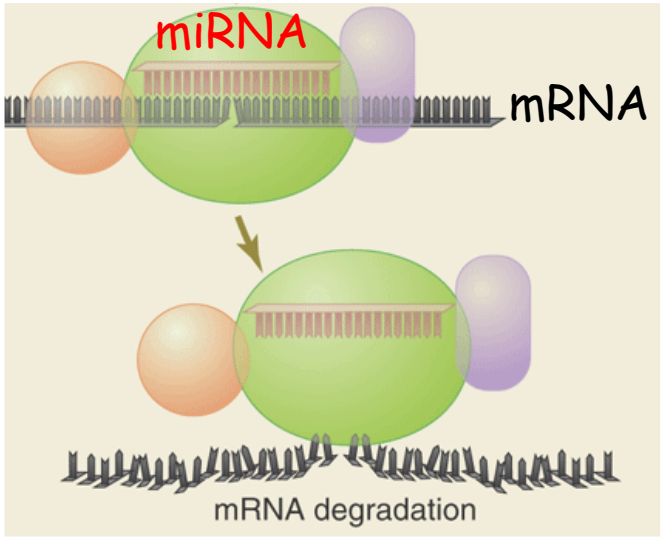
- la repressione da miRNA avviene dopo l'inizio della traduzione (elongazione ?)

Repressione traduzionale

- la repressione dipende solo dal 3'-UTR
- servono piu' siti riconosciuti dal miRNA all'interno del 3'-UTR
- spesso piu' miRNA sono coinvolti nella repressione di un singolo mRNA
- I primi 8-9 nt al 5' del miRNA sono cruciali per il riconoscimento del target

Degradazione del messaggero

- l'appaiamento con il target e' perfetto
- l'appaiamento e' nella sequenza codogenica dell'mRNA



- Il taglio e' effettuato da un fattore proteico del complesso RISC
- l'mRNA e' degradato da esonucleasi cellulari

Piccoli RNA non codificanti regolano molti geni eucariotici: micro RNA e RNAi

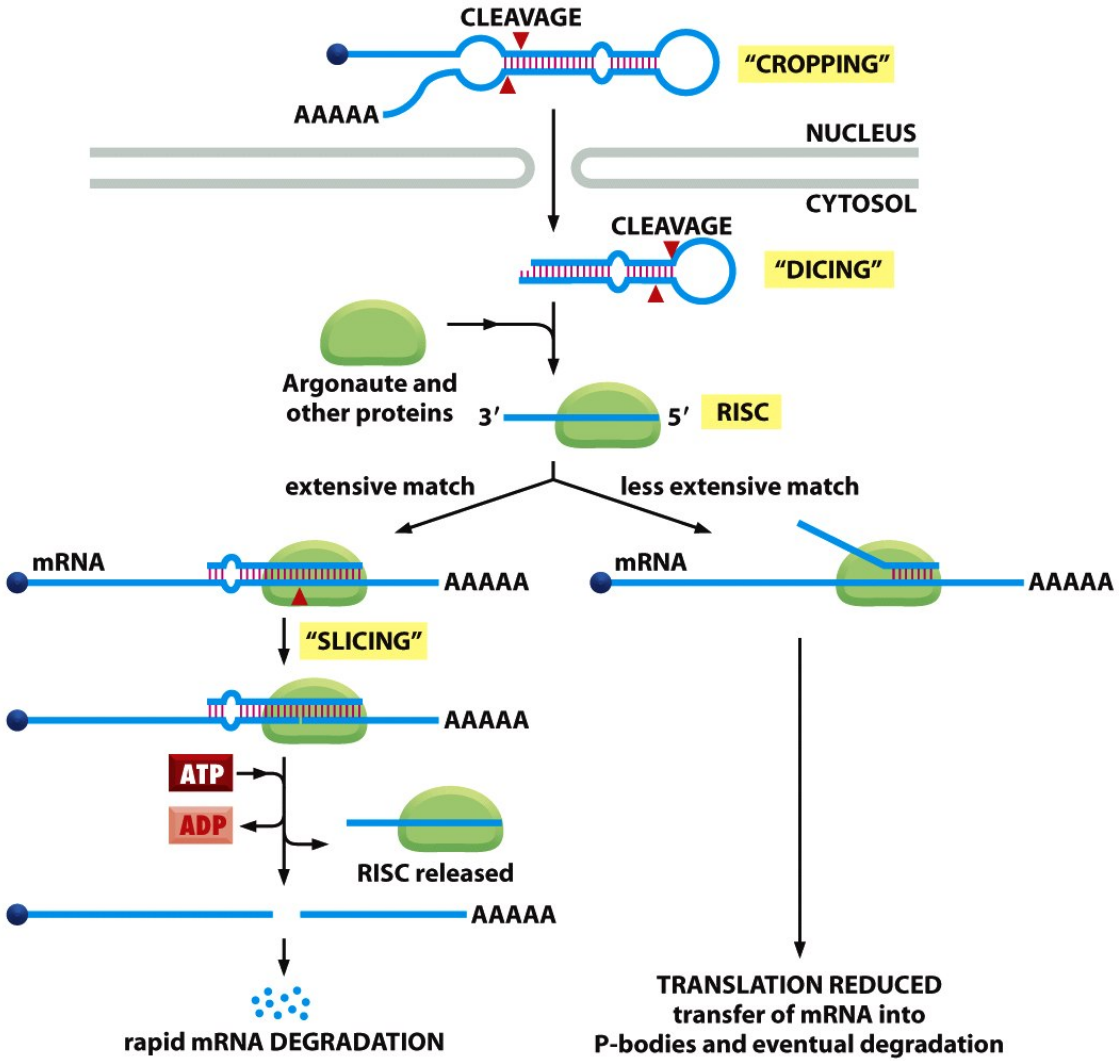
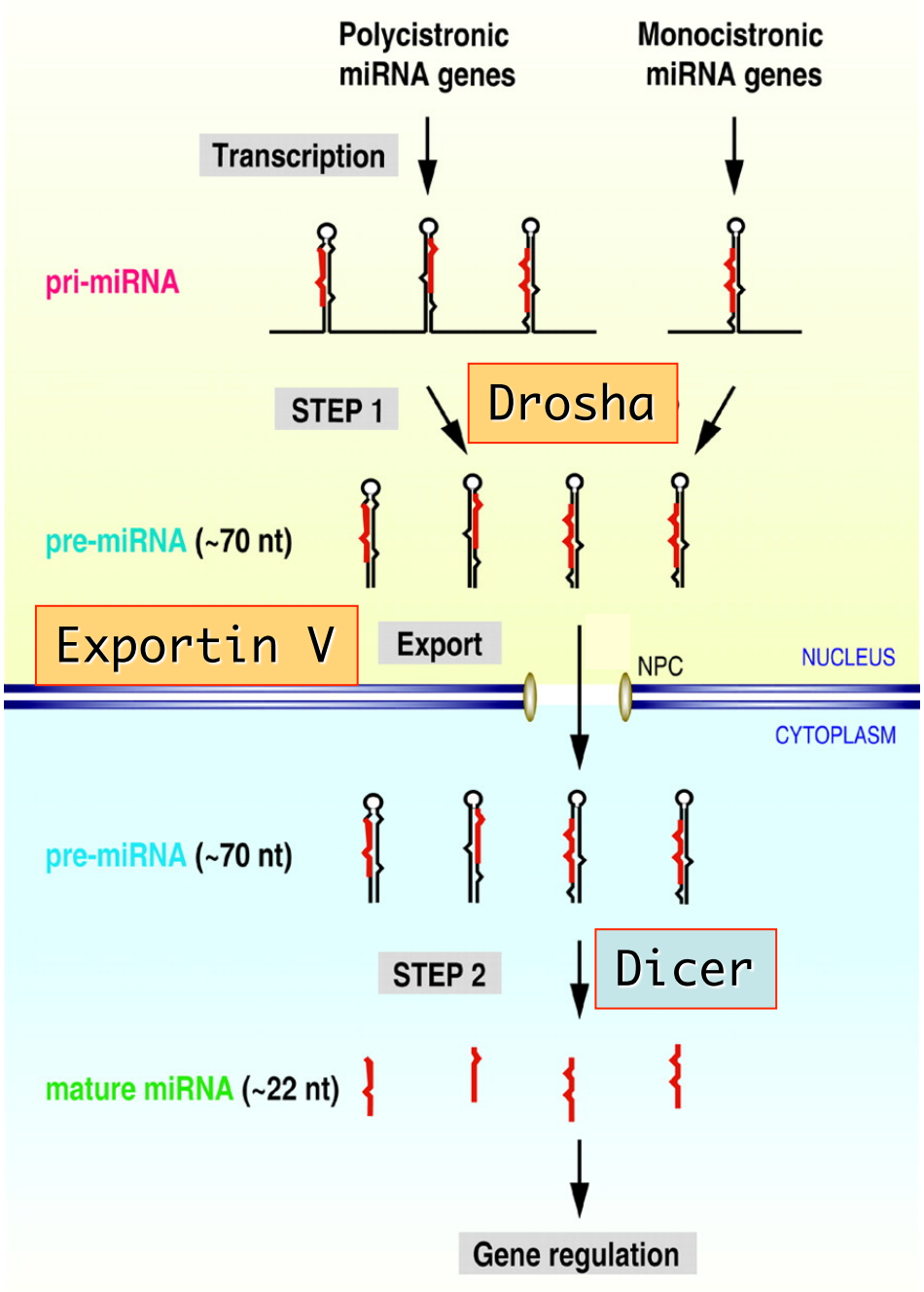


Figure 7-112 *Molecular Biology of the Cell* (© Garland Science 2008)

Biosintesi dei miRNA

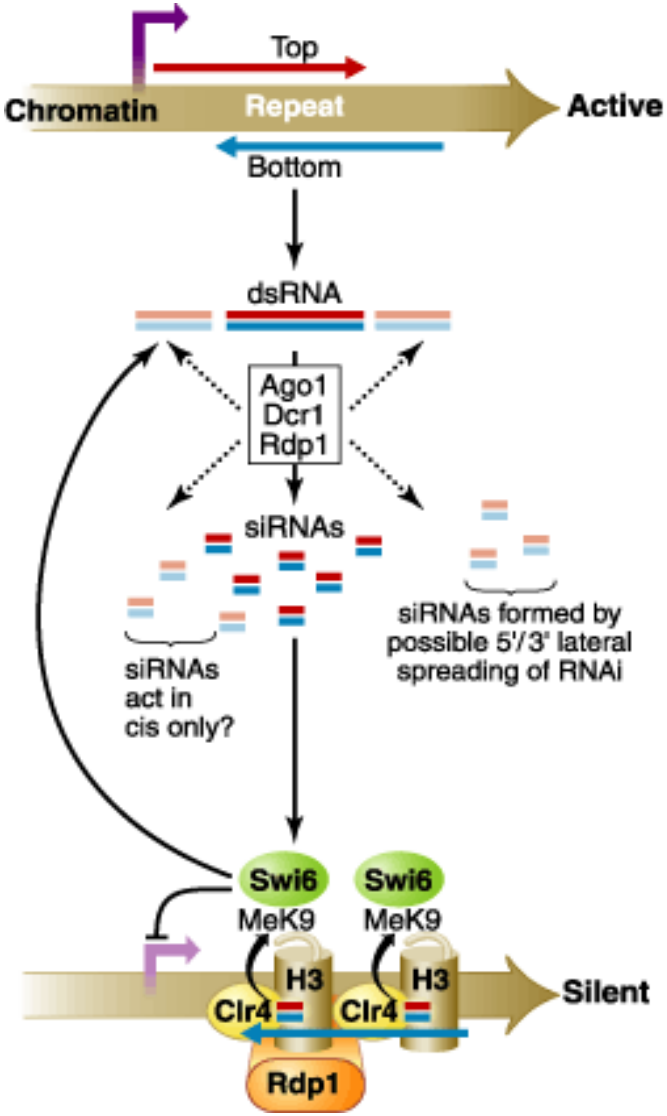


RNAi e silenziamento della cromatina

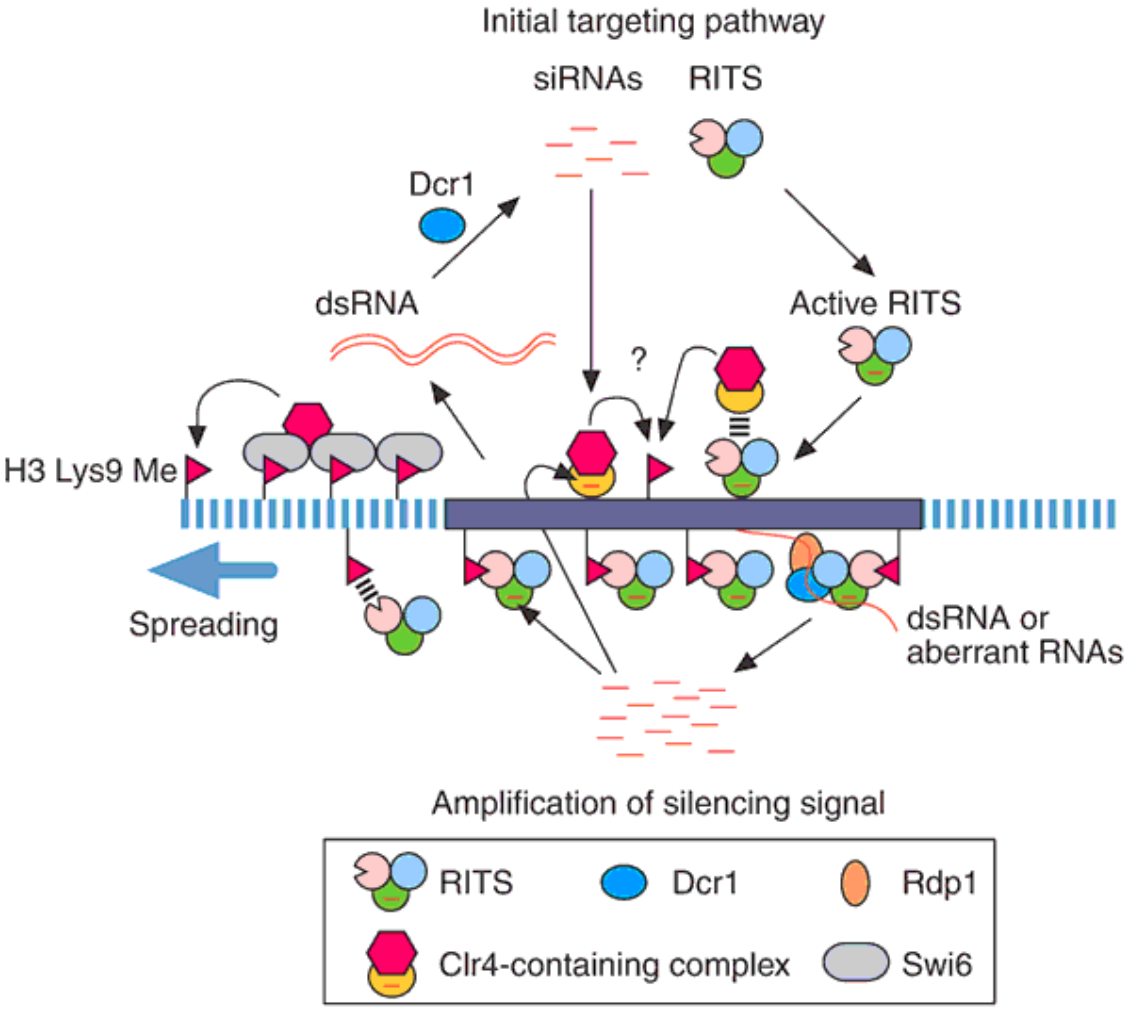
• I centromeri contengono ripetizioni e sono spesso eterocromatici (silenziati)

•Delezioni del macchinario di RNAi causano spesso de-silenziamento dei centromeri

Il complesso RITS
(RNA induced transcriptional silencing)



Il complesso RITS
(RNA induced transcriptional silencing)



S. pombe

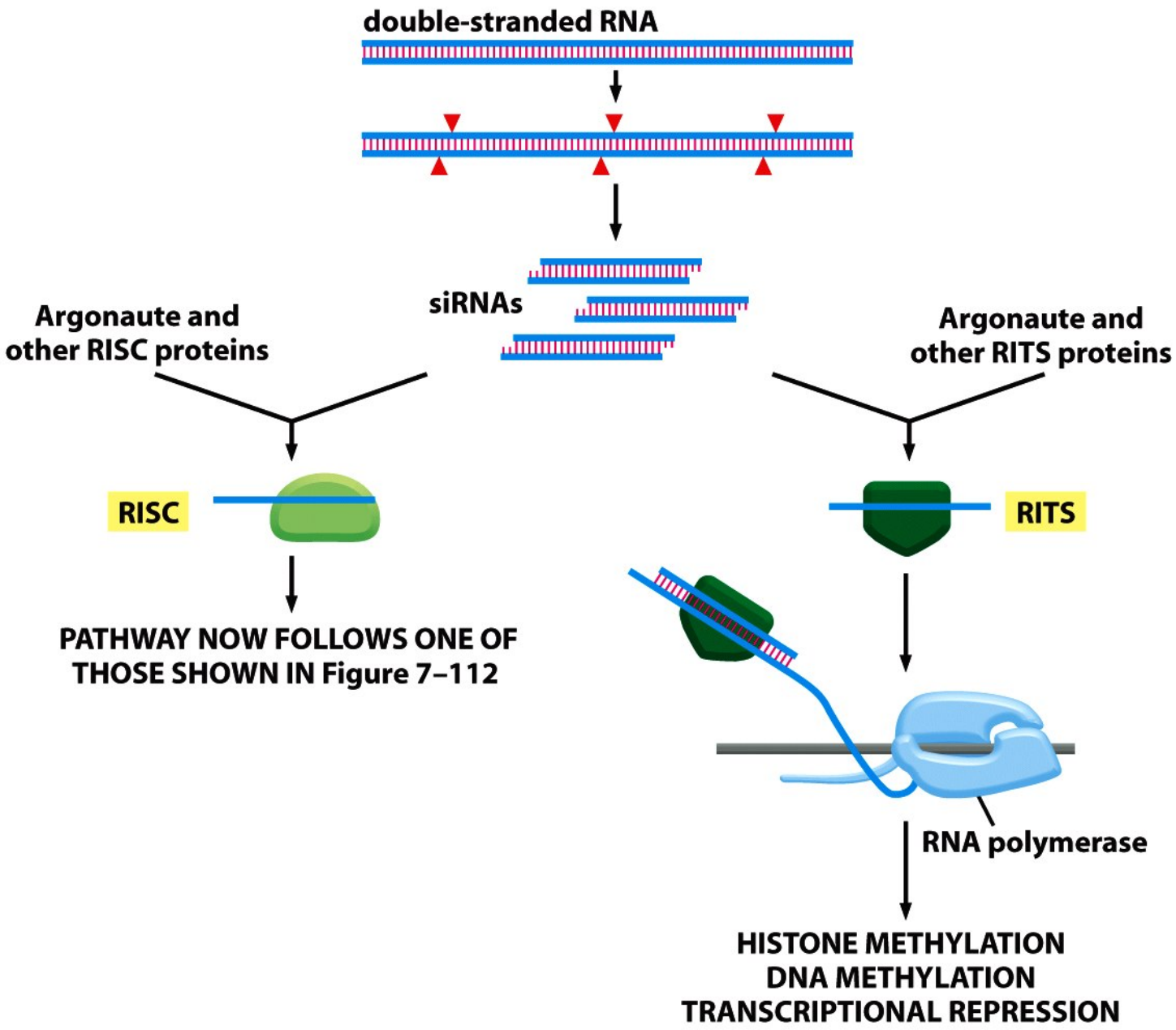


Figure 7-115 *Molecular Biology of the Cell* (© Garland Science 2008)

miRNA e metilazione della cromatina?

Nature. 2004

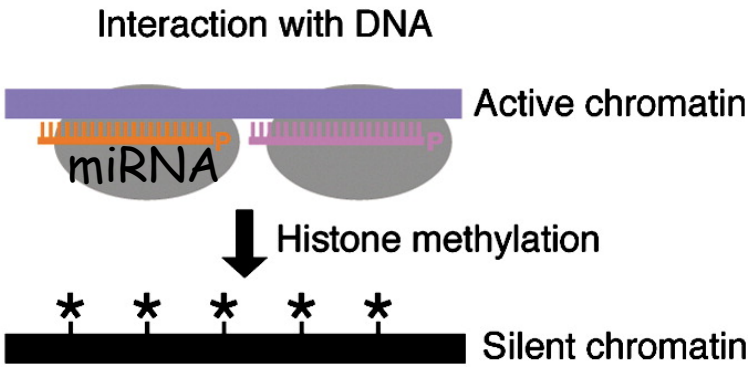
Induction of DNA methylation and gene silencing by short interfering RNAs in human cells.

Kawasaki H, Taira K.

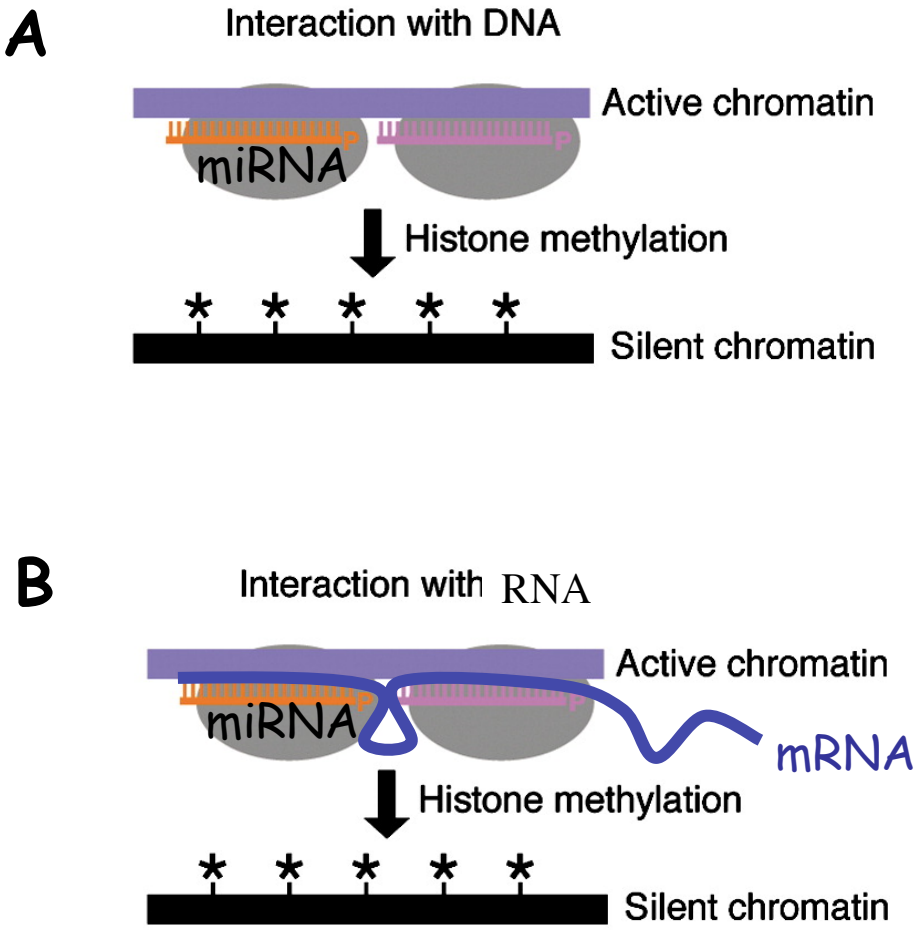
Cell Cycle. 2005

siRNA Induced Transcriptional Gene Silencing in Mammalian Cells.

Kawasaki H, Taira K, Morris KV



Inibizione trascrizionale da miRNA



RNAi e mobilita' dei trasposoni

- I trasposoni sono elementi di DNA ripetuti
- Delezioni del macchinario di RNAi causano attivazione della trasposizione
- L'RNA interference mantiene silenti i trasposoni.