

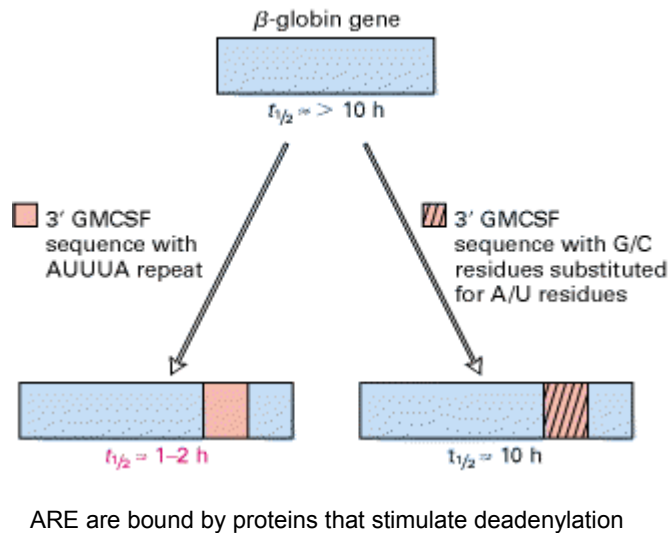
RNA Stability

- Differential Stability
- Regulation of Stability
 - Iron Regulation
- Nonsense Mediated Decay
- RNAi

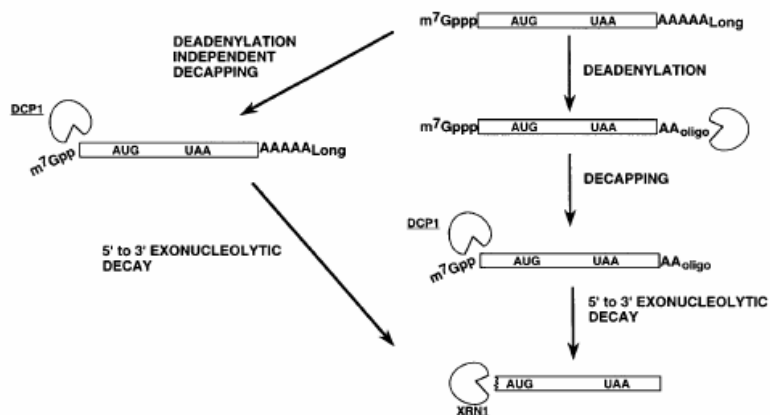
mRNA half life Steady State

| <u>mRNA</u> | <u>Half-Life in Hours</u> |
|-------------------------|---------------------------|
| B-Actin | 60 |
| Dihydrofolate reductase | 97 |
| G3P dehydrogenase | 75-130 |
| Hsp70 | 2 |
| Ornithine decarboxylase | 0.5 |
| C-myc | 0.1 |

AUUUA repeats ARE (A/U Rich Element).



Two Pathways for RNA decay



Regulated turnover

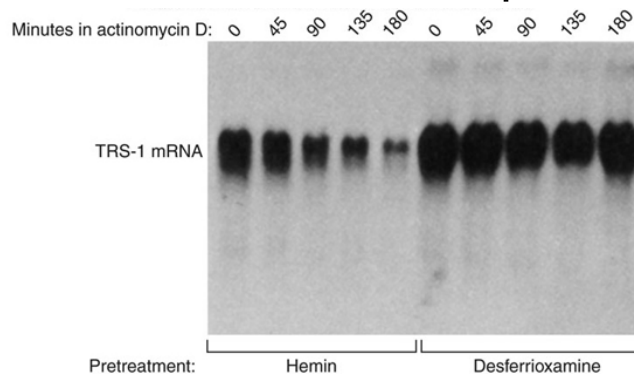
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Table 16.1 Effect of Prolactin on Half-Life of Casein mRNA

| Species of RNA | RNA Half-life (h) | |
|--|-------------------|-------------|
| | – Prolactin | + Prolactin |
| rRNA | >790 | >790 |
| Poly(A) ⁺ RNA (short-lived) | 3.3 | 12.8 |
| Poly(A) ⁺ RNA (long-lived) | 29 | 39 |
| Casein mRNA | 1.1 | 28.5 |

Source: Reprinted from Guyette, W.A., R.J. Matusik, and J.M. Rosen, Prolactin-mediated transcriptional and post-transcriptional control of casein gene expression. *Cell* 17:1013, 1979. Copyright © 1979, with permission from Elsevier Science.

Transferrin Receptor



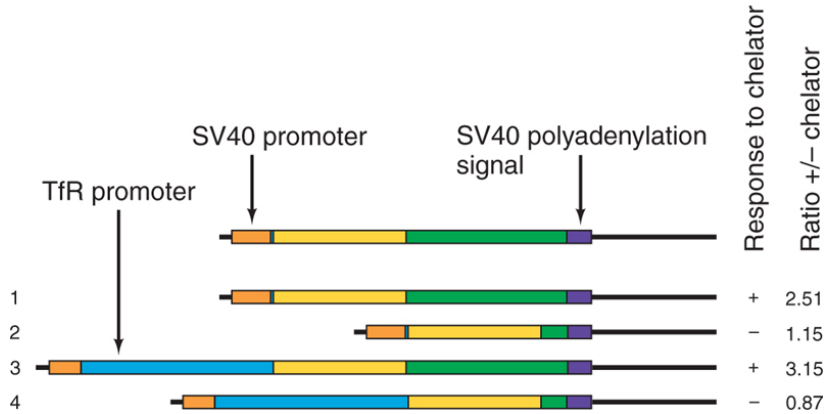
Northern Blot for fibroblast transferrin receptor mRNA over time as a function of two different concentrations of iron.

Hemin treatment of the cells leads to increased intracellular iron concentrations = Shorter half-life of ~45 min.

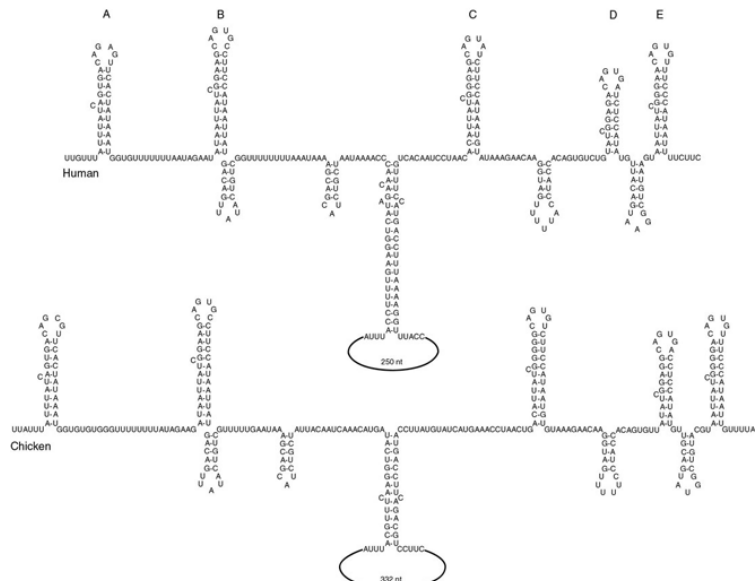
Desferrioxamine treatment of cells leads to lower intracellular iron concentrations = Longer half-life of >180 min.

Deletion analysis of Iron Responsive Region

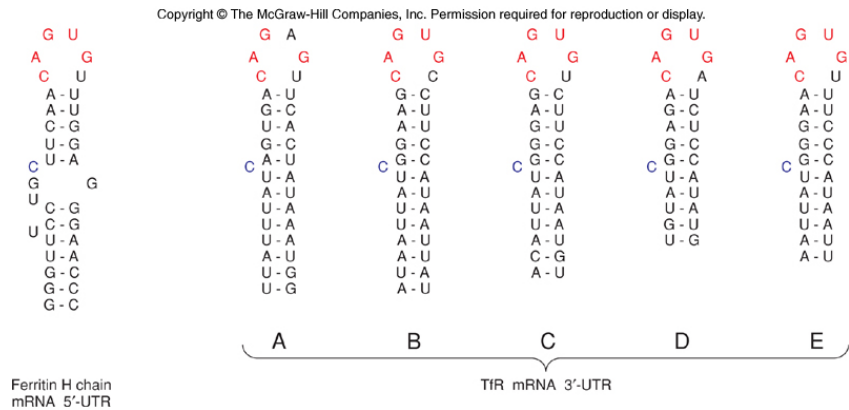
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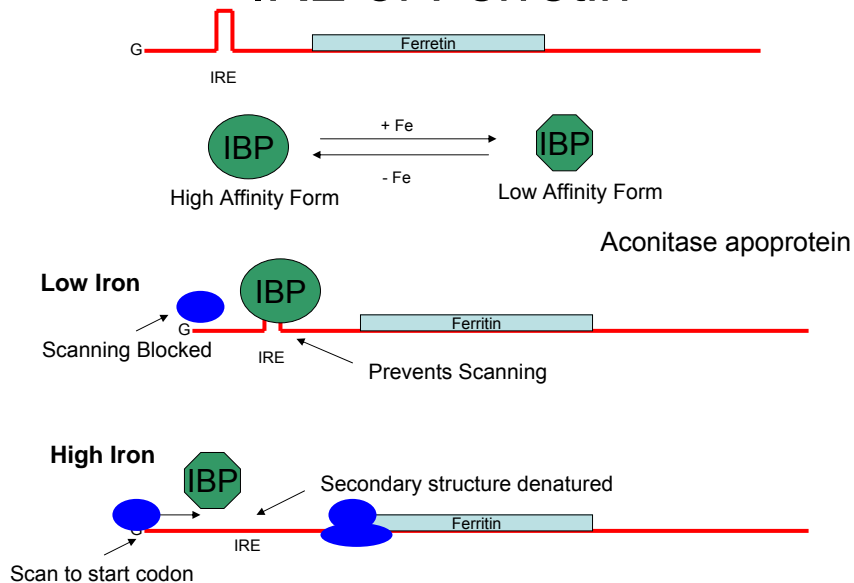
Iron Responsive Region



Comparison to ferretin IRE's

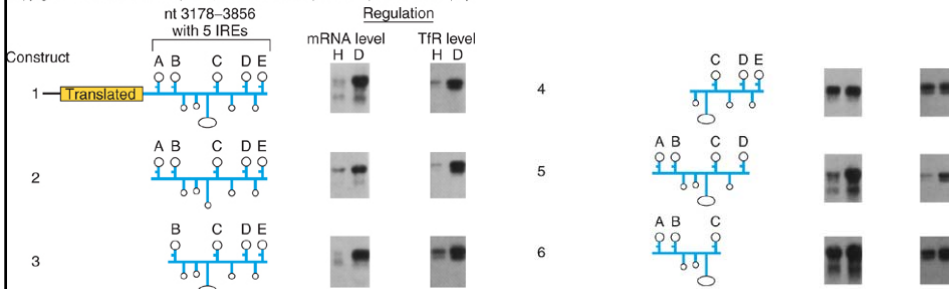


IRE of Ferretin



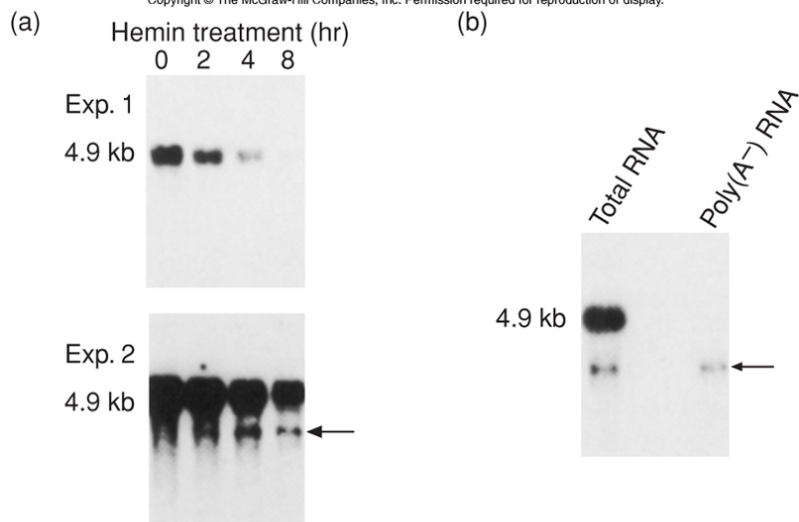
Deletion of IRE like elements

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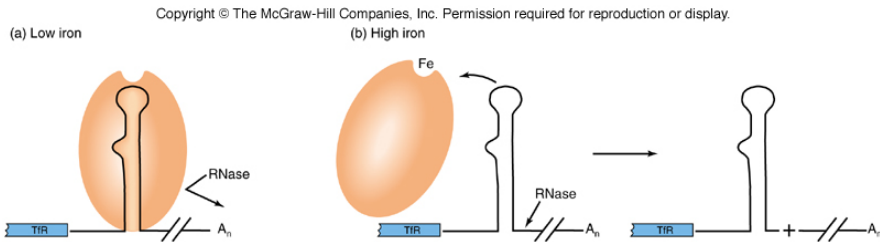
Nuclease Action

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© Binder, R., J.A. Horowitz, J.P. Basilion, D.M. Koeller, R.D. Klausner, and J.B. Harford. Evidence that the pathway of transferrin receptor mRNA degradation involves an endonucleolytic cleavage within the 3' UTR and does not involve poly(A) tail shortening

Transferrin Receptor Regulation The Model



Non-sense Mediate Decay (NMD)

C.I. González et al. / Gene 274 (2001) 15-25

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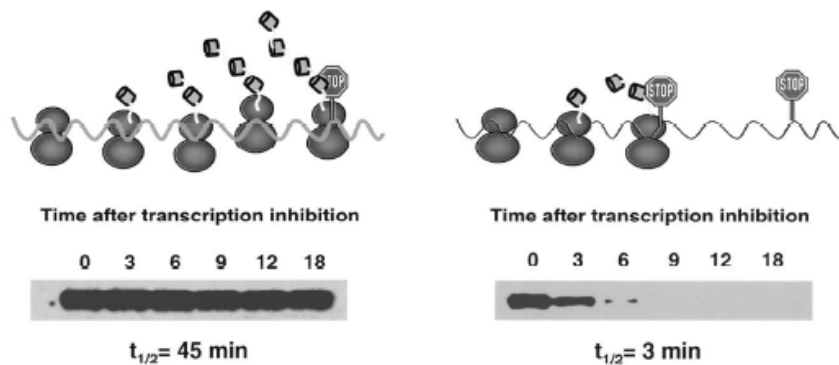
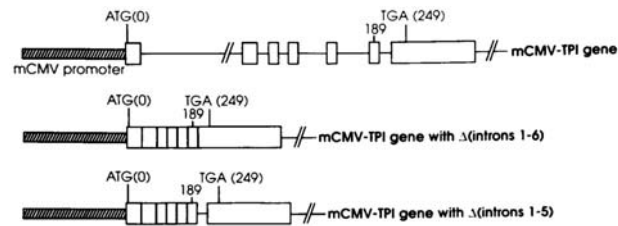
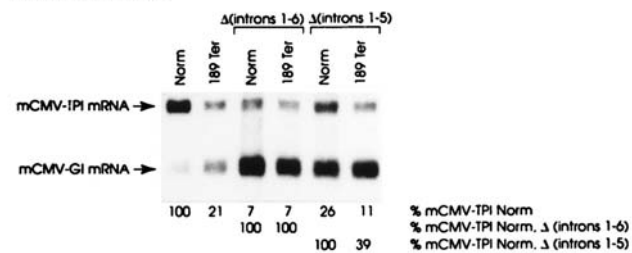


Fig. 1. The effect of a premature termination codon on the decay of the PGK1 mRNA. Decay rates for the mRNAs encoded by the wild-type and the nonsense-containing PGK1 alleles were determined by Northern blot analysis of RNAs isolated at different times after transcription was inhibited by a shift from 24 to 37°C in a yeast strain harboring a temperature-sensitive RNA polymerase II.

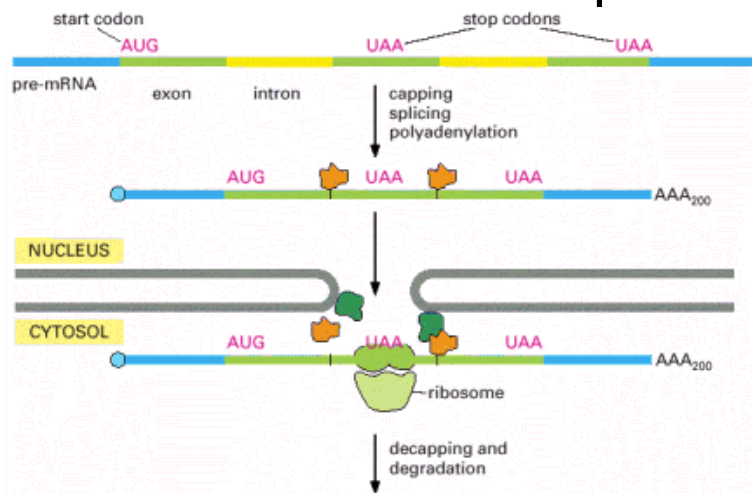
NDM requires an intron



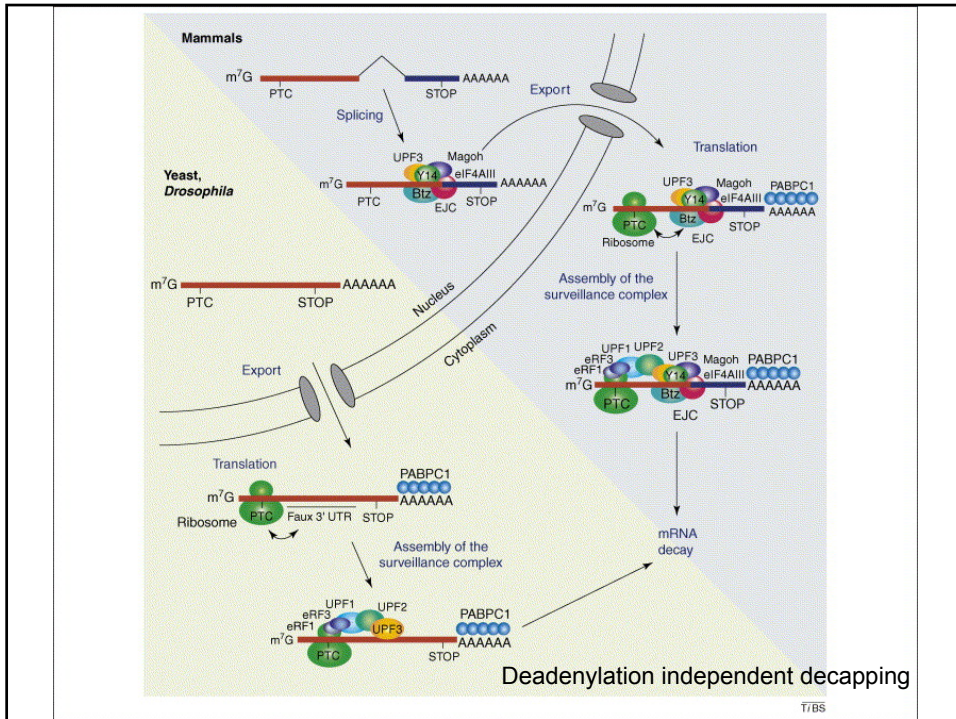
A. Total-Cell RNA



Exon Junction Complex



Alberts



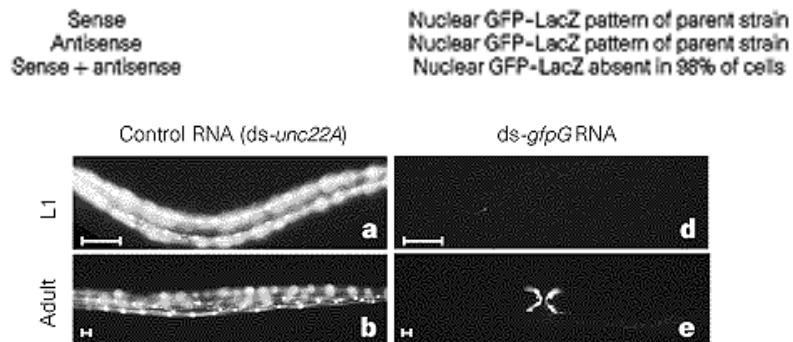
RNA Interference (RNAi)

- Gene silencing with dsRNA
- Generation of miRNA from dsRNA
- RISC targeted mRNA destruction
- Translational Inhibition
- Amplification of RNAi
- Transcriptional Inactivation

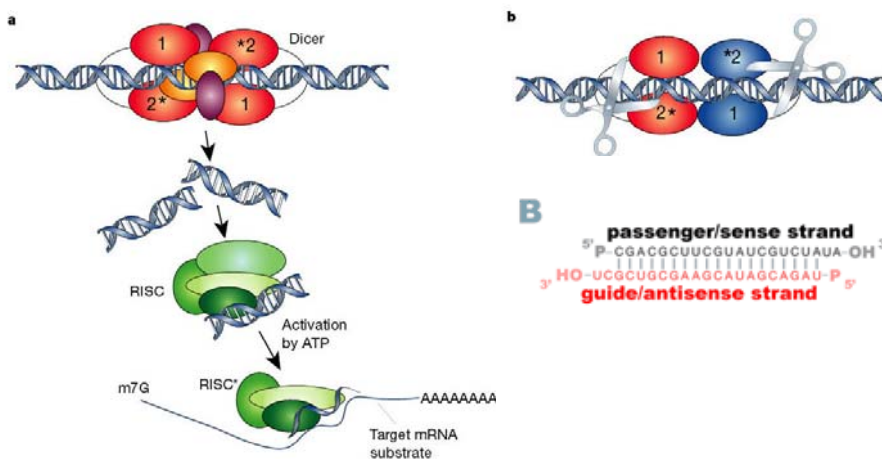


Fire and Mello

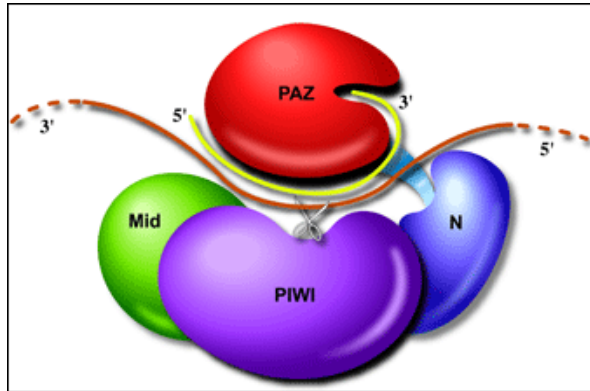
- GFP Transgenic Worms
- Injected Sense RNA, Antisense RNA and Mixture of antisense and sense RNA into ovaries
- Observe GFP expression in offspring



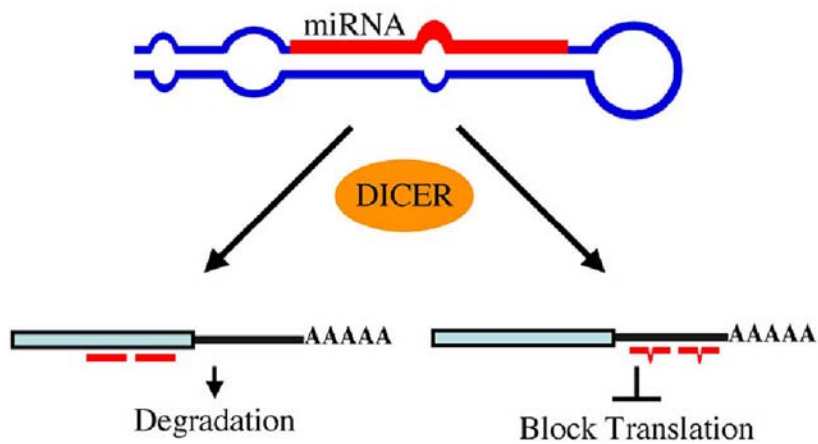
Double Stranded RNA and Dicer



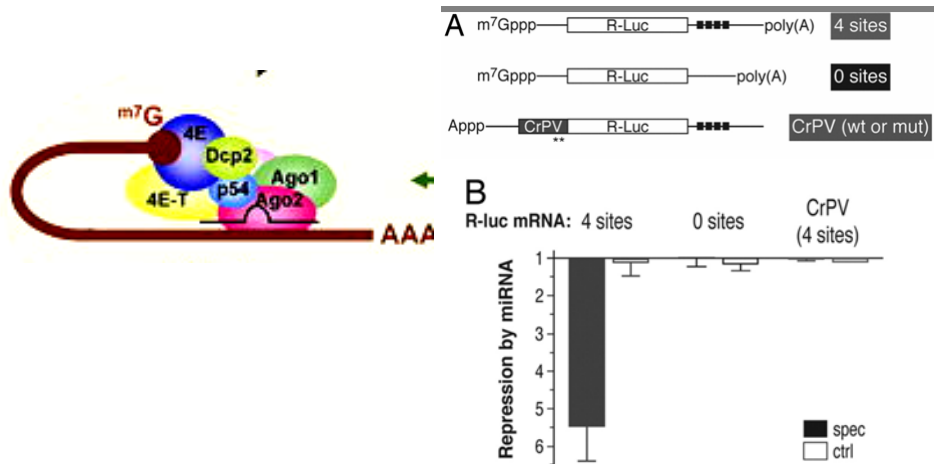
RISC and Argonaute



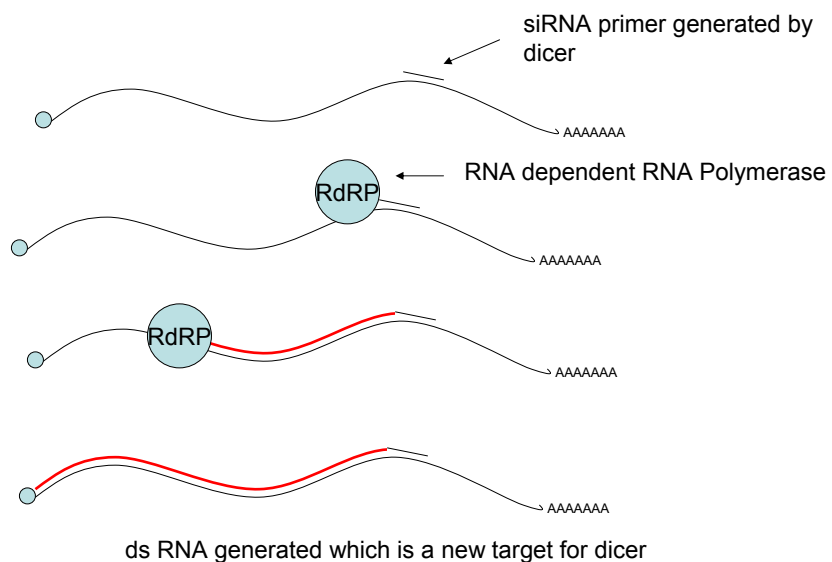
RNAi and Translation

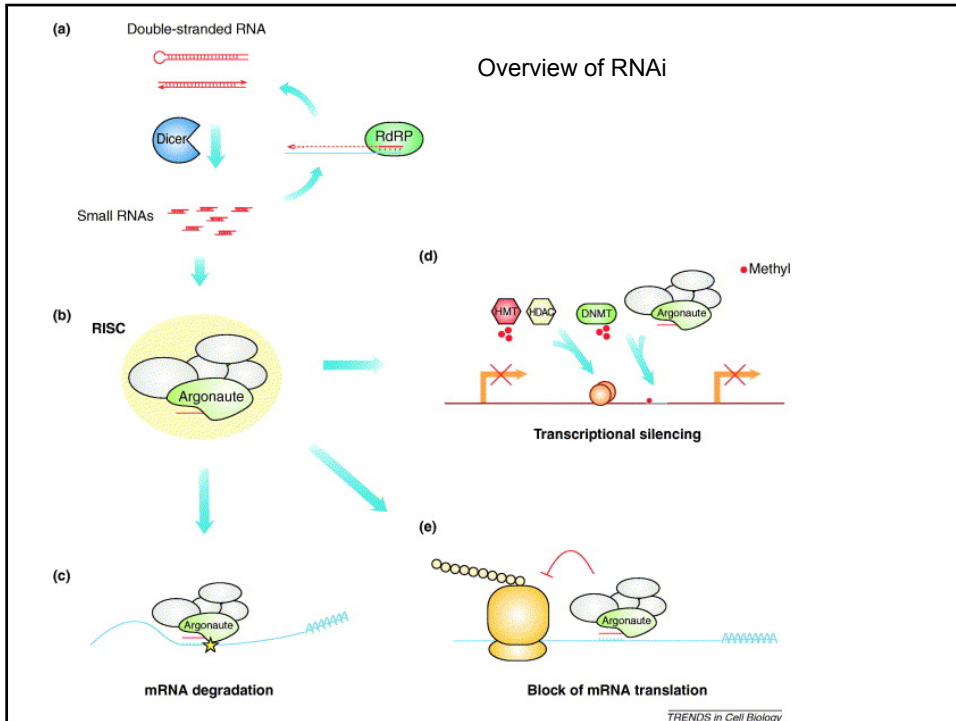


Translational Inhibition

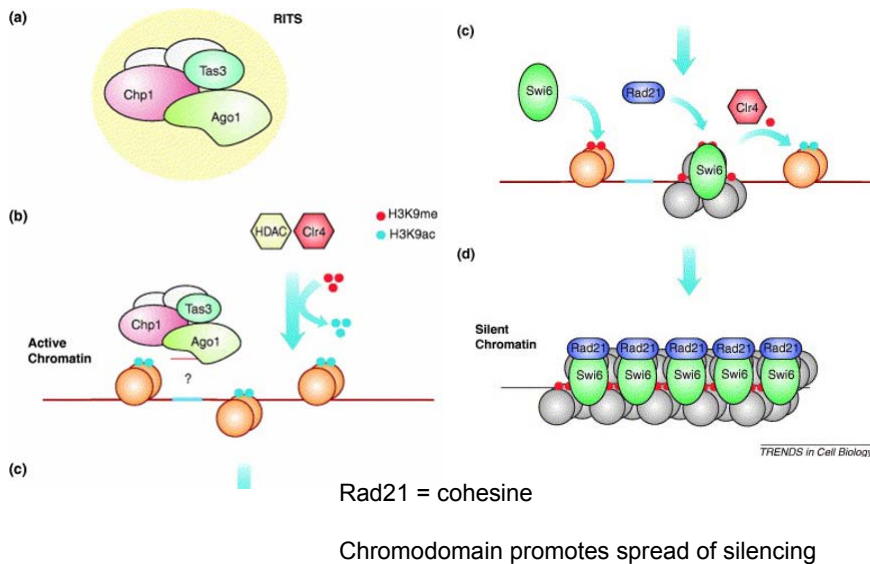


RNAi Amplification by RNA dependent RNA Polymerase





RNAi Transcriptional Silencing



miRNA's

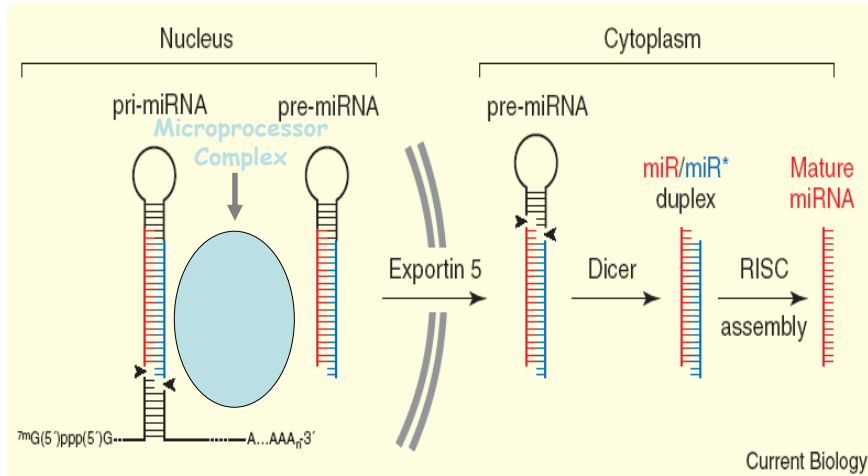
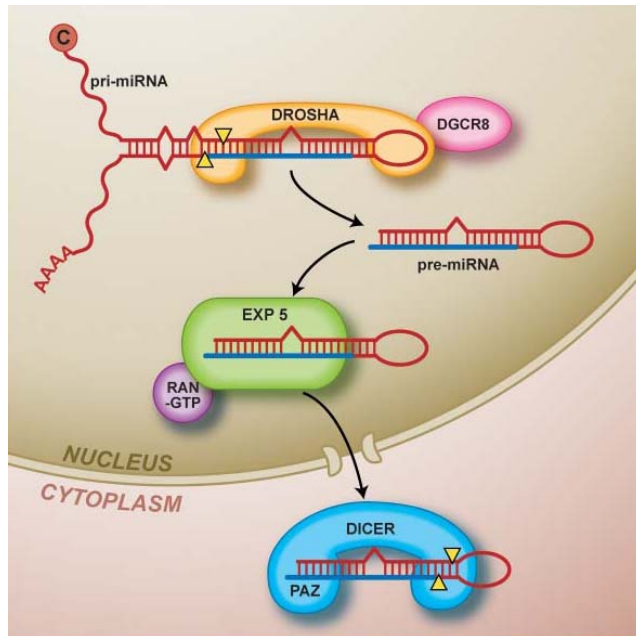


Illustration of miRNA processing



Differences in miRNA Mode of Action

