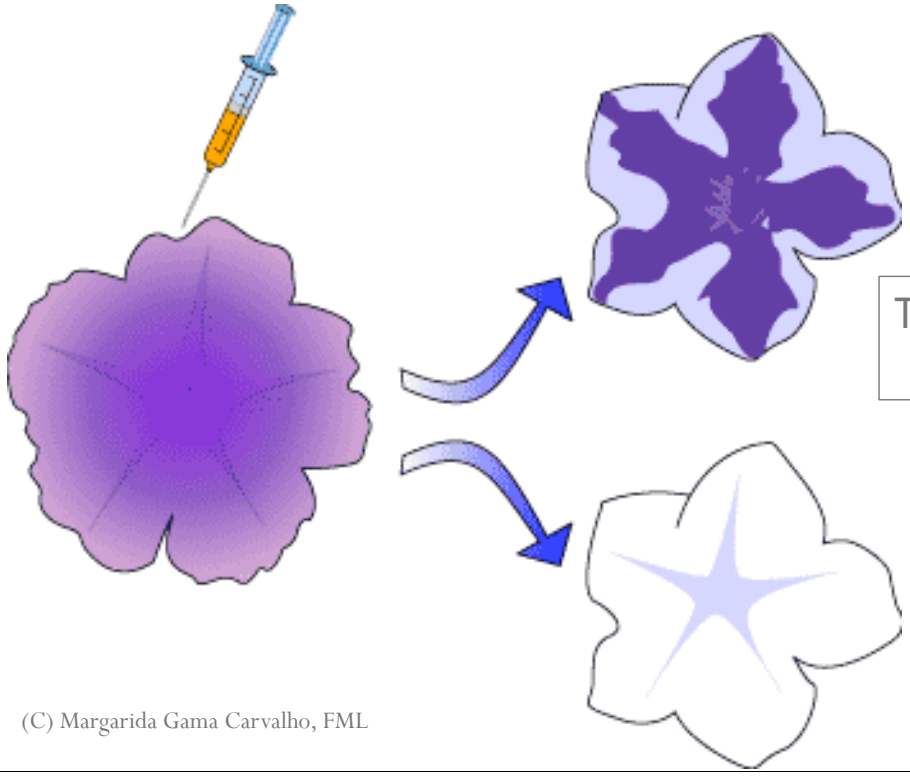
Parte III: Manipulação da informação

RNAi e microRNAs

1990: descoberta do silenciamento pós-transcricional ds expressão génica



Medicine

The Nobel Prize in Physiology or Medicine 2006

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

Photo: L. Cicero

Photo: J. Mottern

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

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† *Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA*
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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

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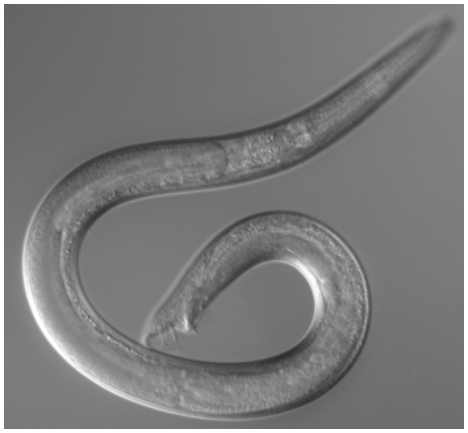
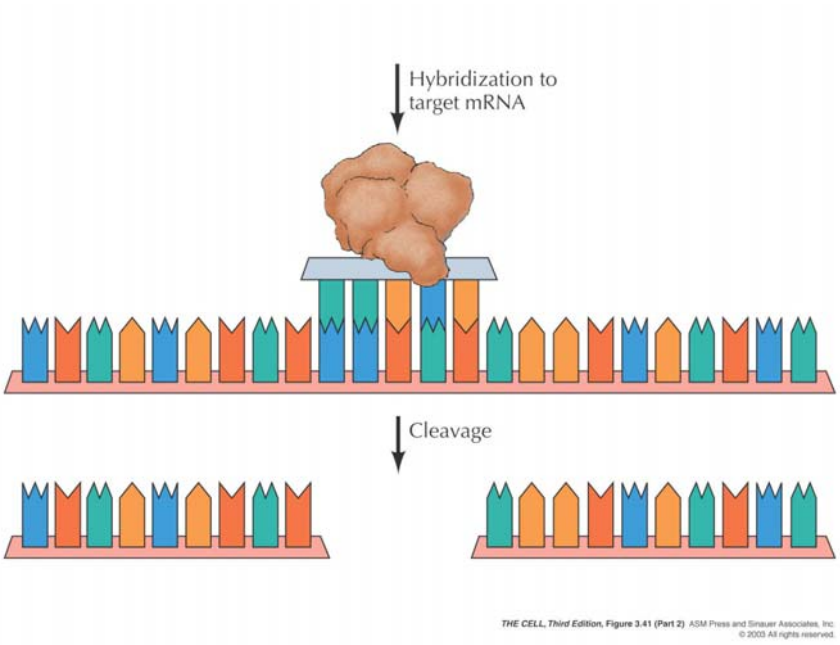


(C) Margarida Gama Carvalho, FML

Interferência de RNA



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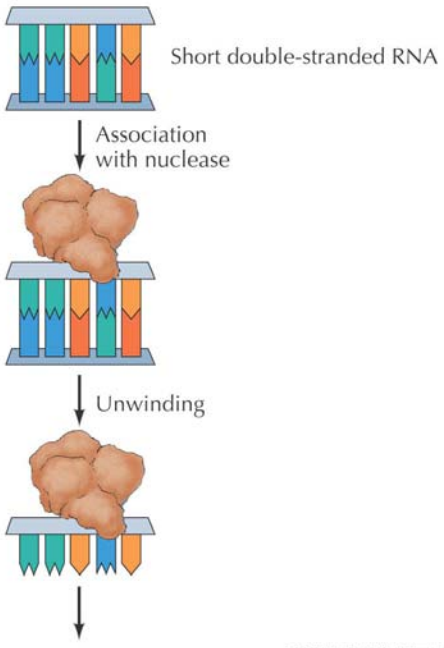
Interferência de RNA



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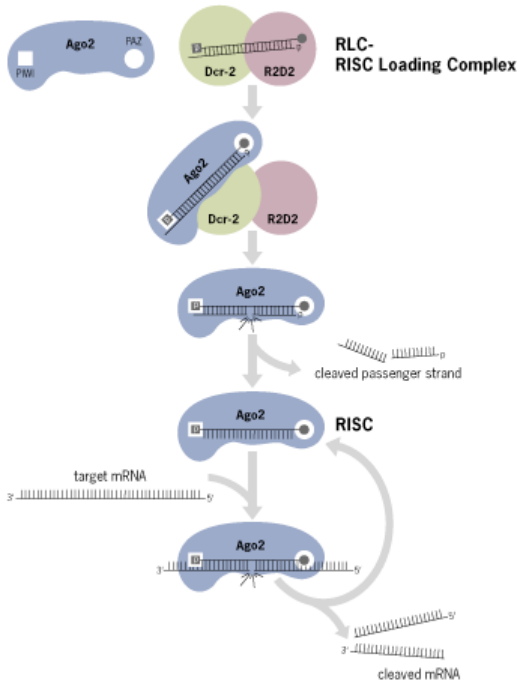
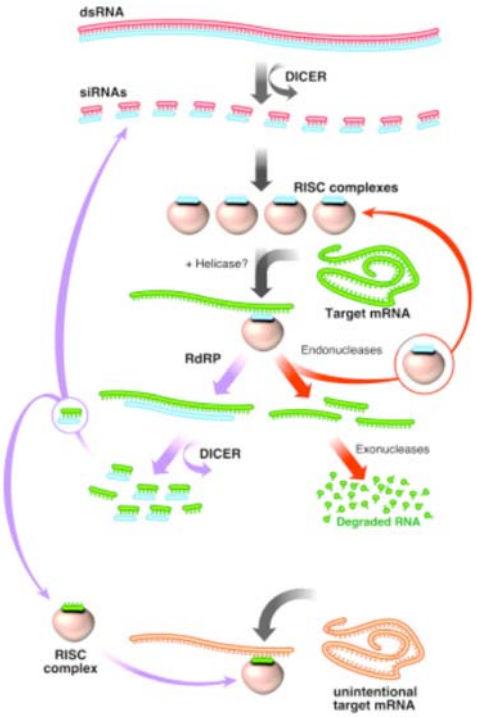
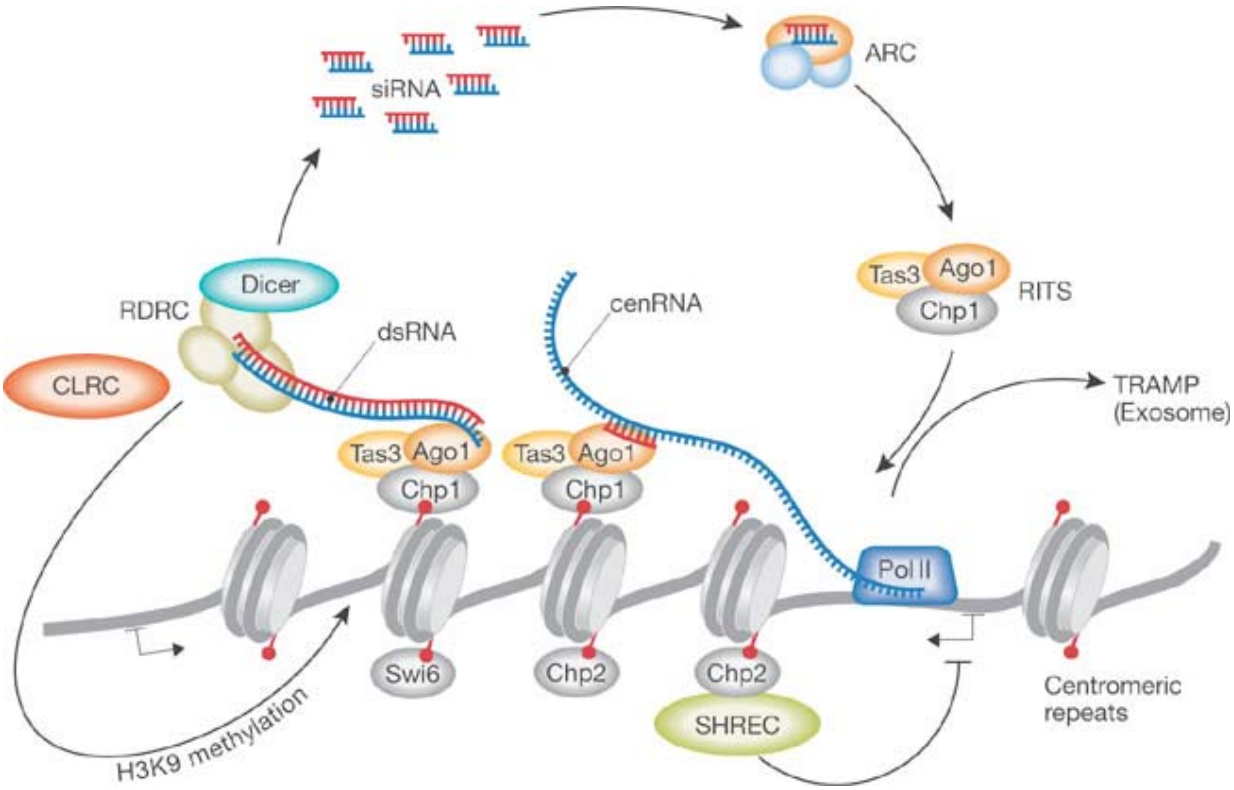
O Complexo RISC



Amplificação da interferência de RNA



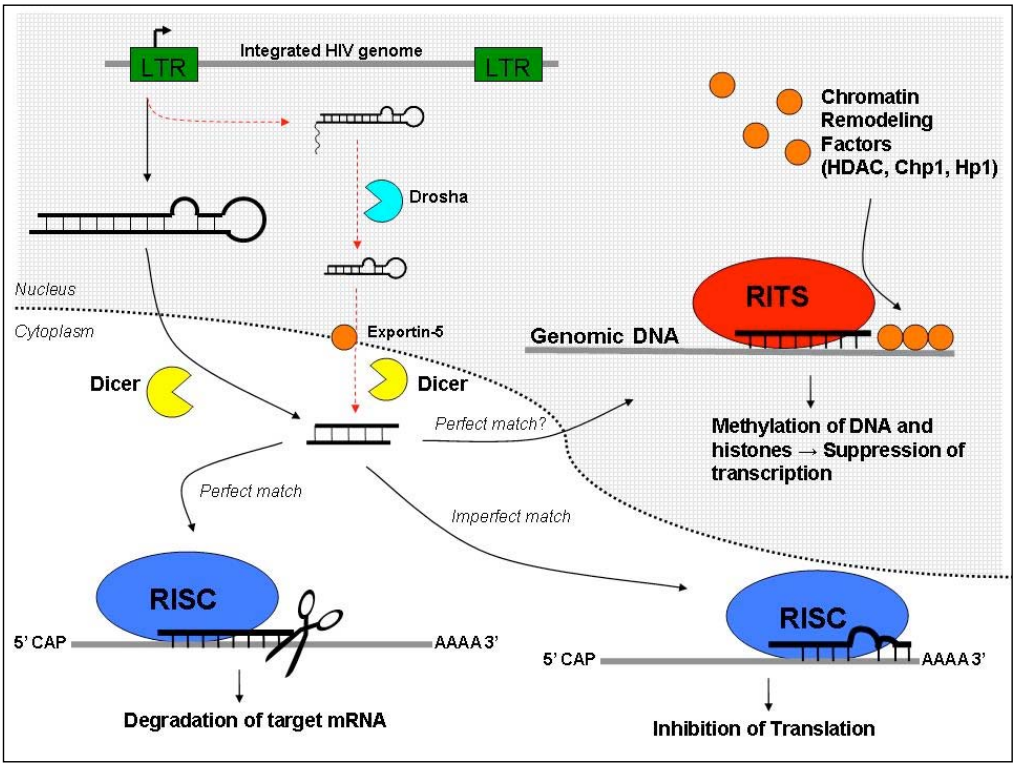
O Complexo RITS



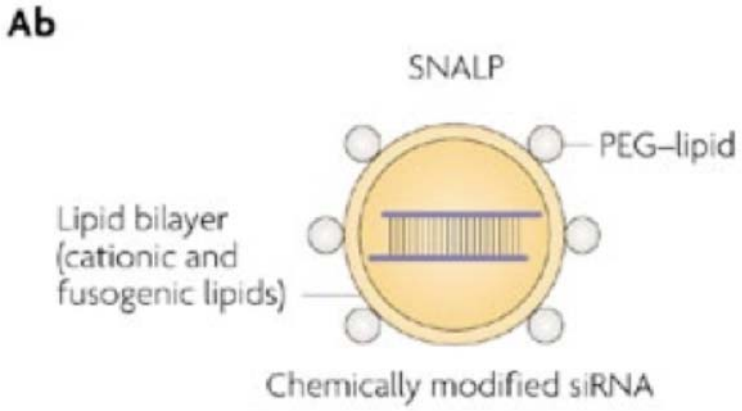
Para que é que isto serve?

- Defesa contra transposões e genomas virais!
- Mas como sempre os vírus evoluíram armas de contra-ataque e aproveitamento dos mecanismos celulares:

O HIV aproveita a via do complexo RITS para induzir a latência dos genomas integrados



siRNAs no silenciamento de genes – uma nova arma na investigação e terapêutica

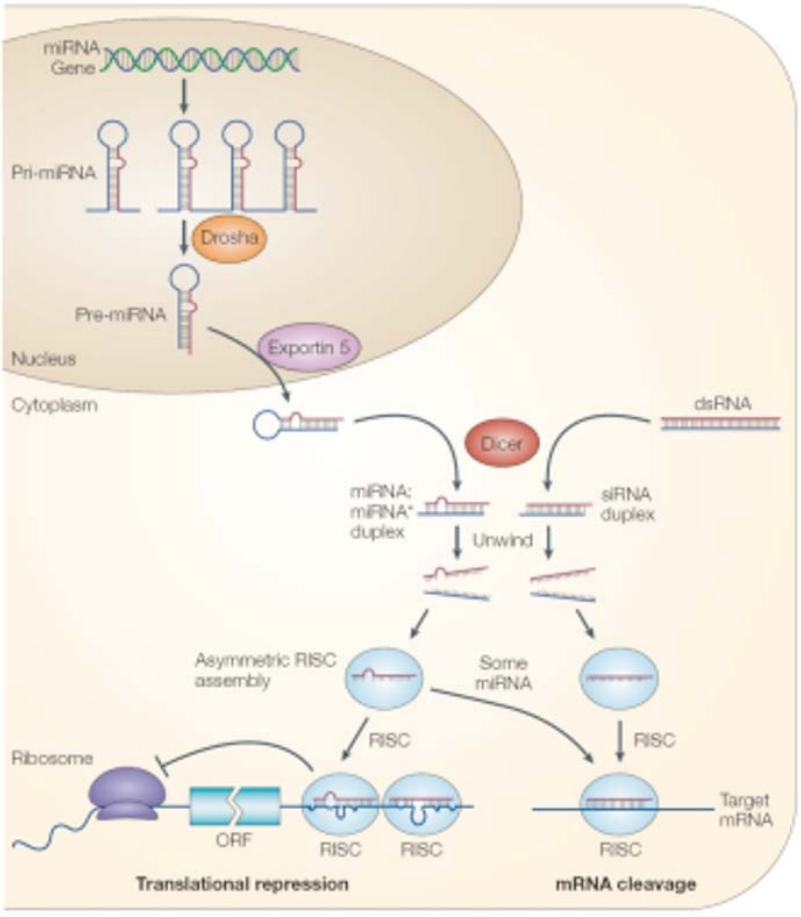


A descoberta dos microRNAs

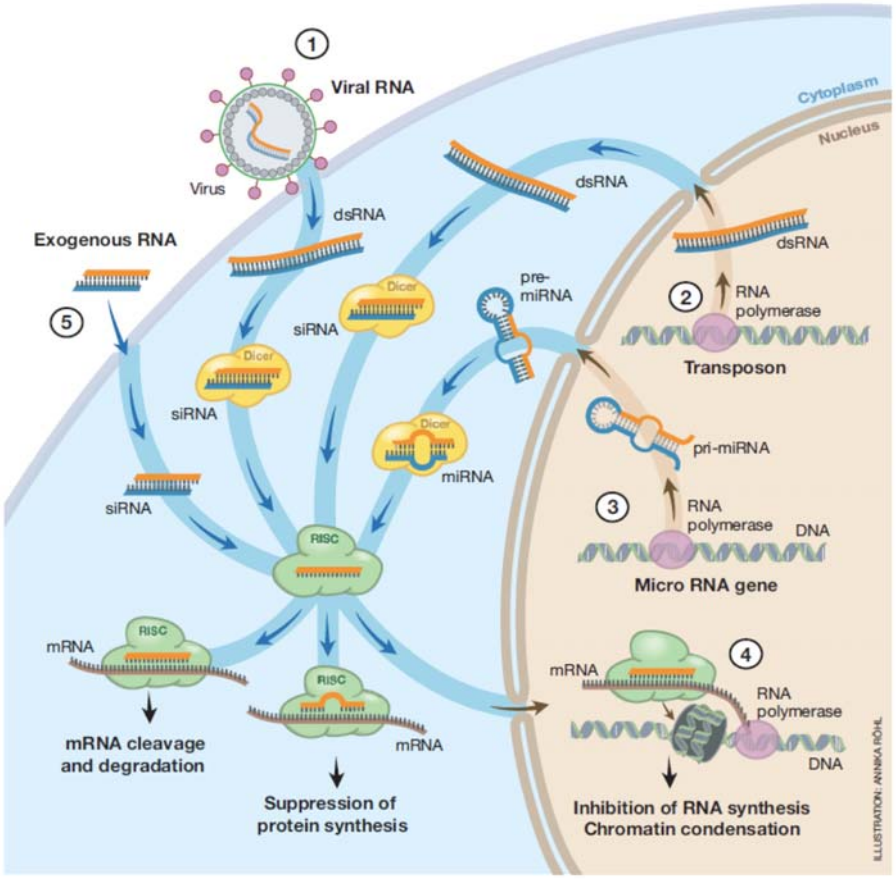
Cell, Vol. 75, 843–854, December 3, 1993, Copyright © 1993 by Cell Press

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

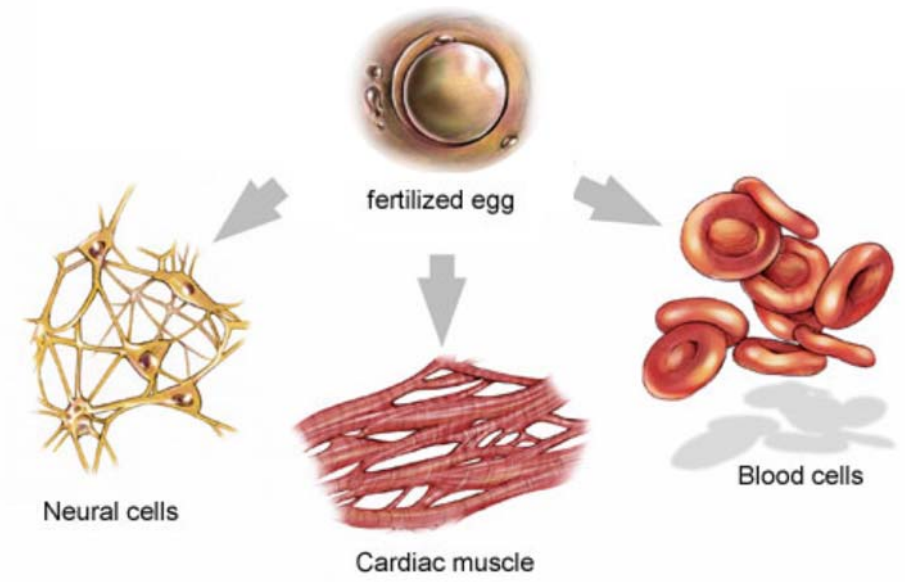
Rosalind C. Lee,*† Rhonda L. Feinbaum,*‡
and Victor Ambros†
Harvard University
Department of Cellular and Developmental Biology
Cambridge, Massachusetts 02138

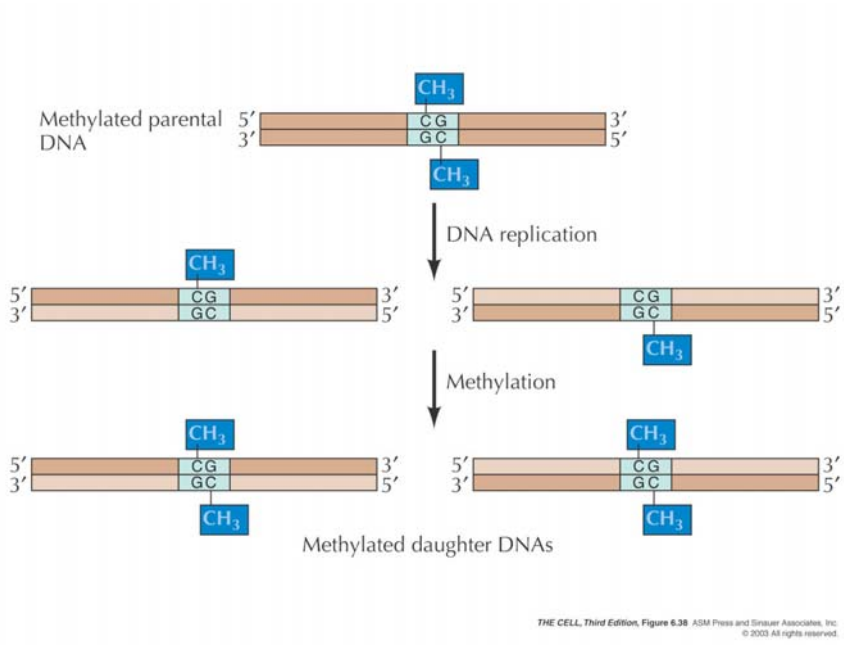


Sumário: mecanismo de acção de siRNAs e miRNAs



RNAs não codificantes e diferenciação celular





Metilação dependente de RNA nas plantas

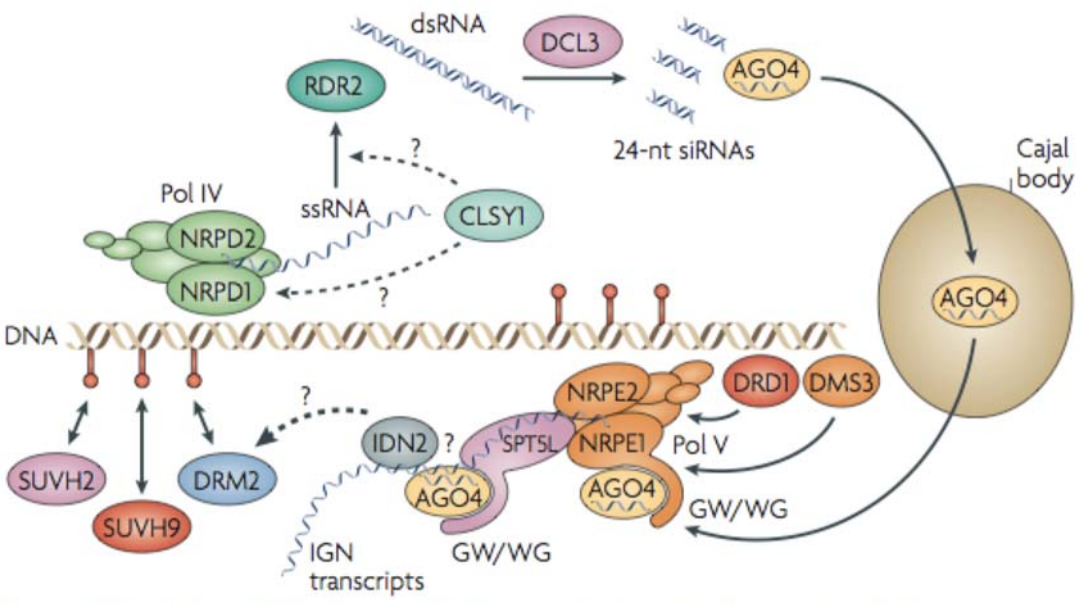


Figure 2 | **Model for RNA-directed DNA methylation.** Single-stranded RNA transcripts

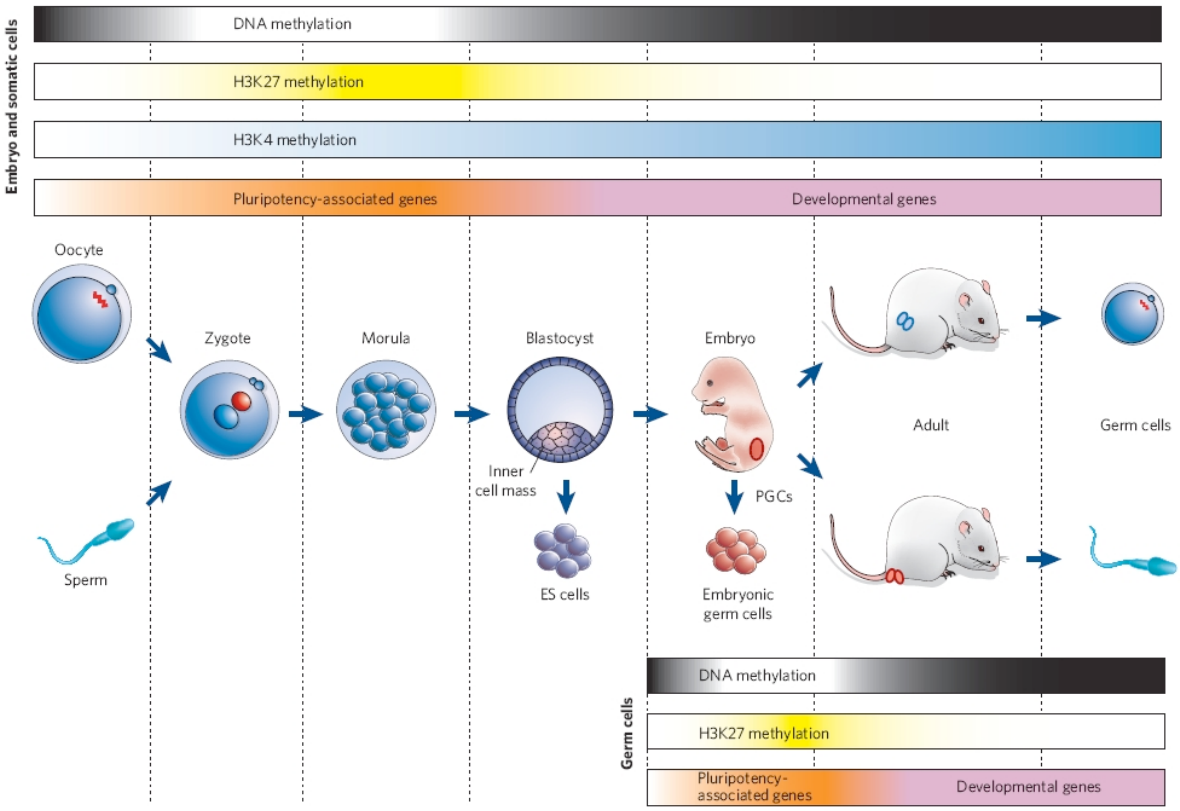
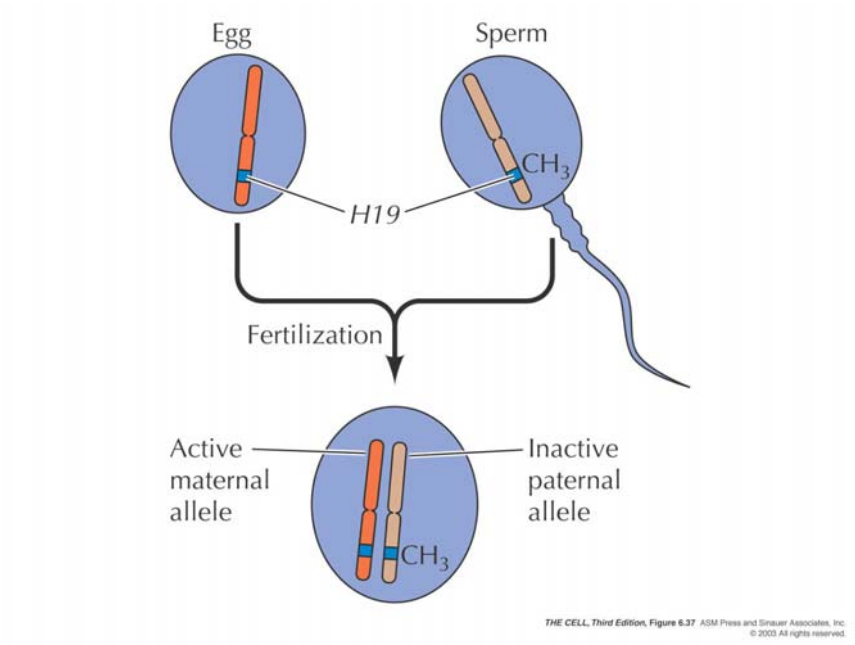


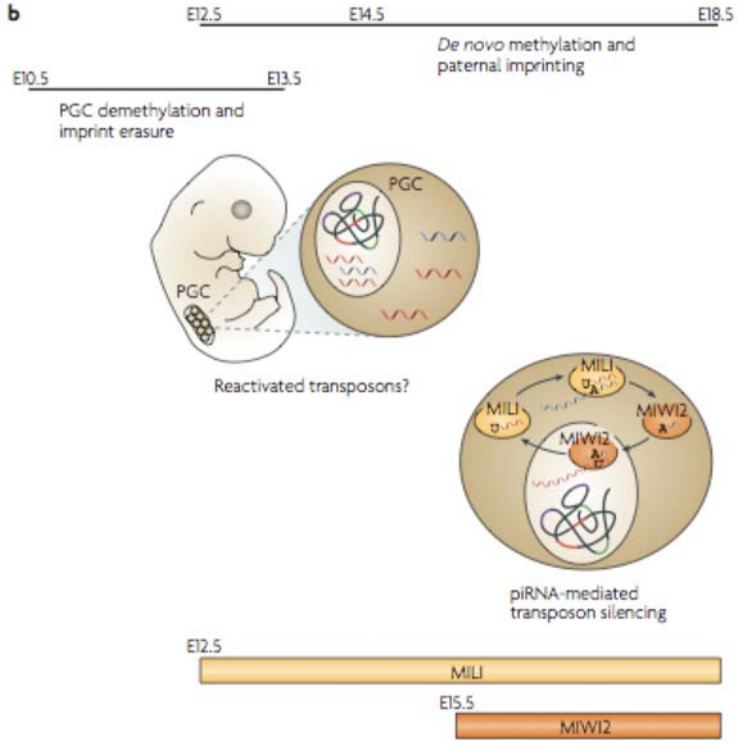
Figure 3: Epigenetic gene regulation during mammalian development.

This figure depicts key developmental events together with global epigenetic modifications and gene expression patterns. Very early in development, DNA methylation is erased. In addition, pluripotency-associated genes begin to be expressed, and developmental genes are repressed by the PcG protein system and H3K27 methylation. During the differentiation of pluripotent cells such as embryonic stem (ES) cells, pluripotency-associated genes are repressed, potentially permanently, as a result of DNA methylation. At the same time, developmental genes begin to be expressed, and there is an increase in H3K4 methylation. During the early development of primordial germ cells (PGCs), DNA methylation and repressive histone modifications (such as H3K9 methylation) are also erased. Pluripotency-associated genes are re-expressed during a time window that allows embryonic germ cells to be derived in culture. Imprinted genes are demethylated during this period, and developmental genes are expressed afterwards. Flexible histone marks such as H3K27 methylation enable developmental genes to be silenced for a short time in pluripotent cells. By contrast, DNA methylation enables the stable silencing of imprinted genes, transposons, and some pluripotency-associated genes.

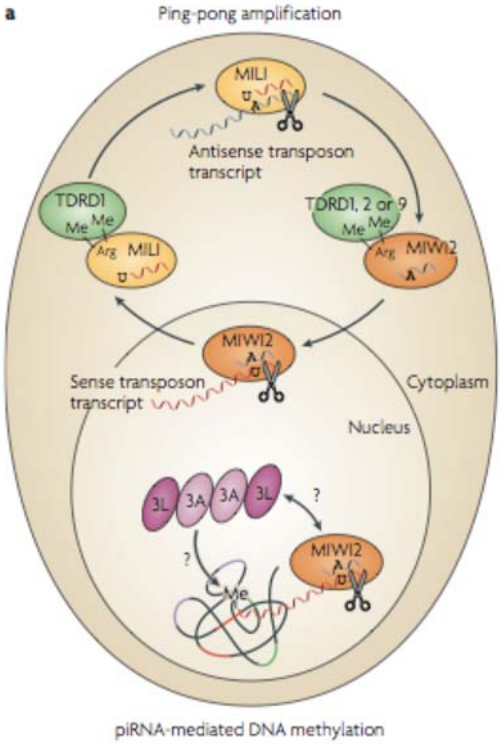
Imprinting de genes paternos e maternos



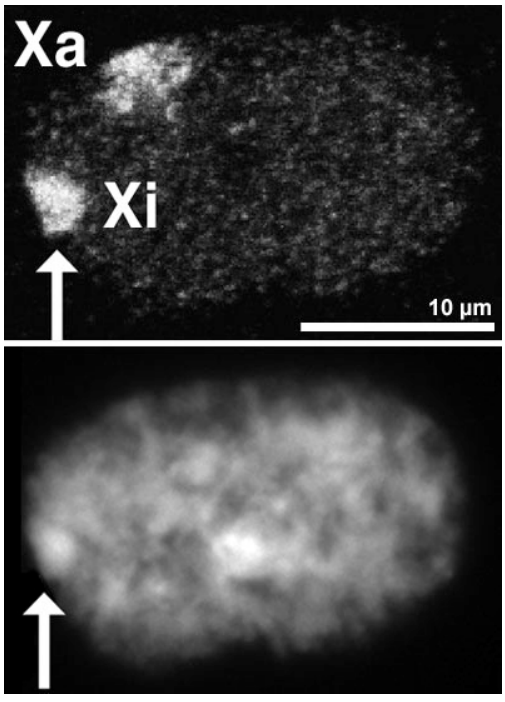
PiRNAs e o silenciamento de transposões



PiRNAs e o silenciamento de transposões



Inactivação do cromossoma X



O RNA Xist